University of Alberta

Development of Isotope Labeling Liquid Chromatography Mass Spectrometry for Metabolome Analysis

by

Kevin Kun Guo

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Department of Chemistry

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Abstract

As the endpoint of the "omics" cascade, metabolomics has attracted much attention in the discovery of diagnostic or prognostic disease biomarkers, and as a powerful tool to understand biological and biochemical processes and mechanisms. The objective of my research was to develop a highly sensitive and reliable method for quantitative and qualitative metabolome analysis, based on a global ^{13}C-/^{12}C-stable isotope labeled internal standards (SIL IS) strategy and reversed-phase liquid chromatography (RPLC) Fourier Transform Ion Cyclotron Resonance (FT ICR) Mass Spectrometry (MS).

In conventional LC-MS-based metabolome analysis, quantification is mainly based on the peak intensities, whilst qualification is based on accurate mass measurement. The SIL IS strategy, which provides isotopic labeled internal standards for every targeted analyte, has been proved to be the most effective approach to overcome ion suppression and matrix effects. Thus, it offers the most accurate quantification and confident identification.

This thesis has focused on four aspects of current metabolomics research: (1) development of chemical derivatization chemistries to improve electrospray ionization (ESI) response and reversed-phase liquid chromatography separation of human metabolites, with particular focus on polar, and/or non-ESI ionizable metabolites; (2) development of a novel ^{13}C-/^{12}C- differential isotope labeling (DIL) strategy for accurate quantification and confident identification of human metabolites; (3) development of a software tool for DIL quantification and putative and definitive identification; and (4) construction of a comprehensive, targeted metabolite library.

^{13}C-/^{12}C-dansylation targeted primary and secondary amines, and phenolic hydroxyl metabolites. An isotope labeling method, based on the use of ^{13}C-/^{12}C-isotope-coded p-dimethylaminophenacyl (DmPA) bromide as a reagent, targeted carboxylic acid-containing metabolites (CAMs). Both ^{13}C-/^{12}C-labeling methods offered several desirable features, including simple and robust experimental procedures, no isotopic effects on reversed-phase separations,

significant ESI enhancement, and improvement of the reversed-phase separation. The construction of a comprehensive ^{13}C-/^{12}C-labeled metabolite library and software tool ensured that accurate quantification and confident identification of metabolites could be carried out in a high-throughput, automatic fashion.

The DIL strategy is a global internal approach that could be applied to many other LC-MS applications beyond metabolome analysis.

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List of Abbreviations

ACN	Acetonitrile
BPC	Base peak chromatogram
CAMs	Carboxylic acid-containing metabolites
CID	Collision-induced dissociation
CSF	Cerebrospinal fluid
DIL	Differential isotope labeling
DMF	N,N-dimethylformamide
DmPABr	p-dimethylaminophenacyl bromide
Dns	Dansyl chloride
EIC	Extracted ion chromatogram
ESI	Electrospray ionization
fmol	Femtomole
FTICR	Fourier-transform ion cyclotron resonance
FWHM	Full width at half maximum
GC	Gas chromatography
GC-MS	Gas chromatography coupled with mass spectrometry
h	Hour
HILIC	Hydrophilic interaction chromatography
HMDB	Human metabolome database
HPLC	High performance liquid chromatography
IP	Ion pair
IT	Ion trap
iTRAQ	isobaric tag for relative and absolute quantitation
LC	Liquid chromatography
m	milli- (10^{-3})
m/z	mass to charge
MeOH	Methanol
min	Minute

MRM	Multiple reaction monitor
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
n	nano- (10^{-9})
NMR	Nuclear magnetic resonance
PBr	Phenacyl bromide
ppm	part(s) per million
QTOF/QqTOF	Quadrupole time-of-flight
RF	Radio frequency
RP	Reversed-phase
RSD	Relative standard derivation
S/N	Signal to noise ratio
SIL	stable-isotope-labeled
SIM	Single ion monitor
TEOA	Triethanolamine
TLC	Thin layer chromatography
TOF	Time-of-flight
μ	micro- (10^{-6})

Chapter 1

Introduction to Mass Spectrometry-Based Metabolomics

1.1 Overview of Metabolomics

Metabolomics, one of the most rapidly growing areas of research in recent years,[1, 2] is defined as the global identification and quantification of all small molecules (<1500 Da), thus excluding proteins and nucleic acids, often referred to as the metabolome, that are present in a biological system. Metabolomics is a global approach in an attempt to collect quantitative and qualitative data from all metabolites to gain a systematic survey of metabolic pathways and metabolic networks of a biological system.[3-8] When compared to "Classical" biochemical approaches, metabolomics will provide a more complete and detailed description of the complex interactions in metabolic networks. Differing from genomics or proteomics, metabolomics focuses on high-throughput measurement of small molecule metabolites, the 'downstream products' of proteins, genes, environmental influences, diseases and drug exposure (Figure 1.1).[3, 4, 8] As the metabolome is believed to be the closest representative of the phenotype, it is the best reporter of phenotype or disease state.[8, 9] As the endpoint of the "omics" cascade, metabolomics should ultimately provide an overall picture of the metabolic pathways involved in the interaction of proteins, encoded by the genome, with environmental factors and drug exposure. Thus, it can potentially lead to a better understanding of biological pathways and to disease biomarker discovery.

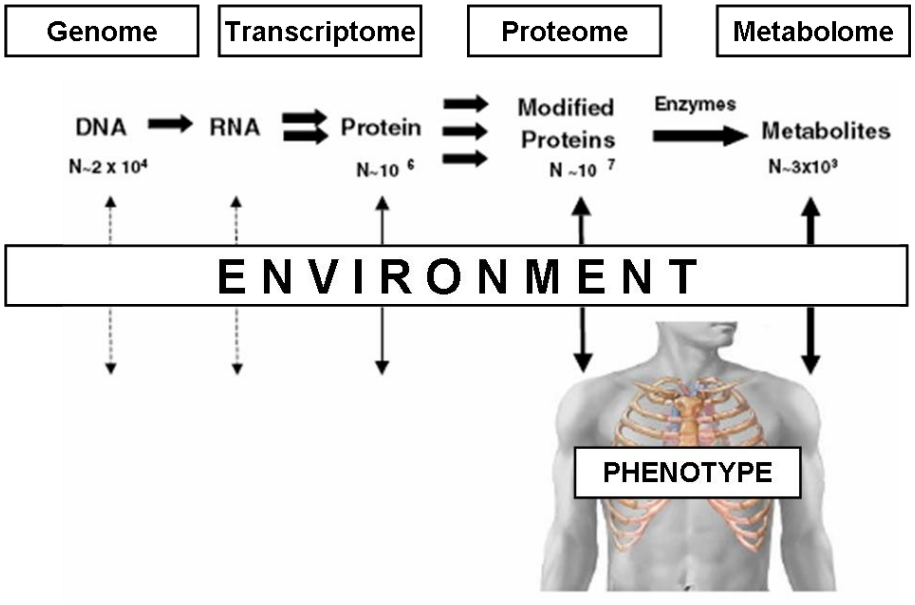

Figure 1.1 Integration of metabolomics with other "Omics" approaches and relationship to phenotype. Reproduction with permission.

The number of endogenous human metabolites was recently estimated at approximately three thousand.[10] The endogenous metabolites are synthesized within the organism, tissue or cell. The exogenous metabolites do not originate in the body, but are consumed as food or produced by host-specific microbes. The number of the exogenous metabolites is far greater.[2] The human metabolome consists of a variety of compound classes whose chemical and physical properties are extremely diverse (Table 1.1).[10, 11] Metabolites present in body fluids or tissues occur in a wide concentration range of approximately 7-9 orders of magnitude (pmolar to mmolar).[5, 12] Currently, no single analytical platform is capable to analyze all metabolites at once.

Systems biology represents the ultimate challenge in that it aims to understand and predict globally the behavior of complex biological systems through integrating the analytical outcomes from all levels of gene products:

genome, transcriptome, proteome and metabolome.[8] Essentially, systems biology looks at the whole picture of how individual pathways or metabolic networks are related. So far, such studies have not been reported. As a critical part of systems biology, the field of metabolomics is still in its infancy and is still facing many analytical and data mining challenges.

Table 1.1 Chemical classes in the human metabolome database (HMDB) (data from Wishart *et al*.[10, 11]) Reproduction with permission.

Acyl glycines	Inorganic ions and gases
Acyl phosphates	Keto acids
Alcohol phosphates	Ketones
Alcohols and polyols	Leukotrienes
Aldehydes	Lipoamides and derivatives
Alkanes and alkenes	Minerals and elements
Amino acid phosphates	Miscellaneous
Amino acids	Nucleosides
Amino alcohols	Nucleotides
Amino ketones	Peptides
Aromatic acids	Phospholipids
Bile acids	Polyamines
Biotin and derivatives	Polyphenols
Carbohydrates	Porphyrins
Carnitines	Prostanoids
Catecholamines and derivatives	Pterins
Cobalamin derivatives	Purines and purine derivatives
Coenzyme A derivatives	Pyridoxals and derivatives
Cyclic amines	Pyrimidines and pyrimidine derivatives
Dicarboxylic acids	Quinones and derivatives
Fatty acids	Retinoids
Glucuronides	Sphingolipids
Glycerolipids	Steroids and steroid derivatives
Glycolipids	Sugar phosphates
Hydroxy acids	Sugar phosphates
Indoles and indole derivatives	Tricarboxylic acids

1.2 NMR Metabolomics

To date, most metabolome analysis has been performed by three major analytical platforms, nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC/MS), and liquid chromatography mass spectrometry (LC-MS).[13-18] NMR is one of the principle techniques used to elucidate the molecular structure information. NMR uses resonant frequencies of the nuclei present in the molecule to determine the number and type of chemical entities in a molecular structure. NMR has been widely used in thousands of applications for "classical" metabolic studies since the early 1970s.[19] The great advantages of metabolomic NMR are that it can provide both quantitative and qualitative information, requires minimal sample preparation, needs no chemical derivatization and is a non-destructive, non-discriminating technique.[19] NMR spectra can be quickly and easily collected. Metabolomic NMR is particularly useful in the case of those metabolites that are less amenable to LC-MS and GC/MS analysis, for example, sugars, amines, and volatile ketones.[19] However, as NMR is a relatively insensitive technique, with a limit of detection of about 1-5μM, it can unambiguously detect only medium to high abundance metabolites.[19] In addition, NMR analysis requires relatively large sample volumes of about 500 μL for a regular probe. The processing and interpretation of metabolomic NMR spectra from a complex biological sample also remain a challenge as a result of the lack of separation prior to analysis.[19]

1.3 GC/MS Based Metabolomics

In contrast to NMR-based metabolomics, mass spectrometry coupled with prior chromatographic separation offers far greater sensitivity by about 3-4 orders of magnitude. MS-based methods are more feasible for the analysis of many biofluids, such as CSF samples, for which often only a limited sample volume is available. Gas chromatography interfaced with electron impact ionization MS (EI-MS) offers high chromatographic separation, and is capable of identifying and quantifying known and unknown metabolites. However, the analysis of polar and non-volatile metabolites, that constitute a large proportion of human metabolites, requires chemical derivatization to reduce polarity and increase thermal stability and volatility. Often it is preferable to dry down biological samples thoroughly prior to many GC derivatizations to avoid contact with moisture that results in derivative degradation or incomplete derivatization reaction. Liquid extraction, lyophilization and often multiple dry-down steps are included in the sample preparation for the GC-MS analysis of biological samples. Some metabolites might be incompletely extracted and losses will occur when the metabolites are highly volatile.[9] Volatile metabolites can include alcohols, aldehydes, furans, ketons, pyrroles, terpenes, and many small carboxylic acids.[9] In addition, the high-energy (typically 70 eV) EI spectra hardly show any molecular ions (often below 1%).

1.4 Reversed-phase Liquid Chromatography

Reversed-phase liquid chromatography (RPLC) interfaced with mass spectrometry via electrospray ionization (ESI) offers quantitative and qualitative analyses with high selectivity and sensitivity and has great potential to be the

mainstream technique for metabolomics.[9, 20, 21] Thousands to tens of thousands of components may be present in a complex metabolomic sample. High resolution separation prior to mass spectrometric analysis is essential to reduce ion suppression and complexity of the mass spectra in a time dimension.[1] RPLC is naturally compatible with electrospray ionization and water-based biological samples, offers the high separation resolution and reproducible compound-specific retention that can be used as a secondary criterion for identification. RPLC using C-18 or C-8 narrow bore columns with particle sizes of 3-5 μm is the standard set-up for separation of moderately polar and non-polar metabolites in metabolomic investigations. To produce statistically meaningful data, quantitative profiling of a metabolome often involves analyzing large numbers of samples. Therefore, fast analysis is highly desirable to improve sample throughput. One approach to high-throughput chromatographic separation is to use a column packed with smaller particles, such as sub-2 μm particle columns used in ultra performance liquid chromatography (UPLC).[22, 23] A downside of using sub-2μm particle columns is the high back-pressure (10,000 to 15,000 psi) generated, and therefore an HPLC pump that can withstand very high back-pressure is required. One consequence of high back-pressure of sub-2 μm particle columns is heat dissipation.[24] A fraction of the mobile phase against the stationary phase is heated.[25] The amount of heat produced increases with increasing pressure drop and flow rate. The dissipation of the heat creates radial and axial temperature gradients. The difference between the temperatures at the center and at the column wall can be as much as 6 K and the difference between the temperatures

at the inlet and the outlet of the column can be up to 20 K. Temperature gradients have a negative impact on the column efficiency due to heterogeneous distribution of the mobile phase linear velocity, viscosity and density along the column.[24, 25] A shorter (for example 5 cm) sub-2 μm particle column can be used to achieve fast separation while still maintaining sufficient column efficiency and peak capacity for separation of a large number of metabolites in a complex biological sample,[26] thus overcoming the downsides of high pressure and heat dissipation generated in long (for example 15 cm) columns. An additional benefit of using a short column is that an ultrahigh pressure pump is no longer mandatory; a conventional HPLC system (with a maximum pressure of 400 bar) can readily handle the pressure produced by a 5 cm sub-2μm particle column. However, care should be taken with sample preparation because finer frits are commonly used in front of these columns.

1.5 Mass Spectrometry

1.5.1 Electrospray Ionization

My thesis focuses on human metabolome analysis by reversed-phase liquid chromatography coupled with electrospray ionization mass spectrometry. Ionization is the critical event because only ions can be measured in mass spectrometry. Electrospray had been used for electrostatic dispersion of liquids for a long time before its application to analytical mass spectrometry. For example, in car manufacture, electrospray is used in the application of paints and coatings to metal surfaces. Very small charged droplets of paint produced by electrospray are attracted to the metal surface of the car. The fine spray deposits as a very smooth

and even film, and thus paint is used more efficiently. Electrospray MS is an analytical technique that transfers ions from solution to the gas phase and then subjects them to mass spectrometry analysis.[27] The electrospray mechanism by which the gas-phase ions are produced from tiny charged droplets is not fully understood. [28, 29] However, the most plausible mechanisms provide a theoretical guide to optimal results by rational choices of experimental parameters and designs that are often neglected by modern mass spectrometer users.[30]

There are four main steps in the production of gas-phase ions from ions in solution.[28, 29] 1) Generation of the charged droplets at the ES capillary tip, shown in Figure 1.2. In positive ion mode, a positive voltage (2-6 kV) is applied to the spray tip where HPLC eluent flows through. To simplify the discussion, only the positive ion mode is considered here. Because the spray capillary tip is very narrow (~1 mm o.d.), a high electric field (~10^6 V/m) at the capillary tip will partially penetrate the liquid at the tip. The solvent near the meniscus of the liquid will be polarized, leading to the separation of positive and negative electrolyte ions in solution under this electric field. Positive ions will move toward the liquid surface and negative ions drift away from the surface (Figure 1.2). As positive charges accumulate at the liquid surface, the surface becomes destabilized. The surface then is drawn out downfield into a conical shape, referred to as the Taylor cone (in honor of Geoffrey Ingram Taylor who first described the phenomenom).[28, 29] If the electric field is high enough, the Taylor cone becomes unstable, and a fine jet is emitted from its tip (Figure 1.2). The downstream jet becomes unstable because the surface of the fine jet is highly charged by an

excess of positive ions. The repulsion among the positive charges breaks up the jet into a mist of droplets. The length of the unbroken liquid jet increases as the electric field decreases. The positively charged droplets fly downfield at atmospheric pressure towards the counter-electrode, commonly held at ground potential (Figure 1.2). For high flow rates (>10 μL/min) of HPLC eluent, nebulizing gas is added to form a more stable spray. Nebulizing gas enters the spray chamber concentrically though a metal tube that is housed inside the ES capillary.

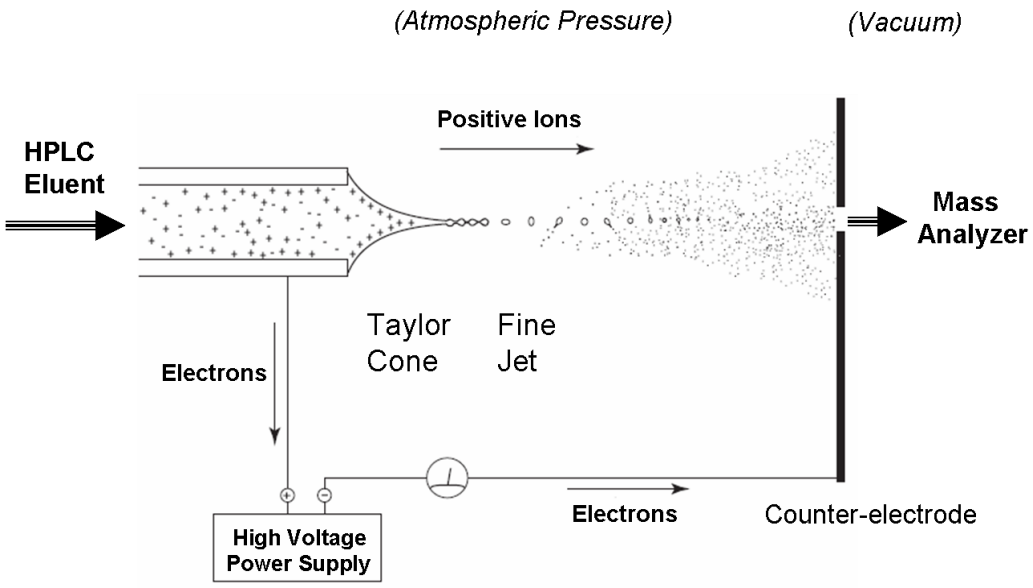

Figure 1.2 Schematic representation of electrospray process

2) Solvent evaporation from droplets causes charged droplet shrinkage. A counter flow of heated dry gas, typically nitrogen, is used to promote evaporation of the solvents from the initially formed droplets. As solvents evaporate, the droplets get smaller.

3) Repeated droplet fission of parent droplets. As droplets continue to shrink by solvent evaporation at a constant rate, the repulsion between the charges at the droplet surface increases until the droplet radius is close to or at the Rayleigh limit where repulsion forces just become sufficient to overcome the surface tension holding the droplet together. The parent droplets become unstable and undergo fission into smaller, charged offspring droplets (Figure 1.3).[27-29, 31]

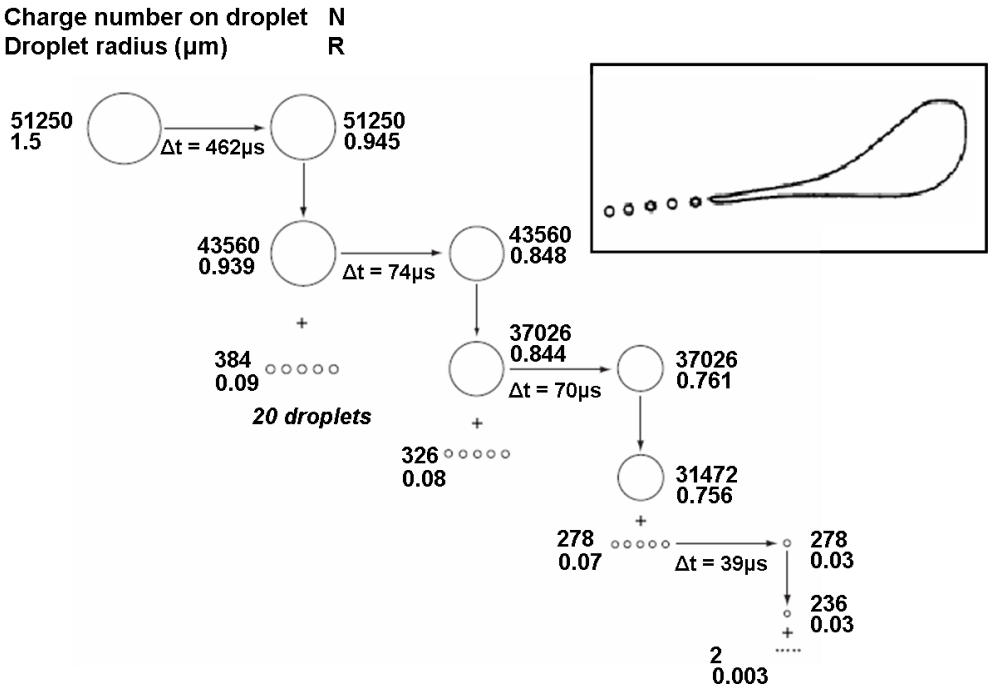

Figure 1.3 Schematic diagram of time history of production of parent and offspring droplets. Reproduction with permission.

Kebarle *et al.* proposed that the resulting fission undergoes an uneven fission process.[27-29] Offspring droplets typically carry about 2% of the mass of the parent droplets, they have 15% of the parent charge. Thus offspring are much smaller and have a much higher charge-to-mass ratio.[28] A schematic representation of the first three fissions of a parent droplet (based on methanol evaporation) is shown in Figure 1.3. About 20 smaller offspring droplets are

produced in each fission process. Δt (in μs) is the estimated time required for droplet shrinkage to the Rayleigh limit where fission occurs. The repeated solvent evaporation and fission cycles, lead to smaller parent droplets and offspring droplets, and ultimately evolve into very small, highly charged droplets that are capable of producing gas-phase ions.[27-29]

4) Generation of gas-phase ions. Two mechanisms have been proposed for the production of gas-phase ions from nanometer-sized, supercharged droplets: the ion evaporation model (IEM)[32] and the charged residue model (CRM).[33] IEM proposed that ions can escape directly from the surface of droplets into gas phase as the droplets reach a certain radius.[32] Because solvent evaporates from the droplets in the strong electric field, the surface of the droplet becomes highly charged. As droplet radius shrinks to less than approximately 10 nm, the field created by the ions at the droplet surface overcomes the surface tension and analyte ions can be emitted directly from the droplet surface into the gas phase. CRM suggests that electrospray ionization is based on continued division of supercharged droplets until a single residual ion remains. Charges on analyte ions originate from the charges at the surfaces of droplets.[29, 33, 34] Both models are feasible according to recent studies. IEM is experimentally well-supported for small organic ions ($m/z < 3300$).[34, 35] A simulation of the ion evaporation is available from Vertes's website: (http://www.gwu.edu/~vertes/publicat.html). CRM is more plausible for very large, multiply charged species, such as proteins.[36]

The highest charge density is at the droplet surface with monotonic descent toward the droplet center. Hydrophobic species prefer the surface of droplets, whereas hydrophilic species reside closer to the droplet center.[37] In general, the more hydrophobic an analyte is in a solvent (yet still soluble in that solvent), the better ions can be transferred into the gas phase and the higher the ESI sensitivity.[37, 38]

1.5.2 Ion Trap Mass Spectrometer

The Paul ion trap functions both as an ion storage device and mass analyzer. As a storage device, gas-phase ions can be confined for a period of time at a pressure of 1 mTorr of helium buffer gas within the trapping volume. The ion trap consists of three hyperbolic electrodes, a ring and two end cap electrodes (Figure 1.4). The end caps have small perforation(s) in the center to allow the ions to enter and exit the ion trap. An oscillating electric potential (RF voltage) is applied to the ring electrode to focus ions toward the center of the trap and two end-cap electrodes are grounded. Ion trapping is accomplished by creating a parabolic potential, saddle-shaped, inside the trapping volume.[39] Trapped ions form a "packet" and only occupy a very small space near the center of the trap as result of dampening collisions with He gas coupled with electric field.[40] As the number of trapped ions increases up to the order of 10^5, a space-charge effect becomes noticeable. Space-charge effects occur when too many ions are trapped in a small space, and lead to losses in mass resolution and/or mass shifts.[41]

Once ions are trapped, the collection of ions can be subjected to additional electric fields from which each ion is ejected in turn to an external detector to

create a mass spectrum of a series of ion signals dispersed in time. For an MS/MS experiment in an ion trap, all the ions except the single selected ion species are ejected first. Then the remaining ion species is fragmented and its product ions are analyzed. The strength of the ion trap instrument is its ability to perform multiple stages of mass spectrometry (MS^n) for detailed fragmentation study, ion chemistry and unknown structure elucidation. Up to 12 stages of tandem mass spectrometry (MS^{12}) have been performed in an ion trap instrument.[39]

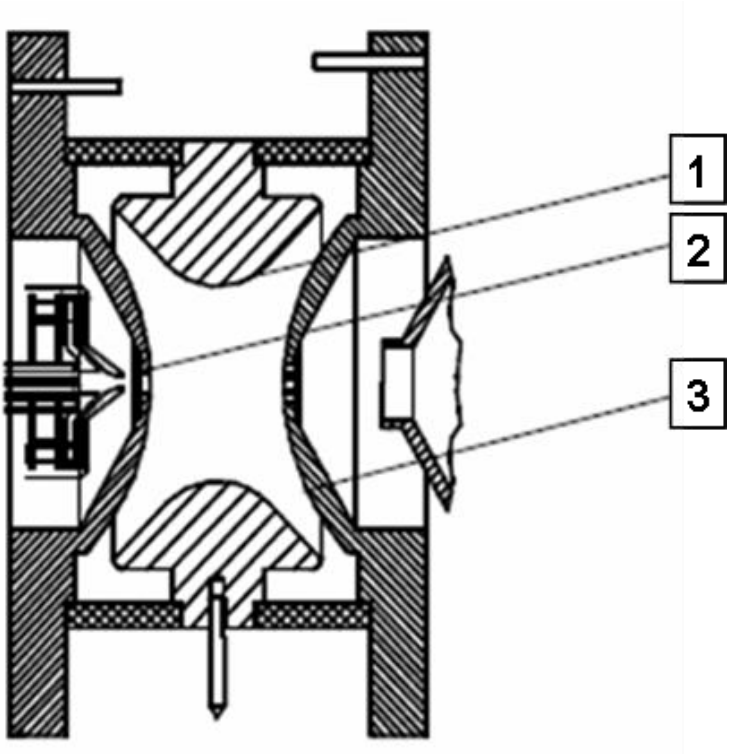

Figure 1.4 Schematic diagram of Bruker EsquireLC ion trap. (1) ring electrode, (2) incoming end cap electrode, (3) outgoing end cap electrode. Reproduction with permission.

The ion trap is a simple, robust, sensitive, and relatively inexpensive MS instrument. However, the ion trap is a low resolution (unit mass resolution), low

mass accuracy (0.1-0.2 Da) mass spectrometer, therefore, it was only used for method development in our metabolomics research. The high confidence identification and accurate quantification metabolomics studies were carried out by a state-of-the-art 9.4-Tesla Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR-MS).

1.5.3 Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS)

FT-ICR-MS is capable of achieving much higher resolution than any other type of mass spectrometer. FT-ICR-MS offers a resolution of 100,000 – 1,000,000 (routinely 50,000 – 100,000) and the highest available mass accuracy (0.01-1 mamu), a low detection limit in the attomole to femtomole range and MS^n capabilities.[42, 43] Ion cyclotron resonance mass spectrometry is fundamentally based on the measurement of an ion's cyclotron frequency which is proportional to its mass-to-charge ratio. An ICR analyzer cell (known as a Penning trap) is located within a strong magnetic field, typically generated by a superconducting magnet. When an ion moves in a spatially uniform magnetic field, it rotates in a plane perpendicular to the direction of the magnetic field (as dictated by Fleming's Left Hand Rule), as shown in Figure 1.5.

This phenomenon is called ion cyclotron motion. The cyclotron frequency, f (in Hz) is expressed in the following equation.

$$f = \frac{qB}{2\pi M} = \frac{1.535611 \times 10^7 B}{m/z}$$

in which q is charge, B is magnetic field (in T), M is mass (in kg), m is mass (in Da), z is elementary charge, m/z is mass-to-charge ratio.

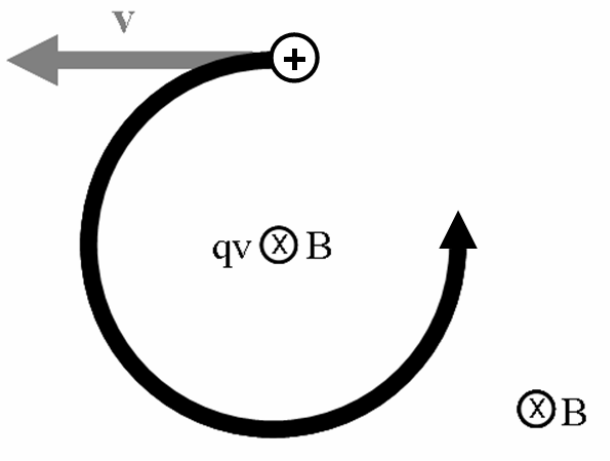

Figure 1.5 Schematic representation of ion cyclotron motion in a magnetic field.

An important feature of the above equation is that ions with same mass-to-charge ratio (m/z) give the same ICR frequency *f*, independent of the ion kinetic energy from ion formation, transfer or later excitation. Translational (kinetic) energy "focusing" is not critically important for precise determination of m/z.[42] However, an ion (for example, a singly charged ion of molecular weight of 300) only has an ICR orbital radius of ~ 0.04 mm at room temperature. Such radii of ICR orbits are too small to be detected, and therefore, the ion cyclotron motion itself is not useful. To measure ICR frequencies accurately, ions must be excited to detectable radii and this process is accomplished by applying a radio frequency (RF) potential to two excitation plates at the resonant frequency (i.e. resonant with the cyclotron frequency) of the ions of interest.[42, 44] Figure 1.6 shows a schematic diagram of a cross section of an ICR cell in which ions are excited by the RF potential applied to the excitation plates.[44] An excited ion of 100 amu can travel up to ~30 kilometers during a 1 second observation time.[42] This is one of the

reasons why ICR as a mass analyzer can provide a much higher resolution than meter-long time-of-flight and magnetic-sector instruments.

The RF potential is often a frequency sweep, known as an "RF chirp", that scans though the range of frequencies corresponding to the different m/z values. During this excitation, ions of different m/z have different cyclotron frequencies, however, they are all excited to orbits of the same radius. This excitation process creates the coherence of the ion packets (i.e. ions moving in phase). As this coherently orbiting ion packet passes two detection plates, the potential change between the detector plates is measured as a function of time, known as "time-domain" data, or "transient" or "free induction decay" ("FID").[42, 44] All the ions with different m/z, and therefore different cyclotron frequencies, are simultaneously detected and collected in "time-domain" raw data. The ion detection is non-destructive (unlike the ions hitting a detector, such as an electron multiplier, used in other types of MS).

The next step is to extract data about the different ion packets by the mathematical procedure known as a "Fourier transform" (FT). Figure 1.7 shows the processing of the time-domain raw data. The time domain raw data (transient or FID) is converted to the frequency domain "Mass spectrum" in Hz by a "Fourier transform". Because the cyclotron frequencies of ions are inversely proportional to their *m/z*, the frequency domain data can be readily converted into mass spectra in *m/z* with mass calibration. It is important to note that the detected ICR signal is proportional to the total charge and proximity of the ions to the detection plates (i.e. orbital radius).

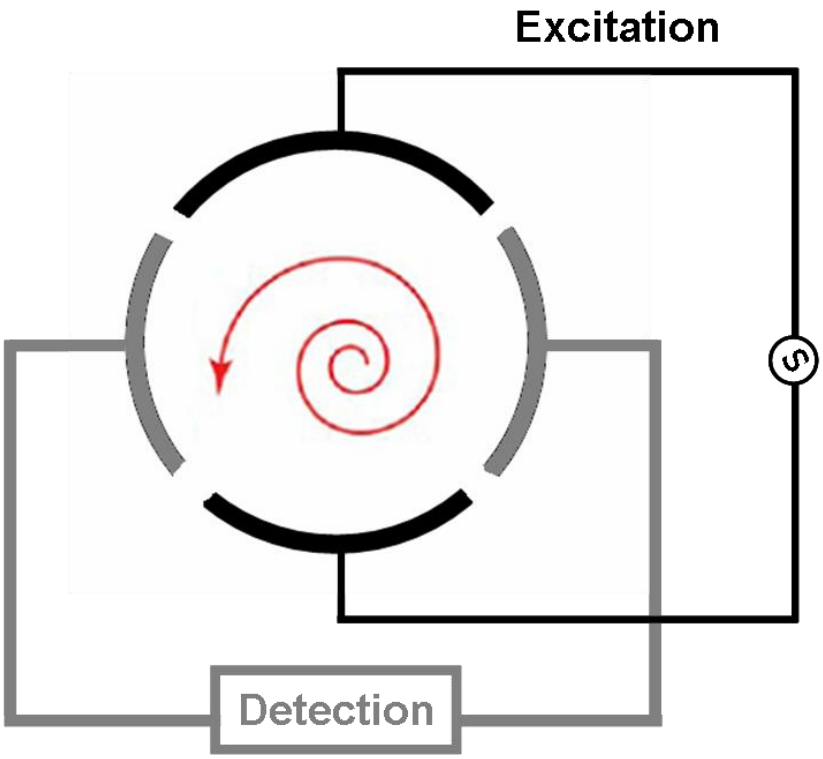

Figure 1.6 Schematic diagram of an ICR analyzer cell in which ions are excited and the image current of the orbiting ions is detected. Reproduction with permission.

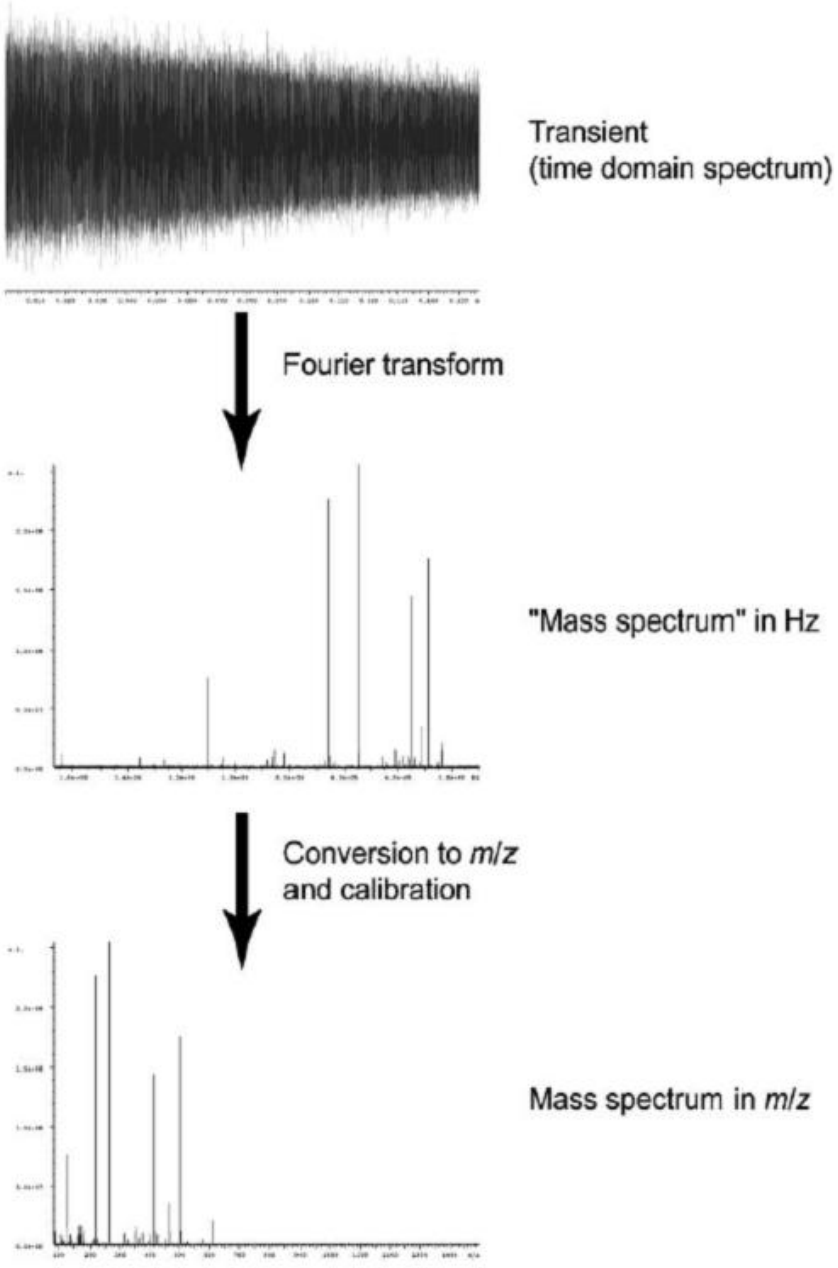

Figure 1.7 Schematic representation of the processing of raw data.

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Thus, ICR is more sensitive for multiply charged ions and more sensitive when ions are excited to a radius which is close to that of the ICR cell.[45] As in the

ion trap, space-charge effects can also occur when too many different ion packets interact with each other through Coulombic repulsion in the ICR cell.[46] Space-charge effects can cause a substantial decrease in mass resolution and accuracy and often are the main reasons for underperformance of FT-ICR-MS. Space-charge effects can be minimized by exciting the ions to larger cyclotron radii or though controlling the number of ions introduced into the ICR cell.[47, 48] The automatic gain control (AGC) in, for example, Thermo LTQ FTMS, is such a device and it carefully regulates the optimum number of ions filling an ICR cell.

The Bruker apex-Qe 9.4-Tesla FT-ICR-MS used in my research consists of four main sections: ion source, the Qh-interface, ion transfer optics and the ICR cell (Figure 1.8). Q stands for Quadrupole, allowing for mass-selection, and "h" for hexapole in which mass-selected ions can be accumulated and collisionally cooled (through multiple collisions with Ar) prior their transfer into the ICR cell. Six differential pumping stages maintained by two roughing pumps, and four turbo-molecular pumps provide an ultra high vacuum of $< 10^{-9}$ mbar.[49]

Resolution of FT-ICR-MS is proportional to magnetic field strength and acquisition time, and is inversely proportional to low m/z cut-off.[42-44, 50] Therefore, increasing the low m/z cut-off improves resolution, and long-lived transients are beneficial in obtaining higher resolution. However, as the long travel path of an ion with longer transient time makes collisions with gas particles in the ICR cell more likely, FT-ICR-MS requires a very high vacuum in the cell region ($\sim 10^{-9}$ to 10^{-10} mbar range) to minimize such unwanted collisions.[51]

The greatest advantage of FT-ICR as a mass analyzer is that the mass-to-charge ratios of ions are measured by their ICR frequency. Since frequency can be measured much more accurately than any other experimental parameter, ICR, as a

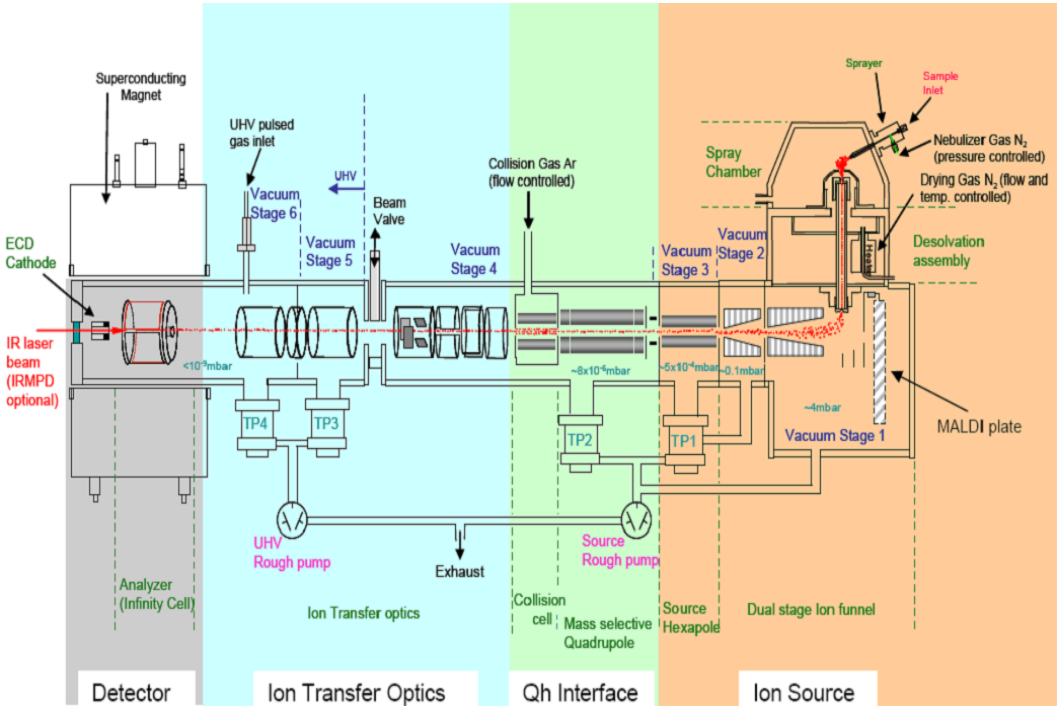

Figure 1.8 Schematic diagram of Bruker apex-Qe FT-ICR-MS. Reproduction with permission.

mass analyzer, offers extraordinarily high resolution and higher mass accuracy than any other type of mass analyzer.[42-44, 50, 51] For a 9.4 T magnetic field, the ICR frequencies of ions are typically within the kHz to MHz range. Commercially available broadband electronics are quite suitable for measurement of such frequencies. Additionally, modern superconducting magnets are much more stable than RF voltage and this too leads to the high resolution and mass accuracy of FT-ICR-MS.

1.6 LC-MS-Based Quantification and Identification of Metabolome

1.6.1 Acquisition Modes LC-MS

Traditionally, before the advent of modern ESI MS, most quantitative analysis was carried out using HPLC and UV detection.[52] However, HPLC/UV analysis suffered from lack of specificity and sensitivity. In particular, the lack of specificity will mislead the investigator from time to time. Over the past decade, quantitative analysis by LC-MS has become a routine practice due to its high sensitivity and exceptional specificity when compared to UV detection.[53, 54]

There are three common operating modes of acquiring LC-MS data:

1) Full scan analysis in which the MS scan is set to scan a wide mass range, for example, to scan 50 – 1500 *m/z* in a typical metabolomics analysis. Full scan acquisition produces a total ion chromatogram (TIC), and compounds of every m/z that are set in full scan are plotted in the TIC. The base peak chromatogram (BPC) and extracted ion chromatogram (EIC) can be extracted from TIC. The BPC represents the intensity of the most intense peaks at any time in the LC-MS analysis. In general, BPCs have a cleaner look than TICs because much of the background and interference peaks will sum together in a TIC. EIC is the reconstructed chromatogram from a larger dataset of TIC, exclusively showing the intensity of a single ion (or, more precisely, a certain m/z window within a reasonable tolerance) plotted against time.

2) Selected ion monitoring (SIM) in which the mass spectrometer is set to scan a very small mass range, typically one *m/z* (also a certain *m/z* window within a reasonable tolerance). Only the compounds with selected *m/z* can be detected

and plotted against time in SIM. SIM will look similar to EIC, but be more sensitive than the full scan MS experiment because the mass spectrometer can dwell for a longer time over a small mass range.[54]

3) Selected reaction monitoring (SRM) which is currently the method most used for MS-based quantitation.[55] The SRM experiment is carried out by tandem mass spectrometry by specifying the parent mass of the compound(s) of interest for MS/MS fragmentation, and then specifically monitoring for one fragment ion originating from the parent compound(s). SRM can be executed for monitoring multiple user defined fragment ions, referred to as multiple reaction monitoring (MRM). SRM experiments are commonly used with scanning instruments where the second mass analysis event is duty cycle limited. SRM experiments increase specificity of detection of known molecules. The increased specificity derives from the additional dimension of information from MS/MS fragment ion(s), i.e. the selection of the parent ion mass, the selection of the fragment ion mass with known relative intensity, as well as the compound-specific retention time, can be involved.[54] SRM plots are very simple, ideally containing only a single peak for every parent compound. These characteristics make SRM and MRM experiments ideal for sensitive and specific quantitation in LC-MS/MS.

1.6.2 Quantitative Analysis of Metabolome by LC-MS

In principle, the quantitation method in LC-MS is not different from a quantitative method used in many other analytical techniques;[54] the signal intensities of compounds of interest in the samples are compared with those from

known amounts of authentic standards or analogues. There are three major ways to execute LC-MS-based quantitation. 1) External standard calibration. Probably the simplest and most commonly used methods for determining the concentration of an unknown sample is to construct a calibration curve using external standards. Standard solutions are prepared as a series of known concentrations. A fixed volume of every standard solution is injected onto LC-MS for analysis. The calibration curves are constructed by the peak area (sometimes ion peak height) plotted *vs*. known amount. The standards used are referred to as external standards because they are prepared and analyzed in separate (or ideally parallel) experiments from those of the unknown sample(s).[53, 54] The slope of the calibration curve is called the sensitivity or calibration factor S. Unknown samples are prepared, injected and analyzed in exactly the same procedure, and then the concentration of unknown sample(s) can be calculated using the calibration factor S or determined graphically from the calibration curve.

As mass spectrometry detection offers unrivaled sensitivity and selectivity, it is a most attractive candidate for quantitative analysis.[56] However, MS quantitative analysis by a straightforward external standard approach may mislead from time to time. The major problem in LC-MS-based quantitative analysis is that signal response of an analyte may vary greatly in different matrices. This occurrence of well-known matrix effects/ion suppression, particularly for complex biological samples, has a major influence on the MS signal intensity and frequently produces significant deterioration of the precision, accuracy and detection capability for the analytes of interest.[56, 57] At total compound

concentrations of $>10^{-5}$ M for ESI, the approximate linearity of the electrospray response will be lost and followed by "saturation" and a small decrease of intensity at higher concentration (10^{-3} M).[28, 58] This phenomenon is caused by the limited number of charges and space available on ESI droplets. In general, compounds with higher surface activity (hydrophobicity) and chargeability (basicity in positive mode) will out-compete others for the limited charge or space on the surface of the ESI droplets, and show higher ESI response.[29, 38, 57] Biological matrices usually contain large numbers of endogenous compounds with possible high surface activity and chargeability. Co-eluting compounds from the HPLC column are introduced into the ion source simultaneously and the limiting concentration of 10^{-5} M can be reached readily, so ion suppression often occurs in such complex samples.[59] In addition, a high proportion of metabolites in biological samples are polar compounds. Polar compounds are poorly retained by the hydrophobic nature of reversed-phase HPLC columns and often will co-elute together at or near the initial void. Consequently, the ion suppression due to co-elution and salts is more frequently observed for polar metabolites.[60, 61] Furthermore, polar analytes are more vulnerable to ion suppression and usually show very low signal intensity in mass spectra because they can be easily out-competed for limited charges and space on ESI droplets by other more ES-active species.[60]

Ion suppression occurs in the early stages of the ionization process in the LC-MS interface.[57, 59] It is worth emphasizing that SRM or SIM are just as susceptible to ion suppression effects as full scan analysis because mass analysis

is after the ion formation, and it is a misleading misconception that ion suppression does not exist in SRM and SIM.[62, 63] Because the *m/z* of only the analyte of interest is recorded in SRM and SIM modes, SRM and SIM acquisition may result in very clean chromatograms. Furthermore, the choice of mass analyzer should not have any influence on ion suppression effects because ion suppression occurs in the ion source region.

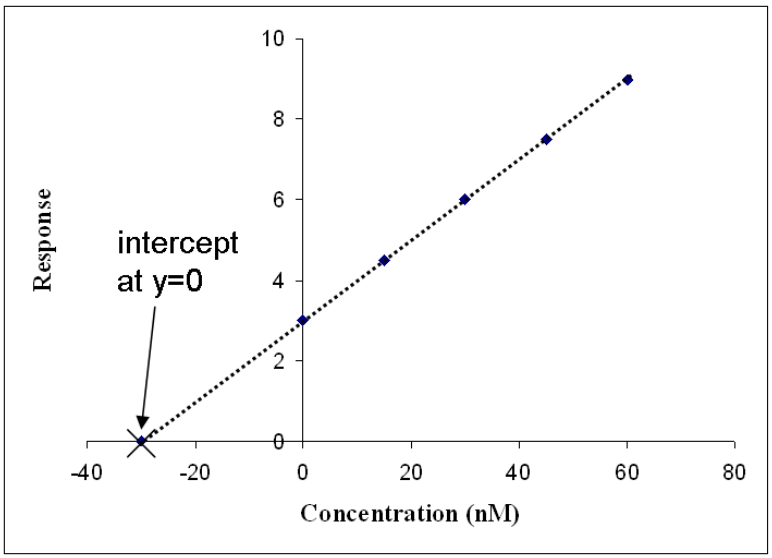

Figure 1.9 Schematic representation of the determination of the concentration of a known analyte by standard addition.

2) Method of Standard addition. Standard addition can be used to compensate for matrix effect/ion suppression. In the standard addition approach, a series of known amounts of analyte standard(s) is added to the sample matrix, which originally contains an unknown amount/concentration of the analyte(s). By this approach a plot of signal response *vs.* amount/concentration of standard, as shown in Figure 1.9, is constructed. By extrapolation of the plot of the response (y) to zero, the intercept of the x axis defines the original concentration in the

unknown sample, which, in this example, is 30 μg/mL (i.e. the x intercept at y=0 in Figure 1.9). The standard addition approach is very effective, offering good results, even with non-reproducible sample matrices. However, standard addition is a very time consuming approach because a series of spiked samples for each unknown must be run. For this reason, standard addition is not commonly used in LC-MS-based metabolomics.

3) Internal Standard Calibration. The internal standard approach is a widely used technique in LC-MS-based quantitation. The use of internal standard(s) is designed to normalize the response of analyte(s), therefore, it (ideally) is capable of compensating for potential variation in sample preparation, injection volume, chromatography, and matrix effect/ion suppression. Structural analogue(s) are commonly used as internal standards. However, matrix effects are strongly compound dependent and the ionization of internal standard structural analogues and the analyte(s) may be differently suppressed by the matrix.[61] Structural analogues are often separated from targeted analytes on an HPLC column, and therefore are introduced into the ion source at different times. This may lead to different levels of ion suppression of the internal standards and targeted analytes.[56, 64] A study has shown that the intra-day precision in the analysis of 2-C-ethynylcytidine in rat plasma as analyte and 3'-C-ethynylcytidine as structural analogue internal standard improved from 16.2% RSD with chromatographic separation between analyte and internal standard to 4.2% RSD without separation using ^{13}C-2-C-ethynylcytidine as an isotopically labeled internal standard.[65]

When the number of analyte(s) of interest is limited, the stable isotopic labeled (SIL) internal standards are obviously better choices than structural analogues. This approach is also referred to as stable isotopic dilution quantification. A SIL internal standard usually containing ^{2}H, ^{13}C, ^{15}N or ^{18}O to replace hydrogen, ^{12}C, ^{14}N or ^{16}O in the analytes of interest, respectively. The SIL internal standards show (almost) identical behavior to the targeted analytes in sample preparation and electrospray ionization.[38, 54, 61, 65] Unfortunately, isotopically labeled internal standards are very expensive and only available for a limited number of common metabolites. Deuterated labeled standards are the least expensive and commonly used as internal standards. Unfortunately, deuterated labeled standards show more isotopic effect in reversed-phase liquid chromatography.[66-68] It was found that protiated compounds bind to the hydrophobic surface of reversed-phase stationary phases more strongly than deuterated ones; therefore, protiated analytes will elute slower and can be partially or even fully separated from their deuterated internal standards.[66, 69] Because the separated analytes and deuterated standards are not introduced and ionized simultaneously in the ion source, they may not experience identical ionization conditions and ion suppression, causing different ESI responses and compromised quantification accuracy and precision. However, the negative effects of chromatographic separation between targeted analytes and deuterated labeled internal standards is often neglected, possibly because the deuterated standards often are the only commercially available SILs.

The bond length difference between the C-D and C-H bond is the main reason that leads to hydrogen/deuterium isotope effect on hydrophobic binding. The amplitude of vibration is smaller for deuterium, result in slightly lower average volumes and polarizabilities for C-D bonds than for the corresponding C-H bonds.[70] Therefore, the attraction binding forces of deuterated standards to the hydrophobic RP stationary phase are less than those of protiated analytes, leading, therefore, to chromatographic separation of protiated and deuterated isotopologue pairs.[66, 69] Because of isotopic effect, the use of deuterated internal standards often is not the optimum choices. Since the ^{13}C-SIL internal standards do not show any isotopic effects in RP chromatography, more accurate quantification and easier spectral interpretation follow. However, using ^{13}C-SILs as internal standards in metabolomics study is simply not practical. Whilst a large number of metabolites would be involved, only a few dozen extremely expensive ^{13}C-SIL metabolites are commercially available.

1.6.3 Qualitative Analysis of Metabolome by LC-MS

Sadly, metabolomics identification has lagged far behind the progress made in proteomics or transcriptomics.[71] MS-based identification of metabolomes employs a range of mass spectral and chromatographic techniques. including accurate mass for atomic composition, MS/MS, MS^n, neutral losses, spectral isotopic pattern and (HPLC, GC, or CE) retention time.[21] There are two types of qualitative analysis, putative (or preliminary) identification and definitive identification.[72] Putative identification uses one or a few molecular properties for identification, and does not compare these properties to that of authentic standards.

For example, accurate mass can be used to define molecular formula and putative metabolite assignments are derived by searching electronic databases, such as HMDB, PubChem, ChemSpider, NIST, KEGG and CAS. Unfortunately, a large proportion of metabolites have isomeric forms in nature. As isomers have the same accurate mass, they require an orthogonal property for differentiation of possible metabolite isomers. Tens of thousands of spectral peak features can be detected in a single LC-MS run of a complex biological sample. In this case, putative identification only serves to narrow down the possible candidates rather than provide an unambiguous metabolite assignment.[73] Definitive identification usually uses at least two properties (typically chromatographic retention time, accurate mass or fragmentation mass spectrum), and then compares these properties to an authentic standard, assuming that they are commercially available. Currently, many metabolome qualitative analysis is putative identification rather than more confident definitive identification. A searchable database with a comprehensive compound list and their LC retention times has yet to be published.

1.7 Chemical Derivatization in LC-MS-Based Metabolomics

Chemical derivatization has been widely used to make targeted analyte(s) more suitable for quantitative and/or qualitative analysis in analytical chemistry. Derivatization is a technique to modify functional group(s) to desired properties by using chemical reaction(s) where essential parts of the parent molecule remain unchanged.[74]

A judiciously chosen derivatization reaction may offer many benefits in LC-MS based metabolomics. (1) Improve quantitative analysis. A large number

of effective methods and commercial reagents have been developed that use chemical derivatization combined with stable isotope-labeling for (mainly relative) quantitative proteomics since Aebersold and co-workers' first application in 1999.[75-95] In the differential isotope labeling (DIL) approach, the relative quantitation in two comparative samples can be carried out by derivatizing one sample using light isotope reagent (for example, ^{1}H-reagent or ^{12}C-reagent) and the other sample with heavy isotope reagent (for example, ^{2}H-reagent or ^{13}C-reagent) , which leads to a mass shift in the mass spectrum for analytes between two comparative samples. Differentially labeled samples are then combined and analyzed by LC-MS or LC-MS/MS. The differences (or ratio) of the peak intensities of the isotope pairs accurately reflect differences (or ratio) in the abundance of the corresponding analytes. The DIL approach is not fundamentally different from classic stable isotopic dilution quantification, and the accuracy and precision of quantification would be close to that in the isotope dilution quantification method, assuming the derivatization reaction yield is high or equivalent in parallel light/heavy-isotope labeling experiments. The significant advantage of the DIL approach is that since it can produce a stable isotope labeled internal standard for every analyte, the purchase or synthesis of SIL internal standards for every analytes is not required as in isotope dilution quantification. This excellent feature makes DIL commonly used method in quantitative proteomics, and therefore a plausible approach for quantitative analysis in metabolomics. However, there are only a limited number of studies (when compared to proteomics) on the use of DIL for MS-based metabolome

quantification.[26, 64, 96-111] Most of these reports were published in the last five years. However, almost all the studies have only focused on the quantitation feature of DIL; the potential capabilities of a derivatization reaction that can be used for increasing ES ionization efficiency, improving chromatographic separation, providing structural information or shifting derivative products into high mass regions have not been fully exploited yet.

(2) Increase ionization efficiency. Unlike GC/MS, there is no need for derivatization to increase the volatility in LC-MS. As stated in electrospray ionization theory, the major disadvantage of ESI MS is that not all types of molecules are applicable to ESI MS analysis. Only those compounds that have good chargeability and surface-activity can be readily detected. However, poor sensitivity in ESI has been often observed for a large proportion of metabolites which lack a chargeable group and/or surface-activity. Derivatization can be a simple, effective solution for detection of those types of metabolite. Derivatization can be readily employed to convert a non-ES-active compound into an "ES-active" structure by making it more easily charged, and by increasing its surface activity (for example, hydrophobicity) or a combination of both enhancement factors.[38] For positive mode detection, ES-active compounds would possess ionic groups or a group that provides in-solution ionization by high proton affinity or chargeability.[112] For example, tertiary amines, quaternary amines and phosphonium compounds are among the most ES-active species. It is generally true that a compound most responsive to ESI analysis would possess hydrophobic region(s), thus, high surface activity.[28, 30, 37, 38, 113] This is because the nonpolar

ions would prefer the droplet air interface, and thus, reside at the electrospray droplet surface.[32] Consequently, these nonpolar ions with chargeability would have higher response by being more competitive for the limited space and charges on the droplet surfaces and would enter the gas phase more favorably than those polar ions in the droplet interior.[37, 38] Furthermore, hydrophobic reagents can convert polar analytes to less polar ones and in an RPC gradient run be eluted in higher percentage organic mobile phase where the ESI desolvation process is much more efficient and electrospray stability improves as the surface tension of the droplet decreases.

(3) Improve chromatographic separation. A large number of metabolites are highly polar compounds. Polar metabolite are poorly retained by the hydrophobic nature of C_{18} stationary phase and are often closely eluted or even co-eluted near or at the initial void volume. Consequently, co-eluting species and salts present in samples will cause severe ion suppression, and thus ESI detection ability of polar metabolites is generally very low. The detection of polar isomers will be even more problematic because MS spectra of co-eluted isomers will look exactly the same. Besides enhancing ionization efficiency, a well designed derivatization would be capable of improving reversed-phase chromatographic separation (in particular, of polar metabolites) and this can be accomplished by introducing a non-polar moiety to polar analytes. After derivatization, reversed-phase chromatographic retention of the polar analytes can be extended, thus, the suppression of ionization related to co-elution and salts can be significantly reduced. In general, the compounds with permanently charged moieties, such as

quaternary amines and phosphonium, show very high ESI responses. However, the RP separation of those pre-charged compounds often tends to be compromised. Both the factors of ionization efficiency and chromatographic separation should be balanced when designing a derivatization reaction for LC-MS.

(4) Shift small analytes to the high-mass region. Heavy reagents have often been used to bind small organic analytes to produce high mass derivatives. In these cases, the signal-to-noise ratio for the small analytes can be improved because the masses of resulting derivatives are shifted out of the low-mass region that is typically complicated by significant background noises from the solvent clusters and other contaminants.[26, 38, 114] Heavy derivatization reagents have additional benefits for FT-ICR-MS analysis because increasing the low m/z cut-off of metabolite derivatives improves resolution in FTMS. The structure and molecular mass of reagent should be chosen for each particular case, although a small reagent brings a small *m/z* increment for derivatives, it has advantages with the derivatization of sterically hindered structures.

(5) Improve compound identification. Assuming the derivatization reaction is specific for a functional group, only the metabolites with the targeted functional group will be differentially labeled in the DIL approach. The resulting mass spectra ideally will contain the (light/heavy) isoform pairs with characteristic mass differences for targeted metabolites, and other metabolites will not show as pairs in the mass spectra. The characteristic mass differences also indicate the number of reactive functional group(s) present in the metabolites. This greatly facilitates the MS spectral interpretation and therefore, metabolites

can be more confidently identified in combination with highly reproducible RP retention times. Furthermore, a well-designed DIL should show simple fragmentation patterns, the added derivative group should not be eliminated during CID and provide better structural information in MS/MS experiments.[74]

There are at least a few hundred published derivatization reactions. Some of them or their reaction variants can also be used in DIL. The choice of ideal DIL reagents and reactions should satisfy a number of basic criteria. 1) The synthesis of heavy-labeled DIL reagents should be simple and (ideally) high yielding. The heavy-labeled starting materials for synthesis of DIL reagents should be as inexpensive as possible. The main cost of the DIL approach (other than LC-MS instrumentation) comes from the synthesis of heavy-labeled DIL reagents. 2) The derivatization reagent should be selective for (ideally) a single functional group. 3) The analytes of interest should be rapidly and quantitatively derivatized under mild conditions and produce a minimum of side-products. 4) A single-stage reaction is preferred. Multiple reaction steps should be avoided unless it is mandatory. 5) The derivatization reagents and products should be nontoxic and stable over a prolonged time. 6) Direct injection of (diluted) reaction mixture is desirable. The derivatives should not require an additional evaporation, lyophilization or purification step prior to analysis by LC-MS. 7) The ideal derivatization reaction should be directly compatible with aqueous biological samples, should tolerate water, or at least small amounts of water should not influence the reaction yield significantly. Otherwise, an indirect derivatization technique should be used.[74] In this technique, the targeted analytes can be first

activated by a suitable activator that provides the possibility of using different labeling reagents for the same functional group. 8) The formation of nonpolar derivatives is advantageous to achieve separation in (the most commonly used) RPC.

Recently, a similar strategy that used isotope enriched media for cell culturing to introduce isotope labeling internal standards has been used for quantitative metabolomics.[115-121] This subject will not be covered in my thesis.

Chemical derivatization is often used to increase volatilities and thermal stability of analytes in GC/MS. The common advantage of LC-MS over GC/MS is the elimination of a derivatization step. When a derivatization step is required for both LC-MS and GC/MS, the LC-MS approach still has advantages.[122] The sample preparation step in LC-MS is usually much simpler than in GC/MS. Trace amounts of water in the sample influences the reaction yield in most GC/MS derivatization reactions and derivatization products often are sensitive to moisture. Consequently, GC/MS-based metabolomics often involves multiple-step sample preparation of biological samples. In this case, highly concentrated biological sample matrix can interfere with analysis. Additionally, the molecular ion can be readily detected in LC-MS, fragment ions information can be collected in LC MS/MS experiments and can be used to facilitate the elucidation of unknown metabolite structures. The fragments in high-energy EI spectra in GC/MS are often dominated by the chemically derivatized groups, ions representing the parent molecular structures are often missing or very small (<1%), thus, *de novo* identification of unknown metabolites becomes a challenge,[8] making the

advantage of high resolution in GC separation not as obvious as the high efficiency of sub-2 μm particle columns commonly used in LC-MS in recent years. The run time for LC-MS (with sub-2μm particle columns) can be shorter than that of GC/MS and furthermore, LC MS/MS (for example, MRM mode) may offer better sensitivity and selectivity than GC/MS.[113]

1.8 Overview of the Thesis

The main objective of this work was to develop novel LC-MS-based methods for quantitative and qualitative analysis of the human metabolome in complex biological samples. As part of the research for the human metabolome database (HMDB) project, most of my efforts were focused on developing simple, efficient, robust, methods, based on a ^{13}C-/^{12}C-differential isotope labeling (^{13}C-/^{12}C-DIL) strategy, that could be routinely performed. Most of the present DIL metabolome studies have only focused on a limited number (often a dozen to a few dozen) of targeted analytes. In this research, our DIL strategy will be focused on expanding targeted metabolites to a relatively large number (a few hundred) of metabolites, establishing ^{13}C-/^{12}C-labeled metabolite libraries and software packages for fast identification and quantification; and judiciously choosing the labeling reaction for optimizing the ionization efficiency, reversed-phase separation and identification of targeted metabolites.

In Chapter 2, our first DIL application of differential ^{13}C-/^{12}C-dimethylation labeling for quantification of amine-containing metabolites in urine is described. In Chapter 3, the method development and validation of differential ^{13}C-/^{12}C-isotope dansylation labeling and fast HPLC FT-ICR-MS for absolute and

relative quantification of targeted amino acids, amines and phenolic hydroxyls in biological samples is demonstrated. In Chapter 4, the focus is on a novel ^{13}C-/^{12}C-DIL library identification strategy which consisted of the construction of a ^{13}C-/^{12}C-dansylation library, software-based ^{13}C-/^{12}C-labeled ion pair picking, and a ^{13}C-/^{12}C-standard library search to achieve fast and confident metabolite identification in cerebrospinal fluid (CSF). In Chapter 5, a novel ^{13}C-/^{12}C-isotope labeling for carboxylic acids and fatty acids, which includes the synthesis of a ^{13}C-derivatization reagent, optimization of the derivatization reaction, construction of a ^{13}C-/^{12}C-labeled compound library, and method validation, is given in detail. In Chapter 6, a two-dimensional separation scheme based on reversed-phase LC fractionation of the metabolites followed by isotope labeling and then reversed-phase LC separation MS analysis is described. Finally, Chapter 7 summarizes my thesis work and also gives my brief comment on future work related to the differential isotope labeling approach to the quantitative and qualitative analysis of the human metabolome.

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Chapter 2

Stable-Isotope Dimethylation Labeling Combined with LC-ESI MS for Quantification of Amine-containing Metabolites in Biological Samples

2.1 Introduction

LC-MS has become an important tool for metabolome analysis. However, generation of accurate quantitative information by LC-MS is not straightforward.[1] One of the most reliable quantitative methods is to use stable-isotope-labeled (SIL) analogs as internal standards for quantifying the metabolites of interest. SIL internal standards are chemically and structurally similar to the analytes with specific atoms in the analytes replaced by their corresponding isotopes, such as deuterium for hydrogen, ^{13}C for ^{12}C, ^{15}N for ^{14}N or ^{18}O for ^{16}O etc.[1, 2] The use of SIL internal standards normalizes the MS intensity of analytes to their isotopic analogs, and therefore effectively compensates for the matrix effect, ion suppression from other co-eluting analytes, and variations caused by sample preparation, injection and instrument parameters.[1-6] Unfortunately, only a limited number of SIL internal standards are commercially available. In the absence of SIL standards, structural analogs are used as the second best choice; but the use of structural analogs may result in poor quantification performance, particularly in analyzing metabolites present in a complex matrix.[7] Errors can be introduced during sample processing (e.g., different recovery rates in the extraction of a

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metabolite and its structural analog) and the LC-MS analysis step. Structural analogs are often not co-eluted with the analyte of interest and, therefore, they would experience different levels of matrix effect from that of analyte. As a result, the relative signal intensities of a metabolite and its analog may not reflect their concentration ratio in the sample.

Another practical strategy for quantification in LC-MS is chemical labeling which has been widely applied to obtain relative quantitative information of proteomes.[8, 9] For metabolome analysis, there are a limited number of reports of using chemical derivatization to introduce a stable isotopic tag to metabolites to facilitate their quantitative analysis by LC-ESI MS.[10-12] Development of facile derivatization methods for stable isotope dilution-based quantification of metabolomes is needed. For an ideal derivatization protocol, the derivatization reagent should be specific for the target functional group, and the analytes of interest need to be rapidly and quantitatively derivatized with minimum byproducts (i.e., high yield). The reaction should be performed under mild conditions with minimum manipulation. For quantitative applications the resulting products need to be stable.

Differential isotopic dimethyl labeling of N-terminal peptides with d(0) and d(2)- or d(0), ^{12}C and d(2), ^{13}C-formaldehyde combined with LC-ESI or LC-MALDI have been successfully used for relative proteome quantification.[13-17] The labeling is carried out by using reductive amination chemistry.[18] In this work, we report our studies of using reductive amination to introduce isotopic tags to amine-containing metabolites, and applying this strategy to the quantification by LC-ESI MS of both primary and secondary amine metabolites in human urine. Amine-containing metabolites play essential roles in biological functions. For examples, amino acids and their derivatives are common biomarkers for human physiological processes.[19] Their identification and quantification in human fluids

provide significant insights related to human health. The polycationic polyamines are essential for eukaryotic cellular growth and viability, rapid tumor growth was associated with polyamine biosynthesis and accumulation.[20] Many studies indicate that significantly higher levels of polyamines and their metabolites were present in the biological fluids and the affected tissues of cancer patients and other hyperproliferative diseases.[20-22] Some therapeutic polyamine analogues are showing exciting potentials to treat cancer and other hyperproliferative disorders.[2018] Thus, quantitative profiling of amine-containing metabolites could potentially be applied for the discovery of new disease biomarkers as well as for the monitoring of tumor growth and regression in cancer study.

2.2 Experimental

2.2.1 Chemicals and Reagents

20 amino acids: L-alanine, L-arginine, L-aspartic acid, L-asparagine, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-tryptophan, and L-valine; 15 amines: 1-ephedrine, 1,4-diaminobutane, (-)-epinephrine, 2-methylbenzylamine, 3-methyl-L-histidine, aniline, benzylamine, cysteamine, dopamine, histamine, L-4-hydroxyproline, p-aminohippuric acid, pyridoxamine, γ-aminobutyric acid, and tyramine were purchased from Sigma Aldrich (Oakville, ON, Canada). Formaldehyde (37 wt % solution in water), sodium cyanoborohydride (95%), ammonium acetate, sodium acetate, LC-MS grade formic acid and acetic acid were also obtained from Sigma-Aldrich. LC-MS grade of water, methanol and acetonitrile (ACN) were purchased from Fisher Scientific Canada (Edmonton, AB, Canada). Formaldehyde-^{13}C (20 wt % solution in water, >99% isotope purity) solution, and

d(2)-formaldehyde (20 wt % solution in deuterated water, >98% isotope purity) were the products of Cambridge Isotope Laboratories, Inc. (Andover, MA).

2.2.2 Dimethylation Labeling Reaction.

The freshly collected human urine was centrifuged for 10 minutes at 12000 rpm. 500 μL of urine supernatant, 20 amino acid, or 15 amine standard solutions were mixed with an equal volume of ammonium acetate buffer (0.2 M, pH 5.3) in a reaction vial. The solutions were vortexed, centrifuged, and mixed with 125 μL freshly prepared sodium cyanoborohydride (1.0 M). After further mixing, centrifugation and the addition of 100 μL of 4% formaldehyde, or formaldehyde-^{13}C or d(2)-formaldehyde solution the mixtures were vortexed and centrifuged again, and the reaction was allowed to proceed for 10 minutes at 37 ^{o}C and 200 rpm in an Innova 4000 benchtop incubator shaker. The pH of mixtures was adjusted to pH 2-3 by adding approximately 25 μL of formic acid. The solutions then were centrifuged or filtered before being injected onto an LC column. The samples for hydrophilic interaction liquid chromatography (HILIC) were diluted with acetonitrile to obtain optimal separation efficiency. Because sodium cyanoborohydride is a highly toxic chemical that will produce hydrogen cyanide gas when exposed to acid, and formaldehyde is a known carcinogen on inhalation exposure, the dimethylation labeling reaction was carried out in a fume hood.

2.2.3 LC-ESI MS.

The HPLC system used in conjunction with the mass spectrometer was an Agilent 1100 series binary system and it was modified to reduce extra column system volume according to an Agilent protocol (Agilent Publication Number: 5988-2682EN). A reversed-phase (RP) Agilent Zorbax XDB C_{18} column (1.0 x 150 mm, 3.5 μm particle size, 80 Å pore size) and a Zorbax XDB C_{18} rapid

resolution high throughput (RRHT) cartridge column (2.1 x 15mm, 1.8 μm, 80 Å) were purchased from Agilent Technologies, Inc. (Palo Alto, CA).

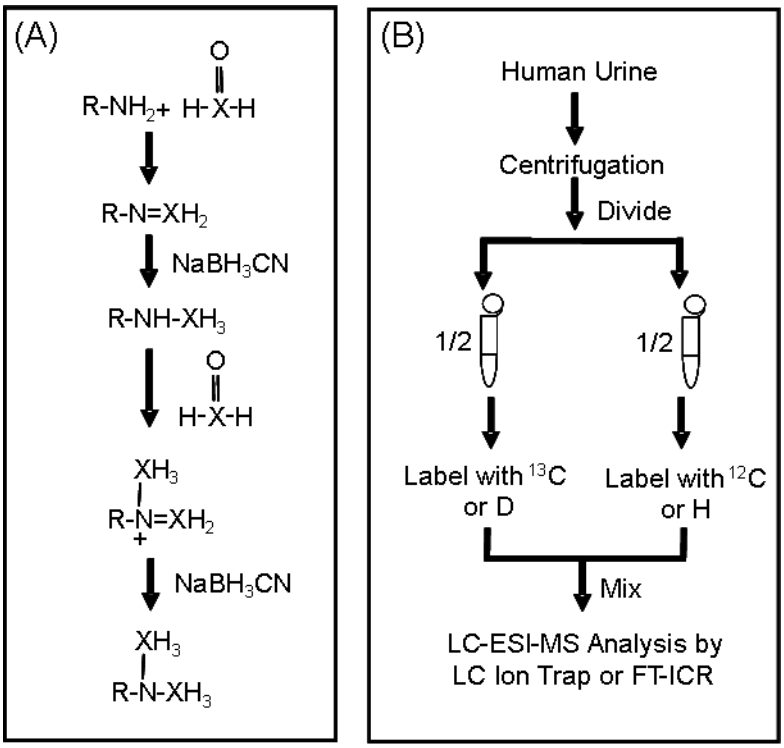

Figure 2.1 Overview of dimethylation labeling strategy for quantitative analysis of amine-containing metabolites: (A) Scheme of reductive amination labeling. X=^{13}C or ^{12}C; in the case of D(2) labeling, two deuterium atoms replace two hydrogen atoms in formaldehyde. (B) Workflow for demonstrating the feasibility and performance of relative quantitative analysis of primary and secondary amine metabolites in urine samples by LC-ESI MS.

For RP chromatography, solvent A was 0.1% formic acid, 5% methanol in water, and solvent B was 0.1% formic acid in methanol. All the formic acid, methanol and water used were LC-MS grade. The 64 minute binary gradient elution profile was as follows: t = 0, 0%B; : t = 6 min, 0%B; : t = 21 min, 30%B; : t = 54 min, 90%B; : t = 64 min, 90%B. The flow rate was 50 μL/min, and sample injection volumes were 10 μL.

For separation of polar derivatives, a TSKgel Amide-80 HILIC (Tosoh Bioscience LLC, Montgomeryville, PA) column (1.0 x 250 mm, 5 μm) was used. For HILIC, solvent A was 10% of 15mM ammonium acetate (pH 5.5) in LC-MS grade acetonitrile, and solvent B was 40% of 15mM ammonium acetate (pH 5.5) in LC-MS grade acetonitrile. The 45 minute binary gradient elution profile was as follows: t = 0, 10%B; t = 30 min, 30%B; t = 37 min, 45%B; t = 42 min, 70%B; t = 45 min, 70%B. The flow rate was 55 μL/min, and sample injection volumes were 3 μL. The flow from RP or HILIC columns was directed to the electrospray ionization (ESI) source of a Bruker Esquire-LC Ion Trap LC-MS system or a Bruker 9.4-Tesla Fourier-transform (FT) ion cyclotron resonance (ICR) mass spectrometer. All MS spectra were obtained in the positive ion mode. Negative ion detection was found to be not as sensitive as the positive ion detection for the labeled amines tested in these instruments.

2.3 Results and Discussion

2.3.1 Dimethyl Isotope Labeling.

When amine-containing molecules are treated with low concentrations of simple aliphatic aldehydes and a small amount of sodium borohydride, amine groups can be converted in high yield into their corresponding mono- or di-alkylamine derivatives.[18] We investigated the reaction of 20 amino acids and 15 amines with formaldehyde and observed the formation of predominantly

dimethylated derivatives (28 Da difference for each labeled site) from primary amines, and mono-methylated derivatives (14 Da difference) from secondary amines, such as proline. Judging from the LC-MS results of the labeled products, the conversion yield is better than 97%. This observation is similar to those found in other reported studies.[23-24] The use of dimethylation labeling is attractive for several reasons. The formaldehyde used as the labeling reagent is inexpensive and ^{13}C or deuterium labeled formaldehyde is commercially available. The experimental conditions for reductive amination are extremely mild, and the reaction is easily completed without any special reagents or reaction equipment. As Figure 2.1A shows, an intermediate Schiff base is formed in reductive amination. The high yield of methylated products observed in our experiment suggests that the Schiff base intermediate was reduced at a reaction rate much greater than that of formaldehyde. Consequently, the reactive intermediate readily reacts with formaldehyde, and is reduced to dimethylated products. The dimethylation labeling reaction appears highly specific for amine groups for the 20 amino acids and the 15 amines we studied.[18]

We also tested the dimethylation reaction for each amino acid and amine, one by one, to examine any possible byproducts from the reaction. In general, no significant amount of side-reaction products were observed, at least for the 20 amino acids and 15 amines tested. For Lys, there were two dimethylated tags introduced to the molecule. The number of tags introduced to an analyte is corresponding to the number of primary or secondary amino-groups present. This is another benefit of using this derivatization chemistry, i.e., one can deduce information about the number of amino-group present in an analyte, which may be useful for unknown metabolite identification or structural analysis. We also observed some ESI signal enhancement (about 1 to 10 folds depending on the compound structures) for the products, compared to the unlabeled analytes. This

can be attributed to the fact that dimethylation converts primary or secondary amine into a more easily protonated tertiary amine in positive mode detection. In addition, in the case of amino acids, the labeled ones are slightly more hydrophobic than the unlabeled ones (e.g., they elute out at a longer retention time in RP LC). The increased hydrophobicity for some of the labeled polar amino acids may enhance the ESI signals.

It should be noted that the dimethylation labeling reaction is pH dependent. Ammonium acetate buffer (pH 5.3) was found to provide the optimal condition resulting in the highest yield possible for all the analytes. The pH of reaction mixtures was carefully controlled and checked when necessary. The labeling reaction is fast. No or very little byproducts were observed from 10 minutes incubation at 37 ℃. Some byproducts could be observed if the reaction time was too long or the incubation temperature was too high. The stability of the labeled products was also studied. LC-MS chromatograms show that the products were stable at room temperature over a period of at least two weeks. The labeled products can be stored at -20 ℃ for long term storage.

In summary, reductive amination provides a simple means of labeling amine-containing compounds, such as amino acids and various amines. The labeling chemistry produces a high yield, requires a short reaction time, and is very specific under mild conditions. Due to its long term stability, an isotope labeled analog can serve as the internal standard for absolute quantification, and differential labeling of comparative samples can be used for relative quantification of amine-containing compounds (see below).

2.3.2 Evaluation of Isotopic Effects.

For quantitative analysis using the isotope dilution method, analytes and their isotopic analogs should co-elute on LC, i.e., there should be no isotopic effect on analyte retention. In this way, a pair of isotope labeled analytes would

experience the same degree of ion suppression or matrix effect in MS analysis. Thus the overall analytical efficiencies for the pair would be the same, which is important for accurate relative quantification. Figure 2.2 shows the results of the isotopic effect studies. In Figure 2.2A, the heavy deuterium dimethylated isoleucine (Ile) and leucine (Leu) eluted noticeably faster than light hydrogen dimethylated Ile and Leu on RP chromatography. This result indicates that there was less interaction between the stationary phase and the deuterium labeled compounds than there was between the stationary phase and the hydrogen labeled counterpart. The origin of the deuterium isotopic effect has been attributed primarily to the differences in the lengths of the C-D and C-H bonds;[25-27] the smaller amplitude of vibrations, and therefore lower average volumes and polarizabilities for C-D bonds. Therefore, van der Waals interactions between the hydrophobic stationary phase and deuterium labeled species, or dispersion forces that result in attraction binding forces of deuterium analogs within the hydrophobic stationary phase is less than that of hydrogen labeled counterparts. Thus, the deuterium labeled internal standard is not the best choice for stable isotope dilution quantitative analysis due to the isotopic effect in RP LC.

In contrast, as shown in Figure 2.2B, the deuterium dimethylated Ile and Leu perfectly co-eluted with their hydrogen counterparts in HILIC mode separation. There appears to be no difference in the magnitude of interactions with the stationary phase of the deuterium labeled species and that of their hydrogen counterparts. In HILIC LC, the combination of the hydrophilic interaction and ion-exchange mechanisms results in the enhanced polar retention, and the hydrophilic interaction is dominated when the mobile phase is above 70% organic solvent. The hydrophilic interaction, the partitioning of polar analytes into and out of the adsorbed water layer for deuterium and the corresponding hydrogen species are approximately the same. Thus, no isotopic effect was

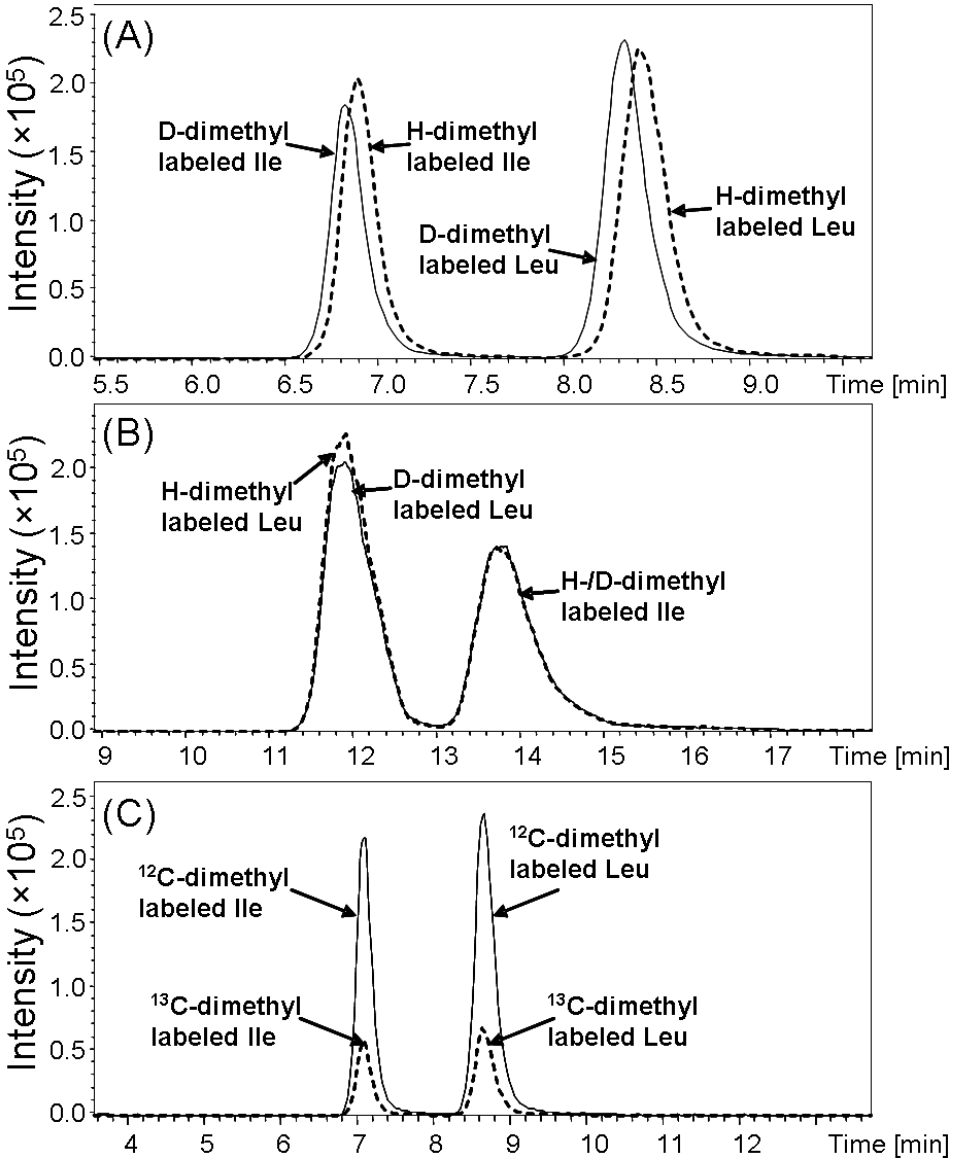

Figure 2.2 Evaluation of isotopic effects: (A) deuterium dimethyl-labeled isoleucine and leucine eluted earlier than those of hydrogen counterparts in RP chromatography. (B) Deuterium/hydrogen dimethyl-labeled isoleucine and leucine co-eluted perfectly in HILIC separation. (C) 1:4 ratio of ^{13}C-/^{12}C-dimethyl-labeled isoleucine and leucine co-eluted perfectly in RP separation.

observed in HILIC LC. Thornton et. al. reported that much less isotopic effect was observed when a less hydrophobic stationary phase (compared to C_{18}) was used, even in reversed-phase LC.[25] Our results are consistent with the notion that increasing hydrophilic interactions decreases the isotopic effect of deuterium labeled compounds.

As expected, the heavy ^{13}C-dimethylated Ile and Leu eluted with exactly the same retention time as the light ^{12}C-dimethylated Ile and Leu in RP LC (see Figure 2.2C). Not surprisingly, ^{13}C-dimethylated standards did not show any isotopic effect in HILIC separation either (data not shown). Thus, ^{13}C-dimethylation is a preferred labeling method for stable isotope quantitative analysis. This reaction produces at least a 2 Da mass difference between the light and heavy labeled analytes except for a secondary amine where the mass difference would be 1 Da. However, the more expensive ^{13}C-labeled analogs may not be needed if a HILIC separation is used for LC-ESI MS. In this case, deuterium labeling is sufficient and provides at least a 4 Da mass difference which can be advantageous in avoiding the overlaps of isotope envelopes, particularly for high mass analytes.

2.3.3 Quantitative Response.

To study the feasibility of our labeling strategy for quantitative analysis, ^{13}C- and ^{12}C-dimethylated amino acid and amine standards were mixed in ratios of 1:8, 1:4, 1:1, 4:1, and 8:1 in aqueous solution. The standard mixtures were then injected onto a RP column followed by ESI-MS. The resulting extracted ion chromatograms were obtained from the corresponding mass for heavy ^{13}C- and light ^{12}C-dimethylated products. The ratios of chromatographic peak areas were calculated for each pair of 20 amino acids and 15 amines from their corresponding extracted ion chromatograms. The linear regression plots of tryptophan, phenylalanine and methionine, as examples, are shown in Figure 2.3(A). An R-

squared value of above 0.99 was obtained for all the retained species, indicating good correlation of the experimental data with the theoretical ratios. Thus the amine-containing compounds were quantitatively and reproducibly derivatized by this protocol. The D- and H-dimethylated standards in water with the same mixing ratios were also examined using HILIC LC-ESI MS. Similar R-squared values for all the polar species were obtained (data not shown).

Matrix effect / ion suppression occurs in early stage of ionization process at LC-MS interface. Matrix effect /ion suppression could severely compromise the quality of quantification. To demonstrate the feasibility of our labeling strategy to overcome the matrix effect in complex biological samples, ^{13}C- and ^{12}C-dimethylated standards were mixed in ratios of 1:8, 1:4, 1:1, 4:1 and 8:1 in water containing 20% unlabeled human urine to mimic a complex matrix. The unlabeled urine would not contribute to the signals of the labeled ion pairs of interest, but would introduce chemical noise and co-eluting components to the analysis. With the same procedure described above, linear regression plots were performed and three examples are shown in Figure 2.3B. Similarly, the D- and H-dimethylated standards in 20% unlabeled urine with the same mixing ratios were also examined for polar species in HILIC separation. In general, the R-squared values for those in 20% unlabeled urine were over 0.99 (Figure 2.3C and 3D), about the same as those in pure water. These results of good linearity indicate that the use of dimethylated analogs as internal standards can effectively overcome the matrix effect.

In general, the performance of stable isotope dilution quantification largely depends on the control of the two labeling reactions, i.e., heavy and light dimethylation, and also the quality of the mass spectra obtained that display the isotope peak pairs. These spectra can be affected by the ionization processes of the analytes. Reductive amination occurs with a minimum change in

hydrophobicity of amino acids and amines. For some of the less polar species which can be retained on C_{18} reversed-phase columns, good quality mass spectra

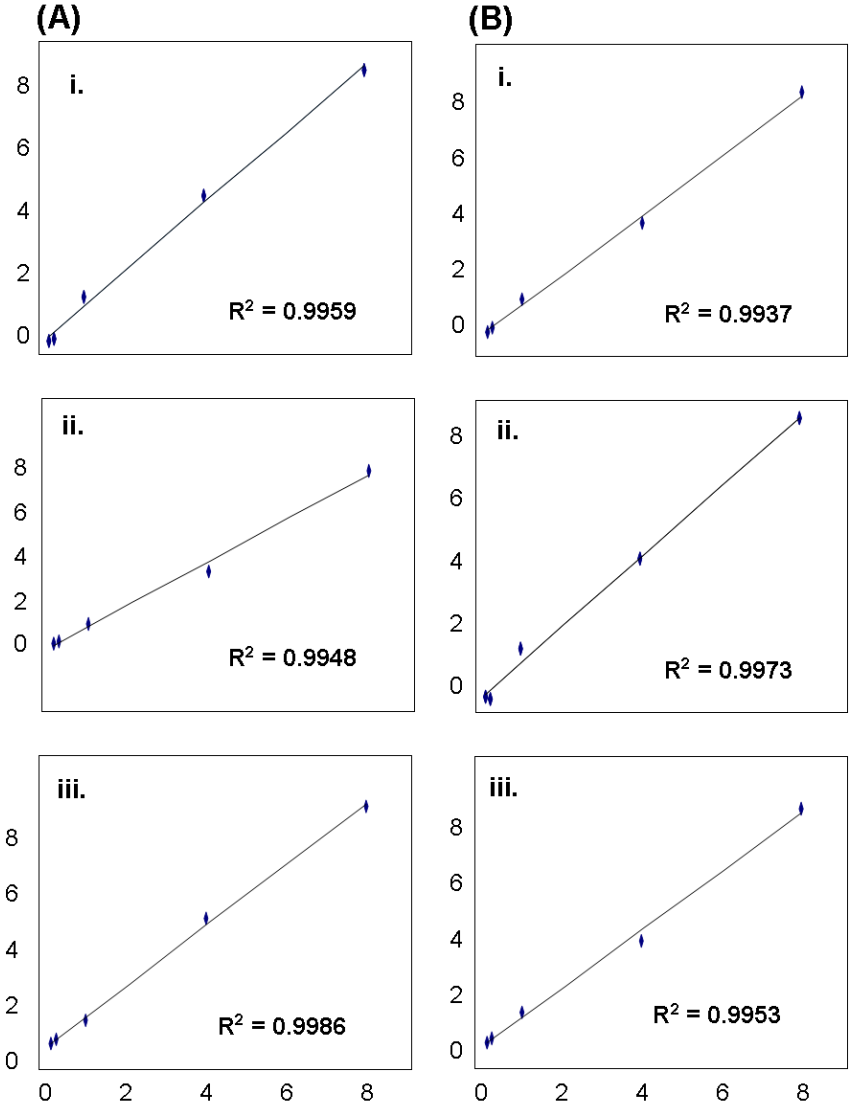

Figure 2.3 (A,B) Linear regression plot of (A) RP chromatography of (i) ^{13}C-/^{12}C-dimethyl labeled tryptophan in water, (ii) ^{13}C-/^{12}C-dimethyl labeled phenylalanine in water, and (iii) ^{13}C-/^{12}C-dimethyl labeled methionine in water. Linear regression plot of (B) HILIC separation of (i) ^{13}C-/^{12}C-dimethyl labeled leucine in water, (ii) ^{13}C-/^{12}C-dimethyl labeled

tyrosine in water, and (iii) ^{13}C-/^{12}C-dimethyl labeled asparagine in water. (x, y are the ratio of ^{13}C-/^{12}C and ^{12}C-/^{13}C)

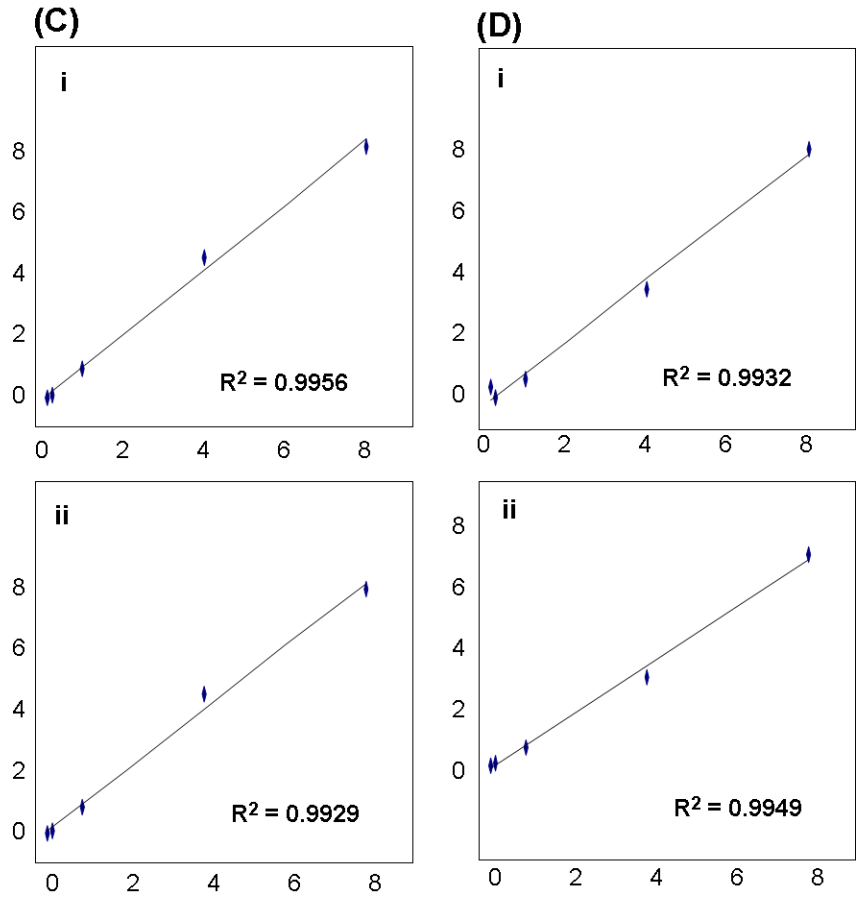

Figure 2.3 (C, D) Linear regression plot of (C) RP chromatography of (i) ^{13}C-/^{12}C-dimethyl labeled tryptophan in 20% urine and (ii) ^{13}C-/^{12}C-dimethyl labeled phenylalanine in 20% urine. Linear regression plot of (D) HILIC separation of (i) ^{13}C-/^{12}C-dimethyl labeled tyrosine in 20% urine and (ii) ^{13}C-/^{12}C-dimethyl labeled asparagine in 20% urine. (x, y are the ratio of ^{13}C-/^{12}C and ^{12}C-/^{13}C)

of the dimethylated products with high signal-to-noise ratios can be obtained. However, many of the amino acids and amines studied in this work are very polar. They are poorly retained on the hydrophobic C_{18} stationary phase, and elute in the initial void volume, resulting in low sensitivity of ESI-MS detection due to analyte ion suppression and high background levels. For those highly polar species, HILIC separation that utilizes high organic mobile phases (>70%) provides excellent complementary selectivity to that of RP chromatography. A polar species generally elutes after a less polar one, and at a high percentage of organic solvent. As a consequence, much higher sensitivity can be obtained from HILIC LC-ESI MS for the polar compounds. This is important for achieving quantitative results from the stable isotope dilution method. Thus, in this work, all the data for the polar species were obtained from the HILIC mode, and that of less polar species were from RP LC. Dimethylated products of some less polar species, such as leucine and isoleucine, were retained in both modes, and could be used for comparison studies. As illustrated above, the linear regression R-squared values of both modes were similar, indicating that both separation modes can be used for quantitative analysis with the dimethylation labeling strategy.

2.3.4 Relative Quantification of Amine-containing Metabolites in Human Urine.

Relative quantification of different metabolome samples is important for many biological studies, including the search for potential biomarkers of diseases. Our labeling strategy combined with LC-ESI MS can potentially be used to determine relative quantities of all the primary and secondary amines in two biological samples. The reproducibility of our method was evaluated by running four replicate experiments of mixtures containing 1:1 ^{12}C- and ^{13}C-dimethylated

20 amino acids in aqueous solution. Relative standard deviation (RSD) of the measured intensity ratio for tryptophan, phenylalanine, leucine, isoleucine, tyrosine, arginine, methionine, valine and lysine was calculated from extracted ion chromatograms of RP LC-MS runs. RSD of other dimethylated amino acids were calculated from extracted ion chromatograms of HILIC LC-MS runs. The results are summarized in Table 2.1. As Table 2.1 shows, the measured relative intensity ratio of an amino acid pair varies from 0.96 to 1.10 with RSD ranging from 2.1 to 9.3 (average RSD = 5.6%).

Table 2.1 LC-ion trap-MS results of four relicate measurements of 1:1 mixtures of 20 isotope labeled amino acids using dimethylation.

Metabolite	Measured ratio of the isotope labeled pair	RSD%
Glycine	1.03	8.0
Alanine	0.96	4.3
Proline	1.02	6.6
Serine	1.08	7.7
Valine	0.97	4.5
Threonine	1.07	8.1
Cysteine	1.10	8.9
Leucine	1.01	4.5
Isoleucine	1.02	5.6
Asparagine	1.09	3.9
Aspartic acid	1.10	4.4
Glutamine	1.05	2.1
Glutamic acid	1.09	9.3
Methionine	1.00	3.1
Histidine	1.07	6.2
Phenylalanine	1.00	2.7
Arginine	1.08	7.0
Lysine	1.05	8.5
Tyrosine	1.07	5.2
Tryptophan	1.02	2.3

To demonstrate the feasibility of comparative quantification by this labeling strategy, we divided a biological sample into two equal fractions (see Figure 2.1B), followed by labeling with heavy and light dimethyl isotopes, and then examined whether the ratio of heavy to light labeled pair was close to 1:1. For this demonstration, a human urine sample was split into four fractions which were ^{13}C- or ^{12}C- and D(2)- or H-dimethyl labeled under the same reaction conditions. Then the fractions from the ^{13}C- and ^{12}C-labeled samples or D(2)- and H-labeled samples, respectively, were well mixed by vortexing. The aliquots of mixtures were injected onto RP or HILIC columns, followed by ESI MS detection. Note that, for the treatment of the urine sample, only centrifugation was applied to remove possible particles in urine. Molecular weight cutoff filters were initially used to remove proteins, but found to not be necessary. The amount of sample injected was very small and the protein concentration in urine should be very low. We have not observed any adverse effect on the life time of a column or the chromatographic performance of the urine metabolites from injecting the urine samples without removal of the proteins. As an example of the urine analysis, Figure 2.4A shows the resulting base peak ion chromatogram from RP LC on the Bruker 9.4-Tesla FT-ICR mass spectrometer. The ratio of integrated peak areas or peak heights for the ^{13}C- and ^{12}C-dimethylated tryptophan pair (m/z 233.12862 and 235.13530, see Figure 2.4B) were 1.02 and 1.04, respectively. The ratio of the phenylalanine pair (m/z 194.11755 and 196.12424, see Figure 2.4C) were 1.00 and 1.02. For the 20 amino acids, the measured ratio of an amino acid pair ranges from 0.96 to 1.10 with an average ratio of 1.04 and a coefficients of variation (CV) of 4.1%. These LC-MS experimental values are in excellent agreement with the expected ratios of 1.00.

Note that, as pointed out earlier, the characteristic mass differences between heavy and light dimethylated compounds in mass spectra indicate the

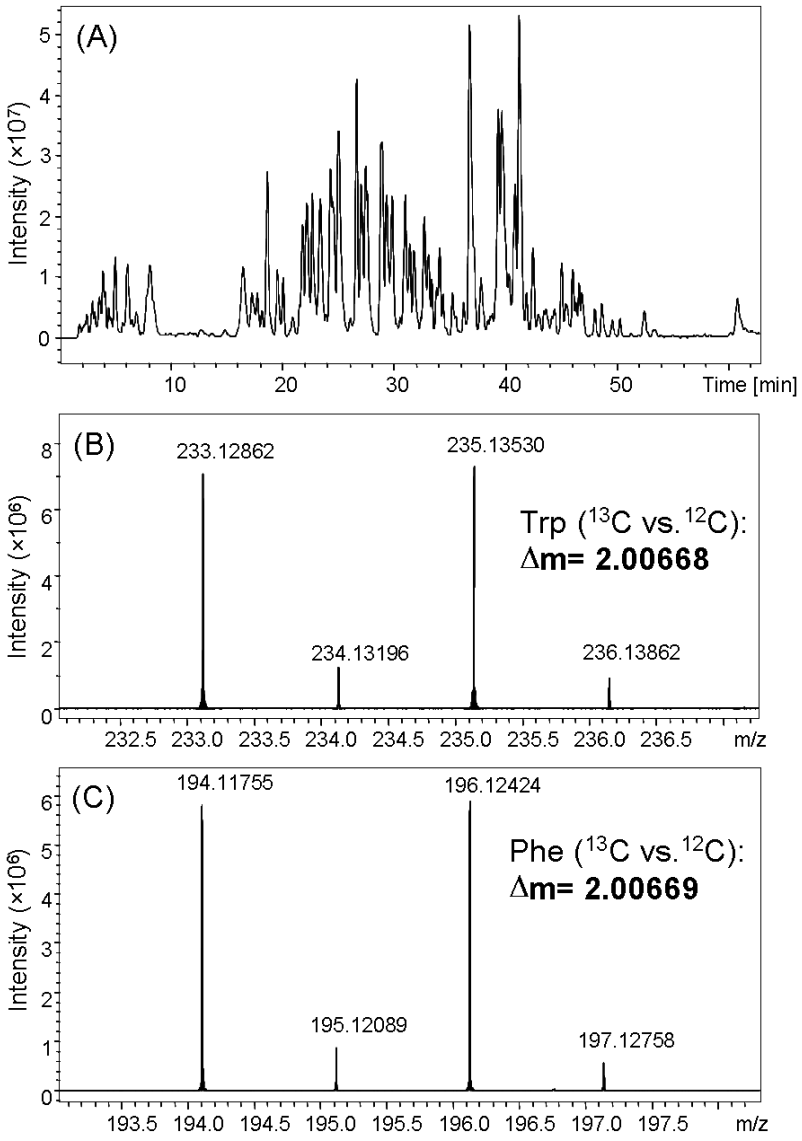

Figure 2.4 Feasibility of relative quantification of metabolites in human urine: (A) Reversed-phase base peak ion chromatogram of equivalent mixing of ^{13}C- and ^{12}C-dimethyl labeled human urine obtained by using RPLC FT-ICR-MS; (B) Mass spectrum of ^{13}C-/^{12}C-dimethyl labeled tryptophan in human urine at the retention time of 22.35 min; (C) Mass spectrum of ^{13}C-/^{12}C-dimethyl labeled phenylalanine in human urine at the retention time of 16.52 min.

existence of primary or secondary amine functional groups in a metabolite, and this could potentially be used for unknown amine identification, in addition to the information gained from retention time, MS/MS, accurate mass, and isotopic pattern by high resolution MS. In our FT-ICR-MS, the mass measurement accuracy for the metabolites is typically less than 2 ppm. This high mass accuracy facilitates the identification of ion pairs of amine-containing metabolites in the urine sample. Supplementary Table S2.1 lists 33 amine-containing metabolites detected by either RP or HILIC LC-ESI FT-ICR-MS with each pair having signal-to-noise ratios of greater than 10. The 20 amino acids plus 13 amines were positively identified based on their accurate mass and retention time in comparison to those of labeled standards. Among them, 16 were found in both RP and HILIC LC-MS runs, 2 was found in RP LC-MS alone and 15 were found in HILIC LC-MS alone.

In the RP LC-ESI FT-ICR-MS analysis of the labeled urine sample, besides the 18 metabolites identified and listed in Supplementary Table S2.1, we actually detected an additional 420 ion pairs (see Supplementary Table S2.2). All these pairs displayed a signal-to-noise ratio of greater than 10. Over 100 ion pairs showed signal-to-noise ratios greater than 80, indicating they are high abundance metabolites. Based on accurate mass and retention time information, we conclude that these ion pairs belong to different metabolites. However, these metabolites remain to be identified. Accurate mass measurement alone does not lead to unambiguous metabolite identification, considering there are many metabolites potentially present in body fluids. Nevertheless, this work demonstrates that using the labeling strategy combined with RP LC-ESI FT-ICR-MS, we can potentially profile over 439 amine-containing metabolites in human urine. One would expect that multiple dimensional separation of the labeled metabolites followed by LC-MS analysis should further expand the metabolome coverage.

2.3.5 Absolute Quantification of 20 Amino Acids and 15 Amines in Human Urine.

Absolute quantification of analytes of interest can provide direct information on the expression of a given metabolite in relation to other metabolites present in a sample. Absolute quantification by stable isotope dilution LC-MS can be done using this dimethylation labeling strategy, as long as the targeted amines are commercially available. The availability of the ^{13}C- or deuterium isotope labeled analog of an amine of interest is no longer an issue, because stable isotopic tags will be introduced onto the parent compounds using the dimethylation labeling reaction to produce the isotope analog, while the same reaction will be done on the sample to be analyzed.

As an example of absolute quantification, aliquots of human urine were heavy dimethyl-labeled with ^{13}C- or D(2)-formaldehyde. The 20 amino acid and 15 amine standard solutions of known concentration were light dimethyl-labeled with normal formaldehyde under exactly the same reaction conditions. Heavy-labeled urine was mixed with light-labeled standards and then injected onto RP and HILIC columns followed by ESI-MS analysis. Figure 2.5 and 2.6 show the base peak ion chromatograms of the ^{13}C- and ^{12}C-dimethyl labeled amino acid standards, amine standards and the heavy-labeled human urine sample mixed with light-labeled standards obtained by RP LC-ESI MS and HILIC LC-ESI MS, respectively. The calculated ratios of heavy- to light-labeled pairs of amines from their extracted ion chromatograms were used to perform absolute quantification, since the concentrations of the standards are known. The absolute concentrations of 20 amino acids and 15 amines in human urine were determined and are listed in Table 2.2. As Table 2.2 shows, the metabolites detected in the urine sample have concentrations ranging from 0.05 mg/L (arginine or tyramine) to 21.2 mg/L

Table 2.2. Absolute quantification of 20 amino acids and 15 amines in human urine.

Metabolites	Concentration (mg/L)	Metabolites	Concentration (mg/L)
Glycine	10.1	Tyrosine	3.00
Alanine	0.97	Tryptophan	4.80
Proline	0.14	Aniline	<D.L.
Serine	1.40	2-Methylbenzylamine	< D.L.
Valine	0.51	Benzylamine	0.09
Threonine	2.20	1-Ephedrine	1.90
Cysteine	0.20	p-Aminohippuric acid	1.40
Leucine	0.37	Tyramine	0.05
Isoleucine	0.27	γ-Aminobutyric acid	0.22
Asparagine	0.96	Histamine	0.26
Aspartic acid	1.40	L-4-hydroxyproline	0.29
Glutamine	21.2	Cysteamine	0.12
Glutamic acid	0.17	1,4-Diaminobutane	0.14
Methionine	0.22	(-)-Epinephrine	0.06
Histidine	16.2	Pyridoxamine	0.25
Phenylalanine	1.00	Dopamine	0.38
Arginine	0.05	3-Methyl-L-histidine	0.08
Lysine	1.60		

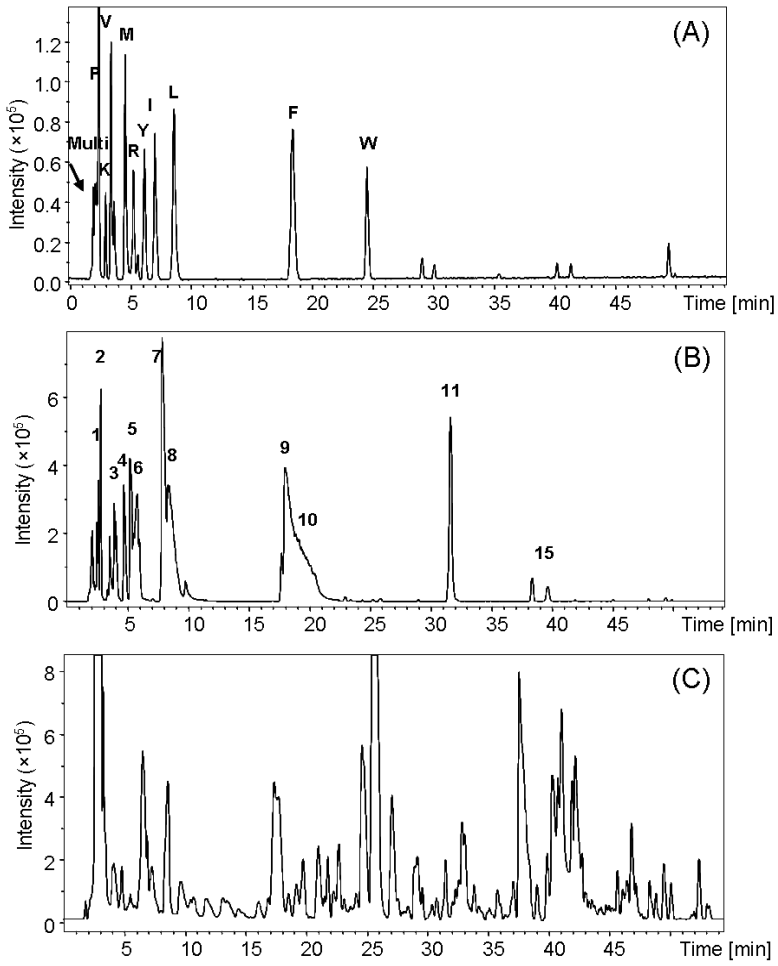

Figure 2.5 Base peak ion chromatograms of RP chromatography of (A) ^{13}C-/^{12}C-dimethyl labeled 20 amino acids; the components that eluted in the void volume are: A, C, E, Q, G, S and T, and (B) ^{13}C-/^{12}C-dimethyl labeled 15 amines. Histamine, diaminobutane, 4-hydroxproline, 3-methyl-histidine, and γ-amino-butyric acid were eluted in the column void volume. 1. pyridoxamine & cysteamine; 2. (-)epinephrine; 3. dopamine; 4. & 5. unknown; 6. tyramine; 7. benzylamine; 8. aniline; 9. 2-methylbenzyl amine; 10. 1-ephedrine; 11. p-amino-hippuric acid; (C) ^{13}C-dimethyl labeled human urine mixed with ^{12}C-dimethyl labeled standards.

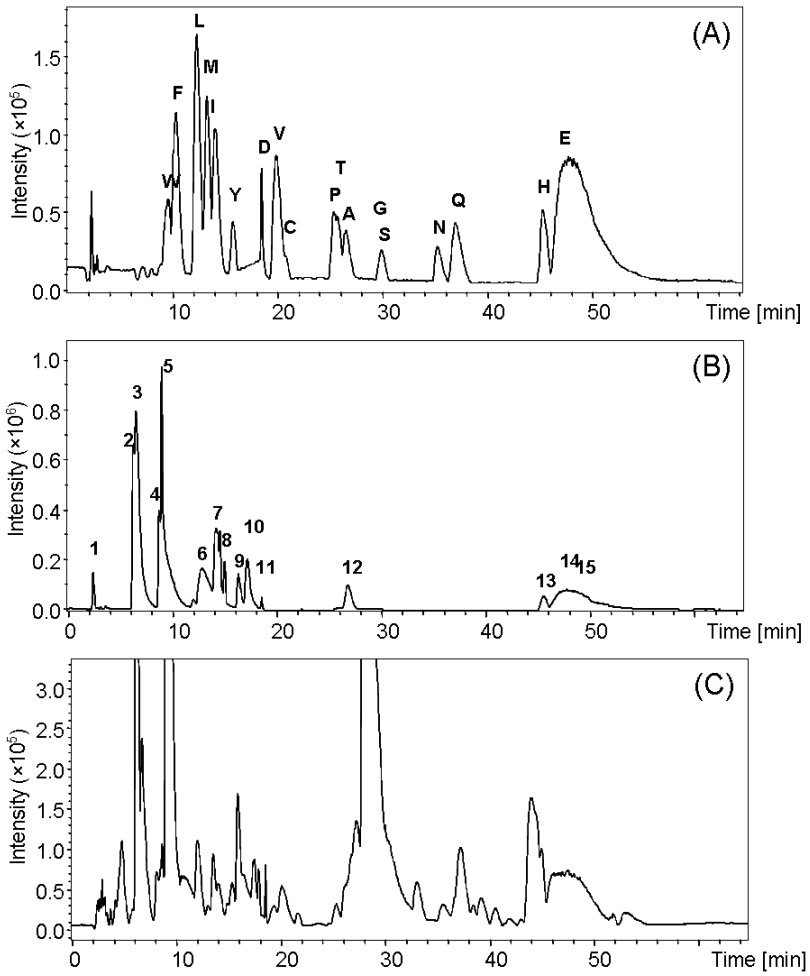

Figure 2.6 Base peak ion chromatograms of hydrophilic interaction chromatography of (A) ^{13}C- /^{12}C dimethyl labeled 20 amino acids, (B) ^{13}C- /^{12}C-dimethyl labeled 15 amines, and (C) ^{13}C--dimethyl labeled human urine mixed with ^{12}C-dimethyl labeled standards. The 15 amines in (B) are: 1. aniline; 2. 2-methylbenzylamine; 3. p-amino-hippuric acid; 4. benzylamine; 5. 1-ephedrine; 6. pyridoxamine; 7. tyramine; 8. histamine; 9. dopamine; 10. cysteamine; 11. (-)-epinephrine; 12. 4-hydroxyproline; 13. diaminobutane; 14. γ-amino-butyric acid; 15. 3-methyl-histidine.

(glutamine) with a concentration dynamic range of at least 424-fold.

The preliminary results shown in Table 2.2 illustrate the possibility of carrying out absolute metabolite quantification using the isotope dilution method with dimethylation. Additional work is required to validate this method. To this end, we are planning to study the analyte recovery issue during sample workup with spiked samples, compare this method with other widely used techniques such as the LC-UV method, and investigate the run-to-run and day-to-day reproducibility of the method. We envisage that, once the method is validated with the amino acid standards, we should be able to extend this method for absolute quantification of many other amine-containing metabolites.

It should be noted that, in addition to allowing absolute quantification, spiking a complex sample with isotopically labeled standards can facilitate the identification of targeted amines. This can be done by looking at the extracted ion chromatogram of the labeled standards: the analytes of interest in urine appear in the same mass spectrum with a characteristic mass difference.

2.4 Conclusions

In this work, we examined the use of reductive amination to provide isotope tags for amine-containing metabolites. The simple and rapid labeling reaction is carried out under very mild condition with minimum sample and reagent manipulation. The targeted amine-containing metabolites could be quantitatively derivatized under controlled reaction conditions. The dimethylated products were stable at room temperature, and the ionization efficiency of the products was not compromised. This labeling strategy essentially produces inexpensive ^{13}C- or deuterium-labeled analogs of targeted amines for stable isotope dilution analysis in RP and HILIC LC-ESI MS. This method was proved to be effective to overcome matrix effects. It was shown to be feasible to quantify

amine-containing metabolites in the complex biofluid, human urine. Absolute quantification can be performed using this strategy as long as the parent standards are available for dimethylation labeling. Relative quantification can be easily performed on biological samples by differential isotope labeling. Another advantage of labeling is that the characteristic mass difference between the heavy and light dimethyl amines provides additional information to facilitate peak and compound identification. Using FT-ICR-MS combined with one-dimensional RP LC separation, we identified a total of 438 ion pairs of amine-containing metabolites in a human urine sample. Although most of them have not been identified, they appear to be different metabolites based on the accurate mass and retention time information. Future work will involve expanding the list of 35 standards to many more biologically relevant amines and apply this isotope dilution strategy to generate quantitative profiles of amine-containing metabolites in different natures of complex samples, such as urine samples collected from disease and control objects and cells of different states. We note that, while the method can potentially be used for high-throughput quantitative profiling of amine-containing metabolome, the major challenge is for the identification of unknown metabolites. To this end, we are in the process of developing a human metabolome MS/MS database which should facilitate the identification of unknown metabolites[28].

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Chapter 3

Differential ^{12}C-/^{13}C-Isotope Dansylation Labeling and Fast Liquid Chromatography Mass Spectrometry for Absolute and Relative Quantification of Metabolome

3.1 Introduction

Liquid chromatography combined with mass spectrometry (LC-MS) has become an increasingly important tool for metabolome profiling.[1-4] An ideal LC-MS platform would identify and quantify all metabolites present in a biological sample such as cell extracts and biofluids. Unfortunately, due to great diversity in chemophysical properties of metabolites, it is very challenging to detect all metabolites at once. One strategy to tackle the diversity issue is to fractionate the metabolome into several groups, according to hydrophobicity, chemical structures or other property, and then analyze them using a combination of several optimized LC-MS methods with each tailored to a group of metabolites. We are currently pursuing an analytical strategy of selectively labeling metabolites containing a certain chemical moiety, followed by LC-MS analysis for metabolite identification and quantification.[5] In this work, we report a facile metabolome profiling technique for analyzing metabolites containing amines and phenol hydroxyls or phenols.

Amines and phenols are major groups of metabolites in a metabolome.[6-14] Quantitative profiling of amine- and phenol-containing metabolites in complex biological samples is important for biological studies and disease biomarker

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discovery using metabolomics. For example, amino acids and their derivatives are common biomarkers for human physiological processes.[15-21] Their identification and quantification in human fluids provides significant insight into human health. Another example is polyamines essential for eukaryotic cellular growth; rapid tumor growth is associated with polyamine biosynthesis and accumulation.[22] Many studies have found significantly higher levels of polyamines and their metabolites present in the biological fluids and affected tissues of cancer patients and other patients with hyperproliferative diseases.[23] Some therapeutic polyamine analogues have been shown to be potentially useful in treating cancer and other hyperproliferative disorders.[22] Quantifying amine-containing metabolites could potentially be applied to monitor tumor growth and regression in cancer studies.[22, 24]

MS-based quantification of a large number of metabolites is not straightforward. The commonly used electrospray ionization (ESI) technique is prone to interference and ion suppression from matrix molecules or co-eluting compounds during the LC-MS runs.[1, 25, 26] For analyzing a small number of metabolites, stable-isotope-labeled (SIL) analogs are often used as the internal standards to overcome the matrix and ion suppression effects.[27] However, for metabolome analysis, the number of available SIL standards is very limited and the synthesis of SIL analog of each metabolite will be very expensive and not practical. Instead of synthesizing an isotope analogy of the analyte of interest, the differential isotope labeling (DIL) method uses a chemical reaction to introduce an isotope tag to the analyte in one sample and another mass-difference isotope tag to the same analyte in another comparative sample (or standard), followed by mixing the two labeled samples for mass spectrometric analysis. The peak intensity ratio of the isotope labeled analyte pair provides the basis of relative quantification of the analyte in two comparative samples or absolute

quantification of the analyte in a sample if the other sample is a standard compound with a known concentration. DIL is widely used for quantitative proteome analysis.[28-30] However, only a few reports are on the use of DIL for quantitative metabolome analysis.

One of the early reports of using DIL for metabolite analysis was the use of the iTRAQ reagent, commonly known as the labeling reagent for peptides for quantitative proteomics, to label amino acids for quantitative analysis of these small molecules in urine and blood samples.[31] Fukusaki *et al.* reported the use of ^{13}C- and ^{12}C-methylation to introduce differential isotope tags to flavonoids for relative quantification.[32] Yang *et al*. described a LC-MS method for amino acid analysis involving derivatization with an *N*-hydroxysuccinimide ester of *N*-alkylnicotinic acid where the alkyl chain can contain deuterium, instead of hydrogen, to provide a differential isotope tag.[33] Shortreed, *et al.* reported the use of heavy and light isotopic forms of methyl acetimidate for the relative quantification of amine-containing species.[34] Guo *et al.* used the reductive amination reaction to label amine-containing metabolites with ^{13}C- and ^{12}C-formaldehyde for relative metabolome quantification.[5] Ji *et al*. reported the use of acetaldehyde-d(4) to label and quantify the monoamine neurotransmitters in rat brain microdialysates.[35] Abello *et al* developed isotope tagged pentafluorophenyl-activated esters of poly(ethylene glycol) to label amine-containing metabolites with multiplexing capability.[36] $^{13}C_4$ labeled succinic anhydride and deuterated (D9) butanol have been used for labeling metabolites for relative metabolome analysis.[37] While LC-MS is commonly used for detecting the differential isotope labeled metabolites, GC/MS has also been combined with chemical derivatization with isotope-coded reagents for metabolome analysis.[38] It should be noted that a related method using isotope enriched media for cell culturing has been used for quantitative metabolomics.[39-45]

While isotope labeling can be useful for MS-based metabolome quantification, another major challenge for metabolome profiling lies in the analysis of the large portion of highly polar metabolites present in a typical metabolome of cells and biofluids.[1, 24] Highly polar, hydrophilic compounds are poorly retained on a reversed phase (RP) LC stationary phase and will elute at or near the initial void. Sensitivity of ESI-MS detection near the void may be significantly reduced due to poor ESI desolvation as a result of the high percentage of aqueous mobile phase in initial RP gradient runs. Severe ion suppression by co-eluted polar species and salts may further decrease the ESI signal of polar analytes. To analyze the polar and ionic metabolites, LC separation based on different separation mechanisms from RPLC, such as HILIC column, has been reported to be useful.[5, 46] However, the separation efficiency of HILIC columns is relatively poor for separating complex mixtures, compared to the RP column. Moreover, the use of different columns for analyzing one sample increases the overall analysis time. Thus, it is highly desirable that chemical properties such as hydrophobicility of the analyte in a sample can be altered to an extent that they all can be separated with high efficiency using RPLC compatible to ESI-MS. In addition, the detectability of these analytes by MS should be similar, averting a bias towards a certain class of analytes, thereby increasing the metabolome coverage. The alteration of the metabolite chromatographic retention properties and MS detectability may be accomplished through chemical derivatization.

In this work, we report a chemical derivatization strategy based on dansylation reaction for absolute and relative quantification of amine- and phenol-containing metabolites in a complex sample. Dansylation is simple, robust and routinely performed for many years as pre-column derivatization for quantification of amino acids, biogenic amines and phenolic hydroxyls by thin

layer chromatography (TLC) and HPLC separation followed by fluorescence or UV detection.[47-52] Dansylation has also been used to form derivatives of targeted analytes, followed by LC-MS analysis, for the detection of p-chlorophenol and amines,[53] four phenol-containing metabolites of a drug,[54] fenfluramine and phentermine,[55] and beta-estradiol and estrone.[56] In a recent conference report, we illustrated that endogenous human metabolites in urine could be sensitively detected by dansylation derivatization and LC-MS.[57] Herein we report the synthesis of an isotope coded reagent for dansylation of amines and phenols for quantitative metabolome profiling and demonstrate that differential isotope labeling via dansylation, combined with fast LC separation of labeled metabolites and Fourier-transform (FT) ion-cyclotron resonance (ICR) MS, can substantially enhance the ESI sensitivity, improve chromatographic retention, and facilitate MS-based quantification and identification of potentially hundreds of amine- and phenol-containing metabolites in a complex biological sample.

3.2 Experimental

3.2.1 Chemicals and Reagents.

All chemicals and reagents were purchased from Sigma-Aldrich Canada (Markham, ON, Canada) except those otherwise noted. The isotope compound, $^{13}C_2$-dimethyl sulfate, used to synthesize the isotope tagged dansylation reagent (^{13}C-danysl chloride) was also purchased from Sigma-Aldrich. LC-MS grade of water, methanol and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Edmonton, AB, Canada). Urine samples were collected from a healthy individual and processed by adding 50% (v/v) LC-MS grade acetonitrile, then stored in a -20 ℃ or -80 ℃ freezer.

3.2.2 Synthesis of Dansyl Chloride-$^{13}C_2$.

The synthesis of ^{13}C-dansyl chloride as a derivatizing reagent was based on a two-step procedure described by Horner and Bergmann.[58, 59] Figure 3.1A

shows the synthesis scheme. In a 25-mL round-bottom flask, 0.78 g of 5-aminonaphthalene-1-sulfonic acid was added slowly in portions to 1.09 g of sodium bicarbonate in 3.5 mL of water. Then 0.77 mL of $^{13}C_2$-dimethyl sulfate was added drop-wise over 30 minutes to the stirred ice-cooled solution. The solution was warmed to 80^{o}C in a hot water-bath for 30 min. After cooling to room temperature, 0.46 mL of concentrated hydrochloric acid was added to the solution, and the pH was adjusted to 4. The precipitated product, 5-dimethylamino-naphthalene-1-sulfonic acid was filtered, washed with a small quantity of water, dried in the air to a constant weight and then further dried at 120^{o}C in an oven. Under ice-cooling, 5-dimethylamino-naphthalene-1-sulfonic acid was grounded into a powder and then mixed with 0.88 g of phosphorus pentachloride. To complete the reaction, the mixture was warmed to 60^{o}C for two hours under the exclusion of moisture. About 12.5 mL of ice water was then poured in. After careful neutralization with 1.75 g of sodium bicarbonate, the product was extracted with Et_2O (4 × 6 mL). The organic layer was dried using sodium sulfate. The residue was purified by flush chromatography (silica gel, 40 × 3 cm, 20 mL AcOEt), and further purified by a semi-preparative Grace Apollo silica normal-phase HPLC column (10 × 150 mm, 5 μm particles). The resulting product of ^{13}C-dansyl chloride was then dried in a Speed Vacuum and stored in a -80^{0}C freezer. The purity and confirmation of ^{13}C-dansyl chloride was tested against the commercial ^{12}C-dansyl chloride using LC-FTICR MS. NMR was also used to characterize the reaction products and confirm the identity and purity of the final product.

3.2.3 Dansylation Labeling Reaction.

Figure 3.1B shows the reaction schedule for dansylation of amine- and phenol-containing compounds. The frozen urine was thawed in an ice-bath and then centrifuged for 10 min at 12000 rpm. About 100 μL of urine supernatant, or

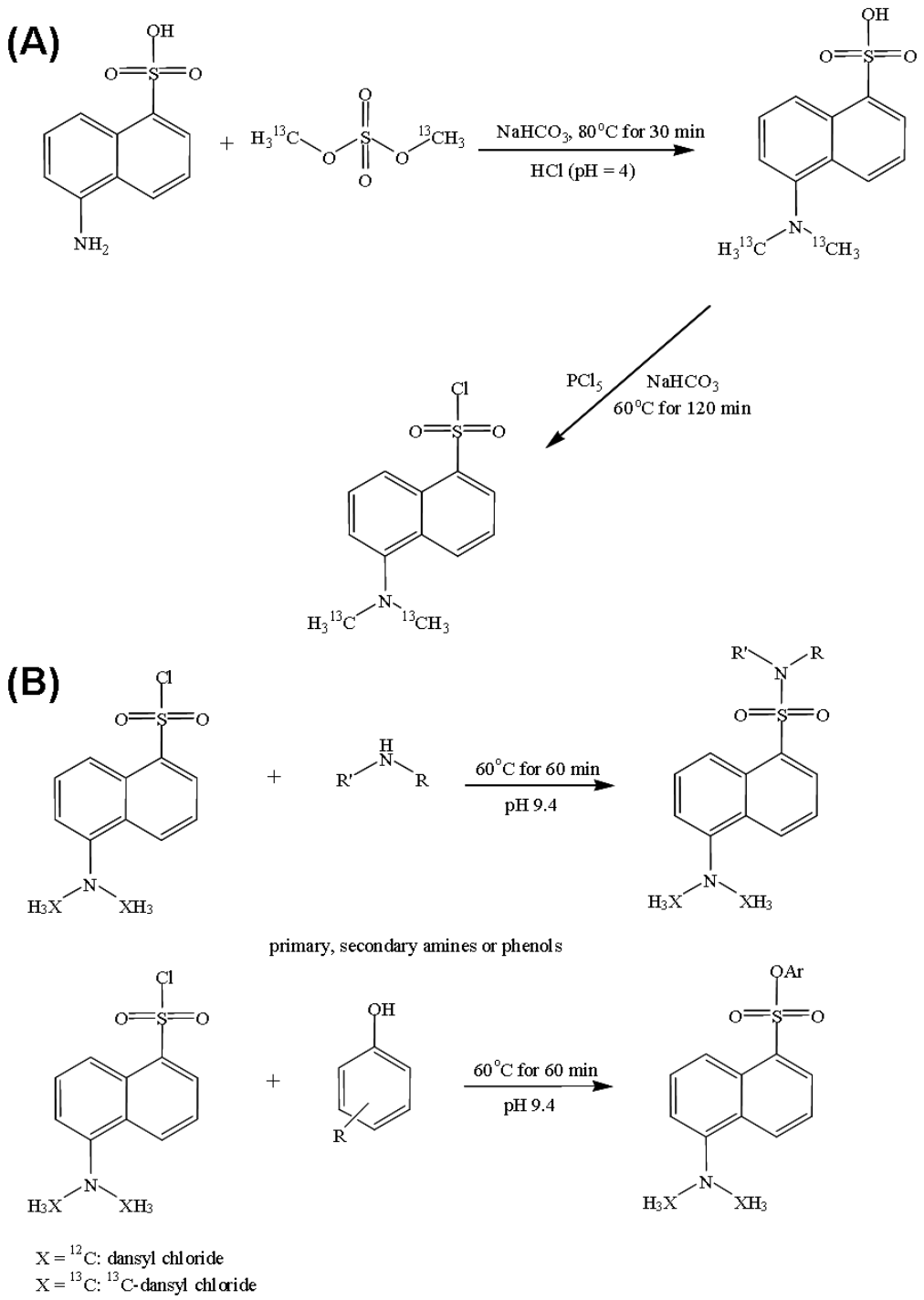

Figure 3.1 Reaction schemes for (A) synthesis of the isotope labeling reagent, dansyl chloride- $^{13}C_2$ and (B) dansylation derivatization.

amino acids, amine, phenolic hydroxyl standard solutions were mixed with an equal volume of sodium carbonate/sodium bicarbonate buffer (0.5 mol/L, pH 9.4) in a reaction vial. The solutions were vortexed, spun down, and mixed with 100 μL of freshly prepared ^{12}C-dansyl chloride solution (20 mg/mL) (for light labeling) or ^{13}C-dansyl chloride (20 mg/mL) (for heavy labeling). The dansylation reaction was allowed to proceed for 60 min at 60^{o}C with shaking at 150 rpm in an Innova-4000 bench top incubator shaker. After 60 min, mixtures were vortexed, spun down and 30 μL of methylamine (0.5 mol/L) was added to the reaction mixture to consume the excess dansyl chloride. The solutions were again vortexed and spun down. After an additional 30 min of 60^{o}C incubation, samples were then centrifuged. The ^{13}C-labeled mixtures were combined with their ^{12}C-labeled counterparts for MS analysis. The reaction vials were carefully washed twice using 50 μL LC-MS grade MeOH and the washing solution was added to the initial mixture to ensure dissolution and transfer of all products for MS analysis. The combined mixtures were centrifuged for 10 min at 12000 rpm and were ready to be injected onto a RPLC column.

3.2.4 LC-MS.

The HPLC system connected to the FTICR MS or ion-trap MS was an Agilent 1100 series binary system (Agilent, Palo Alto, CA) and was modified to reduce extra system solvent volume according to an Agilent protocol (Agilent Publication Number: 5988-2682EN). For the fast (12-min) chromatography runs, a reversed-phase Waters BEH C_{18} column (2.1 × 50 mm, 1.7 μm particle size, 130 Å pore size) was purchased from Waters (Milford, MA). Solvent A was 0.1% (v/v) LC-MS grade formic acid in 5% (v/v) of LC-MS grade acetonitrile, and solvent B was 0.1% (v/v) LC-MS grade formic acid in LC-MS grade acetonitrile. The binary gradient elution profile was as follows: t = 0 min, 20% B; t = 1.5 min, 35% B; t = 8 min, 65% B; t = 9.3 min, 95% B; t = 9.8 min, 95% B; t = 10 min,

99%B. The flow rate was 170 μL/min and the sample injection volume was 1.0 μL.

For the 65-min chromatography experiment, a reversed-phase Agilent Zorbax XDB C_{18} column (1.0 × 150 mm, 3.5 μm particle size, 80 Å pore size) was purchased from Agilent. Solvent A was 0.1% (v/v) formic acid in 5% (v/v) acetonitrile, and solvent B was 0.1% (v/v) formic acid in acetonitrile. The 65-minute binary gradient elution profile was as follows: t = 0 min, 0% B; t = 6 min, 0% B; t = 21 min, 30% B; t = 54 min, 90% B; t = 65 min, 90% B. The flow rate was 50 μL/min and the sample injection volume was 1.0 μL.

The flow from RPLC was directed to the electrospray ionization (ESI) source of a Bruker 9.4-Tesla Apex-Qe FTICR mass spectrometer (Bruker, Billerica, MA, USA) or a Bruker Esquire ion trap mass spectrometer. All MS spectra were obtained in the positive ion mode. It was found that negative ion detection was not as sensitive as the positive ion detection for dansylated derivatives. The Esquire LC-MS system was only used for method development. All the data presented in this work were obtained using the 9.4-Tesla FTICR mass spectrometer.

3.3 Results and Discussion

3.3.1 ^{13}C-/^{12}C-Dansylation Derivatization.

One of the important considerations in developing labeling chemistry to tag the metabolites with differential isotopic group(s) is that the derivatization chemistry must be simple and robust. The differential dansylation labeling technique reported herein is based on well-studied derivatization chemistry.[47-52] Using this chemistry, primary amines, secondary amines and phenolic hydroxyls are dansylated with high yield, while tertiary amines and alkyl hydroxyls cannot be dansylated. The derivatization process is simple with mild reaction conditions and without the need of any special equipment. Because our interest is to profile

as many metabolites as possible, we have examined the performance of this derivatization chemistry on a variety of metabolites. In total, 161 metabolite standards were subjected to dansylation individually and their products were analyzed by LC-ESI MS. Among them, 121 metabolites were found to be derivatized under the chosen reaction condition (see Table 3.1 for the list). The other 40 metabolites including amides and indole-derivatives could not be derivatized (see Supplemental Table S3.1). For the amides, as expected, dansyl chloride does not react with the amide nitrogen. For the indole-derivatives, it appears that, if the targeted nitrogen is a part of a conjugated structure in the metabolite molecule, this nitrogen will not react with dansyl chloride. Except for this special type of amines, our study indicates that dansyl chloride is reactive with a wide range of amines and phenols. For the 121 reactive metabolites, possible impurities, side-reaction products, ESI mass spectral patterns and RPLC retention times of the dansylated derivatives were carefully examined by LC-MS. This compound list forms the current dansylation standard library and, as it will be discussed below, we intend to expand this library in the future for more comprehensive metabolome analysis.

For differential isotope labeling of metabolites containing amine and phenolic hydroxyl or phenol group(s), the light reagent ^{12}C-dansyl chloride is commercially available and the heavy reagent ^{13}C-dansyl chloride can be synthesized according to the reaction scheme shown in Figure 3.1A. We have found that the dansylation labeling process itself does not introduce LC-MS background signals. After the reaction, excess dansyl chloride can be consumed by adding methylamine. The resulting dansyl methylamine does not significantly interfere with the detection of the labeled metabolites, as it can be chromatographically separated from most of the dansylated amino acids, amines, and phenols tested (see, for example, Figure 3.2A). We note that, for the

dansylation reaction, it is crucial to keep the buffer pH at 9.4-9.5. The buffer pH should be sufficiently high so that the amine group is present in the neutral NH_x (x=1-2) form and should be low enough to avoid reagent hydrolysis as a competitive side reaction.[60]

3.3.2 Chromatography Improvement and ESI Signal Enhancement.

Due to diverse chemical structures and chemophysical properties of metabolites, separation and detection of these compounds in a complex biological sample may not be readily accomplished using a single LC-MS method. For example, many biofluids, particularly urine, contain a large number of highly polar and poorly ESI ionizable metabolites.[6, 24] Efficient LC separation and ESI MS detection of these metabolites can be difficult.[25, 61] However, dansylation derivatization can overcome this difficulty by altering the chromatographic retention behavior of very polar metabolites and improving the ESI responsiveness of the analytes.

Figure 3.2A shows the base peak ion chromatograms of two separate RPLC-MS runs from the injections of a mixture of 20 free amino acids (500 pmol each) and a mixture of dansylated amino acids (5 pmol each). Two significant differences are clearly noticeable by comparing the two ion chromatograms. One is related to the chromatographic separation; the 20 dansylated amino acids are separated much better than the underivatized amino acids. This is not surprising in light of the fact that dansylation has been shown to provide better chromatographic separation in RPLC with UV or fluorescence detection than the free amino acids.[47-52] A more striking difference is on the detection sensitivity. Even though the injection amount of the individual dansylated amino acids is 100-fold less than that of the free amino acids, the overall ion signal intensity of the dansylated amino acids is much greater than that of the free amino acids. The signal enhancement factors for the ESI detectable amino acids, such as Phe, Ala,

Leu and Ile, are about one to two orders of magnitude. For the ESI-insensitive amino acids or highly polar ones that do not retain on the RPLC column, a separate set of experiments were carried out to compare the ESI responsiveness where individual dansyl or free amino acids were continuously infused into the ESI mass spectrometer. The MS signal enhancement factors were found to be up to three orders of magnitude, depending on the solvent conditions and MS tuning parameters used. As Figure 3.2A shows, within the LC run of an equal mole mixture, different dansyl amino acids give different peak intensities or different detection sensitivities. However, the sensitivity difference is within the range of two orders of magnitude. Thus, dansylation has a leveling effect on the LC-MS detection of the 20 amino acids. In other words, dansylation makes the vastly different metabolites become more uniform in chromatography retention properties (i.e., they all are retained in the RPLC column) and ESI detectability.

Another example of signal enhancement by dansylation is shown in Figure 3.2B where two ion chromatograms obtained from the LC-MS analysis of plain human urine and dansylated urine are shown. It is clear that, with the injection of the same amount of the samples, the overall ion intensity of the analytes in the dansylated urine is much higher than that of the plain urine.

The significant gain in ion signals by dansylation can be attributed to three major factors. First of all, the relatively hydrophobic naphthalene moiety introduced to an analyte through dansylation allows an otherwise very polar analyte to be eluted in a much higher percentage of organic mobile-phase during the RPLC gradient runs. The ionization desolvation process becomes much more efficient at a higher percentage organic solvent. Electrospray stability is also improved due to decreased surface tension in a droplet containing a higher organic content. Secondly, the hydrophobic naphthalene moiety tagged to an analyte increases the droplet surface affinity of the analyte, resulting in a

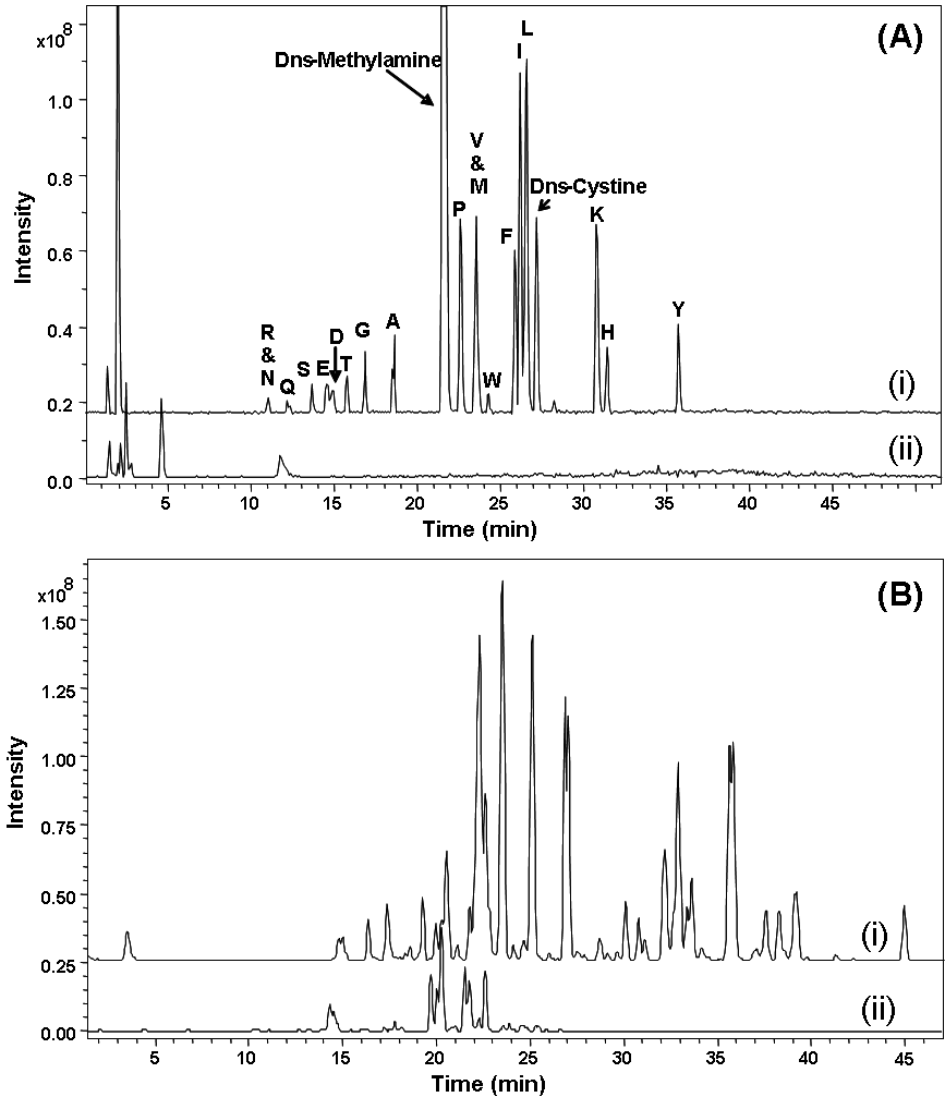

Figure 3.2 (A) Base peak ion chromatograms of a mixture of 20 dansylated amino acids (5 pmol each) (upper chromatogram) and a mixture of free amino acids (500 pmol) (lower chromatogram) obtained by using LC-FTICR MS. (B) Base peak ion chromatograms of dansylated human urine (upper chromatogram) and plain urine (lower chromatogram) obtained by using LC-FTICR MS.

significant enhancement in the ESI surface activity of the analyte. In general, as long as an analyte molecule is chargeable under the ESI condition, the analyte containing more hydrophobic and less polar groups will have a higher electrospray response than that with more polar groups.[25] This is because the less polar ions would prefer the droplet air interface and thus would reside at the droplet surface. As a consequence, the less polar ions would compete more favorably for the limited amount of charges and space on the droplet surfaces and would enter the gas phase more readily than those found in the droplet interior, resulting in a higher ESI response. Finally, the chargeability of the analyte is improved through the introduction of a more easily protonated dimethylamino moiety, resulting in more favorable competition for the limited amount of charges on the droplet surfaces. The conjugated structure of naphthalene may play a role in the stabilization of the protonated charge at the tertiary amine moiety and thus the proton affinities of dansylated derivatives are expected to increase.

Dansylation derivatization is a highly selective ESI enhancement process for both amines and phenols. For example, ion chromatogram ii in Figure 3.2B, the peaks shown are not from the amines or phenols. This was determined by comparing the peak masses of the plain urine and its corresponding dansylated urine; an amine detected in the plain urine would have a characteristic upper mass shift in the mass spectra of the dansylated urine. All the peaks shown in the dansylated urine appear to be the derivatives of amines or phenols (see below for more discussion on peak identification). Thus, with dansylation, amine and phenols in the urine sample were selectively detected by LC-MS. Apparently dansyl derivatives suppressed the other co-eluting non-reactive components in LC-MS. Note that, to profile the non-reactive components, better separation (e.g., using multidimensional LC separation) will be required to lessen co-elution of

labeled and unlabeled metabolites, thereby reducing ion suppression of the non-reactive metabolites.

Another major benefit of dansylation labeling is that the signal-to-background ratio of the analytes can be improved, because the mass-to-charge ratio of the derivatives are shifted out of the low-mass region that typically exhibits significant background noise from solvent clusters and common contaminants.[62] In addition, tuning the FT-ICR mass spectrometer to achieve high sensitivity and high mass measurement accuracy becomes much easier after the m/z values of dansyl derivatives are shifted to the detection mass range of above m/z 250.

Finally, dansylation derivatization increases the stability of metabolites for LC-MS. Some metabolites such as phenylalanine and methionine may fragment in-source or during the transition to the mass analyzer. This reduces the molecular ion peak intensity and makes the spectral interpretation difficult. Since many m/z values are detected in a typical mass spectrum of a LC-MS run of a complex mixture, the fragment ions in the mass spectrum may be mistakenly considered to be from the metabolites present in the sample. With dansyl derivatives, we rarely observed any in-source fragment ions. Thus, a peak observed in the mass spectrum can be confidently assigned to a metabolite ion, not a fragment ion.

3.3.3 No Isotopic Effect in RPLC.

Stable isotope labeled (SIL) compounds are commonly used as the internal standards for targeted metabolite quantification by LC-MS, such as the analysis of metabolites of a drug candidate. Ideally, SILs and analytes should have identical behavior in the sample preparation and ionization process. Because deuterated-SILs often have different retention properties from those of the analytes in RPLC,[63, 64] they may not be co-eluted and ionized simultaneously.[65] Thus, there

is a possibility that the SILs and analytes experience different matrix or ion suppression effect, resulting in different signal responses. This isotopic effect can be mitigated by using ^{13}C-SILs. Unfortunately, for metabolome profiling where a large number of different metabolites are analyzed, the number of commercial available ^{13}C-SILs is very limited. With differential ^{13}C-/^{12}C-dansylation, there is no need to use an expensive or time-consuming route to generate ^{13}C-SIL; dansylation creates a ^{13}C-labeled internal standard for every ^{12}C-labeled analytes. The drawback is that the sample needs to be derivatized via the same reaction used to label the standards. However, sample derivatization, so long as the reaction used is robust and fast, is just another step of sample preparation and should not be a limiting factor in sample throughput in the overall metabolome workflow. Sample derivatization can be done in parallel or in batches while LC-MS analysis is currently done in series and is a bottleneck in sample throughput.

Figure 3.3 shows the extracted ion chromatograms of RPLC-MS from the injection of a 5:1 ^{13}C-/^{12}C-dansylated isoforms of Trp, Ile, Leu and Lys. The four dansylated amino acids chosen are relatively hydrophobic amongst the twenty amino acids and dansylated Lys contains two dansylated tags. The isotope tags of these amino acids would interact more with hydrophobic stationary phases than the relatively polar amino acids. Multiple tags could potentially create even larger isotopic effect.[65] Thus the four dansylated amino acids shown in Figure 3.3 should represent the extreme case of isotopic effect. However, as Figure 3.3 shows, even in these cases, all four of the ^{13}C-dansylated derivatives as internal standards exactly co-elute with their ^{12}C-counterparts in RPLC. Due to the lack of isotopic effect, matrix and ion suppression effect between the ^{12}C-labeled analytes and their corresponding ^{13}C-labeled internal standards are expected to be identical. In principle, the peak abundance ratio of the ^{13}C-/^{12}C-ion pair can be calculated from

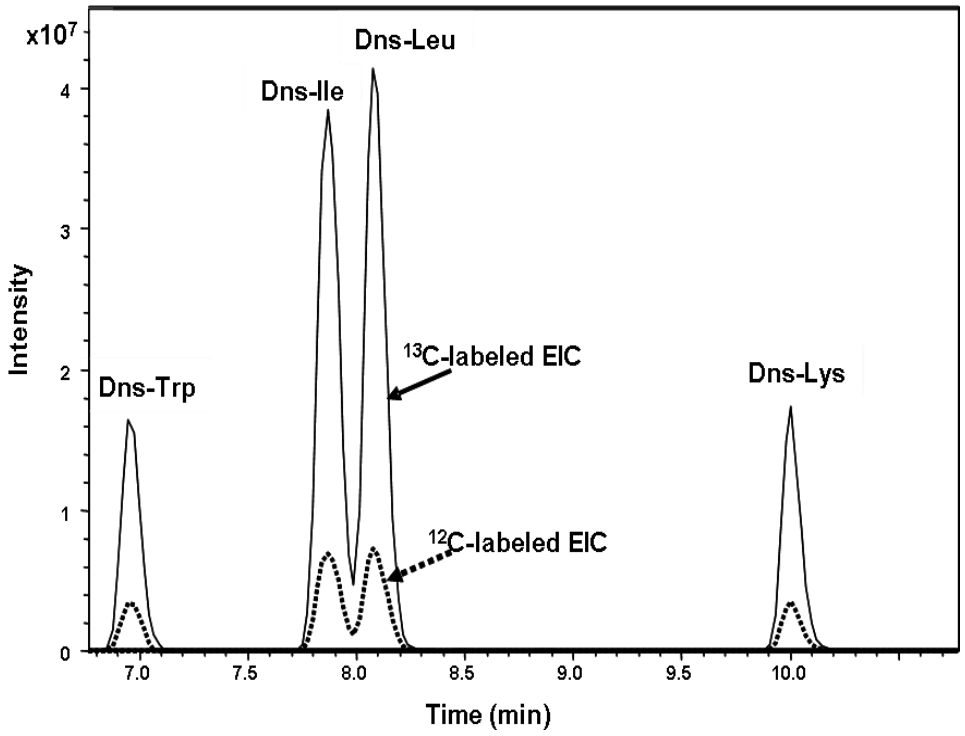

Figure 3.3 Extracted ion chromatograms of 1:5 of ^{12}C-/^{13}C-dansylated Trp, Ile, Leu and Lys obtained by fast gradient RPLC-FTICR MS.

a single mass spectrum taken at any point in the eluting chromatographic peak. In our data analysis, two to four mass spectra, dependent on the signal intensity of the ion pair, were usually averaged to obtain better accuracy and precision in the abundance ratio calculation.

Because of no isotopic effect on RPLC separation of the differential isotope dansyl derivatives, ^{12}C-/^{13}C-dansylated isoforms are always detected as pairs in the mass spectra with characteristic mass differences. This greatly facilitates the spectral interpretation and metabolite identification. Ion pairs with characteristic peak profiles also indicate the existence and number of reactive functional groups. The dansyl tag generates a nominal 234-Da mass shift for the analytes with one reactive functional group and a 467-Da mass shift for the analytes with two functional groups. The ^{12}C-/^{13}C-dansylated ion pair with a mass difference of 2.00671 indicates only one tag added and a mass difference of 4.01342 indicates the presence of two tags added. In the 9.4T FTICR mass spectrometer, the error of mass differences of ^{12}C-/^{13}C-dansylated ion pairs is typically less than 1.5 ppm. We used this level of accuracy as a criterion to confirm the presence of ^{12}C-/^{13}C-labeling ion pairs.

3.3.4 Fast LC-MS.

The ion chromatograms shown in Figure 3.3 were obtained using a short LC column combined with ESI FTICR MS. This was purposely done to gauge the possibility of using a shorter column to reduce the overall analysis time while maintaining a sufficiently high peak capacity for detecting a large number of metabolites. Quantitative profiling of the metabolome in applications such as disease biomarker discovery involves the analysis of a large number of samples to produce statistically meaningful results. Thus, rapid analysis is highly desirable to improve sample throughput. One option to improving the speed of analysis is to use a shorter column packed with small particles. Small particle columns have

been shown to improve separation efficiency in ultra-high pressure liquid chromatography (UPLC).[66] We have investigated the use of a 5-cm Waters reversed phase BEH column packed with sub-micron particles (1.7 μm). The use of this short column does not require any special pumps such as ultra-high pressure pumps; a conventional HPLC system can be used. As Figure 3.3 shows, narrow peak widths (8-12 s) are observed. Compared to a longer column, the overall peak capability is, of course, reduced. However, the enhanced ESI detection sensitivity, the improvement of chromatographic separation and a total elimination of isotopic effect through ^{13}C-/^{12}C-dansylation derivatization compensates for the performance loss due to the use of a shorter column. As illustrated below, quantitative analysis of a large number of amines and phenols can be carried out using fast LC combined with FTICR MS.

3.3.5 Relative Metabolome Quantification.

Relative quantification of amines and phenols in two comparative metabolome samples can be done by making ^{12}C-dansyl derivatives from one sample and ^{13}C-dansyl derivatives from the other sample, followed by mixing the two labeled samples and injecting the mixture into LC-MS for analysis. The intensities of the mass spectral peak pairs are compared to generate information on the relative quantity differences of the metabolites in the two samples. To detect the relative quantity changes, a reasonably large range of linear responses is required. To investigate the capability of our method for accurate relative quantification, ^{12}C- and ^{13}C-dansylated amino acids standards were mixed in ratios of 1:20, 1:10, 1:5, 1:1, 5:1 and 10:1, and then injected into the 5-cm sub-micron RP column followed by FTICR MS. The analyses were done in triplicates. The ratios of the isotope peak areas of the extracted ion chromatograms for 20 amino acids were calculated after subtracting out the natural abundance of the second isotopic peak of a ^{12}C-dansylated analyte ion.

The average isotopic ratios calculated from each pair of ^{13}C- and ^{12}C- dansylated amino acids were determined to be 0.046, 0.093, 0.195, 1.02, 4.66, 10.9 with relative standard derivation (RSD; n=3) 6.9%, 7.4%, 5.4%, 2.3%, 2.8%, 4.6%, respectively, for the 1:20, 1:10, 1:5, 1:1, 5:1 and 10:1 mixtures. In the linear regression plots of the measured values vs. the expected values, the average R-square values obtained for 20 amino acids were 0.999, indicating a good correlation of the experimental data with the theoretical ratios.

The above results illustrate that a linear range from 1:20 to 10:1 can be achieved with the differential isotope dansylation labeling for relative metabolome quantification. The lower limit is governed by the detection dynamic range of the mass spectrometer. For a mixture of <1:20, the peak intensity of the ^{12}C-dansyl derivative cannot be measured accurately. Increasing ion accumulation time in the trap would increase its signal; but the corresponding ^{13}C-dansyl derivative signal becomes saturated, making it impossible to measure the correct intensity ratio of the two ions. For a mixture of >10:1, the natural abundance ^{13}C-isotope peak of the ^{12}C-dansyl derivative starts to interfere with the measurement of the peak intensity of the isotope peak of ^{13}C-dansyal derivative. Nevertheless, the linear range of 1:20 to 10:1 should be adequate for most applications involving relative metabolite quantification, as greater than 10-fold or 20-fold changes of metabolite abundances are the exceptions while most biological events only induce subtle changes.

To further examine the performance of fast LC-MS with dansylation for relative quantification of metabolites, we applied this method to address a real world issue related to the metabolome sample storage. Specifically, for metabolome analysis of urine samples, fresh urines are often stored under certain conditions for a period of time prior to analysis. During the storage, metabolites may change their properties if the storage conditions are not properly controlled.

In principle, samples stored in a -80 ℃ freezer is preferred over a -20 ℃ freezer; but the cost associated with the use of a -20 ℃ freezer is substantially lower. The question here is whether there is any difference on the use of -80 ℃ and -20 ℃ freezers for urine sample storage for metabolome profiling. To address this question, we used a forward and reverse labeling strategy to examine the metabolite abundance changes, if any, between the urine samples stored at two different temperatures. A human urine sample was centrifuged and equally split into two fractions. One fraction was stored in a -20^{o}C freezer and the other in a -80^{o}C freezer for 14 days. After the samples were thawed, four separated isotopic labeling experiments were carried out on each sample (i.e., four replicate labeling experiments). In the forward labeling, the -20 ℃ urine sample was labeled by ^{12}C-dansylation and the -80 ℃ sample was labeled by ^{13}C-dansylation. The two labeled samples were then combined to form a forward labeling mixture. The order was reversed to form a reverse labeling mixture (i.e., ^{12}C-dansylated -80^{o}C urine combined with ^{13}C-dansylated -20^{o}C urine). In total, four forward labeling mixtures and four reverse labeling mixtures were prepared. Each mixture was injected three times onto the fast LC-MS system to gauge the run-to-run reproducibility.

Table 3.2 shows two examples of the measured abundance ratios of 30 metabolites found in urine (i.e., two amino acids, Asn and Gln), while Supplemental Table S3.2 lists the complete results for 20 amino acids, five known biological amines, and five unknowns. As Table 3.2 (and Table S3.2) illustrates, there is no significant difference between the abundance ratios of ^{12}C-dansylated (-20^{o}C urine)/^{13}C-dansylated (-80^{o}C urine) and ^{12}C-dansylated (-80^{o}C urine)/^{13}C-dansylated (-20^{o}C urine). For the 30 compounds studied, the average ratio of ^{12}C-dansylated (-20^{o}C urine)/^{13}C-dansylated (-80^{o}C urine) for the forward labeling mixture is 1.004, while the average ratio is 1.007 for the reverse labeling mixture.

The experimental reproducibility is excellent. The run-to-run RSD ranges from 1.1 to 4.9% with an average of 3.2%. The experimental RSD ranges from 3.1 to 7.7% with an average of 5.3%. These results indicate that, at least for the analytes listed in Tables 3.2 and S3.2, there is no significant difference between -80^{o}C and -20^{o}C urine storages of up to 14 days. It should be noted that the measured masses of the 20 amino acids and 5 amines were within 1.5 ppm from the calculated molecular masses. The average error of mass differences between the ^{13}C- and ^{12}C-dansylated isoforms is 0.028 ppm. With the retention time information for each known metabolite, along with accurate mass measurement of the isotope pairs, we can confidently identify these 25 known compounds in the urine sample. This example demonstrates that dansylation combined with fast LC-MS can provide a means of accurate relative quantification of amines (and phenols, as shown in Table 3.1) with excellent labeling reaction reproducibility and LC-MS run-to-run reproducibility.

3.3.6 Absolute Metabolome Quantification.

With the availability of a dansyl compound library, it is possible to determine the absolute concentration of each metabolite in a biological sample, as long as the dansylated analyte standard is present in the library. We have explored a strategy of measuring absolute metabolite concentrations of individual samples by using a pooled sample as an internal standard (see Figure 3.4). As Figure 3.4A illustrates, a pooled sample is prepared by taking aliquots from individual samples and then combining them to form a composite sample. This sample is labeled with ^{12}C-dansylation. The ^{13}C-dansylated metabolite standards are spiked to an aliquot of the pooled sample, followed by running the mixture in LC-MS. The metabolites present in the pooled sample can be identified based on the retention time match and accurate molecular mass measurement. The metabolite concentration can be determined based on the measured peak

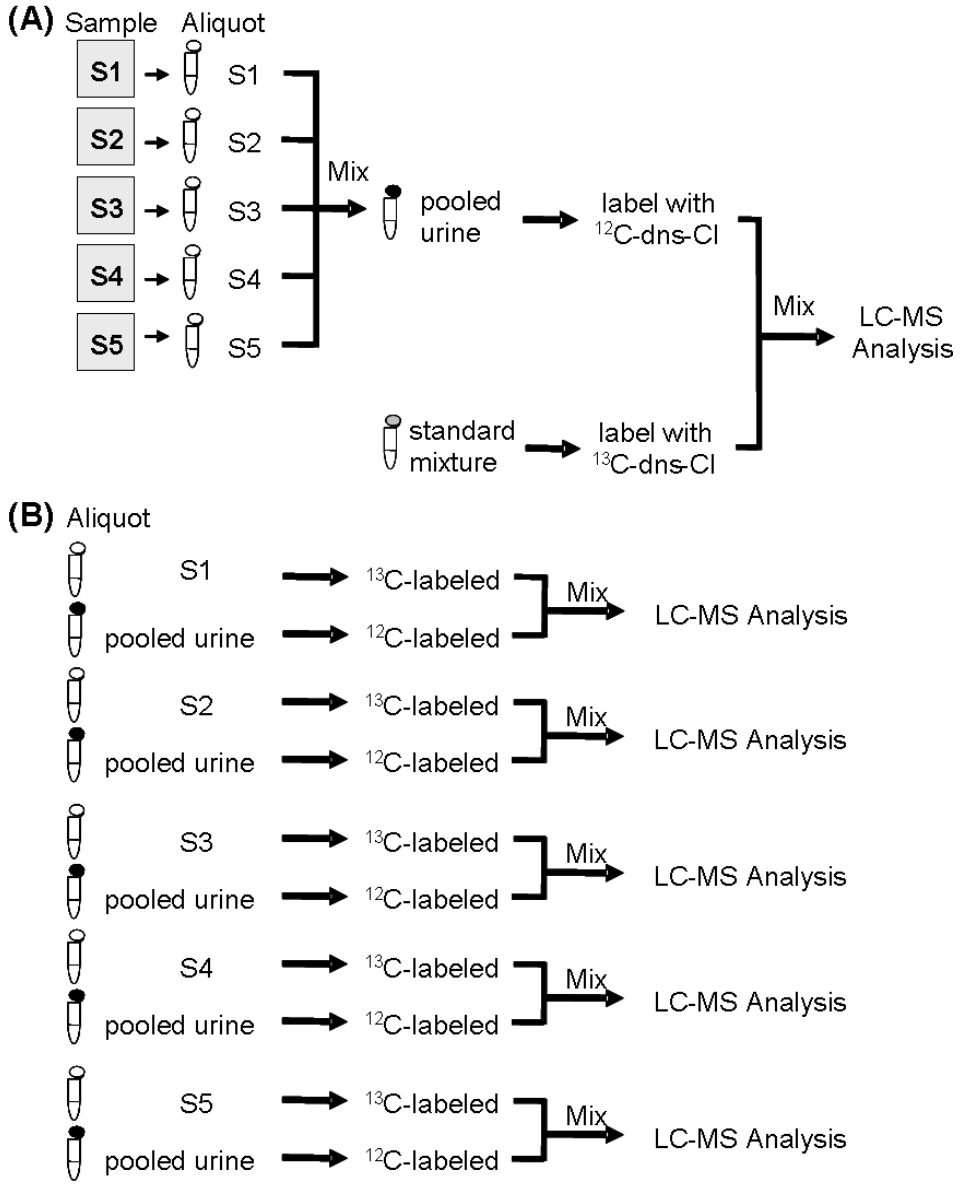

Figure 3.4 Workflow for relative and absolute quantification of metabolites using dansylation fast LC-FTICR MS.

Abundance ratios of ^{13}C-/^{12}C-danzyl derivatives and the amount of the ^{13}C-standards spiked to the sample. To determine the concentrations of metabolites in the individual samples, each sample is labeled by ^{13}C-dansylation and then mixed with an aliquot of the ^{12}C-dansylated pooled sample (see Figure 3.4B). Based on the peak ratio of ^{13}C-/^{12}C-danzyl derivatives and the concentration of individual metabolites already measured in the pooled sample, we can determine the absolute concentration of each metabolite in the individual samples.

To demonstrate the utility of this method of using a pooled sample as an internal standard, human urine samples were collected over five consecutive mornings from the same healthy individual. A pooled urine sample was then prepared by mixing equal volume aliquots of "Day-1" to "Day-5" urine samples. As indicated earlier, our current dansyl library consists of 121 amine and phenol standards. These standards were grouped into five mixtures to minimize the complexity of the samples and reduce the possibility of ion suppression in LC-MS (i.e., the spiked standards may suppress the analyte signals in the urine sample). Note that, depending on the type of biological samples analyzed, the concentrations of individual standards in the mixture may be adjusted so that the ^{13}C-/^{12}C-dansyl peaks do not fall off the linear dynamic range of relative quantification. Each group of mixture was dansylated by ^{13}C-dansyl chloride and then spiked into the ^{12}C-dansylated pooled urine for absolute quantification. Out of 121 standards, 93 compounds were identified and quantified in the pooled urine by fast LC-MS. The results are shown in Table 3.1 (the quantified metabolites are in bold faces).

Figure 3.5A shows a base peak ion chromatogram from the ^{12}C-labeled pooled urine spiked with one of the ^{13}C-dansylated standard mixtures containing a known quantity of 20 amino acids and 19 amines. As an example, Figure 3.5B shows an averaged mass spectrum obtained by the FTICR MS system at the

Table 3.1 List of 121 dansyl derivative standards and the corresponding metabolites (in bold face) identified and quantified in a 5-day pooled human urine sample by using fast LC-FTICR MS.

Compound	Ret. Time (min)	Conc. in Urine (μM)	Compound	Ret. Time (min)	Conc. in Urine (μM)
Dns-o-phospho-L-serine	0.92	<D.L.*	**Dns-Ile**	6.35	25
Dns-o-phospho-L-tyrosine	0.95	<D.L.	**Dns-3-aminosalicylic acid**	6.44	0.5
Dns-adnosine monophosphate	0.99	<D.L.	**Dns-pipecolic acid**	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	**Dns-Leu**	6.54	54
Dns-glucosamine	1.06	22	**Dns-cystathionine**	6.54	0.3
Dns-o-phospho-L-threonine	1.09	<D.L.	**Dns-Leu-Pro**	6.60	0.4
Dns-6-dimethylamine purine	1.20	<D.L.	**Dns-5-hydroxylysine**	6.65	1.6
Dns-3-methyl-histidine	1.22	80	**Dns-Cystine**	6.73	160
Dns-taurine	1.25	834	**Dns-N-norleucine**	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	**Dns-dimethylamine**	7.33	293
Dns-Asn	1.55	133	**Dns-5-HIAA**	7.46	18
Dns-hypotaurine	1.58	10	**Dns-umbelliferone**	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3-diaminoproprionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	**Dns-L-ornithine**	7.70	15
Dns-Gln	1.72	633	**Dns-4-acetyamidophenol**	7.73	51
Dns-allantoin	1.83	3.8	**Dns-procaine**	7.73	8.9
Dns-L-citrulline	1.87	2.9	**Dns-homocystine**	7.76	3.3
Dns-1 (or 3-)-methylhistamine	1.94	1.9	**Dns-acetaminophen**	7.97	82
Dns-adenosine	2.06	2.6	**Dns-Phe-Phe**	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	**Dns-5-methyoxysalicylic acid**	8.04	2.1
Dns-Ser	2.24	511	**Dns-Lys**	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy-proline	2.56	2.3	**Dns-leu-Phe**	8.22	0.3
Dns-Glu	2.57	21	**Dns-His**	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	**Dns-1-ephedrine**	8.50	0.6
Dns-ethanolamine	3.11	471	**Dns-tryptamine**	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl-benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	**Dns-5-hydroxytrptophan**	9.25	0.12
Dns-aminolevulinic acid	3.97	30	**Dns-1,3-diaminopropane**	9.44	0.23
Dns-r-amino-butyric acid	3.98	4.6	**Dns-putrescine**	9.60	0.5
Dns-p-amino-hippuric acid	3.98	2.9	**Dns-1,2-diaminopropane**	9.66	0.1
Dns-5-hydroxymethyluricil	4.58	1.9	**Dns-tyrosinamide**	9.79	29
Dns-tryptophanamide	4.70	5.5	**Dns-dopamine**	10.08	140

Dns-isoguanine	4.75	<D.L.	**Dns-cadaverine**	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	**Dns-histamine**	10.19	0.4
Dns-sarcosine	4.81	7.2	**Dns-3-methoxy-tyramine**	10.19	9.2
Dns-3-amino-isobutyrate	4.81	85	**Dns-Tyr**	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cysteamine	10.44	<D.L.
Dns-Ser-Leu	5.06	<D.L.	**Dns-phenol**	10.52	1.0
Dns-Pro	5.07	13	Dns-desipramine	10.57	<D.L.
Dns-pyridoxine	5.27	<D.L.	Dns-3-chlorotyrosine	10.58	<D.L.
Dns-Val	5.35	75	**Dns-2,3-diaminosalicylic acid**	10.60	0.6
Dns-Met	5.40	16	Dns-octopamine	10.75	<D.L.
Dns-Thr-Leu	5.40	0.6	**Dns-serotonin**	10.85	1.0
Dns-3-hydroxypicolinic acid	5.47	44	**Dns-o-(p or m)-cresol**	10.93	2.1
Dns-salicyluric acid	5.51	7.6	**Dns-metanephrine**	10.97	0.04
Dns-Trp	5.59	120	**Dns-propranolol**	11.00	0.04
Dns-kynurenine	5.66	6.3	Dns-4-aminophenol	11.04	<D.L.
Dns-Gly-Leu	5.79	1.1	**Dns-synephrine**	11.06	0.27
Dns-Gly-Trp	5.85	<D.L.	**Dns-phenylephrine**	11.17	0.03
Dns-norvaline	5.89	0.3	**Dns-tyramine**	11.22	5.1
Dns-Ala-leu	5.89	<D.L.	Dns-hydroquinone	11.28	<D.L.
Dns-ethylamine	5.90	25	**Dns-spermidine**	11.37	0.4
Dns-4-aminobenzoic acid	5.99	1.6	Dns-diiodothyronine	11.59	<D.L.
Dns-Ala-Trp	6.00	0.5	Dns-4-isopropylphenol	11.81	<D.L.
Dns-3-aminobenzoic acid	6.08	1.2	**Dns-spermine**	12.05	0.3
Dns-Phe	6.20	90			

*<D.L. indicates that the amount of analyte, if present, is below the detection limit of the current technique. The detection limit is compound dependent and has not been determined for each compound shown in the list.

Table 3.2 Partial list of the measured abundance ratios of 30 metabolites found in urine (see Supplemental Table S3.2 for the complete list).

Compound	Labeling replicates		Run1	Run2	Run3	Average of three runs	%RSD (Run to run)
Dns-Asn	**-20°C (^{12}C)/-80°C (^{13}C)**	**#1**	1.083	1.035	1.032	1.050	2.7
		#2	0.982	0.975	0.918	0.958	3.7
		#3	1.017	1.061	1.047	1.042	2.2
		#4	0.925	0.978	0.911	0.938	3.8
	Average					**1.00**	3.1
	%RSD (Labeling)					**5.7**	
Dns-Asn	**-80°C (^{12}C)/-20°C (^{13}C)**	**# 1**	0.942	1.015	0.978	0.978	3.7
		#2	0.955	0.996	1.010	0.987	2.9
		#3	1.035	1.010	1.105	1.050	4.7
		#4	0.931	0.945	0.878	0.918	3.8
	Average					**0.98**	3.8
	%RSD (Labeling)					**5.5**	
Dns-Asn	%Diff between -20°C/-80°C Labeling					**1.4**	
Dns-Gln	**-20°C (^{12}C)/-80°C (^{13}C)**	**# 1**	1.032	1.047	1.022	1.034	1.2
		#2	1.005	0.975	1.055	1.012	4.0
		#3	0.962	0.953	0.917	0.944	2.5
		#4	1.060	1.045	0.984	1.030	3.9
	Average					**1.00**	2.9
	%RSD (Labeling)					**4.1**	
Dns-Gln	**-80°C (^{12}C)/-20°C (^{13}C)**	**# 1**	0.999	1.045	1.012	1.019	2.3
		#2	0.998	0.987	1.043	1.009	2.9
		#3	0.998	0.956	0.949	0.968	2.7
		#4	0.964	0.980	0.925	0.956	3.0
	Average					**0.99**	2.7
	%RSD (Labeling)					**3.1**	
Dns-Gln	%Diff between -20°C/-80°C Labeling					**1.7**	

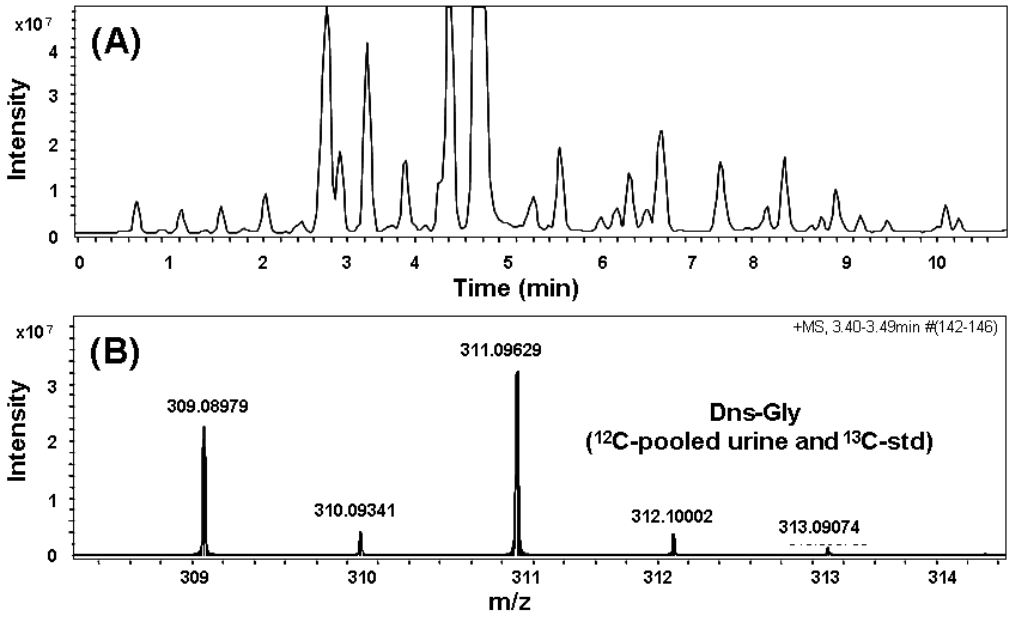

Figure 3.5 (A) Base peak ion chromatogram of ^{12}C-dansylated pooled urine spiked with known quantities of ^{13}C-dansylated 20 amino acids and 19 amines. (B) An expanded mass spectrum showing the dns-Gly ion pair from chromatogram (A).

retention times between 3.40 and 3.49 min. In this case, the peak abundance ratio of ^{12}C-/^{13}C-dns-Gly (dansylated Gly) is calculated to be 0.73. Since the quantity of the spiked ^{13}C-dns-Gly is known, the absolute concentration of Gly in the pooled urine can be readily calculated to be 2510 μM. Table 3.1 summarizes the results from the analysis of the pooled urine sample with spiked dansyl standards. The metabolite concentrations range from 30 nM (phenylephrine) to 2510 μM (Gly), which is about 83700-fold difference in concentrations. The retention times and masses of the labeled derivatives along with their concentrations in the pooled urine shown in this table were subsequently used to identify and quantify the individual metabolites in the Day-1 to Day-5 urine samples.

Figure 3.6A shows a base peak ion chromatogram obtained by fast LC-MS analysis of a mixture of ^{13}C-dansylated "Day-1" urine and ^{12}C-labeled pooled urine. The ion chromatogram displays a number of peaks similar to those shown in Figure 3.5A, although the relative intensities of some of the peaks are noticeably different. Figure 3.6B shows an averaged mass spectrum obtained at the retention times between 3.36 and 3.43 min. The retention time and masses of the peaks match with dns-Gly. The peak abundance ratio of ^{12}C/^{13}C-dns-Gly is calculated to be 1.17. Thus, the absolute concentration of Gly in the "Day-1" urine is 2140 μM.

Figure 3.7 shows the plots of the absolute concentrations of 20 amino acids detected in "Day-1" to "Day-5" urine samples (a summary of the abundance ratios and concentrations is given in Supplementary Table S3.3). Interestingly, for most of the amino acids, day-to-day concentration variations are relatively small. It should be noted that no biological significance should be drawn from this limited sample analysis. However, the significance of this analysis is reflected in the quality of the data obtained. The average concentration of

individual amino acids from the "Day-1" to "Day-5" urine samples is similar to that of the pooled urine,

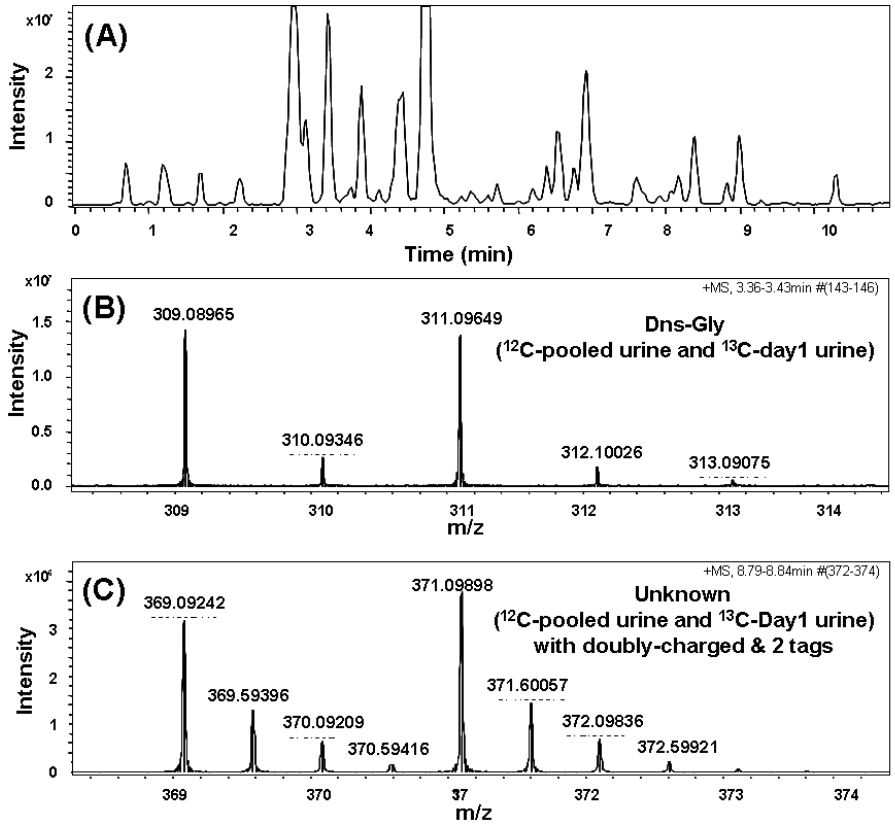

Figure 3.6 (A) Base peak ion chromatogram of ^{12}C-dansylated pooled urine combined with ^{13}C-dansylated "Day-1" urine. (B) An expanded mass spectrum showing the dns-Gly ion pair from chromatogram (A). (C) An expanded mass spectrum showing an unknown ion pair from chromatogram (A).

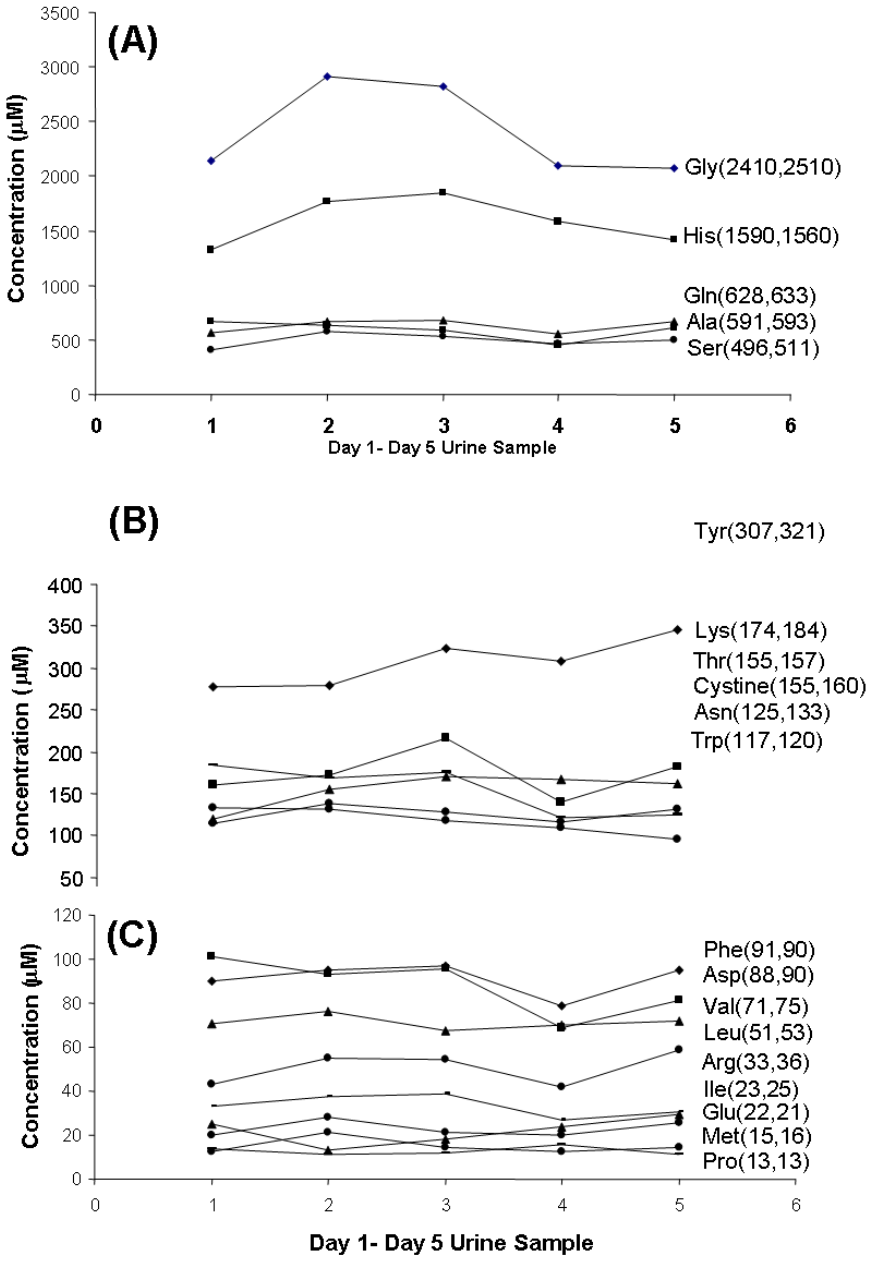

Figure 3.7 Day-to-day relative and absolute concentration changes of 20 amino acids in urine samples collected from a healthy individual in 5 consequent days.

and the calculated percent difference between the average concentration and the pooled urine concentrations ranges from 2.4% to -8%. These data again illustrate that fast LC-MS with dansylation can produce accurate results for both relative and absolute quantification of metabolites.

3.3.7 Comprehensive Analysis of Metabolome.

One major goal of developing LC-MS for metabolome analysis is to detect and quantify as many metabolites as possible. Although the dansylation chemistry described in this work is limited to the analysis of amines and phenols, there are many compounds of this type apparently present in a biological system. Using fast LC-FTICR MS, at least 672 ion pairs of these compounds in human urine samples were detected with a S/N > 20 after taking into account and deleting the common adduct ions, doubly- and triply-charged species (see Supplemental Table S3.4). Among them, 545 ion pairs have S/N > 50. These ion pairs should be from different metabolites as they have either different retention times or different masses at the same retention time. The usual Na^+ or K^+ adduct ions can be readily taken into account. And, as indicated earlier, in-source fragmentation is rarely observed for the dansyl derivatives and thus the ion pairs detected are not from the fragment ions. The use of ion pairing with accurate mass measurement of the ion pairs also increases the confidence of identifying them as metabolites. As an example, Figure 3.6C shows an averaged mass spectrum obtained at the retention times between 8.79 and 8.84 min from the differentially labeled urine mixture. The molecular masses and retention times do not match with any known metabolites in the current dansyl library and, furthermore, searching the human metabolome database by the molecular weight did not result in a match of compound where its structure allows two tags attached. This unknown ion pair contains two dansylated tags showing the

characteristic peaks for a doubly charged, two-tag species. The mass error for an ion pair mass measurement is 0.41 ppm.

The potential of detecting over 672 metabolites using a 12-min LC-MS run opens a possibility of carrying out high throughput metabolome profiling work. However, the above example also illustrates a major analytical challenge currently faced in metabolomics research, i.e., most of the ion pairs detected are from unknown metabolites. Clearly we need to expand the current dansyl library of 121 metabolites. To this end, we have identified about 130 other amine and phenol metabolites that are commercially available and can potentially be labeled with dansyl chloride, with due consideration of the fundamental limitations of the dansylation chemistry (e.g., indoles may not be labeled efficiently).[6] We plan to add these compounds to the current library. Updated information on the dansyl library will be reported in future publications. However, this effort still will not identify all 672 ion pairs detected. Moreover, one can expect that with a longer column separation or multidimensional LC separation, a much greater number of unknown metabolites will be detected. Fractionation and purification of unknown metabolites, followed by MS and NMR characterization, are needed for compound identification. In our future work, we will carry out metabolome profiling work first using the dansylation labeling and fast LC-MS method, followed by statistical analysis of the profiles generated to identify interesting features (e.g., one or a few characteristic metabolites have discriminate power for classification of healthy and diseased individuals). These metabolites of interest, if not present in the dansyl library, will be fractionated and identified by MS and NMR.

3.4 Conclusions

We have developed a quantitative metabolome profiling technique for targeted analysis of metabolites containing amine or phenol functional group(s). It involves the use of differential isotope labeling of amine- and phenol-containing metabolites by dansylation reaction with ^{13}C-/^{12}C-dansyl chloride. Fast LC with a 12-min run combined with FTICR MS can be used to separate and detect the labeled metabolites with high efficiency and sensitivity.

This technique offers the following features for quantitative metabolome analysis. First of all, the ^{13}C-dansyl chloride reagent can be readily synthesized and purified as detailed in this work, where ^{12}C-dansyl chloride is commercially available. Secondly, dansylation reaction with dansyl chloride is simple (no special equipment required), quick (~90 min), widely applicable for a range of amines and phenols, and produces little or no side reaction products. Thirdly, dansylated metabolites can be separated by RPLC, even for polar or ionic metabolites that are normally not retainable on a RP column, thereby alleviating the need of using different columns for analyzing a given sample. Fourthly, dansylated metabolites can be efficiently ionized and detected by ESI MS. In the analysis of 20 amino acids, the ESI signal enhancement by dansylation is shown to be in the order of one to three orders of magnitudes, depending on the type of amino acid and solvent conditions used for ESI MS. Adding a dansyl group to a metabolite (+234 Da for one tag) effectively shifts the metabolite detection mass window to above 250 Da, avoiding any signal interference raised from low-mass background molecules and contaminants commonly present in the ESI process. In-source fragmentation of dansylated metabolites is rarely observed and thus there is no confusion on peak assignment; all peaks in a mass spectrum except the background peaks (if any) should be from the intact metabolite ions, not their fragment ions. Fifthly, the ^{13}C-/^{12}C-dansylated metabolites do not show any

isotopic effect in RPLC. The differential isotope ion pairs are co-eluted and detected by MS and thus are subjected to the same degrees of matrix and/or ion suppression effect, which leads to high precision and accuracy for quantitative metabolite analysis. Finally, dansylated derivatives of metabolites with known structures can form a library of standards from which absolute concentrations of these metabolites can be determined in any biological samples and metabolite identification can be done based on accurate mass and retention time information.

We have demonstrated that, for human urine samples, a total of 672 metabolites can be profiled by using fast LC FTICR MS in 12 min. However, most of these metabolites cannot be confirmed, due to the lack of standards. The current dansylated metabolite library consists of only 121 compounds; but we were able to identify and quantify 93 of them in a pooled urine sample. Our future work will focus on building a larger dansylated metabolite library. In addition, dansylation or other chemical reaction schemes will be developed to label metabolites containing other functional groups than amines and phenols to increase the metabolome coverage. As amines and phenols are important groups of metabolites, profiling these metabolites using the current technique should already be useful for biomarker discovery and biological studies. The applications of this technique in a variety of metabolomics projects will be reported in the near future.

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Chapter 4

Qualitative Metabolome Analysis of Human Cerebrospinal Fluid by ^{13}C-/^{12}C-Isotope Dansylation Labeling Combined with Liquid Chromatography Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

4.1 Introduction

Cerebrospinal fluid (CSF) is an important body fluid for discovery of potential biomarkers of diseases, particularly those related to malfunctions of central nervous system (CNS), because of its proximity to the neuropathology site in the brain [1,2]. Recently, proteomics and metabolomics tools have been applied for the analysis of proteome and metabolome of CSF [3,4]. However, metabolome analysis of CSF remains to be an analytical challenge, because of low concentrations of many metabolites present in CSF and the availability of only small volumes of samples. Through literature mining of more than 2000 books and journal articles, a total of 329 metabolites have been found to be detectable in various studies of human CSF (www.csfmetabolome.ca) [5]. Among them only about 75 metabolites were reported to have concentrations of above 1 μM. Analyzing metabolites in CSF is mostly done by using NMR [6-10], GC-MS [11,12] and LC-MS [10,12-14]. In a report that compares different techniques for human CSF metabolome analysis [5], it was shown that the NMR technique could positively identify about 53 metabolites, GC-MS could identify 41 metabolites and LC-MS

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using Fourier-transform ion cyclotron resonance (FTICR) MS could identify 17 metabolites. The combination of the three techniques identified a total of 70 different metabolites.

Among different analytical techniques available for CSF metabolome analysis, LC-MS is a very promising technique for improving metabolite detectability, thereby expanding the metabolome coverage. However, it, presently, has some limitations. Low concentrations of metabolites (less than 1 μM) are generally difficult to analyze without special sample enrichment in a typical un-targeted metabolome profiling work. Another major problem is related to metabolite identification. Targeted analysis of certain known metabolites in CSF can be carried out by LC-MS using sensitive methods such as multiple reaction monitoring (MRM) or selected reaction monitoring (SRM) in a tandem mass spectrometer. For example, 23 metabolites of neurotransmitters were analyzed using MRM MS/MS [15]. However, there are only a few reports of un-targeted metabolome analysis of CSF by LC-MS [12-14, 16-19]. Because of low abundance of metabolites present in CSF, less than 5000 features could be detected from LC-MS and many of the features were likely from non-metabolite signals. Only a few metabolites could be identified in a LC-MS run.

We have recently developed an isotope labeling chemistry based on dansylation reaction that tags amine- and phenol-containing metabolites [20]. Unlike other chemical derivatization schemes where the main purpose was to introduce a mass tag to the metabolites for quantitative metabolite or metabolome analysis [21-28], dansylation can not only effectively introduce an isotope tag for accurate metabolite quantification, but also improve the chromatographic retention of the metabolites in reversed-phase LC and enhance the efficiency of electrospray ionization (ESI). In our previous work, sensitive detection of metabolites from human urine samples was demonstrated. In particular, we

observed a significant enhancement of detection signals (up to 3 orders of magnitude) in LC-MS after dansylation of metabolites. In this work, we report our studies of applying this strategy for analyzing amine- and phenol-containing metabolites in a more challenging biofluid, CSF and demonstrate that the method of isotope labeling via dansylation can be used for profiling the metabolome of CSF with more extended coverage than previously possible.

4.2 Experimental

4.2.1 Chemicals and Reagents

All chemicals and reagents were purchased from Sigma-Aldrich Canada (Markham, ON, Canada) except those otherwise noted. $^{13}C_2$-dimethyl sulfate that used to synthesize ^{13}C-danysl chloride was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). LC-MS grade of water, methanol and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Edmonton, AB, Canada).

Lumbar CSF samples were collected from patients screened for meningitis in accordance with guidelines established by the University of Alberta Health Research Ethics Board. As part of the disease screening procedure, CSF samples were required to be stored at 4 ℃ for 2 days, after which they were placed in a freezer for long-term storage at −80 ℃. For this work, CSF samples from four individuals were processed by adding LC-MS grade acetonitrile in 1:1 (v/v) ratio, and then stored in a -80 ℃ freezer for further use.

4.2.2 Synthesis of ^{13}C-Dansyl Chloride

The synthesis of ^{13}C-dansyl chloride as a derivatizing reagent was reported in previous work [20]. The purity and confirmation of ^{13}C-dansyl chloride was tested against the commercial ^{12}C-dansyl chloride using LC-FTICR-MS and LC-UV. The purity of ^{13}C-dansyl chloride is over 99% based on the LC-MS and LC-UV

results. NMR was also used to further confirm the identity and purity of the synthesized ^{13}C-dansyl chloride.

4.2.3 Dansylation Labeling Reaction

About 50 μL of the CSF in acetonitrile were mixed with an equal volume of sodium carbonate/sodium bicarbonate buffer (0.5 mol/L, pH 9.4) in reaction vials. 50 μL of freshly prepared ^{12}C-dansyl chloride solution (20 mg/mL) (for light labeling) or ^{13}C-dansyl chloride (20 mg/mL) (for heavy labeling) were then added. The dansylation reaction was allowed to proceed for 60 min at 60^{o}C with shaking at 150 rpm. After 60 min, 20 μL of methylamine (0.5 mol/L) were added to the reaction mixture to consume the excess dansyl chloride and quench the dansylation reaction. After an additional 30 min of 60^{o}C incubation, the ^{13}C-labeled mixture was combined with the ^{12}C-labeled counterpart for LC-MS analysis.

4.2.4 LC-FTICR-MS Measurement

The HPLC system was an Agilent 1100 series binary system (Agilent, Palo Alto, CA) and was modified to reduce extra system solvent volume according to an Agilent protocol (Agilent Publication Number: 5988-2682EN). A reversed-phase Agilent Eclipse plus C_{18} column (2.1 x 100mm, 1.8μm particle size, 95Å pore size) was purchased from Agilent Canada (Mississauga, ON). LC solvent A was 0.1% (v/v) LC-MS grade formic acid in 5% (v/v) LC-MS grade ACN, and solvent B was 0.1% (v/v) LC-MS grade formic acid in LC-MS grade acetonitrile. The gradient elution profile was as follows: t = 0 min, 20% B; t = 3.0 min, 35% B; t = 16 min, 65% B; t = 18.6min, 95% B; t = 21 min, 95% B; t = 21.3 min, 98%B; t = 23.0 min, 98%B; t = 24.0 min, 20%B. The flow rate was 150 μL/min. The flow from RPLC was split in 1:3 and a 50 μL/min flow was loaded to an sample injector and the electrospray ionization (ESI) source of a Bruker 9.4-Tesla Apex-Qe FTICR mass spectrometer (Bruker, Billerica, MA, USA) or an

Applied Biosystems, QStar Pulsar i mass spectrometer while the rest of flow was delivered to the waste. All MS spectra were obtained in the positive ion mode. The QStar Pulsar i LC-MS system was only used for detecting individual dansylated standards for the development of the dansylation library. For each standard, the system was used to generate the MS spectra of the labeled product to assess the purity of the product and labeling efficiency. All of the mass spectral data presented in this work were obtained using the 9.4-Tesla FTICR mass spectrometer.

4.3 Results and Discussion

Figure 4.1 shows the work flow for generating a qualitative metabolome profile from a CSF sample. Briefly, a sample is divided into two equal aliquots, one labeled with the heavy or ^{13}C-dansyl-chloride reagent and another one labeled with the light or ^{12}C-reagent. The labeled aliquots are mixed and then injected into LC-FTICR-MS for analysis. FTICR-MS offers high resolution and high accuracy measurement of the metabolite masses [29]. Thus, the ^{13}C-/^{12}C-labeled ion pairs can be picked based on their characteristic mass differences as well as perfect co-elution of the pairs due to the absence of an isotope effect on chromatography separation. The detected ion pairs can be searched against a database for putative metabolite identification or a library of standards for definitive identification. In this work, putative identification is based on the accurate mass matches of the dansylated metabolites with the human endogenous metabolites found in the Human Metabolome Database (HMDB) (about 8000 metabolite entries) [30]. Definitive identification is based on matches of accurate masses and retention times to a ^{13}C-/^{12}C-labeled authentic standards library (220 standards; see below).

There are several important features of using the above strategy for metabolite identification. First of all, dansylation derivatization improves

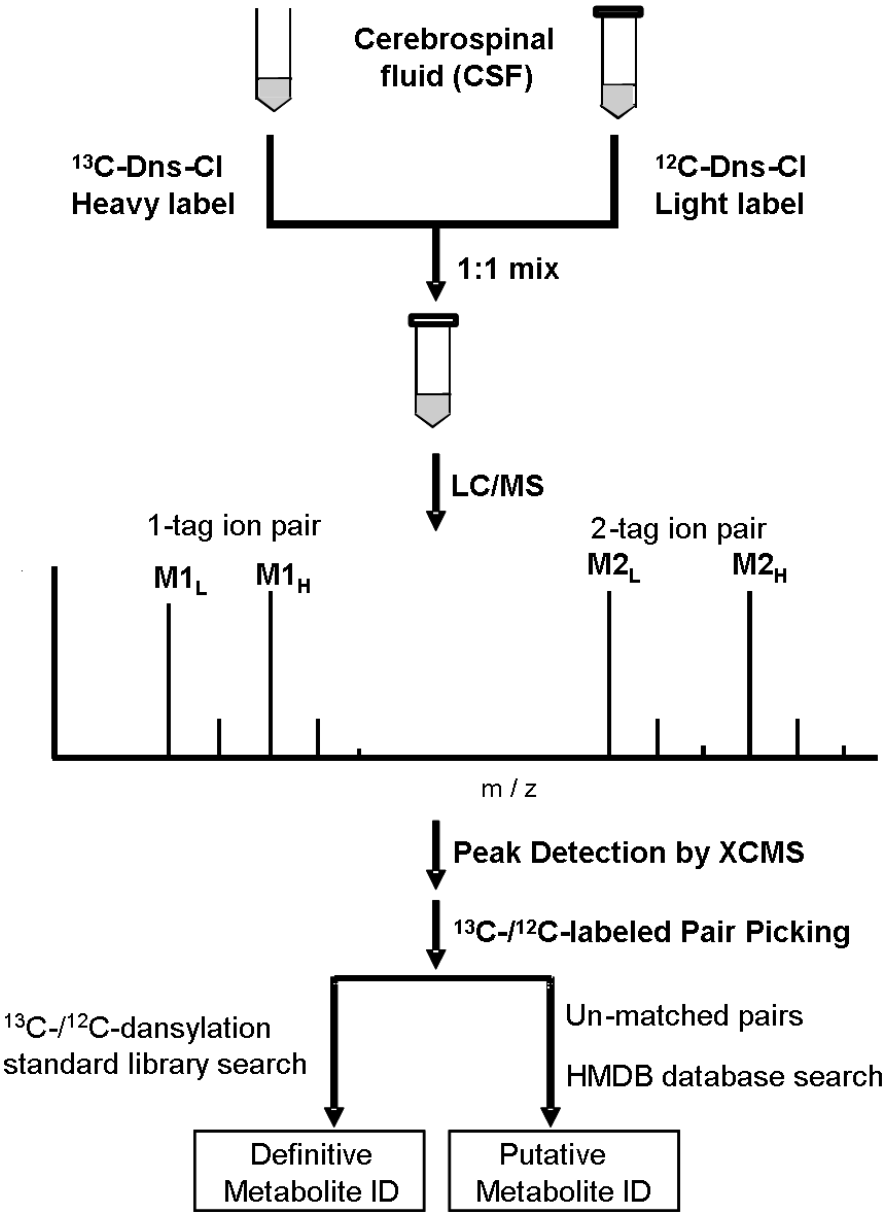


Figure 4.1 Workflow for CSF sample analysis and LC-MS data processing using the ^{12}C-/^{13}C-dansylation strategy.

metabolite detection by enhancing ESI efficiency and improving chromatography retention and separation. This is illustrated in Figure 4.2 where it shows the base peak ion chromatograms of two reversed-phase (RP) LC-MS runs from the injections of the same amount of ^{13}C-/^{12}C-dansylated CSF (A) and un-labeled CSF (B). The injection amount was equivalent to 0.5 μL of the original CSF sample. As Figure 4.2B shows, without any sample pre-concentration, injection of 0.5 μL of un-labeled CSF on a 2.1-mm RP column hardly generates any MS signals. This chromatogram was found to be the same as that of blank injection; the two peaks shown at the beginning of the chromatogram are from the background, not the metabolites in the sample. In contrast, many peaks with signal-to-noise ratios between 3 and 4,500 are observed in Figure 4.2A and they are distributed along the chromatographic elution profile. This is consistent with a previous study where signal enhancement of 1 to 3 orders of magnitude was found in ESI MS analysis of dansylated metabolites over their un-labeled counterparts and the labeled metabolites were better retained and separated in the RP column[20].

The signal enhancement can be attributed to several factors. One is related to the increased propensity of being charged for the labeled amines and phenols due to the presence of the dimethyamine moiety attached to the aromatic ring of the tag where a tertiary amine can be readily formed. The labeled compound has higher hydrophobicity than its unlabeled counterpart, making it easier to stay on the surface of the droplets during ESI. An elution solvent with higher organic solvent content where a labeled compound is eluted out, as opposed to the unlabeled one eluted at void or high water content solvent, also enhances the ionization efficiency. On the chromatographic separation, the addition of the dansyl group containing a hydrophobic aromatic ring to a polar and hydrophilic

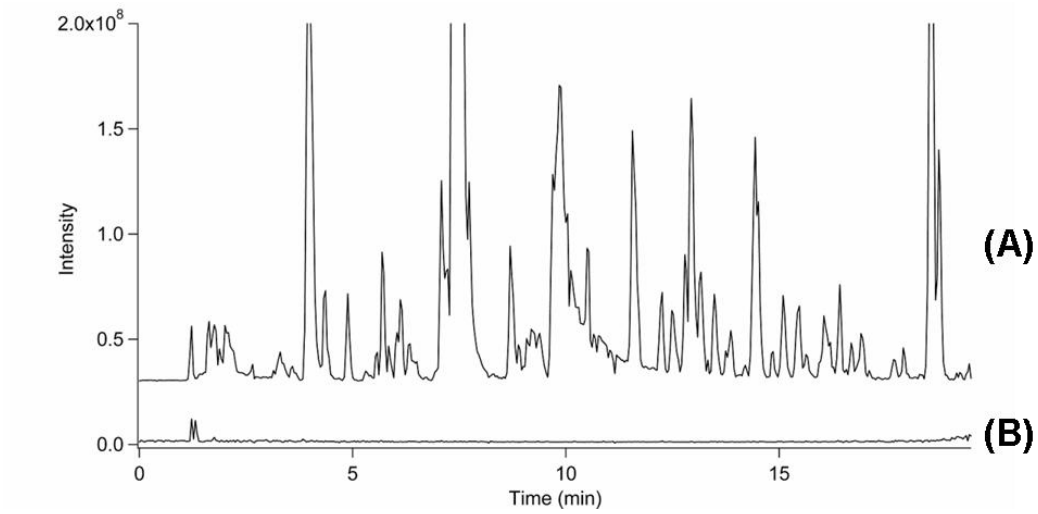


Figure 4.2 Base peak ion chromatograms of (A) 1:1 ^{12}C-/^{13}C-dansylated CSF sample #1 and (B) non-derivatized CSF sample #1 obtained by using LC-FTICR MS. In both cases, the sample amount injected was equivalent to about 0.5 μL of the original CSF sample.

amine or phenol increases hydrophobicity, thereby enhancing its retention on a RP column.

The sensitivity enhancement by dansylation derivatization is particularly important in analyzing CSF samples, because of the limited amount of samples collectable from a patient compared to other body fluids such as blood and urine. The volume or amount of CSF samples available would be even smaller for biological model systems such as rats. Because no metabolite peaks were detected from the injection of the un-labeled CSF, all the metabolite peaks shown in Figure 4.2A that were generated from the injection of an equivalent volume of the labeled CSF should be from the amine and phenol derivatives. The second important feature is that ^{13}C-/^{12}C-dansylated metabolites are perfectly co-eluted in RPLC and always detected as pairs in the same mass spectra. Thus, we can readily automate the process of peak picking and peak pairing (and relative quantification based on differential labeling of two comparative samples, although this is not the focus of the present work). Using isotope ion pairs has been used to distinguish true metabolite signals from many other peaks detected in LC-MS [20, 31-33]. In this work, the accurate mass difference between heavy-labeled and light-labeled compounds indicates the presence of primary and secondary amines or phenol compounds, in addition to the number of reactive functional groups. Error in mass difference is the mass error between the theoretical mass difference and the measured mass difference for ^{13}C-/^{12}C-labeled ion pairs. The theoretical mass difference for one tag and singly charged ion pair is 2.00671 and two-tag singly charged ion pair is 4.01342. Error in mass difference was used as a key criterion to assign a ^{13}C-/^{12}C-labeled ion pair. As an example, Figure 4.3A shows a singly charged spectrum of 1:1 molar ratio of ^{13}C-/^{12}C-dansylated isoforms of 5-hydroxyindolacetic acid (5-HIAA), a physiologically important metabolite of serotonin [34]. The ^{13}C-/^{12}C-labeled ion pair with error in mass difference of 0.47

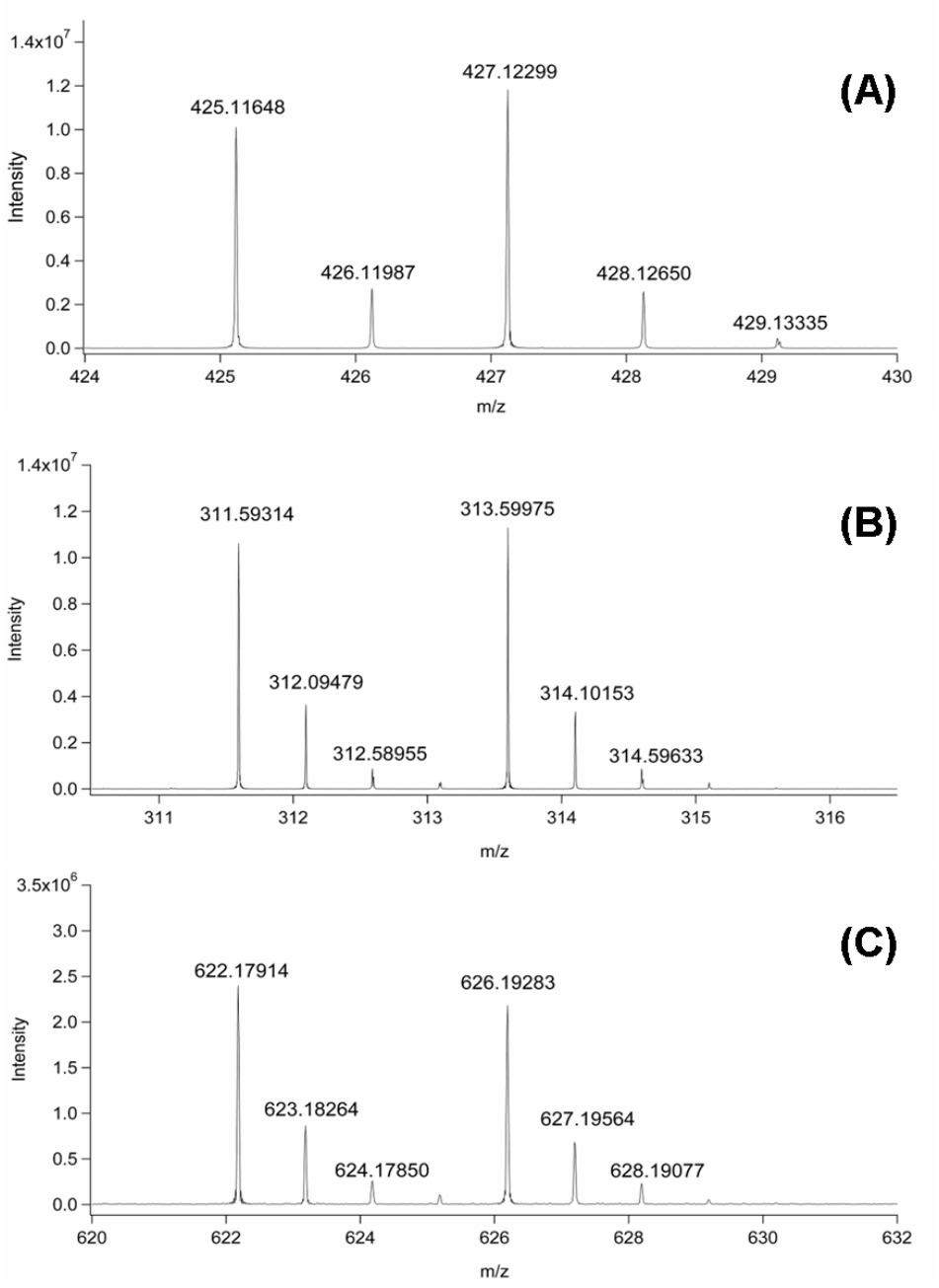


Figure 4.3 Expanded molecular ion regions of the mass spectra of (A) singly charged, one-dansylation-tag ion pair from 5-HIAA, (B) doubly charged, two-dansylation-tag ion pair from histidine, and (C) singly charged, two-dansylation-tag ion pair from histidine.

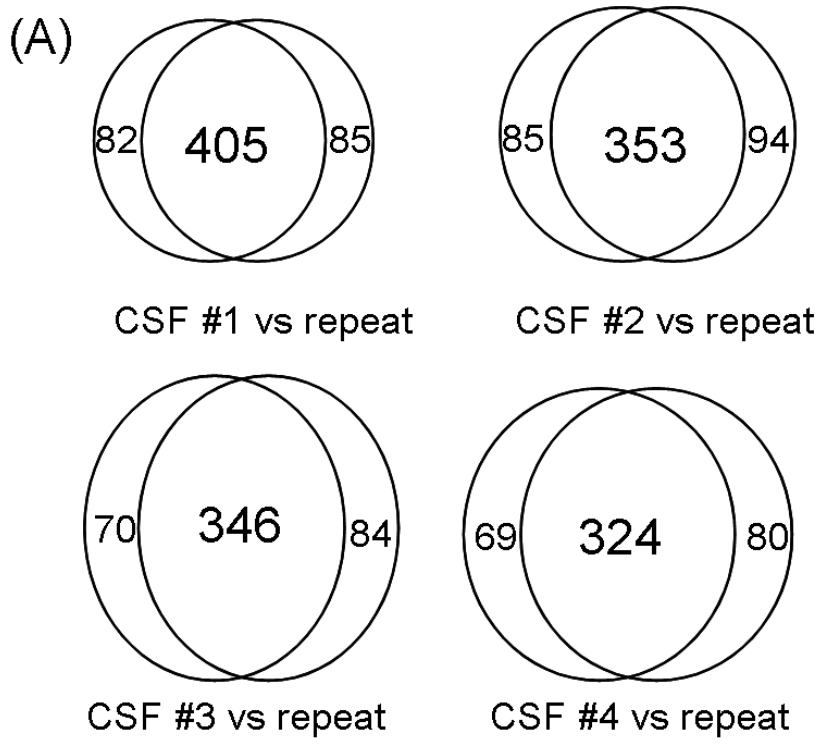
ppm and matched retention time [difference in retention time = 2.4 s; variation of retention time in general is < ±15 s (see Supplemental Tables S4.1 to S4.4)] to standards ensures the identity of 5-HIAA (see below). The spectral pattern clearly indicates only one reactive functional group exists. Figure 4.3B shows a doubly-charged spectrum of histidine and Figure 4.3C shows the corresponding singly charged spectrum. The errors in mass difference are 0.32 ppm for the doubly charged ion pair of histidine and 0.43 ppm for the singly charged ion pair. The difference in retention time to histidine standard is 1.8 s. The accurate mass differences and unique spectra patterns shown in Figure 4.3B and 4.3C unambiguously reveal that histidine has two reactive functional groups. It is interesting to note that the doubly charged ion pairs from the dansylated derivatives with two tags often exhibit very high signal intensity in the mass spectra. This may be due to the readiness of forming doubly charged ions by protonation of both amine groups on the two tags. In these cases, both doubly charged and singly charged ion pairs can be used to validate the presence of the same metabolite.

Another feature is related to the high stability of the dansylated metabolites. Dansylated compounds hardly fragment in-source or during the transfer to the mass analyzer in LC-MS. This enhances the molecular ion intensity and makes it less ambiguous in identifying the molecular ion peaks. Finally, dansylation increases the molecular ion mass by 234 Da for one tag and 467 Da for two tags which effectively shifts their mass-to-charge ratios out of the low-mass region of a mass spectrum that typically contains more background noise from common contaminants and solvent clusters.

Using the workflow shown in Figure 4.1, a typical LC-MS run of a 1:1 ^{13}C-/^{12}C-dansylated CSF sample generated about 14,000 peak features detected by the automated XCMS peak picking software [35]. Obviously, not all the features

belong to the real signals from the metabolites. Isotopic ions, adducts and fragment ions as well as multiply charged ions were treated as separated peak features in XCMS [17]. To pick the ^{13}C-/^{12}C-dansylated ion pairs, the peak features in the XCMS output table were exported into Excel for processing. Because the error in mass difference between the ^{13}C-/^{12}C-dansylated ion pair was found to be generally less than 2 ppm, we used 2 ppm error in mass difference as the first criterion to pick the ion pairs. The 2nd criterion was based on the fact that the ^{13}C-/^{12}C-dansylated pairs were perfectly co-eluted in RPLC; therefore, ^{13}C-/^{12}C-labeled ion pairs must be shown in a same spectrum. At the last step in pair picking, only the protonated ion pair peaks were retained while the ion pairs corresponding to isotopic peaks, common adduct ions, multimers, and multiply charged ions were eliminated. Non-reactive metabolites, interference ions, background ions, instrument noises and electronic noises will not form ^{13}C-/^{12}C-ion pairs with characteristic mass differences. For a given CSF sample, approximately 500 ion pairs can be confirmed to be from the ^{13}C-/^{12}C-dansylated metabolites (see below).

The reproducibility for detecting the amine- and phenol-containing metabolites using dansylation in CSF samples has been examined. In total, four different CSF samples were analyzed with each sample analyzed twice following the workflow shown in Figure 4.1, i.e., two experimental replicates (not repeat injections of the same mixture) were done on each sample. The results are summarized in Supplemental Tables S4.1 to S4.4 with each table listing the compound name (if known), retention time, masses of ion pair, and ion pair signal intensity. Figure 4.4A shows the comparison of the number of ion pairs detected from replicate runs of individual samples. The average number of ion pairs detected from the four samples ranges from 473 to 572 with an average of 519. These ion pairs were detected in the LC gradient time window between 1.6 and



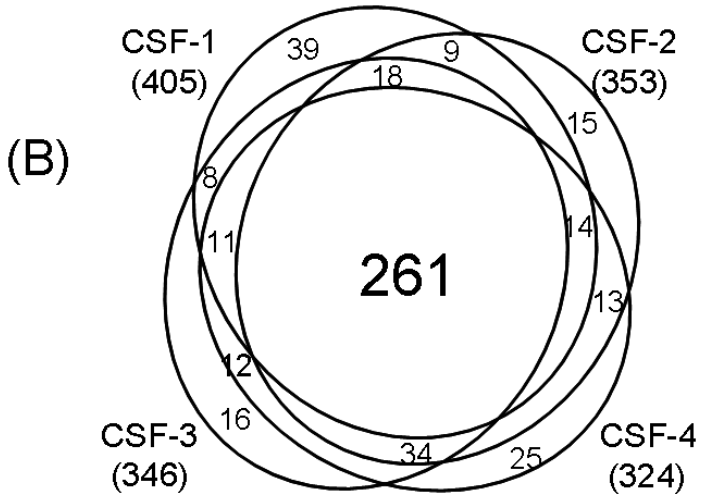


Figure 4.4 Comparison results of the common ion pairs detected among the four samples.

24.5 min. There are 405 (71%), 353 (66%), 346 (69%), and 324 (68%) common ion pairs detected for samples 1 to 4, respectively. In addition, the signal intensities of common ion pairs from run to run are reproducible. The CV of the two dataset for samples 1 to 4 was found to be 3.2, 5.7, 6.8 and 4.4%, respectively. Comparing the common ion pairs detected from the four samples, we found that 261 ion pairs were consistently detected (see Figure 4.4B), representing 64% of the 405 ion pairs found in CSF-1, 74% of the 353 pairs in CSF-2, 75% of the 346 pairs in CSF-3, and 81% of the 324 pairs in CSF-4. There were 15 to 39 unique ion pairs found in individual samples. If we combined all the results obtained from the four samples, there were 1132 unique ion pairs found. The unique ion pairs found in the individual samples are likely the results of abundance differences; only the metabolites with the concentrations above the detection limit (low nanomolar) would be detected by dansylation LC-FTICR-MS. These results also show the diversity of metabolites potentially present in human CSF. It should be noted that the low overlap of metabolites found in replicate runs or between samples is likely the result of under-sampling in one-dimensional-LC MS, much like in the shotgun proteome analysis where an overlap of 60-70% is commonly observed in replicate runs of peptides generated from trypsin digestion of whole cell lysates [36]. It remains to be seen whether multidimensional LC separation of metabolites prior to MS analysis can increase the number of common metabolites detected in replicates.

While the use of ion pairing allows the differentiation of the mass spectral peaks originated from the true metabolites vs. those from other sources, accurate mass measurement of the molecular ions by FTICR-MS offers the possibility of putative metabolite identification based on mass matches with a database of known metabolites. While several databases of chemical compounds are available, we focused on our efforts on using the HMDB for putative

identification of metabolites in CSF, as this database is composed of about 8000 human metabolites reported in the literature, as opposed to other database where many types of chemical compounds, in addition to the endogenous human metabolites, are enclosed. The results of the HMDB database search using the molecular masses of ion pairs (minus the dansyl group) are shown in Supplemental Tables S4.5 to S4.8. For the 1132 unique ion pairs found in the four samples combined, 785 pairs (69%) do not match with any metabolites in the database, while 347 pairs (31%) match with one or more putative metabolites in HMDB database within ±3 mDa in mass tolerance. Among the 347 matches, 90 pairs (26%) match with one metabolite. Because dansylation reaction targets primary and secondary amines as well as phenol groups in metabolites, we can use this information to eliminate some of the matches, i.e., matches with metabolites without an amine or phenol group are deemed to be false positive. Overall, there are 334 pairs (29%) matched with at least one metabolite in HMDB from the 1132 unique ion pairs.

To provide definitive identification of the ion pairs or metabolites detected in CSF, we used an in-house library of dansylation standards and compared the molecular ion masses and retention times of the standards with those of unknown metabolites. Our current library consists of 220 amine- and phenol-containing metabolites, which is almost doubled the number of standards we previously reported [20]. To build this library, for each standard, a dansylation reaction was carried out and the product was examined by LC-MS. Most compounds gave high conversion yields (>90%). A list of these compounds along with their RPLC retention time and measured masses by LC-FTICR-MS are shown in Supplemental Table S4.9. To minimize suppression, we divided the 220 standards into six groups (30-40 compounds per group), largely based on their retention properties and avoidance of closely eluted isomers on RPLC. The

standards within a group were mixed and then ^{13}C- or ^{12}C-dansylated, followed by 1:1 mixing of the ^{13}C-/^{12}C-labeled standard mixtures and injecting them into LC-FTICR-MS for analysis. The accurate masses and retention time of each ion pair were determined from these experiments and the data shown in Supplemental Table S4.9 were used to compare with those found in the CSF samples run under the same LC and column conditions for positive metabolite identification.

In total, 85 metabolites are positively identified from the four samples combined and they are shown in Table 4.1. The results of metabolites identified in individual CSF samples are listed in Supplemental Tables S4.9 to S4.12. As these tables show, 76, 65, 70, 68 metabolites were reproducibly detected in the repeated differential labeling experiments from CSF samples 1 to 4, respectively. As an example, Supplemental Table S4.9 shows a list of identified metabolites from the replicate experiments of 1:1 ^{13}C-/^{12}C-labeled CSF sample #1. There are 76 common metabolites positively identified in both experiments. Only four metabolites in the first labeled experiment and three metabolites in the second experiment are not commonly detected. Note that the signal intensities of these non-common metabolites are relatively low. Most of the high abundant metabolite ion pairs can be observed in both labeling experiments. There are 54 metabolites that are commonly detected in all 4 CSF samples. There are only 8, 2, 1 and 3 metabolites are solely detected in CSF samples 1 to 4, respectively.

It is interesting to compare the 85 metabolites identified to the 329 CSF metabolites reported in the literature (www.csfmetabolome.ca). Our work identified 21 metabolites that have not been reported to be present in human CSF (see Table 4.1 with compound names highlighted in bold). Interestingly, three of them, homoserine, 4-hydroxy-proline and cadaverine, were on the list of the standard compounds in the study of Myint *et al* [18]. However, these compounds

were not identified in their nano-LC-MS analysis of CSF samples. This can be most likely attributed to the detection sensitivity difference of the techniques

Table 4.1 Combined list of identified metabolites from the replicate experiments of 1:1 ^{13}C-/^{12}C-labeled CSF samples #1-4 (the experimental data are shown in Supplemental Tables S4.9 to S4.12).

Compound Name	Compound Name	Compound Name
Phosphoethaolamine	r-aminobutyric acid	L-ornithine
3-Methylhistidine	Hypoxanthine	Acetaminophen / or 4-acetamidophenol
Glucosamine	5-hydroxymethyluracil	Acetaminophen / or 4-acetamidophenol
Taurine	**3-Aminoisobutyric acid**	Homovanillic acid
1-Methylhistidine	**5-Aminopentanoic acid**	3-**/4-hydroxyphenylacetic acid / or 3-Cresotinic acid**
Arginine	2-Aminobutyric acid	Homocarnosine
Homoarginine	Cysteine-glutathione disulfide	Gentisic Acid
Asparagine	**Sarcosine**	Lysine
Glutamine	**Methylcysteine**	4-Hydroxybenzoic acid
L-citrulline	Proline	Histidine
3-sn-Phosphatidylethanolamine	Methylamine	2-aminooctanoic acid
Methylguanidine	Valine	1,3-diaminopropane
Homoserine*	Methionine	L-Tyrosinamide
Methionine sulfoxide	**3-Hydroxypicolinic acid**	**1,4-diaminobutane**
Serine	Tryptophan	**Cadaverine**
Homocitruline	Pipecolic acid	Tyrosine
Glutamic Acid	Phenylalanine	Cysteamine
Aspartic Acid	**3-Hydroxymandelic acid**	**Metoprolol**
4-Hydroxyl-Proline	Isoleucine	Phenol
Aminoadipic acid	Leucine	4-Nitrophenol
Iminodiacetic acid	L-cystathionine	**Octopamine**
Folic acid	L-norleucine	**Tyramine**
Threonine	Cystine	Serotonin
Diethanolamine	Hydroxyphenyllactic acid	Pyrocatechol
Ethanolamine	Homocystine	Spermidine
Glycine	5-Hydroxyindoleacetic acid	**Thymol**
Glycylproline	Dimethylamine	Deoxyepinephrine
Tyrosine methyl ester	Phenylpropanolamine	
Alanine	2,4-Diaminobutyric acid	

*highlighted in bold are the metabolites that have not been previously reported to be present in human CSF.

used. Detection sensitivity improvement afforded by dansylation labeling allowed us to identify these compounds in CSF. The detection limit in their work was in the low micromolar range which is typical for LC-MS without ion selection monitoring, while detection of dansylated metabolites such as amino acids in the low nanomolar range can be achieved [20]. Note that some of the 21 metabolites listed in Table 4.1 (highlighted compounds) are biologically relevant to the neuronal system. For example, tyramine is a suspected neurotransmitter/neuromodulator or co-transmitter with octopamine in the central nerve system.

Identification of many of the remaining unknown ion pairs detected by the dansylation LC-MS method is, however, a major analytical challenge. One strategy of averting this problem is to carry out relative quantification of the metabolomes of a number of comparative samples (e.g., diseased vs. controlled) first to discover one or a few putative biomarkers. Relative quantification is done by a process including the following steps: 1) aliquoting an individual sample into two halves and mixing the aliquots of the individual samples to form a pooled sample, 2) labeling the pooled sample with the heavy chain reagent and the individual sample aliquots with the light chain reagent, 3) mixing the light-mass-tagged individual sample with an aliquot of the heavy-mass-tagged pooled sample, and 4) injecting the mixture into LC-MS to determine peak ratios of ion pairs for relative quantification of metabolites. After the discovery of the putative biomarkers, major efforts are then devoted to the identification of these metabolites using techniques such as tandem MS, NMR and synthesis of standard compounds. A sensitive LC-MS method can then be developed for targeted analysis of these putative biomarkers so that they may be validated by high-throughput analysis of a large number of samples. We envisage that the dansylation LC-MS method described in this work will be useful in the initial

biomarker discovery stage, as it can be used to profile the CSF metabolome in a more comprehensive manner than other techniques.

4.4 Conclusions

We report the development and application of an isotope labeling LC-MS technique for the detection and identification of metabolites in human CSF samples. It is shown that differential isotope labeling using dansylation chemistry is effective in analyzing many low abundance metabolites present in CSF. Without labeling, very few metabolite peaks were detected in LC-MS. With labeling, an average of 519 ion pairs was observed in a 25-min LC-MS run with the injection of an equivalent of 0.5 μL of the original CSF sample. About 261 ion pairs (50% of the total number of pairs found in each run) were commonly detected in four different CSF samples with each sample analyzed twice. Unique ion pairs were found in individual samples and, in total, 1132 unique ion pairs were detected from the combined results. Because dansylated metabolites rarely fragment in the skimmer region of the interface and during the transport into the detection cell of FTIRC-MS, these ion pairs are most likely from the true metabolites. By searching the Human Metabolome Database, 347 unique ion pairs (31%) matched with at least one metabolite in the database. Even if we only consider these matched pairs, this number is already greater than the 329 CSF metabolites reported in the literature (www.csfmetabolome.ca). To provide positive identification of the metabolites in CSF, we have constructed a dansylation library of 220 standard compounds. Using this library, 85 metabolites were identified and, among them, 21 metabolites have not been described in the literature to be present in human CSF.

Future expansion of the standard library will undoubtedly increase the metabolite coverage and our understanding of the CSF metabolome. The ion pair detection technique described in this work is focused on the use of dansylation

chemistry to label amine- and phenol-containing metabolites. Other labeling chemistries targeted at different functional groups are being developed in this laboratory. We envisage that the high performance isotope labeling LC-MS technique with much improved detection sensitivity and ion pair detection specificity should enable comprehensive detection of metabolites in biofluids with unprecedented metabolome coverage.

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Chapter 5

High Performance Isotope Labeling for Profiling Carboxylic Acid-Containing Metabolites in Biofluids by Mass Spectrometry

5.1 Introduction

Metabolomics is a rapidly evolving field for studying biological systems and discovering potential disease biomarkers.[1] For any metabolomics application, metabolome analysis with adequate sensitivity and specificity is essential in defining the metabolome. Ideally, all metabolites present in a biological system are qualitatively and quantitatively profiled. Unfortunately, due to technical limitations, only a fraction of metabolites are currently analyzed by using techniques such as NMR and mass spectrometry (MS).[2, 3] Due to limited metabolome coverage, many important metabolome networks and some subtle changes in the metabolome may not be revealed with current techniques. Herein we report a high performance isotope labeling strategy, in combination with liquid chromatography (LC) MS, for profiling the metabolites containing carboxylic acid moieties in a biological sample including body fluids such as urine.

Isotope labeling of chemicals has been widely used for quantitative analysis of targeted molecules such as drug metabolites by LC-MS.[4] By overcoming matrix and ion suppression effects, the use of an isotope internal standard for the analyte of interest often provides high accuracy of quantitative measurement. However, at present, it is not practical to generate individual isotope standards for all the metabolites of a biological system, or the

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metabolome. An alternative approach is to use a chemical reaction to introduce a mass tag to all the metabolites with common reactive moiety in a sample and apply the same derivatization reaction to the standards with a differential mass tag (e.g., ^{12}C-labeling for the sample and ^{13}C-labeling for the standards).[5] After mixing the labeled sample and the mass-differentially labeled standards, LC-MS is carried out and, based on the accurate mass, retention time, and relative peak intensities of ion pairs detected in the mass spectra, tentative identification and absolute quantification of metabolites in the sample can be attained. Using additional information such as MS/MS spectral match, positive metabolite identification can be made. Without the availability of standards, information on relative quantification of metabolites in different samples can still be obtained.

While chemical derivatization is effective in introducing a mass tag to the metabolites for LC-MS,[5-13] it can also provide an opportunity to enhance the performance of the overall LC-MS analysis process. Within this context, rational design of the tag to be attached to the metabolites becomes important. Considering that the LC-MS metabolome profiling work involves in several sample handling and analysis steps, an ideal tag would provide concurrent improvement in analytical performance of each step. In this work, we report a new isotope labeling method to tag carboxylic acid-containing metabolites (CAMs) and demonstrate its application for LC-MS metabolome profiling of complex samples. Global profiling of these metabolites is significant in metabolomics, as a large portion of the metabolome including a vast number of fatty acids belong to this class. For example, about 65% of the ~5000 known endogenous human metabolites contain at least one carboxylic acid group in a chemical structure.[14]

5.2 Experimental

5.2.1 Chemicals and Reagents.

All chemicals and reagents were purchased from Sigma-Aldrich Canada (Markham, ON, Canada) except those otherwise noted. LC-MS grade of water, methanol and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Edmonton, AB, Canada). Urine samples were collected from a healthy individual and processed by adding 50% (v/v) LC-MS grade acetonitrile, then stored in a -80 ℃ freezer.

5.2.2 Synthesis of DmPa-$^{13}C_2$.

The synthesis of ^{13}C-DmPa as a derivatizing reagent was based on a two-step procedure (Figure 5.1). In the first step, in a 25-mL round-bottom flask, 1.1 p-aminoacetophenone was added to 2.55 g of $NaHCO_3$ in 4.2 mL of H_2O. The flask was placed in a 10^{o}C water bath for 5 min. About 1.5 mL of ^{13}C-dimethyl sulphate was added drop-wise within 90 min to the solution in an ice-water bath at 10^{o}C. The resulting solution was warmed to 50^o for 10 min. About 1.6 mL of saturated KOH solution was added to hydrolyze un-reacted dimethyl sulfate. The resulting solution was subjected to liquid/liquid extraction by water, then chloroform. The precipitate of ternary amine formed by the above treatment was washed a few times with water, then with heptane, and then dissolved in chloroform and dried with magnesium sulfate. Subsequently, chloroform was removed and the ternary amine was dissolved in hot heptane under reflux and filtered through a hot Shott funnel. After cooling, the crystallized amine was filtered, washed a few times with heptane. The residue was purified by flush chromatography (silica gel, 40 × 3 cm, 20 mL AcOEt), and further purified by a semi-preparative Grace Apollo silica normal-phase HPLC column (10 × 150 mm, 5 μm particles).

In the second step, 0.65 g of ^{13}C-N,N-Dimethylamino-p-acetophenone synthesized from the first step was dissolved in 4 mL of concentrated H_2SO_4 and then cooled to 0^{o}C in a water-ice bath, which resulted in a positively charged

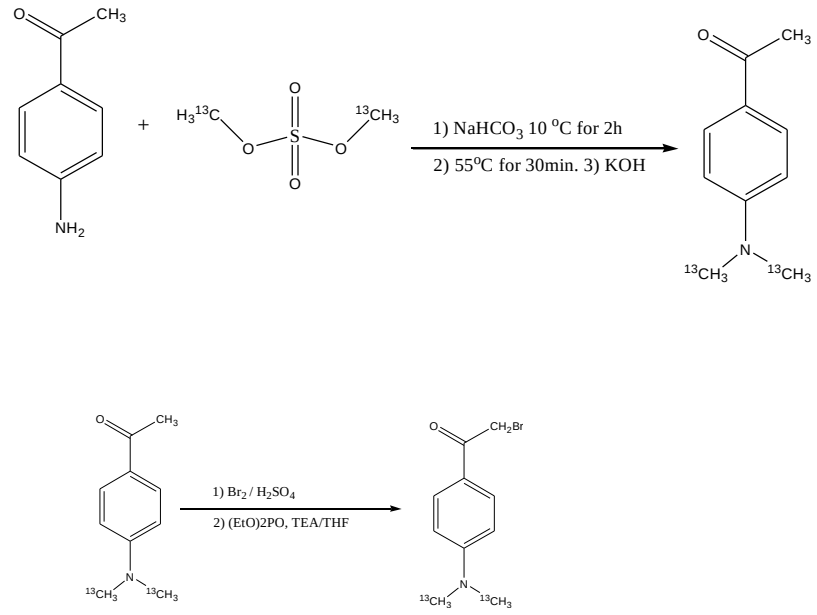

Figure 5.1 Reaction scheme for the synthesis of p-dialkylaminophenacyl (DaPA) bromide

tertianary amine. To the solution, was drop-wise added 0.2 mL of bromine at 0^{o}C and was then gradually warmed to room temperature and stirred for 6 hr. The reaction mixture was poured into ice/water. The yellow precipitate was extracted by liquid/liquid extraction with chloroform, and then dried, then re-dissolved in 5 mL of tetrahydrofuran, and cooled to 0^{o}C in ice-water bath. About 0.45 mL of diethylphosphite and 0.47 mL of triethylamine in 2.5 mL of tetrahydrofurin at 0^{o}C were added drop-wise to the solution. The resulting mixture was gradually warmed to room temperature and stirred for 6 hr. The solution then was extracted

by liquid/liquid extraction with chloroform/cold water, and dried. This material was further purified by the semi-prep RP column (Agilent Zorbax Rx-C18, 9.4 x 250mm, 5 μm particle size). The purity was tested using LC-FTICR-MS and LC/UV. NMR was also used to characterize the reaction products and confirm the identity and purity of the final product. The purity of the labeling reagent was >99.5% by HPLC, UV, MS and NMR analysis.

5.2.3 LC-MS Experiments.

An Agilent 1100 binary system (Agilent, Palo Alto, CA) was combined with the Bruker 9.4-Tesla Apex-Qe FTICR mass spectrometer (Bruker, Billerica, MA, USA) equipped with an electrospray ionization (ESI) source or an Applied Biosystems MDS QSTAR Pulser Quadrupole Time-of-Flight mass spectrometer. A reversed-phase Eclipse Plus C_{18} column (2.1 mm x 100 mm, 1.8 μm particle size, 95 Å pore size) was purchased from Agilent. Solvent A was 0.1% (v/v) LC-MS grade formic acid in 5% (v/v) of LC-MS grade acetonitrile, and solvent B was 0.1% (v/v) LC-MS grade formic acid in LC-MS grade acetonitrile. The binary gradient elution profile was as follows: t = 0 min, 35% B; t = 1.0 min, 40% B; t = 9 min, 52% B; t = 22 min, 65% B; t = 26 min, 80% B; t = 29 min, 98%B, t = 30 min, 98%B. The flow rate was 150 μL/min and the sample injection volume was 1.0 μL. The LC eluent from column was splitted 3:1, and about 50 μL/min was delivered into the ESI mass spectrometer. All MS spectra were obtained in the positive ion mode. It was found that negative ion detection was not as sensitive as the positive ion detection for DmPA derivatives.

The MS conditions used for FTICR-MS were as follows: nitrogen nebulizer gas: 1.8 L/min, dry gas flow: 7.0L/min, dry temperature: 180^{o}C, capillary voltage: 4200V, spray shield: 3700V, acquisition size: 256k, scan range: 70-1400, ion accumulation time: 0.1 sec, TOF (AQS): 0.007 sec, DC Extract Bias: 0.7V. The MS Conditions used for QStar Pulsar i were as follows:. IonSpray

voltage (IS): 5000, ion source gas 1 (GS1): 30, curtain gas (CUR): 18, de-clustering potential (DP): 50V, focusing potential (FP): 245V, scan range: 60-1200.

For the analysis of un-derivatized acids, negative ion mode in LC-FTICR-MS was used. The mobile phase solvent A used was 10 mM ammonium acetate in LC-MS grade water. Solvent B was 10 mM ammonium acetate in 80% LC-MS grade ACN. The gradient was as follows: 0-2min: 5%B, 2-15min: 5-40%B; 15-18min: 40-70%B; 18-20min: 70-90%B, 20-25min: 5%B.

5.2.4 Labeling Reaction.

Figure 5.2 shows the reaction scheme for labeling carboxylic acids using the isotope reagents, ^{13}C- or ^{12}C-DmPABr. For labeling human urine samples, urine in 50% (v:v) of acetonitrile was centrifuged for 10 min at 12 000 rpm. About 50 µL of human urine in acetonitrile (v:v) or 50 µL carboxylic acid standards in acetonitrile (1.2 mM each) were mixed with an equal volume of 750 mM of triethanolamine (TEOA) in a reaction vial. The solutions were vortexed and spun down, then mixed with 50 µL of freshly prepared ^{13}C-DmPA (20 mg/mL) (for heavy labeling) or ^{12}C-DmPA (20 mg/mL) (for light labeling). The derivatization reaction was proceeded for 60 min at 90^{o}C in a water bath. After 60 min, the mixtures were vortexed, spun down and 100 µL of triphenylacetic acid (30 mg/mL) was added to consume the excess labeling reagent, ^{12}C-/^{13}C-DmPA, at 90 ˚C in TEOA for 30 min. The solutions were vortexed and spun down. The labeling reactions were carried out in sealed glass vials with Teflon lined caps. After labeling, the ^{13}C-labeled mixtures were combined with their ^{12}C-labeled mixtures for LC-MS analysis. We note that the labeled sample was usually analyzed within two weeks. No degradation products for the labeled standards were observed after storing the labeled samples in -20 ˚C for up to 2 weeks.

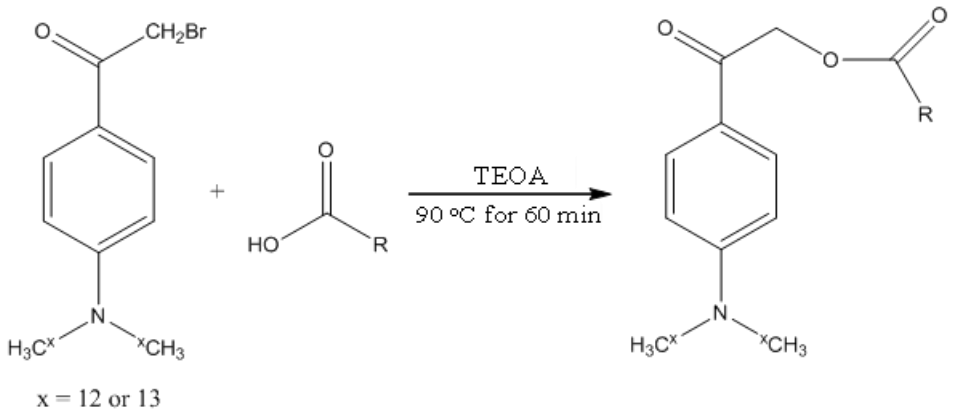

Figure 5.2 Reaction scheme for labeling carboxylic acid-containing metabolites using isotope coded p-dimethylaminophenacyl (DmPA) bromide (light chain, x=12; heavy chain, x=13).

5.3 Results and Discussion

Derivatization of carboxylic acids can be done with a variety of chemical reactions for analytical applications and, among them, phenacyl bromide (PBr) has been used to label the acids to improve the performance of HPLC and UV detection.[15] Our labeling chemistry is based on this reaction. However, to tailor our needs, we designed a new reagent that allows the introduction of a mass tag and concurrent improvement in LC-MS analysis. Figure 5.2 shows the structure of the reagent, p-dimethylaminophenacyl (DmPA) bromide and the reaction scheme for labeling carboxylic acid to form isotope mass-coded derivatives. Triethanolamine (TEOA) was used as a base catalyst for the reaction. The mass difference of the ^{13}C-/^{12}C-labeled products with one tag has a nominal mass of 2 Da.

We have also constructed a standard library of 113 carboxylic acid-containing metabolites (CAMs) by labeling individual compounds with DmPABr one-by-one (See Supplemental Table S5.1). The reaction was found to be complete within 60 min and the yield was in the range of 95 to 99%. Supplemental Table S5.2 shows the reproducibility and reaction yield of ten standards; the average CV was 4.3% with a range from 2.2% to 7.2% for four repeated experiments. This labeling reaction has high specificity towards the acids. We tested a variety of compounds with different functional groups, such as alcohols, thiols, amides, amines, ketones and aldehydes, and did not see reaction products. The reaction can be performed in an aprotic solvent such as acetonitrile, acetone or N,N-dimethylformamide (DMF). A small amount of water (up to 20%) do not significantly affect the reaction yield. To quench the reaction, various acids were tested and it was found that triphenylacetic acid could be used to effectively consume the excess amount of DmPABr and the labeled product eluted often as the last peak in reversed-phase (RP) LC, thereby avoiding the interference of the analyte peaks from the quenching reagent. We note that the reaction product of the hydrophobic quenching reagent, triphenylacetic acid, has low solubility. The majority of the product remained as solid and would not be extracted to the sample and thus not injected onto the column. This could avoid column overloading. Furthermore, derivatized triphenylacetic acid eluted when there was around 90-95% ACN in the mobile phase. Under this condition, the ionization efficiency of the derivatized triphenylacetic acid was low and thus their signal intensity was low.

Figure 5.3 shows a base-peak ion chromatogram (BPC) of a mixture of 10 carboxylic acids labeled with ^{13}C-/^{12}C-DmPABr obtained by LC-MS using the QSTAR mass spectrometry (6 pmol each injected). Figure 5.4 shows the chromatograms of a human urine sample labeled with ^{13}C-/^{12}C-DmPABr (Figure

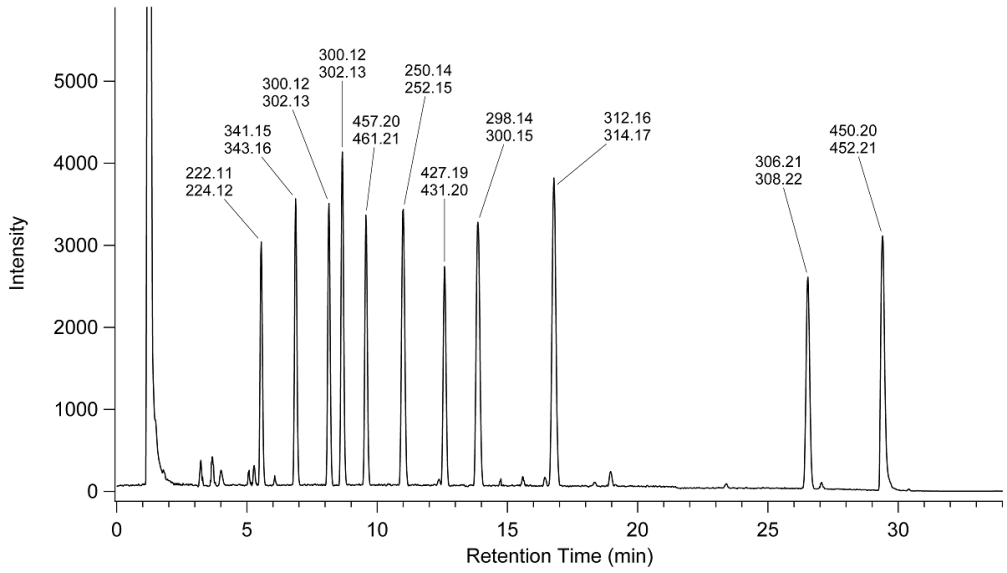

Figure 5.3 Base-peak ion chromatogram of a mixture of 10 carboxylic acid standards (6 pmol each injected). The peaks labeled with masses from left to right are: 1. acetic acid, 2. hippuric acid, 3. 4-hydroxybenzoic acid, 4. 3-hydroxybenzoic acid, 5. malic acid, 6. butyric acid, 7. malonic acid, 8. phenylacetic acid, 9. hydrocinnamic acid, 10. octanoic acid, 11. triphenylacetic acid (triphenylacetic acid was added to consume the remaining labeling reagent and quench the reaction). The standard mixture was labeled with either ^{13}C-DmPABr or ^{12}C-DmPABr and a 1:1 mixture of the labeled compounds was analyzed by RPLC QTOFMS.

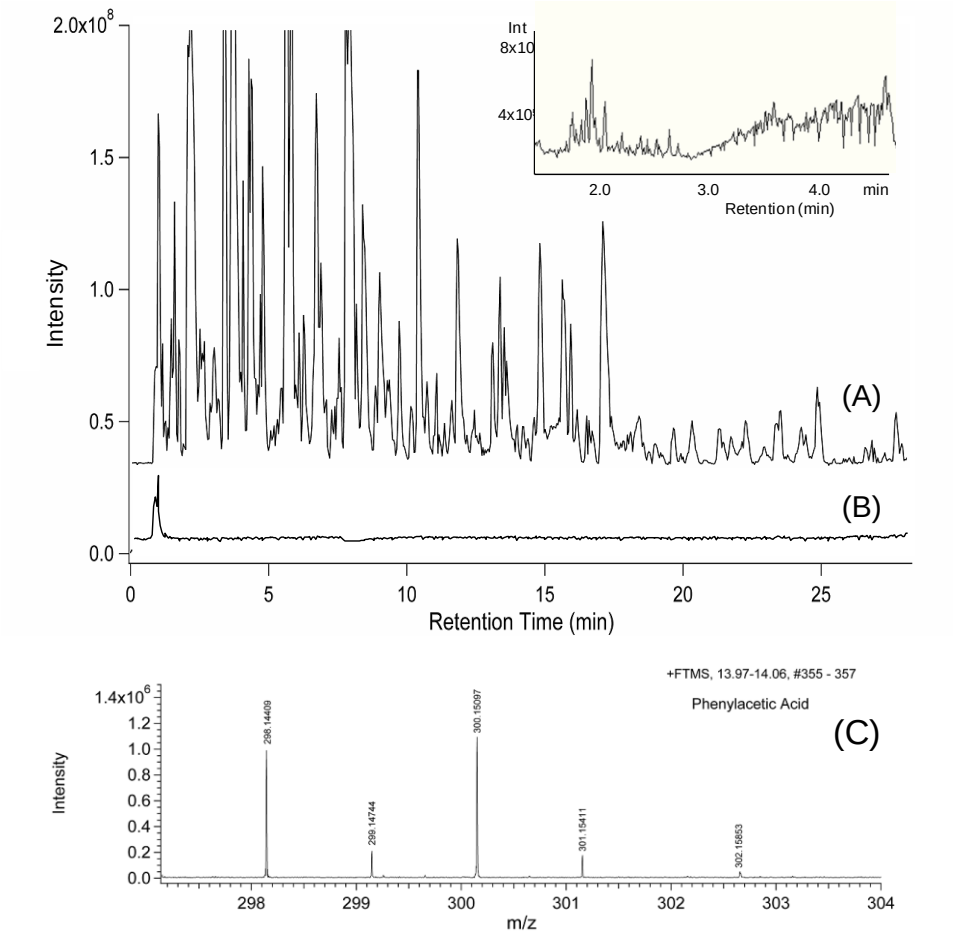

Figure 5.4 Base-peak ion chromatograms of (A) a labeled human urine sample obtained in positive ion mode and (B) a urine sample without labeling obtained in negative ion mode that was optimized for detecting carboxylic acids (the expanded view of the chromatogram at the earlier elution is shown in the inset). The urine sample was labeled with either ^{13}C-DmPABr or ^{12}C--DmPABr and the labeled urine samples were mixed in 1:1. A sample with an amount equivalent to about 20 nL of the original urine was injected into RPLC FTMS for analysis. (C) the molecular ion region of an ESI mass spectrum displaying a pair of ^{13}C-/^{12}C-DmPABr labeled metabolite ions. Some of the metabolites tentatively identified and their retention times from (A) are provided in Supplemental Table S5.3.

5.4A) and the same sample without labeling (Figure 5.4B). In each case, a sample amount of equivalent to 30 nL of urine was injected into a 2.1-mm RPLC combined with the Bruker FTICR mass spectrometer. The molecular region of a mass spectrum obtained from the labeled urine sample is shown in Figure 5.4C.

There are several important features shown in Figures 5.3 and 5.4 that demonstrate the merits of performing DmPA derivatization for acid analysis. First of all, DmPA derivatization improves the metabolite separation by RPLC. While many unlabeled acids do not retain on a RP column, the labeled analytes have sufficient interaction with the column to be separated by RPLC. For the standard mixture shown in Figure 5.3, except the last two acids, the unlabeled acids eluted out in the void or short retention time of less than 5 min (data not shown). For the unlabeled urine sample, most peaks were observed at or near the void volume (Figure 5.4B); many other acids expected to retain on the column were not observed due to low signal intensities (see below). However, the labeled acids were separated over the gradient elution time. The addition of the DmPA tag containing a hydrophobic aromatic ring to a polar and hydrophilic acid increases hydrophobicity, thereby enhancing its retention on a RP column. The major advantage of using RPLC for separating the metabolites is that it has superior separation efficiency to other modes of separation such as hydrophilic interaction (HILIC) chromatography. In addition, the use of one column, i.e., RPLC, instead of using different columns to handle different polarity of metabolites of the same sample, saves the analysis time.

Second, DmPA derivatization enhances the ESI efficiency significantly. As Figure 5.3 shows, 10 chromatographic peaks from the standards are observed with similar responses, despite large differences in chemical structures of these acids. The injection of the same amount of the mixture without derivatization did not produce much detectable signals, even in the optimized negative ion mode.

For the 113 carboxylic acid standards, DmPA derivatization was found to enhance the detection sensitivity by about 2 to 4 order of magnitude, depending on the compound structure. The signal enhancement can be attributed to several factors. One is related to the increased propensity of being charged for the labeled acid due to the presence of the dimethyamine moiety attached the aromatic ring of the tag where a tertiary amine can be readily formed. The labeled acid has higher hydrophobicity than its unlabeled counterpart, making it easier to stay on the surface of the droplets during ESI. An elution solvent with higher organic solvent content where a labeled acid is eluted out, as opposed to the unlabeled one eluted at void or high water content solvent, also enhances the ionization efficiency. Note that the first large peak eluted at or near the void volume in Figure 5.3 was mainly from the base catalyst, TEOA. The small peaks shown were most likely from the unknown impurities present in the standards that were also labeled with DmPA. The ESI signal enhancement is quite dramatic in the analysis of biological samples such as urine. As Figure 5.4A,B illustrates, only a few peaks are observed in the analysis of unlabeled urine (Figure 5.4B and the expanded chromatogram in the inset) while many peaks are detected from the labeled urine sample (Figure 5.4A).

The third advantage of DmPA derivatization is that a proper isotope mass tag can be readily attached to a carboxylic acid-containing metabolite and the labeled metabolite does not display any isotope effect on RPLC. Co-elution of the analyte and its internal standard is a key to achieve accurate quantification by LC-MS. Otherwise, ion suppression can cause significant variation in detectability of analyte and standard, resulting in quantitative errors. The ^{13}C-/^{12}C-labeled metabolites co-elute perfectly. The relative amount of two comparative samples, one labeled with ^{13}C-DmPA and another with ^{12}C-DmPA, can be determined directly from the peak ratio of the ion pair in the mass spectra (e.g., Figure 5.4C),

not in the chromatogram. For example, a linear relation (R^2>0.995) was found in LC-MS analysis of differential isotope labeled carboxylic acid standards, such as malic acid, where the amount ratios of ^{13}C-/^{12}C-labeling were 5:1, 1:1, 1:5, 1:10 and 1:20. The relative standard derivation (RSD) on the ratios of the ion pairs in replicate analysis was less than 6%, indicating that good reproducibility could also be achieved.

Finally, DmPA isotope labeling facilitates the identification of metabolite peaks among many spectral features observed in LC-MS (a feature refers to a mass spectral peak with a specific m/z at a given retention time). There are usually many peaks detected in an LC-MS run (e.g., for the labeled urine samples, over 15,000 features could be seen) and many of these peaks are from impurities present in the solvents, column, tubing, and interface, salts and adducts, electric noises, column coatings, etc. Identification of the true metabolite peaks can be done by analyzing a mixture of equal amount of a sample differentially labeled with ^{13}C- and ^{12}C-DmPABr, such as the urine sample results shown in Figure 5.4A and Supporting Table S5.3. Perfect co-elution of the ion pairs and their characteristic mass difference of 2×n Da for metabolites with n tags or acidic groups with <2 ppm errors in mass difference can be used to identify the metabolite peaks. Other non-metabolite peaks and unlabeled metabolites would show up as singlet peaks. For example, the ion pair at 298.14411 and 300.15098 shown in Figure 5.4C has a mass difference error of -0.53 ppm. The mass difference of 2.00671 Da indicates this metabolite has one carboxylic acid. Comparing the retention time and accurate mass measurement of the standards, the ion pair was identified to be from phenylacetic acid. While using ion pairing of differentially labeled samples to assist in distinguishing the metabolite peaks from the others is not unique to DmPA labeling, this labeling method does provide two additional benefits. One is related to the reduction of interference

from low mass ions in LC-MS. Adding a tag (162 Da) to a metabolite increases the molecular ion mass, effectively shifting away from the low mass region of the mass spectra where high background signals are usually observed at m/z<200. Another benefit is related to the increased stability of the labeled metabolites where, in general, only the molecular ions are observed in the mass spectra. Dissociation of molecular ions of unlabeled metabolites in the interface and during ion translation to the mass analyzer can cause ambiguity in assigning the mass spectra peaks; a number of peaks observed may be from the fragment ions of the metabolites, not the intact metabolite ions. In triplicate experiments of 1:1 ratio of ^{13}C-/^{12}C-DmPA labeled human urine, 2671, 2546 and 2820 ion pairs were detected (see Supporting Information, Table S5.3, for the list). Using the 113 carboxylic acid standard library, we identified 51, 43, and 48 metabolites (see Table S5.3). This example illustrates that a large number of metabolites can be profiled from human urine using the DmPA labeling LC-FTICR-MS method. Of course, positive identification of these ion pairs remains to be challenging. Future work on MS/MS analysis of these labeled ions may result in identifying more metabolites. We also note that in the case of the urine sample, we could not determine if there were any side reactions contributing to the overall number of peaks observed, as the identities of most of the peaks were unknown.

In summary, we have developed a new isotope labeling method for high performance metabolome analysis with a focus on global profiling of carboxylic acid-containing metabolites. This labeling method is demonstrated to be not only effective in introducing an isotope tag for accurate metabolite quantification, but also improving the chromatographic retention of the metabolites in RPLC, enhancing ESI efficiency by 2 to 4 orders of magnitude, and facilitating the identification of metabolite peaks in LC-MS. This method along with other high performance labeling methods, such as dansylation, that are targeted at amine-,[5]

phenol-,[5] and ketone/aldehyde-containing metabolites should cover the majority of the metabolome. We believe that high performance isotope labeling LC-MS should open the possibility of carrying out comprehensive metabolome profiling experiments on any biological samples, thereby increasing the power of metabolomics for investigating subtle changes in the metabolome for biological studies and disease biomarker discovery. Finally, we note that design of multiplex isotope labeling reagents based on a similar reaction scheme of DmPABr is possible and will be reported in the future.

5.4 Conclusions

We have developed a new isotope labeling method, based on the use of isotope-coded p-dimethylaminophenacyl (DmPA) bromide as a reagent, combined with liquid chromatography mass spectrometry (LC-MS) for high performance metabolome analysis with a focus on profiling carboxylic acid-containing metabolites. Derivatization is simple, fast (1 hr plus 30 min for quenching the reaction), and applicable to a wide range of carboxylic acids with high yield and little or no side reaction products. This labeling method is demonstrated to be not only effective in introducing an isotope tag for accurate metabolite quantification, but also improving the chromatographic retention of the metabolites in reversed-phase (RP) LC, enhancing ESI efficiency by 2 to 4 orders of magnitude, and facilitating the identification of metabolite peaks in LC-MS. In triplicate experiments of 1:1 ratio of ^{13}C-/^{12}C-DmPA labeled human urine, we were able to detect 2671, 2546 and 2820 ion pairs from metabolites containing one or more carboxylic acid groups.

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Chapter 6

Double Reversed-phase Liquid Chromatography Fractionation and ^{12}C-/^{13}C- Dansylation Labeling Combined with FTMS for Comprehensive Qualitative Analysis of Urine Metabolome

6.1 Introduction

Liquid chromatography combined with mass spectrometry (LC-MS) has become the routine analytical technique for generating global metabolite profiles due to its high sensitivity and specificity. However, there are a number of analytical challenges in current LC-MS-based metabolome analysis, for example, elucidation of structures of unknown metabolites from tens of thousands of ion features detected in LC-MS, lack of ultra-high chromatographic peak capacity to separate the peak features detected, great diversity in chemical and physical properties of metabolites, and lack of a large-scale, fast, software-based, definitive identification and putative identification approach.

The resolving power of one-dimensional HPLC (1DLC) is often limited, not offering large enough peak capacity to separate all the components in a complex biological sample.[1, 2] In a typical 25 min LC-MS analysis of a human serum or urine sample, up to 10,000 - 15,000 spectral peak features can be generated. Currently, there is no 1DLC that is even close to being able to separate all the components in such a complex sample. For a detailed qualitative study of a limited number of complex biological samples, a multi-dimensional liquid chromatography (LC x LC) would seem to have advantages in further reducing the sample complexity prior to mass spectrometry analysis, thus reducing ion suppression or matrix effects.

NMR is one of the major techniques commonly used to elucidate the metabolite structure in metabolomic analysis. Metabolomic NMR requires minimal sample preparation, is a non-destructive and non-discriminating technique.[3] NMR spectra can be quickly and easily collected. However, the key disadvantage of NMR is that it is a relatively insensitive technique with a limit of detection of about 1-5 μM, and it requires large sample sizes (~500 μL for regular probes).[3] Furthermore, interpretation of metabolomic NMR spectra from a complex biological sample can be highly challenging as a result of the lack of high resolution separation prior to NMR analysis. One useful solution to these problems is to use preparative or semi-preparative LC x LC to produce a large sample size with efficient chromatographic separation and purification for ^{1}H NMR or even 2D NMR, ^{13}C NMR analysis. The small fractions collected from LC x LC can be further analyzed by high resolution, high mass accuracy Fourier transform ion cyclotron resonance (FT-ICR) and/or time-of-flight (TOF) mass spectrometers. The unknown metabolite structural elucidation then can be readily carried out by combining the information from NMR and FTMS or TOFMS.

A variety of combinations of liquid chromatography separating mechanisms, such as ion exchange-reversed phase (IEC-RPLC), size-exclusion-reversed phase (SEC-RPLC), and ion exchange-size exclusion (IEC-SEC) have been routinely used in 2DLC.[4] All these 2DLC modes share a common feature, of containing two orthogonal separation mechanisms. In an ideal situation, all the analytes in a complex sample will be differentially retained by two orthogonal mechanisms, and each mechanism is considered an independent separation dimension.[2] A relatively low resolution separation, such as ion-exchange or size-exclusion is commonly chosen as the first dimensional separation followed by a much higher resolving and more reproducible reversed-phase separation as the second dimension. In these cases, the power to resolve the components is limited

by the selectivity of the stationary phase and the low resolution in first dimension. The peak capacity of this type of 2DLC would be lower than a RPLC x RPLC, assuming the RPLC x RPLC mode is capable of offering true orthogonality. RPLC x RPLC has been reported in research for years, however, in most reports, only limited differences in selectivity of the reversed-phase stationary phase are used for each dimension. The orthogonality offered by such RPLC x RPLC remains questionable.

An alternative strategy of 2DLC could be carried out by altering the hydrophobicity of analytes prior to the 2nd dimension of RPLC separation rather than by attempting to choose stationary phases of differing chemistries for each retention mechanism. A judiciously chosen chemical derivatization prior the 2nd dimension would generate such orthogonality by changing the hydrophobicity of targeted analytes.

A large proportion of human metabolites in biofluids are highly polar compounds, and conventional RPLC often lacks the capability to adequately retain and separate ionic, polar metabolites. Recently HILIC has been frequently used in bioanalytical applications, but poor peak shape and low chromatographic efficiency is often observed.[5, 6] An alternative approach to improve the retention of ionizable polar metabolites and maintain high separation efficiency is to use ion-pairing RPLC in which cationic species, such as amines or amino acids are reported to be successfully separated by ion pairing reversed-phase chromatography using long chain perfluorocarboxylic acids as the volatile ion pairing reagent.[6-14]

As the main cationic metabolites in human biofluids, amines, amino acids and phenolic hydroxyls, metabolites can be first separated by ion-pairing RPLC, then dansylated prior to the 2nd dimension (regular) of RPLC separation. The orthogonality of separation in this strategy relies on the alteration of the

hydrophobicity of the analytes by the dansylation chemical derivatization prior to the second dimension RPLC separation, rather than the different retention mechanisms with a different stationary phase in traditional 2DLC. The amplitude of changes in hydrophobicity of derivatives is directly related to the number of reactive functional groups in the parent compounds, i.e. retention of derivatives in the 2^{nd} dimension of RPLC is largely dependent on the number of hydrophobic dansylation tags added, rather than merely their parent compound structures. Thus, dansylation potentially provides the orthogonality for 2^{nd} dimension a RPLC separation that is needed for RPLC x RPLC. To our knowledge, this is the first report of such strategy for metabolome analysis.

In this research we proposed a novel strategy that applied 2 x RPLC separations with the same semi-preparative C18 reversed-phase column on a complex biological sample. The fractions from two RPLC (1^{st}, IP RPLC and then dansylation followed by a 2^{nd}, regular RPLC) were collected from multiple injections of urine to ensure a large enough sample amount to carry out the relatively insensitive NMR analysis. The highly reproducible IP RPLC chromatography ensured the integrity of the fractionation from multiple injections. The fractions collected from IP RPLC x RPLC were analyzed by NMR and RPLC/FTMS. The definitive identification of the metabolites was carried out by ^{13}C-/^{12}C-dansylation of IP RPLC fractions and matching of the RPLC retention times and accurate masses of ion pairs to a ^{13}C-/^{12}C-dansylation library of compounds (of LC/FTMS). A significant ESI signal enhancement (up to 3 orders of magnitude) and chromatographic improvement (particularly for polar species) in RPLC-MS after dansylation derivatization was observed.[15] The current library includes 220 compounds. The structural elucidation of unknown metabolites was readily carried out by two steps: first, putative identification (searching the HMDB database for an accurate mass match) to narrow down the possible

metabolite structures, and second, the structural elucidation and confirmation by NMR analysis.

6.2 Experimental

6.2.1 Chemicals and Reagents.

All chemicals and reagents were purchased from Sigma-Aldrich Canada (Markham, ON, Canada) except those otherwise noted. The isotopic compound, $^{13}C_2$-dimethyl sulfate, used to synthesize the isotope tagged dansylation reagent (^{13}C-danysl chloride) was purchased from Cambridge Isotope Laboratories (Cambidge, MA, US). LC-MS grade water, methanol and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Edmonton, AB, Canada). Urine samples were collected from a healthy individual and processed by adding 50% (v/v) LC-MS grade acetonitrile, then stored at -20 ℃.

6.2.2 Synthesis of Dansyl Chloride-$^{13}C_2$.

The synthesis of ^{13}C-dansyl chloride as derivatizing reagent was based on a two-step procedure described by Horner and Bergmann.[16, 17] The purity and confirmation of ^{13}C-dansyl chloride was tested against the commercial ^{12}C-dansyl chloride using LC/UV, LC-FTICR MS, and ^{1}H-NMR. The purity of ^{13}C-dansyl chloride was >99%, based on the LC/UV analysis, and its ^{13}C-isotopic purity exceeded 99%, based on the LC-FTICR MS analysis.

6.2.3 Dansylation Labeling Reaction.

Figure 6.1 shows the reaction scheme for dansylation of amine- and phenol-containing compounds. The detailed procedure has been reported previously.[15] The fractionations collected were mixed with an equal volume of sodium carbonate/sodium bicarbonate buffer (0.5 mol/L, pH 9.4) in reaction vials. About a 4-fold molar ratio excess ^{12}C-dansyl chloride solution (20 mg/mL) (for light labeling) or ^{13}C-dansyl chloride (20 mg/mL) (for heavy labeling) was then added, and the reaction was allowed to proceed for 60 min at 60 ^{o}C with shaking

at 150 rpm. After 60 min, methylamine (0.5mol/L) was added to the reaction mixture to consume the excess dansyl chloride and quench the dansylation

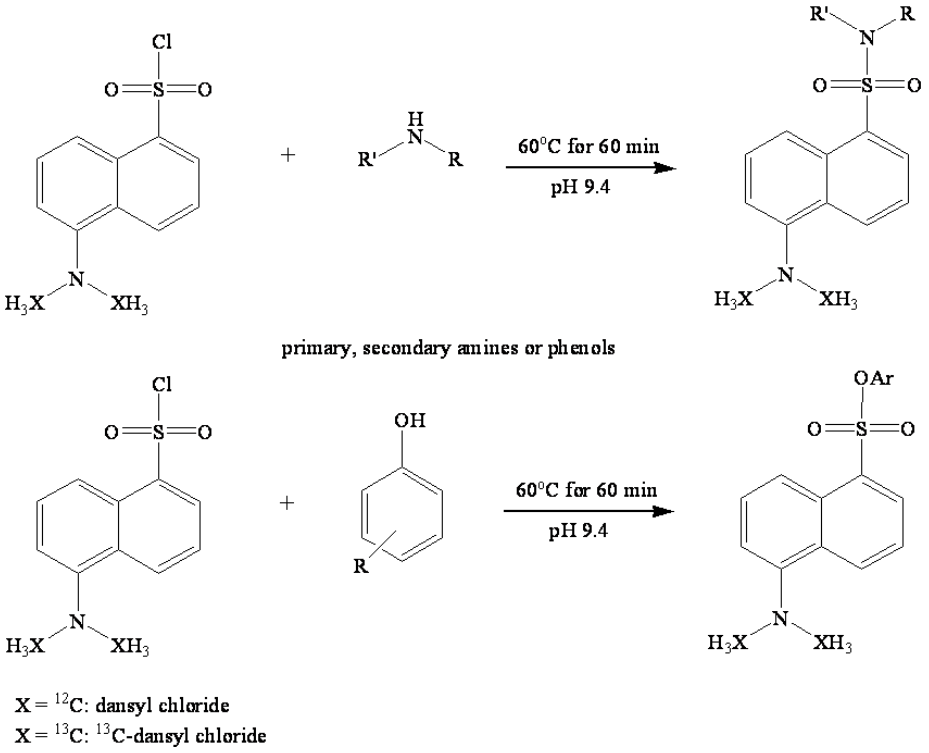

Figure 6.1 Reaction schemes for ^{13}C-/^{12}C-dansylation derivatization of primary, secondary amines and phenols.

reaction. After an additional 30 min of 60 ^{o}C incubation, the ^{13}C-labeled mixture was combined with its ^{12}C-labeled counterpart for LC/FTMS analysis. For the 2nd-dimension of RPLC separation, only regular ^{12}C-dansyl chloride was used for labeling reaction to alter the hydrophobicity of the targeted metabolites.

6.2.4 1st Dimensional Ion Pair Reversed-Phase Liquid Chromatography.

An Agilent 1100 series quaternary HPLC system (Agilent, Palo Alto, CA), and an Agilent Zorbax Rx-C18 column (9.4 x 250 mm, 5 μm particle size) were used in the 1st and 2nd dimensions of separation. Mobile phase A was 12 mM heptafluorobutyric acid (HFBA) and mobile phase B was 100 % acetonitrile. 254 nm was chosen as the UV detector wavelength. The gradient elution profile was as follows: t = 0, 0% B; t = 8 min, 10% B; t = 20 min, 30% B; t = 23 min, 95% B; t = 23.5 min, 0% B; t = 50 min, 0% B. The flow rate was 5 mL/min, and sample injection volumes were 800 μL.

6.2.5 2nd Dimension of Regular Reversed-Phase Liquid Chromatography.

Mobile phase A was 5% acetonitrile and mobile phase B was 100% acetonitrile. 254 nm was chosen as UV detector wavelength. The gradient elution profile was as follows: t = 0, 20% B; t = 4 min, 28% B; t = 16 min, 40% B; t = 21 min, 60% B; t = 24 min, 95% B; t = 24.5 min, 20% B. t = 35 min, 20% B. The flow rate was 5 mL/ min, and sample injection volumes were 800 μL.

6.2.6 LC/FTMS.

The HPLC system was an Agilent 1100 series binary system (Agilent, Palo Alto, CA) that was modified to reduce extra system solvent volume according to an Agilent protocol (Agilent Publication Number: 5988-2682EN). A reversed-phase Agilent Eclipse plus C_{18} column (2.1 x 100 mm, 1.8 μm particle size, 95 Å pore size) was purchased from Agilent Canada (Mississauga, ON). LC solvent A was 0.1% (v/v) LC-MS grade formic acid in 5% (v/v) LC-MS grade ACN, and solvent B was 0.1% (v/v) LC-MS grade formic acid in LC-MS grade

acetonitrile. The gradient elution profile was as follows: t = 0 min, 20% B; t = 3.0 min, 35% B; t = 16 min, 65% B; t = 18.6min, 95% B; t = 21 min, 95% B; t = 21.3 min, 98% B; t = 23.0 min, 98% B; t = 24.0 min, 20% B. The flow rate was 150 μL/min. The flow from RPLC was split 1:3 and a 50 μL/min flow was loaded to the electrospray ionization (ESI) source of a Bruker 9.4 Tesla Apex-Qe FTICR mass spectrometer (Bruker, Billerica, MA, USA) or an Applied Biosystems, QStar Pulsar i mass spectrometer, while the rest of the flow was delivered to waste. All MS spectra were obtained in the positive ion mode. The QStar Pulsar i LC-MS system was only used for detecting individual dansylated standards for the development of the dansylation library. All the mass spectral data presented in this work were obtained using the Bruker 9.4 Tesla FTICR mass spectrometer.

6.3 Results and Discussion

6.3.1 1st dimension, Ion Pairing Reversed-Phase Chromatography.

The most efficient stationary phase is the hydrophobic octadecyl silica support and its derivatives.[7] The majority of amino acids and amine metabolites are highly hydrophilic compounds. Due to the weak hydrophobic character of amino acids and other amines, traditional RPLC is not efficient enough to retain and separate the most polar compounds. Ion-pairing RPLC is an appropriate choice for increasing the retention of the polar compounds while maintaining the RPLC character with high efficiency and peak capacity. It appears that the commonly used ion pair reagent, trifluoroacetic acid (TFA), even at high concentrations, does not promote enough retention and selectivity for the polar amino acids and other amines. In this study, a long chain perfluorocarboxylic acids, heptafluorobutyric acid (HFBA) was chosen as the acid ion pair reagent for increasing retention of cationic metabolites.

The major benefit of ion-pairing with heptafluorobutyric acid in reversed-phase chromatography is that it addresses the issue of chemophysical diversity of

metabolites by generating selective retention of polar cationic compounds. Amines, amino acids and phenolic hydroxyl metabolites represent the majority of cationic metabolites in human biofluids. In IP RPLC, the cationic species form an ion-pair with the negatively charged ion-pairing reagent in the mobile phase to become electrically neutral. With heptafluorobutyric acid the increase in hydrophobic character of the ion-pair leads to a greater affinity for the stationary reversed-phase and thus, results in greater retention of cationic polar compounds in the RPLC column. Thus, ion paring in RPLC can be used to generate selective retention of cationic polar metabolites and elute the anionic polar metabolites at or near void volume.

We observed that a long column equilibration is necessary to maintain reproducible chromatographic separation in IP RPLC. Systems containing 12 mM heptafluorobutyric acid (surfactants with a C_3 side chain) required at least 25 column volumes to be well equilibrated. Superimposed HPLC/UV chromatograms from four consecutive injections are shown in Figure 6.2. All the major chromatographic peaks are perfectly superimposed in both retention times and intensities. This high reproducibility of IP RPLC ensures the integrity of the fraction collections for multiple HPLC injections. In total, 8 HPLC fractions were collected from multiple HPLC injections. The resulting fractions were then checked by HPLC/UV detection (with an analytical column developed with the same gradient), and crossover contamination between adjacent fractions was found to be minimal.

It is well known that a high ionic strength of the mobile phase, particularly many ion-pairing reagents, causes discharges and severe ion suppression at the ESI interface. [18-22] It has been reported that with commonly used volatile ion-pairing reagents, such as perfluoroheptanoic acid, ESI signal intensity decreased about 30 – 80%, compared with formic acid.[9]

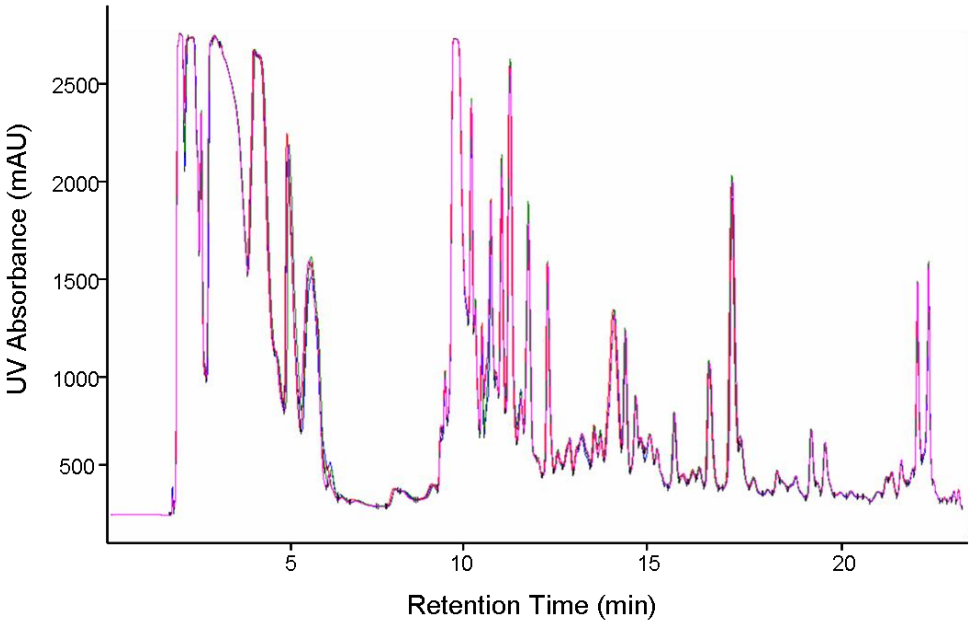

Figure 6.2 Superimposed 1st dimensional IP RPLC/UV chromatograms from four consecutive run.

This inconvenience of ion suppress on ESI from high concentrations of ion pairing reagents did not present a problem because the heptafluorobutyric acid was eluted in the RPLC void volume of our 2nd dimension of LC-MS analysis. It was still preferable to use volatile ion-pairing reagents, such as HFBA, as a non-volatile ion-pairing reagent may have formed crystals and posed a problem by contaminating the interface. The non-volatile sulfonic acids that are commonly used in LC/UV as ion-pairing reagents are not the optimal choice.

6.3.2 Dansylation Derivatization Changes the hydrophobicities of the Cationic Compounds.

In contrast to most current 2DLC separations that employed two columns with two different stationary phases with orthogonal retention mechanisms, in this study, we proposed a novel strategy that generates the orthogonality of RPLC x RPLC by altering the hydrophobicities of analytes through a chemical derivatization prior to the 2nd dimension of separation on the same RPLC column.

As the major components of cationic metabolites, amines, amino acids, and polar phenolic hydroxyl compounds were separated by IP RPLC in the 1st dimension of separation. Their hydrophobicities can be significantly altered by a simple, robust dansylation derivatization procedure. Dansylation is a well-studied derivatization chemistry that targets primary amines, secondary amines and phenolic hydroxyls.[15, 23-28] Tertiary amines and alkyl hydroxyls can not be dansylated. The large hydrophobic dansylation tag attached to the surface of the molecules changes their hydrophobicities, and thus, their retention in the 2nd dimension of RPLC. The degree to which the hydrophobicity changes is mainly dependent on the number of dansylation tag(s) added on, whilst the structures of the polar cationic compounds have less impact on their hydrophobicity and their consequent retention on the 2nd dimensional RPLC. In general, the elution order of dansylated compounds is: derivatives with 1 tag added eluted first, then derivatives with 2 tags, then derivatives with 3 tags and so on. Five to eight fractions were collected from each 1st dimension IP RPLC fractionation, allowing a high proportion of the resulting 2nd-dimension fractions to be subjected to NMR analysis for their identification.

6.3.3 Definitive Identification of ^{13}C-/^{12}C-dansylation in 2nd dimensional RPLC-FTMS.

There were in total, seven fractions collected from the 1st dimensional IP RPLC. A small percentage of the collected 1st dimension IP RPLC fractions were differentially dansylated by ^{13}C-dansyl chloride and ^{12}C-dansyl chloride, and then combined in 1:1 equal molar ratio. The dansylation experimental details have been reported previously.[15]

Two types of metabolite identification, definitive and putative (or preliminary) were carried out in 2nd dimensional RPLC-FTMS (see Figure 6.3). In total, 220 authentic standards were dansylated, analyzed by LC FTICR MS, and

included in a dansylation compound library. (See Supplementary Table S6.1 for complete list). The definitive identification was carried out to match the accurate mass, retention time, and ion pair patterns of ^{13}C-/^{12}C-dansylation ion pairs detected to ^{13}C-/^{12}C-dansylated standards. Error in mass difference was the mass error between the measured mass difference and theoretical mass difference for ^{13}C-/^{12}C-dansylation ion pairs. The theoretical mass difference for one dansylation tag (equivalent to two ^{13}C minus ^{12}C) is 2.00671. Two parts per million (2ppm) for error in mass difference was used as a key criterion to assign the ^{13}C-/^{12}C-dansylation ion pairs. High mass accuracy FTMS measurement ensures the confident assignment of true ^{13}C-/^{12}C-ion pairs. Obviously, non-reactive metabolites, background ions, instrumental and electronic noise will not have characteristic mass differences as ^{13}C-/^{12}C-ion pairs. ^{13}C-/^{12}C-dansylation ion pairs never show isotopic chromatographic separation in reversed-phase chromatography, i.e. ^{13}C-/^{12}C-ion pairs will be shown in the same spectrum. This feature would make software-based ^{13}C-/^{12}C-ion pair picking much easier than D/H ion pairs. A software program based on XCMS was written to pick up the ^{13}C-/^{12}C-ion pairs. The software program eliminated isotopic peaks, common adduct ions, multiply charged ions, and multimers. Only the protonated ion pairs were exported to an Excel table.

The ion pairs from the 7 ^{13}C-/^{12}C-dansylated IP RPLC fractions are combined into supplementary Table S6.2. In total, 3564 ^{13}C-/^{12}C-ion pairs are listed in Table S6.2. The number of ^{13}C-/^{12}C-ion pairs from the 7 fractions of 2D separation is almost three fold that of ion pairs from ^{13}C-/^{12}C-dansyltion of 1D RPLC FTMS of the same urine sample. For definitive identification, 173 ion pairs were found to match the accurate mass, retention time, and ion pair patterns of 220 authentic ^{13}C-/^{12}C-dansylated standards. The list of 173 definitively identified ^{13}C-/^{12}C-ion pairs with their retention time and accurate mass is shown in

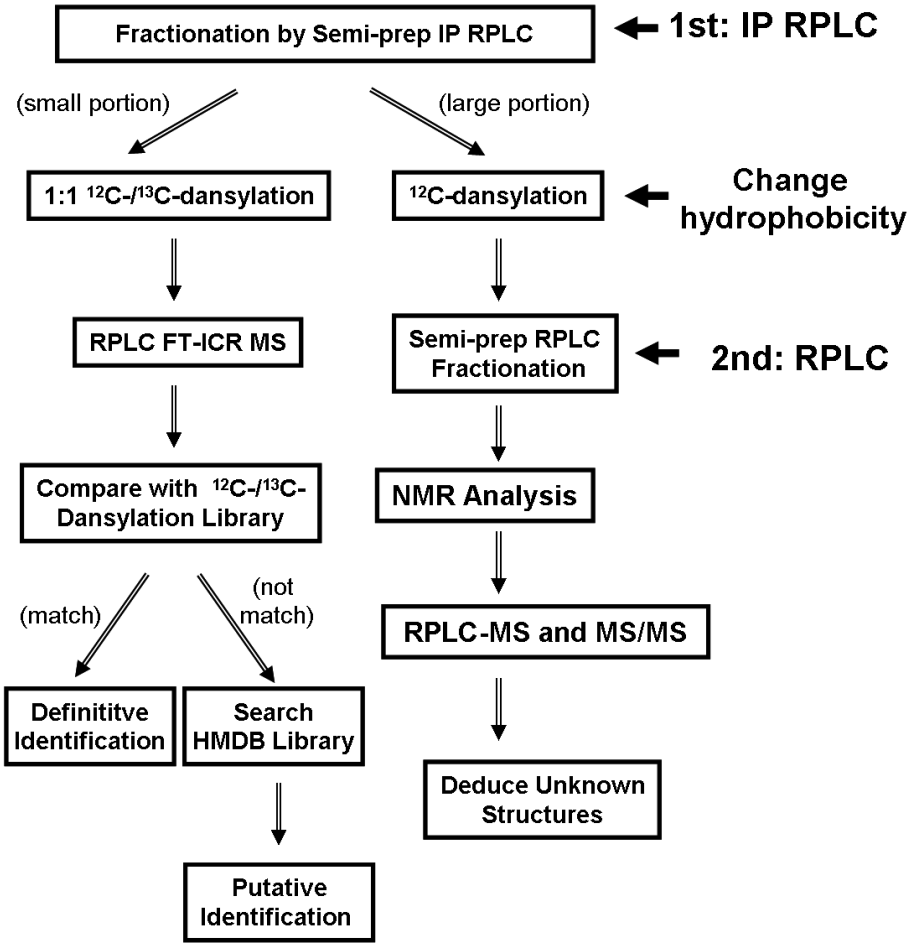

Figure 6.3 Workflow for double RPLC fractionation and ^{12}C-/^{13}C-dansylation labeling for definitive and putative identification

Table 6.1. The number of definitively identify metabolites from the 2D 7 fractions is about 2.5 times the number of metabolites definitively identified by 1D ^{13}C-/^{12}C-dansyltion of the same urine sample. It is interesting to observe that the repeated ion pairs often were in adjacent IP RPLC fractions, and this indicates the orthogonality of this 2D approach. The number of identified metabolites and ion pairs for 2D separation was obviously increased, and this is another obvious proof

of the orthogonality of the 2-dimensional separation. Even though this 2D separation is time-consuming, and labor intensive, it can be easily applied to the identification of unknown metabolites with the combination of RPLC FTICRMS and NMR analysis.

6.3.4 Putative Identification of ^{13}C-/^{12}C-dansylation RPLC/FTMS.

Identification of unknown metabolites has been one of the greatest challenges to the metabolomics community. Another potential application for this 2D approach is that it is capable of handling large volumes of sample for structural elucidation of unknown metabolites. First, the putative identification of unknown ion pairs was used to narrow down the possible metabolite structures, and then second, the structural elucidation and confirmation can be readily carried out by NMR analysis. In this report, all the ion pairs that were not matched to the ^{13}C-/^{12}C-labeled authentic standard library were used to search against the Human Metabolome Database (HMDB) for putative identification. The HMDB database search was based on matching the accurate mass of measured ions (^{12}C-dansyltion ions minus the dansyl group) against the accurate mass of 10364 human metabolites listed in the HMDB database. The search criterion for accurate mass searching was ± 3 mDa. The search result is shown in Supplemental Table S6.3.

Table 6.1 List of compound identified in 2D RPLC FTICR MS from 1:1 ^{12}C-/^{13}C-dansylated human urine sample.

(Ion pair identification is based on matching the accurate mass pairs plus the RPLC retention time against ^{12}C-/^{13}C-dansylated authentic standard LCFTMS library)

Fraction number	Compound Name	Ret. Time (min)	mz_light	mz_heavy	Int.	Error in mass diff. (ppm)
F1	Phospho-tyrosine	1.85	**495.09850**	**497.10511**	3.6E+05	**-0.19**
F 3	Hydroxylamine	2.00	**267.08652**	**269.09343**	4.3E+05	**0.76**
F 2	Hydrochlorothiazide	2.06	**519.02382**	**521.03039**	3.0E+05	**-0.27**
F 1	Phospho-serine	2.20	**419.06826**	**421.07472**	1.9E+05	**-0.59**
F 1	Phosphoethanolamine	2.23	**375.07775**	**377.08439**	4.0E+05	**-0.16**
F 1	Glucosamine	2.29	**413.13870**	**415.14522**	2.3E+06	**-0.47**
F 2	Taurine	2.65	**359.07380**	**361.08058**	5.1E+05	**-0.17**
F 1	Saccharopine	2.67	**510.19188**	**512.19806**	1.1E+06	**-1.03**
F 1	Phospho-threonine	2.79	**433.08363**	**435.08994**	2.7E+05	**-0.30**
F 1	3-methylhistidine	2.82	**403.14421**	**405.15073**	4.0E+07	**-0.45**
F 1	1-methylhistidine	2.94	**403.14418**	**405.15071**	3.4E+05	**-0.44**
F 2	Carnosine	2.96	**460.16571**	**462.17270**	7.0E+05	**0.60**
F 2	Hypotaurine	2.98	**343.07850**	**345.08496**	8.2E+05	**-0.14**
F 2	Arginine	3.24	**408.17050**	**410.17724**	3.6E+06	**0.06**
F 2	Guanidine	3.40	**293.10698**	**295.11364**	7.7E+05	**-0.17**
F 1	Asparagine	3.44	**366.11168**	**368.11832**	3.9E+07	**-0.19**
F 4	Homoarginine	3.54	**422.21118**	**424.21787**	1.2E+07	**-0.05**
F 5	Histamine	3.58	**345.13831**	**347.14520**	8.7E+04	**0.52**
F 1	Glutamine	3.89	**380.12612**	**382.13248**	1.8E+08	**-0.91**
F 1	Citrulline	4.07	**409.15477**	**411.16142**	5.8E+06	**-0.14**
F 5	1-methylhistamine	4.08	**359.15416**	**361.16066**	6.1E+06	**-0.57**
F 7	3-methylhistamine	4.14	**359.15404**	**361.16046**	3.0E+05	**-0.80**
F 2	3-sn-phosphatidylethanolamine	4.23	**484.13241**	**488.14489**	9.0E+07	**-0.97**
F 3	Aspartic acid amide	4.46	**366.10110**	**368.10787**	1.9E+06	**0.14**
F 2	Methylguanidine	4.47	**307.12258**	**309.12937**	1.1E+06	**0.28**
F 5	Homoserine	4.59	**353.11687**	**355.12366**	6.6E+06	**0.21**
F 4	Adenosine	4.59	**501.15547**	**503.16224**	7.3E+06	**0.11**
F 1	Methionine sulfoxide	4.60	**399.10505**	**401.11173**	5.2E+06	**-0.07**
F 1	Homocitrulline	4.71	**423.17063**	**425.17746**	2.3E+07	**0.28**
F 1	Serine	4.79	**339.09993**	**341.10643**	1.9E+08	**-0.62**
F 1	Glutamic acid	4.94	**381.11069**	**383.11748**	4.7E+07	**0.21**
F 1	Aspartic acid	5.01	**367.09603**	**369.10267**	2.1E+07	**-0.19**
F 4	Diglycine	5.20	**366.10114**	**368.10781**	9.4E+06	**-0.11**
F 1	4-hydroxy-proline	5.21	**365.11744**	**367.12388**	7.7E+06	**-0.74**
F 1	Aminoadipic acid	5.54	**395.12767**	**397.13443**	2.7E+07	**0.12**
F 1	Threonine	5.62	**353.11676**	**355.12357**	5.6E+07	**0.29**
F 2	Folic acid	5.76	**338.10241**	**339.10582**	4.8E+06	**0.37**
F 5	Dopamine	5.78	**387.12391**	**389.13057**	1.6E+06	**-0.12**
F 3	Iminodiacetic acid	5.87	**367.13271**	**369.13927**	1.2E+06	**-0.42**
F 4	Diethanolamine	5.88	**339.13767**	**341.14437**	4.0E+05	**-0.03**
F 4	Ethanolamine	5.98	**295.10994**	**297.11671**	8.8E+07	**0.21**
F 2	Epinephrine	6.20	**417.15936**	**419.16609**	1.3E+06	**0.05**
F 5	Glycine	6.46	**309.09057**	**311.09719**	4.0E+06	**-0.27**
F 3	Glycylproline	6.79	**406.14363**	**408.15024**	2.6E+05	**-0.26**
F 2	Beta-alanine	7.04	**323.10651**	**325.11313**	9.7E+05	**-0.28**

F 2	Tyrosine methylester	7.12	**415.13277**	**417.13994**	6.3E+06	**1.11**
F 1	Alanine	7.26	**323.10507**	**325.11134**	2.0E+08	**-1.35**
F 1	r-aminobutyric acid	7.41	**337.12161**	**339.12849**	1.6E+07	**0.50**
F 2	Aminolevulinic acid	7.50	**365.11734**	**367.12392**	2.0E+06	**-0.36**
F 3	Procaine	7.61	**470.21009**	**472.21677**	2.6E+06	**-0.06**
F 4	Pantothenic acid	7.71	**453.16968**	**455.17643**	2.2E+06	**0.10**
F 4	p-aminohippuric acid	8.05	**428.12760**	**430.13432**	2.8E+05	**0.01**
F 5	Salbutamol	8.15	**455.20013**	**457.20712**	5.7E+04	**0.61**
F 3	Hypoxanthine	8.19	**370.09733**	**372.10407**	3.5E+06	**0.09**
F 5	Isoguanine	8.29	**385.10798**	**387.11504**	1.9E+05	**0.91**
F 3	3-aminoisobutyric acid	8.37	**337.12175**	**339.12815**	2.0E+07	**-0.90**
F 2	5-hydroxylmethyluracil	8.43	**376.09617**	**378.10266**	2.5E+06	**-0.58**
F 4	5-aminopentanoic acid	8.49	**351.13765**	**353.14433**	8.1E+06	**-0.09**
F 1	2-minoisobutyric acid	8.69	**337.12280**	**339.12954**	3.9E+06	**0.10**
F 1	2-aminobutyric acid	9.00	**337.12226**	**339.12907**	8.2E+06	**0.29**
F 1	Sarcosine	9.11	**323.10673**	**325.11332**	1.2E+07	**-0.37**
F 3	Pyridoxine	9.29	**403.13269**	**405.13930**	6.9E+05	**0.25**
F 1	Proline	9.82	**349.12201**	**351.12862**	3.4E+06	**-0.28**
F 6	Methylamine	9.83	**265.09967**	**267.10617**	4.9E+07	**-0.77**
F 7	Methylamine	9.84	**265.09989**	**268.10943**	9.6E+06	**-1.31**
F 2	Methylamine	9.85	**265.09944**	**267.10621**	3.7E+07	**0.22**
F 5	Methylamine	9.85	**265.09975**	**267.10614**	2.3E+07	**-1.18**
F 1	Methylamine	9.90	**265.09952**	**267.10611**	5.8E+07	**-0.45**
F 6	Aminocaproic acid	9.91	**365.15129**	**367.15790**	6.7E+06	**-0.28**
F 4	Methylamine	10.12	**265.09932**	**267.10621**	4.9E+07	**0.64**
F 3	Methylamine	10.14	**265.09940**	**267.10628**	4.0E+07	**0.62**
F 4	Valine	10.47	**351.13794**	**353.14471**	2.1E+06	**0.16**
F 5	Salicyluric acid	10.49	**429.11198**	**431.11884**	8.9E+06	**0.36**
F 5	Methionine	10.53	**383.10981**	**385.11632**	1.2E+07	**-0.50**
F 4	3-hydroxyl-picolinic acid	10.55	**373.08521**	**375.09218**	2.9E+06	**0.70**
F 1	Gly-Trp	10.58	**495.16289**	**497.17055**	6.9E+05	**1.92**
F 1	3-nitrotyrosine	10.88	**460.14359**	**462.14961**	1.3E+06	**-1.49**
F 6	Tryptophan	10.96	**438.14630**	**440.15292**	1.4E+08	**-0.21**
F 5	Kynurenine	11.02	**442.14357**	**444.15054**	1.2E+07	**0.59**
F 4	Norvaline	11.08	**351.13518**	**353.14206**	2.3E+06	**0.48**
F 4	Phenylephrine	11.24	**401.15460**	**403.16140**	1.5E+06	**0.22**
F 6	2-phenylglycine	11.35	**385.12168**	**387.12821**	1.6E+05	**-0.44**
F 4	3-aminobenzoic acid	11.51	**371.10659**	**373.11282**	2.3E+06	**-1.29**
F 3	3-aminosalicylic acid	11.91	**387.10076**	**389.10779**	9.8E+05	**0.82**
F 4	Ethylamine	11.97	**279.11564**	**281.12227**	3.7E+07	**-0.31**
F 7	Ethylamine	11.97	**279.11646**	**281.12299**	3.1E+06	**-0.64**
F 2	Ethylamine	11.97	**279.11627**	**281.12288**	1.4E+07	**-0.35**
F 3	Ethylamine	11.98	**279.11610**	**281.12258**	2.4E+07	**-0.83**
F 1	Ethylamine	11.98	**279.11657**	**281.12298**	2.0E+07	**-0.35**
F 6	Ethylamine	12.00	**279.11638**	**281.12298**	5.3E+06	**-0.40**
F 5	Diaminopimelic acid	12.04	**329.10612**	**331.11289**	8.5E+04	**0.19**
F 1	Vanillylmandelic acid	12.09	**432.10865**	**434.11528**	6.0E+07	**-0.18**
F 1	Pipecolic acid	12.16	**363.13813**	**365.14478**	2.2E+06	**-0.17**
F 5	Phenylalanine	12.18	**399.13559**	**401.14161**	1.9E+08	**-1.74**
F 1	Hydroxyphenylacetylglycine	12.20	**443.12738**	**445.13442**	4.0E+06	**0.75**
F 1	Acetyl-tyrosine	12.24	**457.14041**	**459.14719**	4.9E+06	**0.14**

F 5	Leu-Pro	12.37	**462.20605**	**464.21279**	8.5E+05	**0.05**
F 1	3-hydroxymandelic acid	12.43	**402.09929**	**404.10577**	4.1E+07	**-0.55**
F 2	Isoleucine	12.56	**365.15332**	**367.16006**	1.5E+07	**0.09**
F 1	L-cystathionine	12.73	**345.09150**	**347.09822**	1.6E+07	**0.03**
F 2	Leucine	12.79	**365.15168**	**367.15834**	8.6E+07	**-0.16**
F 1	5-hydroxylysine	12.88	**315.10829**	**317.11499**	1.1E+07	**-0.02**
F 1	Cystine	13.10	**354.06996**	**356.07658**	1.1E+08	**-0.26**
F 3	Norlecine	13.23	**365.15131**	**367.15807**	6.0E+06	**0.14**
F 6	4-hydroxy-3-methoxyphenyllactic acid	13.25	**446.12802**	**448.13435**	8.5E+04	**-0.84**
F 2	Phenylethanolamine	13.43	**371.14071**	**373.14711**	2.3E+06	**-0.81**
F 1	Hydroxyphenyllactic acid	13.60	**416.11648**	**418.12343**	2.5E+07	**0.55**
F 1	5-hydroxyindoleacetic acid	14.16	**425.11460**	**427.12148**	5.2E+07	**0.41**
F 4	Dimethylamine	14.35	**279.11542**	**281.12189**	6.0E+07	**-0.83**
F 2	Dimethylamine	14.36	**279.11551**	**281.12212**	6.1E+07	**-0.34**
F 5	Dimethylamine	14.39	**279.11567**	**281.12239**	4.7E+07	**0.03**
F 6	Dimethylamine	14.41	**279.11576**	**281.12262**	4.4E+07	**0.51**
F 7	Dimethylamine	14.41	**279.11586**	**281.12258**	2.8E+07	**0.03**
F 4	2,4-Diaminobutyric acid	14.73	**293.13197**	**295.13865**	3.3E+05	**-0.11**
F 3	Homocystine	14.75	**368.08662**	**370.09333**	1.2E+06	**-0.01**
F 2	Salicylic acid	14.78	**372.09052**	**374.09743**	2.2E+06	**0.54**
F 5	Ornithine	15.38	**300.10355**	**302.11006**	8.8E+06	**-0.65**
F 4	Methyl-phenylalanine	15.47	**413.15412**	**415.16044**	1.1E+07	**-0.94**
F 2	5-Methoxysalicylic acid	15.52	**402.10120**	**404.10802**	1.4E+07	**0.28**
F 2	3-/4-hydroxyphenylacetic acid	15.56	**386.10557**	**388.11289**	1.8E+07	**1.57**
F 5	Homovanillic	15.61	**416.11647**	**418.12342**	6.5E+06	**0.57**
F 5	5-Methoxytryptamine	15.72	**424.16861**	**426.17514**	4.8E+04	**-0.42**
F 2	Syringic acid	15.79	**432.11179**	**434.11864**	6.8E+06	**0.33**
F 2	Homocarnosine	15.94	**354.11996**	**356.12666**	6.6E+06	**-0.04**
F 4	3-Cresotinic acid	16.00	**386.10573**	**388.11240**	1.4E+07	**-0.10**
F 1	Carnosine	16.12	**347.11216**	**349.11885**	3.6E+07	**-0.07**
F 1	Gentisic acid	16.34	**388.08555**	**390.09251**	1.9E+07	**0.64**
F 2	Lysine	16.36	**307.11116**	**309.11755**	1.8E+07	**-1.06**
F 3	3-hydroxybenzoic acid	16.43	**372.09043**	**374.09738**	5.2E+05	**0.63**
F 1	Vanillic acid	16.45	**402.09958**	**404.10611**	5.3E+07	**-0.44**
F 5	Isoferulic acid	16.49	**428.11671**	**430.12332**	2.9E+06	**-0.25**
F 1	4-hydroxybenzoic acid	16.64	**372.08897**	**374.09539**	8.6E+07	**-0.78**
F 4	Aniline	16.70	**327.11227**	**329.11889**	1.5E+07	**-0.28**
F 1	Histidine	16.83	**389.12626**	**391.13290**	1.6E+08	**-0.19**
F 4	Desaminotyrosine	16.93	**400.12180**	**402.12838**	2.5E+07	**-0.33**
F 4	3-hydroxyanthranilic acid	17.04	**387.10138**	**389.10870**	2.5E+05	**1.58**
F 5	Benzylamine	17.20	**341.13229**	**343.13861**	9.4E+04	**-1.14**
F 6	Tryptamine	17.21	**394.15870**	**396.16539**	5.4E+05	**-0.06**
F 4	m-coumaric acid	17.32	**398.10629**	**400.11287**	2.2E+07	**-0.33**
F 4	trans-ferulic acid	17.36	**428.11658**	**430.12359**	1.0E+07	**0.71**
F 4	Ephedrine	17.66	**399.17219**	**401.17883**	1.4E+07	**-0.17**
F 7	2-aminooctanoic acid	18.01	**393.18462**	**395.19131**	1.8E+06	**-0.06**
F 5	Pyridoxamine	18.21	**318.10386**	**320.11020**	9.7E+04	**-1.14**

F 5	5-hydroxyltryptophan	18.70	**344.10065**	**346.10742**	5.3E+05	**0.18**
F 5	1,3-diaminopropane	19.07	**271.10059**	**273.10730**	9.2E+05	**-0.01**
F 2	Tyrosinamide	19.15	**324.10352**	**326.10967**	1.5E+07	**-1.72**
F 5	1,2-diaminopropane	19.46	**271.10037**	**273.10703**	4.6E+05	**-0.17**
F 4	1,4-diaminobutane	19.59	**555.21079**	**559.22317**	4.1E+05	**-0.93**
F 7	o-tyrosine	20.01	**324.59549**	**326.60218**	7.3E+06	**-0.06**
F 3	Thyroxine	20.10	**505.87519**	**506.87854**	3.7E+05	**-0.03**
F 4	3-nitrotyrosine	20.17	**347.10096**	**349.10759**	2.4E+06	**-0.23**
F 5	Cadaverine	20.25	**285.11627**	**287.12306**	5.4E+05	**0.29**
F 2	Tyrosine	20.28	**324.59433**	**326.60092**	2.0E+08	**-0.37**
F 3	Metoprolol	20.34	**501.16260**	**503.17004**	3.4E+06	**1.47**
F 5	Phenol	20.75	**328.10051**	**330.10720**	9.2E+05	**-0.06**
F 5	4-nitrophenol	20.79	**373.08584**	**375.09232**	6.4E+05	**-0.60**
F 2	Cysteamine	20.86	**310.07912**	**312.08674**	6.0E+06	**2.93**
F 5	16b-hydroxyestradiol	20.87	**522.23212**	**524.23907**	7.7E+04	**0.47**
F 5	4,9-dioxa-1,12-dodecanediamine	21.12	**336.15063**	**338.15732**	3.1E+06	**-0.06**
F 1	Octopamine	21.33	**310.58044**	**312.58688**	3.9E+06	**-0.88**
F 3	p-Cresol	21.44	**342.11627**	**344.12306**	2.9E+06	**0.22**
F 7	Protocatechuic acid	21.48	**311.07187**	**313.07863**	4.2E+06	**0.16**
F 5	Gentisic acid	21.50	**311.07180**	**313.07861**	9.9E+05	**0.34**
F 3	o-Cresol	21.53	**342.11485**	**344.12128**	1.3E+08	**-0.81**
F 5	Serotonin	21.54	**322.10629**	**324.11287**	3.2E+05	**-0.42**
F 3	Caffeic acid	21.73	**647.27973**	**649.28652**	4.9E+05	**0.12**
F 4	Metanephrine	21.77	**332.61143**	**334.61808**	1.8E+06	**-0.17**
F 5	Piperazine	21.90	**277.10072**	**279.10737**	1.5E+06	**-0.20**
F 6	Thyronine	21.93	**370.60903**	**372.61599**	2.5E+05	**0.68**
F 4	Phenylephrine/Synephrine	21.96	**317.60605**	**319.61277**	1.5E+06	**0.02**
F 5	Tyramine	22.36	**302.60006**	**304.60681**	2.2E+07	**0.11**
F 6	Spermidine	22.86	**423.16346**	**426.17361**	1.4E+06	**0.14**
F 2	Xanthurenic acid	22.90	**336.57758**	**338.58441**	4.3E+06	**0.36**
F 5	Estradiol	23.04	**506.23675**	**508.24367**	1.3E+06	**0.41**
F 5	3-isopropylphenol	23.12	**370.14743**	**372.15418**	1.9E+07	**0.11**
F 4	Pyrocatechol	23.30	**577.14757**	**581.16065**	2.2E+05	**-0.29**
F 6	Estrone	23.69	**504.22064**	**506.22769**	1.5E+05	**0.68**
F 3	Norepinephrine	23.82	**435.12180**	**438.13167**	9.5E+05	**-0.30**
F 5	Thymol	24.07	**384.16337**	**386.17005**	7.5E+06	**-0.08**
F 4	Hydroquinone	24.17	**577.14805**	**581.16125**	1.0E+05	**-0.18**
F 5	Deoxyepinephrine	24.22	**317.09023**	**319.09701**	1.1E+06	**0.22**
F 5	Desipramine	24.29	**500.23795**	**502.24421**	8.5E+04	**-0.89**

For the 3381 unique ion pairs in Table S6.3, 53% of pairs do not match with any metabolites in HMDB database, 47% of pairs match with one or more putative metabolite, just based on their accurate mass, and about 18% of pairs match to more than one putative metabolite. In the case of multiple chemical formula

results, isotopic spectrum pattern matching can be used for confirmation of the formula assignments.

6.4 Conclusions

The double reversed-phase fractionation strategy presented here offers a novel strategy to comprehensively identify large numbers of amine, amino acid, and phenolic hydroxyl compounds in a complex biological sample. Cationic species in urine were successfully separated and fractionated by ion-pairing semi-preparative RPLC. The highly reproducible IP RPLC runs ensured the integrity of fractionation from multiple injections. Most cationic compounds presented in urine are amines and amino acids which can be easily derivatized by dansyl chloride. The RP chromatographic retention behavior of polar amines, amino acids, and hydroxyl phenols were altered to an extent after dansylation derivatization such that they can be well retained and separated with high efficiency in a 2nd RP fractionation. In total, 3564 ^{13}C-/^{12}C-ion pairs were detected in double reversed-phased 1:1 ^{13}C-/^{12}C-dansylated fractions. In definitive identification, a total of 173 ^{13}C-/^{12}C-ion pairs were matched with their retention time, accurate mass pair to a dansylation standard compound library consisting of 220 known amines and phenols. For the non-matched 3381 ion pairs, an HMDB database containing 10364 human metabolites was used to match the accurate mass of measured ions (^{12}C-dansylted ions minus the mass of dansyl group) to achieve putative (or preliminary) identification. 1588 pairs matched with one or more putative metabolite.

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Chapter 7

Conclusions and Future Work

Liquid chromatography followed by mass spectrometry (LC-MS) has become the workhorses of metabolome analysis, largely due to its widespread availability, reasonable cost, and the compatibility of reversed-phase (RP) separations with biological samples. An ideal LC-MS analytical platform for metabolomics would offer accurate quantification and confident identification of all metabolites present in a biological sample. However, such ideal LC-MS platforms are not achievable with the current state of development in mass spectrometry. A large proportion of metabolites are highly polar compounds whose separation and detection by RPLC MS remains very challenging. Because the ionization efficiency of most metabolites is quite low, their ESI-MS detection sensitivity is poor. In contrast to the well-established, easily searchable protein and gene databases, currently there is no such comprehensive, searchable metabolome database available. Confident confirmation of known metabolites and identification of unknown metabolites remain a time-consuming and a major bottleneck in current metabolomics. LC-MS-based metabolomic experiments generate large and complex datasets. The large number of MS spectra due to chemical noise, instrument noise and contamination become hard to differentiate from low abundance/and or poor ESI-ionizable metabolites. One of the current challenges is the lack of reliable software tools to enable automated data processing and organizing into data matrices for further statistical interpretation and visualization. This Ph.D. research addressed these challenges by developing

a non-traditional, differential isotope labeling (DIL) strategy for a high-throughput LC-MS analysis of human metabolome. Stable isotope labeled internal standards (SIL) has been proved to be the most effective method to compensate ion suppression and matrix effects, and offers the most accurate quantification results for LC-MS analysis. Obviously, it is not practical to purchase all the SIL standards for every metabolite in metabolomic studies. A DIL approach provides global internal standards for every targeted analyte (with minimum cost) and thus, it can potentially be applied to many other LC-MS or LC-MS/MS applications.

In Chapter 1, an overview of metabolomics, chemical derivatization and several key technologies related to qualitative analysis and quantitative analysis of metabolomes by LC-MS are included.

In Chapter 2, the application and development of a novel stable isotope ^{13}C-/^{12}C-dimethylation of amine-containing metabolites is described. This work is regarded as one of the first DIL approaches to be applied to small molecule metabolites. A ^{13}C-/^{12}C-dimethylation reaction was used to introduce a stable isotope tag onto the targeted amines and amino acids. Unlike most other deuterium DIL approaches, ^{13}C-formaldehyde/^{12}C-formaldehyde was used as labeling reagents. Thus, the isotope effect was never observed on RPLC. This ^{13}C-/^{12}C-SIL approach was proven to be effective to compensate matrix effects or ion suppression in both relative and absolute quantification in a complicated biological sample, human urine. Both heavy and light labeling reagent, ^{13}C-formaldehyde and ^{12}C-formaldehyde are commercially available. The simple and rapid dimethylation reaction was carried out under mild conditions. An additional benefit of the ^{13}C-

/^{12}C-SIL approach is that the characteristic mass difference between ^{13}C- and ^{12}C-dimethylated amine and amino acids provides additional structural information, and facilitate the spectral peak pair and compound identification. However, as the increases in hydrophobicity of derivatives are minimal, hydrophilic interaction chromatography (HILIC) has to be carried out to separate the polar derivatives.

In Chapter 3, a global internal standard technique for quantitative metabolome analysis by differential ^{13}C-/^{12}C-dansylation is described. ^{13}C-dansyl chloride was easily synthesized. The simple and robust labeling reaction was proceeded in high yield. 1-3 orders of magnitude RPLC-MS signal enhancement over the non-dansylated metabolites was observed. Dansylation increased the hydrophobicity of polar and ionic amino acids and amines, allowing most of them to be retained and separated by RPLC. 121 authentic standards of amino acids, amines and phenols were included in a dansylation library. As an example, the absolute quantification of 93 metabolites from a pooled human urine sample, and relative quantification of 5 individual urine samples, stored for two weeks at -20 ^{o}C or -80 ^{o}C, were carried out. A reverse and forwarding ^{13}C-/^{12}C-dansylation experiment proved the integrity of the dansylation labeling reaction. There was no isotopic effect on RPLC separation; the ^{13}C-/^{12}C-isoforms co-eluted perfectly and were shown in one spectrum. In the ^{13}C-/^{12}C-SIL approach, the isotopic ratio in a ^{13}C-/^{12}C spectrum was used for quantification, permitting a single spectrum across the chromatographic peak to be used for quantification and identification. The requirement of having a minimum of ten data points to construct a chromatographic peak is not necessary for the ^{13}C-/^{12}C-SIL approach. This

characteristic makes fast LCFTMS feasible, even though FTMS (Apex-Qe FTMS) is a relative slow response MS, and is also critical for software-based spectra interpretation and compound identification.

In Chapter 4, a software-based definitive identification and putative (preliminary) identification is described. Confirmation of known metabolites against a comprehensive authentic standard library and structural elucidation of unknown metabolites has been a major bottleneck in current metabolomic study. In this study, ^{13}C-/^{12}C-dansylated human cerebrospinal fluids (CSF) were analyzed by RPLC FTMS. About 14,000 ion features could be detected in a 25 min LCFTMS run. The LC FTMS data (or batch data) were then analyzed by an open source XCMS software for ^{13}C-/^{12}C-ion pairs picking. About 5-fold data reduction has been achieved. Most ion features due to non-reactive species, impurities, chemical and instrument noise were effectively removed by the ion pair picking process. For definitive identification, the ion pairs were used to match against an in-house ^{13}C-/^{12}C-dansylation library containing 220 authentic standards by a Visual Basic Applications (VBA) micro. A total of 85 metabolites could be positively identified, of which 21 have never been reported in human CSF. For putative identification, the non-matched ion pairs were used to match against the Human Metabolome Database (HMDB). 529 pairs matched with at least one metabolite. The process of ion pair picking and database searching can be applied to either single or batch LC-MS data. The quantitative data output can be readily used to enable further multivariate statistics analysis for biomarker discovery.

In Chapter 5, a novel, high performance stable isotope ^{13}C-/^{12}C-labeling chemistry that targeted carboxylic acids is described. The labeling reagent is ^{13}C-/^{12}C-p-dimethylaminophenacyl (DmPA) bromide. A US patent application based on this chemistry has been filed. The DmPA chemistry, derived from phenacyl bromide derivatization that is the most commonly used labeling chemistry for carboxylic and fatty acids, was followed by LC/UV detection. The dimethylamino group was purposely added to improve the chargeability and ESI sensitivity in positive mode MS detection. ^{13}C-/^{12}C-DmPA labeling of carboxylic acid can be used for accurate (absolute and relative) quantification, and definitive identification. The labeling chemistry was proved to improve the RPLC retention and enhance ESI efficiency of carboxylic acids by 2 to 4 orders of magnitude. A library of 113 ^{13}C-/^{12}C-DmPA labeled authentic standards has been constructed. The ion pairs detected by LC FTMS were picked up by XCMS software and searched against the ^{13}C-/^{12}C-DmPA labeled library for definitive identification.

A conventional 2-dimensional LC separation contains two orthogonal LC separation mechanisms to maximize chromatographic peak capacities. In Chapter 6, a novel 2-dimensional LC strategy is described. 2 x RPLC separation was carried out on the same C18 RPLC column. The first dimension RPLC was an ion-pair RPLC separation for polar cationic compounds. The hydrophobicity of the analytes was then altered by dansylation derivatization prior to the 2nd dimension RPLC separation, rather than by choosing a different stationary phase for each retention mechanism. The retention by the 2nd dimension was largely based on the number of dansylation tags added. To our knowledge, this is the first

report of applying such a strategy for 2D RPLC in metabolome analysis. 173 ^{13}C-/^{12}C-dansylated ion pairs were matched in the ^{13}C-/^{12}C-dansylation library with their accurate mass pairs and retention times. 3564 ^{13}C-/^{12}C-ion pairs were picked up by XCMS software, representing about a three fold increase in the number of ion pairs detected, compared to one dimensional RPLC of 1:1 ^{13}C-/^{12}C-dansylated human urine.

Structural elucidation of unknown metabolites has been the greatest challenge in metabolomic studies. Combinations of NMR, HPLC-FTMS and QTOF-MS/MS with accurate mass, MS/MS, and isotopic pattern information would be the most powerful solution for unambiguous structural elucidation of unknowns. The novel 2D approach described in Chapter 6 was designed to handle large sample volumes that potentially permit its fractions to be submitted to NMR structural elucidation of unknowns.

Identification of metabolites employs a range of mass spectral techniques. MS/MS spectra can be used to confirm known metabolites and facilitate unknown identification. In these studies, tertiary instead of quaternary amines were chosen as labeling agents because both tagged and parent compounds show in MS/MS spectra for the dansylated and DmPA labeled standards, respectively. The most dominant MS/MS spectra for quaternary amines come from the labeled tag with a permanent charge, and often the fragment information from parent compounds is missing. Libraries of MS/MS spectra of dansylated and DmPA standards could be easily constructed to facilitate metabolite identification in the future. When comprehensive libraries of MS/MS, MS, and retention time of dansylated and of

DmPA standards are constructed, the routine quantification of large sets of biological samples could be carried out by high-throughput RPLC followed by triple quadrupole MS to obtain the optimal sensitivity and dynamic range. The massive LC-MS data set would be analyzed by non-supervised Principal Component Analysis (PCA) or supervised analysis techniques, such as Projection to Latent Discriminant Structures Analysis (also called Partial Least-Squares Discriminant Analysis) (PLS-DA) or Orthogonal Projection on Latent Structure Discriminant Analysis (O-PLS-DA).

Dansylation and DmPA tags have strong UV absorbance and the dansyl tag is a good fluorescence emitter. UV and fluorescence detectors could be used as adjuncts with the MS detector. The dansyl tag has strong UV absorbance around 350-360 nm, which matches with the UV Nd:YAG laser (355 nm) found in most commercial MALDI-TOF MS equipment. Another potential application would be the RPLC/MALDI of ^{13}C-/^{12}C-dansylated small molecules and digested (polar) peptides.

The DIL strategy is a global internal technique that would not be limited to only metabolomic applications. The DIL approach can be applied to many other LC-MS applications, such as food, environmental, pharmaceutical, and petroleum analyses.

The driving force of this research was to develop novel approaches to overcome or compensate current limitations of LC-MS quantification and identification. The novel SIL approach developed in this thesis offers a potential solution for the problems associated with ion suppression, matrix effect, and the

catastrophic failure in RPLC separation and detection of highly polar compounds in complex mixtures.

Appendix

Table S2.1. Ion pairs of amine-containing metabolites detected and identified by RP and HILIC LC-ESI FT-ICR-MS from a mixture of 1:1 human urine samples labeled by ^{12}C- and ^{13}C-formaldehyde, respectively. 33 metabolites were identified on the basis of accurate mass and retention time comparison with the labeled standards. 16 of them were found in both RP and HILIC LC-MS runs, 2 were found in RP LC-MS alone and 15 were found in HILIC LC-MS alone.

	RT (min)	^{12}C-labeled (m/z)	^{13}C-labeled (m/z)	Identity	Sep. Mode	Mass Difference	Error (ppm) in Mass Difference
1	16.52	194.11755	196.12424	Phenylalanine	RPLC	2.00669	-0.10
	10.07	194.11756	196.12426	Phenylalanine	HILIC	2.00670	-0.05
2	22.35	233.12862	235.13530	Tryptophan	RPLC	2.00668	-0.13
	8.91	233.12860	235.13529	Tryptophan	HILIC	2.00669	-0.08
3	2.82	130.08691	131.09031	Proline	RPLC	1.00340	0.34
	24.98	130.08625	131.08949	Proline	HILIC	1.00324	-0.88
4	3.87	146.11761	148.12432	Valline	RPLC	2.00671	0.00
	19.63	146.11753	148.12422	Valline	HILIC	2.00669	-0.13
5	6.56	160.13323	162.13991	Isoleucine	RPLC	2.00668	-0.18
	13.91	160.13316	162.13987	Isoleucine	HILIC	2.00671	0.00
6	7.96	160.13323	162.13992	Leucine	RPLC	2.00669	-0.12
	12.01	160.13317	162.13988	Leucine	HILIC	2.00671	0.00
7	4.63	178.08963	180.09635	Methionine	RPLC	2.00672	0.06
	12.93	178.08959	180.09629	Methionine	HILIC	2.00670	-0.05
8	2.60	203.05259	207.06612	Lysine	RPLC	4.01353	0.53
9	3.92	203.13906	205.14577	Arginine	RPLC	2.00671	0.00
	24.63	203.13953	205.14620	Arginine	HILIC	2.00667	-0.19
10	6.21	210.11249	212.11920	Tyrosine	RPLC	2.00671	0.00
	14.79	210.11241	212.11913	Tyrosine	HILIC	2.00672	0.05
11	29.21	104.07061	106.07730	Glycine	HILIC	2.00669	-0.19
12	25.96	118.08623	120.09293	Alanine	HILIC	2.00670	-0.08
13	29.70	134.08117	136.08785	Serine	HILIC	2.00668	-0.22
14	25.12	148.09680	150.10351	Threonine	HILIC	2.00671	0.00
15	19.85	150.05863	152.06540	Cycteine	HILIC	2.00677	0.40
16	35.19	161.09196	163.09867	Asparagine	HILIC	2.00671	0.00
17	18.66	162.13988	164.14656	Aspartic acid	HILIC	2.00668	-0.18
18	35.99	175.10765	177.11435	Glutamine	HILIC	2.00670	-0.05
19	49.85	176.09309	178.09975	Glutamic acid	HILIC	2.00666	-0.28
20	45.30	184.10803	186.11472	Histidine	HILIC	2.00669	-0.11
21	7.89	136.18652	138.19318	Benzylamine	RPLC	2.00666	-0.36
22	11.18	180.10186	181.10519	1-Ephedrine	RPLC	1.00333	-0.14
	7.01	180.10247	181.10578	1-Ephedrine	HILIC	1.00331	-0.25
23	31.41	223.13028	225.13706	p-Aminohippuric acid	RPLC	2.00678	0.31
	6.81	223.13065	225.13737	p-Aminohippuric	HILIC	2.00672	0.05

				acid			
24	6.92	166.12260	168.12931	Tyramine	RPLC	2.00671	0.00
	13.11	166.12261	168.12929	Tyramine	HILIC	2.00668	-0.18
25	27.68	132.10188	134.10860	r-aminobutyric acid	HILIC	2.00661	-0.60
	3.11	132.10192	134.10863	r-aminobutyric acid	RPLC	2.00671	0.00
26	15.06	165.05456	167.06117	Histamine	HILIC	2.00661	-0.60
27	14.04	146.11753	147.12091	L-4-hydroxyproline	HILIC	1.00338	0.17
28	19.44	106.07658	108.08328	Cysteamine	HILIC	2.00670	-0.09
29	44.15	156.16720	159.17732	1,4-diaminobutane	HILIC	3.01012	0.35
30	3.39	198.03735	199.04062	(-)-Epinephrine	RPLC	1.00327	-0.43
	18.27	198.03739	199.04064	(-)-Epinephrine	HILIC	1.00325	-0.53
31	2.64	197.08965	199.09637	Pyridoxamine	RPLC	2.00672	0.05
	36.12	197.08958	199.09631	Pyridoxamine	HILIC	2.00673	0.10
32	6.34	182.11764	184.12434	Dopamine	RPLC	2.00670	-0.05
	15.90	182.11758	184.12440	Dopamine	HILIC	2.00682	0.60
33	45.04	198.12360	200.13035	3-methyl-histindine	HILIC	2.00675	0.20

Table S2.2. Ion pairs of amine-containing metabolites detected by RP LC-ESI FT-ICR-MS from a mixture of 1:1 human urine labeled by ^{12}C- and ^{13}C-formaldehyde, respectively. 440 ion pairs were detected, of which only 18 were positively identified and listed in Table S2.1, and 420 are yet to be identified. Their retention times and measured masses are listed below.

	RT (min)	^{12}C-labeled (m/z)	^{13}C-labeled (m/z)	Identity	Sep. Mode	Mass Difference	Error (ppm) in Mass Difference
1	2.60	385.13240	387.13898	Unknown	RPLC	2.00658	-0.33
2	2.60	405.09767	407.10396	Unknown	RPLC	2.00629	-1.03
3	2.60	230.06353	233.07385	Unknown	RPLC	3.01032	1.10
4	2.68	175.10770	177.11435	Unknown	RPLC	2.00665	-0.34
5	2.68	184.10804	186.11466	Unknown	RPLC	2.00662	-0.48
6	2.68	187.05772	189.06441	Unknown	RPLC	2.00669	-0.10
7	2.68	263.08498	265.09158	Unknown	RPLC	2.00660	-0.41
8	2.73	160.19798	163.20802	Unknown	RPLC	3.01004	-0.15
9	2.73	138.10260	140.10927	Unknown	RPLC	2.00667	-0.28
10	2.73	222.06384	224.07026	Unknown	RPLC	2.00642	-1.29
11	2.73	266.07819	268.08465	Unknown	RPLC	2.00646	-0.93
12	2.78	203.05235	205.05923	Unknown	RPLC	2.00688	0.83
13	2.78	206.08990	208.09665	Unknown	RPLC	2.00675	0.19
14	2.82	198.12362	200.13028	Unknown	RPLC	2.00666	-0.25
15	2.82	235.12865	237.13546	Unknown	RPLC	2.00681	0.42
16	2.82	257.11072	259.11720	Unknown	RPLC	2.00648	-0.89
17	2.82	385.18185	386.18486	Unknown	RPLC	1.00301	-0.89
18	2.86	118.08624	120.09298	Unknown	RPLC	2.00674	0.25

19	2.86	158.11750	160.12421	Unknown	RPLC	2.00671	0.00
20	2.86	203.17538	207.18878	Unknown	RPLC	4.01340	-0.09
21	2.91	297.10276	301.11611	Unknown	RPLC	4.01335	-0.23
22	3.00	132.10198	134.10866	Unknown	RPLC	2.00668	-0.22
23	3.00	143.11794	145.12467	Unknown	RPLC	2.00673	0.14
24	3.00	162.11249	164.11917	Unknown	RPLC	2.00668	-0.18
25	3.00	190.10743	192.11410	Unknown	RPLC	2.00667	-0.21
26	3.00	228.17068	232.18413	Unknown	RPLC	4.01345	0.13
27	3.00	243.13402	244.13712	Unknown	RPLC	1.00310	-1.04
28	3.00	289.13951	291.14619	Unknown	RPLC	2.00668	-0.10
29	3.00	299.17121	300.17515	Unknown	RPLC	1.00394	1.95
30	3.00	304.18683	307.19685	Unknown	RPLC	3.01002	-0.14
31	3.00	318.20239	322.21579	Unknown	RPLC	4.01340	-0.06
32	3.00	330.16617	332.17250	Unknown	RPLC	2.00633	-1.14
33	3.08	154.08384	156.09043	Unknown	RPLC	2.00659	-0.77
34	3.08	**231.18162**	**233.18808**	Unknown	RPLC	2.00646	-1.07
35	3.08	292.15064	294.15710	Unknown	RPLC	2.00646	-0.85
36	3.08	329.15315	333.16644	Unknown	RPLC	4.01329	-0.39
37	3.13	311.10953	315.12289	Unknown	RPLC	4.01336	-0.19
38	3.13	322.16209	324.16867	Unknown	RPLC	2.00658	-0.40
39	3.13	346.16116	348.16761	Unknown	RPLC	2.00645	-0.75
40	3.17	217.15467	219.16142	Unknown	RPLC	2.00675	0.18
41	3.17	233.11327	235.12002	Unknown	RPLC	2.00675	0.17
42	3.17	266.06930	268.07614	Unknown	RPLC	2.00684	0.49
43	3.17	286.07773	288.08441	Unknown	RPLC	2.00668	-0.10
44	3.17	291.11879	293.12552	Unknown	RPLC	2.00673	0.07
45	3.17	306.16608	308.17294	Unknown	RPLC	2.00686	0.49
46	3.17	320.14464	322.15165	Unknown	RPLC	2.00701	0.93
47	3.17	336.15919	340.17217	Unknown	RPLC	4.01298	-1.29
48	3.17	354.11520	358.12824	Unknown	RPLC	4.01304	-1.06
49	3.22	267.05881	269.06537	Unknown	RPLC	2.00656	-0.56
50	3.35	201.12341	203.13025	Unknown	RPLC	2.00684	0.64
51	3.35	231.18162	233.18834	Unknown	RPLC	2.00672	0.04
52	3.35	252.08992	254.09649	Unknown	RPLC	2.00657	-0.55
53	3.35	257.16084	259.16762	Unknown	RPLC	2.00678	0.27
54	3.35	263.12385	265.13050	Unknown	RPLC	2.00665	-0.23
55	3.35	278.06938	280.07609	Unknown	RPLC	2.00671	0.00
56	3.35	**287.17146**	**289.17825**	Unknown	RPLC	2.00679	0.28
57	3.35	300.05135	302.05780	Unknown	RPLC	2.00645	-0.86
58	3.35	329.15317	333.16641	Unknown	RPLC	4.01324	-0.54
59	3.43	159.07655	161.08320	Unknown	RPLC	2.00665	-0.37
60	3.43	181.05840	183.06510	Unknown	RPLC	2.00670	-0.05
61	3.43	**257.16092**	**259.16762**	Unknown	RPLC	2.00670	-0.04
62	3.43	**277.13954**	**279.14632**	Unknown	RPLC	2.00678	0.25
63	3.43	292.08509	294.09176	Unknown	RPLC	2.00667	-0.13
64	3.43	320.11636	322.12297	Unknown	RPLC	2.00661	-0.31
65	3.42	456.20754	458.21381	Unknown	RPLC	2.00627	-0.96
66	3.52	129.06593	131.07262	Unknown	RPLC	2.00669	-0.15

67	3.52	310.09595	312.10235	Unknown	RPLC	2.00640	-0.99
68	3.61	**270.04649**	**272.05322**	Unknown	RPLC	2.00673	0.07
69	3.61	321.09289	323.09979	Unknown	RPLC	2.00690	0.59
70	3.65	102.05497	104.06170	Unknown	RPLC	2.00673	0.20
71	3.74	146.11766	148.12434	Unknown	RPLC	2.00668	-0.20
72	3.74	222.07948	224.08639	Unknown	RPLC	2.00691	0.89
73	3.74	290.06950	292.07612	Unknown	RPLC	2.00662	-0.31
74	3.74	433.22865	434.23260	Unknown	RPLC	1.00395	1.37
75	3.87	247.16533	249.17212	Unknown	RPLC	2.00679	0.32
76	3.87	286.17636	288.18288	Unknown	RPLC	2.00652	-0.66
77	3.87	343.19749	347.21118	Unknown	RPLC	4.01369	0.78
78	4.05	**116.07063**	**118.07738**	Unknown	RPLC	2.00675	0.34
79	4.05	**184.10812**	**186.11482**	Unknown	RPLC	2.00670	-0.05
80	4.05	217.15460	218.15799	Unknown	RPLC	1.00339	0.16
81	4.09	406.21861	410.23260	Unknown	RPLC	4.01399	1.39
82	4.14	217.15464	218.15810	Unknown	RPLC	1.00346	0.48
83	4.14	259.12899	261.13559	Unknown	RPLC	2.00660	-0.42
84	4.14	**311.07316**	**313.07983**	Unknown	RPLC	2.00667	-0.13
85	4.14	**334.16100**	**336.16765**	Unknown	RPLC	2.00665	-0.18
86	4.14	366.16934	370.18291	Unknown	RPLC	4.01357	0.41
87	4.23	259.12892	261.13565	Unknown	RPLC	2.00673	0.08
88	4.23	256.14052	257.14384	Unknown	RPLC	1.00332	-0.14
89	4.23	280.14040	282.14729	Unknown	RPLC	2.00689	0.64
90	4.23	299.20787	303.22128	Unknown	RPLC	4.01341	-0.03
91	4.23	334.16075	336.16768	Unknown	RPLC	2.00693	0.66
92	4.27	**157.13359**	**159.14029**	Unknown	RPLC	2.00670	-0.06
93	4.27	204.18789	207.19784	Unknown	RPLC	3.00995	-0.55
94	4.27	343.16875	347.18204	Unknown	RPLC	4.01329	-0.37
95	4.32	428.20215	432.21465	Unknown	RPLC	4.01250	-2.13
96	4.32	204.18783	207.19782	Unknown	RPLC	3.00999	-0.36
97	4.32	257.22232	259.22908	Unknown	RPLC	2.00676	0.19
98	4.32	273.14452	275.15113	Unknown	RPLC	2.00661	-0.36
99	4.32	360.21257	363.22310	Unknown	RPLC	3.01053	1.28
100	4.41	110.09645	111.09981	Unknown	RPLC	1.00336	0.05
101	4.41	**178.08959**	**180.09637**	Unknown	RPLC	2.00678	0.39
102	4.41	290.17103	292.17759	Unknown	RPLC	2.00656	-0.51
103	4.41	314.17102	316.17771	Unknown	RPLC	2.00669	-0.06
104	4.41	324.13014	326.13730	Unknown	RPLC	2.00716	1.38
105	4.41	325.12459	329.13836	Unknown	RPLC	4.01377	1.07
106	4.45	190.11859	194.13209	Unknown	RPLC	4.01350	0.42
107	4.45	311.07277	313.07918	Unknown	RPLC	2.00641	-0.96
108	4.45	325.12468	329.13844	Unknown	RPLC	4.01376	1.03
109	4.45	378.22307	381.23312	Unknown	RPLC	3.01005	-0.04
110	4.54	173.16484	175.17162	Unknown	RPLC	2.00678	0.40
111	4.63	**138.10260**	**140.10932**	Unknown	RPLC	2.00672	0.07
112	4.63	**182.09237**	**184.09913**	Unknown	RPLC	2.00676	0.27
113	4.63	242.18620	244.19293	Unknown	RPLC	2.00673	0.08
114	4.72	104.55183	105.55521	Unknown	RPLC	1.00338	0.24

115	4.72	143.08154	145.08829	Unknown	RPLC	2.00675	0.28
116	4.72	**209.10331**	**211.11003**	Unknown	RPLC	2.00672	0.05
117	4.72	242.18619	244.19294	Unknown	RPLC	2.00675	0.17
118	4.85	116.07064	118.07734	Unknown	RPLC	2.00670	-0.08
119	4.85	214.15499	217.16509	Unknown	RPLC	3.01010	0.16
120	4.85	**374.15581**	**376.16249**	Unknown	RPLC	2.00668	-0.08
121	4.85	388.17110	390.17767	Unknown	RPLC	2.00657	-0.36
122	4.94	143.08152	145.08825	Unknown	RPLC	2.00673	0.14
123	4.94	223.11891	225.12566	Unknown	RPLC	2.00675	0.18
124	4.94	**228.17064**	**232.18406**	Unknown	RPLC	4.01342	0.00
125	4.94	290.11681	294.13026	Unknown	RPLC	4.01345	0.10
126	4.94	**374.15579**	**376.16253**	Unknown	RPLC	2.00674	0.08
127	4.98	290.11696	294.13040	Unknown	RPLC	4.01344	0.07
128	4.98	299.20785	301.21457	Unknown	RPLC	2.00672	0.03
129	5.03	152.11822	154.12494	Unknown	RPLC	2.00672	0.07
130	5.03	341.08387	343.09078	Unknown	RPLC	2.00691	0.58
131	5.03	379.18919	381.19602	Unknown	RPLC	2.00683	0.32
132	5.03	448.22882	450.23605	Unknown	RPLC	2.00723	1.16
133	5.25	**146.11761**	**147.12097**	Unknown	RPLC	1.00336	0.04
134	5.25	**196.09682**	**197.10017**	Unknown	RPLC	1.00335	-0.02
135	5.25	251.05180	253.05856	Unknown	RPLC	2.00676	0.20
136	5.25	346.16110	348.16673	Unknown	RPLC	2.00563	-3.10
137	5.29	**272.17459**	**274.18120**	Unknown	RPLC	2.00661	-0.36
138	5.29	301.08713	303.09388	Unknown	RPLC	2.00675	0.13
139	5.29	309.12137	311.12799	Unknown	RPLC	2.00662	-0.29
140	5.34	173.09203	175.09877	Unknown	RPLC	2.00674	0.17
141	5.34	184.14426	186.15115	Unknown	RPLC	2.00689	0.97
142	5.34	**200.10293**	**202.10969**	Unknown	RPLC	2.00676	0.25
143	5.34	251.05190	253.05849	Unknown	RPLC	2.00659	-0.47
144	5.38	369.07826	371.08511	Unknown	RPLC	2.00685	0.38
145	5.38	379.18998	381.19642	Unknown	RPLC	2.00644	-0.71
146	5.38	222.08498	224.09150	Unknown	RPLC	2.00652	-0.85
147	5.38	157.13356	159.14028	Unknown	RPLC	2.00672	0.06
148	5.38	150.09131	151.09470	Unknown	RPLC	1.00339	0.23
149	5.55	144.06555	145.06884	Unknown	RPLC	1.00329	-0.45
150	5.73	316.15027	318.15671	Unknown	RPLC	2.00644	-0.85
151	5.73	310.18650	311.18955	Unknown	RPLC	1.00305	-0.98
152	5.81	166.12260	168.12938	Unknown	RPLC	2.00678	0.42
153	5.81	**246.07938**	**248.08608**	Unknown	RPLC	2.00670	-0.04
154	5.90	173.09204	175.09872	Unknown	RPLC	2.00668	-0.17
155	5.90	268.06136	270.06831	Unknown	RPLC	2.00695	0.89
156	5.90	313.15050	315.15722	Unknown	RPLC	2.00672	0.03
157	5.90	316.15038	318.15680	Unknown	RPLC	2.00642	-0.91
158	5.98	**146.11759**	**147.12096**	Unknown	RPLC	1.00337	0.10
159	6.30	**110.07131**	**113.08137**	Unknown	RPLC	3.01006	-0.04
160	6.30	164.10699	166.11377	Unknown	RPLC	2.00678	0.42
161	6.30	182.11761	184.12438	Unknown	RPLC	2.00677	0.33
162	6.30	**262.07442**	**264.08114**	Unknown	RPLC	2.00672	0.04

163	6.30	284.05601	286.06291	Unknown	RPLC	2.00690	0.67
164	6.30	329.15364	333.16694	Unknown	RPLC	4.01330	-0.36
165	6.38	362.19878	364.20580	Unknown	RPLC	2.00702	0.85
166	6.47	**274.07441**	**275.07775**	Unknown	RPLC	1.00334	-0.05
167	6.47	334.19732	336.20405	Unknown	RPLC	2.00673	0.06
168	6.56	194.11752	195.12092	Unknown	RPLC	1.00340	0.23
169	6.56	343.17279	345.17930	Unknown	RPLC	2.00651	-0.58
170	7.09	321.11120	324.12122	Unknown	RPLC	3.01002	-0.14
171	7.40	296.15237	297.15575	Unknown	RPLC	1.00338	0.08
172	7.62	236.09495	238.10168	Unknown	RPLC	2.00673	0.09
173	7.62	273.18064	275.18743	Unknown	RPLC	2.00679	0.29
174	7.70	**114.12775**	**116.13447**	Unknown	RPLC	2.00672	0.09
175	7.70	336.18397	338.19080	Unknown	RPLC	2.00683	0.36
176	7.92	285.24544	287.25191	Unknown	RPLC	2.00647	-0.83
177	7.92	288.25572	290.26227	Unknown	RPLC	2.00655	-0.55
178	8.18	359.14532	360.14848	Unknown	RPLC	1.00316	-0.54
179	8.36	**192.10535**	**194.11207**	Unknown	RPLC	2.00672	0.05
180	8.36	359.14464	360.14807	Unknown	RPLC	1.00343	0.21
181	8.36	390.14053	394.15391	Unknown	RPLC	4.01338	-0.10
182	8.63	320.12739	323.13750	Unknown	RPLC	3.01011	0.14
183	8.76	364.18963	368.20324	Unknown	RPLC	4.01361	0.52
184	8.76	**475.13646**	**477.14320**	Unknown	RPLC	2.00674	0.06
185	8.76	285.19205	289.20562	Unknown	RPLC	4.01357	0.52
186	8.90	304.13259	308.14580	Unknown	RPLC	4.01321	-0.68
187	8.98	322.14306	324.14979	Unknown	RPLC	2.00673	0.06
188	9.25	261.14431	263.15097	Unknown	RPLC	2.00666	-0.19
189	10.17	**318.16575**	**320.17236**	Unknown	RPLC	2.00661	-0.31
190	10.17	256.17676	258.18343	Unknown	RPLC	2.00667	-0.15
191	10.61	282.15583	284.16269	Unknown	RPLC	2.00686	0.53
192	11.01	357.14789	359.15498	Unknown	RPLC	2.00709	1.06
193	11.14	**250.11068**	**252.11724**	Unknown	RPLC	2.00656	-0.59
194	11.18	180.10186	181.10519	Unknown	RPLC	1.00333	-0.14
195	11.18	134.09643	135.09981	Unknown	RPLC	1.00338	0.19
196	11.58	395.18104	397.18768	Unknown	RPLC	2.00664	-0.18
197	11.58	343.19711	347.21009	Unknown	RPLC	4.01298	-1.27
198	11.80	**223.10771**	**225.11446**	Unknown	RPLC	2.00675	0.18
199	12.19	**256.17667**	**258.18340**	Unknown	RPLC	2.00673	0.08
200	12.37	381.16441	382.16811	Unknown	RPLC	1.00370	0.90
201	13.52	287.06939	289.07603	Unknown	RPLC	2.00664	-0.24
202	15.05	226.15478	230.16837	Unknown	RPLC	4.01359	0.74
203	16.06	397.19565	399.20216	Unknown	RPLC	2.00651	-0.50
204	16.11	148.11215	150.11873	Unknown	RPLC	2.00658	-0.86
205	16.19	**275.16010**	**277.16677**	Unknown	RPLC	2.00667	-0.14
206	16.19	274.17610	276.18272	Unknown	RPLC	2.00662	-0.32
207	16.46	182.08121	184.08792	Unknown	RPLC	2.00671	0.00
208	16.46	216.09949	218.10616	Unknown	RPLC	2.00667	-0.18
209	16.68	379.06279	381.06989	Unknown	RPLC	2.00710	1.02
210	16.73	**182.08117**	**184.08789**	Unknown	RPLC	2.00672	0.06

211	16.73	334.19720	336.20384	Unknown	RPLC	2.00664	-0.21
212	16.82	164.07062	166.07741	Unknown	RPLC	2.00679	0.48
213	16.82	379.06272	381.06984	Unknown	RPLC	2.00712	1.08
214	16.82	**399.21231**	**401.21889**	Unknown	RPLC	2.00658	-0.32
215	16.99	**157.09718**	**159.10387**	Unknown	RPLC	2.00669	-0.12
216	17.12	**130.08630**	**132.09300**	Unknown	RPLC	2.00670	-0.07
217	17.12	179.07914	181.08580	Unknown	RPLC	2.00666	-0.27
218	17.12	271.07464	273.08137	Unknown	RPLC	2.00673	0.07
219	17.12	**322.08889**	**326.10234**	Unknown	RPLC	4.01345	0.09
220	17.21	336.18402	338.19050	Unknown	RPLC	2.00648	-0.68
221	17.56	285.09039	286.09373	Unknown	RPLC	1.00334	-0.05
222	17.92	**124.10407**	**127.11416**	Unknown	RPLC	3.01009	0.20
223	17.92	146.08605	149.09606	Unknown	RPLC	3.01001	-0.37
224	17.92	325.13937	327.14584	Unknown	RPLC	2.00647	-0.73
225	17.96	482.20937	484.21575	Unknown	RPLC	2.00638	-0.68
226	17.96	318.19971	320.20644	Unknown	RPLC	2.00673	0.06
227	18.09	272.11483	274.12150	Unknown	RPLC	2.00667	-0.14
228	18.18	141.13065	144.14071	Unknown	RPLC	3.01006	-0.03
229	18.18	184.13644	187.14663	Unknown	RPLC	3.01019	0.67
230	18.18	307.16541	309.17196	Unknown	RPLC	2.00655	-0.52
231	18.36	123.10774	126.11779	Unknown	RPLC	3.01005	-0.12
232	18.36	157.09717	159.10382	Unknown	RPLC	2.00665	-0.38
233	18.45	223.13608	225.14293	Unknown	RPLC	2.00685	0.62
234	18.45	228.13411	230.14078	Unknown	RPLC	2.00667	-0.17
235	18.54	**299.10597**	**301.11264**	Unknown	RPLC	2.00667	-0.13
236	18.54	149.55352	150.55681	Unknown	RPLC	1.00329	-0.43
237	18.54	184.10814	186.11484	Unknown	RPLC	2.00670	-0.05
238	18.54	321.08785	323.09471	Unknown	RPLC	2.00686	0.47
239	18.54	325.13944	327.14619	Unknown	RPLC	2.00675	0.12
240	18.54	355.22244	357.22930	Unknown	RPLC	2.00686	0.42
241	18.88	188.09185	190.09840	Unknown	RPLC	2.00655	-0.84
242	18.88	215.10258	217.10950	Unknown	RPLC	2.00692	0.97
243	18.88	322.17642	324.18277	Unknown	RPLC	2.00635	-1.11
244	19.11	**336.18385**	**338.19061**	Unknown	RPLC	2.00676	0.15
245	19.11	**246.16985**	**248.17646**	Unknown	RPLC	2.00661	-0.40
246	19.11	264.12645	266.13312	Unknown	RPLC	2.00667	-0.15
247	19.11	284.09505	285.09827	Unknown	RPLC	1.00322	-0.47
248	19.37	286.13964	288.14640	Unknown	RPLC	2.00676	0.17
249	19.37	304.18658	306.19324	Unknown	RPLC	2.00666	-0.16
250	19.37	**332.21804**	**334.22471**	Unknown	RPLC	2.00667	-0.12
251	19.76	339.15505	341.16179	Unknown	RPLC	2.00674	0.09
252	19.76	115.10376	118.11385	Unknown	RPLC	3.01009	0.22
253	19.76	124.10409	127.11414	Unknown	RPLC	3.01005	-0.11
254	19.76	137.08572	140.09577	Unknown	RPLC	3.01005	-0.10
255	19.85	175.13597	178.14617	Unknown	RPLC	3.01020	0.76
256	19.85	260.09515	262.10175	Unknown	RPLC	2.00660	-0.42
257	19.94	132.10202	134.10872	Unknown	RPLC	2.00670	-0.07
258	20.16	215.13903	217.14580	Unknown	RPLC	2.00677	0.28

259	20.16	242.14995	244.15674	Unknown	RPLC	2.00679	0.33
260	20.25	**275.16011**	**277.16664**	Unknown	RPLC	2.00653	-0.65
261	20.46	334.19693	336.20382	Unknown	RPLC	2.00689	0.54
262	20.68	**336.18388**	**338.19071**	Unknown	RPLC	2.00683	0.36
263	20.82	**219.11276**	**220.11609**	Unknown	RPLC	1.00333	-0.11
264	20.99	**251.13888**	**253.14566**	Unknown	RPLC	2.00678	0.28
265	20.99	281.14943	283.15630	Unknown	RPLC	2.00687	0.57
266	21.16	303.26421	306.27444	Unknown	RPLC	3.01023	0.54
267	21.16	336.10465	340.11813	Unknown	RPLC	4.01348	0.18
268	21.25	270.19130	271.19429	Unknown	RPLC	1.00299	-1.35
269	21.52	**276.12646**	**278.13316**	Unknown	RPLC	2.00670	-0.03
270	21.65	393.22245	395.22941	Unknown	RPLC	2.00696	0.63
271	21.74	138.06360	139.06694	Unknown	RPLC	1.00334	-0.11
272	21.74	284.17031	285.17367	Unknown	RPLC	1.00336	0.02
273	21.74	338.17107	340.17781	Unknown	RPLC	2.00674	0.09
274	21.96	314.10626	316.11281	Unknown	RPLC	2.00655	-0.51
275	22.09	298.14961	299.15290	Unknown	RPLC	1.00329	-0.22
276	22.32	189.13875	191.14545	Unknown	RPLC	2.00670	-0.05
277	22.53	409.25427	410.25731	Unknown	RPLC	1.00304	-0.77
278	22.62	275.16044	276.16366	Unknown	RPLC	1.00322	-0.49
279	22.79	279.17050	281.17719	Unknown	RPLC	2.00669	-0.07
280	22.79	346.19815	348.20496	Unknown	RPLC	2.00681	0.29
281	22.88	191.11795	193.12467	Unknown	RPLC	2.00672	0.05
282	22.88	286.07441	288.08099	Unknown	RPLC	2.00658	-0.45
283	22.93	308.15265	310.15908	Unknown	RPLC	2.00643	-0.90
284	22.97	238.14388	239.14700	Unknown	RPLC	1.00312	-0.98
285	23.15	132.10186	134.10866	Unknown	RPLC	2.00680	0.67
286	23.15	320.15227	322.15904	Unknown	RPLC	2.00677	0.19
287	23.24	209.10329	211.11012	Unknown	RPLC	2.00683	0.57
288	23.24	397.07084	403.09102	Unknown	RPLC	6.02018	0.13
289	23.32	232.19091	234.19755	Unknown	RPLC	2.00664	-0.30
290	23.32	257.14993	259.15681	Unknown	RPLC	2.00688	0.66
291	23.54	**278.14179**	**280.14854**	Unknown	RPLC	2.00675	0.14
292	23.54	282.13379	284.14067	Unknown	RPLC	2.00688	0.60
293	23.54	314.17093	316.17788	Unknown	RPLC	2.00695	0.76
294	23.63	257.14942	259.15621	Unknown	RPLC	2.00679	0.31
295	23.63	422.09402	424.10096	Unknown	RPLC	2.00694	0.54
296	23.94	329.11648	331.12311	Unknown	RPLC	2.00663	-0.24
297	23.98	332.21807	334.22492	Unknown	RPLC	2.00685	0.42
298	24.07	399.15041	401.15718	Unknown	RPLC	2.00677	0.15
299	24.07	352.18637	354.19283	Unknown	RPLC	2.00646	-0.70
300	24.20	**289.17617**	**291.18287**	Unknown	RPLC	2.00670	-0.03
301	24.38	240.06915	241.07249	Unknown	RPLC	1.00334	-0.06
302	24.47	**346.23338**	**348.24003**	Unknown	RPLC	2.00665	-0.17
303	24.55	366.16576	368.17248	Unknown	RPLC	2.00672	0.03
304	24.55	328.19663	329.19974	Unknown	RPLC	1.00311	-0.74
305	24.86	260.18581	262.19252	Unknown	RPLC	2.00671	0.00
306	24.86	342.17588	343.17934	Unknown	RPLC	1.00346	0.31

307	25.53	**289.17588**	**291.18255**	Unknown	RPLC	2.00667	-0.14
308	25.75	**309.14455**	**311.15120**	Unknown	RPLC	2.00665	-0.19
309	25.75	269.14953	271.15625	Unknown	RPLC	2.00672	0.04
310	25.83	225.15975	226.16305	Unknown	RPLC	1.00330	-0.24
311	25.92	**293.18627**	**295.19273**	Unknown	RPLC	2.00646	-0.85
312	25.96	**220.13658**	**222.14314**	Unknown	RPLC	2.00656	-0.67
313	25.96	264.12617	266.13309	Unknown	RPLC	2.00692	0.79
314	25.96	313.21213	316.22203	Unknown	RPLC	3.00990	-0.52
315	26.01	268.03072	270.03776	Unknown	RPLC	2.00704	1.22
316	26.01	340.17574	341.17880	Unknown	RPLC	1.00306	-0.86
317	26.01	334.16845	336.17519	Unknown	RPLC	2.00674	0.09
318	26.14	295.04183	297.04827	Unknown	RPLC	2.00644	-0.91
319	26.27	290.14209	292.14865	Unknown	RPLC	2.00656	-0.51
320	26.36	**247.14409**	**250.15416**	Unknown	RPLC	3.01007	0.02
321	26.49	291.13403	293.14085	Unknown	RPLC	2.00682	0.38
322	26.49	358.24367	359.24678	Unknown	RPLC	1.00311	-0.68
323	26.81	**239.17558**	**241.18226**	Unknown	RPLC	2.00668	-0.12
324	26.90	205.09742	206.10065	Unknown	RPLC	1.00323	-0.61
325	26.90	**372.22321**	**373.22651**	Unknown	RPLC	1.00330	-0.15
326	26.98	231.11320	232.11653	Unknown	RPLC	1.00333	-0.11
327	26.98	277.15514	278.15835	Unknown	RPLC	1.00321	-0.52
328	27.29	321.14831	322.15164	Unknown	RPLC	1.00333	-0.08
329	27.74	**171.09518**	**173.10190**	Unknown	RPLC	2.00672	0.06
330	27.74	265.15503	267.16184	Unknown	RPLC	2.00681	0.38
331	27.87	**323.16039**	**325.16710**	Unknown	RPLC	2.00671	0.00
332	27.87	360.24951	362.25617	Unknown	RPLC	2.00666	-0.14
333	27.92	380.21782	382.22454	Unknown	RPLC	2.00672	0.03
334	28.00	272.18564	274.19257	Unknown	RPLC	2.00693	0.80
335	28.57	275.10592	277.11269	Unknown	RPLC	2.00677	0.22
336	28.79	**402.27025**	**403.27357**	Unknown	RPLC	1.00332	-0.09
337	29.09	**302.17898**	**304.18564**	Unknown	RPLC	2.00666	-0.16
338	29.57	291.17072	293.17742	Unknown	RPLC	2.00670	-0.03
339	29.97	217.09735	218.10077	Unknown	RPLC	1.00342	0.30
340	29.97	**244.10828**	**245.11164**	Unknown	RPLC	1.00336	0.02
341	29.97	274.20156	276.20832	Unknown	RPLC	2.00676	0.18
342	30.01	**300.15555**	**302.16232**	Unknown	RPLC	2.00677	0.20
343	30.48	**233.12850**	**235.13517**	Unknown	RPLC	2.00667	-0.17
344	30.57	**202.08625**	**203.08961**	Unknown	RPLC	1.00336	0.03
345	30.71	208.09684	210.10365	Unknown	RPLC	2.00681	0.48
346	31.15	**394.23455**	**396.24098**	Unknown	RPLC	2.00643	-0.71
347	31.15	**274.20158**	**276.20832**	Unknown	RPLC	2.00674	0.11
348	31.27	**304.15819**	**306.16484**	Unknown	RPLC	2.00665	-0.19
349	31.50	247.14438	250.15441	Unknown	RPLC	3.01003	-0.14
350	31.50	330.18166	332.18842	Unknown	RPLC	2.00676	0.15
351	31.63	**297.09056**	**299.09722**	Unknown	RPLC	2.00666	-0.17
352	31.63	346.08353	348.09057	Unknown	RPLC	2.00704	0.95
353	31.72	239.17559	240.17921	Unknown	RPLC	1.00362	1.10
354	31.72	324.10183	326.10856	Unknown	RPLC	2.00673	0.06

355	32.11	**302.17842**	**304.18520**	Unknown	RPLC	2.00678	0.23
356	32.11	310.20827	311.21180	Unknown	RPLC	1.00353	0.56
357	32.11	394.23356	396.24062	Unknown	RPLC	2.00706	0.88
358	32.42	**253.19115**	**255.19787**	Unknown	RPLC	2.00672	0.04
359	32.95	258.17024	261.18042	Unknown	RPLC	3.01018	0.44
360	32.95	308.15287	310.15972	Unknown	RPLC	2.00685	0.45
361	34.17	**171.11293**	**173.11968**	Unknown	RPLC	2.00675	0.23
362	34.26	318.18123	320.18751	Unknown	RPLC	2.00628	-1.34
363	34.65	**296.17579**	**297.17915**	Unknown	RPLC	1.00336	0.02
364	35.05	381.16593	382.16975	Unknown	RPLC	1.00382	1.22
365	35.18	201.11235	204.12238	Unknown	RPLC	3.01003	-0.17
366	35.35	**246.15239**	**248.15908**	Unknown	RPLC	2.00669	-0.08
367	35.80	**301.12165**	**303.12833**	Unknown	RPLC	2.00668	-0.10
368	36.20	348.22819	351.23850	Unknown	RPLC	3.01031	0.70
369	36.42	380.20598	382.21245	Unknown	RPLC	2.00647	-0.63
370	36.60	350.16351	352.17007	Unknown	RPLC	2.00656	-0.43
371	36.73	**302.17846**	**304.18512**	Unknown	RPLC	2.00666	-0.16
372	37.48	360.18405	362.19069	Unknown	RPLC	2.00664	-0.19
373	37.92	305.18630	306.18967	Unknown	RPLC	1.00337	0.05
374	38.71	**248.16800**	**250.17469**	Unknown	RPLC	2.00669	-0.08
375	38.71	318.20991	320.21636	Unknown	RPLC	2.00645	-0.81
376	38.84	332.18924	334.19595	Unknown	RPLC	2.00671	0.00
377	39.55	158.11778	160.12450	Unknown	RPLC	2.00672	0.06
378	39.55	185.12871	187.13545	Unknown	RPLC	2.00674	0.16
379	40.21	**258.12383**	**260.13051**	Unknown	RPLC	2.00668	-0.11
380	40.21	231.11297	233.11964	Unknown	RPLC	2.00667	-0.17
381	40.25	280.10568	282.11255	Unknown	RPLC	2.00687	0.57
382	40.69	**192.10201**	**194.10872**	Unknown	RPLC	2.00671	0.00
383	40.69	**219.11299**	**221.11967**	Unknown	RPLC	2.00668	-0.13
384	40.69	241.09487	243.10169	Unknown	RPLC	2.00682	0.45
385	40.74	292.19448	294.20097	Unknown	RPLC	2.00649	-0.75
386	40.74	408.23883	410.24539	Unknown	RPLC	2.00656	-0.36
387	40.87	320.22510	322.23203	Unknown	RPLC	2.00693	0.68
388	40.92	334.20474	336.21140	Unknown	RPLC	2.00666	-0.15
389	41.84	**272.13955**	**275.14960**	Unknown	RPLC	3.01005	-0.05
390	41.84	362.25420	363.25735	Unknown	RPLC	1.00315	-0.56
391	41.93	**386.17881**	**389.18872**	Unknown	RPLC	3.00991	-0.40
392	42.24	**408.23877**	**410.24520**	Unknown	RPLC	2.00643	-0.68
393	42.81	274.18379	276.19054	Unknown	RPLC	2.00675	0.15
394	42.98	301.19121	303.19812	Unknown	RPLC	2.00691	0.66
395	42.98	344.22597	346.23249	Unknown	RPLC	2.00652	-0.55
396	42.98	379.20861	380.21170	Unknown	RPLC	1.00309	-0.70
397	43.29	393.22428	395.23138	Unknown	RPLC	2.00710	0.99
398	43.42	**452.25049**	**453.25403**	Unknown	RPLC	1.00354	0.41
399	43.42	292.15778	294.16434	Unknown	RPLC	2.00656	-0.51
400	43.68	245.13189	247.13857	Unknown	RPLC	2.00668	-0.12
401	44.47	215.12774	216.13110	Unknown	RPLC	1.00336	0.02
402	44.47	**360.22031**	**362.22734**	Unknown	RPLC	2.00703	0.88

403	44.92	300.16291	302.16939	Unknown	RPLC	2.00648	-0.76
404	45.09	318.21004	320.21648	Unknown	RPLC	2.00644	-0.84
405	45.27	**346.24113**	**348.24800**	Unknown	RPLC	2.00687	0.46
406	46.51	**276.19933**	**278.20597**	Unknown	RPLC	2.00664	-0.25
407	46.59	**300.19943**	**302.20611**	Unknown	RPLC	2.00668	-0.10
408	47.70	278.18625	280.19294	Unknown	RPLC	2.00669	-0.07
409	48.72	**318.17323**	**320.18006**	Unknown	RPLC	2.00683	0.38
410	49.20	344.22531	346.23227	Unknown	RPLC	2.00696	0.72
411	49.37	**302.21476**	**304.22141**	Unknown	RPLC	2.00665	-0.20
412	49.76	295.23805	297.24488	Unknown	RPLC	2.00683	0.40
413	50.16	**302.21495**	**304.22162**	Unknown	RPLC	2.00667	-0.13
414	50.16	**326.21487**	**328.22172**	Unknown	RPLC	2.00685	0.43
415	51.48	320.18896	322.19582	Unknown	RPLC	2.00686	0.47
416	52.01	273.16295	275.16971	Unknown	RPLC	2.00676	0.18
417	52.27	**304.23070**	**306.23742**	Unknown	RPLC	2.00672	0.03
418	52.67	**328.23042**	**330.23714**	Unknown	RPLC	2.00672	0.03
419	53.29	372.25669	374.26330	Unknown	RPLC	2.00661	-0.27
420	58.18	**301.19440**	**303.20112**	Unknown	RPLC	2.00672	0.03

Note: 1). The unknown pairs with S/N ratio of > 80 are highlighted in bold.

2). Error (ppm) in mass difference is the mass error between the theoretical mass difference and the measured mass differences for the ^{12}C-/^{13}C-dimethylated derivatives. For example, the theoretical mass difference for the two tags is 2.006709676 Da.

3). All the ion pairs listed have S/N ratios of > 10 and mass error in mass difference of < 1.5 ppm.

Table S3.1. List of compounds tested for dansylation with dansyl chloride that showed little or no derivative products.

Diphenylamine	Dihydrouracil
N-acetyl-aspartic acid	Guanosine
N-acetyl-glutamic acid	Ureidopropionic acid
N-acetyl-glycine	Pterin
Z-gly-pro	Neopterin
Creatine	Aminopterin
Creatinine	Isoxanthopterin
Imidazol	Biopterin
4-nitroaniline	Dyspropterin
Diphenylamine	Sepiapterin
Thiamine	Paraxanthine
Carpylogylglycine	3-methylxanthine
Indole	Thyroxine
Indolelatic acid	Pyroglutamic acid
Indole-3-carboxylic acid	Cytidine
Indoleacetic acid	Cytosine
3-indolebutyric acid	Pyridoxal

3-indolepropionic acid	Hypoanthine
Indoxyl sulphate	Guanidine acetic acid
Kynurenic acid	Urea

Table S3.2. Results of the experiments to gauge technical reproducibility and compare the relative metabolite abundance differences between urine samples stored at -20 ^{o}C and -80 ^{o}C.

		Run1	Run2	Run3	Avg three runs	%RSD (Run to run)
Dns-Asn	**20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.083	1.035	1.032	1.050	2.7
	#2	0.982	0.975	0.918	0.958	3.7
	#3	1.017	1.061	1.047	1.042	2.2
	#4	0.925	0.978	0.911	0.938	3.8
	Average				**1.00**	3.1
	%RSD (Labeling)				**5.7**	
Dns-Asn	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	0.942	1.015	0.978	0.978	3.7
	#2	0.955	0.996	1.010	0.987	2.9
	#3	1.035	1.010	1.105	1.050	4.7
	#4	0.931	0.945	0.878	0.918	3.8
	Average				**0.98**	3.8
	%RSD (Labeling)				**5.5**	
Dns-Asn	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**1.4**	
Dns-Gln	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.032	1.047	1.022	1.034	1.2
	#2	1.005	0.975	1.055	1.012	4.0
	#3	0.962	0.953	0.917	0.944	2.5
	#4	1.060	1.045	0.984	1.030	3.9
	Average				**1.00**	2.9
	%RSD (Labeling)				**4.1**	
Dns-Gln	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	0.999	1.045	1.012	1.019	2.3
	#2	0.998	0.987	1.043	1.009	2.9
	#3	0.998	0.956	0.949	0.968	2.7
	#4	0.964	0.980	0.925	0.956	3.0
	Average				**0.99**	2.7
	%RSD (Labeling)				**3.1**	
Dns-Gln	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**1.7**	
Dns-Ser	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.942	0.938	0.975	0.952	2.1
	#2	0.885	0.958	0.947	0.930	4.2
	#3	0.929	0.943	0.885	0.919	3.3

	#4	1.021	0.991	1.067	1.026	3.7
	Average				**0.96**	3.3
	%RSD (Labeling)				**5.1**	
Dns-Ser	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	0.953	0.987	0.891	0.944	5.2
	#2	1.029	1.045	1.105	1.060	3.8
	#3	1.021	1.019	1.055	1.032	2.0
	#4	1.033	1.058	0.965	1.019	4.7
	Average				**1.01**	3.9
	%RSD (Labeling)				**4.9**	
Dns-Ser	%Diff Between -20 °C/-80 °C Labeling				**-5.8**	
Dns-Glu	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	0.885	0.829	0.891	0.868	3.9
	#2	0.937	0.965	0.975	0.959	2.1
	#3	0.932	0.998	0.916	0.949	4.6
	#4	0.991	0.952	1.023	0.989	3.6
	Average				**0.94**	3.5
	%RSD (Labeling)				**5.5**	
Dns-Glu	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	0.932	0.995	0.947	0.958	3.4
	#2	0.983	0.945	0.909	0.946	3.9
	#3	1.005	1.050	1.016	1.024	2.3
	#4	1.021	1.083	1.111	1.072	4.3
	Average				**1.00**	3.5
	%RSD (Labeling)				**5.9**	
Dns-Glu	%Diff Between -20 °C/-80 °C Labeling				**-6.0**	
Dns-Asp	**-20°C (^{12}C)/-80 °C (^{13}C) # 1**	0.875	0.880	0.920	0.892	2.8
	#2	1.055	1.102	1.015	1.057	4.1
	#3	0.935	0.989	0.993	0.972	3.3
	#4	0.915	0.988	0.968	0.957	3.9
	Average				**0.97**	3.5
	%RSD (Labeling)				**7.0**	
Dns-Asp	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	0.954	0.925	0.974	0.951	2.6
	#2	0.944	0.868	0.915	0.909	4.2
	#3	0.948	0.958	0.983	0.963	1.9
	#4	1.021	1.049	0.987	1.019	3.0
	Average				**0.96**	2.9
	%RSD (Labeling)				**4.7**	
Dns-Asp	%Diff Between -20 °C/-80 °C Labeling				**0.9**	
Dns-Thr	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	1.103	1.028	1.100	1.077	3.9
	#2	1.055	1.056	1.029	1.047	1.5

	#3	1.043	0.988	1.026	1.019	2.8
	#4	0.982	0.956	0.899	0.946	4.5
	Average				**1.02**	3.2
	%RSD (Labeling)				**5.5**	
Dns-Thr	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.087	1.025	1.074	1.062	3.1
	#2	1.044	1.010	1.070	1.041	2.9
	#3	0.953	0.885	0.931	0.923	3.8
	#4	1.089	1.070	1.118	1.092	2.2
	Average				**1.03**	3.0
	%RSD (Labeling)				**7.2**	
Dns-Thr	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**-0.7**	
Dns-Gly	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.147	1.103	1.082	1.111	3.0
	#2	0.899	0.933	0.975	0.936	4.1
	#3	0.976	0.947	0.993	0.972	2.4
	#4	1.047	1.061	1.028	1.045	1.6
	Average				**1.02**	2.8
	%RSD (Labeling)				**7.7**	
Dns-Gly	**-80 ^{o}C (^{12}C)/-20^{o}C (^{13}C) # 1**	0.907	0.889	0.875	0.890	1.8
	#2	1.035	0.998	0.975	1.003	3.0
	#3	0.974	0.965	0.930	0.956	2.4
	#4	1.105	1.029	1.013	1.049	4.7
	Average				**0.97**	3.0
	%RSD (Labeling)				**6.9**	
Dns-Gly	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**4.2**	
Dns-Ala	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.033	1.115	1.085	1.078	3.8
	#2	1.103	1.046	1.011	1.053	4.4
	#3	0.976	0.993	0.955	0.975	2.0
	#4	1.033	1.115	1.102	1.083	4.1
	Average				**1.05**	3.6
	%RSD (Labeling)				**4.8**	
Dns-Ala	**-80 ^{o}C (^{12}C)/-20^{o}C (^{13}C) # 1**	1.105	1.069	1.055	1.076	2.4
	#2	0.898	0.973	0.944	0.938	4.0
	#3	0.891	0.948	0.959	0.933	3.9
	#4	0.931	0.976	0.889	0.932	4.7
	Average				**0.97**	3.8
	%RSD (Labeling)				**7.3**	
Dns-Ala	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**7.7**	
Dns-Pro	**-20 ^{o}C (^{12}C)/-80^{o}C**	0.977	1.035	0.998	1.003	2.9

	(^{13}C) # 1					
	#2	0.928	0.905	0.953	0.929	2.6
	#3	1.005	0.985	1.033	1.008	2.4
	#4	1.105	1.112	1.058	1.092	2.7
	Average				**1.01**	2.6
	%RSD (Labeling)				**6.6**	
Dns-Pro	**-80 ^{o}C (^{12}C)/-20^{o}C (^{13}C) # 1**	0.967	0.974	0.993	0.978	1.4
	#2	0.981	0.955	1.031	0.989	3.9
	#3	1.052	1.075	1.087	1.071	1.7
	#4	0.872	0.886	0.939	0.899	3.9
	Average				**0.98**	2.7
	%RSD (Labeling)				**7.2**	
Dns-Pro	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**2.4**	
Dns-Val	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.925	0.936	0.897	0.919	2.2
	#2	0.996	0.983	1.054	1.011	3.7
	#3	1.042	1.039	0.998	1.026	2.4
	#4	0.977	0.999	1.035	1.004	2.9
	Average				**0.99**	2.8
	%RSD (Labeling)				**4.9**	
Dns-Val	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.038	1.092	1.016	1.049	3.7
	#2	1.103	1.082	1.118	1.101	1.6
	#3	0.976	1.035	1.023	1.011	3.1
	#4	1.105	1.020	1.018	1.048	4.7
	Average				**1.05**	3.3
	%RSD (Labeling)				**3.5**	
Dns-Val	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**-6.1**	
Dns-Met	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.857	0.890	0.903	0.883	2.7
	#2	0.997	0.949	1.025	0.990	3.9
	#3	0.955	0.987	0.947	0.963	2.2
	#4	0.979	0.998	0.950	0.976	2.5
	Average				**0.95**	2.8
	%RSD (Labeling)				**5.0**	
Dns-Met	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.020	0.993	0.935	0.983	4.4
	#2	0.859	0.905	0.886	0.883	2.6
	#3	0.898	0.976	0.965	0.946	4.5
	#4	0.993	1.046	1.058	1.032	3.4
	Average				**0.96**	3.7
	%RSD (Labeling)				**6.5**	
Dns-Met	%Diff Between -20 ^{o}C/-80 ^{o}C				**-0.8**	

	Labeling					
Dns-Trp	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	0.897	0.945	0.951	0.931	3.2
	#2	0.885	0.930	0.853	0.889	4.3
	#3	0.989	0.935	0.967	0.964	2.8
	#4	0.983	1.045	0.975	1.001	3.8
	Average				**0.95**	3.5
	%RSD (Labeling)				**5.0**	
Dns-Trp	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.058	1.143	1.101	1.101	3.9
	#2	0.926	0.983	0.975	0.961	3.2
	#3	1.029	1.038	1.100	1.056	3.7
	#4	0.979	0.991	0.907	0.959	4.7
	Average				**1.02**	3.9
	%RSD (Labeling)				**6.9**	
Dns-Trp	%Diff Between -20 °C/-80 °C Labeling				**-7.4**	
Dns-Phe	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	1.111	1.035	1.093	1.080	3.7
	#2	1.015	1.047	1.092	1.051	3.7
	#3	1.024	0.949	0.954	0.976	4.3
	#4	1.082	1.078	0.998	1.053	4.5
	Average				**1.04**	4.0
	%RSD (Labeling)				**4.3**	
Dns-Phe	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.125	1.043	1.112	1.093	4.0
	#2	1.072	1.088	1.020	1.060	3.4
	#3	1.084	1.125	1.050	1.086	3.5
	#4	0.998	0.943	1.005	0.982	3.5
	Average				**1.06**	3.6
	%RSD (Labeling)				**4.8**	
Dns-Phe	%Diff Between -20 °C/-80 °C Labeling				**-1.5**	
Dns-Ile	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	0.937	0.950	0.970	0.952	1.7
	#2	1.009	1.022	0.959	0.997	3.3
	#3	0.928	0.881	0.921	0.910	2.8
	#4	1.053	1.022	1.003	1.026	2.5
	Average				**0.97**	2.6
	%RSD (Labeling)				**5.2**	
Dns-Ile	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.041	1.020	0.999	1.020	2.1
	#2	1.090	1.033	1.011	1.045	3.9
	#3	0.950	0.995	0.952	0.966	2.6
	#4	1.022	1.030	1.048	1.033	1.3
	Average				**1.02**	2.5
	%RSD (Labeling)				**3.4**	

Dns-Ile	%Diff Between -20 °C/-80 °C Labeling				**-4.5**	
Dns-Leu	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	1.038	1.031	0.991	1.020	2.5
	#2	0.921	0.998	0.967	0.962	4.0
	#3	1.038	1.059	0.998	1.032	3.0
	#4	0.945	0.973	0.970	0.963	1.6
	Average				**0.99**	2.8
	%RSD (Labeling)				**3.7**	
Dns-Lue	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.102	1.147	1.103	1.117	2.3
	#2	1.074	1.016	1.082	1.057	3.4
	#3	1.096	1.042	1.018	1.052	3.8
	#4	0.921	0.975	0.995	0.964	4.0
	Average				**1.05**	3.4
	%RSD (Labeling)				**6.0**	
Dns-Lue	%Diff Between -20 °C/-80 °C Labeling				**-5.2**	
Dns-Cystine	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	0.973	1.047	1.022	1.014	3.7
	#2	1.103	1.015	1.014	1.044	4.9
	#3	1.110	1.128	1.059	1.099	3.3
	#4	1.135	1.064	1.075	1.091	3.5
	Average				**1.06**	3.8
	%RSD (Labeling)				**3.8**	
Dns-Cystine	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.092	1.103	1.015	1.070	4.5
	#2	1.030	0.976	0.949	0.985	4.2
	#3	1.028	1.050	1.103	1.060	3.6
	#4	1.107	1.042	1.029	1.059	3.9
	Average				**1.04**	4.1
	%RSD (Labeling)				**3.8**	
Dns-Cystine	%Diff Between -20 °C/-80 °C Labeling				**1.7**	
Dns-Lys	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	0.975	0.947	1.020	0.981	3.8
	#2	0.993	0.979	1.050	1.007	3.7
	#3	0.941	0.929	0.971	0.947	2.3
	#4	1.103	1.051	1.035	1.063	3.3
	Average				**1.00**	3.3
	%RSD (Labeling)				**4.9**	
Dns-Lys	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	0.857	0.883	0.923	0.888	3.7
	#2	0.953	0.973	0.997	0.974	2.3
	#3	0.928	0.953	0.887	0.923	3.6
	#4	1.029	1.041	1.105	1.058	3.9
	Average				**0.96**	3.4

	%RSD (Labeling)				**7.7**	
Dns-Lys	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**4.0**	
Dns-His	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.041	0.974	0.989	1.001	3.5
	#2	1.092	1.065	1.033	1.063	2.8
	#3	0.996	1.054	1.033	1.028	2.9
	#4	0.977	1.020	0.935	0.977	4.3
	Average				**1.02**	3.4
	%RSD (Labeling)				**3.6**	
Dns-His	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	0.975	1.033	1.029	1.012	3.2
	#2	0.995	0.973	0.929	0.966	3.5
	#3	0.981	0.976	1.010	0.989	1.9
	#4	0.936	0.929	0.885	0.917	3.0
	Average				**0.97**	2.9
	%RSD (Labeling)				**4.2**	
Dns-His	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**4.7**	
Dns-Tyr	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.039	1.016	1.085	1.047	3.4
	#2	1.101	1.042	1.029	1.057	3.6
	#3	0.957	0.946	0.889	0.931	3.9
	#4	0.975	1.036	0.984	0.998	3.3
	Average				**1.01**	3.6
	%RSD (Labeling)				**5.7**	
Dns-Tyr	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.015	0.993	0.970	0.993	2.3
	#2	1.025	1.020	0.955	1.000	3.9
	#3	1.017	1.031	0.985	1.011	2.3
	#4	1.064	1.058	1.115	1.079	2.9
	Average				**1.02**	2.9
	%RSD (Labeling)				**3.9**	
Dns-Tyr	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**-1.2**	
Dns-r-amino-butyric acid	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.075	1.031	1.098	1.068	3.2
	#2	1.112	1.043	1.027	1.061	4.3
	#3	1.013	1.082	1.071	1.055	3.5
	#4	0.932	0.954	0.997	0.961	3.4
	Average				**1.04**	3.6
	%RSD (Labeling)				**4.9**	
Dns-r-amino-butyric acid	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.032	0.970	0.988	0.997	3.2
	#2	0.976	0.967	1.045	0.996	4.3
	#3	0.932	0.961	0.985	0.959	2.8
	#4	1.102	1.029	1.111	1.081	4.2

	Average				**1.01**	3.6
	%RSD (Labeling)				**5.1**	
Dns-r-amino-butyric acid	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**2.7**	
Dns-Cadaverine	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.897	0.923	0.948	0.923	2.8
	#2	0.969	0.970	0.954	0.964	0.9
	#3	0.993	1.030	1.049	1.024	2.8
	#4	0.988	0.974	0.967	0.976	1.1
	Average				**0.97**	1.9
	%RSD (Labeling)				**4.3**	
Dns-Cadaverine	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.024	0.981	0.976	0.994	2.7
	#2	0.895	0.857	0.905	0.886	2.9
	#3	0.902	0.930	0.974	0.935	3.9
	#4	1.018	0.995	0.973	0.995	2.3
	Average				**0.95**	2.9
	%RSD (Labeling)				**5.5**	
Dns-Cadaverine	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**2.0**	
Dns-4-acetyamidophenol	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.886	0.924	0.932	0.914	2.7
	#2	0.974	0.996	0.967	0.979	1.5
	#3	1.062	0.982	1.054	1.033	4.3
	#4	1.029	1.035	0.975	1.013	3.3
	Average				**0.98**	2.9
	%RSD (Labeling)				**5.3**	
Dns-4-acetyamidophenol	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	0.984	0.949	0.994	0.976	2.4
	#2	1.022	1.046	0.993	1.020	2.6
	#3	1.115	1.029	1.100	1.081	4.2
	#4	0.978	0.961	0.935	0.958	2.3
	Average				**1.01**	2.9
	%RSD (Labeling)				**5.5**	
Dns-4-acetyamidophenol	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**-2.4**	
Dns-Tyramine	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.018	1.029	0.989	1.012	2.0
	#2	0.895	0.942	0.963	0.933	3.7
	#3	0.965	0.989	0.949	0.968	2.1
	#4	1.117	1.043	1.103	1.088	3.6
	Average				**1.00**	2.9
	%RSD (Labeling)				**6.7**	
Dns-Tyramine	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	0.983	0.961	0.919	0.954	3.4
	#2	0.972	0.993	1.025	0.997	2.7

	#3	1.033	1.054	0.992	1.026	3.1
	#4	0.857	0.934	0.907	0.899	4.3
	Average				**0.97**	3.4
	%RSD (Labeling)				**5.7**	
Dns-Tyramine	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**3.1**	
Dns-Unknown-#1	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.042	1.051	0.987	1.027	3.4
504.1256/506.1325 (3.56min.)	**#2**	0.963	1.035	0.954	0.984	4.5
	#3	1.010	1.079	1.103	1.064	4.5
	#4	1.027	1.083	1.065	1.058	2.7
	Average				**1.03**	3.8
	%RSD (Labeling)				**3.6**	
Dns-Unknown-#1	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.082	1.131	1.067	1.093	3.1
504.1256/506.1325 (3.56min.)	**#2**	1.008	1.047	0.997	1.017	2.6
	#3	1.057	1.049	1.024	1.043	1.6
	#4	0.997	0.919	0.956	0.957	4.1
	Average				**1.03**	2.8
	%RSD (Labeling)				**5.5**	
Dns-Unknown-#1	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**0.5**	
Dns-Unknown-#2	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	1.084	1.077	1.050	1.070	1.7
402.0862/404.0928 (4.19min.)	**#2**	1.088	1.027	1.069	1.061	2.9
	#3	0.976	0.970	0.996	0.981	1.4
	#4	0.963	0.974	0.996	0.978	1.7
	Average				**1.02**	1.9
	%RSD (Labeling)				**4.9**	
Dns-Unknown-#2	**-80 ^{o}C (^{12}C)/-20 ^{o}C (^{13}C) # 1**	1.105	1.061	1.029	1.065	3.6
402.0862/404.0928 (4.19min.)	**#2**	1.053	1.020	0.987	1.020	3.2
	#3	1.066	1.071	1.007	1.048	3.4
	#4	1.113	1.181	1.101	1.132	3.8
	Average				**1.07**	3.5
	%RSD (Labeling)				**4.5**	
Dns-Unknown-#2	%Diff Between -20 ^{o}C/-80 ^{o}C Labeling				**-4.2**	
Dns-Unknown-#3	**-20 ^{o}C (^{12}C)/-80 ^{o}C (^{13}C) # 1**	0.981	0.972	0.907	0.953	4.2
374.1172/376.1238 (5.85min.)	**#2**	1.032	1.055	1.016	1.034	1.9
	#3	1.091	1.101	1.041	1.078	3.0
	#4	0.979	0.962	1.032	0.991	3.7

	Average				**1.01**	3.2
	%RSD (Labeling)				**5.3**	
Dns-Unknown-#3	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.085	1.046	1.029	1.053	2.7
374.1172/376.1238 (5.85min.)	**#2**	0.979	0.986	1.039	1.001	3.3
	#3	0.978	1.037	1.019	1.011	3.0
	#4	0.997	0.905	0.937	0.946	4.9
	Average				**1.00**	3.5
	%RSD (Labeling)				**4.4**	
Dns-Unknown-#3	%Diff Between -20 °C/-80 °C Labeling				**1.1**	
Dns-Unknown-#4	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	1.008	0.973	1.030	1.004	2.9
386.1056/388.1121(7.92min.)	**#2**	0.975	0.929	1.006	0.970	4.0
	#3	1.036	0.984	1.010	1.010	2.6
	#4	1.112	1.082	1.137	1.110	2.5
	Average				**1.02**	3.0
	%RSD (Labeling)				**5.9**	
Dns-Unknown-#4	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.052	1.091	1.110	1.084	2.7
386.1056/388.1121(7.92min.)	**#2**	1.038	1.115	1.068	1.074	3.6
	#3	0.975	1.023	0.999	0.999	2.4
	#4	0.983	1.029	1.060	1.024	3.8
	Average				**1.05**	3.1
	%RSD (Labeling)				**3.9**	
Dns-Unknown-#4	%Diff Between -20 °C/-80 °C Labeling				**-2.1**	
Dns-Unknown-#5	**-20 °C (^{12}C)/-80 °C (^{13}C) # 1**	1.156	1.089	1.076	1.107	3.9
415.07712/417.0842 (9.26min.)	**#2**	1.057	1.124	1.043	1.075	4.0
	#3	1.028	1.036	0.975	1.013	3.3
	#4	0.886	0.965	0.957	0.936	4.6
	Average				**1.03**	4.0
	%RSD (Labeling)				**7.3**	
Dns-Unknown-#5	**-80 °C (^{12}C)/-20 °C (^{13}C) # 1**	1.043	1.023	1.100	1.055	3.8
415.07712/417.0842 (9.26min.)	**#2**	0.975	1.035	0.992	1.001	3.1
	#3	0.904	0.972	0.957	0.944	3.8
	#4	1.069	1.025	1.006	1.033	3.1
	Average				**1.01**	3.4
	%RSD (Labeling)				**4.8**	
Dns-Unknown-#5	%Diff Between -20 °C/-80 °C Labeling				**2.4**	

Average of the ratios for -20 °C (^{12}C) / -80 °C (^{13}C)				**1.004**		
Average of the ratios for -80 °C (^{12}C) / -20 °C (^{13}C)				**1.007**		
Average of % RSD (run-to-run replicates)				**3.2**		
Average of % RSD (labeling replicates)				**5.3**		
Average of % difference of -20 °C /-80 °C labeling				**-0.4**		

Table S3.3. Relative abundances normalized against the pooled urine sample and absolute concentrations of 20 amino acids detected in "Day-1" to "Day-5" urine samples.

Compound	Urine Day 1	Urine Day 2	Urine Day 3	Urine Day 4	Urine Day 5	Average Day 1-5	Pooled Urine	% Diff.
Gln: Ratio[a)]	1.11	0.95	0.93	1.14	0.95	1.02	1.00	1.6[b)]
Gln: Concentration (μM)	570	666	681	555	666	**628**	**633**	**-0.8**[c)]
Ser: Ratio	1.27	0.89	0.95	1.10	1.02	1.05	1.00	4.6
Ser: Concentration (μM)	402	574	538	465	501	**496**	**511**	**-2.9**
Glu: Ratio	0.86	1.65	1.19	0.89	0.72	1.06	1.00	6.2
Glu: Concentration (μM)	24.8	12.9	17.9	23.9	29.6	**21.8**	**21.3**	**2.4**
Asp: Ratio	0.89	0.97	0.94	1.31	1.11	1.04	1.00	4.4
Asp: Concentration (μM)	101.2	92.9	95.9	68.8	81.2	**88**	**90.1**	**-2.3**
Thr: Ratio	1.32	1.01	0.92	0.94	0.97	1.03	1.00	3.2
Thr: Concentration (μM)	119	155	171	167	162	**155**	**157**	**-1.4**
Gly: Ratio	1.17	0.86	0.89	1.20	1.21	1.07	1.00	6.6
Gly: Concentration (μM)	2144	2916	2818	2090	2073	**2408**	**2508**	**-4.0**
Ala: Ratio	0.88	0.94	1.01	1.32	0.97	1.02	1.00	2.4
Ala: Concentration (μM)	674	631	587	449	611	**590**	**593**	**-0.4**
Asn: Ratio	1.17	0.96	1.04	1.15	1.02	1.07	1.00	6.8
Asn: Concentration(μmol/L)	114	139	128	116	130	**125**	**133**	**-5.8**
Pro: Ratio	0.94	1.20	1.09	0.85	1.18	1.05	1.00	5.2
Pro: Concentration (μM)	14.0	11.0	12.1	15.5	11.2	**12.8**	**13.2**	**-3.2**
Val: Ratio	1.06	0.98	1.11	1.07	1.04	1.05	1.00	5.2
Val: Concentration (μM)	70.5	76.2	67.3	69.8	71.8	**71.1**	**74.7**	**-4.8**
Met: Ratio	1.23	0.73	1.07	1.23	1.10	1.07	1.00	7.2
Met: Concentration (μM)	12.6	21.2	14.5	12.6	14.1	**15.0**	**15.5**	**-3.2**
Trp: Ratio	0.90	0.91	1.02	1.11	1.26	1.04	1.00	4.0
Trp: Concentration (μM)	133	132	118	108	95	**117**	**120**	**-2.3**
Phe: Ratio	1.00	0.95	0.93	1.14	0.95	0.99	1.00	-0.6

Phe: Concentration (μM)	90.0	94.7	96.8	78.9	94.7	**91.0**	**90.0**	**1.2**
Ile: Ratio	1.23	0.87	1.17	1.24	0.95	1.09	1.00	9.2
Ile: Concentration (μM)	19.9	28.2	20.9	19.8	25.8	**22.9**	**24.5**	**-6.5**
Leu: Ratio	1.23	0.97	0.98	1.27	0.91	1.07	1.00	7.2
Leu: Concentration (μM)	43.3	54.9	54.4	42.0	58.6	**50.6**	**53.3**	**-5.0**
Arg: Ratio	1.10	0.97	0.94	1.34	1.18	1.11	1.00	10.6
Arg: Concentration (μM)	33.0	37.4	38.6	27.1	30.8	**33**	**36.3**	**-8.0**
Cystine: Ratio	0.87	0.95	0.91	1.32	1.29	1.07	1.00	6.8
Cystine: Concentration (μM)	184	168	176	121	124	**155**	**160**	**-3.3**
Lys: Ratio	1.15	1.07	0.85	1.32	1.01	1.08	1.00	8.0
Lys: Concentration (μM)	160	172	216	139	182	**174**	**184**	**-5.4**
His: Ratio	1.17	0.88	0.84	0.98	1.1	0.99	1.00	-0.6
His: Concentration (μM)	1329	1767	1851	1587	1414	**1590**	**1555**	**2.2**
Tyr: Ratio	1.16	1.15	0.99	1.04	0.93	1.05	1.00	5.4
Tyr: Concentration (μM)	277	279	324	309	345	**307**	**321**	**-4.4**
Average of % Ratio Diff.								**5.2**
Average of % Conc. Diff.								**-2.9**

Comments:

a) Peak ratio of the pooled sample and an individual day sample.

b) % Ratio Diff. = (average ratio of day 1-5 samples – 1.00)/1.00

c) % Conc. Diff. = (average conc. of day 1-5 samples – pooled urine sample conc.)/(pooled urine sample conc.)

Table S3.4. Ion pairs of amine- and phenol-containing metabolites detected by fast RPLC-FTICR MS from a mixture of 1:1 urine labeled by ^{12}C-/^{13}C-dansylation. 85 metabolites were identified on the basis of accurate mass, retention time and spectra profile compared to those of the labeled standards.

#	RT	^{12}C-Dns-labeled	^{13}C-Dns-labeled	ID	Mass Differences	Mass Diff. Error	Ion Intensity
	(min)	(m/z)	(m/z)			(ppm)	
1	1.02	387.06816	389.07468	Unknown	2.00651	0.5	1.2E+06
2	1.02	521.17022	523.17724	Unknown	2.00702	-0.6	1.2E+06
3	1.06	389.12802	391.13469	Unknown	2.00668	0.1	8.0E+06
4	1.06	424.11749	426.12418	Unknown	2.00668	0.1	1.7E+06
5	1.06	375.07767	377.08429	**Dns-o-phosphoethanolamine**	2.00662	0.3	1.0E+06
6	1.09	389.12800	391.13460	Unknown	2.00661	0.3	4.0E+06
7	1.09	413.13764	415.14462	**Dns-glucosamine**	2.00661	0.3	6.5E+05
8	1.16	388.10771	390.11446	Unknown	2.00675	-0.1	1.8E+06
9	1.16	495.08935	497.09641	Unknown	2.00707	-0.7	2.0E+06

10	1.16	517.07124	519.07836	Unknown	2.00712	-0.8	2.0E+06
11	1.16	526.13127	528.13863	Unknown	2.00736	-1.2	2.5E+06
12	1.16	533.04460	535.05198	Unknown	2.00737	-1.2	5.0E+05
13	1.16	555.12131	557.12830	Unknown	2.00699	-0.5	3.5E+06
14	1.16	569.19122	571.19789	Unknown	2.00667	0.1	3.5E+06
15	1.20	359.07297	361.07979	**Dns-taurine**	2.00683	-0.3	1.5E+07
16	1.20	381.05504	383.06183	Unknown	2.00679	-0.2	1.4E+06
17	1.20	390.11518	392.12206	Unknown	2.00689	-0.4	4.0E+06
18	1.20	560.11343	562.12022	Unknown	2.00679	-0.1	1.1E+06
19	1.26	403.14191	405.14841	**3-methyl-histidine**	2.00650	0.5	2.2E+07
20	1.26	421.15607	423.16273	Unknown	2.00666	0.1	5.5E+05
21	1.26	501.15414	503.16058	Unknown	2.00644	0.5	1.0E+06
22	1.26	512.20725	514.21320	Unknown	2.00595	1.5	5.0E+05
23	1.26	719.14514	721.15073	Unknown	2.00559	1.6	1.0E+06
24	1.26	763.21535	765.22101	Unknown	2.00567	1.4	1.5E+06
25	1.26	779.17712	781.18345	Unknown	2.00633	0.5	5.0E+05
26	1.33	410.09029	412.09703	Unknown	2.00674	-0.1	1.3E+06
27	1.33	425.12601	427.13231	Unknown	2.00631	0.9	6.0E+05
28	1.33	452.18523	454.19187	Unknown	2.00664	0.1	5.0E+05
29	1.33	504.12610	506.13337	Unknown	2.00727	-1.1	1.0E+06
30	1.40	343.07829	345.08513	**Dns-hypotaurine**	2.00685	-0.4	3.5E+05
31	1.40	524.18096	526.18805	Unknown	2.00709	-0.7	1.7E+06
32	1.33	805.27914	807.28613	Unknown	2.00698	-0.3	1.0E+06
33	1.33	807.28613	809.29166	Unknown	2.00553	1.5	1.0E+06
34	1.40	380.16414	382.17066	Unknown	2.00653	0.5	1.7E+06
35	1.40	382.10688	384.11359	Unknown	2.00671	0.0	2.4E+06
36	1.47	424.11763	426.12418	Unknown	2.00655	0.4	5.0E+06
37	1.40	460.16502	462.17197	**Dns-carnosine**	2.00696	-0.5	1.0E+06
38	1.40	542.18003	544.18675	Unknown	2.00671	0.0	2.7E+06
39	1.40	558.15733	560.16420	Unknown	2.00687	-0.3	1.1E+06
40	1.47	345.05776	346.06113	Unknown	1.00337	0.0	3.0E+06
41	1.47	408.19588	409.19917	Unknown	1.00329	0.2	1.0E+06
42	1.47	470.11990	473.12995	Unknown	3.01006	0.0	7.0E+06
43	1.47	501.16228	504.17176	Unknown	3.00948	1.2	4.0E+06
44	1.47	518.13239	520.13961	Unknown	2.00722	-1.0	6.0E+05
45	1.47	531.10357	533.11051	Unknown	2.00693	-0.4	2.2E+06
46	1.55	366.11208	368.11870	Unknown	2.00662	0.3	1.5E+06
47	1.55	408.17040	410.17708	**Dns-Arg**	2.00668	0.1	4.5E+06
48	1.58	422.18605	423.18950	Unknown	1.00345	-0.2	1.5E+06
49	1.58	425.10155	427.10842	Unknown	2.00687	-0.4	2.5E+06
50	1.58	468.14383	470.15068	Unknown	2.00686	-0.3	1.3E+06
51	1.58	474.18076	476.18749	**Dns-homocarnosine**	2.00673	0.0	6.5E+05
52	1.58	494.15844	496.16469	Unknown	2.00626	0.9	2.5E+06

53	1.58	680.15765	682.16422	Unknown	2.00657	0.2	5.0E+06
54	1.58	711.19948	713.20588	Unknown	2.00640	0.4	1.0E+06
55	1.58	366.11190	368.11835	**Dns-Asn**	2.00645	0.7	1.5E+07
56	1.58	388.09417	390.10090	Unknown	2.00673	0.0	6.5E+05
57	1.58	397.15439	399.16091	Unknown	2.00652	0.5	1.4E+06
58	1.68	531.16581	533.17279	Unknown	2.00698	-0.5	2.5E+06
59	1.65	702.13690	704.14332	Unknown	2.00642	0.4	1.0E+06
60	1.65	424.15376	426.16063	Unknown	2.00688	-0.4	1.5E+06
61	1.68	408.19532	410.20200	Unknown	2.00668	0.1	7.0E+06
62	1.72	380.12772	382.13438	Unknown	2.00666	0.1	7.0E+06
63	1.72	422.20920	424.21579	Unknown	2.00659	0.3	4.0E+07
64	1.72	444.18990	446.19708	Unknown	2.00718	-1.1	7.0E+05
65	1.75	471.13648	473.14295	Unknown	2.00646	0.5	1.4E+07
66	1.72	517.13012	519.13677	Unknown	2.00665	0.1	8.0E+05
67	1.72	535.18570	537.19198	Unknown	2.00628	0.8	1.6E+06
68	1.75	380.12344	382.12992	**Dns-Gln**	2.00648	0.6	2.5E+07
69	1.75	402.10521	404.11221	Unknown	2.00700	-0.7	7.0E+05
70	1.75	411.16512	413.17194	Unknown	2.00682	-0.3	2.5E+06
71	1.75	438.16293	440.16989	Unknown	2.00696	-0.6	2.5E+06
72	1.75	493.17851	495.18542	Unknown	2.00691	-0.4	1.0E+06
73	1.75	502.17826	504.18509	Unknown	2.00683	-0.2	2.0E+06
74	1.75	611.17865	613.18500	Unknown	2.00635	0.6	1.2E+06
75	1.75	680.15194	682.15817	Unknown	2.00623	0.7	5.0E+05
76	1.75	761.24685	763.25360	Unknown	2.00623	0.6	1.6E+06
77	1.82	321.09084	323.09753	Unknown	2.00669	0.1	8.5E+05
78	1.82	385.08905	387.09567	Unknown	2.00662	0.2	6.5E+06
79	1.82	416.13114	418.13820	Unknown	2.00706	-0.8	8.0E+05
80	1.82	515.16903	517.17562	Unknown	2.00659	0.2	1.4E+07
81	1.82	556.19470	558.20147	Unknown	2.00677	-0.1	2.5E+06
82	1.89	409.15429	411.16050	**Dns-L-citrulline**	2.00621	1.2	7.0E+05
83	1.89	545.18154	547.18850	Unknown	2.00696	-0.5	3.0E+06
84	1.96	388.10786	390.11464	Unknown	2.00678	-0.2	2.0E+06
85	1.96	504.14320	506.14998	Unknown	2.00678	-0.1	9.0E+06
86	1.96	562.09764	564.10460	Unknown	2.00696	-0.4	1.2E+06
87	1.96	574.09709	576.10429	Unknown	2.00720	-0.8	8.0E+05
88	2.03	436.20049	438.20719	Unknown	2.00670	0.0	3.6E+07
89	2.03	359.15408	361.16075	**Dns-1 (or 3-)-methylhistamine**	2.00667	0.1	8.0E+05
90	2.03	450.20317	452.21008	Unknown	2.00691	-0.4	1.1E+06
91	2.03	458.18143	460.18853	Unknown	2.00710	-0.9	7.0E+05
92	2.03	479.15815	481.16503	Unknown	2.00687	-0.3	1.2E+06
93	2.10	501.15483	503.16159	**Dns-adenosine**	2.00676	-0.1	1.1E+06
94	2.03	504.14211	506.14882	Unknown	2.00671	0.0	2.6E+06
95	2.03	525.13579	527.14272	Unknown	2.00693	-0.4	8.5E+05

96	2.10	353.11701	355.12373	Unknown	2.00672	0.0	8.0E+05
97	2.10	366.11223	368.11890	**Dns-aspartic acid amide**	2.00667	0.1	1.0E+06
98	2.10	421.22639	423.23366	Unknown	2.00727	-1.3	1.3E+06
99	2.10	471.08832	473.09507	Unknown	2.00675	-0.1	1.0E+06
100	2.17	424.10592	426.11263	Unknown	2.00670	0.0	8.0E+05
101	2.17	661.22733	663.23440	Unknown	2.00707	-0.5	7.5E+05
102	2.24	484.13198	488.14512	Unknown	4.01314	0.6	3.5E+07
103	2.24	515.17366	519.18743	Unknown	4.01377	-0.7	1.0E+07
104	2.24	544.16585	548.17936	Unknown	4.01351	-0.2	1.4E+06
105	2.24	830.29820	834.31176	Unknown	4.01356	-0.2	1.3E+06
106	2.31	339.10099	341.10760	**Dns-Ser**	2.00661	0.3	3.0E+07
107	2.31	370.14371	372.15011	Unknown	2.00640	0.8	7.5E+05
108	2.13	506.11769	510.13141	Unknown	4.01372	-0.6	1.4E+06
109	2.31	583.18551	585.19313	Unknown	2.00761	-1.5	6.5E+05
110	2.31	677.19452	679.20091	Unknown	2.00639	0.5	1.5E+06
111	2.31	679.20091	681.20771	Unknown	2.00680	-0.1	1.0E+06
112	2.31	685.26362	687.27056	Unknown	2.00694	-0.3	1.5E+06
113	2.31	822.22966	824.23523	Unknown	2.00557	1.4	6.0E+05
114	2.31	826.24221	828.24904	Unknown	2.00683	-0.1	6.0E+05
115	2.38	423.17003	425.17707	Unknown	2.00704	-0.8	3.5E+06
116	2.38	492.14325	494.14999	Unknown	2.00674	-0.1	9.0E+05
117	2.38	593.19132	595.19836	Unknown	2.00704	-0.5	6.0E+05
118	2.45	579.17541	581.18204	Unknown	2.00663	0.1	3.0E+06
119	2.45	608.27490	610.28192	Unknown	2.00702	-0.5	5.0E+05
120	2.52	353.11683	355.12354	Unknown	2.00671	0.0	2.6E+06
121	2.52	408.15897	410.16566	Unknown	2.00669	0.0	1.0E+06
122	2.58	381.11168	383.11835	**Dns-Glu**	2.00667	0.1	5.0E+06
123	2.58	499.17448	501.18147	Unknown	2.00698	-0.5	2.5E+06
124	2.62	392.20035	394.20702	Unknown	2.00667	0.1	8.5E+06
125	2.62	516.15495	518.16175	Unknown	2.00681	-0.2	6.0E+06
126	2.69	365.11689	367.12355	**Dns-4-hydroxyproline**	2.00665	0.1	4.0E+06
127	2.69	367.09609	369.10286	**Dns-Asp**	2.00678	-0.2	3.2E+06
128	2.72	484.12801	486.13547	Unknown	2.00747	-1.6	1.2E+06
129	2.72	646.19209	648.19948	Unknown	2.00740	-1.1	1.0E+06
130	2.72	519.19090	521.19792	Unknown	2.00703	-0.6	7.5E+05
131	2.72	560.16988	562.17696	Unknown	2.00709	-0.7	6.5E+05
132	2.76	498.13681	500.14393	Unknown	2.00712	-0.8	9.0E+05
133	2.76	439.15355	441.16041	Unknown	2.00686	-0.3	8.0E+05
134	2.79	379.01859	380.02191	Unknown	1.00332	0.1	3.5E+06
135	2.86	471.08908	473.09620	Unknown	2.00713	-0.9	1.4E+06
136	2.86	341.09898	343.10569	Unknown	2.00671	0.0	1.8E+06
137	2.86	372.14137	374.14788	Unknown	2.00651	0.5	8.5E+05
138	2.86	462.16936	464.17613	Unknown	2.00677	-0.1	7.0E+05

139	2.93	442.11669	444.12381	Unknown	2.00712	-0.9	5.5E+05
140	2.93	452.18536	454.19212	Unknown	2.00676	-0.1	5.5E+05
141	2.93	462.16958	464.17634	Unknown	2.00675	-0.1	6.5E+05
142	2.93	574.09836	576.10552	Unknown	2.00717	-0.8	7.0E+05
143	2.93	621.21085	623.21769	Unknown	2.00684	-0.2	1.4E+06
144	3.00	251.08473	253.09117	**Dns-NH_3**	2.00644	1.1	2.8E+07
145	3.00	487.14876	489.15482	Unknown	2.00607	1.3	2.0E+06
146	3.00	488.15162	490.15788	Unknown	2.00626	0.9	2.5E+06
147	3.00	501.16307	503.16956	Unknown	2.00649	0.4	1.2E+07
148	3.00	503.16956	505.17703	Unknown	2.00747	-1.5	1.0E+07
149	3.00	531.17346	533.18019	Unknown	2.00673	0.0	1.5E+06
150	3.00	532.17672	534.18396	Unknown	2.00724	-1.0	1.8E+06
151	3.00	590.30063	592.30737	Unknown	2.00674	-0.1	2.8E+06
152	3.07	422.17494	424.18157	Unknown	2.00664	0.2	3.0E+06
153	3.07	475.06648	477.07307	Unknown	2.00659	0.3	4.0E+06
154	3.07	523.14372	525.15073	Unknown	2.00701	-0.6	1.5E+06
155	3.07	525.15073	527.15816	Unknown	2.00743	-1.4	1.0E+06
156	3.11	353.11699	355.12366	**Dns-Thr**	2.00667	0.1	7.0E+06
157	3.17	603.19451	605.20106	Unknown	2.00655	0.3	2.0E+06
158	3.11	605.20106	607.20805	Unknown	2.00700	-0.5	2.0E+06
159	3.11	395.12738	397.13426	**Dns-aminoadipic acid**	2.00689	-0.4	3.5E+06
160	3.14	516.09042	518.09749	Unknown	2.00706	-0.7	2.8E+06
161	3.14	295.11089	297.11754	**Dns-ethanolamine**	2.00665	0.2	3.0E+07
162	3.17	336.13804	338.14464	Unknown	2.00660	0.3	1.0E+07
163	3.17	358.11985	360.12669	Unknown	2.00684	-0.4	1.0E+06
164	3.17	367.18011	369.18688	Unknown	2.00677	-0.2	1.0E+06
165	3.17	378.22152	380.22806	Unknown	2.00654	0.4	2.0E+06
166	3.17	545.18885	547.19555	Unknown	2.00670	0.0	4.0E+06
167	3.17	547.19555	549.20257	Unknown	2.00702	-0.6	4.0E+06
168	3.17	589.21431	591.22106	Unknown	2.00675	-0.1	3.0E+06
169	3.17	591.22106	593.22746	Unknown	2.00640	0.5	3.0E+06
170	3.17	611.19565	613.20216	Unknown	2.00651	0.3	3.0E+06
171	3.17	613.20216	615.20880	Unknown	2.00664	0.1	3.0E+06
172	3.24	462.16936	464.17611	Unknown	2.00675	-0.1	1.5E+07
173	3.24	481.11018	483.11688	Unknown	2.00671	0.0	3.5E+06
174	3.24	493.21128	495.21830	Unknown	2.00702	-0.6	2.0E+06
175	3.24	512.15214	514.15977	Unknown	2.00763	-1.8	9.0E+05
176	3.24	600.19870	602.20509	Unknown	2.00639	0.5	8.0E+06
177	3.31	418.21600	420.22286	Unknown	2.00686	-0.4	7.0E+06
178	3.35	577.14704	579.15379	Unknown	2.00675	-0.1	5.0E+06
179	3.35	295.07492	296.07822	Unknown	1.00331	0.2	3.0E+06
180	3.35	400.07322	401.07649	Unknown	1.00327	0.2	1.0E+06
181	3.35	491.19533	493.20207	Unknown	2.00674	-0.1	9.0E+05

182	3.35	515.16761	517.17490	Unknown	2.00730	-1.1	1.7E+06
183	3.35	535.12105	538.13174	Unknown	3.01069	-1.2	1.3E+06
184	3.35	577.14704	579.15379	Unknown	2.00675	-0.1	4.0E+06
185	3.41	389.12796	391.13450	Unknown	2.00654	0.4	3.0E+07
186	3.41	415.13291	417.13927	Unknown	2.00636	0.8	2.5E+06
187	3.41	425.08378	427.09102	Unknown	2.00724	-1.2	1.4E+06
188	3.41	449.08408	451.09104	Unknown	2.00697	-0.6	3.5E+06
189	3.41	504.07982	507.08983	Unknown	3.01001	0.1	5.0E+06
190	3.41	519.14129	521.14777	Unknown	2.00648	0.5	3.5E+06
191	3.41	535.12098	538.13104	Unknown	3.01006	0.0	9.0E+05
192	3.41	584.20935	586.21595	Unknown	2.00661	0.2	7.0E+05
193	3.49	309.08975	311.09647	**Dns-Gly**	2.00672	0.0	6.0E+07
194	3.49	478.12808	480.13488	Unknown	2.00680	-0.2	1.0E+07
195	3.49	559.16889	561.17553	Unknown	2.00664	0.1	2.5E+06
196	3.45	617.17395	619.18073	Unknown	2.00679	-0.1	5.0E+06
197	3.45	619.18073	621.18714	Unknown	2.00640	0.5	4.0E+06
198	3.49	786.21129	788.21765	Unknown	2.00636	0.4	1.0E+06
199	3.49	788.21765	790.22427	Unknown	2.00662	0.1	1.0E+06
200	3.49	851.36405	853.37074	Unknown	2.00669	0.0	7.5E+05
201	3.59	463.23760	465.24462	Unknown	2.00702	-0.7	1.0E+06
202	3.59	464.14904	466.15499	Unknown	2.00595	1.6	1.0E+06
203	3.56	504.12567	506.13250	Unknown	2.00683	-0.2	6.0E+06
204	3.56	602.09988	604.10631	Unknown	2.00643	0.5	6.5E+05
205	3.62	350.15356	352.16039	Unknown	2.00683	-0.3	6.5E+05
206	3.62	423.06811	425.07495	Unknown	2.00684	-0.3	6.5E+05
207	3.69	520.12091	522.12756	Unknown	2.00665	0.1	2.7E+06
208	3.59	561.15201	563.15914	Unknown	2.00713	-0.7	7.5E+06
209	3.66	325.62852	326.63186	Unknown	1.00334	0.0	1.2E+06
210	3.66	365.11695	367.12366	Unknown	2.00671	0.0	1.2E+06
211	3.66	406.14338	408.14999	Unknown	2.00661	0.2	1.0E+06
212	3.66	427.09957	429.10624	Unknown	2.00668	0.1	1.3E+06
213	3.66	650.24903	652.25563	Unknown	2.00659	0.2	2.2E+06
214	3.69	348.13798	350.14451	Unknown	2.00653	0.5	1.1E+07
215	3.73	347.11756	349.12414	Unknown	2.00658	0.4	1.2E+07
216	3.73	364.16903	366.17531	Unknown	2.00629	1.2	2.5E+07
217	3.73	359.04666	360.05004	Unknown	1.00338	-0.1	1.2E+06
218	3.73	379.18003	381.18697	Unknown	2.00695	-0.6	4.0E+06
219	3.73	395.21179	397.21819	Unknown	2.00640	0.8	3.0E+06
220	3.73	422.17498	424.18134	Unknown	2.00636	0.8	8.5E+06
221	3.73	438.11544	440.12236	Unknown	2.00692	-0.5	6.0E+05
222	3.73	452.18569	454.19253	Unknown	2.00684	-0.3	1.0E+06
223	3.73	456.07223	458.07947	Unknown	2.00724	-1.2	8.0E+05
224	3.73	489.08165	491.08910	Unknown	2.00744	-1.5	1.8E+06

225	3.80	309.12686	311.13375	Unknown	2.00689	-0.6	1.0E+06
226	3.80	436.18995	438.19676	Unknown	2.00681	-0.2	1.0E+07
227	3.80	467.23248	469.23916	Unknown	2.00668	0.1	2.5E+06
228	3.80	480.14322	482.14963	Unknown	2.00641	0.6	2.2E+06
229	3.80	531.14727	533.15420	Unknown	2.00693	-0.4	3.0E+06
230	3.80	573.18240	575.19013	Unknown	2.00774	-1.8	1.0E+06
231	3.80	575.19013	577.19760	Unknown	2.00746	-1.3	1.0E+06
232	3.80	663.37927	665.38618	Unknown	2.00691	-0.3	8.0E+05
233	3.80	673.36373	675.37044	Unknown	2.00671	0.0	7.0E+05
234	3.87	588.17521	590.18190	Unknown	2.00669	0.0	1.6E+06
235	3.87	537.20153	539.20820	Unknown	2.00667	0.1	1.2E+06
236	3.87	541.20360	543.21060	Unknown	2.00699	-0.5	1.4E+06
237	3.87	502.17578	504.18285	Unknown	2.00707	-0.7	1.4E+06
238	3.94	323.10542	325.11234	**Dns-Ala**	2.00692	-0.6	6.0E+07
239	3.94	365.11666	367.12351	Unknown	2.00685	-0.4	3.0E+06
240	3.94	395.12747	397.13456	Unknown	2.00710	-1.0	2.0E+06
241	3.94	445.05618	447.06334	Unknown	2.00716	-1.0	2.3E+06
242	4.01	379.13253	381.13936	Unknown	2.00683	-0.3	8.5E+05
243	4.01	546.13662	548.14392	Unknown	2.00731	-1.1	6.5E+05
244	4.04	337.12189	339.12853	**Dns-r-amino-butyric acid**	2.00663	0.2	2.6E+06
245	4.04	342.13560	343.13906	Unknown	1.00346	-0.3	2.5E+06
246	4.07	481.07328	483.08058	Unknown	2.00730	-1.2	1.1E+07
247	4.07	401.11697	403.12367	Unknown	2.00670	0.0	1.3E+06
248	4.07	428.12768	430.13453	**Dns-p-amino-hippuric acid**	2.00686	-0.3	1.3E+06
249	4.07	503.05494	505.06218	Unknown	2.00724	-1.0	2.6E+06
250	4.07	512.11503	514.12223	Unknown	2.00719	-0.9	2.5E+06
251	4.07	519.02817	521.03502	Unknown	2.00685	-0.3	6.0E+05
252	4.07	541.10442	543.11157	Unknown	2.00716	-0.8	4.5E+06
253	4.07	571.18341	573.18995	Unknown	2.00655	0.3	1.2E+06
254	4.11	624.15663	626.16374	Unknown	2.00711	-0.6	6.5E+05
255	4.14	363.17420	365.18069	Unknown	2.00649	0.6	3.0E+06
256	4.14	405.15939	407.16608	Unknown	2.00669	0.1	5.0E+06
257	4.18	469.14654	471.15379	Unknown	2.00725	-1.2	1.5E+06
258	4.21	487.10256	489.11003	Unknown	2.00747	-1.6	2.0E+06
259	4.14	574.18724	576.19460	Unknown	2.00736	-1.1	1.1E+06
260	4.18	402.08637	404.09302	Unknown	2.00664	0.2	1.0E+07
261	4.18	405.15841	407.16519	Unknown	2.00678	-0.2	1.2E+07
262	4.18	358.12247	360.12906	Unknown	2.00659	0.3	1.2E+06
263	4.21	504.14391	506.15099	Unknown	2.00707	-0.7	1.0E+06
264	4.21	386.09213	388.09862	Unknown	2.00649	0.6	4.0E+06
265	4.21	560.18027	562.18731	Unknown	2.00704	-0.6	1.2E+06
266	4.25	502.17082	504.17735	Unknown	2.00653	0.4	5.0E+07
267	4.25	378.18501	380.19176	Unknown	2.00675	-0.1	1.0E+06

268	4.25	477.16099	479.16727	Unknown	2.00628	0.9	5.0E+06
269	4.25	642.76110	644.76791	Unknown	2.00681	-0.2	1.4E+06
270	4.25	689.15800	691.16405	Unknown	2.00604	1.0	6.0E+06
271	4.25	953.31560	957.32934	Unknown	4.01374	-0.3	1.0E+06
272	4.28	411.10451	413.11136	Unknown	2.00686	-0.4	1.5E+06
273	4.28	465.11501	467.12210	Unknown	2.00709	-0.8	1.4E+06
274	4.32	525.14667	527.15291	Unknown	2.00625	0.9	1.5E+06
275	4.32	578.16803	580.17469	Unknown	2.00666	0.1	8.5E+05
276	4.35	323.14252	325.14936	Unknown	2.00684	-0.4	1.3E+06
277	4.35	376.16925	378.17586	**Dns-5-hydroxymethyluricil**	2.00662	0.2	1.5E+06
278	4.35	398.15119	400.15757	Unknown	2.00638	0.8	1.0E+06
279	4.35	407.21219	409.21816	Unknown	2.00597	1.8	8.5E+05
280	4.35	455.13089	457.13775	Unknown	2.00686	-0.3	2.2E+06
281	4.39	428.11861	430.12499	Unknown	2.00638	0.8	1.1E+06
282	4.39	480.18001	482.18706	Unknown	2.00704	-0.7	2.5E+06
283	4.39	577.14868	579.15557	Unknown	2.00689	-0.3	1.2E+06
284	4.39	689.15870	691.16464	Unknown	2.00594	1.1	7.0E+05
285	4.39	693.22666	695.23377	Unknown	2.00710	-0.6	8.0E+05
286	4.42	347.11658	349.12281	Unknown	2.00624	1.4	8.0E+06
287	4.42	337.12075	339.12713	Unknown	2.00639	1.0	8.0E+07
288	4.42	368.16332	370.16985	Unknown	2.00653	0.5	1.3E+06
289	4.42	402.08660	404.09338	Unknown	2.00678	-0.2	1.2E+07
290	4.42	450.18143	452.18805	Unknown	2.00662	0.2	8.0E+05
291	4.42	475.12903	477.13613	Unknown	2.00710	-0.8	5.0E+06
292	4.42	516.19252	518.19951	Unknown	2.00699	-0.5	1.5E+06
293	4.42	589.20855	591.21537	Unknown	2.00682	-0.2	1.5E+06
294	4.49	370.09667	372.10318	Unknown	2.00651	0.5	3.5E+06
295	4.56	433.08896	435.09612	Unknown	2.00716	-1.0	1.0E+06
296	4.56	494.19607	496.20312	Unknown	2.00705	-0.7	1.2E+06
297	4.56	593.16994	595.17696	Unknown	2.00702	-0.5	1.7E+06
298	4.56	675.24175	677.24771	Unknown	2.00596	1.1	4.0E+06
299	4.59	562.12636	564.13207	Unknown	2.00571	1.8	2.0E+07
300	4.59	471.10441	474.11466	Unknown	3.01025	-0.4	1.0E+07
301	4.59	386.09216	388.09876	Unknown	2.00660	0.3	1.2E+06
302	4.59	590.12180	592.12894	Unknown	2.00714	-0.7	1.6E+06
303	4.66	514.16106	516.16783	Unknown	2.00677	-0.1	2.7E+06
304	4.69	351.10132	353.10815	**Dns-5-aminopentanoic acid**	2.00683	-0.3	9.0E+05
305	4.73	337.12200	339.12865	**Dns-tryptophanamide**	2.00665	0.2	3.0E+06
306	4.73	431.13872	433.14553	Unknown	2.00681	-0.2	6.5E+06
307	4.73	393.18461	395.19134	Unknown	2.00673	0.0	1.7E+06
308	4.73	436.19020	438.19687	Unknown	2.00668	0.1	3.5E+06
309	4.73	446.17416	448.18109	Unknown	2.00692	-0.5	8.0E+05
310	4.73	520.12724	522.13395	Unknown	2.00671	0.0	2.2E+06

311	4.76	323.10641	325.11304	**Dns-sarcosine**	2.00663	0.3	5.0E+05
312	4.79	265.10045	267.10719	**Dns-Met-amine**	2.00674	-0.1	8.0E+07
313	4.93	390.11200	392.11857	Unknown	2.00657	0.4	2.0E+07
314	4.83	369.09400	371.10066	Unknown	2.00666	0.1	1.1E+06
315	4.83	401.13909	403.14606	Unknown	2.00696	-0.6	1.5E+06
316	4.89	370.09691	372.10308	Unknown	2.00617	1.4	4.0E+07
317	4.93	424.05246	426.05902	Unknown	2.00656	0.4	5.0E+06
318	4.93	430.12941	432.13609	Unknown	2.00668	0.1	2.8E+06
319	4.93	439.09555	441.10260	Unknown	2.00705	-0.8	5.0E+06
320	4.93	468.17018	470.17715	Unknown	2.00697	-0.6	1.8E+06
321	4.93	483.15585	485.16241	Unknown	2.00656	0.3	1.0E+06
322	4.93	484.08467	486.09173	Unknown	2.00707	-0.7	8.5E+05
323	4.93	741.19351	743.19999	Unknown	2.00647	0.3	1.4E+06
324	4.96	795.14913	797.15428	Unknown	2.00516	1.9	2.0E+06
325	4.96	661.16315	663.16991	Unknown	2.00676	-0.1	3.5E+06
326	4.96	362.15375	364.16041	Unknown	2.00666	0.1	1.0E+07
327	4.96	522.08899	524.09623	Unknown	2.00725	-1.0	1.2E+06
328	4.96	629.26383	631.27075	Unknown	2.00692	-0.3	8.0E+05
329	4.96	791.26316	795.27628	Unknown	4.01312	0.4	2.0E+06
330	5.00	396.13386	398.14038	Unknown	2.00652	0.5	1.2E+07
331	5.03	481.14617	483.15333	Unknown	2.00716	-0.9	1.4E+06
332	5.03	622.17903	625.18916	Unknown	3.01014	-0.1	1.3E+06
333	5.10	337.12198	339.12861	**Dns-2-aminobutyric acid**	2.00664	0.2	1.1E+06
334	5.10	491.09362	493.10077	Unknown	2.00716	-0.9	1.2E+06
335	5.13	349.12187	351.12861	**Dns-Pro**	2.00674	-0.1	1.0E+07
336	5.13	578.10791	581.11776	Unknown	3.00985	0.4	6.0E+06
337	5.13	545.15211	547.15914	Unknown	2.00702	-0.6	2.2E+06
338	5.20	534.14324	536.14977	Unknown	2.00653	0.3	2.5E+06
339	5.20	550.13867	552.14483	Unknown	2.00617	1.0	1.3E+06
340	5.30	576.14626	578.15355	Unknown	2.00729	-1.0	1.3E+06
341	5.30	351.13744	353.14410	Unknown	2.00666	0.1	6.5E+06
342	5.30	370.09682	372.10349	Unknown	2.00667	0.1	9.0E+05
343	5.30	373.11925	375.12575	**Dns-3-hydroxypicolinic acid**	2.00651	0.5	8.0E+05
344	5.30	454.14358	456.15028	Unknown	2.00670	0.0	1.3E+06
345	5.37	514.16249	516.16892	Unknown	2.00643	0.5	8.0E+06
346	5.37	351.13730	353.14388	**Dns-Val**	2.00659	0.3	4.0E+07
347	5.37	418.13241	420.13896	Unknown	2.00655	0.4	2.0E+06
348	5.37	448.09995	450.10702	Unknown	2.00707	-0.8	1.9E+06
349	5.37	484.19063	486.19733	Unknown	2.00670	0.0	8.5E+05
350	5.37	563.13085	565.13816	Unknown	2.00731	-1.1	1.2E+06
351	5.41	546.13155	548.13725	Unknown	2.00570	1.8	7.0E+07
352	5.41	383.10986	385.11666	**Dns-Met**	2.00681	-0.3	3.0E+06
353	5.44	659.19293	661.19931	Unknown	2.00638	0.5	1.0E+06

354	5.54	346.08600	348.09260	Unknown	2.00660	0.3	4.0E+06
355	5.54	360.10169	362.10814	Unknown	2.00645	0.7	8.0E+06
356	5.54	402.14850	404.15514	Unknown	2.00663	0.2	1.2E+07
357	5.54	415.11843	417.12527	Unknown	2.00684	-0.3	3.5E+06
358	5.54	429.11171	431.11852	**Dns-salicyluric acid**	2.00681	-0.2	2.0E+06
359	5.64	438.14718	440.15366	**Dns-Trp**	2.00647	0.5	3.2E+07
360	5.61	469.18960	471.19627	Unknown	2.00667	0.1	6.5E+06
361	5.64	621.15435	625.16869	Unknown	4.01434	-1.5	6.5E+06
362	5.64	311.08323	313.09018	Unknown	2.00695	-0.8	6.5E+05
363	5.64	505.10942	507.11664	Unknown	2.00722	-1.0	2.0E+06
364	5.64	546.13744	548.14336	Unknown	2.00592	1.4	1.5E+06
365	5.64	442.14738	444.15396	**Dns-kynurenine**	2.00658	0.3	1.0E+06
366	5.71	397.12489	399.13111	Unknown	2.00622	1.2	4.5E+06
367	5.71	466.19871	468.20570	**Dns-Thr-Leu**	2.00699	-0.6	4.5E+05
368	5.71	563.13215	565.13909	Unknown	2.00694	-0.4	1.7E+06
369	5.75	545.18170	547.18848	Unknown	2.00678	-0.1	2.0E+06
370	5.75	575.20521	577.21174	Unknown	2.00653	0.3	2.0E+06
371	5.75	363.13785	365.14449	Unknown	2.00664	0.2	1.1E+06
372	5.75	351.13780	353.14470	**Dns-norvaline**	2.00690	-0.5	6.0E+05
373	5.78	379.13251	381.13929	Unknown	2.00678	-0.2	1.5E+06
374	5.78	428.12734	430.13426	Unknown	2.00692	-0.5	2.5E+06
375	5.78	474.16956	476.17628	Unknown	2.00673	0.0	6.5E+05
376	5.78	624.15386	626.16058	Unknown	2.00671	0.0	6.5E+05
377	5.78	683.22717	685.23399	Unknown	2.00683	-0.2	8.0E+05
378	5.85	374.11714	376.12370	Unknown	2.00656	0.4	6.5E+06
379	5.81	401.11700	403.12357	Unknown	2.00657	0.3	1.5E+06
380	5.88	378.06757	380.07452	Unknown	2.00695	-0.6	6.0E+06
381	5.88	400.08513	402.10227	Unknown	2.01715	-26.0	6.0E+06
382	5.95	454.06309	456.07011	Unknown	2.00703	-0.7	2.7E+06
383	5.95	383.09153	385.09845	Unknown	2.00692	-0.5	8.5E+05
384	5.95	432.11144	434.11814	Unknown	2.00670	0.0	5.0E+06
385	5.95	550.18240	552.18904	Unknown	2.00664	0.1	1.6E+06
386	5.95	371.08327	373.08958	**Dns-4-aminobenzoic acid**	2.00631	1.1	3.0E+05
387	5.99	279.11619	281.12294	**Dns-ethylamine**	2.00675	-0.1	1.1E+06
388	6.06	349.15830	361.16506	Unknown	12.00676	-27688.3	1.5E+06
389	6.06	364.62490	366.63149	Unknown	2.00659	0.3	3.7E+06
390	6.06	510.16954	512.17650	Unknown	2.00696	-0.5	7.0E+05
391	6.06	561.13499	563.14132	Unknown	2.00633	0.7	7.0E+06
392	6.06	675.16115	678.17138	Unknown	3.01024	-0.3	1.1E+06
393	6.06	712.24081	714.24799	Unknown	2.00718	-0.7	2.0E+06
394	6.06	728.24056	732.25338	Unknown	4.01282	0.8	2.6E+06
395	6.12	407.20024	409.20714	Unknown	2.00690	-0.5	6.5E+05
396	6.12	509.17647	511.18368	**Dns-Ala-Trp**	2.00721	-1.0	2.0E+06

397	6.12	560.15793	562.16450	Unknown	2.00657	0.2	2.5E+06
398	6.16	585.14650	588.15653	Unknown	3.01004	0.1	9.0E+05
399	6.16	455.12715	457.13374	Unknown	2.00659	0.3	8.0E+06
400	6.16	382.58146	384.58826	Unknown	2.00680	-0.2	8.0E+05
401	6.16	693.11795	696.12793	Unknown	3.00998	0.1	9.0E+05
402	6.16	720.23254	722.23960	Unknown	2.00705	-0.5	9.0E+05
403	6.23	399.13568	401.14244	**Dns-Phe**	2.00676	-0.1	7.0E+07
404	6.23	430.17818	432.18465	Unknown	2.00647	0.5	1.7E+06
405	6.23	454.12408	457.13364	Unknown	3.00956	1.1	5.0E+06
406	6.23	504.12482	506.13194	Unknown	2.00712	-0.8	5.0E+06
407	6.23	553.21120	555.21836	Unknown	2.00716	-0.8	8.0E+05
408	6.23	671.28692	673.29337	Unknown	2.00645	0.4	1.3E+06
409	6.23	674.22757	676.23424	Unknown	2.00667	0.1	9.0E+04
410	6.30	388.12183	390.12858	Unknown	2.00675	-0.1	7.5E+05
411	6.30	432.11057	434.11713	Unknown	2.00656	0.3	2.6E+07
412	6.30	459.11978	461.12659	Unknown	2.00681	-0.2	1.3E+07
413	6.30	487.15322	489.16024	Unknown	2.00703	-0.6	2.5E+06
414	6.30	494.17404	496.18000	Unknown	2.00596	1.5	1.5E+06
415	6.30	511.19321	513.19995	Unknown	2.00674	-0.1	1.3E+06
416	6.30	560.15803	562.16456	Unknown	2.00653	0.3	1.1E+07
417	6.33	338.08460	340.09133	Unknown	2.00673	-0.1	5.0E+06
418	6.33	399.13783	401.14464	Unknown	2.00682	-0.3	2.2E+06
419	6.36	675.16104	679.17427	Unknown	4.01324	0.3	2.6E+06
420	6.36	365.15319	367.15982	**Dns-Ile**	2.00663	0.2	2.0E+07
421	6.40	446.12449	448.13127	Unknown	2.00677	-0.1	4.0E+07
422	6.40	462.20279	464.20985	**Dns-Leu-Pro**	2.00706	-0.7	7.0E+05
423	6.43	507.19480	509.20091	Unknown	2.00611	1.2	5.0E+07
424	6.43	335.14289	337.14952	**Dns-Spermine**	2.00663	0.2	4.0E+06
425	6.43	379.13283	381.13948	Unknown	2.00665	0.2	9.0E+05
426	6.43	385.12192	387.12871	**Dns-pipecolic acid**	2.00679	-0.2	1.7E+06
427	6.43	402.10037	404.10703	Unknown	2.00666	0.1	1.2E+07
428	6.47	576.18759	578.19495	Unknown	2.00736	-1.1	1.2E+06
429	6.47	707.13321	711.14705	Unknown	4.01384	-0.6	9.0E+05
430	6.50	429.11007	431.11642	Unknown	2.00635	0.8	1.5E+08
431	6.50	542.16877	544.17543	Unknown	2.00666	0.1	2.0E+06
432	6.50	365.15287	367.15942	**Dns-Leu**	2.00654	0.5	3.2E+06
433	6.50	475.09679	477.10398	Unknown	2.00719	-1.0	2.0E+06
434	6.64	315.10916	317.11574	**Dns-5-hydroxylysine**	2.00658	0.4	1.5E+06
435	6.64	345.09255	347.09954	**Dns-cystathionine**	2.00699	-0.8	6.0E+05
436	6.64	379.13261	381.13931	Unknown	2.00670	0.0	4.4E+06
437	6.71	491.23324	493.24016	Unknown	2.00692	-0.4	8.0E+06
438	6.71	371.10645	373.11299	Unknown	2.00654	0.5	4.5E+06
439	**6.71**	416.11395	418.12102	Unknown	2.00707	-0.9	3.0E+07

440	6.71	468.15528	470.16221	Unknown	2.00693	-0.5	1.0E+06
441	6.71	507.19491	509.20203	Unknown	2.00712	-0.8	3.0E+06
442	6.71	517.14365	519.15051	Unknown	2.00686	-0.3	9.0E+06
443	6.71	558.13477	561.14485	Unknown	3.01008	0.0	1.7E+06
444	6.71	587.15077	589.15783	Unknown	2.00705	-0.6	2.0E+06
445	6.74	354.07049	356.07719	**Dns-Cystine**	2.00670	0.0	1.4E+07
446	6.78	365.15341	367.15976	**Dns-N-norleucine**	2.00635	1.0	1.3E+05
447	6.81	302.09637	304.10287	Unknown	2.00649	0.7	6.0E+05
448	6.81	429.11149	431.11848	Unknown	2.00699	-0.6	1.3E+06
449	6.81	457.14315	459.14982	Unknown	2.00667	0.1	1.0E+06
450	6.81	485.13756	487.14472	Unknown	2.00716	-0.9	2.5E+06
451	6.81	664.14905	666.15596	Unknown	2.00690	-0.3	1.5E+06
452	6.81	689.17747	693.19046	Unknown	4.01299	0.6	1.4E+06
453	6.85	385.15833	387.16505	**Dns-3-aminosalicylic acid**	2.00672	0.0	1.0E+07
454	6.88	345.09121	347.09769	Unknown	2.00648	0.7	2.5E+07
455	6.92	279.11550	281.12233	**Dns-ethylamine**	2.00683	-0.4	4.0E+07
456	6.92	369.10698	371.11382	Unknown	2.00684	-0.4	1.6E+06
457	6.92	653.25184	655.25801	Unknown	2.00617	0.8	8.0E+06
458	6.99	304.58542	306.09038	Unknown	1.50496	0.2	5.0E+06
459	6.99	416.11644	418.12330	Unknown	2.00685	-0.3	8.0E+06
460	6.99	482.17486	484.18212	Unknown	2.00726	-1.1	2.6E+06
461	6.99	737.20349	741.21671	Unknown	4.01321	0.3	1.2E+06
462	7.06	708.21049	710.21637	Unknown	2.00589	1.2	4.5E+06
463	7.12	361.07860	363.08522	Unknown	2.00662	0.3	2.1E+06
464	7.12	415.07845	417.08530	Unknown	2.00685	-0.3	1.0E+06
465	7.12	455.12706	457.13387	Unknown	2.00680	-0.2	4.0E+06
466	7.16	425.11684	427.12367	Unknown	2.00683	-0.3	1.2E+07
467	7.16	555.17016	558.18045	Unknown	3.01029	-0.4	2.5E+06
468	**7.16**	485.13457	487.14104	Unknown	2.00647	0.5	4.0E+07
469	7.16	599.19875	602.20894	Unknown	3.01020	-0.2	8.0E+05
470	7.23	399.13749	401.14423	Unknown	2.00673	-0.1	4.0E+06
471	7.23	414.11171	416.11784	**Dns-umbelliferone**	2.00613	1.4	9.0E+05
472	7.23	415.07783	417.08488	Unknown	2.00706	-0.8	1.0E+06
473	7.23	542.14142	544.14847	Unknown	2.00705	-0.6	1.0E+06
474	7.23	690.25825	692.26520	Unknown	2.00694	-0.3	6.5E+05
475	7.30	336.11419	338.12072	Unknown	2.00653	0.5	3.5E+06
476	7.30	389.12808	391.13472	Unknown	2.00663	0.2	3.5E+06
477	7.30	612.23119	616.24475	Unknown	4.01356	-0.2	6.0E+05
478	7.30	622.17919	626.19246	Unknown	4.01327	0.2	3.5E+06
479	7.30	664.14554	666.15094	Unknown	2.00540	2.0	3.7E+06
480	7.30	700.14317	704.15671	Unknown	4.01355	-0.2	8.0E+05
481	7.30	724.17835	726.18517	Unknown	2.00682	-0.2	1.2E+06
482	7.40	514.14523	517.15546	Unknown	3.01023	-0.3	6.0E+06

483	7.40	393.14814	395.15482	Unknown	2.00668	0.1	7.0E+06
484	7.40	464.08342	466.09057	Unknown	2.00715	-0.9	2.0E+06
485	7.44	343.05168	345.05821	Unknown	2.00654	0.5	3.0E+06
486	7.44	472.14263	474.14935	**Dns-procaine**	2.00673	0.0	1.0E+06
487	7.44	472.14263	474.14935	Unknown	2.00673	0.0	1.1E+06
488	7.50	621.19344	625.20649	Unknown	4.01305	0.6	7.0E+06
489	7.50	266.08456	268.09132	Unknown	2.00676	-0.2	1.5E+06
490	7.50	279.11625	281.12302	Unknown	2.00676	-0.2	1.5E+06
491	7.54	528.12590	531.13601	Unknown	3.01011	-0.1	5.5E+06
492	7.54	368.08543	370.09192	**Dns-homocystine**	2.00650	0.6	6.0E+05
493	7.58	362.08414	363.58905	Unknown	1.50492	0.3	1.0E+07
494	7.58	469.14306	471.15006	Unknown	2.00700	-0.6	1.6E+06
495	7.61	546.13584	548.14257	**Dns-Phe-Phe**	2.00673	0.0	2.0E+05
496	7.65	385.11983	387.12663	**Dns-4-acetyamidophenol**	2.00680	-0.2	5.0E+07
497	7.65	300.10353	302.11025	**Dns-L-ornithine**	2.00672	0.0	4.5E+06
498	7.65	407.10188	409.10880	Unknown	2.00692	-0.5	1.4E+06
499	7.65	416.16232	418.16949	Unknown	2.00717	-1.1	3.2E+06
500	7.65	423.07684	425.08424	Unknown	2.00740	-1.6	2.2E+06
501	7.65	285.04617	287.05273	Unknown	2.00656	0.5	4.2E+06
502	7.68	473.11987	475.12700	Unknown	2.00714	-0.9	8.5E+06
503	7.68	599.20020	603.21344	Unknown	4.01324	0.3	7.0E+06
504	7.68	425.15668	427.16345	**Dns-5-HIAA**	2.00677	-0.1	5.0E+06
505	7.72	377.08999	379.09655	Unknown	2.00655	0.4	1.3E+06
506	7.72	444.14790	446.15467	Unknown	2.00677	-0.1	1.7E+06
507	7.68	495.10185	497.10937	Unknown	2.00752	-1.6	3.0E+06
508	7.72	504.15931	506.16709	Unknown	2.00779	-2.1	4.0E+06
509	7.82	385.15863	387.16548	**Dns-acetaminophen**	2.00685	-0.4	4.0E+06
510	7.82	608.16326	611.17314	Unknown	3.00988	0.3	8.0E+06
511	7.82	416.11498	418.12172	Unknown	2.00674	-0.1	2.0E+07
512	7.82	441.11032	443.11725	Unknown	2.00694	-0.5	1.0E+06
513	7.82	700.14525	704.15900	Unknown	4.01375	-0.5	1.3E+06
514	7.92	470.11742	473.12800	Unknown	3.01058	-1.1	6.0E+07
515	7.92	386.10558	388.11205	Unknown	2.00647	0.6	3.0E+07
516	7.92	318.10960	320.11628	Unknown	2.00669	0.1	4.0E+05
517	7.92	305.13230	307.13906	Unknown	2.00677	-0.2	1.7E+06
518	7.92	557.17690	559.18412	Unknown	2.00722	-0.9	1.3E+06
519	7.95	579.16030	581.16704	Unknown	2.00674	-0.1	8.0E+05
520	7.95	723.16137	726.17171	Unknown	3.01034	-0.4	1.6E+06
521	7.95	741.17905	744.18916	Unknown	3.01011	-0.1	1.0E+06
522	7.95	753.17253	757.18581	Unknown	4.01327	0.2	1.2E+06
523	8.09	347.11198	349.11856	Unknown	2.00658	0.4	1.3E+07
524	8.09	354.11985	356.12643	Unknown	2.00659	0.3	5.0E+06
525	8.09	415.07830	417.08517	Unknown	2.00688	-0.4	3.5E+06

526	8.09	430.13169	432.13823	Unknown	2.00654	0.4	2.5E+06
527	8.09	450.14847	452.15539	Unknown	2.00692	-0.5	1.0E+06
528	8.09	460.16499	462.17174	Unknown	2.00675	-0.1	5.5E+06
529	8.09	474.18020	476.18703	Unknown	2.00683	-0.3	1.5E+06
530	8.09	693.21619	697.22945	Unknown	4.01326	0.2	4.0E+06
531	8.09	707.23092	711.24500	Unknown	4.01408	-0.9	1.3E+06
532	8.16	307.11112	309.11763	**Dns-Lys**	2.00650	0.7	3.5E+07
533	8.16	321.56591	323.07106	Unknown	1.50515	-0.4	3.0E+06
534	8.16	376.13297	378.13977	Unknown	2.00680	-0.2	1.3E+06
535	8.16	428.15285	430.15985	Unknown	2.00699	-0.7	2.0E+06
536	8.16	693.21531	697.22864	Unknown	4.01333	0.1	1.8E+06
537	8.23	556.15707	559.16715	Unknown	3.01009	0.0	1.0E+07
538	8.23	402.10062	404.10727	**Dns-5-methyoxysalicylic acid**	2.00666	0.1	1.1E+07
539	8.23	350.12987	352.13666	Unknown	2.00678	-0.2	1.0E+06
540	8.23	371.08316	373.08944	Unknown	2.00628	1.2	3.0E+06
541	8.23	633.15880	636.16832	Unknown	3.00952	0.9	2.2E+06
542	8.23	642.12084	645.13000	Unknown	3.00916	1.4	4.0E+06
543	8.33	394.15830	396.16545	**Dns-Tryptamine**	2.00715	-1.1	2.2E+05
544	8.33	654.27218	658.28496	Unknown	4.01278	1.0	8.0E+06
545	8.33	676.25358	680.26695	Unknown	4.01337	0.1	2.0E+06
546	8.33	372.09029	374.09691	Unknown	2.00662	0.2	8.0E+06
547	8.36	311.59293	313.59941	**Dns-His**	2.00649	0.7	3.5E+07
548	8.36	389.12814	391.13465	**Dns-His**	2.00650	0.5	7.0E+07
549	8.46	352.10010	354.10688	Unknown	2.00678	-0.2	1.2E+06
550	8.46	368.60031	370.60711	Unknown	2.00679	-0.2	3.2E+06
551	8.46	400.12155	402.12835	Unknown	2.00681	-0.2	1.5E+06
552	8.46	458.12702	460.13386	Unknown	2.00684	-0.3	6.0E+05
553	8.50	673.09638	677.11013	Unknown	4.01375	-0.5	1.0E+06
554	8.50	399.17377	401.18051	**Dns-1-Ephedrine**	2.00674	-0.1	2.0E+06
555	8.50	387.10113	389.10769	**Dns-Dopamine**	2.00656	0.4	3.0E+06
556	8.63	642.12224	645.13157	Unknown	3.00932	1.1	6.0E+06
557	8.63	335.12437	337.13114	Unknown	2.00677	-0.2	2.3E+06
558	8.70	354.12000	356.12651	Unknown	2.00650	0.6	1.3E+06
559	8.70	428.11584	430.12290	Unknown	2.00706	-0.8	7.0E+06
560	8.70	440.11548	442.12213	Unknown	2.00665	0.1	9.0E+05
561	8.70	480.15047	482.15900	Unknown	2.00854	-3.8	2.0E+06
562	8.73	400.11932	402.12589	Unknown	2.00656	0.4	3.4E+07
563	8.80	369.09113	371.09785	Unknown	2.00672	0.0	5.0E+07
564	8.80	504.12586	506.13339	Unknown	2.00753	-1.6	2.2E+06
565	8.87	335.14271	337.14934	Unknown	2.00663	0.2	1.0E+06
566	8.87	379.06344	380.56845	Unknown	1.50501	0.1	7.0E+06
567	8.87	388.07240	389.57748	Unknown	1.50508	-0.1	1.0E+06
568	8.87	430.21590	432.22304	Unknown	2.00714	-1.0	1.1E+06

569	8.87	451.17221	453.17944	Unknown	2.00724	-1.2	1.5E+06
570	8.87	566.17501	569.18584	Unknown	3.01083	-1.3	1.2E+06
571	8.87	737.17810	741.19050	Unknown	4.01239	1.4	4.5E+06
572	8.90	399.17141	401.17825	Unknown	2.00683	-0.3	1.0E+08
573	8.90	314.11933	316.12600	Unknown	2.00667	0.1	6.5E+05
574	8.90	421.15281	423.15986	Unknown	2.00705	-0.8	1.2E+06
575	8.90	512.23207	514.23912	**Dns-leu-Phe**	2.00705	-0.7	1.4E+06
576	8.93	638.17482	642.18785	Unknown	4.01303	0.6	1.1E+06
577	9.00	318.59119	320.59800	Unknown	2.00682	-0.3	1.5E+06
578	9.00	319.59101	321.59764	Unknown	2.00663	0.3	1.7E+06
579	9.00	378.10075	380.10737	Unknown	2.00662	0.2	3.0E+06
580	9.09	634.16818	637.17804	Unknown	3.00986	0.3	3.5E+06
581	9.09	376.12172	378.12833	Unknown	2.00662	0.2	1.0E+06
582	9.19	503.17628	505.18358	Unknown	2.00730	-1.2	1.1E+06
583	9.19	541.19299	545.20582	**Dns-1,3-diaminopropane**	4.01283	1.1	3.5E+05
584	9.29	541.15838	545.16103	**Dns-1,2-diaminopropane**	4.00264	19.8	1.5E+05
585	9.19	726.16160	730.17507	Unknown	4.01348	-0.1	9.0E+05
586	9.26	415.07712	417.08417	Unknown	2.00705	-0.8	2.2E+07
587	9.26	472.13394	474.14095	Unknown	2.00701	-0.6	1.5E+07
588	9.26	494.11557	496.12290	Unknown	2.00733	-1.2	2.5E+06
589	9.26	503.17625	505.18356	Unknown	2.00731	-1.2	2.2E+05
590	9.29	510.09101	512.09826	Unknown	2.00725	-1.0	1.7E+06
591	9.29	635.15190	638.16217	Unknown	3.01028	-0.3	1.3E+06
592	9.36	414.13718	416.14376	Unknown	2.00657	0.3	1.2E+06
593	9.46	473.11352	475.12038	Unknown	2.00685	-0.3	2.8E+06
594	9.46	314.11904	316.12579	**Dns-Tryptamine**	2.00676	-0.1	1.8E+06
595	9.46	376.59218	378.59887	Unknown	2.00669	0.1	7.0E+05
596	9.46	394.21848	396.22495	Unknown	2.00646	0.6	8.0E+05
597	9.57	278.10843	280.11464	**Dns- Putrescine**	2.00621	1.8	2.0E+05
598	9.60	319.14738	321.15397	Unknown	2.00658	0.4	2.2E+07
599	9.60	324.10331	326.10983	**Dns-tyrosinamide**	2.00652	0.6	3.2E+06
600	9.60	458.10862	460.11573	Unknown	2.00711	-0.9	2.0E+06
601	9.60	539.16720	541.17461	Unknown	2.00741	-1.3	2.7E+06
602	9.60	561.14929	563.15621	Unknown	2.00692	-0.4	8.5E+05
603	9.67	498.15168	501.16181	Unknown	3.01013	-0.1	4.0E+06
604	9.67	369.09248	371.09920	Unknown	2.00672	0.0	7.0E+05
605	9.67	463.58829	466.09673	Unknown	2.50845	-0.1	1.0E+06
606	9.73	453.18732	455.19424	Unknown	2.00691	-0.5	7.0E+06
607	9.73	333.08172	335.08859	Unknown	2.00687	-0.5	1.2E+06
608	9.73	513.15123	515.15795	Unknown	2.00672	0.0	1.5E+06
609	9.73	535.13352	537.14101	Unknown	2.00749	-1.5	1.6E+06
610	9.76	400.12138	402.12824	Unknown	2.00686	-0.4	4.5E+06
611	9.80	519.16797	522.17765	Unknown	3.00968	0.7	6.0E+05

612	9.83	344.10108	346.10762	**Dns-5-hydroxytrptophan**	2.00654	0.5	5.5E+05
613	9.86	370.11112	372.11780	Unknown	2.00668	0.1	2.0E+06
614	9.96	483.10438	487.11783	Unknown	4.01345	-0.1	2.0E+06
615	9.99	494.16604	496.17357	Unknown	2.00753	-1.7	5.0E+06
616	10.03	466.67899	469.68921	Unknown	3.01022	-0.3	1.0E+06
617	10.09	297.08568	299.09230	Unknown	2.00662	0.3	1.1E+06
618	10.09	311.08305	313.08986	Unknown	2.00681	-0.3	8.0E+05
619	10.09	317.58765	319.59446	**Dns-3-methoxy-tyramine**	2.00681	-0.3	1.3E+06
620	10.09	593.16323	597.17662	Unknown	4.01338	0.1	1.3E+06
621	10.09	605.14075	608.15077	Unknown	3.01002	0.1	8.0E+05
622	10.13	668.12788	671.13817	Unknown	3.01028	-0.4	1.1E+06
623	10.16	634.18969	638.20347	Unknown	4.01377	-0.6	4.5E+06
624	10.16	289.59888	291.60531	**Dns-Histamine**	2.00643	1.0	5.0E+05
625	10.16	345.13907	347.14534	**Dns-Histamine**	2.00627	1.3	4.0E+05
626	10.19	334.56832	336.07348	Unknown	1.50516	-0.4	2.5E+06
627	10.19	498.15042	501.16016	Unknown	3.00974	1.0	9.0E+06
628	10.19	549.24527	551.25248	Unknown	2.00721	-0.9	6.5E+05
629	10.19	601.18581	603.19331	Unknown	2.00750	-1.3	1.0E+06
630	10.22	324.59532	326.60191	**Dns-Tyr**	2.00659	0.4	3.0E+07
631	10.26	318.07278	320.07946	Unknown	2.00668	0.1	1.2E+06
632	10.26	500.13161	504.14430	Unknown	4.01269	1.4	9.0E+05
633	10.29	451.11892	453.12572	Unknown	2.00679	-0.2	5.0E+06
634	10.29	472.14252	474.14950	Unknown	2.00698	-0.6	1.2E+06
635	10.29	479.20367	481.21081	Unknown	2.00713	-0.9	9.0E+05
636	10.29	485.11990	488.12983	Unknown	3.00993	0.3	9.0E+05
637	10.29	416.11642	418.12335	Unknown	2.00692	-0.5	1.0E+07
638	10.29	467.17107	470.18100	Unknown	3.00993	0.3	4.5E+06
639	10.36	673.09726	677.11083	Unknown	4.01357	-0.2	3.5E+06
640	10.36	484.17701	486.18410	Unknown	2.00709	-0.8	2.0E+06
641	10.36	346.09853	348.10541	Unknown	2.00688	-0.5	1.4E+06
642	10.36	711.20315	715.21686	Unknown	4.01371	-0.4	5.5E+05
643	10.43	478.60910	481.11780	Unknown	2.50870	-0.7	1.7E+06
644	10.43	310.08049	312.08731	**Dns-Cysteamine**	2.00682	-0.4	5.5E+05
645	10.43	635.15178	638.16220	Unknown	3.01042	-0.6	7.0E+05
646	10.46	386.10585	388.11249	Unknown	2.00664	0.2	6.0E+06
647	10.56	620.20001	624.21351	**Dns-2,3-diaminosalicylic acid**	4.01350	-0.1	3.0E+05
648	10.82	285.08033	287.08681	**Dns-cadaverine**	2.00648	0.8	1.0E+05
649	10.79	643.20424	647.21668	**Dns-serotonin**	4.01244	1.5	6.0E+05
649	10.79	322.10615	324.11285	**Dns-serotonin**	2.00670	0.0	1.2E+06
650	10.89	332.61144	334.61811	**Dns-metanephrine**	2.00667	0.1	1.8E+05
650	10.89	664.21406	668.22815	**Dns-metanephrine**	4.01409	-1.0	3.0E+05
651	11.02	325.08750	327.09412	**Dns-Epinephrine**	2.00662	0.3	3.5E+06
652	11.02	340.09279	342.09948	Unknown	2.00669	0.0	1.5E+06

653	11.02	427.12375	430.13367	Unknown	3.00991	0.4	1.3E+06
654	11.02	317.60595	319.61277	**Dns-synephrine**	2.00681	-0.3	5.0E+05
655	11.06	531.23453	533.24176	Unknown	2.00723	-1.0	5.5E+05
656	11.06	679.17693	683.19017	Unknown	4.01324	0.3	6.0E+05
657	11.06	692.15486	696.16864	Unknown	4.01378	-0.5	1.2E+06
658	11.09	311.07177	313.07858	Unknown	2.00681	-0.3	3.2E+06
659	11.13	302.60058	304.60727	Unknown	2.00669	0.1	3.4E+06
660	11.13	324.07974	326.08624	**Dns-o-(p or m)-cresol**	2.00650	0.6	2.0E+06
661	11.13	344.11918	346.12608	Unknown	2.00690	-0.6	1.0E+06
662	11.13	425.18970	427.19638	Unknown	2.00668	0.1	1.1E+06
663	11.16	507.23484	509.24215	Unknown	2.00732	-1.2	5.0E+06
664	11.16	302.60051	304.60713	**Dns-Tyramine**	2.00663	0.3	6.0E+06
665	11.19	507.23475	509.24216	Unknown	2.00742	-1.4	2.4E+06
666	11.23	577.15762	581.17148	Dns-hydroquinone	4.01387	-3453.6	1.0E+06
667	11.26	282.44444	284.45128	**Dns-spermidine**	2.00685	-0.5	1.0E+05
668	11.29	356.55413	358.05957	Unknown	1.50543	-1.1	1.3E+06
669	11.36	346.58129	348.58796	Unknown	2.00668	0.1	2.0E+07
670	11.36	443.11162	446.12146	Unknown	3.00984	0.5	5.0E+05
671	11.36	575.15035	578.16054	Unknown	3.01019	-0.2	8.0E+05
672	11.52	435.09375	436.09715	Unknown	1.00340	-0.1	7.0E+05

Note:

Error (ppm) in mass difference is the mass error between the theoretical mass difference and the measured mass difference for the $^{12}C_2$-/$^{13}C_2$-dansylated derivatives. The theoretical mass difference for the 1 tag is 2.0067096 and 2 tags is 4.0134192.

Table S4.1. Ion pairs detected and identified by RPLC FTICR MS from repeatedly 1:1 ^{12}C-/^{13}C-dansylated CSF sample #1. Ion pairs detected in both repeatedly labeled CSF sample are highlighted in bold.

CSF - #1		**CSF - #1**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	**1.63**	**252.06908**	**254.07579**	1.0E+07
unknown	1.63	283.11153	285.11818	1.0E+06
unknown	1.63	314.15384	316.16051	3.0E+05
unknown	**1.63**	**534.17395**	**536.18042**	4.3E+06
unknown	1.63	598.26595	600.27240	5.6E+05
unknown	1.63	658.18429	660.19037	8.4E+05
unknown	**1.63**	**783.71391**	**785.72132**	7.5E+05
unknown	1.63	789.24792	792.25680	4.5E+06
unknown	1.63	921.24188	923.24841	4.7E+05
unknown	1.63	924.76428	926.77209	3.5E+05

unknown	**1.68**	**280.04791**	**282.05458**	5.3E+05
unknown	**1.68**	**331.04324**	**333.04999**	1.7E+05
unknown	**1.68**	**336.02214**	**338.02865**	5.3E+05
unknown	**1.68**	**349.05359**	**351.06028**	2.3E+05
unknown	**1.68**	**356.09225**	**358.09887**	3.8E+05
unknown	1.68	373.07352	375.08017	2.0E+05
unknown	1.68	515.12170	517.12772	1.8E+05
unknown	1.68	566.12598	568.13285	1.8E+05
unknown	**1.68**	**569.13562**	**571.14154**	2.5E+05
unknown	**1.68**	**582.10508**	**584.11194**	3.2E+05
unknown	**1.68**	**585.11523**	**587.12207**	2.9E+05
unknown	**1.68**	**794.14835**	**796.15425**	7.2E+05
unknown	1.72	253.07244	255.07921	1.7E+06
unknown	**1.72**	**271.04262**	**273.04931**	1.7E+06
unknown	**1.72**	**389.58878**	**391.59558**	4.1E+05
unknown	**1.72**	**396.57414**	**398.58100**	5.4E+05
unknown	**1.72**	**503.13217**	**505.13855**	2.0E+06
unknown	**1.72**	**523.10869**	**525.11452**	6.1E+05
unknown	**1.72**	**526.11747**	**528.12367**	1.4E+06
unknown	1.72	641.65776	643.66357	3.6E+05
unknown	1.72	649.64306	651.64930	9.3E+05
unknown	1.72	652.65093	654.65716	9.9E+05
unknown	1.72	661.63511	663.64070	4.7E+05
unknown	**1.72**	**669.61927**	**671.62469**	3.6E+05
unknown	**1.72**	**754.19452**	**756.19985**	3.4E+05
unknown	1.72	797.15625	803.17505	6.9E+05
unknown	**1.76**	**252.06911**	**254.07566**	1.1E+07
unknown	1.76	802.17113	804.17865	6.1E+05
unknown	**1.80**	**364.02069**	**366.02728**	4.0E+05
unknown	1.80	547.09583	549.10246	5.3E+05
unknown	1.80	550.10554	552.11257	5.3E+05
unknown	1.84	606.09369	608.10028	9.4E+04
unknown	1.84	778.18317	782.19531	1.3E+05
unknown	1.84	846.13751	848.14307	1.4E+05
unknown	**1.88**	**252.06910**	**254.07582**	6.7E+06
unknown	1.88	296.03314	299.04336	1.7E+06
unknown	1.88	432.00848	435.01858	1.0E+06
unknown	**1.88**	**499.99626**	**502.00288**	4.5E+05
unknown	1.88	546.06337	548.07007	1.5E+05
unknown	1.88	558.16258	560.16978	3.6E+05
unknown	**1.88**	**567.98382**	**569.99094**	3.6E+05
unknown	**1.88**	**569.07729**	**571.08491**	4.6E+05
unknown	1.88	578.13715	582.15155	1.6E+05
unknown	1.88	635.97138	637.97733	2.2E+05
unknown	1.88	637.06476	639.07181	1.6E+05
unknown	**1.88**	**707.05859**	**709.06451**	2.7E+05
unknown	**1.92**	**364.02082**	**366.02748**	1.1E+06
unknown	1.92	399.98854	404.00131	9.4E+05
unknown	1.92	593.09274	595.09912	6.9E+05
unknown	**2.01**	**555.12275**	**557.12976**	3.1E+05
unknown	2.05	524.08093	526.08836	3.9E+05
unknown	**2.17**	**558.03257**	**562.04526**	4.1E+05
unknown	2.17	573.05167	577.06586	3.9E+05
unknown	**2.21**	**414.12222**	**416.12909**	5.6E+05

unknown	**2.21**	**632.22684**	**634.23242**	2.5E+05
unknown	**2.25**	**274.05092**	**277.06126**	1.6E+06
unknown	2.25	601.18414	603.18993	8.9E+04
unknown	**2.25**	**605.04364**	**607.05084**	9.1E+04
unknown	2.29	297.03616	299.04306	9.8E+04
unknown	**2.29**	**364.02049**	**366.02718**	3.4E+05
unknown	**2.29**	**372.06922**	**374.07606**	1.3E+05
phosphoethaolamine	**2.29**	**375.07777**	**377.08443**	9.4E+05
unknown	**2.29**	**499.99512**	**502.00220**	9.2E+04
unknown	2.29	571.08403	573.09058	8.0E+04
unknown	2.33	296.03290	302.05273	7.4E+05
unknown	2.33	504.05315	506.05939	7.0E+04
unknown	2.41	353.99149	355.99851	8.2E+04
unknown	**2.49**	**366.14845**	**368.15506**	8.9E+04
unknown	**2.53**	**274.05087**	**277.06110**	1.1E+06
unknown	**2.53**	**438.20573**	**440.21282**	1.5E+05
3-methylhistidine	**2.61**	**403.14383**	**405.15060**	8.3E+05
unknown	**2.61**	**501.11652**	**503.12399**	7.4E+04
unknown	2.66	277.08373	279.09027	6.1E+05
unknown	**2.66**	**320.04418**	**322.05098**	2.8E+05
Taurine	**2.66**	**359.07329**	**361.07999**	5.3E+06
unknown	**2.66**	**380.08026**	**382.08673**	2.7E+05
unknown	**2.66**	**388.10783**	**390.11454**	2.5E+06
unknown	2.66	551.12921	553.13538	1.1E+05
1-methylhistidine	**2.74**	**403.14382**	**405.15041**	1.6E+06
unknown	**2.78**	**252.06903**	**254.07573**	1.1E+06
unknown	2.78	364.02014	367.02994	7.9E+04
unknown	2.86	380.16403	383.17358	4.0E+05
unknown	**2.90**	**363.10113**	**365.10773**	2.1E+05
unknown	**2.90**	**509.17043**	**511.17709**	1.2E+06
unknown	**2.94**	**366.14864**	**368.15525**	2.7E+05
unknown	**2.94**	**424.11714**	**426.12355**	1.1E+05
unknown	2.98	296.08664	299.09708	7.3E+04
unknown	**3.02**	**345.13833**	**347.14493**	1.1E+05
unknown	**3.02**	**376.18052**	**378.18716**	3.3E+05
unknown	**3.06**	**414.12178**	**416.12861**	1.3E+06
unknown	3.10	383.12722	386.13736	1.4E+05
Arginine	**3.22**	**408.17022**	**410.17693**	6.3E+06
unknown	**3.39**	**319.11114**	**321.11810**	2.1E+05
unknown	**3.55**	**314.09566**	**316.10283**	2.7E+05
unknown	**3.55**	**402.14581**	**404.15282**	7.5E+04
Homoarginine	**3.55**	**422.21097**	**424.21773**	5.5E+05
unknown	**3.55**	**502.13953**	**504.14616**	3.0E+05
unknown	**3.55**	**547.15494**	**549.16187**	9.8E+04
Asparagine	**3.59**	**366.11201**	**368.11873**	6.0E+06
unknown	**3.59**	**380.16400**	**382.17071**	1.3E+06
unknown	**3.67**	**365.12807**	**367.13462**	2.3E+06
unknown	3.67	424.15382	426.16072	2.6E+05
unknown	**3.88**	**363.10114**	**365.10797**	3.7E+05
Glutamine	**3.96**	**380.12579**	**382.13227**	1.8E+08
unknown	**3.96**	**411.16800**	**413.17486**	3.8E+06
unknown	**4.00**	**378.14097**	**384.16086**	2.0E+07
unknown	**4.12**	**381.13043**	**383.13743**	6.3E+06
unknown	**4.12**	**402.10899**	**404.11588**	1.3E+06

unknown	**4.16**	**363.10128**	**365.10791**	2.3E+05
L-citrulline	**4.16**	**409.15427**	**411.16062**	2.1E+06
unknown	**4.20**	**364.08533**	**366.09195**	1.2E+05
unknown	**4.20**	**392.12747**	**394.13413**	2.4E+05
unknown	**4.20**	**436.20175**	**438.20838**	1.3E+05
unknown	4.24	427.15344	429.16064	1.4E+05
unknown	4.28	271.03882	273.04537	1.0E+05
unknown	**4.32**	**270.03521**	**272.04182**	4.6E+05
unknown	**4.32**	**302.02506**	**304.03179**	5.6E+05
unknown	**4.32**	**377.08154**	**379.08838**	4.7E+05
unknown	**4.32**	**515.17577**	**519.18936**	1.0E+06
unknown	4.36	484.13345	487.14368	2.8E+07
unknown	4.36	486.12941	490.14310	2.8E+06
unknown	**4.40**	**291.06374**	**293.07046**	2.1E+05
3-sn-Phosphatidylethanolamine	**4.40**	**484.13616**	**488.14960**	1.1E+07
unknown	4.45	270.03517	273.04518	2.6E+05
Methylguanidine	**4.53**	**307.12234**	**309.12925**	1.5E+05
unknown	4.53	504.14375	506.15046	2.4E+05
Homoserine	**4.60**	**353.11683**	**355.12352**	5.0E+05
unknown	4.60	347.05546	349.06190	7.0E+04
unknown	**4.68**	**319.11117**	**321.11798**	2.2E+05
Methionine sulfoxide	**4.68**	**399.10470**	**401.11132**	9.6E+05
unknown	4.68	412.13260	414.13998	7.6E+04
unknown	**4.68**	**465.18049**	**467.18727**	1.4E+06
unknown	**4.81**	**360.05719**	**362.06415**	7.9E+04
unknown	**4.81**	**394.14317**	**396.14997**	2.4E+05
unknown	**4.81**	**499.17474**	**501.18148**	1.7E+05
Serine	**4.89**	**339.10079**	**341.10731**	3.6E+07
Glutamic Acid	**4.93**	**381.11184**	**383.11847**	2.1E+06
unknown	**4.97**	**263.21193**	**265.21874**	3.1E+05
Aspartic Acid	**4.97**	**367.09605**	**369.10280**	1.1E+06
unknown	5.00	408.15841	410.16514	8.6E+04
unknown	5.08	479.23184	481.23898	7.6E+04
unknown	**5.16**	**394.17965**	**396.18645**	9.2E+04
unknown	**5.20**	**367.13315**	**370.14310**	1.9E+05
unknown	**5.20**	**404.12732**	**406.13433**	1.9E+05
unknown	**5.20**	**422.13836**	**424.14508**	1.1E+05
unknown	**5.20**	**433.22638**	**435.23364**	9.8E+04
unknown	5.23	362.10165	364.10876	1.1E+05
unknown	5.31	455.10981	458.11958	2.3E+05
4-Hydroxyl-Proline	**5.31**	**365.11684**	**367.12341**	2.1E+06
unknown	5.34	442.11627	445.12717	1.3E+05
unknown	**5.34**	**452.18549**	**454.19235**	7.7E+04
unknown	**5.34**	**582.19019**	**584.19696**	1.4E+05
unknown	**5.34**	**600.20139**	**602.20822**	5.5E+05
unknown	**5.39**	**442.11692**	**444.12389**	6.7E+05
unknown	5.39	492.04425	495.05437	7.4E+04
unknown	**5.43**	**466.16447**	**468.17146**	1.5E+06
unknown	**5.50**	**422.17483**	**424.18123**	1.8E+05
unknown	**5.54**	**336.13792**	**338.14465**	2.1E+06
Iminodiacetic acid	**5.54**	**367.18013**	**369.18688**	2.2E+05
Aminoadipic acid	**5.54**	**395.12734**	**397.13404**	1.5E+05
unknown	**5.58**	**505.07591**	**509.08833**	2.6E+05
unknown	**5.65**	**363.14883**	**365.15548**	1.2E+06

unknown	**5.65**	**457.08922**	**459.09591**	3.4E+05
Folic acid	**5.69**	**338.09350**	**339.09650**	1.0E+05
unknown	5.73	389.12711	391.13379	5.7E+05
unknown	**5.73**	**480.18019**	**482.18719**	9.9E+05
Threonine	**5.77**	**353.11667**	**355.12338**	1.3E+07
unknown	**5.84**	**337.15823**	**339.16493**	1.8E+06
unknown	**5.88**	**379.13237**	**381.13927**	7.6E+05
Diethanolamine	**5.93**	**339.13776**	**341.14436**	3.0E+05
unknown	5.93	361.13311	363.13955	2.2E+05
unknown	5.97	515.17175	517.17767	7.7E+04
unknown	**6.00**	**478.12805**	**480.13499**	2.3E+06
Ethanolamine	**6.04**	**295.11099**	**297.11773**	1.5E+07
unknown	**6.04**	**611.19739**	**613.20418**	3.6E+05
unknown	**6.08**	**463.23807**	**465.24460**	4.3E+05
unknown	6.08	569.17767	571.18524	4.8E+05
unknown	**6.12**	**251.08468**	**253.09131**	1.8E+07
unknown	**6.12**	**523.14486**	**525.15170**	2.1E+06
unknown	**6.19**	**396.11150**	**398.11829**	6.9E+05
unknown	**6.19**	**427.15397**	**429.16033**	3.1E+05
unknown	6.19	501.16333	503.16999	2.7E+05
unknown	**6.23**	**317.13200**	**319.13879**	1.6E+06
unknown	**6.23**	**351.10127**	**353.10777**	1.8E+05
unknown	**6.23**	**415.13254**	**417.13927**	1.4E+06
unknown	**6.27**	**348.17435**	**350.18103**	3.8E+05
unknown	6.38	349.10556	351.11175	1.1E+05
unknown	**6.38**	**398.12735**	**400.13393**	3.9E+06
unknown	**6.38**	**464.14889**	**466.15579**	1.7E+05
unknown	**6.42**	**348.10154**	**350.10814**	8.9E+05
unknown	**6.47**	**375.14881**	**377.15550**	1.4E+06
unknown	**6.47**	**422.17457**	**424.18164**	1.4E+05
Glycine	**6.51**	**309.09042**	**311.09694**	9.3E+06
unknown	**6.51**	**381.14816**	**383.15491**	1.2E+06
unknown	6.55	334.29532	336.30173	2.6E+06
unknown	**6.59**	**398.12695**	**400.13363**	2.5E+05
unknown	**6.70**	**364.16918**	**366.17579**	1.7E+06
unknown	**6.74**	**344.10656**	**346.11322**	1.6E+06
unknown	**6.74**	**477.16158**	**479.16834**	7.1E+05
unknown	**6.74**	**515.19493**	**517.20135**	7.1E+04
unknown	**6.78**	**348.13785**	**350.14451**	5.0E+05
Glycylproline	**6.85**	**406.08077**	**408.08714**	8.0E+04
unknown	**6.97**	**396.11125**	**398.11800**	2.1E+05
unknown	6.97	436.19033	439.20071	3.5E+05
unknown	**7.04**	**362.11707**	**364.12341**	3.3E+07
unknown	**7.08**	**362.11586**	**364.12200**	5.6E+07
unknown	7.16	287.03345	289.04005	5.7E+05
unknown	**7.16**	**347.11775**	**349.12437**	1.1E+06
unknown	**7.16**	**362.11726**	**364.12391**	1.2E+07
Tyrosine methyl ester	**7.20**	**415.13271**	**417.13951**	1.8E+06
unknown	**7.23**	**344.10667**	**346.11339**	9.1E+05
Alanine	**7.35**	**323.10652**	**325.11314**	1.2E+07
r-aminobutyric acid	**7.50**	**337.12209**	**339.12886**	8.0E+05
unknown	**7.58**	**473.10718**	**475.11400**	5.7E+06
unknown	7.65	493.08432	496.09406	2.0E+06
unknown	**7.81**	**473.11019**	**475.11680**	8.1E+05

unknown	**7.88**	**396.11141**	**398.11828**	1.3E+06
unknown	**7.88**	**402.08700**	**404.09361**	6.8E+05
unknown	7.92	488.13034	490.13686	2.4E+05
unknown	7.96	322.07427	324.08127	1.6E+05
Tryptophanamide	8.15	437.13699	439.14456	4.8E+04
Hypoxanthine	**8.15**	**370.09713**	**372.10397**	2.5E+05
unknown	8.19	474.08917	477.09845	1.9E+06
unknown	**8.27**	**450.20563**	**452.21242**	8.5E+04
unknown	8.31	395.12730	397.13406	1.8E+05
unknown	**8.39**	**378.06743**	**380.07455**	4.3E+05
unknown	**8.43**	**431.13843**	**433.14540**	1.1E+06
3-Aminoisobutyric acid	**8.43**	**337.12185**	**339.12867**	1.7E+05
unknown	**8.46**	**279.07995**	**281.08655**	6.3E+05
unknown	8.46	362.15309	364.16001	7.3E+04
unknown	**8.46**	**386.09177**	**388.09863**	2.2E+05
5-Aminopentanoic acid	**8.50**	**351.13757**	**353.14412**	6.0E+05
unknown	**8.50**	**396.13509**	**398.14153**	3.1E+05
unknown	8.54	391.13260	393.13881	1.5E+05
unknown	8.54	485.12009	488.13063	4.0E+05
2-Aminobutyric acid	**8.89**	**337.12168**	**339.12827**	1.7E+07
unknown	9.12	371.10094	373.10764	4.1E+05
Sarcosine	**9.12**	**323.10454**	**325.11294**	2.0E+05
unknown	**9.19**	**363.10121**	**365.10801**	6.5E+05
unknown	**9.19**	**415.09629**	**417.10296**	4.1E+05
unknown	9.19	507.07083	509.07799	5.6E+05
Methylcysteine	**9.34**	**369.09411**	**371.10085**	5.6E+05
unknown	**9.38**	**492.05266**	**494.05996**	3.3E+05
unknown	9.49	242.28428	244.29116	4.8E+06
unknown	**9.49**	**396.11140**	**398.11815**	6.6E+05
unknown	**9.61**	**266.10711**	**268.11353**	1.8E+06
unknown	**9.65**	**321.12709**	**323.13374**	1.1E+06
unknown	**9.69**	**266.10341**	**268.10976**	2.7E+06
unknown	**9.69**	**287.08189**	**290.09180**	4.5E+06
unknown	**9.69**	**554.18585**	**556.19223**	3.9E+06
unknown	**9.73**	**551.17639**	**553.18256**	1.1E+06
unknown	9.77	287.08089	289.08779	1.5E+07
Proline	**9.84**	**349.12130**	**351.12787**	2.0E+05
unknown	9.88	550.17478	552.18113	1.8E+06
unknown	**9.96**	**287.08178**	**290.09173**	4.3E+06
unknown	**9.96**	**551.17590**	**553.18229**	1.2E+06
unknown	**9.96**	**554.18539**	**556.19128**	4.2E+06
Methylamine	**10.00**	**265.10039**	**267.10674**	1.2E+07
unknown	**10.08**	**287.09045**	**290.10086**	5.7E+05
unknown	**10.15**	**531.20111**	**533.20748**	7.9E+05
unknown	10.19	370.09715	372.10389	9.1E+05
unknown	**10.27**	**311.08298**	**313.08971**	3.5E+05
unknown	**10.38**	**321.11441**	**323.12118**	1.8E+06
unknown	**10.42**	**265.10058**	**268.11055**	4.1E+06
unknown	**10.42**	**412.16928**	**414.17601**	3.8E+05
Valine	**10.50**	**351.13622**	**353.14252**	6.2E+07
Methionine	**10.54**	**383.10828**	**385.11508**	5.2E+06
3-Hydroxypicolinic acid	**10.54**	**373.11820**	**375.12478**	5.0E+05
unknown	**10.57**	**378.18503**	**380.19165**	3.1E+05
unknown	**10.61**	**266.10504**	**268.11209**	4.1E+05

unknown	**10.73**	**400.08510**	**402.09196**	1.1E+06
unknown	10.77	365.11690	368.12686	6.1E+05
unknown	**10.92**	**378.06767**	**380.07469**	1.8E+06
Tryptophan	**10.96**	**438.14839**	**440.15508**	1.1E+07
Ethylamine	11.49	279.09359	281.12300	2.0E+05
unknown	11.75	301.06900	303.07539	1.1E+05
unknown	**11.75**	**387.61865**	**389.62545**	1.9E+05
unknown	**11.75**	**406.09589**	**408.10269**	1.3E+05
unknown	**11.79**	**418.13205**	**420.13885**	6.6E+05
unknown	**11.83**	**315.09051**	**317.09721**	3.1E+05
unknown	**11.83**	**372.12451**	**374.13123**	1.1E+05
unknown	**12.10**	**295.10262**	**297.10924**	9.0E+04
unknown	12.10	400.14249	402.14886	1.2E+05
unknown	**12.14**	**364.14124**	**366.14772**	8.4E+04
Pipecolic acid	**12.18**	**363.13759**	**365.14422**	5.5E+05
unknown	**12.22**	**265.10074**	**268.11090**	8.0E+05
Phenylalanine	**12.26**	**399.13455**	**401.14093**	4.2E+07
unknown	12.33	266.10400	268.11061	4.8E+05
unknown	**12.41**	**406.61708**	**408.62374**	2.3E+05
3-Hydroxymandelic acid	**12.48**	**402.10091**	**404.10795**	2.7E+05
unknown	**12.48**	**265.10074**	**268.11093**	6.6E+05
unknown	12.48	365.15323	368.16360	5.2E+06
unknown	**12.56**	**313.60899**	**315.61556**	3.1E+06
Isoleucine	**12.56**	**365.15306**	**367.15953**	2.1E+07
unknown	**12.56**	**393.15915**	**395.16584**	4.0E+05
unknown	**12.64**	**399.13790**	**401.14474**	1.3E+05
unknown	**12.67**	**501.11515**	**505.12850**	1.3E+06
unknown	12.71	380.13629	382.14294	1.1E+05
unknown	12.71	489.07053	492.08065	8.5E+04
Leucine	**12.75**	**365.15304**	**367.15950**	2.3E+07
unknown	**12.75**	**379.13215**	**381.13901**	7.1E+05
L-norleucine	**12.86**	**365.15219**	**367.15885**	1.6E+07
Cystine	**13.09**	**354.07065**	**356.07673**	6.0E+05
unknown	**13.13**	**317.13200**	**319.13880**	8.3E+05
unknown	13.13	357.12400	359.13064	1.2E+06
unknown	**13.17**	**335.14225**	**337.14864**	3.1E+07
unknown	**13.24**	**335.14250**	**337.14909**	1.1E+07
unknown	**13.29**	**362.06969**	**364.07617**	2.7E+05
unknown	13.37	368.15245	370.15933	7.5E+04
unknown	13.37	376.11474	378.12115	2.0E+05
unknown	**13.40**	**342.62981**	**344.63653**	2.7E+05
unknown	**13.48**	**378.10075**	**380.10727**	7.9E+06
unknown	**13.56**	**378.09103**	**380.09750**	2.5E+06
unknown	**13.59**	**404.08025**	**406.08686**	1.3E+05
unknown	13.63	356.15353	358.16064	7.3E+04
Hydroxyphenyllactic acid	**13.63**	**416.11625**	**418.12322**	2.9E+06
unknown	**13.75**	**307.09297**	**309.09962**	6.1E+06
unknown	13.79	474.06775	476.07478	2.9E+05
unknown	**13.94**	**402.10083**	**404.10753**	2.9E+05
Homocystine	**13.98**	**368.09877**	**370.10571**	1.9E+05
unknown	**14.02**	**322.07448**	**324.08128**	2.2E+05
unknown	**14.09**	**378.10092**	**380.10748**	1.1E+06
unknown	14.09	549.18787	551.19501	9.0E+04
unknown	**14.13**	**342.62992**	**344.63645**	2.5E+05

unknown	**14.21**	**349.63769**	**351.64431**	4.4E+06
5-HIAA	**14.21**	**425.11684**	**427.12361**	5.5E+05
unknown	**14.21**	**511.13806**	**513.14446**	1.7E+05
unknown	**14.25**	**336.11409**	**338.12084**	3.2E+05
unknown	**14.29**	**346.06573**	**348.07247**	1.7E+05
unknown	**14.36**	**301.09807**	**305.11169**	1.2E+06
Dimethylamine	**14.40**	**279.11588**	**281.12248**	2.1E+07
unknown	14.40	561.23910	565.25261	2.5E+06
unknown	**14.44**	**579.20736**	**583.22123**	1.2E+06
unknown	**14.44**	**584.22385**	**587.23386**	2.0E+06
Phenylpropanolamine	**14.51**	**385.12193**	**387.12867**	1.6E+05
unknown	**14.51**	**301.09785**	**305.11153**	1.8E+06
unknown	14.55	280.11929	284.13279	1.3E+06
unknown	14.59	279.11633	283.12962	3.8E+06
unknown	**14.63**	**345.09556**	**347.10223**	2.2E+05
unknown	**14.63**	**349.15825**	**351.16487**	4.3E+05
2,4-Diaminobutyric acid	**14.74**	**293.13174**	**295.13882**	9.8E+04
unknown	14.86	337.23519	339.24191	3.9E+06
unknown	**14.86**	**513.15322**	**515.16036**	2.9E+05
unknown	**14.93**	**350.16177**	**352.16853**	8.7E+04
unknown	**15.02**	**586.29906**	**588.30493**	1.4E+06
unknown	**15.05**	**437.19370**	**439.19979**	1.5E+07
unknown	**15.05**	**446.25380**	**448.26024**	1.3E+07
unknown	**15.09**	**363.65344**	**365.66036**	4.5E+05
unknown	**15.09**	**415.21160**	**417.21784**	4.1E+07
unknown	**15.09**	**446.25264**	**448.25931**	1.7E+07
unknown	**15.17**	**437.19359**	**439.19972**	1.5E+07
unknown	**15.17**	**446.25396**	**448.26024**	9.9E+06
unknown	15.21	349.15824	351.16497	2.2E+05
unknown	15.21	393.14871	395.15494	1.0E+05
unknown	**15.32**	**334.02638**	**336.03278**	7.2E+04
unknown	15.32	381.26140	383.26782	2.2E+06
unknown	15.32	390.32164	392.32778	2.0E+06
unknown	**15.35**	**344.60343**	**346.61019**	1.6E+05
unknown	**15.35**	**360.63566**	**362.64228**	4.0E+05
L-ornithine	**15.39**	**300.10357**	**302.11034**	5.7E+06
unknown	**15.39**	**313.62717**	**315.63407**	4.2E+05
unknown	**15.39**	**416.16440**	**418.17099**	2.5E+05
Acetaminophen	**15.48**	**385.12033**	**387.12690**	3.3E+07
or 4-acetamidophenol				
unknown	**15.52**	**415.21056**	**417.21738**	5.4E+06
unknown	**15.52**	**425.61181**	**427.61821**	3.6E+05
unknown	15.52	443.17449	445.18170	2.4E+05
unknown	**15.52**	**556.20995**	**558.21587**	2.9E+05
unknown	**15.55**	**300.06526**	**302.07186**	6.3E+05
unknown	15.55	549.13059	551.13760	4.9E+05
Homovanillic	**15.63**	**416.11635**	**418.12328**	2.6E+06
unknown	**15.67**	**371.10639**	**373.11306**	2.8E+05
unknown	**15.71**	**266.08461**	**268.09126**	3.9E+06
unknown	**15.78**	**468.14099**	**471.15134**	1.1E+06
unknown	15.82	324.09015	327.10037	8.6E+04
unknown	**15.93**	**415.21165**	**417.21759**	5.9E+06
unknown	**15.97**	**265.10275**	**267.10974**	2.4E+05
Homocarnosine	**16.01**	**354.11951**	**356.12626**	1.3E+07

3-/4-hydroxyphenylacetic acid	**16.01**	**386.10585**	**388.11278**	7.4E+05
or 3-Cresotinic acid				
unknown	**16.05**	**419.47889**	**421.48561**	3.7E+05
Gentisic Acid	**16.20**	**388.10786**	**390.11464**	1.6E+05
unknown	**16.31**	**350.12972**	**352.13646**	1.7E+06
Lysine	**16.42**	**307.11035**	**309.11708**	3.6E+07
unknown	**16.42**	**423.10144**	**425.10829**	7.8E+05
unknown	**16.54**	**354.06333**	**356.07008**	3.7E+05
unknown	**16.58**	**415.21159**	**417.21785**	5.6E+06
unknown	**16.65**	**354.06357**	**356.07037**	1.7E+05
unknown	16.69	264.08841	266.09507	1.8E+05
unknown	**16.69**	**327.64286**	**329.64962**	5.5E+05
4-Hydroxybenzoic acid	**16.69**	**372.09030**	**374.09715**	3.0E+06
unknown	**16.69**	**407.16389**	**409.17058**	2.6E+05
unknown	**16.69**	**548.11822**	**552.13121**	2.7E+05
unknown	**16.73**	**348.63005**	**350.63677**	5.8E+05
unknown	**16.84**	**415.21159**	**417.21768**	8.4E+06
Histidine	**16.96**	**311.59289**	**313.59910**	1.0E+07
unknown	**16.92**	**622.17924**	**626.19295**	2.8E+06
unknown	16.99	382.10886	384.11571	1.5E+05
unknown	**17.03**	**622.17850**	**626.19205**	2.3E+05
unknown	**17.23**	**373.15816**	**375.16503**	1.0E+05
unknown	17.34	531.08352	535.09607	7.4E+04
unknown	**17.38**	**311.08286**	**313.08929**	1.2E+05
unknown	**17.38**	**359.62775**	**361.63433**	8.8E+04
unknown	**17.42**	**407.16361**	**409.17036**	2.1E+05
unknown	**17.57**	**324.10559**	**326.11206**	3.6E+05
unknown	**17.57**	**359.10488**	**361.11159**	8.8E+04
unknown	**17.57**	**390.10081**	**392.10743**	1.0E+06
unknown	**17.61**	**329.09537**	**331.10205**	1.1E+05
unknown	**17.65**	**282.10701**	**284.11371**	3.9E+05
unknown	**17.88**	**413.11681**	**415.12361**	1.9E+05
unknown	**17.88**	**527.16916**	**529.17600**	9.5E+04
unknown	**17.95**	**419.47878**	**421.48549**	4.6E+05
unknown	**18.03**	**330.18301**	**332.18952**	2.2E+06
2-aminooctanoic acid	18.03	393.18458	395.19103	6.2E+04
unknown	**18.14**	**356.09334**	**358.10001**	9.3E+05
unknown	18.14	396.10987	398.11652	1.6E+05
unknown	**18.14**	**455.16405**	**457.17072**	7.6E+04
unknown	**18.18**	**314.11909**	**316.12581**	1.8E+06
unknown	18.18	522.35580	524.36201	1.2E+06
unknown	**18.29**	**353.11173**	**355.11846**	2.6E+05
unknown	18.33	498.37005	500.37696	6.4E+05
unknown	18.36	370.11056	372.11757	1.4E+05
unknown	**18.44**	**307.14766**	**309.15437**	8.7E+05
unknown	18.44	355.05965	357.06674	1.3E+05
unknown	18.44	505.08478	507.09125	8.1E+04
unknown	**18.48**	**354.06331**	**356.07008**	1.4E+06
unknown	18.71	300.06485	302.07146	5.5E+05
unknown	**18.81**	**421.17952**	**423.18634**	4.4E+05
unknown	18.90	265.10370	267.11017	2.2E+05
unknown	**18.94**	**321.06669**	**323.07351**	3.5E+05
1,3-diaminopropane	**19.09**	**271.10055**	**273.10716**	7.0E+04
unknown	**19.16**	**314.11907**	**316.12583**	4.0E+06

L-Tyrosinamide	**19.16**	**324.10306**	**326.10997**	5.6E+04
unknown	19.53	546.10414	548.11078	1.9E+05
1,4-diaminobutane	**19.57**	**278.10854**	**280.11548**	2.0E+06
unknown	**19.57**	**528.17236**	**530.17928**	1.7E+05
unknown	19.65	357.45755	359.46425	2.7E+05
unknown	**19.65**	**419.15663**	**421.16376**	1.6E+05
unknown	**19.72**	**264.58490**	**266.59146**	2.1E+05
unknown	**19.72**	**340.13418**	**342.14094**	1.4E+05
unknown	**19.72**	**386.10576**	**388.11258**	4.3E+05
unknown	**19.76**	**319.14781**	**321.15430**	1.8E+05
unknown	**19.76**	**335.46238**	**337.46915**	1.4E+05
unknown	**19.83**	**316.09288**	**318.09943**	6.5E+05
unknown	**19.83**	**356.09544**	**358.10195**	4.1E+06
unknown	19.87	335.12456	337.13129	2.3E+05
unknown	**19.87**	**564.14984**	**566.15583**	2.3E+05
unknown	**19.91**	**331.11128**	**333.11805**	1.1E+06
unknown	**19.91**	**520.10371**	**522.11061**	3.3E+05
unknown	**19.95**	**319.08743**	**321.09426**	2.3E+05
unknown	**20.02**	**292.10595**	**294.11266**	1.1E+06
unknown	**20.10**	**486.11461**	**488.12112**	5.2E+05
unknown	20.17	349.15808	351.16508	1.9E+05
Cadaverine	**20.21**	**285.11646**	**287.12299**	7.9E+04
unknown	20.21	325.59158	327.59823	5.0E+05
Tyrosine	**20.25**	**324.59517**	**326.60123**	4.2E+07
unknown	20.33	318.07275	320.07945	4.1E+05
unknown	**20.33**	**577.13471**	**579.14038**	5.6E+06
Cysteamine	**20.36**	**310.07502**	**312.08197**	1.4E+05
unknown	20.40	297.08578	299.09248	1.7E+06
Metoprolol	**20.40**	**501.16218**	**503.16891**	1.2E+06
unknown	**20.56**	**315.08491**	**317.09172**	1.2E+06
unknown	**20.59**	**311.78333**	**313.78989**	7.4E+04
unknown	**20.63**	**306.06134**	**308.06795**	1.2E+05
unknown	**20.63**	**379.11136**	**381.11813**	2.0E+05
Phenol	**20.75**	**328.10051**	**330.10702**	1.5E+06
unknown	20.86	365.18545	369.19863	3.6E+05
unknown	20.90	352.32877	354.33551	1.5E+05
unknown	**20.98**	**315.08422**	**317.09096**	2.5E+05
unknown	21.05	363.17367	365.18055	5.0E+05
unknown	21.05	448.35126	450.35792	8.3E+04
unknown	**21.05**	**639.40833**	**641.41479**	2.4E+06
unknown	**21.09**	**298.10617**	**300.11266**	1.5E+05
Octopamine	**21.13**	**310.57723**	**312.58400**	1.2E+05
Caffeic acid	21.16	324.21598	326.26920	1.0E+05
unknown	**21.16**	**301.14130**	**303.14779**	9.3E+06
unknown	21.16	462.13766	464.14450	3.3E+05
Tyramine	**21.20**	**302.58626**	**304.59310**	1.0E+05
unknown	21.20	420.31982	422.32692	1.6E+05
unknown	21.20	584.06614	586.07269	1.0E+05
unknown	21.25	573.39983	575.40643	1.1E+06
unknown	**21.25**	**590.42678**	**592.43268**	1.6E+06
unknown	21.32	344.10671	346.11350	7.3E+05
unknown	**21.32**	**397.20128**	**399.20765**	6.1E+06
unknown	**21.32**	**454.15497**	**456.16177**	3.4E+05
unknown	**21.35**	**299.07196**	**301.07866**	1.4E+06

unknown	**21.35**	**335.17913**	**337.18591**	6.3E+05
unknown	21.35	394.20506	396.21182	7.6E+05
unknown	**21.35**	**551.35591**	**553.36213**	1.3E+07
unknown	**21.35**	**560.41589**	**562.42220**	3.2E+06
unknown	**21.43**	**310.57995**	**312.58664**	3.1E+05
Serotonin	**21.47**	**322.19971**	**324.21451**	4.0E+04
unknown	**21.51**	**507.32992**	**509.33634**	1.9E+07
unknown	21.51	516.39014	518.39646	4.2E+06
unknown	**21.55**	**257.57712**	**259.58368**	2.6E+05
unknown	**21.66**	**354.11627**	**356.12298**	8.4E+06
unknown	**21.66**	**419.27735**	**421.28374**	2.2E+07
unknown	21.69	428.33747	430.34393	6.9E+06
unknown	**21.73**	**375.25103**	**377.25727**	1.1E+07
unknown	21.88	498.15251	501.16354	1.4E+07
unknown	21.88	529.19366	533.20648	2.6E+06
unknown	**21.88**	**598.21594**	**602.22959**	2.2E+05
unknown	**21.88**	**669.23996**	**673.25275**	3.7E+05
unknown	**22.07**	**352.33975**	**354.34629**	3.1E+06
unknown	**22.07**	**420.32019**	**422.32683**	1.1E+05
unknown	22.18	284.10846	286.11500	1.4E+05
unknown	**22.18**	**287.07921**	**289.08569**	6.1E+05
unknown	22.18	352.08944	354.09617	2.6E+05
unknown	22.18	561.39697	563.40307	1.4E+05
unknown	**22.22**	**450.20673**	**452.21353**	1.7E+05
unknown	**22.26**	**316.05247**	**318.05933**	4.6E+05
unknown	**22.30**	**305.57103**	**307.57772**	2.1E+05
unknown	22.30	450.20670	453.21667	8.3E+05
unknown	22.37	315.58995	317.59689	1.3E+05
unknown	22.49	387.21025	389.21769	2.7E+07
unknown	**22.56**	**388.07713**	**390.08446**	1.5E+07
unknown	**22.60**	**344.10672**	**346.11348**	4.4E+05
Pyrocatechol	**22.60**	**577.15951**	**581.17244**	1.2E+05
unknown	**22.68**	**404.13200**	**406.13889**	8.7E+04
unknown	**22.68**	**455.24463**	**457.25171**	1.4E+05
unknown	22.68	541.19397	545.20798	4.4E+05
unknown	**22.68**	**572.23639**	**576.24963**	1.0E+05
unknown	**22.71**	**360.57920**	**362.58608**	1.5E+06
unknown	**22.75**	**298.06411**	**300.07081**	8.1E+04
unknown	22.79	377.19150	383.21169	2.1E+05
unknown	**22.87**	**282.44449**	**284.45123**	2.9E+05
Spermidine	**22.87**	**423.16330**	**426.17345**	3.8E+05
unknown	**22.90**	**289.08239**	**291.08924**	2.6E+05
unknown	**22.94**	**388.07736**	**390.08458**	2.0E+06
unknown	**22.94**	**455.24460**	**457.25198**	1.1E+05
unknown	22.94	648.26212	650.26971	1.2E+05
unknown	23.02	284.06649	286.07319	1.5E+05
unknown	**23.02**	**315.59005**	**317.59683**	5.2E+05
unknown	**23.10**	**447.34706**	**450.35721**	2.3E+05
unknown	**23.33**	**421.15846**	**425.17194**	6.6E+05
unknown	**23.51**	**363.21047**	**365.21718**	2.2E+06
unknown	**23.59**	**281.40863**	**283.41511**	1.3E+05
unknown	**23.59**	**421.60902**	**424.61891**	1.9E+05
unknown	**23.59**	**447.34781**	**450.35706**	2.5E+05
unknown	**23.67**	**305.06801**	**307.07483**	2.0E+06

Thymol	**23.86**	**384.16349**	**386.17009**	1.1E+06
unknown	**23.90**	**288.74786**	**290.75497**	2.0E+05
unknown	**23.90**	**432.61900**	**435.62909**	4.0E+05
Hydroquinone	23.90	289.08891	291.08922	1.4E+05
unknown	23.94	648.26327	650.27112	3.1E+05
unknown	**24.01**	**288.07625**	**290.08279**	4.4E+05
unknown	**24.01**	**431.61105**	**434.62105**	6.3E+05
unknown	24.01	445.12127	449.13429	3.4E+05
unknown	24.01	530.72112	532.72765	3.2E+05
unknown	**24.14**	**281.40830**	**283.41518**	1.5E+05
unknown	24.14	318.10913	320.11586	1.5E+05
unknown	**24.14**	**421.60914**	**424.61912**	4.0E+05
unknown	24.14	434.63531	437.64542	1.8E+05
unknown	24.14	522.59827	524.60516	1.4E+06
unknown	24.14	694.49799	696.50394	1.3E+06
unknown	24.18	625.20172	627.20721	1.1E+05
unknown	24.25	562.31702	564.32385	8.4E+06
unknown	24.29	562.31494	564.32199	1.4E+07
unknown	**24.29**	**685.43631**	**687.44385**	2.4E+06
unknown	24.37	562.31655	564.32343	8.1E+06
unknown	24.37	603.16174	607.17590	1.0E+06
unknown	**24.44**	**288.07619**	**290.08269**	2.1E+05
unknown	24.48	302.08482	304.09130	1.9E+07
unknown	24.48	557.09602	560.10717	4.2E+05

CSF - #1 Repeat		**CSF - #1 Repeat**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	1.62	280.04782	282.05451	4.6E+05
unknown	1.62	349.05387	351.06049	4.4E+05
unknown	1.62	356.09230	358.09894	4.0E+05
unknown	1.62	372.06987	374.07651	1.7E+06
unknown	1.62	534.17402	536.18077	2.8E+06
unknown	1.62	561.09572	563.10287	5.3E+05
unknown	1.62	568.13452	570.14081	5.6E+05
unknown	1.62	574.12781	576.13348	2.4E+05
unknown	1.62	615.15491	617.16205	4.6E+05
unknown	1.62	669.61884	671.62491	4.6E+05
unknown	1.62	825.19098	829.20282	1.7E+05
unknown	1.65	263.05350	265.06053	2.0E+05
unknown	1.65	271.04266	273.04933	2.3E+06
unknown	1.65	331.04305	333.04992	2.8E+05
unknown	1.65	336.02210	338.02865	5.9E+05
unknown	1.65	389.58876	391.59540	3.8E+05
unknown	1.65	396.57422	398.58099	9.9E+05
unknown	1.65	413.09865	415.10500	2.8E+05
unknown	1.65	416.55105	418.55828	4.1E+05
unknown	1.65	523.10883	525.11467	9.5E+05
unknown	1.65	526.11759	528.12421	1.8E+06
unknown	1.65	582.10492	584.11179	5.3E+05
unknown	1.65	585.11512	587.12183	4.8E+05
unknown	1.65	648.64005	650.64579	5.0E+05
unknown	1.65	660.63194	662.63900	3.7E+05
unknown	1.65	794.14895	796.15433	1.7E+06
unknown	1.69	503.13222	505.13859	2.2E+06
unknown	1.69	641.65983	644.66864	3.6E+05
unknown	1.69	754.19476	756.20001	4.0E+05
unknown	1.69	757.20369	759.21143	9.0E+05
unknown	1.69	783.71478	785.72260	2.9E+05
unknown	1.69	788.16449	792.17944	2.5E+05

unknown	1.73	252.06917	254.07567	1.3E+07
unknown	1.73	672.62677	674.63226	2.7E+05
unknown	1.73	800.16364	803.17371	4.0E+05
unknown	1.77	372.06960	374.07627	4.3E+05
unknown	1.77	536.18060	538.18765	1.7E+06
unknown	1.77	675.63568	677.64374	3.5E+05
unknown	1.77	778.18164	780.18939	4.0E+05
unknown	1.81	503.13202	506.14246	1.9E+05
unknown	1.81	580.14492	582.15145	6.1E+05
unknown	1.81	633.05316	637.06549	1.3E+05
unknown	1.81	639.07212	641.07852	4.7E+05
unknown	1.81	707.05963	709.06529	2.5E+05
unknown	1.85	567.98415	569.99019	2.0E+05
unknown	1.85	590.02608	592.03281	3.3E+05
unknown	1.85	605.09009	607.09661	3.8E+05
unknown	1.85	617.20709	619.21306	1.2E+05
unknown	1.89	274.05091	276.05748	6.6E+06
unknown	1.89	336.02155	338.02872	1.6E+05
unknown	1.89	364.02070	366.02735	1.2E+06
unknown	1.89	372.06937	374.07620	3.7E+05
unknown	1.89	388.04141	390.04888	1.0E+05
unknown	1.89	404.01366	406.02045	1.8E+05
unknown	1.89	421.97951	425.99370	1.8E+05
unknown	1.89	432.00831	434.01521	1.1E+06
unknown	1.89	499.99588	502.00259	5.1E+05
unknown	1.89	536.18033	538.18755	1.2E+06
unknown	1.93	268.04645	270.05283	1.1E+05
unknown	1.97	555.12256	557.12982	3.8E+05
unknown	2.09	543.01318	547.02595	3.7E+05
unknown	2.09	584.97266	586.97961	4.1E+05
unknown	2.17	539.09935	541.10701	2.8E+05
unknown	2.17	558.03252	562.04538	4.6E+05
unknown	2.17	632.22696	634.23277	2.1E+05
unknown	2.21	414.12210	416.12906	5.3E+05
unknown	2.25	374.07559	376.08164	9.7E+04
unknown	2.29	274.05092	277.06114	1.2E+06
unknown	2.29	389.12829	391.13496	2.9E+06
unknown	2.29	537.08370	539.09032	1.2E+05
unknown	2.33	397.05960	399.06623	8.3E+04
unknown	2.33	501.11610	503.12247	1.1E+05
unknown	2.37	274.05087	276.05759	1.3E+06
unknown	2.37	364.02046	366.02719	3.1E+05
phosphoethaolamine	2.37	375.07770	377.08444	1.0E+06
unknown	2.37	432.00792	434.01498	2.1E+05
unknown	2.40	360.11282	362.11981	9.6E+04
unknown	2.40	390.08011	392.08683	7.5E+04
unknown	2.48	364.02023	366.02711	2.5E+05
unknown	2.52	252.06903	254.07571	1.2E+06
unknown	2.52	375.07760	377.08435	8.2E+05
unknown	2.52	438.20575	440.21237	1.2E+05
Taurine	2.64	359.07330	361.07997	5.9E+06
Glucosamine	2.64	413.13859	415.14466	1.0E+05
3-methylhistidine	2.64	403.14391	405.15052	1.0E+06
unknown	2.68	276.08014	278.08687	2.8E+06
unknown	2.68	320.04393	322.05100	1.9E+05
unknown	2.68	375.07795	377.08432	4.1E+05
unknown	2.68	380.07991	382.08671	2.5E+05
unknown	2.68	388.10780	390.11444	2.7E+06
1-methylhistidine	2.75	403.14383	405.15044	1.2E+06
unknown	2.83	363.10115	365.10770	3.5E+05
unknown	2.87	380.16451	382.17093	7.8E+05

unknown	2.87	509.17040	511.17704	1.1E+06
unknown	2.91	424.11661	426.12403	9.0E+04
unknown	2.95	366.14856	368.15506	2.7E+05
unknown	3.03	345.13821	347.14491	8.6E+04
unknown	3.03	376.18035	378.18710	2.8E+05
unknown	3.07	275.15041	277.15692	1.0E+05
unknown	3.07	414.12176	416.12858	1.6E+06
unknown	3.10	383.12759	385.13413	1.5E+05
unknown	3.10	388.10922	390.11584	1.0E+05
Arginine	3.22	408.17021	410.17688	4.7E+06
unknown	3.38	319.11119	321.11805	2.2E+05
unknown	3.45	253.07254	255.07886	9.6E+04
unknown	3.49	408.19494	410.20201	1.3E+05
Homoarginine	3.53	422.21098	424.21773	4.4E+05
unknown	3.53	502.13934	504.14605	3.4E+05
unknown	3.53	547.15489	549.16190	9.1E+04
unknown	3.57	314.09565	316.10274	2.3E+05
Asparagine	3.61	366.11197	368.11872	5.4E+06
unknown	3.61	380.16398	382.17073	1.2E+06
unknown	3.65	365.12806	367.13458	1.5E+06
unknown	3.72	380.08000	382.08667	1.8E+05
unknown	3.88	363.10114	365.10785	3.5E+05
unknown	3.92	402.10775	404.11435	3.3E+06
Glutamine	3.96	380.12579	382.13242	1.7E+08
unknown	3.96	411.16795	413.17479	3.5E+06
unknown	4.00	378.14093	384.16093	2.0E+07
unknown	4.12	402.10898	404.11586	1.2E+06
unknown	4.15	392.12753	394.13427	2.7E+05
L-citrulline	4.15	409.15425	411.16064	2.1E+06
unknown	4.19	364.08539	366.09189	1.3E+05
unknown	4.19	436.20158	438.20801	1.4E+05
unknown	4.32	270.03513	272.04189	5.5E+05
unknown	4.32	291.06372	293.07044	3.8E+05
unknown	4.32	302.02507	304.03177	6.4E+05
unknown	4.32	380.12802	383.13837	2.1E+06
unknown	4.32	424.10669	426.11349	3.4E+05
unknown	4.36	242.57191	244.57858	2.6E+06
unknown	4.36	377.08153	379.08843	4.4E+05
unknown	4.36	485.13807	489.15096	8.3E+06
unknown	4.36	506.11527	510.12882	1.2E+06
unknown	4.41	381.13174	383.13815	2.2E+05
unknown	4.45	388.10797	390.11423	1.7E+05
3-sn-Phosphatidylethanolamine	4.45	484.13635	488.14962	9.2E+06
Unknown	4.45	515.17786	519.19182	2.8E+05
Methylguanidine	4.50	307.12245	309.12899	1.3E+05
Unknown	4.50	366.10782	369.11752	1.5E+05
Unknown	4.54	252.06975	254.07634	2.2E+05
Homoserine	4.63	353.11679	355.12352	4.0E+05
Unknown	4.67	319.11127	321.11805	3.2E+05
Methionine sulfoxide	4.67	399.10447	401.11121	1.2E+06
Unknown	4.67	465.18051	467.18725	2.3E+06
Unknown	4.80	360.05728	362.06384	8.7E+04
L-aspartic acid amide	4.80	366.10046	368.10745	9.3E+04
Unknown	4.80	394.14344	396.15001	2.5E+05
Unknown	4.80	499.17458	501.18180	2.1E+05
Unknown	4.80	617.16608	619.17236	1.6E+05
Serine	4.88	339.10075	341.10751	3.4E+07
Aspartic Acid	4.88	367.09595	369.10264	8.0E+05
Glutamic Acid	4.88	381.11179	383.11843	1.7E+06
Unknown	5.00	263.21197	265.21862	2.8E+05
Unknown	5.00	339.10110	342.11151	1.6E+06

Unknown	5.12	380.12329	382.12997	2.7E+05
Unknown	5.16	394.17964	396.18683	1.4E+05
Unknown	5.19	367.13306	370.14297	2.0E+05
Unknown	5.19	422.13809	424.14455	8.2E+04
Unknown	5.19	433.22733	435.23340	1.1E+05
Unknown	5.23	404.12761	406.13427	2.2E+05
Unknown	5.23	502.14563	504.15266	1.2E+05
4-Hydroxy-proline	5.27	365.11680	367.12335	2.4E+06
Unknown	5.31	404.12689	406.13403	8.5E+04
Unknown	5.35	452.18588	454.19238	7.4E+04
Unknown	5.35	455.14843	457.15558	1.4E+05
Unknown	5.35	582.19049	584.19716	1.7E+05
Unknown	5.35	600.20124	602.20792	6.1E+05
Unknown	5.39	442.11694	444.12392	8.4E+05
Unknown	5.42	466.16440	468.17134	1.3E+06
Unknown	5.42	491.04097	495.05399	5.3E+05
Aminoadipic acid	5.50	395.12727	397.13404	1.8E+05
Unknown	5.50	422.17484	424.18146	2.4E+05
Unknown	5.54	336.13792	338.14463	2.5E+06
Iminodiacetic acid	5.54	367.18015	369.18673	2.8E+05
Unknown	5.58	505.07604	509.08853	2.5E+05
Unknown	5.62	458.09137	460.09896	7.5E+04
Unknown	5.66	354.12057	356.12709	4.2E+06
Unknown	5.66	363.14880	365.15527	8.7E+05
Unknown	5.66	457.08942	459.09595	3.7E+05
Unknown	5.66	488.13065	490.13827	3.8E+05
Folic acid	5.69	338.09320	339.09644	1.3E+05
Unknown	5.69	353.11620	356.12596	4.2E+07
Unknown	5.73	480.18027	482.18736	9.4E+05
Threonine	5.77	353.11671	355.12328	1.7E+07
Unknown	5.81	337.15820	339.16498	2.1E+06
Unknown	5.89	379.13243	381.13924	1.1E+06
Diethanolamine	5.93	339.13770	341.14436	2.9E+05
Unknown	5.93	469.12565	473.13834	1.3E+05
Unknown	6.01	296.11457	298.12108	2.9E+06
Unknown	6.01	478.12802	480.13503	2.7E+06
Ethanolamine	6.04	295.11090	297.11766	1.5E+07
Unknown	6.04	611.19741	613.20427	4.3E+05
Unknown	6.08	463.23798	465.24469	4.8E+05
Unknown	6.08	567.17126	569.17749	2.2E+05
Unknown	6.12	251.08469	253.09135	1.9E+07
Unknown	6.12	523.14481	525.15172	2.3E+06
Unknown	6.20	351.10122	353.10758	9.4E+04
Unknown	6.20	396.11145	398.11828	3.4E+05
Unknown	6.20	427.15353	429.16031	1.5E+05
Unknown	6.20	501.16284	504.17307	1.7E+05
Unknown	6.24	317.13198	319.13880	2.0E+06
Unknown	6.24	348.17439	350.18106	5.1E+05
Unknown	6.24	415.13250	417.13951	1.9E+06
Unknown	6.39	348.10144	350.10815	9.4E+05
Unknown	6.39	398.12724	400.13386	3.6E+06
Unknown	6.39	464.14879	466.15546	2.0E+05
Unknown	6.43	398.12756	400.13370	5.9E+05
Unknown	6.43	422.17487	424.18130	1.9E+05
Glycine	6.47	309.09047	311.09710	9.5E+06
Unknown	6.47	375.14867	377.15547	1.8E+05
Unknown	6.47	381.14815	383.15486	1.1E+06
Unknown	6.67	344.10655	346.11320	1.4E+06
Unknown	6.71	364.16915	366.17576	1.4E+06
Unknown	6.75	477.16163	479.16839	8.5E+05
Unknown	6.79	348.13770	350.14457	5.2E+05

Unknown	6.87	515.16815	517.17580	7.6E+04
Unknown	6.94	396.11124	398.11798	2.3E+05
Unknown	6.94	415.09186	417.09914	9.4E+04
Unknown	7.07	362.11499	364.12168	5.2E+07
Glycylproline	7.07	406.08050	408.08667	9.0E+04
Unknown	7.15	347.11760	349.12418	8.9E+05
Unknown	7.15	362.11716	364.12366	1.2E+07
Tyrosine methyl ester	7.19	415.13272	417.13968	1.9E+06
Unknown	7.23	344.10657	346.11322	8.2E+05
Unknown	7.23	367.13278	369.13940	5.1E+05
Unknown	7.31	354.14816	356.15500	3.1E+05
Alanine	7.35	323.10618	325.11267	3.2E+07
r-aminobutyric acid	7.54	337.12207	339.12882	5.8E+05
Unknown	7.90	396.11146	398.11829	1.3E+06
Unknown	7.90	402.08708	404.09363	7.7E+05
Unknown	7.90	473.11057	475.11783	4.5E+05
Unknown	8.05	364.16906	366.17579	2.0E+05
Hypoxanthine	8.21	370.09704	372.10375	1.0E+06
Unknown	8.21	396.11085	398.11782	3.3E+05
Unknown	8.25	450.20566	452.21280	1.6E+05
Unknown	8.32	494.19609	496.20233	7.2E+04
Unknown	8.36	378.06738	380.07459	3.7E+05
3-Aminoisobutyric acid	8.40	337.12195	339.12866	1.9E+05
Unknown	8.40	431.13848	433.14541	1.4E+06
Unknown	8.44	279.07996	281.08658	5.4E+05
Unknown	8.48	386.09193	388.09869	3.2E+05
Unknown	8.52	396.13504	398.14183	3.6E+05
5-Aminopentanoic acid	8.52	351.13754	353.14416	6.0E+05
Unknown	8.56	485.11992	489.13348	4.9E+05
Unknown	8.59	295.13837	297.14514	9.6E+04
2-Aminobutyric acid	8.90	337.12167	339.12824	1.6E+07
unknown	9.02	321.12686	324.13751	1.9E+05
Sarcosine	9.10	323.09902	325.11297	1.5E+05
unknown	9.14	370.09709	372.10385	4.0E+06
unknown	9.17	363.10120	365.10797	6.4E+05
unknown	9.17	415.09622	417.10304	5.4E+05
Methylcysteine	9.37	369.09417	371.10086	8.2E+05
unknown	9.37	492.05249	494.06002	3.1E+05
unknown	9.49	397.11453	399.12156	9.6E+04
unknown	9.53	322.13082	324.13715	1.1E+05
unknown	9.53	396.11132	398.11810	4.9E+05
unknown	9.56	451.18006	453.18744	1.1E+05
unknown	9.60	266.10696	268.11353	3.6E+06
unknown	9.60	553.18384	555.19012	2.1E+05
unknown	9.64	321.12708	323.13358	8.0E+05
unknown	9.67	266.10332	268.10977	4.5E+06
unknown	9.71	343.10893	345.11562	9.7E+05
unknown	9.71	551.17646	553.18264	4.0E+06
unknown	9.75	287.08085	290.09108	3.4E+07
unknown	9.87	417.12477	419.13156	1.4E+06
unknown	9.87	549.17154	551.17771	1.6E+06
unknown	9.87	615.15422	617.16101	1.6E+06
Proline	9.87	349.12142	351.12768	9.0E+04
unknown	9.94	552.17978	554.18616	1.6E+06
unknown	9.98	266.10358	268.11011	4.6E+06
unknown	9.98	287.08177	290.09170	9.8E+06
unknown	9.98	551.17636	553.18227	4.0E+06
Methylamine	10.10	265.10035	267.10665	2.1E+07
unknown	10.10	531.20099	533.20770	1.3E+06
unknown	10.17	266.10377	268.11048	3.2E+06
unknown	10.28	311.08304	313.08966	3.2E+05

unknown	10.36	303.05661	305.06324	2.8E+05
unknown	10.36	321.11439	323.12112	1.7E+06
unknown	10.40	412.16930	414.17594	4.3E+05
Valine	10.51	351.13605	353.14257	7.0E+07
3-Hydroxypicolinic acid	10.51	373.11799	375.12462	6.0E+05
unknown	10.54	265.10403	268.11392	2.5E+06
unknown	10.54	553.17566	555.18274	4.2E+05
unknown	10.58	378.18494	380.19165	3.9E+05
Methionine	10.58	383.10980	385.11637	3.5E+06
unknown	10.73	400.08521	402.09196	1.5E+06
unknown	10.92	378.06765	380.07469	1.6E+06
Tryptophan	10.96	438.14851	440.15513	1.4E+07
unknown	11.11	380.11646	382.12319	1.6E+05
unknown	11.26	380.61057	382.61749	1.2E+05
unknown	11.66	382.58144	384.58808	9.4E+05
unknown	11.74	387.61865	389.62541	2.5E+05
unknown	11.74	406.09583	408.10254	1.8E+05
unknown	11.77	418.13204	420.13893	7.3E+05
unknown	11.82	315.09058	317.09720	1.9E+05
unknown	11.86	372.12512	374.13133	9.0E+04
unknown	12.09	295.10220	297.10923	9.9E+04
unknown	12.12	364.14111	366.14757	1.1E+05
Pipecolic acid	12.16	363.13763	365.14431	6.2E+05
Phenylalanine	12.23	399.13555	401.14221	4.3E+07
unknown	12.39	406.61714	408.62401	3.0E+05
3-Hydroxymandelic acid	12.50	402.10112	404.10782	1.5E+05
Isoleucine	12.54	365.15293	367.15934	1.8E+07
unknown	12.57	313.60903	315.61563	3.9E+06
unknown	12.57	393.15902	395.16556	3.3E+05
unknown	12.65	265.09318	268.10352	1.7E+05
unknown	12.69	320.06616	322.07312	8.0E+04
unknown	12.73	379.13216	381.13879	7.4E+05
unknown	12.73	501.11527	505.12857	1.3E+06
Leucine	12.80	365.15127	367.15780	6.4E+07
L-norleucine	12.88	365.15276	367.15930	1.7E+07
unknown	13.03	342.62976	344.63641	3.2E+05
Cystine	13.11	354.07015	356.07650	8.2E+05
unknown	13.15	317.13197	319.13878	8.1E+05
unknown	13.15	335.14230	337.14858	3.0E+07
unknown	13.22	335.14249	338.15280	1.4E+07
unknown	13.29	362.06957	364.07617	3.6E+05
unknown	13.37	369.10655	371.11328	7.5E+04
unknown	13.41	342.63004	344.63659	2.7E+05
unknown	13.48	378.10066	380.10710	9.0E+06
unknown	13.52	265.10072	268.11093	1.3E+06
unknown	13.60	404.07982	406.08696	1.4E+05
Hydroxyphenyllactic acid	13.64	416.11627	418.12320	3.0E+06
unknown	13.75	307.09301	309.09968	4.5E+06
unknown	13.75	474.06768	477.07832	3.6E+05
unknown	13.79	415.21170	417.21811	2.5E+06
unknown	13.94	402.10070	404.10754	3.2E+05
Homocystine	13.97	368.09869	370.10575	1.8E+05
unknown	14.01	322.07444	324.08125	2.2E+05
unknown	14.08	378.10091	380.10746	1.0E+06
unknown	14.16	342.62990	344.63653	2.8E+05
unknown	14.20	349.63770	351.64426	6.2E+06
5-HIAA	14.20	425.11680	427.12362	7.2E+05
unknown	14.20	511.13792	513.14452	2.0E+05
unknown	14.27	336.11408	338.12080	4.9E+05
unknown	14.31	346.06556	348.07235	1.2E+05
unknown	14.35	281.12275	283.12931	2.6E+06

unknown	14.35	301.09808	305.11165	1.1E+06
Dimethylamine	14.42	279.11577	281.12223	2.2E+07
unknown	14.42	579.20755	583.22139	1.2E+06
unknown	14.42	584.22399	587.23395	2.0E+06
Phenylpropanolamine	14.50	385.12229	387.12875	3.0E+05
unknown	14.54	280.11949	283.12926	1.3E+06
unknown	14.54	301.09807	305.11159	7.8E+05
unknown	14.57	279.11631	282.12646	4.5E+06
unknown	14.57	349.15833	351.16492	3.0E+05
unknown	14.66	345.09553	347.10230	1.3E+05
unknown	14.73	350.16202	352.16858	1.1E+05
unknown	14.73	371.14026	373.14706	9.5E+04
2,4-Diaminobutyric acid	14.77	293.13143	295.13872	4.3E+04
unknown	14.84	513.15308	515.16014	3.9E+05
unknown	14.87	437.19358	439.19963	4.2E+06
unknown	14.94	281.12324	284.13342	7.6E+04
unknown	15.09	415.21164	417.21802	3.8E+07
unknown	15.09	446.25344	448.25998	1.5E+07
unknown	15.13	363.65348	365.66038	5.6E+05
unknown	15.13	586.29938	588.30627	3.5E+06
unknown	15.21	378.10110	380.10755	2.1E+05
unknown	15.21	471.12308	473.13050	9.7E+04
unknown	15.29	334.02586	336.03277	7.4E+04
unknown	15.33	344.60356	346.61017	1.0E+05
unknown	15.37	360.63564	362.64221	4.0E+05
L-ornithine	15.40	300.10354	302.11021	7.8E+06
unknown	15.40	313.62731	315.63410	6.3E+05
Acetaminophen	15.44	385.12108	387.12747	3.9E+07
or 4-acetamidophenol				
unknown	15.44	416.16339	418.17023	6.4E+05
unknown	15.48	425.61229	427.61936	3.1E+05
unknown	15.48	556.20966	558.21585	4.5E+05
unknown	15.52	300.06526	302.07187	7.1E+05
o-Hydroxyphenylacetic acid	15.52	386.11008	388.11649	2.6E+05
unknown	15.52	407.10398	409.11077	1.4E+06
unknown	15.55	343.12183	345.12874	1.2E+05
Homovanillic	15.59	416.11638	418.12327	2.3E+06
unknown	15.67	371.10663	373.11312	2.8E+05
unknown	15.70	266.08458	268.09109	6.8E+05
unknown	15.74	468.14110	471.15150	1.6E+06
unknown	15.81	265.10806	267.11459	1.5E+05
unknown	15.81	385.12540	387.13254	9.7E+04
unknown	15.93	437.19366	439.20004	3.2E+06
Homocarnosine	16.00	354.11911	356.12569	1.9E+07
3-/4-hydroxyphenylacetic acid	16.00	386.10590	388.11279	9.9E+05
or 3-Cresotinic acid				
Unknown	16.00	474.18096	476.18799	1.6E+06
Unknown	16.05	318.10928	320.11589	1.5E+05
Unknown	16.09	402.15958	404.16643	2.0E+05
Unknown	16.09	419.47884	421.48560	4.4E+05
Unknown	16.12	345.57367	347.58012	6.1E+05
Gentisic Acid	16.20	388.10785	390.11456	1.5E+05
Unknown	16.27	350.12968	352.13638	1.7E+06
Unknown	16.38	423.10138	425.10811	5.5E+05
Lysine	16.42	307.11042	309.11706	4.4E+07
Unknown	16.50	308.10767	310.11472	9.4E+05
Unknown	16.61	354.06333	356.07007	3.3E+05
Unknown	16.65	327.64281	329.64961	6.3E+05
Unknown	16.65	407.16418	409.17065	1.1E+05
4-Hydroxybenzoic acid	16.69	372.09038	374.09716	3.0E+06
Unknown	16.69	548.11826	552.13140	3.1E+05

Unknown	16.72	348.63006	350.63672	4.3E+05
Unknown	16.83	415.21161	417.21779	6.9E+06
Histidine	16.94	311.59329	313.59985	1.2E+07
Unknown	17.09	496.33991	498.34630	2.1E+06
Unknown	17.24	373.15793	375.16494	1.3E+05
Unknown	17.35	311.08292	313.08963	1.6E+05
Unknown	17.39	347.08799	349.09457	9.3E+04
Unknown	17.39	359.62772	361.63445	1.9E+05
Unknown	17.43	407.16345	409.17051	2.0E+05
Unknown	17.54	297.08580	299.09221	5.0E+05
Unknown	17.54	324.10552	326.11198	3.7E+05
Unknown	17.54	359.10472	361.11165	1.1E+05
Unknown	17.54	390.10076	392.10739	1.0E+06
Unknown	17.58	305.09552	307.10269	2.4E+05
Unknown	17.62	282.10698	284.11350	3.7E+05
Unknown	17.62	329.09551	331.10217	1.1E+05
Unknown	17.88	413.11668	415.12361	2.6E+05
Unknown	17.88	527.16949	529.17580	1.2E+05
Unknown	17.95	419.47860	421.48536	6.0E+05
Unknown	17.99	330.18300	332.18915	1.8E+06
Unknown	18.02	393.18478	399.20442	8.6E+04
Unknown	18.10	356.09333	358.10001	9.3E+05
Unknown	18.13	395.10612	397.11285	5.5E+05
Unknown	18.13	455.16431	457.17153	7.3E+04
Unknown	18.20	314.11907	316.12575	1.9E+06
Unknown	18.32	321.12284	323.12904	4.8E+06
Unknown	18.32	330.18303	332.18921	2.4E+06
Unknown	18.32	353.11198	355.11853	6.7E+05
Unknown	18.43	307.14765	309.15432	6.8E+05
Unknown	18.50	354.06335	356.07008	1.0E+06
Unknown	18.64	354.06342	356.07010	1.0E+06
Unknown	18.83	421.17945	423.18633	4.8E+05
Unknown	18.93	321.06677	323.07346	4.3E+05
Unknown	19.04	358.12222	360.12878	8.0E+04
1,3-diaminopropane	19.08	271.10039	273.10718	1.0E+05
Unknown	19.15	314.11924	316.12575	1.8E+06
L-Tyrosinamide	19.22	324.10370	326.11029	1.1E+05
Unknown	19.44	279.10743	281.11399	2.6E+05
Unknown	19.44	530.18824	532.19452	5.2E+05
Unknown	19.55	528.17278	530.17920	2.3E+05
1,4-diaminobutane	19.59	278.10849	280.11569	2.7E+06
Unknown	19.63	419.15639	421.16337	1.2E+05
Unknown	19.70	264.58491	266.59156	2.1E+05
Unknown	19.73	340.13386	342.14097	1.4E+05
Unknown	19.73	386.10572	388.11256	3.0E+05
Unknown	19.77	319.14734	321.15437	1.1E+05
Unknown	19.77	335.46222	337.46887	1.6E+05
Unknown	19.84	316.09296	318.09946	6.5E+05
Unknown	19.84	356.09545	358.10192	4.8E+06
Unknown	19.88	564.14932	566.15531	4.4E+05
Unknown	19.91	331.11128	333.11806	1.5E+06
Unknown	19.91	520.10384	522.11063	3.1E+05
Unknown	19.99	365.24533	367.25223	1.9E+06
Unknown	19.99	373.08571	375.09193	1.8E+05
Unknown	20.02	292.10599	294.11263	1.3E+06
Unknown	20.10	486.11463	488.12109	4.8E+05
Cadaverine	20.22	285.11636	287.12296	8.6E+04
Unknown	20.22	412.80347	414.81019	8.5E+04
Tyrosine	20.26	324.59427	326.60101	5.8E+07
Unknown	20.34	577.13476	579.14081	3.5E+06
Cysteamine	20.38	310.07497	312.08199	1.4E+05

Metoprolol	20.42	501.16156	503.16872	1.0E+06
Unknown	20.54	381.12724	383.13443	6.1E+05
Unknown	20.58	315.08492	317.09169	1.8E+06
Unknown	20.62	306.06097	308.06805	7.4E+04
Unknown	20.62	311.78311	313.78998	1.1E+05
Unknown	20.67	379.11151	381.11814	2.2E+05
Phenol	20.75	328.10039	330.10700	1.1E+06
Unknown	20.99	315.08419	317.09097	2.5E+05
Unknown	20.99	706.18237	708.18771	1.9E+05
Unknown	21.11	298.10605	300.11282	2.0E+05
Unknown	21.11	416.30673	420.31989	8.1E+04
Unknown	21.11	639.40862	641.41507	4.0E+06
Unknown	21.11	648.46902	650.47571	7.6E+05
Unknown	21.20	301.14129	303.14773	1.0E+07
Tyramine	21.20	302.58655	304.59313	1.5E+05
Unknown	21.24	590.42678	592.43439	1.5E+06
Unknown	21.24	595.38180	597.38840	7.7E+06
Unknown	21.28	454.15492	456.16161	4.8E+05
Unknown	21.32	299.07200	301.07868	1.7E+06
Unknown	21.32	336.18304	338.18931	8.3E+04
Unknown	21.32	397.20134	399.20786	4.8E+06
Unknown	21.32	598.14026	601.15014	1.1E+05
Unknown	21.36	335.17911	337.18590	3.8E+05
Unknown	21.36	551.35595	553.36227	1.2E+07
Unknown	21.36	560.41601	562.42258	2.5E+06
Serotonin	21.40	322.19853	324.21428	6.0E+04
Octopamine	21.44	310.57997	312.58656	4.1E+05
Unknown	21.52	257.57695	259.58356	2.2E+05
Unknown	21.52	507.32987	509.33622	1.6E+07
Unknown	21.52	515.14969	518.16012	2.8E+05
Unknown	21.56	280.08777	282.09451	8.6E+04
Unknown	21.56	405.16379	409.17697	7.1E+04
Unknown	21.60	463.30332	465.30975	2.1E+07
Unknown	21.60	472.36362	474.37006	5.4E+06
Unknown	21.68	354.11628	356.12273	4.6E+06
Unknown	21.68	419.27730	421.28373	2.4E+07
Unknown	21.72	375.25109	377.25746	1.1E+07
Unknown	21.80	296.42377	298.43045	7.6E+04
Unknown	21.80	444.13214	447.14191	7.7E+04
Unknown	21.84	670.24408	674.25647	1.6E+05
Unknown	21.88	498.15235	502.16565	1.9E+07
Unknown	21.88	530.19592	533.20587	1.1E+06
Unknown	21.88	556.20496	560.21846	8.1E+05
Unknown	21.88	588.12180	592.13489	4.7E+05
Unknown	21.88	598.21608	602.22925	3.7E+05
Unknown	21.88	669.23968	673.25317	5.2E+05
Unknown	21.95	249.57945	251.58630	2.1E+06
Unknown	21.95	333.16364	335.16989	8.2E+04
Unknown	21.95	521.13778	524.14781	7.1E+05
Unknown	21.95	536.10788	540.12133	4.3E+05
Unknown	21.95	670.24333	674.25708	2.0E+05
Unknown	22.04	350.26678	352.27361	5.5E+06
Unknown	22.08	307.08737	309.09429	2.0E+05
Unknown	22.08	420.31969	422.32620	9.6E+04
Unknown	22.08	499.15601	502.16617	1.1E+05
Unknown	22.16	287.07925	289.08581	4.6E+05
Unknown	22.24	316.05238	318.05922	4.9E+05
Unknown	22.28	305.57116	307.57784	1.8E+05
Unknown	22.28	450.20661	452.21355	6.7E+05
Unknown	22.32	276.07655	278.08344	1.2E+05
Unknown	22.41	352.32837	354.33554	2.4E+05

Unknown	22.56	388.07717	390.08444	1.2E+07
Pyrocatechol	22.64	577.15845	581.17148	2.5E+05
Unknown	22.64	344.10669	346.11340	8.4E+05
Unknown	22.64	599.13995	603.15314	7.3E+04
Unknown	22.68	404.13211	406.13858	9.4E+04
Unknown	22.68	541.19403	544.20441	7.7E+05
Unknown	22.68	572.23671	576.24973	1.5E+05
Unknown	22.72	360.57915	362.58589	1.4E+06
Unknown	22.80	298.06400	300.07082	1.5E+05
Unknown	22.88	282.44452	284.45128	5.0E+05
Spermidine	22.88	423.16323	426.17326	5.8E+05
Unknown	22.92	289.08246	291.08914	6.1E+05
Unknown	22.92	344.10668	346.11344	2.7E+05
Unknown	22.96	685.43506	687.44092	2.8E+05
Unknown	23.04	315.59016	317.59681	3.4E+05
Unknown	23.08	567.12598	571.13940	8.0E+04
Unknown	23.40	420.31975	422.32670	1.1E+05
Unknown	23.40	421.15838	425.17168	5.3E+05
Unknown	23.44	447.34745	450.35704	2.0E+05
Unknown	23.48	419.31591	422.32634	4.1E+05
Unknown	23.52	363.21041	365.21711	2.4E+06
Unknown	23.52	466.16179	470.17561	2.5E+05
Unknown	23.61	685.43603	687.44281	7.8E+05
Unknown	23.65	281.40823	283.41501	1.1E+05
Unknown	23.65	421.60922	424.61863	1.6E+05
Unknown	23.69	305.06797	307.07477	3.7E+06
Unknown	23.69	318.57373	320.58021	9.5E+04
Unknown	23.69	633.11792	635.12441	1.4E+05
Unknown	23.73	466.31928	470.33284	1.6E+05
Unknown	23.81	685.43604	687.44250	9.7E+05
Unknown	23.85	431.61075	434.62109	2.4E+05
Thymol	23.89	384.16343	386.17015	4.3E+05
Unknown	23.93	288.74810	290.75485	1.9E+05
Unknown	23.93	432.61919	435.62922	3.6E+05
Unknown	24.01	450.11938	453.12986	1.7E+05
Unknown	24.05	288.07632	290.08286	5.5E+05
Unknown	24.05	431.61108	434.62110	9.6E+05
Unknown	24.05	530.72137	532.72792	4.4E+05
Unknown	24.09	685.43677	687.44443	2.5E+06
Unknown	24.17	421.60915	424.61908	3.0E+05
Unknown	24.17	434.63568	437.64603	1.9E+05
Unknown	24.29	625.20135	629.21423	1.4E+05
Unknown	24.29	694.49751	696.50333	1.4E+06
Unknown	24.33	531.27460	533.28128	2.1E+06
Unknown	24.33	562.31528	564.32250	1.4E+07
Unknown	24.33	624.20034	628.21498	2.1E+05
Unknown	24.33	685.43656	687.44389	3.1E+06
Unknown	24.41	298.31090	300.31762	6.6E+06
Unknown	24.41	370.06854	372.07527	2.3E+05
Unknown	24.41	603.16224	607.17611	2.9E+06
Unknown	24.45	431.61141	434.62146	4.6E+05
Unknown	24.45	606.16672	609.17604	2.6E+05
Unknown	24.49	302.08480	304.09131	2.4E+07

Table S4.2. Ion pairs detected and identified by RPLC FTICR MS from repeatedly 1:1 ^{12}C-/^{13}C-dansylated CSF sample #2. Ion pairs detected in both repeatedly labeled CSF sample are highlighted in bold.

CSF - #2		CSF - #2		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
Unknown	**1.63**	**280.04773**	**282.05456**	6.2E+05
Unknown	1.63	396.57416	398.58095	1.3E+06
Unknown	1.63	523.10867	526.11776	1.1E+06
Unknown	1.63	669.61950	671.62516	8.2E+05
Unknown	1.67	503.13231	505.13854	2.1E+06
unknown	1.67	528.12372	530.13058	1.2E+06
unknown	**1.67**	**534.17392**	**536.18086**	1.8E+06
unknown	1.67	780.18914	782.19566	1.3E+06
unknown	**1.71**	**252.06915**	**254.07567**	1.2E+07
unknown	1.71	653.65364	655.65936	7.0E+05
unknown	**1.75**	**280.04822**	**282.05470**	3.8E+05
unknown	1.75	408.56844	410.57536	2.9E+05
unknown	**1.75**	**547.09604**	**549.10242**	5.6E+05
unknown	1.79	296.03309	298.03992	1.0E+06
unknown	**1.79**	**558.16266**	**560.17002**	3.1E+05
unknown	**1.79**	**572.08774**	**574.09497**	3.6E+05
unknown	**1.83**	**274.05096**	**276.05749**	5.2E+06
unknown	**1.83**	**336.02197**	**338.02840**	5.7E+05
unknown	**1.83**	**349.05372**	**351.06029**	3.5E+05
unknown	1.83	364.02083	366.02750	6.1E+05
unknown	1.83	372.06961	374.07629	1.1E+06
unknown	1.83	425.99193	427.99838	3.8E+05
unknown	**1.83**	**432.00841**	**434.01577**	5.8E+05
unknown	**1.83**	**493.97948**	**495.98599**	3.0E+05
unknown	**1.83**	**561.96715**	**563.97419**	1.8E+05
unknown	**1.83**	**567.98383**	**569.99017**	1.2E+05
unknown	**1.83**	**589.20510**	**591.21194**	2.0E+05
unknown	**1.83**	**606.09291**	**608.09995**	2.0E+05
unknown	**1.88**	**524.08191**	**526.08842**	1.8E+05
unknown	1.88	586.01221	590.02576	1.1E+05
unknown	1.88	605.09006	607.09722	3.4E+05
unknown	1.88	617.20770	619.21334	1.4E+05
unknown	1.92	536.18060	538.18754	7.9E+05
unknown	**1.96**	**449.11490**	**451.12197**	4.1E+05
unknown	1.96	546.06244	548.06927	3.1E+05
unknown	2.00	620.02600	622.03198	2.5E+05
unknown	**2.04**	**524.08154**	**526.08867**	2.2E+05
unknown	2.04	703.00665	705.01215	4.8E+05
unknown	2.04	838.01001	841.02012	2.1E+05
unknown	**2.08**	**555.12272**	**557.13006**	3.3E+05
unknown	**2.16**	**558.03253**	**562.04550**	4.2E+05
unknown	**2.20**	**632.22694**	**634.23288**	2.5E+05
unknown	2.23	299.03548	305.05601	1.4E+05
unknown	**2.23**	**414.12228**	**416.12905**	3.7E+06
unknown	**2.27**	**389.12829**	**391.13502**	3.5E+06
phosphoethaolamine	**2.38**	**375.07778**	**377.08445**	5.8E+05
unknown	2.42	372.06905	376.08212	1.4E+05
unknown	**2.42**	**390.07981**	**392.08655**	9.4E+04
unknown	**2.49**	**366.14870**	**368.15530**	3.0E+05
unknown	2.53	374.07825	377.08810	7.2E+04

unknown	**2.57**	**438.20595**	**440.21303**	3.0E+05
unknown	**2.61**	**252.06902**	**254.07576**	1.2E+06
unknown	**2.61**	**375.07770**	**377.08437**	2.9E+05
unknown	**2.61**	**390.08013**	**392.08703**	8.8E+04
unknown	**2.65**	**276.08016**	**278.08703**	1.0E+06
Taurine	**2.65**	**359.07327**	**361.07996**	8.7E+06
unknown	2.65	456.05811	458.06449	9.2E+04
unknown	**2.69**	**388.10774**	**390.11440**	1.6E+06
1-methylhistidine	**2.76**	**403.14374**	**405.15045**	8.3E+05
unknown	**2.76**	**501.15400**	**503.16079**	2.6E+05
unknown	**2.84**	**501.15464**	**503.16226**	9.3E+04
unknown	**2.88**	**363.10117**	**365.10788**	6.0E+05
unknown	2.88	380.16408	382.17075	3.7E+05
unknown	2.88	492.14327	495.15333	9.5E+04
unknown	**2.88**	**509.17044**	**511.17713**	2.1E+06
unknown	**2.91**	**366.14845**	**368.15515**	7.1E+05
unknown	2.95	296.03235	298.03946	1.0E+05
unknown	2.95	385.08899	387.09559	9.0E+04
unknown	2.95	558.15912	561.16949	6.1E+04
unknown	2.99	377.18442	379.19104	7.1E+04
unknown	**3.03**	**376.18032**	**378.18712**	3.1E+05
unknown	**3.07**	**414.12174**	**416.12853**	5.1E+06
unknown	3.22	382.10729	384.11387	1.0E+05
Arginine	**3.22**	**408.17024**	**410.17694**	6.8E+06
unknown	**3.37**	**319.11127**	**321.11792**	3.4E+05
unknown	**3.37**	**408.16994**	**410.17606**	2.3E+05
unknown	**3.41**	**446.06738**	**448.07409**	8.8E+04
unknown	**3.52**	**408.19520**	**410.20216**	8.4E+05
unknown	3.56	314.09561	316.10266	1.9E+05
Homoarginine	**3.56**	**422.21100**	**424.21779**	8.6E+05
unknown	**3.56**	**502.13946**	**504.14656**	2.1E+05
unknown	**3.56**	**547.15560**	**549.16142**	1.9E+05
Asparagine	**3.59**	**366.11200**	**368.11875**	7.9E+06
unknown	**3.59**	**380.16399**	**382.17081**	1.0E+06
unknown	3.67	365.12812	367.13454	9.9E+05
unknown	**3.67**	**424.15403**	**426.16093**	2.0E+05
unknown	**3.83**	**471.14322**	**473.15018**	2.8E+05
unknown	3.87	381.13214	383.13889	1.6E+06
unknown	3.87	485.19651	488.20709	2.5E+05
unknown	**3.95**	**378.13989**	**384.16066**	7.4E+06
Glutamine	**3.99**	**380.12571**	**382.13218**	1.8E+08
unknown	4.10	402.10877	404.11615	1.2E+06
unknown	**4.18**	**363.10098**	**365.10734**	1.3E+05
unknown	4.18	364.08535	366.09229	1.2E+05
unknown	**4.18**	**392.12760**	**394.13439**	6.5E+05
L-citrulline	**4.18**	**409.15428**	**411.16085**	5.2E+06
unknown	**4.22**	**424.11667**	**426.12420**	1.1E+05
unknown	**4.22**	**436.20129**	**438.20797**	1.5E+05
unknown	4.29	271.03851	273.04529	7.0E+04
unknown	4.29	303.02924	305.03547	8.4E+04
unknown	**4.33**	**242.57196**	**244.57865**	1.9E+06
unknown	**4.33**	**270.03523**	**272.04191**	5.5E+05
unknown	**4.33**	**291.06370**	**293.07048**	4.5E+05
unknown	**4.33**	**302.02500**	**304.03180**	6.2E+05
unknown	4.33	377.08151	379.08835	5.2E+05
unknown	**4.33**	**424.10655**	**426.11335**	5.9E+05
unknown	**4.33**	**515.17601**	**519.18954**	8.5E+05

unknown	**4.37**	**483.12626**	**487.14017**	7.7E+05
unknown	**4.37**	**506.11464**	**510.12855**	1.1E+06
unknown	4.45	270.03510	273.04520	3.0E+05
unknown	**4.45**	**302.02489**	**304.03168**	4.4E+05
3-sn-Phosphatidylethanolamine	**4.45**	**484.13615**	**488.14934**	9.6E+06
unknown	**4.45**	**515.17761**	**519.19147**	2.4E+05
unknown	4.48	388.10797	390.11412	1.7E+05
Methylguanidine	4.52	307.12236	309.12930	7.0E+04
unknown	**4.52**	**397.14274**	**399.14977**	1.4E+05
Homoserine	**4.63**	**353.11673**	**355.12368**	3.3E+05
unknown	**4.67**	**465.18055**	**467.18732**	2.6E+05
unknown	**4.67**	**468.14338**	**470.15058**	9.9E+04
unknown	4.71	362.16478	364.17193	2.1E+05
unknown	4.71	393.11164	395.11900	1.1E+05
Methionine sulfoxide	**4.71**	**399.10450**	**401.11123**	7.1E+05
unknown	**4.82**	**360.05734**	**362.06397**	1.4E+05
unknown	**4.82**	**499.17446**	**501.18152**	2.4E+05
Serine	**4.90**	**339.09961**	**341.10608**	4.3E+07
Homocitruline	**4.90**	**423.05352**	**425.06080**	1.4E+05
unknown	**4.97**	**263.21192**	**265.21869**	3.2E+05
Aspartic Acid	5.02	367.09606	369.10276	9.8E+05
unknown	**5.06**	**408.15876**	**410.16576**	1.9E+05
unknown	5.06	519.19075	521.19806	1.3E+05
unknown	**5.09**	**353.11674**	**355.12360**	1.4E+06
unknown	**5.09**	**479.23227**	**481.23984**	1.9E+05
unknown	**5.17**	**394.17978**	**396.18633**	3.3E+05
unknown	5.21	367.13286	370.14249	5.3E+05
unknown	5.21	433.22687	435.23401	2.7E+05
unknown	5.21	516.15524	518.16180	1.1E+05
unknown	5.25	502.14602	504.15298	2.2E+05
4-Hydroxy-proline	**5.28**	**365.11686**	**367.12336**	9.6E+06
Glutamic Acid	**5.32**	**381.11190**	**383.11856**	3.4E+05
unknown	**5.32**	**455.10983**	**458.11967**	3.7E+05
unknown	**5.36**	**396.11134**	**398.11819**	2.2E+05
unknown	**5.36**	**582.19049**	**584.19726**	3.3E+05
unknown	**5.36**	**600.20144**	**602.20819**	1.3E+06
unknown	**5.39**	**442.11694**	**444.12386**	1.2E+06
unknown	**5.43**	**466.16439**	**468.17145**	3.8E+05
unknown	5.47	269.03088	272.04133	9.1E+04
unknown	**5.58**	**336.13790**	**338.14464**	1.2E+06
Aminoadipic acid	5.58	395.12711	397.13413	1.7E+05
unknown	5.58	505.07585	509.08831	2.2E+05
unknown	**5.66**	**363.14867**	**365.15559**	9.4E+05
unknown	**5.66**	**457.08902**	**459.09602**	3.7E+05
Threonine	**5.70**	**353.11644**	**355.12313**	4.8E+07
Folic acid	**5.70**	**338.09362**	**339.09621**	1.2E+05
unknown	**5.74**	**462.16994**	**464.17686**	1.2E+06
unknown	**5.84**	**337.15826**	**339.16492**	3.3E+06
unknown	**5.88**	**379.13244**	**381.13925**	5.6E+05
Diethanolamine	**5.96**	**339.13781**	**341.14429**	3.8E+05
unknown	**5.96**	**515.17127**	**517.17835**	1.1E+05
unknown	**6.00**	**478.12806**	**480.13505**	3.4E+06
Ethanolamine	**6.03**	**295.11116**	**297.11793**	1.4E+07
unknown	6.07	464.24075	466.24820	2.5E+05
unknown	**6.07**	**494.27972**	**496.28671**	2.3E+05
unknown	**6.07**	**611.19788**	**613.20404**	1.3E+05
unknown	6.11	252.08814	254.09501	3.2E+06

unknown	**6.11**	**463.23788**	**465.24472**	1.1E+06
unknown	**6.11**	**523.14487**	**525.15174**	1.9E+06
unknown	**6.15**	**251.08471**	**253.09138**	1.7E+07
unknown	**6.22**	**317.13199**	**319.13881**	1.0E+06
unknown	**6.22**	**348.17434**	**350.18114**	2.3E+05
unknown	**6.22**	**411.15872**	**413.16489**	8.5E+04
unknown	**6.26**	**415.13255**	**417.13923**	1.3E+06
unknown	**6.30**	**388.16876**	**390.17599**	1.3E+05
unknown	**6.30**	**406.17984**	**408.18658**	1.9E+05
unknown	6.30	454.15503	456.16165	1.4E+05
unknown	**6.38**	**398.12736**	**400.13390**	1.4E+06
unknown	**6.41**	**348.10150**	**350.10817**	1.3E+06
unknown	**6.41**	**464.14893**	**466.15585**	5.9E+05
unknown	**6.45**	**422.17493**	**424.18127**	2.9E+05
Glycine	**6.49**	**309.09050**	**311.09710**	1.3E+07
unknown	**6.49**	**363.13766**	**365.14430**	3.9E+05
unknown	**6.49**	**381.14811**	**383.15484**	4.0E+06
unknown	**6.68**	**344.10658**	**346.11323**	1.5E+06
unknown	6.72	322.15824	324.16556	6.8E+04
unknown	**6.72**	**364.16922**	**366.17582**	6.7E+06
unknown	**6.72**	**395.21106**	**397.21788**	3.0E+05
unknown	**6.72**	**422.22243**	**424.22898**	1.1E+05
unknown	**6.76**	**477.16163**	**479.16838**	2.3E+06
unknown	**6.80**	**348.13776**	**350.14449**	5.6E+05
unknown	**6.80**	**361.12231**	**363.12832**	8.3E+04
unknown	**6.95**	**415.09153**	**417.09905**	7.6E+04
unknown	**7.03**	**362.11713**	**364.12381**	8.5E+06
unknown	7.03	363.17389	365.18062	2.5E+06
unknown	**7.10**	**362.11579**	**364.12245**	3.4E+07
unknown	7.14	362.11680	365.12718	1.2E+07
unknown	**7.18**	**347.11767**	**349.12432**	5.0E+05
Tyrosine methyl ester	**7.18**	**415.13281**	**417.13956**	2.6E+06
unknown	**7.21**	**287.03350**	**289.04013**	5.1E+05
unknown	**7.21**	**344.10675**	**346.11347**	6.3E+05
unknown	**7.21**	**367.13268**	**369.13924**	4.4E+05
Alanine	**7.36**	**323.10647**	**325.11306**	2.4E+07
r-aminobutyric acid	**7.51**	**337.12179**	**399.12916**	1.3E+05
unknown	**7.55**	**473.10696**	**475.11374**	5.8E+06
unknown	7.73	493.08453	496.09496	8.6E+05
unknown	**7.81**	**473.11115**	**475.11746**	5.2E+05
unknown	**7.88**	**396.11137**	**398.11825**	7.6E+05
unknown	**7.88**	**471.10492**	**473.11080**	2.1E+05
unknown	**7.96**	**368.11667**	**370.12327**	1.6E+05
unknown	7.99	322.07447	325.08459	2.4E+05
unknown	8.14	395.12738	397.13390	1.5E+05
Hypoxanthine	**8.18**	**370.09701**	**372.10381**	7.7E+05
unknown	**8.25**	**450.20612**	**452.21319**	9.0E+04
5-hydroxymethyluracil	**8.29**	**376.09651**	**378.10324**	8.4E+04
unknown	**8.37**	**378.06752**	**380.07446**	2.6E+05
unknown	**8.40**	**431.13849**	**433.14549**	3.0E+06
unknown	8.40	537.10221	540.11179	2.6E+05
3-Aminoisobutyric acid	**8.44**	**337.12165**	**339.12846**	1.1E+05
unknown	**8.44**	**279.07999**	**281.08659**	8.0E+05
unknown	8.49	362.15366	365.16415	6.2E+04
unknown	**8.49**	**386.09192**	**388.09871**	4.0E+05
5-Aminopentanoic acid	**8.52**	**351.13755**	**353.14427**	2.2E+05
unknown	**8.52**	**396.13522**	**398.14194**	2.1E+06

unknown	**8.52**	**485.12002**	**488.13049**	4.8E+05
unknown	8.86	367.16888	369.17560	7.9E+04
2-Aminobutyric acid	**8.89**	**337.12179**	**339.12847**	1.0E+07
unknown	**9.09**	**321.09069**	**323.09754**	2.1E+05
Cysteine-glutathione disulfide	**9.09**	**447.10238**	**449.10938**	3.3E+05
unknown	**9.13**	**351.10115**	**354.11126**	2.3E+05
unknown	**9.13**	**364.10443**	**366.11148**	1.1E+05
unknown	**9.13**	**370.09711**	**372.10386**	4.4E+06
unknown	**9.21**	**363.10126**	**365.10802**	1.2E+06
unknown	9.21	507.07063	509.07756	5.7E+05
Methylcysteine	**9.32**	**369.09411**	**371.10086**	1.7E+06
unknown	9.36	492.05244	494.06001	2.9E+05
unknown	**9.47**	**242.28428**	**244.29099**	4.7E+06
unknown	**9.47**	**382.64887**	**384.65548**	2.3E+05
unknown	**9.51**	**396.11134**	**398.11817**	4.1E+05
unknown	**9.58**	**266.10708**	**268.11373**	5.6E+05
unknown	**9.62**	**321.12715**	**323.13379**	5.7E+06
unknown	**9.62**	**381.14817**	**383.15491**	6.7E+06
Methylamine	**9.67**	**265.10051**	**267.10712**	1.3E+07
unknown	**9.67**	**531.20111**	**533.20770**	8.9E+05
unknown	9.70	551.17572	555.18966	1.4E+06
unknown	9.70	609.20953	611.21643	3.9E+05
unknown	**9.78**	**265.10012**	**268.10991**	1.6E+07
unknown	**9.82**	**551.17630**	**553.18262**	1.8E+06
unknown	**9.82**	**554.18578**	**556.19215**	4.5E+06
unknown	**9.86**	**287.08254**	**289.08874**	4.2E+06
unknown	9.86	572.12305	575.13275	6.1E+05
Proline	**9.89**	**349.12206**	**351.12857**	7.6E+06
unknown	9.93	531.20126	534.21155	7.6E+05
unknown	10.12	405.14802	407.15481	2.3E+05
unknown	10.20	266.10405	268.11053	6.1E+05
unknown	**10.20**	**370.09700**	**372.10375**	9.6E+05
unknown	10.20	460.11734	462.12411	1.6E+05
unknown	**10.24**	**265.09364**	**268.10394**	3.4E+05
unknown	**10.28**	**311.08302**	**313.08974**	5.7E+05
unknown	10.31	458.07316	460.07956	2.3E+05
unknown	10.35	336.14616	338.15285	1.1E+05
unknown	**10.39**	**321.11441**	**323.12120**	2.2E+06
Valine	**10.46**	**351.13736**	**353.14381**	4.9E+07
unknown	10.54	352.14053	354.14707	1.2E+07
Methionine	**10.54**	**383.10923**	**385.11608**	1.1E+07
3-Hydroxypicolinic acid	**10.54**	**373.11862**	**375.12535**	5.5E+05
unknown	10.69	474.23891	476.24485	2.6E+05
unknown	**10.73**	**400.08514**	**402.09170**	4.2E+06
unknown	**10.81**	**361.12178**	**363.12870**	1.5E+05
unknown	10.85	324.09030	326.09697	9.1E+04
unknown	10.88	364.62473	366.63170	3.2E+05
unknown	**10.92**	**378.06754**	**380.07452**	8.9E+05
Tryptophan	**10.99**	**438.14838**	**440.15520**	1.5E+07
unknown	11.03	346.08585	348.09248	5.5E+05
2-Phenylglycine	11.48	385.12168	387.12880	1.2E+05
unknown	11.67	382.58134	384.58796	3.8E+05
unknown	**11.78**	**418.13208**	**420.13890**	1.0E+06
unknown	**11.82**	**315.09067**	**317.09727**	1.4E+05
unknown	11.82	372.12453	374.13115	8.4E+04
unknown	**12.13**	**295.10261**	**297.10926**	7.4E+04
unknown	12.16	400.14105	402.14768	3.2E+06

Phenylalanine	**12.20**	**399.13742**	**401.14411**	3.5E+07
unknown	12.32	266.10396	269.11412	4.1E+05
unknown	12.35	395.12236	397.12967	1.1E+05
unknown	**12.40**	**406.61705**	**408.62387**	3.5E+05
3-Hydroxymandelic acid	**12.47**	**402.10080**	**404.10775**	2.6E+05
unknown	**12.55**	**313.60899**	**315.61561**	3.7E+06
Isoleucine	**12.55**	**365.15294**	**367.15950**	4.4E+07
unknown	12.58	324.09012	326.09677	3.9E+05
unknown	**12.58**	**501.11554**	**505.12897**	5.1E+05
unknown	12.62	265.10956	267.11664	1.6E+05
unknown	12.66	320.06635	322.07331	1.7E+05
unknown	12.69	491.07867	493.08447	1.0E+05
unknown	**12.69**	**501.11522**	**505.12854**	1.8E+06
unknown	**12.73**	**379.13226**	**381.13907**	6.4E+05
L-cystathionine	**12.77**	**345.09248**	**347.09923**	3.1E+05
Leucine	**12.81**	**365.15121**	**367.15799**	1.2E+08
unknown	**12.88**	**300.06537**	**302.07166**	9.2E+05
L-norleucine	**12.88**	**365.15317**	**367.15962**	2.0E+07
unknown	13.00	342.62981	344.63654	1.1E+06
unknown	13.07	336.14623	338.15301	1.0E+06
Cystine	**13.18**	**354.07009**	**356.07688**	3.5E+05
unknown	**13.18**	**317.13194**	**319.13880**	8.4E+05
unknown	**13.18**	**335.14225**	**337.14871**	2.7E+07
unknown	**13.25**	**335.14252**	**338.15284**	5.6E+06
unknown	13.29	362.06947	364.07630	2.0E+05
unknown	**13.48**	**378.10087**	**380.10745**	5.2E+06
unknown	**13.48**	**409.14324**	**411.14980**	1.8E+05
unknown	**13.48**	**549.18815**	**551.19521**	2.6E+05
Hydroxyphenyllactic acid	**13.63**	**416.11627**	**418.12331**	4.3E+06
unknown	**13.75**	**307.09297**	**309.09962**	3.3E+06
unknown	13.90	311.18550	313.19162	1.1E+06
unknown	13.90	402.10075	404.10754	3.9E+05
unknown	**14.01**	**322.07454**	**324.08145**	2.2E+05
Homocystine	**14.01**	**368.09850**	**370.10578**	1.0E+05
unknown	14.12	396.07249	399.08195	5.7E+05
unknown	**14.15**	**511.13803**	**513.14450**	6.1E+04
5-HIAA	**14.19**	**425.11677**	**427.12355**	2.1E+06
unknown	14.23	456.17057	458.17709	6.1E+04
unknown	**14.27**	**336.11406**	**338.12075**	2.6E+05
unknown	**14.27**	**349.15821**	**351.16491**	1.0E+06
unknown	**14.27**	**367.16911**	**369.17586**	9.0E+04
unknown	**14.31**	**346.06583**	**348.07231**	1.0E+05
unknown	14.34	279.11618	283.12935	1.6E+07
Dimethylamine	**14.42**	**279.11575**	**281.12241**	3.3E+07
unknown	14.42	561.23914	565.25271	3.0E+06
unknown	14.46	579.20747	583.22131	2.5E+06
unknown	14.46	584.22387	587.23387	2.5E+06
Phenylpropanolamine	**14.49**	**385.12213**	**387.12849**	3.0E+05
unknown	14.53	280.11944	284.13287	2.2E+06
unknown	**14.53**	**301.09800**	**305.11131**	1.1E+06
unknown	**14.53**	**371.14045**	**373.14679**	3.9E+05
unknown	14.53	562.24231	565.25238	4.2E+05
unknown	14.57	279.11628	282.12654	6.4E+06
unknown	**14.57**	**349.15824**	**351.16495**	1.3E+06
unknown	**14.66**	**345.09543**	**347.10223**	2.9E+05
unknown	**14.66**	**367.16886**	**369.17562**	1.4E+05
2,4-Diaminobutyric acid	14.73	293.13164	295.13887	3.8E+04

unknown	**14.73**	**520.24585**	**522.25227**	8.8E+04
unknown	**14.77**	**350.16191**	**352.16858**	3.3E+05
unknown	**14.81**	**513.15346**	**515.16031**	2.0E+05
unknown	**14.88**	**437.19362**	**439.19971**	5.0E+06
unknown	**14.92**	**349.15824**	**351.16496**	1.5E+06
unknown	**15.07**	**437.19363**	**439.19978**	1.8E+07
unknown	**15.07**	**446.25368**	**448.26018**	1.5E+07
unknown	**15.11**	**415.21175**	**417.21816**	3.4E+07
unknown	**15.15**	**363.65358**	**365.66013**	3.6E+05
unknown	**15.18**	**393.14825**	**395.15485**	1.3E+05
unknown	**15.22**	**349.15826**	**352.16885**	7.1E+05
unknown	**15.37**	**360.63577**	**362.64227**	4.0E+05
L-ornithine	**15.41**	**300.10336**	**302.11004**	1.8E+07
unknown	15.41	315.08401	317.09075	6.4E+05
Acetaminophen	**15.45**	**385.12246**	**387.12898**	1.2E+05
or 4-acetamidophenol				
unknown	**15.49**	**425.61268**	**427.61931**	2.8E+05
unknown	15.52	265.10150	267.10834	2.0E+05
Homovanillic	**15.63**	**416.11629**	**418.12317**	9.1E+06
unknown	15.70	266.08458	268.09123	3.1E+06
unknown	**15.74**	**468.14088**	**471.15136**	5.7E+05
unknown	**15.89**	**300.10355**	**302.11075**	1.2E+05
unknown	15.93	355.11581	357.12267	8.3E+05
3-/4-hydroxyphenylacetic acid	**16.00**	**386.10577**	**388.11268**	1.4E+06
or 3-Cresotinic acid				
unknown	**16.00**	**474.18114**	**476.18829**	2.1E+06
unknown	**16.08**	**419.47881**	**421.48549**	4.9E+05
unknown	**16.11**	**345.57366**	**347.58016**	4.9E+05
Homocarnosine	**16.11**	**354.11946**	**356.12622**	1.8E+06
unknown	**16.15**	**391.16896**	**393.17566**	1.0E+06
unknown	**16.30**	**350.12977**	**352.13654**	1.1E+06
unknown	16.33	450.13078	454.14406	7.0E+05
unknown	**16.37**	**423.10144**	**425.10817**	2.0E+06
Lysine	**16.45**	**307.11045**	**309.11759**	4.7E+07
unknown	**16.53**	**614.21765**	**617.22760**	7.2E+04
unknown	**16.64**	**264.08835**	**266.09507**	1.1E+05
unknown	**16.64**	**327.64280**	**329.64962**	8.4E+05
4-Hydroxybenzoic acid	**16.64**	**372.09025**	**374.09706**	7.1E+06
unknown	16.76	498.37092	500.37726	1.8E+06
unknown	**16.83**	**415.21161**	**417.21782**	6.9E+06
unknown	**16.87**	**319.62732**	**321.63390**	9.1E+04
Histidine	**16.95**	**311.59334**	**313.59998**	1.2E+07
unknown	**16.98**	**382.10890**	**384.11557**	1.5E+05
unknown	**17.10**	**415.21160**	**417.21771**	4.3E+06
unknown	**17.14**	**329.08253**	**331.08908**	1.9E+05
unknown	**17.37**	**311.08289**	**313.08967**	1.4E+05
unknown	17.40	347.08815	349.09477	1.5E+05
unknown	**17.40**	**359.62783**	**361.63444**	1.3E+05
unknown	**17.40**	**407.16366**	**409.17041**	6.0E+05
unknown	**17.52**	**297.08573**	**299.09220**	4.6E+05
unknown	**17.52**	**324.10555**	**326.11196**	1.7E+05
unknown	**17.55**	**390.10083**	**392.10740**	3.5E+05
unknown	17.64	282.10699	284.11353	3.5E+05
unknown	17.64	420.31921	422.32574	9.7E+04
unknown	**17.82**	**413.11693**	**415.12363**	5.0E+05
unknown	17.82	484.12640	486.13353	7.9E+04
unknown	17.97	321.12285	323.12943	2.1E+06

unknown	**17.97**	**419.47864**	**421.48546**	7.0E+05
unknown	17.97	498.37085	500.37747	1.7E+06
2-aminooctanoic acid	**18.01**	**393.18441**	**395.19109**	1.6E+05
unknown	18.08	357.08980	359.09682	9.0E+04
unknown	**18.12**	**356.09334**	**358.10007**	1.2E+06
unknown	**18.12**	**395.10619**	**397.11298**	8.9E+05
unknown	18.16	315.06486	317.07148	5.8E+05
unknown	18.20	522.35598	524.36230	1.1E+06
unknown	18.27	353.11198	355.11855	5.8E+05
unknown	**18.34**	**370.11083**	**372.11760**	2.7E+05
unknown	**18.38**	**307.14767**	**309.15437**	8.0E+05
unknown	**18.46**	**354.06343**	**356.07008**	3.5E+06
unknown	**18.46**	**486.14307**	**488.14963**	5.0E+05
unknown	**18.64**	**354.06339**	**356.07010**	1.4E+06
unknown	**18.64**	**486.14200**	**488.14828**	5.3E+05
unknown	**18.72**	**300.06512**	**302.07176**	4.2E+05
unknown	**18.83**	**421.17940**	**423.18632**	7.9E+05
unknown	**18.91**	**321.06667**	**323.07343**	3.9E+05
1,3-diaminopropane	19.10	271.09950	273.10748	4.0E+04
unknown	**19.17**	**314.11895**	**316.12575**	1.3E+07
unknown	19.21	326.11004	328.11671	4.0E+05
unknown	**19.32**	**401.12793**	**403.13483**	2.1E+05
unknown	19.36	390.10383	392.11113	1.2E+05
unknown	**19.40**	**279.10741**	**281.11398**	3.6E+05
unknown	19.40	530.18860	532.19449	5.4E+05
unknown	**19.48**	**361.07752**	**363.08414**	3.3E+06
unknown	19.51	546.10437	548.11110	2.6E+05
unknown	**19.55**	**528.17279**	**530.17935**	3.8E+05
unknown	19.59	328.11693	330.12319	9.5E+04
unknown	19.59	408.12466	410.13086	8.9E+04
unknown	19.59	417.14801	421.16156	1.1E+05
1,4-diaminobutane	**19.59**	**555.20966**	**559.22241**	1.8E+05
unknown	**19.66**	**357.45737**	**359.46415**	2.5E+05
unknown	**19.70**	**264.58500**	**266.59154**	1.3E+05
unknown	**19.74**	**386.10584**	**388.11265**	4.7E+05
unknown	19.81	356.09531	358.10172	1.5E+07
unknown	19.84	316.09238	318.09955	3.0E+05
unknown	19.88	335.12479	337.13107	1.8E+05
unknown	19.88	339.60089	341.60760	1.1E+06
unknown	19.88	564.15029	566.15596	2.2E+05
unknown	**19.92**	**331.11137**	**333.11807**	8.1E+05
unknown	19.92	520.10387	522.11084	2.7E+05
unknown	**20.03**	**292.10603**	**294.11269**	1.2E+06
unknown	**20.11**	**486.11475**	**488.12107**	5.9E+05
Tyrosine	**20.25**	**324.59412**	**326.60066**	1.1E+08
Cysteamine	**20.37**	**310.07526**	**312.08204**	1.4E+05
Metoprolol	**20.40**	**501.16198**	**503.16912**	3.1E+06
unknown	20.51	346.09857	348.10513	5.0E+05
unknown	20.58	311.78333	313.78992	9.2E+04
unknown	20.65	340.12448	343.13514	1.1E+06
unknown	**20.65**	**379.11146**	**381.11818**	4.5E+05
Phenol	**20.73**	**328.10043**	**330.10703**	8.9E+05
4-Nitrophenol	**20.80**	**373.08569**	**375.09225**	1.3E+05
unknown	20.84	727.46139	729.46667	8.1E+05
unknown	20.88	448.35138	451.36224	7.1E+04
unknown	**20.91**	**279.10736**	**281.11423**	1.4E+05
unknown	20.95	706.18127	708.18750	2.6E+05

unknown	**21.09**	**298.10603**	**300.11283**	2.2E+05
unknown	**21.09**	**310.07510**	**312.08206**	7.1E+05
unknown	**21.09**	**639.40895**	**641.41523**	3.0E+06
unknown	**21.09**	**784.18842**	**786.19618**	1.8E+05
unknown	**21.32**	**299.07200**	**301.07866**	1.3E+06
unknown	**21.32**	**335.17915**	**337.18588**	2.1E+05
unknown	**21.32**	**344.10672**	**346.11344**	6.6E+05
unknown	**21.32**	**397.20132**	**399.20775**	6.4E+06
unknown	**21.32**	**454.15475**	**456.16168**	2.8E+05
unknown	21.36	394.20526	396.21170	2.2E+05
unknown	**21.36**	**551.35608**	**553.36238**	9.9E+06
unknown	**21.36**	**560.41597**	**562.42175**	2.0E+06
unknown	21.40	345.60087	347.60751	2.0E+05
unknown	21.44	354.33655	358.35039	8.7E+04
unknown	21.44	372.10104	375.11133	1.1E+05
unknown	**21.51**	**507.33001**	**509.33622**	1.6E+07
unknown	**21.55**	**514.14685**	**518.16037**	7.0E+05
unknown	**21.59**	**463.30371**	**465.31013**	1.8E+07
unknown	**21.59**	**472.36408**	**474.37057**	4.6E+06
unknown	**21.66**	**354.11641**	**356.12312**	4.3E+06
unknown	**21.66**	**419.27754**	**421.28394**	2.1E+07
unknown	**21.74**	**375.25122**	**377.25758**	1.1E+07
unknown	21.78	419.31612	422.32671	4.7E+05
unknown	21.81	523.14441	525.15088	2.0E+05
unknown	**21.89**	**498.15260**	**501.16189**	8.0E+05
unknown	**22.00**	**521.13769**	**524.14775**	3.2E+05
unknown	22.19	287.07957	289.08623	5.6E+05
unknown	22.22	316.05245	318.05934	2.5E+05
unknown	**22.26**	**450.20692**	**452.21397**	5.4E+05
unknown	**22.26**	**499.14688**	**501.15342**	2.5E+05
unknown	22.33	617.01548	621.02936	3.5E+05
unknown	22.37	315.59013	317.59700	2.3E+05
unknown	**22.56**	**388.07720**	**390.08417**	5.9E+06
unknown	**22.59**	**344.10674**	**346.11357**	6.1E+05
unknown	22.63	567.19004	569.19644	3.4E+06
unknown	**22.66**	**360.57928**	**362.58602**	7.7E+05
unknown	22.66	545.20813	547.21497	5.7E+06
unknown	22.66	576.24991	578.25687	1.4E+06
unknown	**22.70**	**454.24186**	**457.25195**	4.9E+05
unknown	22.70	716.29535	718.30127	1.4E+05
unknown	**22.78**	**377.19139**	**383.21166**	1.8E+05
unknown	**22.78**	**458.42087**	**460.42761**	1.5E+06
unknown	22.78	650.26971	652.27698	9.2E+05
unknown	**22.81**	**282.44469**	**284.45134**	1.4E+05
Spermidine	**22.81**	**423.16359**	**426.17352**	2.5E+05
Pyrocatechol	**22.85**	**289.08245**	**291.08922**	5.0E+05
unknown	**22.85**	**289.08248**	**291.08925**	4.0E+05
unknown	**22.85**	**454.24179**	**456.24881**	9.3E+05
unknown	**22.89**	**388.07738**	**390.08440**	8.5E+05
unknown	22.89	391.08830	394.09809	6.3E+05
unknown	**22.93**	**315.59006**	**317.59721**	1.2E+05
unknown	**22.93**	**454.24149**	**457.25174**	7.1E+05
unknown	**22.93**	**648.26196**	**650.26982**	9.3E+04
unknown	**23.00**	**454.24182**	**457.25185**	9.3E+05
unknown	23.00	567.12528	571.13915	2.3E+05
unknown	**23.08**	**455.24507**	**457.25201**	2.6E+05
unknown	23.16	649.26663	651.27350	8.0E+04

unknown	**23.23**	**454.24181**	**456.24872**	6.9E+05
unknown	**23.31**	**421.15826**	**425.17197**	1.0E+05
unknown	**23.31**	**427.11545**	**429.12262**	8.9E+04
unknown	23.46	363.21061	365.21723	2.3E+06
unknown	23.50	550.62920	552.63608	7.2E+06
unknown	**23.61**	**305.06799**	**307.07474**	3.9E+06
unknown	23.61	611.12701	615.14093	4.6E+05
unknown	**23.69**	**466.31937**	**469.32980**	1.5E+05
unknown	23.76	420.32013	422.32730	1.4E+05
unknown	**23.80**	**685.43737**	**687.44532**	2.5E+06
unknown	23.80	694.49850	696.50398	1.5E+06
Thymol	**23.84**	**384.16357**	**386.17032**	2.8E+05
unknown	23.95	649.26753	651.27420	1.6E+05
unknown	**23.99**	**288.07647**	**290.08291**	3.1E+05
unknown	**23.99**	**431.61113**	**434.62119**	4.9E+05
unknown	**23.99**	**530.72113**	**532.72748**	4.1E+05
unknown	24.22	454.11133	457.12134	5.4E+05
unknown	24.26	553.25558	555.26177	9.6E+06
unknown	24.26	562.31411	564.32067	1.5E+07
unknown	24.30	594.12837	598.14225	1.1E+06
unknown	24.34	298.31095	300.31764	6.2E+06
unknown	24.38	302.08483	304.09119	1.8E+07
unknown	24.38	625.14535	629.15849	3.0E+06
unknown	24.38	693.13416	697.14630	3.1E+05
unknown	**24.42**	**431.61093**	**434.62144**	2.4E+05
unknown	24.45	302.08418	304.09064	3.1E+07
unknown	24.49	302.08486	304.09122	2.1E+07
unknown	24.49	550.62967	552.63691	6.1E+06
unknown	24.49	608.17832	610.18408	2.2E+06
CSF - #2 Repeat		**CSF - #2 Repeat**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	**1.62**	**252.06918**	**254.07573**	9.2E+06
unknown	1.62	526.11783	528.12395	1.5E+06
unknown	**1.62**	**534.17416**	**536.18089**	1.9E+06
unknown	1.62	672.62721	674.63397	3.6E+05
unknown	1.62	756.20034	758.20834	7.3E+05
unknown	1.62	781.19154	783.19757	6.6E+05
unknown	**1.65**	**547.09621**	**549.10260**	6.7E+05
unknown	1.69	571.08480	573.09137	1.4E+06
unknown	1.69	580.14519	582.15154	4.2E+05
unknown	**1.69**	**606.09472**	**608.10119**	2.9E+05
unknown	1.69	633.05464	635.06226	2.4E+05
unknown	**1.73**	**558.16272**	**560.16953**	2.8E+05
unknown	1.73	569.07737	572.08766	4.1E+05
unknown	1.73	637.06476	639.07235	1.5E+05
unknown	1.73	707.05894	709.06512	2.4E+05
unknown	1.77	297.03656	299.04334	3.0E+05
unknown	**1.77**	**336.02146**	**338.02807**	3.0E+05
unknown	**1.77**	**349.05361**	**351.06022**	1.6E+05
unknown	1.77	364.02076	367.03068	1.1E+06
unknown	1.77	373.07309	375.07989	1.5E+05
unknown	**1.77**	**432.00835**	**434.01522**	1.0E+06
unknown	**1.77**	**493.98001**	**495.98582**	2.5E+05
unknown	1.77	499.99597	502.00264	4.7E+05
unknown	1.77	505.13945	507.14542	3.7E+05
unknown	**1.77**	**567.98374**	**569.99036**	3.3E+05

unknown	**1.77**	**589.20535**	**591.21213**	1.7E+05
unknown	1.77	629.95343	631.96096	1.5E+05
unknown	**1.81**	**274.05099**	**276.05758**	6.2E+06
unknown	1.81	421.98003	423.98731	2.1E+05
unknown	1.81	489.96823	491.97481	1.9E+05
unknown	1.81	525.11340	528.12317	2.9E+05
unknown	**1.81**	**561.96575**	**563.97314**	1.7E+05
unknown	**1.81**	**572.08707**	**574.09372**	2.5E+05
unknown	**1.89**	**252.06912**	**254.07569**	3.6E+06
unknown	**1.89**	**589.20468**	**591.21176**	2.1E+05
unknown	1.89	631.95968	635.97205	1.2E+05
unknown	1.93	268.04639	270.05286	1.7E+05
unknown	**1.93**	**449.11465**	**451.12167**	2.9E+05
unknown	1.97	584.97309	587.98215	8.7E+05
unknown	**2.01**	**555.12318**	**557.12992**	3.5E+05
unknown	2.05	583.97616	585.98193	3.7E+05
unknown	2.05	702.00625	704.01367	5.2E+05
unknown	**2.17**	**558.03260**	**562.04532**	5.3E+05
unknown	**2.21**	**414.12231**	**416.12909**	4.2E+06
unknown	**2.25**	**389.12839**	**391.13506**	3.0E+06
unknown	2.25	601.18243	603.19028	1.0E+05
unknown	**2.25**	**632.22616**	**634.23282**	2.4E+05
unknown	**2.29**	**432.00777**	**434.01474**	1.0E+05
unknown	2.29	510.17285	512.17970	6.6E+04
unknown	**2.29**	**558.03162**	**562.04480**	9.0E+04
phosphoethaolamine	**2.33**	**375.07789**	**377.08438**	4.4E+05
unknown	**2.33**	**438.13293**	**440.13976**	1.0E+05
unknown	**2.41**	**363.10123**	**365.10796**	4.0E+05
unknown	**2.41**	**390.08035**	**392.08679**	8.7E+04
unknown	**2.41**	**509.17070**	**511.17730**	1.9E+06
unknown	2.49	278.09602	280.10223	1.5E+05
unknown	**2.49**	**366.14854**	**368.15521**	1.9E+05
unknown	**2.53**	**376.10788**	**378.11441**	1.3E+05
unknown	**2.53**	**438.20602**	**440.21285**	6.1E+05
unknown	2.53	495.08988	497.09654	1.1E+05
Taurine	**2.61**	**359.07327**	**361.07996**	4.8E+06
unknown	**2.69**	**276.08010**	**278.08708**	7.5E+05
unknown	**2.69**	**388.10779**	**390.11435**	1.7E+06
unknown	**2.73**	**252.06905**	**254.07571**	9.5E+05
1-methylhistidine	**2.73**	**403.14384**	**405.15052**	8.2E+05
unknown	**2.93**	**366.14851**	**368.15512**	6.7E+05
unknown	3.01	345.13822	347.14497	1.3E+05
unknown	**3.01**	**376.18010**	**378.18713**	4.3E+05
unknown	**3.09**	**414.12180**	**416.12858**	6.7E+06
unknown	3.09	445.16402	447.17111	1.1E+05
Arginine	**3.21**	**408.17030**	**410.17702**	7.8E+06
unknown	**3.36**	**319.11125**	**321.11808**	3.3E+05
unknown	3.36	474.18211	476.18799	4.5E+05
unknown	**3.40**	**408.16979**	**410.17625**	2.1E+05
unknown	**3.44**	**446.06687**	**448.07410**	8.8E+04
Asparagine	**3.52**	**366.11201**	**368.11876**	5.4E+06
unknown	**3.52**	**408.19522**	**410.20220**	9.8E+05
unknown	**3.52**	**502.13923**	**504.14623**	1.9E+05
Homoarginine	**3.55**	**422.21098**	**424.21777**	1.1E+06
unknown	**3.55**	**547.15605**	**549.16203**	2.0E+05
unknown	**3.59**	**380.16400**	**382.17082**	7.9E+05
unknown	**3.70**	**380.07997**	**382.08658**	1.1E+05

unknown	**3.74**	**424.15386**	**426.16028**	9.2E+04
unknown	**3.74**	**471.14316**	**473.14997**	1.8E+05
unknown	**3.85**	**363.10136**	**365.10812**	7.2E+05
Glutamine	**3.93**	**380.12562**	**382.13217**	1.6E+08
unknown	**3.96**	**378.14036**	**384.16056**	1.6E+07
unknown	**4.11**	**363.10129**	**365.10802**	3.9E+05
L-citrulline	**4.15**	**409.15419**	**411.16066**	3.8E+06
unknown	**4.19**	**392.12770**	**394.13453**	4.7E+05
unknown	**4.19**	**436.20168**	**438.20828**	1.6E+05
unknown	**4.27**	**270.03515**	**272.04186**	4.5E+05
unknown	**4.27**	**302.02485**	**304.03163**	7.2E+05
unknown	**4.31**	**424.10654**	**426.11339**	4.5E+05
unknown	4.31	483.12805	485.13492	4.5E+05
unknown	**4.34**	**242.57192**	**244.57859**	3.2E+06
unknown	**4.34**	**483.12534**	**487.13873**	7.4E+05
unknown	4.34	486.12701	490.13950	3.9E+06
unknown	**4.34**	**506.11460**	**510.12812**	1.4E+06
unknown	**4.34**	**515.17555**	**519.18928**	1.2E+06
unknown	**4.42**	**270.03517**	**272.04182**	2.7E+05
unknown	**4.42**	**291.06378**	**293.07053**	4.6E+05
unknown	4.42	381.11211	383.11872	1.5E+06
unknown	4.42	402.57886	404.58563	1.8E+05
3-sn-Phosphatidylethanolamine	**4.42**	**484.13587**	**488.14957**	1.2E+07
unknown	**4.46**	**397.14284**	**399.14995**	1.4E+05
L-aspartic acid amide	4.53	366.10706	368.11307	1.1E+05
unknown	**4.53**	**380.12186**	**382.12842**	5.9E+05
unknown	4.65	400.10806	402.11472	1.5E+05
unknown	**4.65**	**468.14362**	**470.15049**	1.8E+05
Homoserine	**4.65**	**353.11677**	**355.12367**	3.2E+05
Methionine sulfoxide	**4.68**	**399.10448**	**401.11126**	7.3E+05
unknown	**4.68**	**465.18054**	**467.18713**	3.8E+05
unknown	**4.76**	**499.17452**	**501.18177**	1.8E+05
unknown	**4.79**	**360.05704**	**362.06384**	1.3E+05
unknown	**4.79**	**394.14344**	**396.15008**	2.9E+05
Homocitruline	**4.87**	**423.05395**	**425.06031**	1.0E+05
Serine	**4.87**	**339.10082**	**341.10742**	3.5E+07
unknown	**4.98**	**263.21199**	**265.21868**	4.8E+05
unknown	4.98	277.10047	279.10746	6.9E+04
unknown	**5.02**	**408.15874**	**410.16551**	1.2E+05
unknown	5.06	395.12748	398.13788	1.2E+05
unknown	**5.10**	**353.11679**	**355.12354**	1.5E+06
unknown	**5.10**	**479.23254**	**481.23969**	1.5E+05
unknown	**5.17**	**394.17973**	**396.18627**	3.2E+05
4-Hydroxy-proline	**5.25**	**365.11691**	**367.12340**	6.2E+06
Glutamic acid	**5.32**	**381.11195**	**383.11853**	2.0E+05
unknown	**5.32**	**455.10989**	**458.11975**	4.4E+05
unknown	**5.36**	**396.11153**	**398.11833**	2.9E+05
unknown	**5.36**	**466.16491**	**468.17162**	3.9E+05
unknown	**5.36**	**582.19078**	**584.19754**	5.4E+05
unknown	**5.36**	**600.20170**	**602.20836**	2.1E+06
unknown	**5.40**	**442.11704**	**444.12399**	1.2E+06
unknown	5.48	356.10433	358.11123	3.0E+05
unknown	5.52	422.17508	424.18133	1.9E+05
unknown	5.52	473.08439	475.09086	1.0E+05
unknown	**5.56**	**336.13798**	**338.14468**	1.9E+06
Iminodiacetic acid	5.56	367.18036	369.18684	2.0E+05
unknown	5.63	364.15268	366.15866	8.7E+04

unknown	**5.63**	**457.08929**	**459.09590**	2.5E+05
unknown	**5.67**	**363.14835**	**365.15486**	1.6E+06
unknown	5.71	375.09819	377.10486	6.5E+05
unknown	**5.71**	**462.16999**	**464.17687**	1.4E+06
Folic acid	**5.71**	**338.09287**	**339.09664**	1.0E+05
Threonine	**5.78**	**353.11660**	**355.12361**	1.4E+07
unknown	**5.82**	**337.15824**	**339.16494**	5.3E+06
unknown	**5.89**	**379.13246**	**381.13921**	5.1E+05
unknown	5.93	282.09889	284.10567	2.0E+05
Diethanolamine	**5.93**	**339.13769**	**341.14440**	5.3E+05
unknown	5.97	488.11853	491.12817	1.2E+05
unknown	**5.97**	**515.17133**	**517.17786**	1.9E+05
unknown	**6.01**	**478.12806**	**480.13508**	3.4E+06
Ethanolamine	**6.05**	**295.11108**	**297.11786**	1.3E+07
unknown	**6.05**	**611.19714**	**613.20447**	2.3E+05
unknown	6.08	273.06693	275.07351	5.9E+05
unknown	**6.08**	**463.23796**	**465.24466**	1.7E+06
unknown	**6.08**	**494.27960**	**496.28665**	3.8E+05
unknown	**6.12**	**251.08472**	**253.09134**	2.1E+07
unknown	**6.12**	**515.16998**	**517.17634**	4.3E+05
unknown	**6.12**	**523.14498**	**525.15193**	2.7E+06
unknown	6.20	396.11118	398.11846	1.7E+05
unknown	**6.20**	**411.15887**	**413.16541**	1.7E+05
unknown	6.20	501.16315	503.17012	3.4E+05
unknown	**6.23**	**317.13200**	**319.13888**	1.3E+06
unknown	**6.23**	**348.17443**	**350.18108**	2.8E+05
unknown	**6.23**	**415.13263**	**417.13943**	2.6E+06
unknown	**6.31**	**388.16919**	**390.17587**	1.5E+05
unknown	**6.31**	**406.17981**	**408.18655**	2.4E+05
unknown	**6.35**	**464.14900**	**466.15579**	5.2E+05
unknown	**6.39**	**398.12753**	**400.13403**	2.9E+06
unknown	**6.43**	**348.10153**	**350.10820**	1.5E+06
unknown	6.43	480.14545	482.15192	9.2E+04
Glycine	**6.50**	**309.09052**	**311.09711**	1.2E+07
unknown	**6.50**	**363.13778**	**365.14445**	3.9E+05
unknown	**6.50**	**381.14816**	**383.15490**	4.3E+06
unknown	6.54	334.29541	336.30200	2.1E+06
unknown	**6.54**	**464.14828**	**466.15475**	8.4E+04
unknown	6.65	369.12714	371.13354	8.9E+04
unknown	**6.73**	**344.10662**	**346.11330**	1.2E+06
unknown	**6.73**	**364.16922**	**366.17583**	6.2E+06
unknown	**6.73**	**395.21130**	**397.21811**	2.6E+05
unknown	**6.73**	**422.22220**	**424.22925**	9.8E+04
unknown	6.73	535.25644	537.26308	1.2E+05
unknown	6.76	315.11636	317.12350	7.0E+04
unknown	6.76	420.15855	422.16549	8.6E+04
unknown	**6.76**	**477.16184**	**479.16860**	2.7E+06
unknown	**6.80**	**348.13787**	**350.14458**	8.6E+05
unknown	**6.80**	**361.12234**	**363.12886**	1.1E+05
unknown	6.80	509.12274	511.12914	1.4E+05
unknown	6.95	396.11135	398.11813	1.7E+05
unknown	**6.95**	**415.09213**	**417.09912**	6.7E+04
unknown	**7.14**	**287.03347**	**289.04009**	5.3E+05
unknown	**7.18**	**347.11795**	**349.12442**	5.3E+05
unknown	**7.18**	**362.11735**	**364.12405**	6.3E+06
Tyrosine methyl ester	**7.18**	**415.13284**	**417.13963**	4.0E+06
unknown	**7.21**	**367.13290**	**369.13934**	5.7E+05

unknown	**7.25**	**344.10651**	**346.11332**	6.4E+05
Alanine	**7.33**	**323.10637**	**325.11291**	2.7E+07
unknown	**7.36**	**473.10822**	**475.11406**	1.0E+07
r-aminobutyric acid	**7.51**	**337.12211**	**339.12908**	1.3E+05
unknown	**7.67**	**473.10623**	**475.11378**	3.2E+06
unknown	**7.75**	**473.10972**	**475.11672**	1.2E+06
unknown	7.75	493.08602	497.09854	6.4E+05
unknown	**7.78**	**471.10400**	**473.11081**	4.5E+05
unknown	**7.89**	**396.11149**	**398.11831**	9.0E+05
unknown	**7.93**	**368.11658**	**370.12317**	1.6E+05
unknown	8.00	322.07453	324.08135	2.0E+05
unknown	8.04	499.13819	505.15781	7.8E+05
unknown	8.12	460.11683	462.12437	2.6E+05
Hypoxanthine	**8.20**	**370.09709**	**372.10380**	9.0E+05
unknown	**8.20**	**396.11101**	**398.11814**	1.9E+05
unknown	**8.24**	**450.20595**	**452.21323**	9.7E+04
5-hydroxymethyluracil	**8.31**	**376.09658**	**378.10302**	1.2E+05
unknown	**8.39**	**378.06744**	**380.07456**	2.9E+05
3-Aminoisobutyric acid	**8.42**	**337.12182**	**339.12856**	1.3E+05
unknown	**8.42**	**279.08000**	**281.08662**	6.2E+05
unknown	**8.42**	**431.13855**	**433.14551**	3.8E+06
unknown	**8.46**	**386.09196**	**388.09874**	5.0E+05
5-Aminopentanoic acid	**8.53**	**351.13755**	**353.14435**	2.0E+05
unknown	**8.53**	**396.13522**	**398.14182**	1.9E+06
unknown	**8.53**	**485.11951**	**488.13052**	4.6E+05
unknown	8.61	293.13216	295.13884	6.1E+04
unknown	8.87	338.12541	340.13219	1.3E+06
2-Aminobutyric acid	**8.91**	**337.12181**	**339.12853**	7.2E+06
unknown	9.02	460.11899	462.12494	6.6E+04
unknown	**9.06**	**321.09063**	**323.09769**	2.3E+05
Cysteine-glutathione disulfide	**9.06**	**447.10246**	**449.10955**	4.6E+05
unknown	**9.10**	**351.10116**	**354.11081**	2.2E+05
unknown	**9.14**	**364.10431**	**366.11084**	7.8E+04
unknown	**9.14**	**370.09714**	**372.10389**	2.9E+06
unknown	**9.22**	**363.10134**	**365.10807**	1.0E+06
Methylcysteine	**9.37**	**369.09419**	**371.10091**	1.7E+06
unknown	**9.44**	**382.64871**	**384.65550**	4.0E+05
unknown	**9.48**	**242.28429**	**244.29100**	5.7E+06
unknown	**9.52**	**396.11135**	**398.11821**	4.1E+05
unknown	9.52	451.17952	453.18669	8.2E+04
unknown	9.59	288.08915	290.09540	6.2E+04
unknown	**9.63**	**266.10738**	**268.11388**	2.1E+06
unknown	**9.63**	**287.08255**	**289.08920**	2.9E+05
unknown	**9.63**	**381.14819**	**383.15493**	6.9E+06
unknown	9.63	532.20654	534.21313	3.7E+05
unknown	**9.67**	**321.12714**	**323.13380**	5.4E+06
unknown	**9.71**	**265.10010**	**268.10982**	2.6E+07
unknown	**9.71**	**551.17645**	**553.18282**	3.1E+06
unknown	9.85	550.17468	552.18064	3.3E+06
unknown	9.85	615.15448	617.16119	1.1E+06
Methylamine	**9.89**	**265.09992**	**267.10615**	3.0E+07
Proline	**9.89**	**349.12204**	**351.12862**	1.0E+06
unknown	9.89	683.22510	685.23059	9.7E+05
unknown	**10.01**	**531.20114**	**533.20777**	1.5E+06
unknown	**10.12**	**287.08246**	**289.08908**	6.9E+05
unknown	**10.19**	**370.09717**	**372.10391**	9.2E+05
unknown	**10.23**	**265.10053**	**268.11059**	9.3E+06

unknown	**10.27**	**311.08304**	**313.08963**	5.8E+05
unknown	**10.30**	**531.19818**	**533.20508**	1.8E+05
unknown	**10.30**	**551.17334**	**553.18005**	1.2E+05
unknown	**10.30**	**554.18341**	**556.19092**	1.7E+05
unknown	**10.38**	**321.11438**	**323.12113**	2.1E+06
unknown	**10.42**	**287.08251**	**289.08928**	2.4E+05
unknown	10.42	412.16934	414.17592	2.7E+05
Valine	**10.45**	**351.13703**	**353.14370**	3.9E+07
3-Hydroxypicolinic acid	**10.52**	**373.11838**	**375.12507**	5.5E+05
Methionine	**10.52**	**383.10841**	**385.11531**	1.0E+07
unknown	10.56	378.18489	380.19156	3.0E+05
unknown	**10.71**	**400.08517**	**402.09192**	5.1E+06
unknown	**10.83**	**361.12177**	**363.12871**	1.8E+05
unknown	10.87	266.11078	268.11780	2.2E+05
unknown	**10.90**	**378.06758**	**380.07455**	1.2E+06
Tryptophan	**10.98**	**438.14849**	**440.15511**	1.6E+07
unknown	11.20	311.59366	313.59979	7.0E+04
unknown	11.46	414.15942	416.16635	8.6E+04
unknown	11.50	286.02780	290.04156	3.3E+05
unknown	**11.77**	**418.13214**	**420.13895**	9.1E+05
unknown	**11.80**	**315.09055**	**317.09725**	1.4E+05
unknown	12.02	482.15682	486.16957	5.4E+05
unknown	**12.10**	**295.10235**	**297.10921**	1.4E+05
unknown	12.17	399.13732	402.14719	2.8E+07
Phenylalanine	**12.29**	**399.13739**	**401.14416**	2.9E+07
unknown	12.32	266.10394	268.11063	7.8E+05
unknown	**12.36**	**406.61710**	**408.62380**	4.4E+05
unknown	12.48	314.60544	316.61221	2.0E+05
3-Hydroxymandelic acid	**12.48**	**402.10089**	**404.10763**	4.3E+05
unknown	**12.52**	**313.60904**	**315.61565**	3.7E+06
Isoleucine	**12.52**	**365.15302**	**367.15969**	3.2E+07
unknown	12.52	393.15878	395.16512	4.9E+05
unknown	12.59	365.15326	368.16327	2.2E+07
unknown	**12.66**	**399.13885**	**401.14505**	9.5E+04
unknown	**12.66**	**501.11528**	**505.12864**	2.2E+06
unknown	12.70	489.07044	492.08080	8.7E+04
unknown	**12.74**	**379.13110**	**381.13794**	7.1E+05
Leucine	**12.78**	**365.15133**	**367.15806**	8.8E+07
L-cystathionine	**12.78**	**345.09233**	**347.09880**	2.3E+05
L-norleucine	**12.85**	**365.15248**	**367.15903**	2.6E+07
unknown	**13.00**	**300.06543**	**302.07165**	6.4E+05
unknown	13.08	357.12460	359.13119	4.4E+05
unknown	13.08	635.65399	637.65948	5.2E+05
unknown	**13.12**	**317.13195**	**319.13883**	8.1E+05
unknown	**13.12**	**335.14209**	**338.15236**	2.8E+07
Cystine	**13.12**	**354.06970**	**356.07642**	5.3E+05
unknown	**13.23**	**335.14244**	**337.14892**	1.4E+07
Phenylethanolamine	13.38	371.14139	373.14755	3.3E+04
unknown	**13.42**	**549.18860**	**551.19525**	8.1E+04
unknown	**13.49**	**378.10090**	**380.10749**	6.1E+06
unknown	**13.49**	**409.14325**	**411.15009**	3.7E+05
Hydroxyphenyllactic acid	**13.64**	**416.11638**	**418.12329**	5.1E+06
unknown	13.71	297.08574	299.09244	2.5E+05
unknown	**13.75**	**307.09299**	**309.09972**	3.1E+06
unknown	13.75	474.06777	476.07494	4.1E+05
unknown	13.83	501.13210	503.13837	4.4E+05
unknown	13.94	402.10085	405.11099	3.0E+05

Homocystine	**13.98**	**368.09866**	**370.10562**	1.0E+05
unknown	13.98	371.14030	373.14711	1.9E+05
unknown	**14.02**	**322.07462**	**324.08148**	2.4E+05
unknown	14.09	693.15039	695.15654	7.5E+04
unknown	**14.13**	**520.24725**	**522.25346**	7.0E+04
5-HIAA	**14.20**	**425.11691**	**427.12368**	2.8E+06
unknown	**14.20**	**511.13807**	**513.14428**	5.0E+05
unknown	**14.27**	**336.11422**	**338.12093**	3.0E+05
unknown	**14.27**	**350.16194**	**352.16881**	2.6E+05
unknown	**14.27**	**520.24554**	**522.25244**	7.8E+04
unknown	**14.31**	**346.06578**	**348.07248**	1.4E+05
unknown	**14.31**	**349.15828**	**351.16498**	1.2E+06
Dimethylamine	**14.38**	**279.11586**	**281.12260**	3.4E+07
unknown	14.38	585.22656	588.23755	7.6E+05
Phenylpropanolamine	**14.45**	**385.12208**	**387.12881**	4.0E+05
unknown	**14.45**	**301.09731**	**305.11041**	5.0E+06
unknown	14.45	557.22607	561.23921	9.9E+05
unknown	14.45	585.22628	587.23382	1.6E+06
unknown	14.53	584.22418	588.23755	9.4E+05
unknown	**14.62**	**349.15834**	**351.16499**	1.7E+06
unknown	**14.65**	**345.09561**	**347.10234**	3.6E+05
unknown	**14.65**	**367.16896**	**369.17571**	1.3E+05
unknown	14.73	371.14042	374.15041	3.4E+05
unknown	14.77	306.61930	308.62598	1.4E+05
unknown	**14.77**	**513.15344**	**515.16040**	3.0E+05
unknown	**14.85**	**513.15232**	**515.15840**	8.9E+05
unknown	**14.88**	**349.15837**	**352.16872**	1.5E+06
unknown	**14.88**	**513.15342**	**515.16040**	5.3E+05
unknown	**14.96**	**371.14054**	**373.14697**	2.7E+05
unknown	**14.96**	**520.24556**	**522.25165**	6.1E+04
unknown	15.00	381.26152	383.26898	1.0E+06
unknown	**15.00**	**415.21167**	**417.21791**	2.5E+06
unknown	15.00	586.29899	588.30603	6.3E+05
unknown	**15.11**	**363.65357**	**365.66037**	4.3E+05
unknown	**15.11**	**415.21192**	**417.21829**	3.8E+07
unknown	**15.11**	**446.25400**	**448.26033**	1.7E+07
unknown	**15.18**	**350.16205**	**352.16895**	1.9E+05
unknown	**15.18**	**393.14874**	**395.15500**	1.6E+05
unknown	15.22	471.12423	473.13044	3.4E+05
unknown	15.33	344.60379	346.61027	9.7E+04
unknown	**15.37**	**360.63585**	**362.64236**	7.4E+05
L-ornithine	**15.40**	**300.10337**	**302.10998**	1.6E+07
unknown	15.40	313.62702	315.63374	3.1E+05
Acetaminophen	**15.45**	**385.12225**	**387.13013**	7.0E+04
or 4-acetamidophenol				
unknown	**15.48**	**425.61277**	**427.61965**	3.3E+05
unknown	**15.52**	**300.06526**	**302.07186**	1.1E+06
unknown	**15.52**	**415.21184**	**417.21810**	5.5E+06
unknown	15.52	549.13071	551.13748	1.2E+06
Homovanillic	**15.64**	**416.11642**	**418.12326**	1.0E+07
unknown	15.71	399.13783	403.15111	5.7E+05
unknown	15.71	530.18829	532.19506	1.5E+05
unknown	**15.75**	**468.14114**	**471.15145**	1.2E+06
unknown	15.97	319.11152	321.11838	3.4E+05
unknown	**15.97**	**474.18121**	**476.18840**	2.4E+06
3-/4-hydroxyphenylacetic acid	**16.01**	**386.10577**	**388.11276**	1.4E+06
or 3-Cresotinic acid				

unknown	**16.05**	**345.57376**	**347.58032**	4.6E+05
unknown	**16.05**	**419.47900**	**421.48572**	6.5E+05
Homocarnosine	**16.08**	**354.11957**	**356.12634**	2.0E+06
unknown	**16.15**	**391.16905**	**393.17588**	9.0E+05
unknown	**16.19**	**345.57397**	**347.58032**	2.2E+05
unknown	**16.31**	**350.12983**	**352.13657**	1.2E+06
unknown	16.35	424.10461	426.11166	2.3E+05
Lysine	**16.42**	**307.11081**	**309.11733**	4.0E+07
unknown	**16.42**	**423.10157**	**425.10824**	1.5E+06
unknown	**16.50**	**614.21796**	**617.22852**	1.0E+05
unknown	**16.61**	**327.64290**	**329.64965**	8.8E+05
unknown	**16.69**	**264.08798**	**266.09471**	5.2E+05
4-Hydroxybenzoic acid	**16.69**	**372.09045**	**374.09726**	5.5E+06
unknown	**16.84**	**415.21176**	**417.21808**	1.2E+07
unknown	**16.88**	**319.62723**	**321.63382**	1.9E+05
Histidine	**16.92**	**311.59342**	**313.60003**	9.6E+06
unknown	16.92	623.18294	627.19628	1.1E+06
unknown	**16.99**	**382.10880**	**384.11575**	1.4E+05
unknown	17.03	622.17859	626.19229	2.0E+05
unknown	17.06	544.11374	550.13424	1.0E+06
unknown	**17.10**	**415.21168**	**417.21798**	5.4E+06
unknown	**17.14**	**329.08243**	**331.08912**	2.8E+05
unknown	17.25	373.15828	375.16495	8.7E+04
unknown	**17.32**	**407.16315**	**409.17010**	7.1E+04
unknown	**17.35**	**311.08300**	**313.08974**	2.3E+05
unknown	**17.35**	**359.62772**	**361.63441**	4.0E+05
unknown	**17.43**	**407.16374**	**409.17052**	4.7E+05
unknown	**17.54**	**297.08583**	**299.09224**	4.6E+05
unknown	**17.54**	**324.10566**	**326.11183**	2.5E+05
unknown	17.54	359.10514	361.11205	8.9E+04
unknown	**17.54**	**390.10095**	**392.10757**	3.5E+05
unknown	**17.87**	**413.11706**	**415.12387**	7.6E+05
unknown	**17.90**	**528.16992**	**530.17559**	1.6E+06
unknown	**17.98**	**419.47898**	**421.48596**	4.8E+05
2-aminooctanoic acid	**18.02**	**393.18472**	**395.19107**	2.0E+05
unknown	**18.02**	**528.17277**	**530.17941**	5.6E+05
unknown	**18.13**	**356.09346**	**358.10014**	2.1E+06
unknown	18.16	314.06145	316.06802	5.0E+06
unknown	**18.16**	**395.10634**	**397.11308**	8.6E+05
unknown	**18.35**	**370.11103**	**372.11784**	3.7E+05
unknown	18.39	308.15109	310.15793	1.5E+05
unknown	**18.42**	**307.14782**	**309.15449**	1.1E+06
unknown	**18.42**	**354.06340**	**356.07010**	2.8E+06
unknown	**18.71**	**300.06519**	**302.07143**	5.8E+05
unknown	18.75	520.10284	522.11046	8.3E+05
unknown	**18.82**	**421.17967**	**423.18657**	7.9E+05
unknown	**18.93**	**321.06665**	**323.07358**	3.8E+05
unknown	**19.15**	**314.11909**	**316.12582**	1.1E+07
L-Tyrosinamide	19.15	324.10352	326.11003	1.1E+05
unknown	19.19	504.10919	506.11576	3.4E+05
unknown	**19.33**	**401.12832**	**403.13531**	2.3E+05
unknown	19.37	501.16234	503.16978	2.9E+06
unknown	**19.44**	**279.10717**	**281.11376**	1.0E+06
unknown	**19.48**	**361.07770**	**363.08429**	2.7E+06
unknown	**19.63**	**357.45756**	**359.46445**	2.3E+05
1,4-diaminobutane	**19.63**	**555.20928**	**559.22302**	2.4E+05
unknown	**19.70**	**264.58514**	**266.59159**	1.5E+05

unknown	**19.74**	**386.10601**	**388.11284**	4.0E+05
unknown	19.74	603.00671	605.01306	1.7E+05
unknown	19.81	357.09901	359.10585	2.6E+06
unknown	**19.81**	**528.17401**	**530.17978**	5.3E+05
unknown	**19.92**	**331.11159**	**333.11815**	1.5E+06
unknown	**20.04**	**292.10607**	**294.11272**	1.3E+06
unknown	20.07	499.14725	501.15333	4.3E+05
unknown	**20.11**	**486.11466**	**488.12087**	8.3E+05
unknown	20.22	749.21576	752.22632	4.3E+06
Tyrosine	**20.26**	**324.59429**	**326.60076**	8.7E+07
Cysteamine	**20.37**	**310.07522**	**312.08202**	2.0E+05
Metoprolol	**20.37**	**501.16201**	**503.16866**	3.0E+06
unknown	20.52	360.08231	362.08932	3.9E+05
unknown	20.52	466.16241	470.17593	2.7E+05
unknown	**20.56**	**315.08502**	**317.09183**	7.0E+05
unknown	**20.63**	**379.11163**	**381.11853**	4.1E+05
unknown	20.67	719.18604	721.19151	3.2E+05
Phenol	**20.75**	**328.10059**	**330.10720**	1.0E+06
4-Nitrophenol	**20.82**	**373.08624**	**375.09262**	1.5E+05
unknown	**20.90**	**279.10752**	**281.11411**	5.2E+05
unknown	20.93	350.62256	352.62934	2.0E+05
unknown	**21.01**	**315.08432**	**317.09106**	4.2E+05
unknown	**21.09**	**298.10617**	**300.11286**	3.1E+05
unknown	**21.09**	**310.07531**	**312.08214**	6.9E+05
unknown	21.09	323.09610	325.10284	1.7E+05
unknown	**21.09**	**639.40931**	**641.41563**	3.8E+06
unknown	**21.09**	**784.18457**	**786.19141**	5.8E+05
unknown	**21.27**	**454.15532**	**456.16162**	4.7E+05
unknown	21.27	595.37926	597.38562	2.4E+06
unknown	**21.32**	**344.10675**	**346.11356**	8.1E+05
unknown	**21.32**	**397.20162**	**399.20824**	5.8E+06
unknown	**21.35**	**299.07232**	**301.07891**	1.2E+06
unknown	**21.35**	**335.17947**	**337.18623**	3.1E+05
unknown	**21.35**	**560.41523**	**562.42163**	2.2E+06
unknown	21.39	471.16108	473.16838	6.2E+05
unknown	**21.39**	**551.35585**	**553.36234**	1.1E+07
unknown	**21.50**	**507.33017**	**509.33645**	2.0E+07
unknown	21.54	280.08798	282.09461	3.1E+05
unknown	**21.54**	**514.14696**	**518.16012**	3.2E+06
unknown	**21.58**	**463.30382**	**465.31017**	1.9E+07
unknown	**21.58**	**472.36423**	**474.37079**	4.2E+06
unknown	21.61	520.10358	522.10989	6.8E+05
unknown	**21.65**	**354.11649**	**356.12316**	2.9E+06
unknown	**21.69**	**419.27755**	**421.28386**	1.3E+07
unknown	**21.72**	**375.25123**	**377.25765**	6.6E+06
unknown	**21.72**	**463.30411**	**465.31079**	3.4E+06
unknown	21.83	501.16293	503.16911	8.7E+05
unknown	**21.83**	**520.13422**	**524.14697**	1.3E+06
unknown	**21.83**	**529.19409**	**533.20660**	6.0E+05
unknown	**21.87**	**498.15239**	**501.16186**	6.3E+06
unknown	**21.99**	**520.13436**	**524.14679**	9.4E+05
unknown	**21.99**	**529.19373**	**533.20650**	3.6E+05
unknown	**22.06**	**521.13819**	**524.14782**	2.8E+05
unknown	22.17	618.02478	620.03253	7.9E+05
unknown	**22.29**	**450.20686**	**452.21390**	4.6E+05
unknown	**22.29**	**499.14618**	**501.15349**	1.8E+05
unknown	22.36	355.07062	361.09007	1.2E+05

unknown	22.55	390.08437	394.09781	1.3E+07
unknown	**22.59**	**388.07698**	**390.08358**	8.2E+06
unknown	**22.62**	**344.10685**	**346.11360**	1.3E+06
unknown	**22.62**	**572.23688**	**576.25012**	1.9E+05
unknown	**22.66**	**388.07761**	**390.08475**	1.5E+06
unknown	22.66	541.19493	545.20844	2.4E+06
unknown	**22.70**	**572.23615**	**576.24904**	7.7E+05
unknown	**22.74**	**360.57934**	**362.58616**	6.7E+06
unknown	22.74	564.17971	567.19025	3.1E+05
unknown	22.78	298.06420	300.07083	2.1E+05
unknown	**22.78**	**572.23730**	**576.25013**	2.0E+05
unknown	**22.81**	**377.19165**	**383.21189**	1.4E+05
unknown	**22.81**	**648.26379**	**650.26984**	3.8E+05
unknown	22.85	294.08844	296.09540	1.1E+05
unknown	22.85	372.10179	375.11149	1.3E+05
unknown	**22.85**	**458.42106**	**460.42800**	2.9E+06
unknown	22.85	521.13836	524.14905	3.0E+05
unknown	**22.89**	**282.44465**	**284.45141**	2.6E+05
Spermidine	**22.89**	**423.16368**	**426.17382**	3.7E+05
unknown	22.89	440.62964	443.63970	9.9E+04
Pyrocatechol	**22.93**	**289.08247**	**291.08926**	9.4E+05
unknown	**22.93**	**344.10687**	**346.11362**	4.1E+05
unknown	**22.96**	**388.07746**	**390.08445**	1.1E+06
unknown	**22.96**	**648.26285**	**650.26974**	5.4E+05
unknown	**23.04**	**315.59026**	**317.59709**	1.2E+06
unknown	**23.04**	**454.24158**	**456.24890**	8.8E+05
unknown	23.08	284.06658	286.07337	4.1E+05
unknown	**23.19**	**648.26321**	**650.27001**	5.1E+05
unknown	**23.23**	**455.24487**	**457.25223**	1.9E+05
unknown	23.34	298.12415	300.13105	1.1E+05
unknown	23.38	382.60888	384.61556	5.0E+05
unknown	**23.38**	**421.15815**	**425.17202**	2.0E+05
unknown	**23.38**	**427.11573**	**429.12274**	2.5E+05
unknown	**23.38**	**648.26303**	**650.27034**	3.2E+05
unknown	23.42	444.33743	448.35126	8.9E+04
unknown	23.50	364.21415	366.22071	4.8E+05
unknown	23.50	385.19241	387.19937	1.1E+05
unknown	**23.50**	**466.31969**	**469.32892**	1.2E+06
unknown	**23.65**	**305.06804**	**307.07482**	4.1E+06
unknown	23.69	610.10376	614.11829	8.6E+05
unknown	23.72	609.13007	613.14367	1.6E+06
unknown	23.80	486.16050	488.16813	5.1E+05
unknown	**23.83**	**431.61157**	**434.62154**	1.6E+05
unknown	23.83	521.13840	524.14936	3.2E+05
Thymol	**23.87**	**384.16375**	**386.17041**	6.3E+05
unknown	23.87	613.11707	617.13098	1.4E+05
unknown	**23.91**	**432.61946**	**435.62931**	3.2E+05
unknown	**23.98**	**648.26376**	**650.27057**	1.1E+06
unknown	23.98	679.30603	681.31371	4.7E+05
unknown	**24.02**	**530.72102**	**532.72781**	2.9E+05
unknown	**24.02**	**648.26353**	**650.27100**	3.3E+05
unknown	**24.06**	**288.07642**	**290.08292**	2.3E+05
unknown	**24.06**	**431.61104**	**434.62114**	5.1E+05
unknown	24.13	290.09242	292.09920	5.8E+05
unknown	24.13	318.10947	320.11621	2.4E+06
unknown	24.13	434.63549	437.64539	3.1E+06
unknown	**24.13**	**685.43775**	**687.44391**	1.9E+06

unknown	**24.25**	**432.61383**	**435.62415**	1.5E+05
unknown	24.32	360.07021	362.07682	8.9E+05
unknown	24.32	562.31638	564.32309	1.3E+07
unknown	24.32	594.12817	598.14203	2.7E+06
unknown	**24.44**	**431.61127**	**434.62150**	3.7E+05

Table S4.3. Ion pairs detected and identified by RPLC FTICR MS from repeatedly 1:1 ^{12}C-/^{13}C-dansylated CSF sample #3. Ion pairs detected in both repeatedly labeled CSF sample are highlighted as bold.

CSF - #3		**CSF - #3**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	1.62	503.13233	505.13874	2.4E+06
unknown	1.62	534.17424	536.18090	2.2E+06
unknown	1.62	648.64042	650.64597	3.9E+05
unknown	1.62	754.19498	757.20380	5.9E+05
unknown	1.62	789.24916	791.25442	1.2E+06
unknown	**1.69**	**524.11157**	**526.11733**	4.5E+05
unknown	1.73	547.09596	549.10238	7.9E+05
unknown	1.73	559.09070	562.09991	1.8E+05
unknown	1.73	570.08075	572.08742	2.2E+05
unknown	1.73	672.62634	674.63281	1.6E+05
unknown	1.76	275.05460	277.06125	1.3E+06
unknown	1.76	336.02146	338.02813	2.5E+05
unknown	1.76	580.14493	582.15142	5.7E+05
unknown	1.76	639.07198	641.07858	5.0E+05
unknown	1.80	296.03308	298.03979	2.6E+06
unknown	**1.80**	**353.99181**	**355.99869**	3.7E+05
unknown	1.80	364.02078	366.02739	1.4E+06
unknown	**1.80**	**404.01413**	**406.02030**	1.8E+05
unknown	1.80	421.97961	423.98715	1.9E+05
unknown	**1.80**	**432.00839**	**434.01525**	1.1E+06
unknown	**1.80**	**499.99590**	**502.00284**	4.3E+05
unknown	1.80	567.98373	569.99012	2.9E+05
unknown	**1.80**	**589.20459**	**591.21173**	2.1E+05
unknown	**1.88**	**252.06914**	**254.07579**	3.7E+06
unknown	**1.88**	**546.06299**	**548.06940**	2.5E+05
unknown	**1.88**	**555.12331**	**557.12939**	1.5E+05
unknown	1.88	590.02584	592.03277	2.2E+05
unknown	1.88	605.08969	607.09642	3.9E+05
unknown	1.88	617.20727	619.21404	1.6E+05
unknown	1.92	268.04630	270.05286	9.5E+04
unknown	1.92	449.11530	451.12183	1.4E+05
unknown	**1.99**	**555.12260**	**557.12996**	3.5E+05
unknown	1.99	701.00728	703.01385	4.1E+05
unknown	**2.03**	**524.08139**	**526.08872**	3.6E+05
unknown	**2.11**	**558.03252**	**562.04524**	6.0E+05
unknown	**2.14**	**414.12231**	**416.12907**	2.8E+05
unknown	**2.22**	**252.06905**	**254.07577**	1.7E+06
unknown	**2.22**	**389.12831**	**391.13497**	4.1E+06
unknown	2.22	632.22427	634.23157	1.1E+05

unknown	**2.26**	**432.00798**	**434.01480**	6.4E+04
unknown	2.26	537.08284	539.09045	9.5E+04
unknown	**2.30**	**376.08160**	**378.08846**	1.0E+05
phosphoethaolamine	**2.33**	**375.07778**	**377.08436**	6.0E+05
unknown	**2.41**	**252.06903**	**254.07570**	1.1E+06
unknown	2.48	296.02985	298.03693	1.1E+05
unknown	**2.52**	**274.05087**	**276.05768**	7.7E+05
unknown	**2.52**	**364.02025**	**366.02686**	9.3E+04
unknown	**2.56**	**252.06905**	**254.07576**	1.2E+06
3-methylhistidine	**2.56**	**403.14370**	**406.15320**	4.3E+05
Glucosamine	2.56	413.13797	415.14510	5.3E+04
unknown	**2.60**	**276.08021**	**278.08701**	1.3E+06
unknown	2.60	311.19704	313.20333	1.0E+05
Taurine	**2.60**	**359.07326**	**361.07997**	4.9E+06
unknown	**2.64**	**388.10775**	**390.11437**	2.0E+06
1-methylhistidine	**2.68**	**403.14389**	**405.15050**	7.1E+05
unknown	2.84	380.16415	382.17073	4.6E+05
unknown	2.88	363.10118	365.10786	3.8E+05
unknown	**2.88**	**366.14843**	**368.15511**	4.2E+05
unknown	2.88	509.17049	511.17714	1.4E+06
unknown	**2.95**	**345.13849**	**347.14487**	7.5E+04
unknown	**2.95**	**376.18034**	**378.18711**	2.4E+05
unknown	**2.99**	**414.12182**	**416.12862**	1.3E+06
unknown	**3.02**	**275.15036**	**277.15711**	1.8E+05
Arginine	**3.14**	**408.17021**	**410.17691**	6.0E+06
unknown	**3.33**	**319.11111**	**321.11807**	3.7E+05
unknown	3.33	474.18205	476.18813	4.7E+05
unknown	**3.44**	**408.19503**	**410.20190**	2.7E+05
unknown	3.51	314.09548	316.10279	3.0E+05
unknown	**3.51**	**380.16401**	**382.17082**	1.2E+06
Homoarginine	**3.51**	**422.21105**	**424.21776**	3.1E+05
unknown	3.51	502.13964	504.14629	2.6E+05
Asparagine	**3.55**	**366.11201**	**368.11875**	5.5E+06
unknown	**3.63**	**365.12811**	**367.13456**	1.1E+06
unknown	3.63	424.15374	426.16085	2.9E+05
unknown	3.78	471.14300	473.14932	6.8E+04
unknown	3.81	485.19669	487.20315	3.0E+05
unknown	**3.85**	**363.10120**	**365.10794**	2.5E+05
Glutamine	**3.93**	**380.12560**	**382.13206**	1.5E+08
unknown	**3.93**	**411.16817**	**413.17501**	2.7E+06
unknown	**4.08**	**363.10130**	**365.10791**	2.7E+05
unknown	4.16	392.12749	394.13443	2.6E+05
L-citrulline	**4.16**	**409.15435**	**411.16088**	1.9E+06
unknown	**4.16**	**436.20152**	**438.20849**	1.2E+05
unknown	4.23	424.11756	426.12457	6.4E+04
unknown	**4.27**	**253.56268**	**255.56931**	4.7E+05
unknown	**4.27**	**291.06383**	**293.07056**	5.6E+05
unknown	**4.27**	**380.12813**	**382.13492**	1.1E+06
unknown	4.27	505.11313	507.11968	3.5E+05
unknown	**4.27**	**515.17576**	**519.18939**	1.0E+06
unknown	**4.31**	**377.08157**	**379.08848**	4.6E+05
unknown	4.38	242.06802	244.07455	3.0E+05
unknown	**4.38**	**302.02482**	**304.03170**	5.1E+05
3-sn-Phosphatidylethanolamine	**4.38**	**484.13622**	**488.14933**	1.1E+07

unknown	**4.38**	**515.17813**	**519.19104**	2.9E+05
Methylguanidine	4.46	307.12228	309.12935	7.8E+04
Homoserine	**4.60**	**353.11684**	**355.12346**	2.2E+05
unknown	4.64	319.11116	321.11826	1.6E+05
unknown	**4.64**	**465.18050**	**467.18723**	8.6E+05
unknown	4.68	393.11135	395.11777	6.3E+04
Methionine sulfoxide	**4.68**	**399.10450**	**401.11123**	1.4E+06
unknown	4.79	360.05722	362.06409	8.8E+04
unknown	4.79	499.17446	501.18166	1.1E+05
unknown	4.83	380.12347	382.13037	1.8E+05
Serine	**4.87**	**339.10044**	**341.10681**	3.1E+07
Homocitruline	**4.87**	**423.05400**	**425.06122**	9.0E+04
unknown	**4.94**	**263.21194**	**265.21866**	1.2E+06
unknown	4.94	340.09357	343.10359	1.2E+05
Aspartic Acid	**4.98**	**367.09604**	**369.10274**	1.6E+06
Glutamic Acid	**4.98**	**381.11177**	**383.11842**	**5.4E+06**
unknown	5.02	408.15886	410.16559	3.0E+05
unknown	**5.13**	**394.17973**	**396.18632**	4.3E+05
unknown	**5.17**	**367.13283**	**369.13916**	2.9E+05
unknown	5.21	502.14585	504.15289	8.6E+04
4-Hydroxy-proline	**5.28**	**365.11686**	**367.12335**	1.9E+06
unknown	**5.28**	**455.10973**	**458.11963**	1.9E+05
unknown	**5.32**	**600.20113**	**602.20825**	3.2E+05
unknown	**5.36**	**442.11674**	**444.12404**	3.1E+05
unknown	**5.40**	**466.16445**	**468.17147**	7.4E+05
unknown	**5.47**	**422.17490**	**424.18173**	1.0E+05
unknown	**5.51**	**336.13796**	**338.14467**	1.9E+06
Iminodiacetic acid	**5.51**	**367.18005**	**369.18691**	1.8E+05
Aminoadipic acid	5.55	395.12737	397.13419	1.6E+05
unknown	**5.55**	**505.07592**	**509.08849**	2.5E+05
unknown	5.63	345.13821	347.14493	8.2E+04
unknown	**5.63**	**363.14877**	**365.15540**	1.3E+06
unknown	**5.63**	**457.08920**	**459.09597**	2.4E+05
Threonine	**5.67**	**353.11645**	**355.12281**	4.8E+07
Folic acid	**5.67**	**338.09322**	**339.09608**	1.3E+05
unknown	**5.71**	**389.12714**	**391.13406**	7.2E+05
unknown	5.74	480.18036	482.18747	3.4E+05
unknown	**5.82**	**337.15823**	**339.16493**	2.9E+06
unknown	**5.85**	**379.13241**	**381.13929**	3.7E+05
Diethanolamine	**5.89**	**339.13812**	**341.14430**	1.3E+05
unknown	5.93	475.11899	478.12829	6.5E+04
unknown	5.97	479.13199	481.13841	4.5E+05
unknown	**5.97**	**524.19659**	**526.20383**	8.0E+05
Ethanolamine	**6.01**	**295.11084**	**297.11751**	3.1E+07
unknown	6.04	318.09656	320.10355	2.5E+05
unknown	**6.04**	**463.23807**	**465.24454**	2.7E+05
unknown	**6.08**	**251.08472**	**253.09133**	1.7E+07
unknown	6.08	503.17028	505.17676	5.6E+05
unknown	**6.08**	**523.14495**	**525.15188**	1.8E+06
unknown	**6.19**	**317.13200**	**319.13883**	1.6E+06
unknown	**6.19**	**348.17440**	**350.18105**	4.5E+05
unknown	**6.23**	**415.13255**	**417.13927**	2.4E+06
unknown	6.27	454.15485	456.16160	1.3E+05
unknown	**6.37**	**348.10152**	**350.10819**	1.1E+06

unknown	**6.37**	**398.12728**	**400.13399**	9.2E+05
unknown	**6.41**	**464.14878**	**466.15561**	2.2E+05
Glycine	**6.45**	**309.09053**	**311.09711**	8.3E+06
unknown	**6.45**	**363.13776**	**365.14421**	1.7E+05
unknown	**6.45**	**381.14815**	**383.15488**	2.0E+06
unknown	6.45	541.20453	543.21106	1.3E+05
unknown	**6.64**	**344.10663**	**346.11335**	1.5E+06
unknown	6.64	367.09242	369.09886	6.8E+04
unknown	**6.68**	**364.16921**	**366.17579**	1.4E+06
unknown	**6.72**	**477.16178**	**479.16842**	5.5E+05
unknown	**6.75**	**348.13777**	**350.14453**	5.4E+05
unknown	6.83	395.12722	397.13387	1.3E+05
unknown	**6.94**	**396.11136**	**398.11811**	1.4E+05
unknown	**7.05**	**362.11661**	**364.12315**	3.4E+07
unknown	**7.12**	**347.11761**	**349.12431**	6.2E+05
unknown	**7.12**	**362.11719**	**364.12380**	1.4E+07
Tyrosine methyl ester	**7.17**	**415.13278**	**417.13946**	2.3E+06
unknown	**7.21**	**344.10651**	**346.11325**	7.0E+05
unknown	**7.21**	**367.13290**	**369.13940**	6.1E+05
unknown	7.28	287.03336	289.04010	4.0E+05
Alanine	**7.32**	**323.10483**	**325.11142**	4.7E+07
unknown	**7.36**	**473.10770**	**475.11472**	6.8E+06
r-aminobutyric acid	**7.51**	**337.12197**	**339.12881**	2.0E+05
unknown	**7.66**	**473.10636**	**475.11384**	2.9E+06
unknown	**7.82**	**473.11107**	**475.11770**	5.3E+05
unknown	**7.86**	**396.11143**	**398.11831**	8.3E+05
unknown	**7.97**	**322.07431**	**324.08135**	1.2E+05
Hypoxanthine	**8.20**	**370.09699**	**372.10374**	8.5E+05
unknown	**8.20**	**396.11099**	**398.11792**	1.1E+05
unknown	8.20	450.20551	452.21213	6.4E+04
5-hydroxymethyluracil	**8.29**	**376.09622**	**378.10303**	7.7E+04
unknown	8.29	395.12747	397.13448	8.6E+04
3-Aminoisobutyric acid	**8.40**	**337.12164**	**339.12881**	1.2E+05
unknown	**8.40**	**431.13852**	**433.14548**	1.9E+06
unknown	**8.44**	**279.07997**	**281.08659**	6.3E+05
unknown	**8.44**	**386.09197**	**388.09863**	1.6E+05
5-Aminopentanoic acid	**8.47**	**351.13762**	**353.14428**	3.2E+05
unknown	**8.47**	**396.13517**	**398.14190**	2.8E+05
unknown	8.51	321.12650	324.13626	1.3E+05
unknown	**8.51**	**485.12006**	**488.13046**	3.9E+05
unknown	8.55	293.13204	296.14206	1.5E+05
2-Aminobutyric acid	**8.88**	**337.12177**	**339.12845**	1.2E+07
unknown	**9.04**	**524.19598**	**526.20358**	3.0E+05
unknown	9.07	524.19638	527.20709	1.5E+06
Sarcosine	**9.11**	**323.10603**	**325.11305**	4.0E+05
unknown	9.11	351.10126	354.11124	1.5E+05
unknown	**9.11**	**370.09704**	**372.10378**	3.0E+06
unknown	**9.19**	**363.10122**	**365.10799**	7.1E+05
unknown	**9.19**	**507.07104**	**509.07850**	6.0E+05
Methylcysteine	**9.34**	**369.09407**	**371.10086**	5.2E+05
unknown	**9.37**	**492.05254**	**494.06004**	2.5E+05
unknown	**9.48**	**242.28436**	**244.29094**	2.9E+06
unknown	**9.48**	**396.11129**	**398.11821**	5.4E+05
unknown	9.56	510.07251	513.08356	2.5E+05

unknown	**9.60**	**266.10733**	**268.11384**	1.3E+06
unknown	9.60	321.12709	324.13722	2.8E+06
unknown	**9.60**	**554.18719**	**556.19495**	3.1E+05
unknown	**9.64**	**531.19806**	**533.20465**	8.2E+05
Methylamine	**9.67**	**265.10002**	**267.10625**	1.8E+07
unknown	9.67	343.10897	345.11559	1.6E+06
unknown	**9.67**	**554.18582**	**556.19260**	4.4E+06
unknown	**9.71**	**551.17658**	**553.18277**	1.6E+06
unknown	**9.75**	**529.18622**	**531.19275**	1.1E+06
unknown	**9.75**	**554.18619**	**556.19363**	1.5E+06
unknown	**9.79**	**287.08086**	**289.08772**	1.9E+07
unknown	**9.86**	**551.17600**	**553.18230**	1.2E+06
unknown	**9.86**	**554.18549**	**556.19292**	3.4E+06
unknown	9.86	572.12335	574.13025	5.5E+05
unknown	**9.90**	**265.10038**	**268.11041**	1.3E+07
Proline	**9.90**	**349.12208**	**351.12859**	1.4E+06
unknown	**9.90**	**531.20032**	**533.20769**	7.9E+05
unknown	**10.01**	**265.10764**	**268.11789**	4.7E+05
unknown	**10.16**	**266.10373**	**268.11002**	6.2E+05
unknown	10.16	335.14377	337.15048	3.9E+05
unknown	10.16	553.16376	555.17078	3.3E+05
unknown	**10.20**	**370.09711**	**372.10399**	7.3E+05
unknown	**10.23**	**311.08295**	**313.08968**	3.9E+05
unknown	**10.34**	**321.11445**	**323.12122**	2.0E+06
unknown	**10.49**	**265.10074**	**268.11090**	1.9E+06
Valine	**10.49**	**351.13656**	**353.14283**	7.6E+07
Methionine	**10.57**	**383.10980**	**385.11615**	5.6E+06
3-Hydroxypicolinic acid	**10.59**	**373.11839**	**375.12536**	6.0E+05
Methylamine	**10.61**	**265.10065**	**267.10721**	2.3E+06
unknown	10.68	335.13574	337.14307	1.6E+05
unknown	**10.72**	**400.08516**	**402.09193**	2.7E+06
unknown	**10.76**	**365.11685**	**368.12687**	5.5E+05
unknown	**10.91**	**378.06763**	**380.07459**	1.0E+06
Tryptophan	**10.95**	**438.14844**	**440.15518**	1.9E+07
unknown	**11.02**	**346.08605**	**348.09261**	2.1E+05
unknown	11.09	439.15128	441.15805	1.2E+05
unknown	**11.24**	**418.64157**	**420.64761**	7.4E+04
unknown	**11.66**	**382.58127**	**384.58805**	4.0E+05
unknown	11.73	387.61842	389.62538	1.5E+05
unknown	**11.77**	**418.13204**	**420.13891**	4.3E+05
unknown	**11.81**	**315.09059**	**317.09726**	1.1E+05
unknown	12.07	399.13776	402.14822	1.3E+05
unknown	**12.10**	**295.10272**	**297.10917**	6.6E+04
unknown	**12.10**	**388.12155**	**390.12821**	3.4E+05
unknown	12.14	364.14109	366.14775	1.1E+05
Pipecolic acid	**12.18**	**363.13772**	**365.14433**	6.9E+05
Phenylalanine	**12.22**	**399.13658**	**401.14312**	4.6E+07
unknown	**12.33**	**266.10383**	**268.11063**	3.9E+05
unknown	**12.37**	**406.61714**	**408.62350**	1.5E+05
unknown	**12.37**	**470.08429**	**474.09808**	8.8E+04
unknown	**12.44**	**313.60897**	**315.61575**	2.1E+06
3-Hydroxymandelic acid	**12.48**	**402.10093**	**404.10768**	3.4E+05
Isoleucine	**12.55**	**365.15103**	**367.15784**	3.7E+07
unknown	**12.59**	**313.60905**	**315.61575**	2.3E+06

unknown	12.67	320.06668	322.07339	7.2E+04
unknown	12.67	332.10668	334.11324	2.4E+05
unknown	12.67	489.07022	491.07715	6.7E+04
unknown	**12.67**	**501.11530**	**505.12864**	1.2E+06
unknown	**12.70**	**379.13201**	**381.13918**	4.3E+05
Leucine	**12.81**	**365.15108**	**367.15779**	8.1E+07
L-norleucine	**12.89**	**365.15326**	**367.15977**	1.1E+07
unknown	**13.04**	**265.09460**	**267.10150**	2.1E+05
unknown	**13.15**	**317.13202**	**319.13885**	9.0E+05
unknown	**13.15**	**335.14232**	**337.14881**	3.4E+07
Cystine	**13.15**	**354.06986**	**356.07653**	1.2E+06
unknown	13.23	357.12475	359.13131	3.2E+05
unknown	**13.38**	**376.11473**	**378.12147**	1.4E+05
unknown	**13.49**	**378.10097**	**380.10752**	6.8E+06
unknown	**13.49**	**409.14312**	**411.15001**	2.4E+05
unknown	13.49	549.18830	551.19526	2.5E+05
Hydroxyphenyllactic acid	**13.63**	**416.11628**	**418.12328**	2.5E+06
unknown	**13.71**	**277.10072**	**279.10733**	1.2E+05
unknown	**13.74**	**307.09303**	**309.09973**	3.6E+06
unknown	13.78	437.19365	439.19978	3.7E+06
unknown	13.93	306.09036	309.09991	1.1E+05
unknown	**13.93**	**402.10068**	**404.10761**	4.5E+05
Homocystine	**13.97**	**368.09889**	**370.10576**	1.2E+05
unknown	**14.01**	**322.07454**	**324.08130**	1.3E+05
unknown	14.08	378.10087	380.10746	7.3E+05
unknown	**14.13**	**342.62983**	**344.63667**	1.2E+05
5-HIAA	**14.20**	**425.11688**	**427.12363**	4.7E+05
unknown	**14.20**	**511.13798**	**513.14454**	1.0E+05
unknown	14.24	520.24640	522.25348	6.4E+04
unknown	14.27	346.06563	348.07254	7.6E+04
unknown	14.31	349.15826	351.16496	1.3E+06
unknown	14.35	301.09833	305.11175	1.0E+06
unknown	14.39	561.23928	565.25287	2.5E+06
Dimethylamine	**14.43**	**279.11584**	**281.12256**	2.7E+07
unknown	**14.43**	**579.20785**	**583.22181**	1.3E+06
unknown	**14.43**	**584.22443**	**587.23433**	1.7E+06
Phenylpropanolamine	**14.46**	**385.12213**	**387.12878**	3.3E+05
unknown	14.54	281.12283	283.12956	1.4E+06
unknown	14.57	279.10565	285.12533	1.3E+05
unknown	**14.57**	**371.14036**	**373.14703**	2.9E+05
unknown	**14.61**	**345.09563**	**347.10203**	8.3E+04
unknown	**14.61**	**349.15826**	**352.16860**	1.6E+06
unknown	**14.64**	**367.16895**	**369.17567**	1.6E+05
unknown	**14.68**	**387.11382**	**389.12068**	6.3E+04
2,4-Diaminobutyric acid	**14.75**	**293.13191**	**295.13866**	7.1E+04
unknown	**14.83**	**367.16919**	**369.17542**	1.3E+05
unknown	**14.83**	**513.15313**	**515.16037**	1.6E+05
unknown	**14.83**	**520.24601**	**522.25320**	8.3E+04
unknown	**14.87**	**437.19364**	**439.19965**	3.6E+06
unknown	14.90	531.08301	535.09656	2.2E+05
unknown	14.94	350.16195	352.16861	3.6E+05
unknown	**15.10**	**415.21178**	**417.21817**	3.7E+07
unknown	**15.10**	**446.25389**	**448.26038**	1.4E+07
unknown	15.10	586.29955	588.30646	3.5E+06

unknown	**15.21**	**349.15824**	**352.16868**	9.9E+05
unknown	**15.21**	**393.14847**	**395.15506**	8.9E+04
unknown	**15.36**	**360.63579**	**362.64233**	4.3E+05
L-ornithine	**15.40**	**300.10353**	**302.11020**	1.0E+07
unknown	**15.40**	**313.62742**	**315.63403**	1.8E+05
unknown	**15.47**	**415.21175**	**417.21786**	3.7E+06
unknown	**15.47**	**425.61249**	**427.61944**	2.2E+05
unknown	**15.50**	**300.06519**	**302.07177**	5.2E+05
Homovanillic	**15.62**	**416.11633**	**418.12326**	3.6E+06
unknown	15.65	371.10636	373.11317	3.6E+05
unknown	**15.65**	**379.16861**	**381.17553**	1.3E+05
unknown	15.69	266.08459	268.09130	2.0E+06
unknown	15.69	388.13275	390.13962	3.2E+05
unknown	15.76	468.14097	471.15141	4.0E+05
unknown	**15.88**	**474.18069**	**476.18777**	1.0E+05
Homocarnosine	**15.99**	**354.11938**	**356.12593**	2.4E+07
unknown	**15.99**	**474.18113**	**476.18818**	1.9E+06
3-/4-hydroxyphenylacetic acid	**16.03**	**386.10591**	**388.11276**	1.2E+06
or 3-Cresotinic acid				
unknown	**16.07**	**345.57379**	**347.58030**	5.6E+05
unknown	**16.25**	**378.18468**	**380.19153**	2.4E+05
unknown	16.29	350.12983	352.13660	7.4E+05
unknown	**16.40**	**423.10161**	**425.10830**	8.7E+05
Lysine	**16.48**	**307.11098**	**309.11759**	1.9E+07
unknown	**16.59**	**415.21167**	**417.21797**	4.9E+06
unknown	**16.59**	**430.13116**	**432.13864**	8.8E+04
unknown	**16.59**	**498.37094**	**500.37689**	1.8E+06
unknown	**16.67**	**327.64291**	**329.64957**	2.2E+05
unknown	16.67	348.63005	350.63673	1.2E+05
4-Hydroxybenzoic acid	**16.67**	**372.09026**	**374.09706**	6.1E+06
unknown	16.67	376.09220	378.09964	2.7E+05
unknown	**16.67**	**407.16373**	**409.17072**	1.6E+05
unknown	16.67	437.19372	439.19987	4.1E+06
unknown	**16.67**	**548.11847**	**552.13116**	1.0E+05
unknown	**16.74**	**305.13199**	**307.13854**	3.3E+05
unknown	**16.82**	**415.21167**	**417.21792**	6.0E+06
Histidine	**16.93**	**311.59333**	**313.60000**	1.1E+07
unknown	17.00	382.10898	384.11546	6.5E+04
Desaminotyrosine	17.04	400.12057	402.12704	3.0E+04
unknown	**17.04**	**622.17834**	**626.19183**	9.6E+04
unknown	**17.23**	**407.16321**	**409.17029**	1.1E+05
unknown	**17.38**	**347.08819**	**349.09475**	1.1E+05
unknown	**17.38**	**359.62784**	**361.63427**	9.0E+04
unknown	**17.42**	**407.16376**	**409.17039**	3.3E+05
unknown	**17.53**	**297.08577**	**299.09228**	4.5E+05
unknown	**17.53**	**324.10551**	**326.11207**	1.9E+05
unknown	17.53	359.10503	361.11124	1.1E+05
unknown	**17.64**	**332.10645**	**334.11323**	1.4E+05
unknown	**17.64**	**509.09266**	**511.09958**	1.4E+05
unknown	17.80	499.37412	501.38116	4.2E+05
unknown	**17.95**	**419.47878**	**421.48543**	1.7E+05
unknown	**17.98**	**321.12283**	**323.12906**	3.2E+06
unknown	**18.02**	**370.11103**	**372.11782**	1.3E+05
2-aminooctanoic acid	**18.02**	**393.18440**	**395.19113**	2.5E+05

unknown	**18.13**	**356.09342**	**358.10002**	5.0E+05
unknown	18.13	395.10622	397.11298	1.2E+06
unknown	**18.13**	**455.16354**	**457.17065**	7.9E+04
unknown	**18.17**	**314.06143**	**316.06799**	3.8E+06
unknown	**18.37**	**370.11106**	**372.11784**	3.5E+05
unknown	**18.40**	**307.14764**	**309.15431**	7.9E+05
unknown	**18.48**	**354.06339**	**356.07005**	2.1E+06
unknown	**18.62**	**354.06340**	**356.07010**	8.2E+05
unknown	18.74	300.06494	302.07151	6.9E+05
unknown	**18.85**	**421.17945**	**423.18648**	2.5E+05
unknown	**19.01**	**440.20040**	**442.20718**	4.0E+05
unknown	**19.08**	**379.12749**	**381.13430**	7.1E+05
unknown	**19.15**	**314.11898**	**316.12561**	2.1E+07
unknown	19.19	326.10955	328.11642	1.8E+05
unknown	**19.30**	**317.59328**	**319.59998**	8.0E+05
unknown	**19.34**	**333.16336**	**335.16984**	1.2E+05
unknown	**19.34**	**401.12807**	**403.13481**	8.2E+05
unknown	19.34	436.16891	438.17590	9.4E+04
unknown	**19.41**	**390.10367**	**392.11080**	1.3E+05
unknown	**19.41**	**530.18862**	**532.19457**	1.6E+05
unknown	**19.53**	**546.10448**	**548.11079**	3.5E+05
unknown	19.57	420.31861	422.32587	7.0E+04
1,4-diaminobutane	**19.57**	**555.20947**	**559.22298**	7.0E+04
unknown	**19.64**	**294.11620**	**296.12268**	4.7E+05
unknown	**19.71**	**264.58494**	**266.59143**	3.0E+05
unknown	**19.75**	**335.46249**	**337.46924**	8.2E+04
unknown	**19.82**	**316.09301**	**318.09952**	2.5E+05
unknown	**19.82**	**356.09540**	**358.10185**	1.5E+07
unknown	**19.86**	**335.12476**	**337.13127**	2.5E+05
unknown	**19.86**	**339.60077**	**341.60754**	2.6E+05
unknown	19.86	379.12755	381.13436	3.8E+05
unknown	19.86	404.07193	406.07938	4.2E+05
unknown	**19.86**	**564.15002**	**566.15580**	8.9E+04
unknown	**19.90**	**331.11125**	**333.11803**	6.0E+05
unknown	**19.90**	**520.10388**	**522.11070**	9.0E+04
unknown	**20.01**	**292.10599**	**294.11264**	1.5E+06
unknown	**20.09**	**486.11448**	**488.12115**	3.6E+05
unknown	**20.17**	**346.60864**	**348.61505**	1.1E+05
unknown	20.20	375.11087	377.11713	2.8E+05
Tyrosine	**20.24**	**324.59406**	**326.60071**	8.2E+07
unknown	20.32	577.13484	579.14154	1.9E+06
Cysteamine	**20.35**	**310.07501**	**312.08184**	1.1E+05
unknown	**20.39**	**297.08573**	**299.09239**	1.2E+06
Metoprolol	**20.39**	**501.16180**	**503.16876**	5.9E+05
unknown	**20.54**	**315.08491**	**317.09178**	6.4E+05
unknown	20.61	379.11145	382.12143	2.7E+05
unknown	20.65	265.10327	267.11029	1.1E+05
Phenol	**20.73**	**328.10056**	**330.10719**	8.8E+05
unknown	**21.06**	**363.17387**	**365.18073**	1.3E+06
unknown	**21.10**	**639.40856**	**641.41492**	1.7E+06
unknown	21.17	537.15857	539.16528	3.0E+05
unknown	21.17	550.62067	552.62769	2.4E+06
unknown	**21.21**	**595.38186**	**597.38818**	4.6E+06
unknown	**21.29**	**344.10671**	**346.11342**	1.3E+06

unknown	**21.29**	**448.35129**	**450.35817**	1.0E+05
unknown	**21.29**	**454.15477**	**456.16167**	4.7E+05
unknown	**21.33**	**335.17908**	**337.18583**	1.4E+05
unknown	**21.33**	**397.20131**	**399.20747**	5.6E+06
unknown	**21.37**	**546.40058**	**548.40674**	1.5E+06
unknown	**21.37**	**551.35587**	**553.36217**	1.0E+07
unknown	**21.37**	**560.41589**	**562.42152**	1.9E+06
unknown	21.40	345.60077	347.60751	1.8E+05
unknown	**21.40**	**448.35067**	**450.35764**	1.0E+05
unknown	**21.44**	**419.27719**	**421.28342**	1.7E+06
unknown	21.44	427.37847	431.39236	8.9E+04
unknown	**21.52**	**507.32977**	**509.33608**	8.9E+06
unknown	21.55	257.57718	259.58365	1.6E+05
unknown	**21.59**	**463.30356**	**465.30993**	1.5E+07
unknown	**21.59**	**472.36388**	**474.36981**	3.2E+06
unknown	21.63	360.58476	362.59106	6.9E+06
unknown	21.63	520.10498	522.11136	1.8E+05
unknown	**21.67**	**354.11632**	**356.12302**	3.8E+06
unknown	**21.67**	**419.27743**	**421.28376**	2.1E+07
unknown	**21.74**	**375.25111**	**377.25745**	1.1E+07
unknown	**21.78**	**419.27727**	**421.28342**	4.2E+06
unknown	**21.78**	**448.35085**	**450.35690**	7.2E+04
unknown	**21.78**	**499.22659**	**501.23377**	9.5E+04
unknown	21.85	521.13728	524.14757	2.5E+05
unknown	**21.89**	**498.15248**	**502.16584**	1.1E+06
unknown	**21.89**	**529.19465**	**533.20736**	1.6E+05
unknown	**22.11**	**448.35087**	**450.35735**	8.1E+04
unknown	**22.15**	**284.10862**	**286.11530**	1.2E+05
unknown	**22.15**	**499.22639**	**501.23390**	8.1E+04
unknown	**22.19**	**287.07937**	**289.08614**	8.0E+05
unknown	**22.19**	**352.08987**	**354.09640**	1.1E+05
unknown	**22.30**	**450.20672**	**452.21365**	6.0E+05
unknown	**22.30**	**499.22663**	**501.23401**	6.2E+04
unknown	**22.38**	**315.59030**	**317.59718**	3.5E+05
unknown	22.38	460.29972	462.30630	1.0E+05
unknown	**22.38**	**499.22585**	**501.23344**	7.2E+04
unknown	**22.56**	**388.07735**	**390.08433**	6.6E+06
unknown	**22.60**	**344.10676**	**346.11351**	3.3E+05
unknown	**22.67**	**313.12947**	**315.13626**	1.2E+05
unknown	**22.67**	**404.13198**	**406.13863**	8.8E+04
unknown	**22.67**	**541.19391**	**545.20797**	3.1E+05
unknown	**22.67**	**572.23645**	**576.24925**	1.0E+05
Spermidine	**22.82**	**423.16360**	**426.17365**	1.6E+05
unknown	22.86	282.44467	284.45139	1.3E+05
Pyrocatechol	**22.90**	**289.08262**	**291.08928**	2.0E+05
unknown	22.90	420.31943	422.32694	1.6E+05
unknown	22.93	348.11625	350.12277	1.7E+06
unknown	22.93	418.11855	421.12870	2.6E+05
unknown	**22.93**	**447.34744**	**450.35836**	3.0E+05
unknown	22.97	489.31418	491.32178	1.2E+05
unknown	**23.01**	**315.59024**	**317.59712**	3.5E+05
unknown	23.05	351.27026	355.28348	1.4E+05
unknown	23.13	440.22503	442.23252	7.9E+04
unknown	**23.13**	**454.24174**	**457.25165**	1.2E+05

unknown	23.16	440.22559	443.23607	1.2E+05
unknown	23.16	648.26221	650.26947	7.3E+04
unknown	**23.35**	**421.15825**	**425.17185**	1.6E+05
unknown	**23.42**	**447.34767**	**450.35793**	3.2E+05
unknown	23.42	522.59781	524.60497	1.9E+06
unknown	23.46	466.31948	469.32956	4.5E+05
unknown	**23.46**	**550.62873**	**552.63563**	8.3E+06
unknown	23.50	364.21416	366.22054	4.9E+05
unknown	23.50	385.19266	387.19958	1.2E+05
unknown	**23.61**	**281.40823**	**283.41504**	6.6E+04
unknown	**23.61**	**323.60615**	**325.61261**	3.3E+05
unknown	**23.65**	**305.06801**	**307.07479**	4.0E+06
unknown	**23.69**	**542.12213**	**548.14277**	1.9E+06
unknown	23.69	550.62911	553.63934	9.4E+06
unknown	23.80	284.29510	286.30185	4.6E+05
unknown	**23.80**	**419.31638**	**422.32701**	5.5E+05
unknown	**23.80**	**447.34768**	**450.35848**	2.8E+05
Thymol	**23.84**	**384.16345**	**386.17007**	4.7E+05
unknown	**23.95**	**550.62806**	**552.63518**	9.3E+06
unknown	23.95	670.24512	672.25299	1.4E+05
unknown	**24.03**	**288.07624**	**290.08286**	3.4E+05
unknown	24.10	522.59778	524.60445	2.0E+06
unknown	24.14	440.22601	442.23286	2.8E+05
unknown	24.14	685.43634	687.44366	8.5E+05
unknown	24.18	522.59802	524.60498	1.9E+06
unknown	24.18	551.63165	553.63902	4.1E+06
unknown	24.22	488.33175	490.33808	1.0E+05
unknown	24.33	562.31319	564.31996	8.4E+06
unknown	24.40	298.31048	300.31702	4.1E+07
unknown	24.44	625.14533	629.15872	1.6E+06

CSF - #3 Repeat		CSF - #3 Repeat		
Compound Name	Rt	mz_light	mz_heavy	int
unknown	1.63	274.05119	276.05762	7.7E+06
unknown	1.68	527.12063	529.12733	3.0E+06
unknown	1.68	548.09827	550.10554	2.4E+05
unknown	1.71	353.99182	355.99867	2.2E+05
unknown	1.71	537.18384	539.19159	2.3E+05
unknown	1.71	569.07734	571.08467	5.1E+05
unknown	1.71	572.08757	574.09419	5.1E+05
unknown	1.71	589.20544	591.21212	1.8E+05
unknown	1.71	631.04785	633.05377	1.6E+05
unknown	1.71	637.06476	639.07236	2.0E+05
unknown	1.75	364.02077	367.03099	1.6E+06
unknown	1.75	423.98733	425.99392	3.9E+05
unknown	1.75	432.00842	434.01527	1.5E+06
unknown	1.75	499.99607	502.00283	7.6E+05
unknown	1.79	331.07285	333.07962	1.8E+05
unknown	1.79	489.96759	491.97485	2.3E+05
unknown	1.79	561.96392	567.98382	1.6E+05
unknown	1.79	625.94295	627.94889	1.6E+05
unknown	1.79	629.95038	635.97094	1.5E+05
unknown	1.83	404.01422	406.02030	1.9E+05
unknown	1.87	252.06912	254.07572	4.2E+06
unknown	1.99	524.08139	526.08858	2.7E+05
unknown	1.99	555.12256	557.12998	2.8E+05
unknown	1.99	585.98432	588.99363	2.5E+05
unknown	2.06	252.07140	254.07832	5.0E+05

unknown	2.10	546.06220	548.06969	4.4E+05
unknown	2.14	558.03273	562.04540	5.5E+05
unknown	2.22	414.12225	416.12909	5.8E+05
unknown	2.22	509.17157	511.17773	1.3E+05
unknown	2.25	376.08162	378.08863	7.1E+04
unknown	2.25	389.12833	391.13498	2.5E+06
phosphoethaolamine	2.29	375.07786	377.08440	4.3E+05
unknown	2.29	336.02064	338.02765	6.3E+04
unknown	2.29	501.11566	505.12854	9.0E+04
unknown	2.29	536.17980	538.18674	1.2E+05
unknown	2.33	509.17073	511.17736	1.0E+06
unknown	2.41	376.08156	378.08775	6.5E+04
unknown	2.49	274.05095	276.05756	1.5E+06
unknown	2.49	376.10789	378.11455	1.1E+05
unknown	2.53	364.02029	366.02720	1.3E+05
3-methylhistidine	2.57	403.14367	405.15048	2.4E+05
unknown	2.57	468.14310	470.15060	6.3E+04
unknown	2.64	276.08016	278.08699	1.6E+06
Taurine	2.64	359.07333	361.08006	3.9E+06
unknown	2.64	410.08948	412.09660	1.1E+05
unknown	2.68	252.06906	254.07574	1.2E+06
unknown	2.68	375.07801	377.08453	1.8E+05
unknown	2.68	388.10783	390.11447	2.1E+06
unknown	2.68	501.15506	503.16110	7.2E+04
unknown	2.72	364.02003	366.02643	7.8E+04
1-methylhistidine	2.72	403.14384	405.15041	6.0E+05
unknown	2.90	366.14853	368.15526	2.7E+05
unknown	2.98	345.13835	347.14499	1.2E+05
unknown	2.98	376.17975	378.18711	2.9E+05
unknown	3.07	275.15037	277.15731	2.0E+05
unknown	3.07	414.12185	416.12870	1.8E+06
unknown	3.14	482.15906	484.16534	6.4E+04
Arginine	3.18	408.17029	410.17700	7.8E+06
unknown	3.37	319.11129	321.11806	3.5E+05
unknown	3.52	367.11559	369.12223	2.0E+06
unknown	3.52	408.19526	410.20202	2.1E+05
unknown	3.57	336.07794	338.08452	1.1E+05
unknown	3.57	380.16405	382.17079	1.0E+06
Homoarginine	3.57	422.21093	424.21777	3.5E+05
Asparagine	3.60	366.11206	368.11877	4.2E+06
unknown	3.64	365.12813	367.13455	9.3E+05
unknown	3.79	363.10122	365.10803	5.8E+05
unknown	3.79	381.13124	383.13771	7.3E+06
Glutamine	3.87	380.12566	382.13211	1.2E+08
unknown	3.87	411.16835	413.17516	1.9E+06
unknown	3.99	378.13998	384.16012	6.9E+06
unknown	4.10	363.10114	365.10794	3.4E+05
unknown	4.14	402.10976	404.11641	2.1E+05
L-citrulline	4.18	409.15438	411.16082	9.4E+05
unknown	4.18	436.20126	438.20825	1.1E+05
unknown	4.25	270.03512	272.04187	4.4E+05
unknown	4.25	302.02496	304.03164	6.1E+05
unknown	4.29	253.56285	255.56941	4.4E+05
unknown	4.29	291.06372	293.07043	5.6E+05
unknown	4.29	377.08170	379.08841	3.7E+05
unknown	4.29	483.12796	485.13528	4.5E+05
unknown	4.33	242.57191	244.57870	2.7E+06
unknown	4.33	507.11786	510.12732	7.4E+05
unknown	4.33	515.17493	519.18854	9.6E+05
Glutamic acid	4.37	381.11216	383.11879	2.6E+06
unknown	4.41	291.06374	293.07048	3.7E+05

Aspartic acid	4.41	367.09654	369.10293	8.1E+05
3-sn-Phosphatidylethanolamine	4.41	484.13607	488.14960	1.3E+07
Homoserine	4.63	353.11675	355.12366	1.2E+05
Methionine sulfoxide	4.63	399.10455	401.11124	1.1E+06
unknown	4.63	466.18360	468.19083	2.1E+05
unknown	4.67	465.18054	467.18752	8.9E+05
unknown	4.74	394.14325	396.14996	1.8E+05
Homocitruline	4.82	423.05421	425.06105	1.0E+05
Serine	4.85	339.10074	341.10736	3.5E+07
unknown	4.97	263.21189	265.21866	1.1E+06
unknown	5.00	340.10205	342.10880	1.2E+05
Aminoadipic acid	5.04	395.12749	397.13418	1.6E+05
unknown	5.16	394.17971	396.18638	4.9E+05
4-Hydroxy-proline	5.19	365.11686	367.12334	2.5E+06
unknown	5.23	404.12742	406.13428	8.1E+04
unknown	5.31	455.11002	458.11971	2.6E+05
unknown	5.35	466.16459	468.17151	1.2E+06
unknown	5.35	582.19073	584.19763	1.3E+05
unknown	5.35	600.20178	602.20826	5.4E+05
unknown	5.39	442.11687	444.12395	3.1E+05
unknown	5.43	466.16512	468.17120	1.3E+05
Diethanolamine	5.43	339.10069	341.10778	1.0E+05
unknown	5.50	422.17514	424.18146	1.2E+05
unknown	5.54	336.13792	338.14467	1.7E+06
Iminodiacetic acid	5.54	367.18011	369.18674	1.5E+05
unknown	5.61	354.12067	356.12735	9.7E+05
unknown	5.61	457.08936	459.09602	2.6E+05
unknown	5.61	505.07678	509.08926	7.5E+04
Folic acid	5.65	338.09370	339.09602	1.0E+05
unknown	5.65	363.14840	365.15512	1.5E+06
Threonine	5.69	353.11569	355.12203	4.1E+07
unknown	5.69	389.12748	391.13416	7.7E+05
unknown	5.72	462.17004	464.17694	4.5E+05
unknown	5.80	337.15823	339.16492	4.6E+06
unknown	5.88	379.13248	381.13925	5.2E+05
unknown	5.99	478.12823	480.13521	1.6E+06
unknown	5.99	524.19657	526.20366	6.8E+05
Ethanolamine	6.03	295.11089	297.11748	2.9E+07
unknown	6.07	463.23798	465.24481	3.8E+05
unknown	6.07	547.19641	549.20367	3.5E+05
unknown	6.07	567.17133	569.17804	3.1E+05
unknown	6.11	251.08474	253.09130	2.2E+07
unknown	6.11	523.14502	525.15196	2.5E+06
unknown	6.18	395.12760	397.13406	2.1E+05
unknown	6.18	396.11163	398.11835	2.0E+05
unknown	6.18	501.16327	504.17279	3.0E+05
unknown	6.22	317.13202	319.13886	1.1E+06
unknown	6.22	348.17443	350.18106	3.8E+05
unknown	6.22	415.13261	417.13952	2.8E+06
unknown	6.29	464.14850	466.15561	1.5E+05
unknown	6.37	398.12723	400.13393	1.3E+06
unknown	6.40	348.10149	350.10815	1.3E+06
Glycine	6.48	309.09055	311.09713	8.9E+06
unknown	6.48	363.13760	365.14425	1.6E+05
unknown	6.48	381.14817	383.15488	2.1E+06
unknown	6.55	292.11167	294.11859	2.5E+05
unknown	6.70	344.10665	346.11327	8.3E+05
unknown	6.70	364.16916	366.17579	8.6E+05
unknown	6.74	477.16165	479.16857	6.4E+05
unknown	6.78	348.13783	350.14454	5.1E+05
unknown	6.92	396.11136	398.11807	1.6E+05

unknown	7.04	362.11712	364.12346	3.0E+07
unknown	7.15	347.11776	349.12435	5.6E+05
Tyrosine methyl ester	7.19	415.13280	417.13962	2.5E+06
unknown	7.22	344.10662	346.11347	7.5E+05
unknown	7.22	367.13272	369.13931	5.0E+05
Aalanine	7.30	323.10474	325.11115	3.4E+07
r-aminobutyric acid	7.49	337.12196	339.12889	2.5E+05
unknown	7.65	473.10615	475.11377	2.8E+06
unknown	7.76	493.08496	496.09542	5.5E+05
unknown	7.80	473.11065	475.11770	7.4E+05
unknown	7.87	396.11129	398.11835	6.3E+05
unknown	7.94	322.07448	324.08133	1.3E+05
Tryptophanamide	8.13	437.13788	439.14465	4.3E+04
unknown	8.17	306.12724	310.14079	6.6E+04
Hypoxanthine	8.17	370.09708	372.10385	8.0E+05
unknown	8.21	396.11095	398.11799	1.8E+05
5-hydroxymethyluracil	8.28	376.09634	378.10304	9.2E+04
3-Aminoisobutyric acid	8.36	337.12161	339.12876	5.0E+04
unknown	8.40	431.13851	433.14552	2.6E+06
unknown	8.43	279.08001	281.08661	4.8E+05
unknown	8.43	359.11772	361.12376	9.4E+04
unknown	8.47	386.09196	388.09870	2.6E+05
unknown	8.51	396.13537	398.14162	3.4E+05
unknown	8.51	485.12033	488.13055	4.5E+05
5-Aminopentanoic acid	8.51	351.13746	353.14420	3.0E+05
unknown	8.58	293.13225	295.13867	9.6E+04
2-Aminobutyric acid	8.88	337.12178	339.12848	1.1E+07
unknown	8.92	321.12680	323.13397	1.7E+05
unknown	9.03	321.09071	323.09768	2.2E+05
unknown	9.07	370.09705	373.10770	9.1E+05
Sarcosine	9.11	323.10606	325.11297	3.0E+05
unknown	9.11	524.19651	526.20385	3.1E+06
unknown	9.14	370.09717	372.10388	3.6E+06
unknown	9.18	363.10123	365.10802	7.0E+05
unknown	9.18	507.07128	509.07845	6.4E+05
Methylcysteine	9.34	369.09416	371.10091	6.1E+05
unknown	9.34	492.05271	494.06009	2.8E+05
unknown	9.49	242.28430	244.29094	5.0E+06
unknown	9.49	396.11137	398.11822	5.2E+05
unknown	9.60	266.10730	268.11353	3.0E+06
unknown	9.60	554.18890	556.19586	2.2E+05
unknown	9.63	321.12703	323.13373	2.3E+06
unknown	9.67	265.10034	268.10991	2.0E+07
unknown	9.67	551.17639	553.18262	2.3E+06
unknown	9.67	554.18570	556.19238	4.9E+06
unknown	9.79	287.08093	289.08767	2.2E+07
unknown	9.83	529.18620	531.19281	1.5E+06
unknown	9.83	550.17482	552.18100	3.2E+06
unknown	9.86	265.09976	268.10971	2.5E+07
unknown	9.94	287.08213	290.09180	6.8E+06
unknown	9.94	551.17618	553.18224	2.6E+06
unknown	9.94	554.18534	556.19159	5.2E+06
Proline	9.94	349.12209	351.12872	3.0E+05
Methylamine	9.98	265.10037	267.10669	1.8E+07
unknown	10.17	266.10387	268.11060	1.9E+06
unknown	10.17	370.09720	372.10391	1.0E+06
unknown	10.24	311.08324	313.08975	4.9E+05
unknown	10.28	531.18750	533.19501	2.5E+05
unknown	10.36	321.11446	323.12125	2.4E+06
unknown	10.51	352.14050	354.14741	9.5E+06
3-Hydroxypicolinic acid	10.51	373.11907	375.12565	3.6E+05

Valine	10.55	351.13723	353.14428	3.8E+07
unknown	10.55	384.11234	386.11887	1.5E+06
Methionine	10.58	383.10968	385.11644	4.1E+06
unknown	10.74	365.11695	368.12695	6.5E+05
unknown	10.74	400.08524	402.09197	2.8E+06
unknown	10.92	378.06770	380.07464	9.7E+05
Tryptophan	10.96	438.14855	440.15501	1.9E+07
Norvaline	11.00	351.13776	353.14481	1.9E+05
unknown	11.03	346.08594	348.09263	1.9E+05
unknown	11.26	351.13861	353.14484	1.2E+05
unknown	11.26	418.64111	420.64749	1.0E+05
unknown	11.64	382.58145	384.58808	8.6E+05
unknown	11.76	418.13215	420.13891	3.4E+05
unknown	11.79	315.09061	317.09728	8.6E+04
unknown	11.91	524.19607	526.20306	2.3E+05
unknown	12.06	388.12163	390.12830	2.9E+05
unknown	12.10	295.10259	297.10928	7.7E+04
Pipecolic acid	12.14	363.13775	365.14432	8.8E+05
unknown	12.14	400.14114	402.14748	2.7E+06
Phenylalanine	12.21	399.13653	401.14279	4.1E+07
unknown	12.29	266.10393	268.11069	5.2E+05
unknown	12.33	470.08394	474.09757	1.6E+05
unknown	12.36	395.12252	397.12914	1.4E+05
unknown	12.36	406.61702	408.62396	2.6E+05
unknown	12.39	335.62231	337.62918	8.0E+04
3-Hydroxymandelic acid	12.47	402.10095	404.10769	4.8E+05
Isoleucine	12.52	365.15263	367.15925	2.9E+07
unknown	12.56	313.60904	315.61573	2.9E+06
unknown	12.59	393.15899	395.16577	1.8E+05
unknown	12.63	251.06087	253.06786	1.0E+05
unknown	12.67	265.11139	267.11823	1.2E+05
unknown	12.67	335.14413	337.15030	7.4E+04
unknown	12.67	399.14014	401.14694	6.2E+04
unknown	12.71	379.13214	381.13895	6.8E+05
unknown	12.74	501.11532	505.12863	9.9E+05
Leucine	12.78	365.15155	367.15815	8.3E+07
L-norleucine	12.90	365.15305	367.15967	4.0E+06
unknown	13.01	300.06534	302.07194	4.8E+05
unknown	13.09	335.14244	337.14906	1.9E+07
unknown	13.13	317.13182	319.13874	7.8E+05
unknown	13.13	335.14110	337.14792	4.2E+07
Cystine	13.13	354.06967	356.07636	1.6E+06
unknown	13.17	265.10078	268.11096	7.5E+05
unknown	13.21	335.14208	337.14924	1.8E+07
unknown	13.36	317.13211	319.13846	1.0E+05
unknown	13.47	265.10072	268.11096	8.4E+05
unknown	13.47	378.10088	380.10741	6.8E+06
unknown	13.47	409.14307	411.14998	3.7E+05
unknown	13.54	425.11668	427.12411	1.4E+05
Hydroxyphenyllactic acid	13.61	416.11633	418.12328	3.5E+06
unknown	13.73	277.10074	279.10738	1.6E+05
unknown	13.73	297.08584	299.09253	2.4E+05
unknown	13.73	474.06793	477.07839	2.3E+05
unknown	13.76	307.09307	309.09973	2.6E+06
unknown	13.80	501.13370	503.14115	2.4E+05
unknown	13.91	403.10464	405.11080	1.3E+05
unknown	13.94	402.10082	404.10739	4.6E+05
unknown	13.98	322.07473	324.08126	1.4E+05
Homocystine	13.98	368.09891	370.10574	1.6E+05
unknown	14.02	371.14066	374.15137	1.6E+05
unknown	14.13	342.62988	344.63686	1.3E+05

unknown	14.17	350.63416	352.64072	1.0E+05
unknown	14.17	511.13762	513.14452	1.3E+05
unknown	14.21	349.63786	351.64451	1.2E+06
5-HIAA	14.21	425.11702	427.12374	2.9E+05
unknown	14.25	336.11404	338.12093	2.2E+05
unknown	14.25	487.11090	490.12120	1.1E+07
unknown	14.32	349.15837	352.16871	1.4E+06
unknown	14.36	557.22589	561.23930	5.0E+05
Dimethylamine	14.43	279.11583	281.12267	3.1E+07
unknown	14.43	579.20768	583.22156	1.8E+06
unknown	14.43	584.22406	587.23395	2.0E+06
unknown	14.47	487.11141	490.12157	9.4E+06
Phenylpropanolamine	14.47	385.12224	387.12862	3.6E+05
unknown	14.54	279.11626	283.12939	9.1E+06
unknown	14.54	371.14053	373.14699	4.2E+05
unknown	14.58	349.15834	352.16867	1.8E+06
unknown	14.58	367.16895	369.17538	1.5E+05
unknown	14.65	345.09565	347.10242	1.2E+05
unknown	14.74	520.24658	522.25336	1.1E+05
2,4-Diaminobutyric acid	14.78	293.13187	295.13879	9.9E+04
unknown	14.85	513.15339	515.16052	2.7E+05
unknown	14.89	337.23523	339.24130	3.0E+06
unknown	14.89	437.19368	439.19992	6.3E+06
unknown	14.93	349.15829	352.16864	2.0E+06
unknown	14.97	367.16919	369.17564	1.1E+05
unknown	15.00	381.26147	383.26801	2.3E+06
unknown	15.00	390.32173	392.32819	1.4E+06
unknown	15.04	265.10388	267.11050	4.4E+05
unknown	15.04	349.15411	351.16074	1.1E+06
unknown	15.04	498.36435	500.37140	1.7E+06
unknown	15.11	415.21179	417.21818	3.5E+07
unknown	15.11	446.25388	448.26023	1.5E+07
unknown	15.19	349.15826	352.16887	1.2E+06
unknown	15.19	393.14861	395.15463	1.3E+05
unknown	15.31	381.26143	383.26774	2.8E+06
unknown	15.31	390.32170	392.32786	1.8E+06
unknown	15.38	360.63573	362.64228	5.1E+05
unknown	15.38	599.19924	603.21343	2.4E+05
L-ornithine	15.42	300.10343	302.11008	1.2E+07
unknown	15.42	313.62719	315.63395	2.5E+05
unknown	15.49	415.21177	417.21795	5.5E+06
unknown	15.49	425.61261	427.61947	3.0E+05
unknown	15.53	300.06526	303.07534	6.8E+05
unknown	15.53	549.13062	551.13733	2.2E+05
unknown	15.57	265.10318	267.11002	4.9E+05
unknown	15.64	285.03649	288.04651	1.7E+05
Homovanillic	15.64	416.11644	418.12336	4.0E+06
unknown	15.68	379.16876	381.17518	1.4E+05
unknown	15.72	399.13773	403.15094	4.2E+05
unknown	15.72	469.14545	471.15137	1.1E+05
unknown	15.76	468.14105	472.15482	7.0E+05
unknown	15.91	355.12457	357.13122	3.8E+05
unknown	15.95	415.21176	417.21805	6.3E+06
Homocarnosine	15.98	354.11868	356.12521	2.7E+07
unknown	15.98	319.11148	321.11838	3.8E+05
unknown	15.98	474.18127	476.18838	2.5E+06
3-/4-hydroxyphenylacetic acid	16.02	386.10569	388.11261	1.1E+06
or 3-Cresotinic acid				
unknown	16.06	345.57369	347.58021	4.3E+05
unknown	16.13	354.11954	356.12624	1.1E+06
unknown	16.17	345.57385	347.58036	3.4E+05

unknown	16.28	378.18485	380.19153	2.7E+05
unknown	16.28	498.15192	502.16541	7.0E+05
unknown	16.39	423.10151	425.10822	9.6E+05
Lysine	16.43	307.11055	309.11715	5.4E+07
unknown	16.50	613.21558	617.22791	5.8E+05
unknown	16.54	308.10786	310.11475	3.8E+05
unknown	16.58	415.21176	417.21800	7.0E+06
unknown	16.65	327.64290	329.64968	1.8E+05
4-Hydroxybenzoic acid	16.69	372.09037	374.09721	4.8E+06
unknown	16.69	407.16415	409.17089	1.9E+05
unknown	16.69	498.37122	500.37845	4.1E+06
unknown	16.69	548.11827	552.13130	2.0E+05
unknown	16.72	264.08838	266.09507	1.3E+05
unknown	16.76	305.13200	307.13858	3.5E+05
unknown	16.84	415.21172	417.21806	1.0E+07
unknown	16.84	498.37101	500.37766	4.1E+06
Histidine	16.91	311.59335	313.60001	9.3E+06
unknown	17.03	622.17903	626.19342	3.9E+05
unknown	17.14	415.21164	417.21775	5.7E+06
unknown	17.39	347.08834	349.09479	1.7E+05
unknown	17.39	359.62765	361.63443	3.7E+05
unknown	17.43	407.16377	409.17056	4.3E+05
unknown	17.54	297.08574	299.09227	4.4E+05
unknown	17.58	324.10556	326.11208	2.5E+05
unknown	17.65	282.10702	284.11366	3.6E+05
unknown	17.65	332.10644	334.11342	2.2E+05
unknown	17.65	509.09286	511.09953	2.0E+05
unknown	17.87	413.11678	415.12357	5.3E+05
unknown	17.94	528.17272	530.17907	3.5E+05
unknown	17.98	321.12287	323.12982	2.6E+06
unknown	17.98	419.47857	421.48553	2.5E+05
2-aminooctanoic acid	18.02	393.18428	395.19122	1.8E+05
unknown	18.06	314.06122	316.06842	7.3E+04
unknown	18.14	356.09343	358.10017	7.2E+05
unknown	18.14	395.10631	398.11652	1.3E+06
unknown	18.14	455.16504	457.17102	7.1E+04
unknown	18.18	314.06144	316.06805	4.4E+06
unknown	18.29	353.11202	355.11865	9.2E+05
unknown	18.36	370.11103	372.11782	3.8E+05
unknown	18.36	489.31085	491.31686	3.0E+06
unknown	18.40	307.14767	309.15438	7.3E+05
unknown	18.44	498.36343	500.37039	2.7E+06
unknown	18.48	354.06346	356.07013	2.7E+06
unknown	18.66	354.06351	356.07016	6.2E+05
unknown	18.85	421.17956	423.18656	5.8E+05
unknown	18.89	498.37085	500.37767	5.4E+06
unknown	19.04	411.26614	413.27279	7.9E+06
unknown	19.04	440.20043	442.20737	4.4E+05
unknown	19.04	489.31094	491.31713	5.0E+06
unknown	19.04	498.37095	500.37762	6.8E+06
unknown	19.12	379.12761	381.13446	1.2E+06
unknown	19.19	314.11885	316.12557	1.3E+07
unknown	19.19	328.11660	330.12372	1.5E+05
unknown	19.19	638.41608	640.42383	7.9E+05
L-Tyrosinamide	19.23	324.10352	326.10999	6.0E+04
unknown	19.34	317.59330	319.60010	9.5E+05
unknown	19.34	401.12812	403.13496	9.3E+05
unknown	19.38	333.16331	335.17032	2.1E+05
unknown	19.42	279.10749	281.11411	2.0E+05
unknown	19.42	390.10357	392.11081	1.3E+05
unknown	19.42	530.18877	532.19481	2.5E+05

unknown	19.53	277.07557	279.08250	3.6E+05
unknown	19.53	546.10452	548.11130	4.3E+05
1,4-diaminobutane	19.60	555.21008	559.22345	9.4E+04
unknown	19.64	294.11621	296.12272	5.1E+05
unknown	19.71	264.58491	266.59153	3.8E+05
unknown	19.75	340.13420	342.14087	2.0E+05
unknown	19.75	386.10587	388.11272	3.6E+05
unknown	19.79	335.46265	337.46948	1.0E+05
unknown	19.82	356.09549	358.10193	1.6E+07
unknown	19.86	316.09304	318.09955	3.2E+05
unknown	19.86	335.12464	337.13141	2.3E+05
unknown	19.86	564.14966	566.15594	2.1E+05
unknown	19.90	339.60097	341.60807	2.2E+05
unknown	19.90	520.10366	522.11085	2.3E+05
unknown	19.94	331.11136	333.11810	1.2E+06
unknown	20.06	292.10601	294.11267	8.8E+05
unknown	20.13	486.11476	488.12128	3.7E+05
unknown	20.17	346.60861	348.61523	1.0E+05
Tyrosine	20.28	324.59436	326.60091	8.7E+07
Cysteamine	20.35	310.07508	312.08207	2.3E+05
unknown	20.39	297.08577	299.09242	1.5E+06
Metoprolol	20.39	501.16222	503.16883	7.8E+05
unknown	20.54	381.12727	383.13383	6.7E+05
unknown	20.58	315.08498	317.09173	1.0E+06
unknown	20.65	379.11150	381.11817	4.2E+05
Phenol	20.77	328.10071	330.10739	7.4E+05
unknown	20.85	352.33985	354.34702	2.6E+06
unknown	21.00	363.17337	365.18076	6.3E+04
unknown	21.03	363.17398	366.18436	9.1E+05
unknown	21.07	298.10605	300.11282	1.6E+05
unknown	21.07	639.40872	641.41515	2.4E+06
unknown	21.15	301.14131	303.14791	8.8E+06
unknown	21.22	447.34772	450.35779	3.5E+05
unknown	21.22	590.42693	592.43298	1.1E+06
unknown	21.22	595.38188	597.38843	5.8E+06
unknown	21.29	344.10672	346.11345	1.6E+06
unknown	21.29	454.15474	456.16163	5.7E+05
unknown	21.33	335.17904	337.18587	2.9E+05
unknown	21.33	397.20130	399.20754	5.8E+06
unknown	21.37	546.40060	548.40643	1.3E+06
unknown	21.37	551.35554	553.36180	8.8E+06
unknown	21.37	560.41518	562.42114	1.9E+06
unknown	21.40	507.32938	509.33548	5.8E+05
unknown	21.52	507.33005	509.33639	1.5E+07
unknown	21.55	355.11993	357.12636	5.0E+05
unknown	21.55	514.14700	518.16051	1.1E+06
unknown	21.59	463.30367	465.31002	2.1E+07
unknown	21.59	472.36394	474.37030	4.8E+06
unknown	21.63	361.58179	363.58858	9.9E+05
unknown	21.67	354.11629	356.12294	4.7E+06
unknown	21.67	419.27748	421.28387	2.2E+07
unknown	21.74	375.25106	377.25751	9.5E+06
unknown	21.85	523.14368	525.15074	8.8E+04
unknown	21.89	454.21654	456.22352	2.1E+05
unknown	21.89	498.15249	502.16590	1.2E+07
unknown	21.89	529.19415	533.20739	1.9E+06
unknown	21.89	543.21002	547.22364	1.9E+05
unknown	21.89	556.20505	560.21795	4.7E+05
unknown	21.89	669.23998	673.25255	4.0E+05
unknown	22.04	352.32820	354.33556	2.5E+05
unknown	22.15	284.10852	286.11519	9.3E+04

unknown	22.19	287.07933	289.08608	9.5E+05
unknown	22.19	352.08964	354.09636	2.1E+05
unknown	22.22	382.60905	384.61547	1.6E+05
unknown	22.26	450.20674	452.21370	4.2E+05
unknown	22.30	276.07660	278.08322	1.6E+05
unknown	22.30	305.57105	307.57773	6.5E+04
unknown	22.30	413.61139	416.62179	2.2E+05
unknown	22.38	315.59017	317.59698	2.6E+05
unknown	22.38	419.31564	422.32632	3.2E+05
unknown	22.41	450.20652	452.21315	8.2E+04
unknown	22.60	388.07728	390.08441	7.7E+06
unknown	22.63	344.10674	346.11355	7.1E+05
unknown	22.63	404.13185	406.13846	8.4E+04
unknown	22.67	313.12967	315.13631	1.1E+05
unknown	22.72	360.57920	362.58598	1.2E+06
unknown	22.72	430.32241	433.33173	1.5E+05
unknown	22.72	541.19353	545.20771	4.7E+05
unknown	22.72	572.23594	576.24914	1.1E+05
unknown	22.75	454.24180	456.24838	5.4E+05
unknown	22.82	377.19164	383.21162	1.7E+05
Spermidine	22.86	423.16369	426.17337	9.2E+04
Pyrocatechol	22.90	289.08241	291.08923	4.5E+05
unknown	22.90	455.24496	457.25177	3.0E+05
unknown	22.90	855.20648	857.21289	1.0E+05
unknown	22.98	323.41873	325.42563	7.8E+04
unknown	23.02	315.59030	317.59713	7.0E+05
unknown	23.02	419.31614	422.32693	7.7E+05
unknown	23.05	356.31588	359.32617	6.8E+04
unknown	23.12	454.24157	457.25169	1.1E+06
unknown	23.24	454.24173	456.24843	1.3E+06
unknown	23.36	421.15852	425.17192	2.9E+05
unknown	23.40	454.24182	456.24843	1.0E+06
unknown	23.51	363.21050	365.21722	3.3E+06
unknown	23.54	454.24182	456.24853	6.2E+05
unknown	23.58	281.40829	283.41502	1.1E+05
unknown	23.58	323.60617	325.61278	3.5E+05
unknown	23.58	421.60878	424.61903	1.7E+05
unknown	23.66	305.06802	307.07479	4.1E+06
unknown	23.66	420.32069	422.32785	1.3E+05
unknown	23.70	454.24256	456.24899	1.3E+05
unknown	23.70	542.11841	548.13923	2.1E+06
Thymol	23.85	384.16351	386.17024	8.0E+05
unknown	23.85	419.31638	422.32694	5.6E+05
unknown	23.85	447.34782	450.35815	4.1E+05
unknown	23.93	420.31996	422.32724	1.5E+05
unknown	23.93	447.34756	450.35852	3.0E+05
unknown	23.96	648.26398	650.27026	2.2E+05
unknown	24.01	288.07640	290.08284	1.9E+05
unknown	24.01	431.61095	434.62100	3.0E+05
unknown	24.08	454.24240	456.24872	1.6E+05
unknown	24.16	447.34766	450.35770	3.4E+05
unknown	24.16	685.43713	687.44432	2.3E+06
unknown	24.30	419.31625	422.32700	4.7E+05
unknown	24.30	488.33159	490.33834	7.5E+05
unknown	24.30	553.25579	555.26197	6.4E+06
unknown	24.30	562.31329	564.32017	1.1E+07
unknown	24.30	685.43648	687.44415	2.2E+06
unknown	24.34	522.59802	524.60427	1.4E+06
unknown	24.34	603.16150	607.17560	7.7E+05
unknown	24.45	302.08486	304.09128	2.1E+07
unknown	24.45	369.10365	371.11105	4.2E+05

unknown	24.45	604.16305	607.17316	2.4E+06
unknown	24.49	685.43723	687.44515	2.9E+06
unknown	24.49	694.49805	696.50342	1.5E+06

Table S4.4. Ion pairs detected and identified by RPLC FTICR MS from repeatedly 1:1 ^{12}C-/^{13}C-dansylated CSF sample #4. Ion pairs detected in both repeatedly labeled CSF sample are highlighted as bold.

CSF - #4		CSF - #4		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	1.63	271.04272	273.04937	1.2E+06
unknown	1.63	396.57423	398.58105	5.1E+05
unknown	**1.63**	**503.13236**	**505.13877**	2.2E+06
unknown	1.63	523.10869	525.11447	4.4E+05
unknown	**1.63**	**526.11754**	**528.12386**	1.3E+06
unknown	1.63	641.65963	643.66510	5.1E+05
unknown	1.63	648.63977	650.64580	2.7E+05
unknown	**1.63**	**652.65136**	**654.65764**	1.3E+06
unknown	1.63	754.19501	756.20022	4.4E+05
unknown	1.63	758.20804	760.21391	4.7E+06
unknown	1.63	768.19336	770.19946	2.7E+05
unknown	1.63	783.71517	785.72249	4.2E+05
unknown	1.71	538.11128	540.11755	3.9E+05
unknown	1.71	663.64119	665.64822	4.5E+05
unknown	1.71	674.63263	676.63968	3.3E+05
unknown	1.71	800.16354	802.17101	4.5E+05
unknown	1.75	547.09613	550.10546	6.5E+05
unknown	1.79	296.03311	299.04331	2.5E+06
unknown	**1.79**	**558.16258**	**560.16988**	3.2E+05
unknown	**1.79**	**572.08775**	**574.09439**	7.5E+05
unknown	**1.79**	**589.20526**	**591.21226**	2.3E+05
unknown	1.79	629.04205	631.04828	2.3E+05
unknown	**1.79**	**639.07243**	**641.07857**	6.2E+05
unknown	**1.79**	**707.05936**	**709.06549**	3.3E+05
unknown	1.79	778.18219	780.18994	2.6E+05
unknown	1.82	274.05104	276.05761	7.7E+06
unknown	1.82	364.02080	366.02744	1.5E+06
unknown	1.82	372.06964	374.07625	4.1E+05
unknown	**1.82**	**404.01436**	**406.02060**	1.8E+05
unknown	**1.82**	**421.97958**	**423.98708**	2.3E+05
unknown	**1.82**	**432.00841**	**434.01537**	1.3E+06
unknown	**1.82**	**489.96722**	**491.97443**	1.6E+05
unknown	1.82	499.99624	502.00290	5.6E+05
unknown	**1.82**	**534.17365**	**537.18381**	3.2E+05
unknown	**1.82**	**567.98393**	**569.99062**	3.4E+05
unknown	**1.82**	**635.97072**	**637.97724**	2.0E+05
unknown	**1.86**	**524.08118**	**526.08832**	1.9E+05
unknown	1.86	590.02582	592.03309	3.6E+05
unknown	1.86	605.08999	607.09661	3.4E+05
unknown	1.86	617.20784	619.21466	1.4E+05
unknown	**1.94**	**252.06913**	**254.07570**	3.7E+06
unknown	**1.94**	**449.11459**	**451.12195**	1.1E+05
unknown	1.94	546.06254	548.06952	4.6E+05
unknown	**1.94**	**555.12224**	**557.12990**	1.6E+05

unknown	**2.09**	**524.08121**	**526.08856**	3.1E+05
unknown	**2.20**	**414.12225**	**416.12915**	1.5E+05
unknown	**2.25**	**389.12839**	**391.13515**	1.2E+06
unknown	2.25	537.08411	539.09132	9.5E+04
unknown	**2.29**	**252.06905**	**254.07575**	2.2E+06
phosphoethaolamine	**2.29**	**375.07782**	**377.08455**	6.2E+05
unknown	**2.29**	**536.17987**	**538.18691**	1.5E+05
unknown	**2.36**	**364.02008**	**366.02713**	1.5E+05
unknown	**2.47**	**274.05090**	**276.05758**	1.1E+06
unknown	**2.51**	**376.10851**	**378.11457**	1.9E+05
unknown	2.51	501.15387	503.16083	1.3E+05
unknown	**2.59**	**364.02014**	**366.02701**	1.1E+05
unknown	2.59	374.07559	376.08179	9.7E+04
Taurine	**2.62**	**359.07327**	**361.07996**	4.4E+06
Glucosamine	**2.62**	**413.13742**	**415.14457**	6.0E+04
unknown	2.66	276.08008	278.08705	3.7E+05
unknown	**2.66**	**388.10776**	**390.11438**	2.0E+06
1-methylhistidine	**2.74**	**403.14384**	**405.15059**	5.0E+05
unknown	2.86	363.10124	365.10782	3.2E+05
unknown	2.86	375.07748	377.08475	1.4E+05
unknown	2.86	380.16423	382.17088	4.1E+05
unknown	2.86	509.17059	511.17720	1.1E+06
unknown	2.93	385.08911	387.09555	8.7E+04
unknown	**2.97**	**345.13810**	**347.14503**	9.4E+04
unknown	**2.97**	**376.18033**	**378.18713**	2.3E+05
unknown	2.97	424.11739	426.12475	8.1E+04
unknown	**3.04**	**414.12183**	**416.12872**	3.8E+05
Arginine	**3.19**	**408.17024**	**410.17698**	4.2E+06
unknown	**3.49**	**408.19518**	**410.20221**	4.2E+05
Homoarginine	**3.52**	**422.21107**	**424.21767**	3.9E+05
unknown	**3.52**	**502.13938**	**504.14573**	2.3E+05
unknown	**3.52**	**547.15503**	**549.16187**	1.0E+05
unknown	**3.56**	**314.09556**	**316.10289**	1.1E+05
Asparagine	**3.56**	**366.11200**	**368.11874**	4.4E+06
unknown	**3.56**	**380.16406**	**382.17085**	6.7E+05
unknown	3.64	425.15770	427.16474	7.8E+04
unknown	**3.67**	**365.12808**	**367.13461**	9.5E+05
unknown	3.67	424.15385	426.16077	3.2E+05
unknown	3.97	378.14016	384.16010	1.2E+07
unknown	3.97	411.16828	413.17520	2.5E+06
unknown	**4.05**	**363.10138**	**365.10800**	5.6E+05
Glutamine	**4.05**	**380.12753**	**382.13387**	4.3E+07
unknown	**4.18**	**392.12740**	**394.13483**	1.3E+05
L-citrulline	**4.18**	**409.15433**	**411.16076**	1.2E+06
unknown	4.21	424.11740	426.12436	7.0E+04
unknown	4.21	436.20143	438.20869	2.5E+05
unknown	**4.29**	**515.17834**	**519.19165**	4.1E+05
unknown	**4.33**	**291.06375**	**293.07053**	4.7E+05
unknown	**4.33**	**380.12803**	**382.13492**	1.0E+06
unknown	**4.33**	**515.17600**	**519.18920**	8.8E+05
unknown	4.37	253.56275	255.56949	3.3E+05
unknown	**4.37**	**270.03534**	**272.04191**	4.4E+05
unknown	**4.37**	**302.02498**	**304.03175**	6.0E+05
3-sn-Phosphatidylethanolamine	**4.41**	**484.13636**	**488.14956**	8.9E+06
unknown	**4.41**	**515.17791**	**519.19166**	2.6E+05
Methylguanidine	**4.48**	**307.12232**	**309.12923**	7.5E+04
unknown	**4.48**	**504.14381**	**506.15049**	2.2E+05

Homoserine	**4.59**	**353.11684**	**355.12373**	2.7E+05
Methionine sulfoxide	**4.67**	**399.10459**	**401.11133**	9.2E+05
unknown	**4.67**	**465.18047**	**467.18719**	5.1E+05
unknown	4.79	360.05664	362.06402	8.7E+04
unknown	**4.79**	**394.14319**	**396.14996**	3.0E+05
Homocitrulline	4.79	423.17029	425.17770	4.4E+04
unknown	4.79	499.17469	501.18138	1.7E+05
Serine	**4.86**	**339.10070**	**341.10728**	3.7E+07
unknown	**4.98**	**263.21221**	**265.21887**	4.7E+05
Aspartic Acid	**4.98**	**367.09610**	**369.10267**	1.4E+06
unknown	**5.02**	**408.15881**	**410.16551**	1.2E+05
unknown	**5.06**	**479.23203**	**481.23901**	7.5E+04
unknown	**5.13**	**394.17975**	**396.18641**	5.3E+05
unknown	**5.21**	**394.18002**	**396.18719**	8.8E+04
4-Hydroxy-proline	**5.28**	**365.11686**	**367.12336**	2.8E+06
Glutamic Acid	**5.28**	**381.11181**	**383.11846**	1.0E+06
unknown	**5.32**	**455.11002**	**458.11965**	1.7E+05
unknown	5.32	601.20420	603.21021	9.5E+04
unknown	**5.36**	**600.20148**	**602.20840**	2.5E+05
unknown	**5.40**	**442.11684**	**444.12398**	2.5E+05
unknown	**5.40**	**466.16432**	**468.17142**	4.8E+05
unknown	5.43	291.00953	297.02917	1.4E+06
unknown	5.51	422.17475	424.18155	1.4E+05
unknown	**5.55**	**336.13794**	**338.14468**	6.4E+05
Aminoadipic acid	**5.55**	**395.12737**	**397.13418**	1.6E+05
unknown	5.55	505.07596	509.08855	2.2E+05
unknown	**5.66**	**363.14883**	**365.15553**	5.8E+05
unknown	5.66	457.08912	459.09584	2.1E+05
Threonine	**5.70**	**353.11650**	**355.12311**	2.8E+07
unknown	5.73	480.18029	482.18692	2.6E+05
unknown	**5.81**	**337.15828**	**339.16497**	3.3E+06
unknown	**5.85**	**379.13247**	**381.13930**	3.2E+05
Diethanolamine	5.93	339.13803	341.14438	2.2E+05
Ethanolamine	**6.00**	**295.11063**	**297.11738**	2.6E+07
unknown	**6.00**	**478.12824**	**480.13522**	2.5E+06
unknown	**6.08**	**463.23807**	**465.24469**	6.3E+05
unknown	**6.08**	**567.17102**	**569.17810**	2.5E+05
unknown	**6.12**	**251.08483**	**253.09145**	1.6E+07
unknown	**6.12**	**523.14498**	**525.15189**	1.8E+06
unknown	**6.19**	**317.13200**	**319.13887**	1.3E+06
unknown	**6.19**	**348.17441**	**350.18118**	3.6E+05
unknown	**6.19**	**396.11122**	**398.11816**	1.5E+05
unknown	**6.23**	**415.13255**	**417.13933**	1.8E+06
unknown	**6.38**	**398.12730**	**400.13398**	1.4E+06
unknown	**6.42**	**348.10155**	**350.10818**	9.2E+05
unknown	6.42	464.14903	466.15589	2.2E+05
Glycine	**6.50**	**309.09050**	**311.09712**	1.2E+07
unknown	**6.50**	**381.14818**	**383.15489**	1.3E+06
unknown	6.61	350.29026	352.29745	7.9E+05
unknown	**6.69**	**344.10660**	**346.11327**	6.7E+05
unknown	**6.69**	**364.16919**	**366.17578**	7.1E+05
unknown	**6.73**	**477.16170**	**479.16833**	5.6E+05
unknown	**6.77**	**348.13765**	**350.14452**	3.5E+05
N-Methylaspartic acid	6.96	381.11215	383.11926	4.7E+04
unknown	**6.96**	**396.11104**	**398.11809**	1.1E+05
unknown	6.96	436.19037	439.20063	4.2E+05
unknown	**7.04**	**362.11713**	**364.12376**	1.5E+07

unknown	**7.11**	**362.11709**	**364.12360**	2.4E+07
unknown	**7.15**	**347.11774**	**349.12432**	5.9E+05
Tyrosine methyl ester	**7.19**	**415.13281**	**417.13958**	1.8E+06
unknown	**7.22**	**367.13276**	**369.13930**	5.6E+05
Alanine	**7.30**	**323.10654**	**325.11287**	1.7E+07
r-aminobutyric acid	**7.49**	**337.12205**	**339.12906**	9.0E+05
unknown	7.49	493.08389	496.09306	3.6E+06
unknown	**7.58**	**473.10719**	**475.11411**	5.3E+06
unknown	**7.65**	**473.10625**	**475.11372**	2.6E+06
unknown	**7.80**	**473.11072**	**475.11737**	6.6E+05
unknown	**7.92**	**402.08679**	**404.09363**	1.4E+05
unknown	8.18	309.12578	312.13626	1.9E+05
Hypoxanthine	**8.18**	**370.09700**	**372.10378**	6.0E+05
unknown	**8.22**	**450.20577**	**452.21274**	1.0E+05
unknown	**8.26**	**308.10638**	**310.11332**	1.8E+05
unknown	8.30	395.12720	397.13408	1.5E+05
unknown	**8.42**	**431.13860**	**433.14565**	7.3E+05
3-Aminoisobutyric acid	**8.42**	**337.12209**	**339.12866**	1.4E+05
unknown	**8.45**	**279.07988**	**281.08662**	2.3E+05
unknown	**8.45**	**386.09174**	**388.09874**	1.2E+05
unknown	**8.49**	**396.13518**	**398.14182**	7.3E+05
5-Aminopentanoic acid	**8.50**	**351.13753**	**353.14427**	2.5E+05
unknown	8.53	485.12029	489.13359	2.8E+05
unknown	8.57	295.13854	297.14505	1.0E+05
unknown	**8.72**	**251.08496**	**253.09154**	9.0E+05
unknown	**8.72**	**524.14878**	**526.15479**	6.8E+05
2-Aminobutyric acid	**8.88**	**337.12183**	**339.12853**	4.0E+06
unknown	9.07	321.09065	323.09756	1.6E+05
Sarcosine	**9.11**	**323.10617**	**325.11307**	5.5E+05
unknown	9.11	351.10098	354.11124	1.0E+05
unknown	9.11	371.10087	373.10739	3.5E+05
unknown	**9.18**	**363.10132**	**365.10805**	1.0E+06
unknown	9.18	507.07121	509.07829	6.0E+05
Methylcysteine	**9.34**	**369.09418**	**371.10089**	6.2E+05
unknown	**9.34**	**492.05262**	**494.06015**	3.0E+05
unknown	**9.49**	**242.28435**	**244.29105**	3.5E+06
unknown	**9.49**	**396.11128**	**398.11820**	4.1E+05
unknown	**9.61**	**266.10707**	**268.11377**	4.4E+06
unknown	**9.64**	**303.05666**	**305.06325**	5.3E+05
unknown	**9.64**	**321.12716**	**323.13383**	2.7E+06
unknown	**9.64**	**529.19440**	**531.20115**	7.6E+05
unknown	9.73	423.05823	425.06500	8.6E+05
unknown	**9.81**	**287.08085**	**289.08755**	4.9E+07
unknown	**9.81**	**527.17958**	**529.18632**	9.8E+05
unknown	9.81	550.17468	556.19397	4.2E+06
unknown	**9.85**	**266.10342**	**268.10994**	7.1E+06
unknown	9.85	554.18646	556.19290	4.0E+06
unknown	9.85	682.22156	684.22760	1.2E+06
unknown	**9.85**	**793.27994**	**795.28606**	7.7E+05
unknown	**9.89**	**287.08215**	**289.08871**	1.3E+07
Proline	**9.89**	**349.12212**	**351.12868**	8.4E+05
unknown	**9.89**	**551.17633**	**553.18207**	1.1E+07
unknown	**9.89**	**572.12402**	**574.13098**	1.6E+06
unknown	**9.93**	**529.19439**	**531.20128**	6.5E+05
unknown	**10.01**	**287.08218**	**289.08909**	2.4E+06
unknown	**10.01**	**303.05658**	**305.06323**	4.9E+05
unknown	**10.09**	**529.19436**	**531.20142**	4.7E+05

unknown	**10.13**	**405.14828**	**407.15513**	2.5E+05
unknown	**10.17**	**370.09718**	**372.10393**	7.1E+05
unknown	**10.22**	**265.10052**	**268.11054**	1.5E+07
unknown	**10.26**	**311.08307**	**313.08978**	3.3E+05
unknown	10.34	529.18335	533.19672	8.9E+04
unknown	**10.38**	**321.11449**	**323.12127**	1.5E+06
unknown	10.38	412.16915	414.17594	1.7E+05
unknown	**10.42**	**265.10060**	**268.11055**	9.0E+06
3-Hydroxypicolinic acid	**10.50**	**373.11875**	**375.12538**	5.0E+05
Valine	**10.50**	**351.13704**	**353.14361**	4.1E+07
Methionine	**10.54**	**383.10960**	**385.11626**	5.5E+06
unknown	10.58	378.18475	380.19116	1.3E+05
unknown	**10.71**	**400.08516**	**402.09193**	1.8E+06
Methylamine	**10.83**	**265.10059**	**267.10715**	6.3E+06
unknown	**10.96**	**378.06758**	**380.07493**	2.1E+05
Tryptophan	**10.96**	**438.14852**	**440.15537**	1.1E+07
unknown	**11.04**	**346.08588**	**348.09259**	1.9E+05
unknown	**11.28**	**397.12506**	**399.13232**	7.6E+04
unknown	**11.65**	**382.58146**	**384.58808**	4.8E+05
unknown	**11.73**	**387.61892**	**389.62538**	1.2E+05
unknown	11.77	418.13212	420.13892	5.4E+05
unknown	**11.81**	**315.09069**	**317.09728**	8.3E+04
unknown	11.81	372.12514	374.13134	9.4E+04
unknown	11.93	367.07843	369.08582	1.1E+05
Pipecolic acid	**12.15**	**363.13774**	**365.14432**	9.0E+05
unknown	12.15	376.16931	378.17577	1.4E+05
unknown	12.19	265.10071	268.11090	1.9E+06
Phenylalanine	**12.19**	**399.13733**	**401.14413**	3.6E+07
unknown	**12.39**	**335.62192**	**337.62884**	1.3E+05
unknown	**12.39**	**406.61720**	**408.62395**	2.1E+05
unknown	12.47	393.15955	396.16956	1.5E+05
3-Hydroxymandelic acid	**12.47**	**402.10091**	**404.10774**	3.6E+05
Isoleucine	**12.56**	**365.15310**	**367.15970**	3.4E+07
unknown	12.64	320.06640	322.07318	9.6E+04
unknown	**12.68**	**501.11529**	**505.12864**	9.0E+05
unknown	**12.71**	**366.15694**	**368.16357**	2.7E+06
unknown	12.71	379.13249	381.13866	2.0E+05
unknown	**12.75**	**366.15658**	**368.16311**	8.4E+06
Leucine	**12.79**	**365.15100**	**367.15817**	5.3E+07
L-norleucine	**12.87**	**365.15344**	**367.16006**	1.3E+07
unknown	13.08	300.06567	302.07207	1.5E+05
Cystine	**13.12**	**354.07011**	**356.07690**	1.0E+06
unknown	**13.17**	**317.13205**	**319.13886**	7.0E+05
unknown	**13.17**	**335.14235**	**337.14869**	3.0E+07
unknown	**13.37**	**342.63007**	**344.63692**	8.7E+04
unknown	**13.45**	**265.09689**	**267.10379**	6.9E+05
unknown	**13.53**	**378.10079**	**380.10750**	4.8E+06
unknown	**13.53**	**409.14307**	**411.14996**	1.7E+05
unknown	**13.53**	**549.18838**	**551.19517**	2.6E+05
Hydroxyphenyllactic acid	**13.61**	**416.11636**	**418.12335**	2.3E+06
unknown	**13.73**	**277.10076**	**279.10735**	1.2E+05
unknown	**13.73**	**307.09302**	**309.09973**	2.2E+06
unknown	**13.73**	**474.06769**	**476.07459**	1.1E+05
unknown	13.77	437.19367	439.19957	4.3E+06
unknown	13.82	378.64044	380.64639	8.8E+04
unknown	13.82	586.29919	588.30664	1.1E+06
unknown	**13.86**	**501.13374**	**503.14112**	1.9E+05

unknown	**13.94**	**402.10076**	**404.10756**	3.7E+05
unknown	**13.98**	**349.15827**	**352.16849**	1.4E+06
Homocystine	**13.98**	**368.09899**	**370.10611**	1.2E+05
unknown	**14.03**	**322.07444**	**324.08111**	9.4E+04
unknown	14.07	378.10092	380.10753	6.7E+05
unknown	14.11	342.62978	344.63649	7.3E+04
5-HIAA	**14.19**	**425.11688**	**427.12372**	5.9E+05
unknown	**14.23**	**349.15827**	**352.16852**	1.8E+06
unknown	**14.27**	**336.11404**	**338.12095**	3.8E+05
unknown	**14.31**	**301.09833**	**305.11175**	9.8E+04
unknown	**14.31**	**346.06595**	**348.07236**	8.7E+04
unknown	**14.31**	**367.16898**	**369.17546**	1.7E+05
unknown	14.31	371.14034	374.14978	3.3E+05
unknown	**14.35**	**349.15834**	**351.16498**	1.9E+06
unknown	**14.39**	**561.23917**	**565.25269**	2.4E+06
Dimethylamine	**14.44**	**279.11598**	**281.12248**	1.9E+07
Phenylpropanolamine	**14.52**	**385.12238**	**387.12763**	2.0E+05
unknown	14.56	282.12644	284.13299	6.3E+05
unknown	**14.61**	**349.15829**	**351.16494**	3.2E+06
unknown	**14.61**	**367.16902**	**369.17565**	2.5E+05
unknown	14.65	345.09547	347.10242	1.5E+05
unknown	**14.65**	**520.24573**	**522.25238**	1.3E+05
unknown	**14.69**	**523.15314**	**525.16040**	7.8E+04
unknown	**14.73**	**387.11431**	**389.12130**	1.3E+05
2,4-Diaminobutyric acid	**14.78**	**293.13179**	**295.13887**	3.9E+04
unknown	**14.86**	**337.23522**	**339.24167**	3.6E+06
unknown	14.86	513.15308	515.16038	1.4E+05
unknown	**14.86**	**520.24595**	**522.25244**	1.2E+05
unknown	**14.90**	**437.19373**	**439.19962**	5.8E+06
unknown	**14.98**	**350.16199**	**352.16876**	5.3E+05
unknown	**14.98**	**367.16903**	**369.17545**	1.8E+05
unknown	**14.98**	**371.14035**	**373.14695**	3.5E+05
unknown	**15.02**	**586.29905**	**588.30590**	1.5E+06
unknown	**15.10**	**363.65358**	**365.66037**	6.0E+05
unknown	**15.10**	**415.21184**	**417.21816**	3.6E+07
unknown	**15.10**	**446.25369**	**448.26005**	1.4E+07
unknown	**15.26**	**349.15829**	**352.16865**	1.3E+06
unknown	**15.35**	**344.60359**	**346.61031**	1.1E+05
L-ornithine	**15.42**	**300.10344**	**302.11008**	9.6E+06
unknown	15.42	315.08403	317.09082	6.3E+05
Acetaminophen	**15.47**	**385.12210**	**387.12877**	1.5E+06
or 4-acetamidophenol				
unknown	**15.47**	**425.61257**	**427.61946**	3.5E+05
unknown	**15.59**	**343.12198**	**345.12852**	1.9E+05
Homovanillic	**15.63**	**416.11636**	**418.12327**	3.4E+06
unknown	**15.67**	**371.10672**	**373.11313**	1.2E+05
unknown	**15.67**	**379.16888**	**381.17542**	2.9E+05
unknown	15.71	266.08459	268.09123	2.8E+06
unknown	**15.75**	**468.14099**	**471.15160**	7.9E+05
unknown	**15.96**	**415.21171**	**417.21788**	5.3E+06
unknown	**16.00**	**345.57367**	**347.58031**	2.9E+05
Homocarnosine	**16.00**	**354.11958**	**356.12635**	4.9E+06
3-/4-hydroxyphenylacetic acid	**16.00**	**386.10593**	**388.11282**	5.6E+05
or 3-Cresotinic acid				
unknown	**16.17**	**345.57383**	**347.58018**	3.5E+05
unknown	16.29	307.61325	309.61975	2.7E+05
unknown	**16.29**	**350.12977**	**352.13653**	1.5E+06

unknown	16.29	378.18475	381.19513	3.0E+05
unknown	16.29	498.15187	502.16525	6.2E+05
unknown	**16.37**	**423.10147**	**425.10815**	4.1E+05
Lysine	**16.42**	**307.11079**	**309.11744**	3.9E+07
unknown	16.54	378.10081	380.10753	2.9E+05
unknown	**16.58**	**415.21170**	**417.21805**	5.9E+06
unknown	16.70	327.64294	329.64972	2.5E+05
unknown	16.70	348.63008	350.63653	1.1E+05
4-Hydroxybenzoic acid	**16.70**	**372.09028**	**374.09712**	3.8E+06
unknown	16.70	548.11829	552.13073	8.8E+04
unknown	16.70	586.29943	588.30621	1.3E+06
unknown	**16.82**	**415.21164**	**417.21793**	8.4E+06
Histidine	**16.94**	**311.59332**	**313.59999**	5.2E+06
unknown	**16.99**	**382.10866**	**384.11576**	1.7E+05
unknown	**17.27**	**407.16446**	**409.17120**	8.3E+04
unknown	**17.40**	**347.08794**	**349.09487**	8.6E+04
unknown	**17.44**	**407.16358**	**409.17045**	2.2E+05
unknown	**17.56**	**324.10551**	**326.11208**	2.9E+05
unknown	17.56	359.10480	361.11134	8.6E+04
unknown	**17.65**	**282.10695**	**284.11357**	3.0E+05
unknown	17.89	414.12069	416.12795	9.2E+04
unknown	**17.93**	**419.47865**	**421.48583**	1.3E+05
unknown	17.93	528.17283	530.17945	1.5E+05
2-aminooctanoic acid	**18.02**	**393.18454**	**395.19126**	3.9E+05
unknown	**18.10**	**356.09345**	**358.10009**	5.2E+05
unknown	**18.18**	**314.06139**	**316.06799**	5.7E+06
unknown	**18.18**	**395.10624**	**397.11300**	5.0E+05
unknown	**18.40**	**370.11106**	**372.11765**	3.5E+05
unknown	**18.44**	**307.14767**	**309.15438**	7.3E+05
unknown	**18.48**	**354.06335**	**356.07003**	1.8E+06
unknown	**18.52**	**486.14318**	**488.14918**	8.2E+05
unknown	18.56	484.13373	486.13960	1.1E+06
unknown	**18.68**	**297.08582**	**299.09235**	2.3E+05
unknown	**18.68**	**486.14297**	**488.14959**	1.9E+05
unknown	**18.76**	**300.06505**	**302.07166**	5.0E+05
unknown	**18.85**	**421.17943**	**423.18611**	1.2E+05
1,3-diaminopropane	19.09	271.10103	273.10723	7.5E+04
unknown	**19.17**	**314.11898**	**316.12566**	2.3E+07
unknown	19.25	265.10181	267.10886	3.8E+05
unknown	19.25	498.36633	500.37369	1.3E+06
unknown	**19.32**	**317.59324**	**319.60001**	7.5E+05
unknown	**19.36**	**401.12799**	**403.13486**	6.1E+05
unknown	**19.44**	**390.10360**	**392.11091**	1.4E+05
unknown	19.44	530.18903	532.19469	8.6E+04
unknown	**19.55**	**277.07561**	**279.08234**	6.1E+05
unknown	**19.55**	**546.10409**	**548.11113**	2.7E+05
1,4-diaminobutane	**19.59**	**278.10856**	**280.11554**	3.7E+05
unknown	**19.63**	**294.11613**	**296.12265**	8.3E+05
unknown	19.63	301.07489	307.09473	7.2E+04
unknown	19.71	264.58489	266.59151	3.1E+05
unknown	**19.75**	**340.13429**	**342.14067**	2.8E+05
unknown	**19.75**	**386.10592**	**388.11259**	3.3E+05
unknown	**19.83**	**356.09544**	**358.10188**	8.0E+06
unknown	**19.87**	**316.09282**	**318.09950**	2.5E+05
unknown	19.87	404.07212	406.07920	1.8E+05
unknown	**19.90**	**331.11128**	**333.11806**	6.8E+05
unknown	**19.90**	**339.60081**	**341.60770**	1.8E+05

unknown	**20.02**	**292.10597**	**294.11267**	6.7E+05
unknown	**20.13**	**486.11445**	**488.12106**	3.4E+05
unknown	20.17	346.60843	348.61525	8.1E+04
Tyrosine	**20.25**	**324.59506**	**326.60115**	4.9E+07
unknown	**20.33**	**577.13482**	**579.14038**	2.3E+06
unknown	**20.40**	**297.08569**	**299.09240**	5.4E+05
unknown	20.40	502.16525	504.17210	2.5E+05
unknown	20.44	399.25086	401.25690	7.6E+06
Metoprolol	**20.44**	**501.16163**	**503.16876**	2.6E+05
unknown	20.59	311.78331	313.78982	1.3E+05
unknown	20.59	352.32835	354.33490	1.1E+05
unknown	**20.59**	**447.34725**	**450.35666**	1.7E+05
Phenol	**20.74**	**328.10043**	**330.10710**	1.5E+06
4-Nitrophenol	**20.82**	**373.08597**	**375.09238**	1.2E+05
unknown	20.88	309.13037	313.14371	1.5E+05
unknown	**20.88**	**365.18551**	**369.19906**	5.2E+05
unknown	20.88	386.10632	388.11272	1.9E+05
unknown	**21.07**	**363.17378**	**365.18060**	5.6E+05
unknown	**21.07**	**639.40860**	**641.41514**	2.2E+06
unknown	**21.14**	**301.14130**	**303.14769**	1.1E+07
unknown	**21.14**	**448.35074**	**450.35806**	1.2E+05
unknown	**21.22**	**595.38197**	**597.38836**	6.0E+06
unknown	**21.30**	**344.10651**	**346.11324**	1.3E+05
unknown	**21.34**	**335.17899**	**337.18582**	3.0E+05
unknown	**21.34**	**397.20123**	**399.20776**	5.0E+06
unknown	**21.38**	**546.40060**	**548.40704**	1.2E+06
unknown	21.38	551.35577	553.36198	8.3E+06
unknown	**21.38**	**560.41570**	**562.42223**	1.7E+06
unknown	**21.49**	**507.33003**	**509.33630**	1.7E+07
unknown	**21.57**	**463.30340**	**465.30986**	1.9E+07
unknown	**21.57**	**472.36378**	**474.37033**	4.4E+06
unknown	**21.64**	**354.11626**	**356.12301**	6.7E+06
unknown	**21.64**	**419.27733**	**421.28368**	2.1E+07
unknown	21.68	550.62323	552.63025	3.7E+06
unknown	**21.72**	**419.27728**	**421.28359**	5.8E+06
unknown	21.76	312.32636	314.33307	3.3E+05
unknown	21.83	530.19781	533.20740	9.2E+04
unknown	21.87	260.06679	262.07344	1.3E+05
unknown	21.87	498.15255	502.16590	1.0E+07
unknown	21.87	530.19745	534.21088	5.2E+05
unknown	21.87	543.20951	547.22360	1.5E+05
unknown	21.87	556.20508	560.21815	4.5E+05
unknown	21.87	588.12167	592.13506	2.3E+05
unknown	21.87	598.21583	602.22944	1.9E+05
unknown	21.91	670.24321	674.25715	1.8E+05
unknown	**22.06**	**307.08752**	**309.09439**	9.6E+04
unknown	**22.18**	**287.07925**	**289.08601**	9.1E+05
unknown	22.21	550.61157	552.61804	3.8E+06
unknown	**22.29**	**450.20672**	**452.21340**	6.1E+05
unknown	22.37	315.59030	317.59721	2.6E+05
unknown	**22.60**	**388.07725**	**390.08439**	8.6E+06
unknown	22.60	419.31564	422.32642	4.4E+05
unknown	**22.68**	**313.12973**	**315.13628**	3.0E+05
unknown	**22.68**	**404.13194**	**406.13897**	1.7E+05
unknown	22.68	541.19401	545.20827	9.6E+05
unknown	22.71	360.57916	362.58617	9.4E+05
unknown	**22.79**	**309.20383**	**311.21055**	4.1E+06

unknown	22.83	377.19146	383.21167	1.7E+05
unknown	22.87	282.44437	284.45108	1.2E+05
unknown	**22.87**	**356.31605**	**359.32610**	8.9E+04
Spermidine	**22.87**	**423.16330**	**426.17371**	1.8E+05
unknown	**23.02**	**315.59035**	**317.59665**	4.8E+05
unknown	**23.10**	**356.31700**	**359.32648**	8.2E+04
unknown	23.14	466.31891	469.32924	1.4E+05
unknown	**23.21**	**352.32828**	**354.33545**	2.0E+05
unknown	23.56	363.21044	365.21713	1.7E+06
unknown	**23.60**	**466.31972**	**470.33334**	6.7E+05
unknown	23.64	281.40851	283.41512	1.3E+05
unknown	**23.64**	**323.60623**	**325.61270**	3.7E+05
unknown	**23.64**	**421.60925**	**424.61898**	1.5E+05
unknown	**23.68**	**305.06803**	**307.07478**	3.4E+06
unknown	23.68	551.63259	553.63937	4.6E+06
unknown	23.68	611.12626	615.14001	3.9E+05
unknown	23.68	632.11408	635.12513	1.5E+05
unknown	23.83	541.12128	545.13402	5.0E+06
Thymol	**23.87**	**384.16345**	**386.17024**	8.1E+05
unknown	**23.99**	**648.26398**	**650.27060**	1.7E+05
unknown	**24.03**	**288.07611**	**290.08277**	1.5E+05
unknown	**24.07**	**550.62806**	**552.63558**	1.1E+07
unknown	**24.18**	**522.59764**	**524.60400**	2.0E+06
Deoxyepinephrine	**24.22**	**317.09012**	**319.09683**	1.1E+05
unknown	24.29	563.31787	565.32544	4.9E+06
unknown	**24.33**	**553.25530**	**555.26174**	7.3E+06
unknown	**24.37**	**685.43725**	**687.44440**	2.5E+06
unknown	**24.41**	**431.61105**	**434.62087**	1.5E+05
unknown	**24.41**	**625.14441**	**629.15786**	1.7E+06
unknown	24.44	443.33420	447.34692	8.4E+04
unknown	24.48	302.08490	304.09142	1.6E+07
unknown	24.48	693.13332	697.14618	2.8E+05
CSF - #4 Repeat		**CSF - #4 Repeat**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**
unknown	**1.61**	**503.13239**	**505.13875**	1.3E+06
unknown	1.61	528.12400	530.13088	1.5E+06
unknown	1.61	534.17432	536.18116	1.8E+06
unknown	**1.61**	**652.65135**	**654.65826**	7.1E+05
unknown	1.61	756.20065	758.20879	6.0E+05
unknown	1.61	777.17957	781.19160	2.0E+05
unknown	1.61	789.24872	791.25403	6.6E+05
unknown	**1.65**	**252.06914**	**254.07572**	9.1E+06
unknown	1.65	372.06989	375.07998	1.2E+06
unknown	**1.69**	**526.11734**	**528.12360**	3.9E+05
unknown	**1.69**	**547.09618**	**549.10268**	5.9E+05
unknown	**1.69**	**639.07184**	**641.07870**	2.5E+05
unknown	**1.73**	**572.08785**	**574.09455**	4.9E+05
unknown	1.73	606.09463	608.10104	2.3E+05
unknown	1.77	297.03660	299.04337	3.4E+05
unknown	1.77	336.02153	338.02810	2.9E+05
unknown	**1.77**	**432.00839**	**434.01528**	1.0E+06
unknown	1.77	493.97972	495.98597	2.4E+05
unknown	1.77	561.96711	563.97394	2.0E+05
unknown	**1.77**	**567.98423**	**569.99063**	3.0E+05
unknown	**1.77**	**635.97217**	**637.97761**	1.8E+05
unknown	**1.77**	**707.05996**	**709.06561**	2.8E+05

unknown	1.81	365.02430	367.03099	2.0E+05
unknown	**1.81**	**421.98012**	**423.98724**	2.0E+05
unknown	**1.81**	**489.96840**	**491.97504**	1.8E+05
unknown	**1.81**	**547.09538**	**549.10223**	2.5E+05
unknown	**1.81**	**558.16278**	**560.16952**	3.2E+05
unknown	1.85	349.05323	351.06014	1.4E+05
unknown	**1.85**	**534.17358**	**537.18373**	3.3E+05
unknown	**1.85**	**589.20524**	**591.21191**	2.6E+05
unknown	1.89	252.06913	255.07931	4.6E+06
unknown	**1.89**	**404.01435**	**406.02031**	1.4E+05
unknown	**1.89**	**536.18054**	**538.18760**	1.1E+06
unknown	1.89	569.07675	571.08432	2.3E+05
unknown	**1.93**	**449.11520**	**451.12207**	1.4E+05
unknown	1.97	583.97687	585.98358	2.6E+05
unknown	2.02	543.01343	547.02584	4.3E+05
unknown	**2.02**	**555.12285**	**557.12992**	4.5E+05
unknown	2.02	702.00642	704.01415	4.7E+05
unknown	**2.10**	**252.06989**	**254.07654**	5.8E+05
unknown	2.14	558.03276	562.04546	6.1E+05
phosphoethaolamine	**2.18**	**375.07807**	**377.08462**	3.4E+05
unknown	**2.22**	**414.12231**	**416.12933**	1.8E+05
unknown	2.22	632.22720	634.23285	2.8E+05
unknown	2.26	275.05490	277.06120	1.6E+05
unknown	**2.26**	**389.12839**	**391.13503**	1.2E+06
unknown	2.30	374.07602	378.08875	2.6E+05
unknown	2.34	509.17082	511.17756	9.4E+05
unknown	**2.50**	**376.10822**	**378.11461**	1.5E+05
unknown	**2.54**	**274.05086**	**276.05758**	8.6E+05
unknown	2.54	438.20569	440.21310	1.1E+05
unknown	**2.59**	**252.06908**	**254.07570**	1.3E+06
unknown	2.59	468.14357	470.15069	7.4E+04
Taurine	**2.63**	**359.07330**	**361.08000**	5.9E+06
unknown	**2.63**	**388.10780**	**390.11448**	1.8E+06
unknown	2.63	412.09680	414.10410	1.6E+05
Glucosamine	**2.63**	**413.13842**	**415.14441**	8.0E+04
1-methylhistidine	**2.74**	**403.14377**	**405.15043**	5.0E+05
unknown	2.82	274.05085	277.06100	5.7E+05
unknown	2.86	366.14865	368.15510	1.1E+05
unknown	2.90	366.14848	369.15793	2.4E+05
unknown	**3.03**	**345.13824**	**347.14502**	1.5E+05
unknown	**3.03**	**376.18034**	**378.18717**	5.0E+05
unknown	**3.07**	**388.10950**	**390.11608**	7.8E+04
unknown	**3.07**	**414.12197**	**416.12867**	5.2E+05
Arginine	**3.19**	**408.17026**	**410.17701**	5.5E+06
unknown	**3.39**	**408.16995**	**410.17643**	2.0E+05
unknown	3.43	367.11565	369.12230	1.6E+06
Asparagine	**3.47**	**366.11210**	**368.11882**	3.6E+06
unknown	**3.47**	**502.13953**	**504.14677**	1.5E+05
unknown	**3.51**	**408.19521**	**410.20210**	4.2E+05
Homoarginine	**3.55**	**422.21112**	**424.21780**	4.4E+05
unknown	**3.55**	**547.15479**	**549.16224**	1.1E+05
unknown	**3.59**	**314.09599**	**316.10281**	2.0E+05
unknown	**3.59**	**380.16402**	**382.17087**	7.1E+05
unknown	**3.63**	**365.12814**	**367.13459**	1.2E+06
unknown	3.67	424.15417	427.16445	2.0E+05
unknown	**3.78**	**363.10123**	**365.10815**	3.3E+05
unknown	3.78	381.13160	383.13830	3.8E+06

unknown	3.90	402.10781	404.11442	2.9E+06
Glutamine	**4.09**	**380.12744**	**382.13372**	3.9E+07
unknown	**4.16**	**392.12787**	**394.13461**	9.1E+04
L-citrulline	**4.16**	**409.15433**	**411.16059**	7.4E+05
unknown	**4.28**	**270.03520**	**272.04186**	4.9E+05
unknown	**4.28**	**302.02498**	**304.03173**	7.5E+05
unknown	4.32	242.57188	244.57854	3.1E+06
unknown	4.32	487.14346	489.14998	1.3E+06
unknown	**4.35**	**291.06380**	**293.07054**	5.4E+05
unknown	4.35	381.11212	383.11877	4.9E+06
unknown	4.35	506.11527	510.12928	1.2E+06
unknown	**4.35**	**515.17586**	**519.18950**	1.1E+06
unknown	4.39	377.08145	379.08850	2.4E+05
unknown	4.39	402.57922	404.58551	2.4E+05
unknown	**4.43**	**302.02502**	**304.03185**	4.0E+05
Aspartic acid	**4.43**	**367.09646**	**369.10289**	8.3E+05
3-sn-Phosphatidylethanolamine	**4.43**	**484.13638**	**488.14972**	1.0E+07
unknown	**4.43**	**515.17852**	**519.19179**	3.8E+05
Methylguanidine	**4.51**	**307.12238**	**309.12927**	1.5E+05
unknown	**4.51**	**504.14420**	**506.15090**	2.7E+05
Homoserine	**4.58**	**353.11600**	**355.12277**	1.3E+05
L-aspartic acid amide	4.58	366.11096	368.11743	6.6E+04
unknown	**4.63**	**484.13589**	**488.14948**	4.7E+05
Methionine sulfoxide	**4.66**	**399.10457**	**401.11135**	7.0E+05
unknown	**4.66**	**465.18056**	**467.18735**	6.7E+05
unknown	4.66	468.14377	470.15073	1.2E+05
unknown	**4.78**	**394.14315**	**396.15010**	2.7E+05
Serine	**4.86**	**339.10084**	**341.10754**	3.7E+07
unknown	**4.97**	**263.21197**	**265.21869**	9.9E+05
unknown	4.97	339.10114	342.11142	4.5E+06
unknown	5.01	403.07446	407.08786	1.1E+05
unknown	**5.01**	**408.15838**	**410.16548**	1.3E+05
Aminoadipic acid	**5.05**	**395.12710**	**397.13434**	1.5E+05
unknown	**5.09**	**479.23257**	**481.24009**	7.7E+04
4-Hydroxy-proline	**5.16**	**365.11684**	**367.12332**	1.6E+06
unknown	**5.16**	**394.17980**	**396.18643**	6.2E+05
Glutamic Acid	**5.31**	**381.11187**	**383.11850**	9.2E+05
unknown	**5.31**	**455.10994**	**458.11976**	4.3E+05
4-Hydroxy-proline	**5.36**	**365.11694**	**367.12335**	1.2E+06
unknown	**5.36**	**408.15796**	**410.16501**	7.2E+04
unknown	**5.36**	**466.16461**	**468.17138**	5.6E+05
unknown	**5.36**	**600.20168**	**602.20834**	5.5E+05
unknown	**5.40**	**442.11696**	**444.12438**	2.5E+05
unknown	**5.55**	**336.13799**	**338.14469**	8.9E+05
unknown	**5.66**	**363.14854**	**365.15523**	1.6E+06
Threonine	**5.70**	**353.11667**	**355.12322**	2.6E+07
unknown	5.70	462.16995	464.17682	3.6E+05
unknown	**5.85**	**337.15825**	**339.16490**	4.7E+06
unknown	**5.89**	**379.13243**	**381.13926**	4.3E+05
unknown	5.97	488.11847	491.12790	1.6E+05
unknown	5.97	515.17188	517.17828	7.8E+04
unknown	**6.01**	**478.12820**	**480.13525**	2.2E+06
Ethanolamine	**6.04**	**295.11077**	**297.11727**	3.7E+07
unknown	**6.08**	**463.23792**	**465.24472**	7.7E+05
unknown	6.08	494.28000	496.28735	1.7E+05
unknown	**6.08**	**567.17139**	**569.17788**	2.1E+05
unknown	**6.12**	**251.08474**	**253.09146**	1.6E+07

unknown	**6.12**	**523.14500**	**525.15191**	1.7E+06
unknown	6.16	503.17008	505.17670	7.2E+05
unknown	**6.19**	**396.11148**	**398.11836**	2.2E+05
unknown	6.19	427.15356	429.16071	1.0E+05
unknown	6.19	501.16290	504.17285	1.9E+05
unknown	**6.23**	**317.13206**	**319.13892**	1.2E+06
unknown	**6.23**	**348.17456**	**350.18108**	2.9E+05
unknown	**6.23**	**415.13270**	**417.13946**	1.7E+06
unknown	**6.38**	**398.12739**	**400.13402**	2.2E+06
unknown	**6.43**	**348.10158**	**350.10826**	1.2E+06
Glycine	**6.47**	**309.09052**	**311.09702**	1.2E+07
unknown	**6.47**	**381.14824**	**383.15496**	1.5E+06
unknown	6.54	311.08690	313.09363	2.1E+05
unknown	6.54	334.29542	336.30222	2.6E+06
unknown	6.54	409.14298	411.14999	1.2E+05
unknown	6.62	292.98094	295.99118	1.6E+06
unknown	6.70	345.11006	347.11697	1.3E+05
unknown	**6.70**	**364.16919**	**366.17575**	9.6E+05
unknown	**6.73**	**344.10659**	**346.11332**	6.1E+05
unknown	**6.73**	**477.16165**	**479.16847**	8.3E+05
unknown	**6.77**	**348.13784**	**350.14448**	4.3E+05
unknown	**6.96**	**396.11141**	**398.11825**	2.0E+05
unknown	**7.03**	**362.11711**	**364.12366**	2.4E+07
unknown	**7.07**	**362.11611**	**364.12227**	3.7E+07
unknown	7.10	393.15829	395.16544	6.3E+05
unknown	**7.14**	**347.11772**	**349.12427**	6.7E+05
unknown	**7.14**	**362.11740**	**364.12402**	1.4E+07
Tyrosine methyl ester	**7.18**	**415.13281**	**417.13952**	1.3E+06
unknown	7.22	287.03345	289.04019	5.4E+05
unknown	**7.22**	**367.13277**	**369.13929**	6.6E+05
unknown	7.26	344.10675	346.11343	3.8E+05
Alanine	**7.33**	**323.10662**	**325.11323**	1.2E+07
r-aminobutyric acid	**7.49**	**337.12253**	**339.12899**	8.0E+05
unknown	**7.61**	**473.10640**	**475.11385**	4.0E+06
unknown	**7.79**	**473.11021**	**475.11679**	9.8E+05
unknown	**7.87**	**473.11122**	**475.11792**	4.4E+05
unknown	7.87	488.13028	490.13754	2.6E+05
unknown	**7.90**	**396.11151**	**398.11830**	1.0E+06
unknown	**7.90**	**402.08704**	**404.09366**	1.9E+05
unknown	7.94	368.11639	370.12311	1.3E+05
Hypoxanthine	**8.17**	**370.09712**	**372.10386**	5.0E+05
unknown	**8.20**	**396.11087**	**398.11810**	1.5E+05
unknown	**8.24**	**450.20564**	**452.21274**	9.2E+04
unknown	**8.28**	**308.10647**	**310.11322**	2.8E+05
5-hydroxymethyluracil	8.28	376.09610	378.10303	5.3E+04
3-Aminoisobutyric acid	**8.39**	**337.12203**	**339.12862**	1.5E+05
unknown	**8.39**	**431.13864**	**433.14561**	1.0E+06
unknown	**8.43**	**279.07999**	**281.08669**	3.1E+05
unknown	**8.47**	**386.09204**	**388.09861**	2.8E+05
5-Aminopentanoic acid	**8.47**	**351.13758**	**353.14422**	2.5E+05
unknown	**8.51**	**396.13525**	**398.14186**	6.4E+05
unknown	**8.51**	**485.12028**	**488.13066**	6.3E+05
unknown	**8.62**	**485.11942**	**488.12958**	9.4E+04
unknown	**8.73**	**251.08494**	**253.09166**	6.8E+05
unknown	**8.73**	**524.14855**	**526.15484**	5.2E+05
2-Aminobutyric acid	**8.88**	**337.12183**	**339.12852**	3.7E+06
unknown	9.07	370.09696	373.10776	5.6E+05

Cysteine-glutathione disulfide	9.07	447.10282	449.10953	6.5E+04
Sarcosine	**9.11**	**323.10623**	**325.11311**	4.0E+05
unknown	9.15	370.09729	372.10401	2.3E+06
unknown	**9.19**	**363.10133**	**365.10808**	9.6E+05
Methylcysteine	**9.34**	**369.09418**	**371.10093**	7.4E+05
unknown	**9.34**	**492.05272**	**494.05994**	3.3E+05
unknown	**9.50**	**396.11137**	**398.11827**	5.5E+05
unknown	**9.54**	**242.28432**	**244.29065**	4.6E+06
unknown	**9.61**	**266.10707**	**268.11392**	1.2E+06
unknown	**9.65**	**321.12710**	**323.13375**	4.8E+06
unknown	**9.65**	**529.19421**	**531.20125**	6.2E+05
unknown	9.69	265.09994	268.11005	4.4E+07
unknown	**9.73**	**551.17651**	**553.18292**	9.5E+06
unknown	**9.76**	**287.08088**	**289.08771**	3.9E+07
unknown	**9.76**	**793.27936**	**795.28583**	5.5E+05
unknown	**9.80**	**527.17955**	**529.18615**	7.0E+05
unknown	**9.84**	**287.08197**	**289.08895**	1.7E+07
unknown	**9.84**	**551.17598**	**553.18164**	1.0E+07
Proline	**9.88**	**349.12205**	**351.12849**	1.7E+06
unknown	**9.88**	**529.19427**	**531.20142**	6.8E+05
unknown	**9.88**	**572.12434**	**574.13101**	2.2E+06
unknown	9.95	531.20123	533.20842	1.1E+06
unknown	**10.10**	**405.14806**	**407.15520**	2.9E+05
unknown	**10.18**	**370.09711**	**372.10383**	1.0E+06
unknown	10.22	460.11703	462.12444	1.4E+05
unknown	**10.26**	**311.08306**	**313.08969**	7.2E+05
unknown	10.26	388.10767	390.11514	2.4E+05
Methylamine	**10.38**	**265.10062**	**267.10725**	5.1E+06
unknown	**10.38**	**321.11446**	**323.12116**	2.3E+06
Wrong Pickup	**10.41**	**352.14132**	**354.14807**	1.6E+06
Wrong Pickup	**10.49**	**352.14079**	**354.14741**	9.6E+06
3-Hydroxypicolinic acid	**10.49**	**373.11956**	**375.12600**	4.0E+05
Methionine	**10.53**	**383.10929**	**385.11608**	4.9E+06
Valine	**10.53**	**351.13737**	**353.14389**	4.0E+07
unknown	**10.72**	**400.08517**	**402.09193**	2.4E+06
unknown	10.79	400.08508	403.09551	9.9E+05
unknown	10.83	456.05704	458.06385	7.0E+04
unknown	**10.91**	**378.06748**	**380.07463**	5.4E+05
Tryptophan	**10.95**	**438.14825**	**440.15527**	8.4E+06
unknown	**11.02**	**346.08599**	**348.09281**	2.5E+05
unknown	11.10	380.11652	382.12339	1.0E+05
unknown	11.21	365.11646	367.12387	1.4E+05
unknown	**11.29**	**397.12540**	**399.13166**	8.7E+04
unknown	**11.64**	**382.58142**	**384.58810**	9.0E+05
unknown	**11.75**	**387.61882**	**389.62537**	9.8E+04
unknown	11.75	418.13209	421.14288	4.9E+05
unknown	11.75	506.06033	510.07394	1.4E+05
unknown	**11.78**	**315.09053**	**317.09728**	1.0E+05
unknown	12.13	400.14127	402.14772	8.5E+05
Pipecolic acid	**12.17**	**363.13764**	**365.14424**	7.9E+05
Phenylalanine	**12.21**	**399.13719**	**401.14374**	4.4E+07
unknown	12.36	266.10384	268.11063	2.4E+05
unknown	**12.40**	**335.62200**	**337.62885**	1.2E+05
unknown	**12.40**	**406.61726**	**408.62412**	2.2E+05
unknown	12.48	314.60551	316.61203	3.2E+05
3-Hydroxymandelic acid	**12.48**	**402.10075**	**404.10759**	3.7E+05
unknown	12.52	313.60894	315.61542	4.3E+06

unknown	12.52	393.15923	395.16580	4.6E+05
Isoleucine	**12.55**	**365.15315**	**367.15970**	2.9E+07
unknown	**12.67**	**501.11527**	**505.12858**	1.4E+06
unknown	12.71	489.07040	492.08071	9.3E+04
unknown	12.71	491.07825	493.08433	1.1E+05
Leucine	**12.74**	**365.15321**	**367.15973**	2.3E+07
unknown	**12.78**	**501.11575**	**505.12909**	4.8E+05
L-norleucine	**12.90**	**365.15344**	**367.16003**	7.6E+06
unknown	**13.13**	**317.13203**	**319.13882**	9.0E+05
Cystine	**13.13**	**354.06969**	**356.07658**	9.8E+05
unknown	**13.17**	**335.14233**	**337.14873**	3.4E+07
unknown	**13.36**	**342.62982**	**344.63715**	7.5E+04
unknown	**13.40**	**265.10144**	**267.10791**	4.2E+05
unknown	13.40	349.15610	351.16254	2.3E+05
unknown	13.48	550.19151	552.19836	1.2E+05
unknown	**13.52**	**378.10092**	**380.10751**	7.0E+06
unknown	**13.52**	**409.14321**	**411.14991**	2.2E+05
unknown	**13.52**	**549.18848**	**551.19521**	4.4E+05
Hydroxyphenyllactic acid	**13.63**	**416.11635**	**418.12340**	2.3E+06
unknown	13.71	297.08572	299.09231	1.9E+05
unknown	**13.75**	**277.10072**	**279.10741**	1.5E+05
unknown	**13.75**	**307.09306**	**309.09970**	3.5E+06
unknown	**13.75**	**474.06792**	**476.07474**	1.7E+05
unknown	**13.86**	**501.13461**	**503.14108**	7.8E+04
unknown	**13.94**	**402.10078**	**404.10760**	4.2E+05
unknown	**13.94**	**520.24596**	**523.25610**	7.5E+04
Homocystine	**13.98**	**368.09876**	**370.10579**	1.5E+05
unknown	**14.01**	**322.07448**	**324.08138**	1.3E+05
unknown	14.01	520.24670	522.25311	7.6E+04
unknown	**14.09**	**520.24540**	**523.25549**	1.0E+05
5-HIAA	**14.20**	**425.11682**	**427.12363**	6.8E+05
unknown	**14.24**	**336.11414**	**338.12077**	4.3E+05
unknown	**14.28**	**346.06584**	**348.07256**	1.7E+05
unknown	**14.39**	**371.14050**	**373.14696**	1.1E+06
Dimethylamine	**14.43**	**279.11594**	**281.12244**	2.6E+07
unknown	**14.43**	**561.23924**	**565.25276**	2.9E+06
unknown	14.43	579.20760	583.22140	1.6E+06
unknown	14.43	584.22394	587.23391	2.4E+06
unknown	**14.51**	**371.14052**	**373.14707**	1.2E+06
Phenylpropanolamine	**14.51**	**385.12207**	**387.12829**	2.5E+05
unknown	**14.55**	**301.09784**	**305.11135**	7.9E+05
unknown	14.59	279.11635	282.12658	4.0E+06
unknown	**14.62**	**367.16889**	**369.17555**	2.8E+05
unknown	**14.62**	**371.14036**	**373.14688**	5.0E+05
unknown	**14.62**	**387.11412**	**389.12118**	1.3E+05
unknown	**14.62**	**561.23840**	**565.25177**	7.2E+04
unknown	**14.66**	**520.24603**	**522.25243**	1.2E+05
unknown	**14.66**	**523.15344**	**525.16119**	7.1E+04
unknown	14.70	372.14382	374.15083	1.0E+05
2,4-Diaminobutyric acid	**14.78**	**293.13152**	**295.13878**	6.9E+04
unknown	**14.78**	**387.11421**	**389.12149**	1.2E+05
unknown	**14.85**	**337.23522**	**339.24152**	1.5E+06
unknown	**14.89**	**349.15833**	**352.16863**	2.9E+06
unknown	**14.89**	**437.19370**	**439.19990**	5.9E+06
unknown	**14.97**	**367.16900**	**369.17551**	2.3E+05
unknown	**14.97**	**371.14033**	**373.14698**	4.5E+05
unknown	**15.01**	**349.15829**	**352.16867**	2.7E+06

unknown	**15.01**	**586.29884**	**588.30560**	1.0E+06
unknown	**15.08**	**415.21177**	**417.21815**	3.9E+07
unknown	**15.12**	**363.65361**	**365.66048**	8.2E+05
unknown	**15.12**	**446.25347**	**448.25980**	1.6E+07
unknown	**15.16**	**586.29919**	**588.30554**	1.5E+06
unknown	**15.20**	**350.16198**	**352.16873**	3.7E+05
unknown	**15.35**	**344.60337**	**346.61015**	1.2E+05
unknown	15.39	600.20264	603.21318	1.6E+05
L-ornithine	**15.43**	**300.10344**	**302.11008**	7.8E+06
unknown	15.43	316.08771	318.09430	7.6E+05
Acetaminophen	**15.47**	**385.12213**	**387.12877**	1.6E+06
or 4-acetamidophenol				
unknown	**15.47**	**425.61246**	**427.61941**	3.5E+05
unknown	**15.55**	**300.06515**	**302.07177**	3.4E+05
unknown	**15.58**	**343.12201**	**345.12860**	2.1E+05
Homovanillic	**15.62**	**416.11634**	**418.12328**	3.6E+06
unknown	**15.66**	**371.10649**	**373.11312**	1.6E+05
unknown	**15.66**	**379.16883**	**381.17553**	3.0E+05
unknown	**15.77**	**468.14098**	**471.15152**	4.6E+05
unknown	15.81	265.10233	267.10938	1.2E+05
unknown	**15.96**	**415.21176**	**417.21792**	4.9E+06
Homocarnosine	**16.00**	**354.11942**	**356.12617**	6.3E+06
3-/4-hydroxyphenylacetic acid	**16.00**	**386.10601**	**388.11282**	6.2E+05
or 3-Cresotinic acid				
unknown	**16.03**	**345.57379**	**347.58019**	5.0E+05
unknown	16.26	378.18481	380.19155	3.8E+05
unknown	**16.30**	**350.12981**	**352.13659**	1.3E+06
unknown	16.34	265.09290	267.10007	1.5E+05
Lysine	**16.41**	**307.11081**	**309.11744**	3.7E+07
unknown	**16.41**	**423.10146**	**425.10812**	4.2E+05
unknown	16.56	498.37100	500.37689	7.7E+05
4-Hydroxybenzoic acid	**16.68**	**372.09019**	**374.09700**	5.7E+06
unknown	**16.83**	**415.21169**	**417.21803**	7.5E+06
unknown	16.87	319.62692	321.63391	9.0E+04
unknown	16.91	312.58981	314.59653	4.3E+05
Histidine	**16.94**	**311.59333**	**313.60003**	5.2E+06
unknown	**16.98**	**382.10896**	**384.11559**	1.8E+05
unknown	**17.10**	**415.21166**	**417.21814**	4.8E+06
unknown	**17.40**	**347.08810**	**349.09472**	1.7E+05
unknown	17.40	359.62792	361.63440	8.9E+04
unknown	**17.44**	**407.16382**	**409.17051**	2.4E+05
unknown	17.56	297.08576	299.09237	3.2E+05
unknown	**17.56**	**324.10544**	**326.11202**	3.2E+05
unknown	**17.64**	**282.10697**	**284.11363**	3.5E+05
unknown	17.64	305.09549	307.10266	2.4E+05
unknown	17.86	413.11673	416.12705	4.6E+05
unknown	17.98	393.18457	399.20445	1.7E+05
unknown	**17.98**	**419.47853**	**421.48547**	1.7E+05
unknown	18.02	321.12284	323.12901	4.2E+06
2-aminooctanoic acid	**18.05**	**393.18445**	**395.19114**	4.7E+05
unknown	**18.13**	**356.09345**	**358.10014**	1.4E+06
unknown	**18.17**	**395.10629**	**397.11310**	9.0E+05
unknown	**18.21**	**314.06139**	**316.06801**	6.2E+06
unknown	18.32	353.11173	355.11868	1.5E+05
unknown	**18.40**	**370.11102**	**372.11777**	3.9E+05
unknown	**18.44**	**307.14767**	**309.15434**	2.7E+06
unknown	18.48	355.05969	357.06687	2.8E+05

unknown	**18.52**	**354.06332**	**356.07004**	2.7E+06
unknown	**18.71**	**297.08577**	**299.09228**	3.5E+05
unknown	18.71	415.21164	417.21786	3.5E+06
unknown	**18.79**	**300.06505**	**302.07173**	2.9E+05
unknown	**18.83**	**421.17957**	**423.18645**	1.9E+05
unknown	**19.16**	**314.11905**	**316.12572**	2.3E+07
unknown	**19.32**	**317.59331**	**319.60004**	1.2E+06
unknown	**19.36**	**401.12802**	**403.13490**	1.2E+06
unknown	**19.44**	**390.10341**	**392.11035**	1.8E+05
unknown	**19.55**	**277.07555**	**279.08235**	6.3E+05
unknown	**19.55**	**546.10428**	**548.11101**	5.0E+05
1,4-diaminobutane	**19.59**	**278.10850**	**280.11551**	4.9E+05
unknown	**19.67**	**294.11614**	**296.12270**	1.1E+06
unknown	19.70	528.16232	532.17603	3.5E+05
unknown	**19.74**	**340.13405**	**342.14117**	2.0E+05
unknown	**19.74**	**386.10573**	**388.11258**	3.0E+05
unknown	**19.82**	**356.09543**	**358.10191**	9.0E+06
unknown	**19.86**	**316.09284**	**318.09953**	5.3E+05
unknown	**19.90**	**331.11127**	**333.11807**	3.2E+05
unknown	**19.90**	**339.60081**	**341.60752**	2.5E+05
unknown	20.01	387.22729	389.23357	3.2E+06
unknown	**20.05**	**292.10599**	**294.11270**	1.2E+06
unknown	**20.13**	**486.11436**	**488.12109**	4.8E+05
Tyrosine	**20.28**	**324.59402**	**326.60121**	6.4E+07
unknown	**20.36**	**577.13463**	**579.14050**	2.6E+06
unknown	**20.43**	**297.08574**	**299.09239**	1.1E+06
Metoprolol	**20.43**	**501.16214**	**503.16868**	2.0E+05
unknown	20.47	421.23295	423.23899	1.0E+07
unknown	20.54	381.12725	383.13400	5.2E+05
unknown	20.58	315.08497	317.09175	1.2E+06
unknown	20.66	379.11151	381.11890	2.7E+05
Phenol	**20.78**	**328.10054**	**330.10721**	1.7E+06
4-Nitrophenol	**20.82**	**373.08574**	**375.09234**	2.5E+05
unknown	**20.89**	**365.18551**	**369.19855**	4.7E+05
unknown	20.97	693.49701	695.50311	1.1E+05
unknown	**21.09**	**363.17394**	**365.18069**	9.1E+05
unknown	**21.09**	**447.34734**	**450.35804**	3.7E+05
unknown	21.09	551.63239	553.64014	2.4E+06
unknown	21.09	559.13576	562.14643	8.2E+04
unknown	21.09	634.45358	636.46082	7.7E+05
unknown	**21.09**	**639.40873**	**641.41504**	3.9E+06
unknown	21.09	648.46897	650.47479	6.8E+05
unknown	21.13	310.07521	312.08202	8.0E+05
unknown	21.16	265.10309	267.11017	1.3E+05
unknown	21.16	550.62244	552.62885	3.5E+06
unknown	21.20	462.13773	464.14471	3.7E+05
unknown	**21.24**	**301.14135**	**303.14770**	1.0E+07
unknown	**21.24**	**363.17413**	**365.18091**	4.0E+05
unknown	21.24	419.31600	423.33002	6.5E+05
unknown	**21.24**	**447.34734**	**450.35777**	3.5E+05
unknown	21.24	590.42697	592.43408	1.3E+06
unknown	**21.24**	**595.38197**	**597.38837**	7.3E+06
unknown	21.24	604.44224	606.44859	1.5E+06
unknown	**21.32**	**344.10666**	**346.11347**	1.7E+05
unknown	**21.35**	**335.17909**	**337.18594**	5.0E+05
unknown	21.35	394.20522	396.21176	2.6E+05
unknown	**21.35**	**397.20128**	**399.20759**	7.2E+06

unknown	21.35	551.35559	554.36487	1.2E+07
unknown	**21.39**	**546.40060**	**548.40717**	1.7E+06
unknown	**21.39**	**560.41568**	**562.42208**	2.5E+06
unknown	**21.43**	**448.35062**	**450.35767**	8.0E+04
unknown	**21.51**	**507.33001**	**509.33636**	2.3E+07
unknown	21.51	516.39017	518.39638	4.3E+06
unknown	**21.62**	**463.30360**	**465.30983**	2.9E+07
unknown	**21.62**	**472.36378**	**474.37031**	7.6E+06
unknown	**21.66**	**354.11635**	**356.12303**	5.6E+06
unknown	**21.66**	**419.27753**	**421.28393**	2.8E+07
unknown	21.66	428.33763	430.34414	6.8E+06
unknown	21.74	375.25103	377.25752	1.8E+07
unknown	21.78	447.34746	450.35785	3.3E+05
unknown	21.89	498.15257	501.16273	1.5E+06
unknown	21.89	529.19429	533.20751	2.7E+05
unknown	**22.08**	**307.08771**	**309.09431**	1.1E+05
unknown	**22.16**	**287.07937**	**289.08613**	1.3E+06
unknown	22.16	358.23801	360.24445	1.3E+06
unknown	22.20	352.08978	354.09647	4.0E+05
unknown	**22.28**	**450.20674**	**452.21351**	7.6E+05
unknown	22.31	522.59796	524.60494	1.2E+06
unknown	**22.58**	**388.07722**	**390.08436**	9.8E+06
unknown	22.62	344.10675	346.11350	4.5E+05
unknown	**22.66**	**313.12964**	**315.13638**	4.2E+05
unknown	**22.66**	**404.13182**	**406.13871**	2.1E+05
unknown	22.66	542.19678	545.20730	1.2E+05
unknown	**22.81**	**309.20382**	**311.21027**	4.3E+06
Spermidine	**22.88**	**423.16343**	**426.17276**	1.4E+05
Pyrocatechol	22.92	289.08225	291.08918	2.4E+05
unknown	22.92	458.42070	460.42757	1.1E+06
unknown	**23.03**	**315.59027**	**317.59723**	1.2E+06
unknown	**23.03**	**352.33984**	**354.34690**	2.7E+06
unknown	**23.22**	**466.31976**	**470.33227**	9.0E+04
unknown	**23.36**	**466.31992**	**470.33241**	1.1E+05
unknown	23.52	364.21400	366.22057	7.6E+05
unknown	**23.59**	**550.62892**	**552.63596**	8.2E+06
unknown	**23.62**	**323.60608**	**325.61257**	3.3E+05
unknown	**23.62**	**421.60892**	**424.61881**	2.4E+05
unknown	**23.70**	**305.06794**	**307.07471**	4.8E+06
unknown	**23.73**	**466.31931**	**470.33289**	1.9E+05
unknown	23.73	542.12162	548.14242	2.2E+06
unknown	**23.73**	**550.62868**	**552.63594**	8.7E+06
unknown	23.84	419.31636	422.32712	7.9E+05
unknown	23.88	308.58904	310.59595	3.0E+05
Thymol	**23.88**	**384.16348**	**386.17016**	1.6E+06
unknown	**23.88**	**431.61092**	**434.62119**	2.5E+05
unknown	**23.88**	**522.59780**	**524.60388**	1.4E+06
unknown	23.88	694.49769	696.50537	1.2E+06
unknown	23.95	432.61921	435.62922	1.6E+05
unknown	**23.95**	**550.62884**	**552.63576**	8.8E+06
unknown	**24.02**	**648.26331**	**650.27034**	4.3E+05
unknown	**24.06**	**288.07617**	**290.08291**	3.5E+05
unknown	**24.10**	**431.61109**	**434.62098**	6.2E+05
unknown	24.17	537.16641	541.18066	3.0E+06
unknown	**24.17**	**550.62707**	**552.63357**	8.2E+06
Deoxyepinephrine	**24.24**	**317.09006**	**319.09690**	1.6E+05
unknown	24.27	562.31450	564.32019	5.3E+06

unknown	**24.27**	**685.43697**	**687.44452**	2.2E+06
unknown	**24.31**	**553.25439**	**555.26029**	6.7E+06
unknown	**24.37**	**553.25529**	**555.26141**	6.0E+06
unknown	**24.44**	**625.14389**	**629.15731**	2.5E+06
unknown	24.48	303.08128	305.08778	1.9E+06
unknown	24.48	369.10362	371.11087	7.2E+05
unknown	**24.48**	**431.61116**	**434.62119**	4.6E+05
unknown	24.48	604.16603	607.17649	4.9E+06

Table S4.9. List of Positively Identified Metabolites in CSF Sample #1.

		CSF - #1				CSF - #1 Repeat		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**	**Rt**	**mz_light**	**mz_heavy**	**int**
phosphoethaolamine	2.29	375.07777	377.08443	9.4E+05	2.37	375.07770	377.08444	1.0E+06
3-methylhistidine	2.61	403.14383	405.15060	8.3E+05	2.64	403.14391	405.15052	1.0E+06
Taurine	2.66	359.07329	361.07999	5.3E+06	2.64	359.07330	361.07997	5.9E+06
1-methylhistidine	2.74	403.14382	405.15041	1.6E+06	2.75	403.14383	405.15044	1.2E+06
Arginine	3.22	408.17022	410.17693	6.3E+06	3.22	408.17021	410.17688	4.7E+06
Homoarginine	3.55	422.21097	424.21773	5.5E+05	3.53	422.21098	424.21773	4.4E+05
Asparagine	3.59	366.11201	368.11873	6.0E+06	3.61	366.11197	368.11872	5.4E+06
Glutamine	3.96	380.12579	382.13227	1.8E+08	3.96	380.12579	382.13242	1.7E+08
L-citrulline	4.16	409.15427	411.16062	2.1E+06	4.15	409.15425	411.16064	2.1E+06
3-sn-Phosphatidylethanolamine	4.40	484.13616	488.14960	1.1E+07	4.45	484.13635	488.14962	9.2E+06
Methylguanidine	4.53	307.12234	309.12925	1.5E+05	4.50	307.12245	309.12899	1.3E+05
Homoserine	4.60	353.11683	355.12352	5.0E+05	4.63	353.11679	355.12352	4.0E+05
Methionine sulfoxide	4.68	399.10470	401.11132	9.6E+05	4.67	399.10447	401.11121	1.2E+06
Serine	4.89	339.10079	341.10731	3.6E+07	4.88	339.10075	341.10751	3.4E+07
Glutamic Acid	4.93	381.11184	383.11847	2.1E+06	4.88	381.11179	383.11843	1.7E+06
Aspartic Acid	4.97	367.09605	369.10280	1.1E+06	4.88	367.09595	369.10264	8.0E+05
4-Hydroxyl-Proline	5.31	365.11684	367.12341	2.1E+06	5.27	365.11680	367.12335	2.4E+06
Iminodiacetic acid	5.54	367.18013	369.18688	2.2E+05	5.54	367.18015	369.18673	2.8E+05
Aminoadipic acid	5.54	395.12734	397.13404	1.5E+05	5.50	395.12727	397.13404	1.8E+05
Folic acid	5.69	338.09350	339.09650	1.0E+05	5.69	338.09320	339.09644	1.3E+05
Threonine	5.77	353.11667	355.12338	1.3E+07	5.77	353.11671	355.12328	1.7E+07
Diethanolamine	5.93	339.13776	341.14436	3.0E+05	5.93	339.13770	341.14436	2.9E+05
Ethanolamine	6.04	295.11099	297.11773	1.5E+07	6.04	295.11090	297.11766	1.5E+07
Glycine	6.51	309.09042	311.09694	9.3E+06	6.47	309.09047	311.09710	9.5E+06
Glycylproline	6.85	406.08077	408.08714	8.0E+04	7.07	406.08050	408.08667	9.0E+04
Tyrosine methyl ester	7.20	415.13271	417.13951	1.8E+06	7.19	415.13272	417.13968	1.9E+06
Alanine	7.35	323.10652	325.11314	1.2E+07	7.35	323.10618	325.11267	3.2E+07
r-aminobutyric acid	7.50	337.12209	339.12886	8.0E+05	7.54	337.12207	339.12882	5.8E+05
Hypoxanthine	8.15	370.09713	372.10397	2.5E+05	8.21	370.09704	372.10375	1.0E+06
3-Aminoisobutyric acid	8.43	337.12185	339.12867	1.7E+05	8.40	337.12195	339.12866	1.9E+05
5-Aminopentanoic acid	8.50	351.13757	353.14412	6.0E+05	8.52	351.13754	353.14416	6.0E+05
2-Aminobutyric acid	8.89	337.12168	339.12827	1.7E+07	8.90	337.12167	339.12824	1.6E+07

Sarcosine	9.12	323.10454	325.11294	2.0E+05	9.10	323.09902	325.11297	1.5E+05
Methylcysteine	9.34	369.09411	371.10085	5.6E+05	9.37	369.09417	371.10086	8.2E+05
Proline	9.84	349.12130	351.12787	2.0E+05	9.87	349.12142	351.12768	9.0E+04
Methylamine	10.00	265.10039	267.10674	1.2E+07	10.10	265.10035	267.10665	2.1E+07
Valine	10.50	351.13622	353.14252	6.2E+07	10.51	351.13605	353.14257	7.0E+07
Methionine	10.54	383.10828	385.11508	5.2E+06	10.58	383.10980	385.11637	3.5E+06
3-Hydroxypicolinic acid	10.54	373.11820	375.12478	5.0E+05	10.51	373.11799	375.12462	6.0E+05
Tryptophan	10.96	438.14839	440.15508	1.1E+07	10.96	438.14851	440.15513	1.4E+07
Pipecolic acid	12.18	363.13759	365.14422	5.5E+05	12.16	363.13763	365.14431	6.2E+05
Phenylalanine	12.26	399.13455	401.14093	4.2E+07	12.23	399.13555	401.14221	4.3E+07
3-Hydroxymandelic acid	12.48	402.10091	404.10795	2.7E+05	12.50	402.10112	404.10782	1.5E+05
Isoleucine	12.56	365.15306	367.15953	2.1E+07	12.54	365.15293	367.15934	1.8E+07
Leucine	12.75	365.15304	367.15950	2.3E+07	12.80	365.15127	367.15780	6.4E+07
L-norleucine	12.86	365.15219	367.15885	1.6E+07	12.88	365.15276	367.15930	1.7E+07
Cystine	13.09	354.07065	356.07673	6.0E+05	13.11	354.07015	356.07650	8.2E+05
Hydroxyphenyllactic acid	13.63	416.11625	418.12322	2.9E+06	13.64	416.11627	418.12320	3.0E+06
Homocystine	13.98	368.09877	370.10571	1.9E+05	13.97	368.09869	370.10575	1.8E+05
5-HIAA	14.21	425.11684	427.12361	5.5E+05	14.20	425.11680	427.12362	7.2E+05
Dimethylamine	14.40	279.11588	281.12248	2.1E+07	14.42	279.11577	281.12223	2.2E+07
Phenylpropanolamine	14.51	385.12193	387.12867	1.6E+05	14.50	385.12229	387.12875	3.0E+05
2,4-Diaminobutyric acid	14.74	293.13174	295.13882	9.8E+04	14.77	293.13143	295.13872	4.3E+04
L-ornithine	15.39	300.10357	302.11034	5.7E+06	15.40	300.10354	302.11021	7.8E+06
Acetaminophen	15.48	385.12033	387.12690	3.3E+07	15.44	385.12108	387.12747	3.9E+07
or 4-acetamidophenol								
Homovanillic	15.63	416.11635	418.12328	2.6E+06	15.59	416.11638	418.12327	2.3E+06
Homocarnosine	16.01	354.11951	356.12626	1.3E+07	16.00	354.11911	356.12569	1.9E+07
3-/4-hydroxyphenylacetic acid	16.01	386.10585	388.11278	7.4E+05	16.00	386.10590	388.11279	9.9E+05
or 3-Cresotinic acid								
Gentisic Acid	16.20	388.10786	390.11464	1.6E+05	16.20	388.10785	390.11456	1.5E+05
Lysine	16.42	307.11035	309.11708	3.6E+07	16.42	307.11042	309.11706	4.4E+07
4-Hydroxybenzoic acid	16.69	372.09030	374.09715	3.0E+06	16.69	372.09038	374.09716	3.0E+06
Histidine	16.96	311.59289	313.59910	1.0E+07	16.94	311.59329	313.59985	1.2E+07
1,3-diaminopropane	19.09	271.10055	273.10716	7.0E+04	19.08	271.10039	273.10718	1.0E+05
L-Tyrosinamide	19.16	324.10306	326.10997	5.6E+04	19.22	324.10370	326.11029	1.1E+05
1,4-diaminobutane	19.57	278.10854	280.11548	2.0E+06	19.59	278.10849	280.11569	2.7E+06
Cadaverine	20.21	285.11646	287.12299	7.9E+04	20.22	285.11636	287.12296	8.6E+04
Tyrosine	20.25	324.59517	326.60123	4.2E+07	20.26	324.59427	326.60101	5.8E+07
Cysteamine	20.36	310.07502	312.08197	1.4E+05	20.38	310.07497	312.08199	1.4E+05
Metoprolol	20.40	501.16218	503.16891	1.2E+06	20.42	501.16156	503.16872	1.0E+06
Phenol	20.75	328.10051	330.10702	1.5E+06	20.75	328.10039	330.10700	1.1E+06
Octopamine	21.13	310.57723	312.58400	1.2E+05	21.44	310.57997	312.58656	4.1E+05
Tyramine	21.20	302.58626	304.59310	1.0E+05	21.20	302.58655	304.59313	1.5E+05
Serotonin	21.47	322.19971	324.21451	4.0E+04	21.40	322.19853	324.21428	6.0E+04
Pyrocatechol	22.60	577.15951	581.17244	1.2E+05	22.64	577.15845	581.17148	2.5E+05
Spermidine*	22.87	423.16330	426.17345	3.8E+05	22.88	423.16323	426.17326	5.8E+05

Thymol	23.86	384.16349	386.17009	1.1E+06	23.89	384.16343	386.17015	4.3E+05
Glucosamine					2.64	413.13859	415.14466	1.0E+05
L-aspartic acid amide					4.80	366.10046	368.10745	9.3E+04
Ethylamine	11.49	279.09359	281.12300	2.0E+05				
o-Hydroxyphenylacetic acid					15.52	386.11008	388.11649	2.6E+05
2-aminooctanoic acid	18.03	393.18458	395.19103	6.2E+04				
Caffeic acid	21.16	324.21598	326.26920	1.0E+05				
Hydroquinone	23.90	289.08891	291.08922	1.4E+05				

Note:

1) Total 76 metabolites (bold) are detected in both repeated differential labeling experiments. The metabolite assignment is based on accurate ion pair mass and retention time matched with labeled standards.
2) 7 compounds (unbold) are not seen in both repeated labeling experiments, and they are relatively low intensity ion pairs.
3) Error in mass difference is the mass error between the theoretical mass difference and the Measured mass differences for the 13C-/12C-labeled ion pairs. The error of mass differences for the metabolites listed are less than 2ppm, and thus ensure that all ion pairs are the true ion pairs due to the 13C-/12C-labeling.
(see Supplemental Table S4.1 for the complete list of ion pairs).

*The observed peak was from doubly charged ion. The m/z difference between the ion pair was 3, corresponding to 6 Da mass difference. Spermidine has three active sites to be dansylated and thus we expect a mass difference of 6 Da for the ion pair.

Table S4.10. List of Positively Identified Metabolites in CSF Sample #2.

		CSF - #2				**CSF - #2 Repeat**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**	**Rt**	**mz_light**	**mz_heavy**	**int**
phosphoethaolamine	2.38	375.07778	377.08445	5.8E+05	2.33	375.07789	377.08438	4.4E+05
Taurine	2.65	359.07327	361.07996	8.7E+06	2.61	359.07327	361.07996	4.8E+06
1-methylhistidine	2.76	403.14374	405.15045	8.3E+05	2.73	403.14384	405.15052	8.2E+05
Arginine	3.22	408.17024	410.17694	6.8E+06	3.21	408.17030	410.17702	7.8E+06
Homoarginine	3.56	422.21100	424.21779	8.6E+05	3.55	422.21098	424.21777	1.1E+06
Asparagine	3.59	366.11200	368.11875	7.9E+06	3.52	366.11201	368.11876	5.4E+06
Glutamine	3.99	380.12571	382.13218	1.8E+08	3.93	380.12562	382.13217	1.6E+08
L-citrulline	4.18	409.15428	411.16085	5.2E+06	4.15	409.15419	411.16066	3.8E+06
3-sn-Phosphatidylethanolamine	4.45	484.13615	488.14934	9.6E+06	4.42	484.13587	488.14957	1.2E+07
Homoserine	4.63	353.11673	355.12368	3.3E+05	4.65	353.11677	355.12367	3.2E+05
Methionine sulfoxide	4.71	399.10450	401.11123	7.1E+05	4.68	399.10448	401.11126	7.3E+05
Serine	4.90	339.09961	341.10608	4.3E+07	4.87	339.10082	341.10742	3.5E+07
Homocitruline	4.90	423.05352	425.06080	1.4E+05	4.87	423.05395	425.06031	1.0E+05
4-Hydroxy-proline	5.28	365.11686	367.12336	9.6E+06	5.25	365.11691	367.12340	6.2E+06
Glutamic Acid	5.32	381.11190	383.11856	3.4E+05	5.32	381.11195	383.11853	2.0E+05

Folic acid	5.70	338.09362	339.09621	1.2E+05	5.71	338.09287	339.09664	1.0E+05
Threonine	5.70	353.11644	355.12313	4.8E+07	5.78	353.11660	355.12361	1.4E+07
Diethanolamine	5.96	339.13781	341.14429	3.8E+05	5.93	339.13769	341.14440	5.3E+05
Ethanolamine	6.03	295.11116	297.11793	1.4E+07	6.05	295.11108	297.11786	1.3E+07
Glycine	6.49	309.09050	311.09710	1.3E+07	6.50	309.09052	311.09711	1.2E+07
Tyrosine methyl ester	7.18	415.13281	417.13956	2.6E+06	7.18	415.13284	417.13963	4.0E+06
Alanine	7.36	323.10647	325.11306	2.4E+07	7.33	323.10637	325.11291	2.7E+07
r-aminobutyric acid	7.51	337.12179	399.12916	1.3E+05	7.51	337.12211	339.12908	1.3E+05
Hypoxanthine	8.18	370.09701	372.10381	7.7E+05	8.20	370.09709	372.10380	9.0E+05
3-Aminoisobutyric acid	8.44	337.12165	339.12846	1.1E+05	8.42	337.12182	339.12856	1.3E+05
5-Aminopentanoic acid	8.52	351.13755	353.14427	2.2E+05	8.53	351.13755	353.14435	2.0E+05
2-Aminobutyric acid	8.89	337.12179	339.12847	1.0E+07	8.91	337.12181	339.12853	7.2E+06
Cysteine-glutathione disulfide	9.09	447.10238	449.10938	3.3E+05	9.06	447.10246	449.10955	4.6E+05
Methylcysteine	9.32	369.09411	371.10086	1.7E+06	9.37	369.09419	371.10091	1.7E+06
Methylamine	9.67	265.10051	267.10712	1.3E+07	9.89	265.09992	267.10615	3.0E+07
Valine	10.46	351.13736	353.14381	4.9E+07	10.45	351.13703	353.14370	3.9E+07
Methionine	10.54	383.10923	385.11608	1.1E+07	10.52	383.10841	385.11531	1.0E+07
3-Hydroxypicolinic acid	10.54	373.11862	375.12535	5.5E+05	10.52	373.11838	375.12507	5.5E+05
Tryptophan	10.99	438.14838	440.15520	1.5E+07	10.98	438.14849	440.15511	1.6E+07
Phenylalanine	12.20	399.13742	401.14411	3.5E+07	12.29	399.13739	401.14416	2.9E+07
3-Hydroxymandelic acid	12.47	402.10080	404.10775	2.6E+05	12.48	402.10089	404.10763	4.3E+05
Isoleucine	12.55	365.15294	367.15950	4.4E+07	12.52	365.15302	367.15969	3.2E+07
L-cystathionine	12.77	345.09248	347.09923	3.1E+05	12.78	345.09233	347.09880	2.3E+05
Leucine	12.81	365.15121	367.15799	1.2E+08	12.78	365.15133	367.15806	8.8E+07
L-norleucine	12.88	365.15317	367.15962	2.0E+07	12.85	365.15248	367.15903	2.6E+07
Cystine	13.18	354.07009	356.07688	3.5E+05	13.12	354.06970	356.07642	5.3E+05
Hydroxyphenyllactic acid	13.63	416.11627	418.12331	4.3E+06	13.64	416.11638	418.12329	5.1E+06
Homocystine	14.01	368.09850	370.10578	1.0E+05	13.98	368.09866	370.10562	1.0E+05
5-HIAA	14.19	425.11677	427.12355	2.1E+06	14.20	425.11691	427.12368	2.8E+06
Dimethylamine	14.42	279.11575	281.12241	3.3E+07	14.38	279.11586	281.12260	3.4E+07
Phenylpropanolamine	14.49	385.12213	387.12849	3.0E+05	14.45	385.12208	387.12881	4.0E+05
L-ornithine	15.41	300.10336	302.11004	1.8E+07	15.40	300.10337	302.10998	1.6E+07
Acetaminophen	15.45	385.12246	387.12898	1.2E+05	15.45	385.12225	387.13013	7.0E+04
or 4-acetamidophenol								
Homovanillic	15.63	416.11629	418.12317	9.1E+06	15.64	416.11642	418.12326	1.0E+07
3-/4-hydroxyphenylacetic acid	16.00	386.10577	388.11268	1.4E+06	16.01	386.10577	388.11276	1.4E+06
or 3-Cresotinic acid								
Homocarnosine	16.11	354.11946	356.12622	1.8E+06	16.08	354.11957	356.12634	2.0E+06
Lysine	16.45	307.11045	309.11759	4.7E+07	16.42	307.11081	309.11733	4.0E+07
4-Hydroxybenzoic acid	16.64	372.09025	374.09706	7.1E+06	16.69	372.09045	374.09726	5.5E+06
Histidine	16.95	311.59334	313.59998	1.2E+07	16.92	311.59342	313.60003	9.6E+06
2-aminooctanoic acid	18.01	393.18441	395.19109	1.6E+05	18.02	393.18472	395.19107	2.0E+05
1,4-diaminobutane	19.59	555.20966	559.22241	1.8E+05	19.63	555.20928	559.22302	2.4E+05
Tyrosine	20.25	324.59412	326.60066	1.1E+08	20.26	324.59429	326.60076	8.7E+07
Cysteamine	20.37	310.07526	312.08204	1.4E+05	20.37	310.07522	312.08202	2.0E+05
Metoprolol	20.40	501.16198	503.16912	3.1E+06	20.37	501.16201	503.16866	3.0E+06
4-Nitrophenol	20.80	373.08569	375.09225	1.3E+05	20.82	373.08624	375.09262	1.5E+05
Spermidine	22.81	423.16359	426.17352	2.5E+05	22.89	423.16368	426.17382	3.7E+05
Pyrocatechol	22.85	289.08245	291.08922	5.0E+05	22.93	289.08247	291.08926	9.4E+05
Thymol	23.84	384.16357	386.17032	2.8E+05	23.87	384.16375	386.17041	6.3E+05
Methylguanidine	4.52	307.12236	309.12930	7.0E+04				
L-aspartic acid amide					4.53	366.10706	368.11307	1.1E+05
Aspartic Acid	5.02	367.09606	369.10276	9.8E+05				
Aminoadipic acid	5.58	395.12711	397.13413	1.7E+05				
Iminodiacetic acid					5.56	367.18036	369.18684	2.0E+05
5-hydroxymethyluracil					8.31	376.09658	378.10302	1.2E+05

Proline					9.89	349.12204	351.12862	1.0E+06
Phenylethanolamine					13.38	371.14139	373.14755	3.3E+04
2-Phenylglycine	11.48	385.12168	387.12880	1.2E+05				
L-Tyrosinamide					19.15	324.10352	326.11003	1.1E+05
2,4-Diaminobutyric acid	14.73	293.13164	295.13887	3.8E+04				
Phenol					20.75	328.10059	330.10720	1.0E+06
1,3-diaminopropane	19.10	271.09950	273.10748	4.0E+04				

Table S4.11. List of Positively Identified Metabolites in CSF Sample #3.

		CSF - #3				CSF - #3 Repeat		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**	**Rt**	**mz_light**	**mz_heavy**	**int**
phosphoethaolamine	2.33	375.07778	377.08436	6.0E+05	2.29	375.07786	377.08440	4.3E+05
3-methylhistidine	2.56	403.14370	406.15320	4.3E+05	2.57	403.14367	405.15048	2.4E+05
Taurine	2.60	359.07326	361.07997	4.9E+06	2.64	359.07333	361.08006	3.9E+06
1-methylhistidine	2.68	403.14389	405.15050	7.1E+05	2.72	403.14384	405.15041	6.0E+05
Arginine	3.14	408.17021	410.17691	6.0E+06	3.18	408.17029	410.17700	7.8E+06
Homoarginine	3.51	422.21105	424.21776	3.1E+05	3.57	422.21093	424.21777	3.5E+05
Asparagine	3.55	366.11201	368.11875	5.5E+06	3.60	366.11206	368.11877	4.2E+06
Glutamine	3.93	380.12560	382.13206	1.5E+08	3.87	380.12566	382.13211	1.2E+08
L-citrulline	4.16	409.15435	411.16088	1.9E+06	4.18	409.15438	411.16082	9.4E+05
3-sn-Phosphatidylethanolamine	4.38	484.13622	488.14933	1.1E+07	4.41	484.13607	488.14960	1.3E+07
Homoserine	4.60	353.11684	355.12346	2.2E+05	4.63	353.11675	355.12366	1.2E+05
Methionine sulfoxide	4.68	399.10450	401.11123	1.4E+06	4.63	399.10455	401.11124	1.1E+06
Serine	4.87	339.10044	341.10681	3.1E+07	4.85	339.10074	341.10736	3.5E+07
Homocitruline	4.87	423.05400	425.06122	9.0E+04	4.82	423.05421	425.06105	1.0E+05
Aspartic Acid	4.98	367.09604	369.10274	1.6E+06	4.41	367.09654	369.10293	8.1E+05
Glutamic Acid	4.98	381.11177	383.11842	5.4E+06	4.37	381.11216	383.11879	2.6E+06
4-Hydroxy-proline	5.28	365.11686	367.12335	1.9E+06	5.19	365.11686	367.12334	2.5E+06
Iminodiacetic acid	5.51	367.18005	369.18691	1.8E+05	5.54	367.18011	369.18674	1.5E+05
Aminoadipic acid	5.55	395.12737	397.13419	1.6E+05	5.04	395.12749	397.13418	1.6E+05
Threonine	5.67	353.11645	355.12281	4.8E+07	5.69	353.11569	355.12203	4.1E+07
Folic acid	5.67	338.09322	339.09608	1.3E+05	5.65	338.09370	339.09602	1.0E+05
Diethanolamine	5.89	339.13812	341.14430	1.3E+05	5.43	339.10069	341.10778	1.0E+05
Ethanolamine	6.01	295.11084	297.11751	3.1E+07	6.03	295.11089	297.11748	2.9E+07
Glycine	6.45	309.09053	311.09711	8.3E+06	6.48	309.09055	311.09713	8.9E+06
Tyrosine methyl ester	7.17	415.13278	417.13946	2.3E+06	7.19	415.13280	417.13962	2.5E+06
Alanine	7.32	323.10483	325.11142	4.7E+07	7.30	323.10474	325.11115	3.4E+07
r-aminobutyric acid	7.51	337.12197	339.12881	2.0E+05	7.49	337.12196	339.12889	2.5E+05
Hypoxanthine	8.20	370.09699	372.1037	8.5E+0	8.17	370.0970	372.1038	8.0E+0

			4	5		8	5	5
5-hydroxymethyluracil	8.29	376.09622	378.10303	7.7E+04	8.28	376.09634	378.10304	9.2E+04
3-Aminoisobutyric acid	8.40	337.12164	339.12881	1.2E+05	8.36	337.12161	339.12876	5.0E+04
5-Aminopentanoic acid	8.47	351.13762	353.14428	3.2E+05	8.51	351.13746	353.14420	3.0E+05
2-Aminobutyric acid	8.88	337.12177	339.12845	1.2E+07	8.88	337.12178	339.12848	1.1E+07
Sarcosine	9.11	323.10603	325.11305	4.0E+05	9.11	323.10606	325.11297	3.0E+05
Methylcysteine	9.34	369.09407	371.10086	5.2E+05	9.34	369.09416	371.10091	6.1E+05
Methylamine	9.67	265.10002	267.10625	1.8E+07	9.98	265.10037	267.10669	1.8E+07
Proline	9.90	349.12208	351.12859	1.4E+06	9.94	349.12209	351.12872	3.0E+05
Valine	10.49	351.13656	353.14283	7.6E+07	10.55	351.13723	353.14428	3.8E+07
Methionine	10.57	383.10980	385.11615	5.6E+06	10.58	383.10968	385.11644	4.1E+06
3-Hydroxypicolinic acid	10.59	373.11839	375.12536	6.0E+05	10.51	373.11907	375.12565	3.6E+05
Tryptophan	10.95	438.14844	440.15518	1.9E+07	10.96	438.14855	440.15501	1.9E+07
Pipecolic acid	12.18	363.13772	365.14433	6.9E+05	12.14	363.13775	365.14432	8.8E+05
Phenylalanine	12.22	399.13658	401.14312	4.6E+07	12.21	399.13653	401.14279	4.1E+07
3-Hydroxymandelic acid	12.48	402.10093	404.10768	3.4E+05	12.47	402.10095	404.10769	4.8E+05
Isoleucine	12.55	365.15103	367.15784	3.7E+07	12.52	365.15263	367.15925	2.9E+07
Leucine	12.81	365.15108	367.15779	8.1E+07	12.78	365.15155	367.15815	8.3E+07
L-norleucine	12.89	365.15326	367.15977	1.1E+07	12.90	365.15305	367.15967	4.0E+06
Cystine	13.15	354.06986	356.07653	1.2E+06	13.13	354.06967	356.07636	1.6E+06
Hydroxyphenyllactic acid	13.63	416.11628	418.12328	2.5E+06	13.61	416.11633	418.12328	3.5E+06
Homocystine	13.97	368.09889	370.10576	1.2E+05	13.98	368.09891	370.10574	1.6E+05
5-HIAA	14.20	425.11688	427.12363	4.7E+05	14.21	425.11702	427.12374	2.9E+05
Dimethylamine	14.43	279.11584	281.12256	2.7E+07	14.43	279.11583	281.12267	3.1E+07
Phenylpropanolamine	14.46	385.12213	387.12878	3.3E+05	14.47	385.12224	387.12862	3.6E+05
2,4-Diaminobutyric acid	14.75	293.13191	295.13866	7.1E+04	14.78	293.13187	295.13879	9.9E+04
L-ornithine	15.40	300.10353	302.11020	1.0E+07	15.42	300.10343	302.11008	1.2E+07
Homovanillic	15.62	416.11633	418.12326	3.6E+06	15.64	416.11644	418.12336	4.0E+06
Homocarnosine	15.99	354.11938	356.12593	2.4E+07	15.98	354.11868	356.12521	2.7E+07
3-/4-hydroxyphenylacetic acid	16.03	386.10591	388.11276	1.2E+06	16.02	386.10569	388.11261	1.1E+06
or 3-Cresotinic acid								
Lysine	16.48	307.11098	309.11759	1.9E+07	16.43	307.11055	309.11715	5.4E+07
4-Hydroxybenzoic acid	16.67	372.09026	374.09706	6.1E+06	16.69	372.09037	374.09721	4.8E+06
Histidine	16.93	311.59333	313.60000	1.1E+07	16.91	311.59335	313.60001	9.3E+06
2-aminooctanoic acid	18.02	393.18440	395.19113	2.5E+05	18.02	393.18428	395.19122	1.8E+05
1,4-diaminobutane	19.57	555.20947	559.22298	7.0E+04	19.60	555.21008	559.22345	9.4E+04
Tyrosine	20.24	324.59406	326.60071	8.2E+07	20.28	324.59436	326.60091	8.7E+07
Cysteamine	20.35	310.07501	312.08184	1.1E+05	20.35	310.07508	312.08207	2.3E+05
Metoprolol	20.3	501.16180	503.1687	5.9E+0	20.3	501.1622	503.1688	7.8E+0

	9		6	5	9	2	3	5
Phenol	20.73	328.10056	330.10719	8.8E+05	20.77	328.10071	330.10739	7.4E+05
Spermidine	22.82	423.16360	426.17365	1.6E+05	22.86	423.16369	426.17337	9.2E+04
Pyrocatechol	22.90	289.08262	291.08928	2.0E+05	22.90	289.08241	291.08923	4.5E+05
Thymol	23.84	384.16345	386.17007	4.7E+05	23.85	384.16351	386.17024	8.0E+05
Glucosamine	2.56	413.13797	415.14510	5.3E+04				
Methylguanidine	4.46	307.12228	309.12935	7.8E+04				
Tryptophanamide					8.13	437.13788	439.14465	4.3E+04
Norvaline					11.00	351.13776	353.14481	1.9E+05
Desaminotyrosine	17.04	400.12057	402.12704	3.0E+04				
L-Tyrosinamide					19.23	324.10352	326.10999	6.0E+04

Table S4.12. List of Positively Identified Metabolites in CSF Sample #4.

		CSF - #4				**CSF - #4 Repeat**		
Compound Name	**Rt**	**mz_light**	**mz_heavy**	**int**	**Rt**	**mz_light**	**mz_heavy**	**int**
phosphoethaolamine	2.29	375.07782	377.08455	6.2E+05	2.18	375.07807	377.08462	3.4E+05
Taurine	2.62	359.07327	361.07996	4.4E+06	2.63	359.07330	361.08000	5.9E+06
Glucosamine	2.62	413.13742	415.14457	6.0E+04	2.63	413.13842	415.14441	8.0E+04
1-methylhistidine	2.74	403.14384	405.15059	5.0E+05	2.74	403.14377	405.15043	5.0E+05
Arginine	3.19	408.17024	410.17698	4.2E+06	3.19	408.17026	410.17701	5.5E+06
Homoarginine	3.52	422.21107	424.21767	3.9E+05	3.55	422.21112	424.21780	4.4E+05
Asparagine	3.56	366.11200	368.11874	4.4E+06	3.47	366.11210	368.11882	3.6E+06
Glutamine	4.05	380.12753	382.13387	4.3E+07	4.09	380.12744	382.13372	3.9E+07
L-citrulline	4.18	409.15433	411.16076	1.2E+06	4.16	409.15433	411.16059	7.4E+05
3-sn-Phosphatidylethanolamine	4.41	484.13636	488.14956	8.9E+06	4.43	484.13638	488.14972	1.0E+07
Methylguanidine	4.48	307.12232	309.12923	7.5E+04	4.51	307.12238	309.12927	1.5E+05
Homoserine	4.59	353.11684	355.12373	2.7E+05	4.58	353.11600	355.12277	1.3E+05
Methionine sulfoxide	4.67	399.10459	401.11133	9.2E+05	4.66	399.10457	401.11135	7.0E+05
Serine	4.86	339.10070	341.10728	3.7E+07	4.86	339.10084	341.10754	3.7E+07
Aspartic Acid	4.98	367.09610	369.10267	1.4E+06	4.43	367.09646	369.10289	8.3E+05
4-Hydroxy-proline	5.28	365.11686	367.12336	2.8E+06	5.16	365.11684	367.12332	1.6E+06
Glutamic Acid	5.28	381.11181	383.11846	1.0E+06	5.31	381.11187	383.11850	9.2E+05
Aminoadipic acid	5.55	395.12737	397.13418	1.6E+05	5.05	395.12710	397.13434	1.5E+05
Threonine	5.70	353.11650	355.12311	2.8E+07	5.70	353.11667	355.12322	2.6E+07
Ethanolamine	6.00	295.11063	297.11738	2.6E+07	6.04	295.11077	297.11727	3.7E+07
Glycine	6.50	309.09050	311.09712	1.2E+07	6.47	309.09052	311.09702	1.2E+07

Tyrosine methyl ester	7.19	415.13281	417.13958	1.8E+06	7.18	415.13281	417.13952	1.3E+06
Alanine	7.30	323.10654	325.11287	1.7E+07	7.33	323.10662	325.11323	1.2E+07
r-aminobutyric acid	7.49	337.12205	339.12906	9.0E+05	7.49	337.12253	339.12899	8.0E+05
Hypoxanthine	8.18	370.09700	372.10378	6.0E+05	8.17	370.09712	372.10386	5.0E+05
3-Aminoisobutyric acid	8.42	337.12209	339.12866	1.4E+05	8.39	337.12203	339.12862	1.5E+05
5-Aminopentanoic acid	8.50	351.13753	353.14427	2.5E+05	8.47	351.13758	353.14422	2.5E+05
2-Aminobutyric acid	8.88	337.12183	339.12853	4.0E+06	8.88	337.12183	339.12852	3.7E+06
Sarcosine	9.11	323.10617	325.11307	5.5E+05	9.11	323.10623	325.11311	4.0E+05
Methylcysteine	9.34	369.09418	371.10089	6.2E+05	9.34	369.09418	371.10093	7.4E+05
Proline	9.89	349.12212	351.12868	8.4E+05	9.88	349.12205	351.12849	1.7E+06
3-Hydroxypicolinic acid	10.50	373.11875	375.12538	5.0E+05	10.49	373.11956	375.12600	4.0E+05
Valine	10.50	351.13704	353.14361	4.1E+07	10.53	351.13737	353.14389	4.0E+07
Methionine	10.54	383.10960	385.11626	5.5E+06	10.53	383.10929	385.11608	4.9E+06
Methylamine	10.83	265.10059	267.10715	6.3E+06	10.38	265.10062	267.10725	5.1E+06
Tryptophan	10.96	438.14852	440.15537	1.1E+07	10.95	438.14825	440.15527	8.4E+06
Pipecolic acid	12.15	363.13774	365.14432	9.0E+05	12.17	363.13764	365.14424	7.9E+05
Phenylalanine	12.19	399.13733	401.14413	3.6E+07	12.21	399.13719	401.14374	4.4E+07
3-Hydroxymandelic acid	12.47	402.10091	404.10774	3.6E+05	12.48	402.10075	404.10759	3.7E+05
Isoleucine	12.56	365.15310	367.15970	3.4E+07	12.55	365.15315	367.15970	2.9E+07
Leucine	12.79	365.15100	367.15817	5.3E+07	12.74	365.15321	367.15973	2.3E+07
L-norleucine	12.87	365.15344	367.16006	1.3E+07	12.90	365.15344	367.16003	7.6E+06
Cystine	13.12	354.07011	356.07690	1.0E+06	13.13	354.06969	356.07658	9.8E+05
Hydroxyphenyllactic acid	13.61	416.11636	418.12335	2.3E+06	13.63	416.11635	418.12340	2.3E+06
Homocystine	13.98	368.09899	370.10611	1.2E+05	13.98	368.09876	370.10579	1.5E+05
5-HIAA	14.19	425.11688	427.12372	5.9E+05	14.20	425.11682	427.12363	6.8E+05
Dimethylamine	14.44	279.11598	281.12248	1.9E+07	14.43	279.11594	281.12244	2.6E+07
Phenylpropanolamine	14.52	385.12238	387.12763	2.0E+05	14.51	385.12207	387.12829	2.5E+05
2,4-Diaminobutyric acid	14.78	293.13179	295.13887	3.9E+04	14.78	293.13152	295.13878	6.9E+04
L-ornithine	15.42	300.10344	302.11008	9.6E+06	15.43	300.10344	302.11008	7.8E+06
Acetaminophen	15.47	385.12210	387.12877	1.5E+06	15.47	385.12213	387.12877	1.6E+06
or 4-acetamidophenol								
Homovanillic	15.63	416.11636	418.12327	3.4E+06	15.62	416.11634	418.12328	3.6E+06
Homocarnosine	16.00	354.11958	356.12635	4.9E+06	16.00	354.11942	356.12617	6.3E+06
3-/4-hydroxyphenylacetic acid	16.00	386.10593	388.11282	5.6E+05	16.00	386.10601	388.11282	6.2E+05
or 3-Cresotinic acid								
Lysine	16.42	307.11079	309.11744	3.9E+07	16.41	307.11081	309.11744	3.7E+07
4-Hydroxybenzoic acid	16.70	372.09028	374.09712	3.8E+06	16.68	372.09019	374.09700	5.7E+06
Histidine	16.94	311.59332	313.59999	5.2E+06	16.94	311.59333	313.60003	5.2E+06

2-aminooctanoic acid	18.02	393.18454	395.19126	3.9E+05	18.05	393.18445	395.19114	4.7E+05
1,4-diaminobutane	19.59	278.10856	280.11554	3.7E+05	19.59	278.10850	280.11551	4.9E+05
Tyrosine	20.25	324.59506	326.60115	4.9E+07	20.28	324.59402	326.60121	6.4E+07
Metoprolol	20.44	501.16163	503.16876	2.6E+05	20.43	501.16214	503.16868	2.0E+05
Phenol	20.74	328.10043	330.10710	1.5E+06	20.78	328.10054	330.10721	1.7E+06
4-Nitrophenol	20.82	373.08597	375.09238	1.2E+05	20.82	373.08574	375.09234	2.5E+05
Spermidine	22.87	423.16330	426.17371	1.8E+05	22.88	423.16343	426.17276	1.4E+05
Thymol	23.87	384.16345	386.17024	8.1E+05	23.88	384.16348	386.17016	1.6E+06
Deoxyepinephrine	24.22	317.09012	319.09683	1.1E+05	24.24	317.09006	319.09690	1.6E+05
Homocitrulline	4.79	423.17029	425.17770	4.4E+04				
L-aspartic acid amide					4.58	366.11096	368.11743	6.6E+04
Diethanolamine	5.93	339.13803	341.14438	2.2E+05				
N-Methylaspartic acid	6.96	381.11215	383.11926	4.7E+04				
5-hydroxymethyluracil					8.28	376.09610	378.10303	5.3E+04
Cysteine-glutathione disulfide					9.07	447.10282	449.10953	6.5E+04
1,3-diaminopropane	19.09	271.10103	273.10723	7.5E+04				
Pyrocatechol					22.92	289.08225	291.08918	2.4E+05

Table S5.1. List of Carboxylic Acid Standards and Their Classes

Standard	Classs
Malonic acid	Dicarboxylic Acids
Benzoic acid	Aromatic Acids
3,4-Dihydroxybenzoic acid	Aromatic Acids
2,5-Dihydroxybenzoic acid	Aromatic Acids
3-Hydroxybenzoic acid	Aromatic Acids
Malic acid	Dicarboxylic Acids
Citric acid	Tricarboxylic Acids
Hippuric acid	Acyl Glycines
Homovanillic acid	Aromatic Acids
Octanoic acid	Fatty Acids
3-Indolylacetic acid	Indoles and Indole Derivatives
Hydrocinnamic acid	Aromatic Acids
Methylsuccinic acid	2-methylbutanedioic acid
Guanidineacetic acid	Amino Acids
2,3-Pyridinedicarboxylic acid	Aromatic dicarboxylic acids

4-Hydroxy-3-methoxymandelic acid	Aromatic acids
Pantothenic acid	Peptides
1,5-Dihydroxynaphthalene	Phenols
Phenylacetic acid	Aromatic Acids
Butyric acid	Fatty Acids
Propionic acid	Fatty Acids
Maleic acid	Dicarboxylic Acids
Nicotinic acid	Aromatic Acids
Vanillic acid	Aromatic Acids
Acetylsalicylic acid	Aromatic Acids
Salicylic acid	Aromatic Acids
Mucic acid; (Galactaric acid)	Dicarboxylic Acids
4-Hydroxybenzoic acid	Aromatic Acid
Trans-aconitic acid	Tricarboxylic Acids
Pyroglutamic acid	Amino Acids
a-Ketoglutaric acid	Dicarboxylic Acids
L-Tartaric acid	Dicarboxylic Acids
Kynurenic acid	Aromatic Acid
Oxaloacetic acid	Dicarboxylic Acids
Glycolic acid	Hydroxy Acids
2-Hydroxyisobutyric acid	Hydroxy Acids
2-Aminoadipic acid	Amino Acids
Retinoic acid	Retinoids
Folic acid	Pterins
γ-Aminobutyric acid	Amino Acids
p-Aminohippuric acid	Acyl Glycines
9-Anthracenecarboxylic acid	Aromatic Acids
Triphenylacetic acid	Aromatic Acids
γ-Oxo-1-pyrene-butyric acid	Aromatic Acids
N-Acetyl-L-glutamic acid	Amino Acids
N-acetyl-L-aspartic acid	Amino Acids
N-acetylglycine	Amino Acids
Biotin	Biotin and Derivatives
Creatine	Amino Acids
O-Phospho-L-tyrosine	Amino Acid Phosphates
O-phosphate-L-Threonine	Amino Acid Phosphates
O-Phospho-L-serine	Amino Acid Phosphates
L-Kynurenine	Amino Acids
Taurine	Amino Acids
L-Carnosine	Peptides
Carnitine	Carnitines
L-Ascorbic acid	Carbohydrates
DL-Ornithine	Amino Acids

3-Methyl-L-histidine	Peptides
***p*-Acetamidophenyl β-D-glucuronide**	Carboxylic Acids
Homocystine	Amino Acids
Homocysteine	Amino Acids
S-(2-Aminoethyl)-L-cysteine	Amino Acids
5-Methoxysalicylic acid	Aromatic Acids
3-Hydroxypicolinic acid	Aromatic Acids
L-Aspartic acid amide	Amino Acids
Norvaline	Amino Acids
Citrulline	Amino Acids
5-Hydroxyindole-3-acetic acid	Indoles and Indole Derivatives
Gly-Leu	Dipeptides
4-Aminobenzoic acid	Amino Acids
3-Aminobenzoic acid	Amino Acids
L-2-Aminobutyric acid	Amino Acids
L-5-Hydroxytrptophan	Amino Acids
Oxalic acid	Dicarboxylic Acids
Succinic acid	Dicarboxylic Acids
Itaconic acid	Dicarboxylic Acids
p-Coumaric acid (4-hydroxycinnamic acid)	Aromatic Acids
Uric acid	Purines and Purine Derivatives
Lactic acid	Hydroxy Acids
Chloroacetic acid	Carboxylic Acids
Formic acid	Carboxylic Acids
3,4-Dihydroxyphenylacetic acid	Aromatic Acids
Ferulic acid	Aromatic Acids
Sinapic acid; (3,5-Dimethoxy-4-hydroxycinnamic acid)	Aromatic Acids
4-Hydrazinobenzoic acid	Aromatic Acids
2-4(-hydroxyphenylazo)-benzoic acid (HABA)	Aromatic Acids
2-Ethoxybenzoic acid	Aromatic Acids
2-Amino-3-methylbenzoic acid	Aromatic Acids
3-amino-4-hydroxybenzoic acid	Aromatic Acids
Penafluorobenzoic acid	Aromatic Acids
2,5-Bis-(trifluoromethyl)benzoic acid	Aromatic Acids
1-Octanesulfonic acid	Sulfonic Acid
Taurine	Amino Acids
Cysteamine	thiols
Docosahexaenoic acid	Fatty Acids
Palmitoleic acid	Fatty Acids
Oleic acid	Fatty Acids
Linoleic acid	Fatty Acids
Linolenic acid	Fatty Acids

Erucic acid	Fatty Acids
5,8,11,14-Eiscosatetraenoic acid	Fatty Acids
Elaidic acid	Fatty Acids
Nervonic acid	Fatty Acids
Petroselinic acid	Fatty Acids
acetic acid	Carboxylic Acids
Heptadecanoic acid	Fatty Acids
Tricosanoic acid	Fatty Acids
Dodecanoic acid	Fatty Acids
Palmitic acid	Fatty Acids
Decanoic acid	Fatty Acids
Stearic acid	Fatty Acids
Myristic acid	Fatty Acids
Hexanoic acid	Fatty Acids

Table S5.2. Labeling Reproducibility and Yield of 10 Carboxylic Acid Standards

Compound	Ret Time	m/z	m/z	Reproducibility	Reaction Yield
	(min)	(light)	(heavy)	(CV%)	(%)
Acetic acid	5.54	222.112	224.119	7.2	96
Hippuric acid	6.87	341.148	343.156	3.4	98
4-Hydroxybenzoic acid	8.14	300.122	302.129	4.1	97
3-Hydroxybenzoic acid	8.66	300.122	302.129	2.2	99
Malic acid	9.50	457.197	461.210	3.6	95
Butyric acid	11.00	250.143	252.150	2.8	97
Malonic acid	12.60	427.187	431.201	6.1	95
Phenylacetic acid	13.80	298.144	300.150	4.5	96
Hydrocinnamic acid	16.80	312.159	314.166	3.7	99
Octanoic acid	26.53	306.206	308.212	5.1	95

Note: Reproducibility was calculated based on 4 repeated labeling experiments.

Table S6.1. List of ^{12}C-/^{13}C-Dansylated Standard Library Followed by LC/FTICR MS Detection

Compound Name	Ret. Time	mz_light	mz_heavy	Error in mass
	(min)			diff. (ppm)
Phospho-tyrosine	1.78	**495.09856**	**497.10503**	-0.5
Hydrochlorothiazide	2.01	**519.02323**	**521.03035**	0.8
Histidinol	2.05	**375.14900**	**377.15568**	-0.1
Phospho-serine	2.17	**419.06761**	**421.07428**	-0.1
O-phophoethaolamine	2.17	**375.07768**	**377.08431**	-0.2

Adenosine monophosphate	2.30	**581.11890**	**583.12488**	-1.2
Glucosamine	2.32	**413.13803**	**415.14459**	-0.3
r-glutamylcysteine	2.50	**366.58763**	**367.59120**	0.6
3-methyl-histidine	2.55	**403.14374**	**405.15042**	-0.1
Taurine	2.59	**359.07325**	**361.08000**	0.1
1-methylhistidine	2.60	**403.03694**	**405.04373**	0.2
Saccharopine	2.71	**510.19062**	**512.19755**	0.4
Phospho-threonine	2.77	**433.08309**	**435.08990**	0.2
Anserine	2.83	**474.18078**	**476.18792**	0.9
Carnosine	2.96	**460.16571**	**462.17270**	0.60
Hypotaurine	2.97	**343.07822**	**345.08480**	-0.4
Arginine	3.06	**408.17010**	**410.17675**	-0.1
Guanidine	3.37	**293.10688**	**295.11346**	-0.4
Histamine	3.38	**345.13823**	**347.14474**	-0.6
Asparagine	3.59	**366.11166**	**368.11810**	-0.7
Homoarginine	3.79	**422.18625**	**424.19241**	-1.3
1-methyl-histamine	3.98	**359.15390**	**361.16046**	-0.4
Glutamine	4.00	**380.12751**	**382.13371**	-1.3
Citrulline	4.19	**409.15430**	**411.16087**	-0.3
3-methyl- histamine	4.21	**359.15414**	**361.16066**	-0.5
3-sn-phosphatidylethanolamine	4.29	**484.13619**	**488.14917**	-0.9
Methylguanidine	4.45	**307.12241**	**309.12921**	0.3
Aspartic acid amide	4.56	**366.11191**	**368.11869**	0.2
Adenosine	4.60	**501.15540**	**503.16229**	0.4
Homoserine	4.61	**353.11569**	**355.12228**	-0.3
Triglycine	4.62	**423.13345**	**425.14013**	-0.1
Methionine sulfoxide	4.67	**399.10457**	**401.11122**	-0.1
Homocitrulline	4.80	**423.16988**	**425.17661**	0.1
Serine	4.89	**339.10076**	**341.10742**	-0.2
Glutamic acid	5.22	**381.11002**	**383.11642**	-0.8
Diglycine	5.27	**366.11183**	**368.11845**	-0.2
4-hydroxy-proline	5.32	**365.11536**	**367.12179**	-0.7
Glutathione	5.36	**234.05843**	**236.06510**	-0.2
Aspartic Acid	5.37	**367.09582**	**369.10262**	0.3
Iminodiacetic acid	5.63	**367.09581**	**369.10236**	-0.4
Threonine	5.73	**353.11635**	**355.12256**	-1.4
Aminoadipic acid	5.78	**395.12712**	**397.13365**	-0.5
Folic acid	5.78	**338.10290**	**339.10624**	0.0
Dopamine	5.85	**387.12369**	**389.13039**	0.0
Diethanolamine	5.90	**339.13571**	**341.14243**	0.0
Ethanolamine	5.96	**295.11095**	**297.11714**	-1.8
Epinephrine	6.20	**417.15926**	**419.16617**	0.5
Glycine	6.53	**309.09022**	**311.09641**	-1.7
Glycylproline	6.85	**406.14319**	**408.14964**	-0.6
Beta-alanine	7.06	**323.10508**	**325.11115**	-1.9
Tyrosine methyl ester	7.10	**415.13233**	**417.13910**	0.1
Lisinopril	7.12	**320.14584**	**321.14900**	-0.6
N-methyl-aspartic acid	7.14	**381.11167**	**383.11818**	-0.5
Alanine	7.42	**323.10504**	**325.11155**	-0.6
Aminolevulinic acid	7.50	**365.11651**	**367.12346**	0.7
r-aminobutyric acid	7.51	**337.12016**	**339.12669**	-0.5
Procaine	7.64	**470.21094**	**472.21783**	0.4
Pantothenic acid	7.68	**453.16937**	**455.17630**	0.5
Pyridoxal 5'-phosphate	7.90	**481.08290**	**483.08961**	0.0
p-aminohippuric acid	7.93	**428.12771**	**430.13450**	0.2
Salbutamol	8.12	**455.20001**	**457.20618**	-1.2
Hypoxanthine	8.16	**370.09691**	**372.10367**	0.1

Tryptophanamide	8.22	**437.16375**	**439.17035**	-0.2
Isoguanine	8.29	**385.10790**	**387.11458**	-0.1
5-hydroxymethyluracil	8.47	**376.09622**	**378.10274**	-0.5
5-aminopentanoic acid	8.49	**351.13560**	**353.14218**	-0.4
2-aminoisobutyric acid	8.60	**337.12084**	**339.12744**	-0.3
3-aminoisobutyric acid	8.65	**337.12120**	**339.12754**	-1.1
Ser-Leu	8.73	**452.18501**	**454.19143**	-0.6
2-aminobutyric acid	8.92	**337.12151**	**339.12792**	-0.9
Cysteine-glutathione disulfide	9.03	**447.10243**	**449.10928**	0.3
Sarcosine	9.12	**323.10494**	**325.11127**	-1.2
Pyridoxine	9.22	**403.13206**	**405.13913**	0.9
5-methoxytryptophan	9.36	**468.15837**	**470.16512**	0.1
Thr-Leu	9.48	**466.20079**	**468.20757**	0.1
Methylcysteine	9.56	**369.09373**	**371.10051**	0.2
Aminocaproic acid	9.85	**365.15130**	**367.15769**	-0.9
Proline	9.97	**349.12003**	**351.12646**	-0.8
Gly-Leu	10.49	**422.17276**	**424.17889**	-1.4
Salicyluric acid	10.49	**429.10866**	**431.11509**	-0.6
Valine	10.58	**351.13638**	**353.14254**	-1.6
3-hydroxylpicolinic acid	10.61	**373.08490**	**375.09203**	1.1
Methionine	10.70	**383.10958**	**385.11601**	-0.7
Gly-Trp	10.75	**495.16979**	**497.17677**	0.5
Ala-Leu	10.78	**436.18979**	**438.19643**	-0.1
Tryptophan	11.00	**438.14756**	**440.15359**	-1.5
Kynurenine	11.01	**442.14325**	**444.15000**	0.1
Norvaline	11.01	**351.13574**	**353.14237**	-0.2
4-aminobenzoic acid	11.01	**371.10572**	**373.11212**	-0.8
Ala-Trp	11.13	**509.18562**	**511.19179**	-1.1
Atenolol	11.16	**500.21732**	**502.22408**	0.1
Phenylephrine	11.24	**401.15310**	**403.15978**	-0.1
3-aminobenzoic acid	11.32	**371.10590**	**373.11225**	-1.0
2-phenylglycine	11.32	**385.11923**	**387.12579**	-0.4
Selenomethionine	11.48	**431.05159**	**433.05832**	0.1
3-aminosalicylic acid	11.85	**387.10094**	**389.10733**	-0.8
Ethylamine	11.85	**279.11627**	**281.12268**	-1.0
Diaminopimelic acid	12.08	**329.10576**	**331.11260**	0.4
Vanillylmandelic acid	12.17	**432.11118**	**434.11768**	-0.5
Hydroxyphenylacetylglycine	12.21	**443.12406**	**445.13079**	0.1
Phenylalanine	12.28	**399.13524**	**401.14126**	-1.7
Leu-Pro	12.29	**462.20548**	**464.21199**	-0.4
Acetyl-tyrosine	12.30	**457.13923**	**459.14597**	0.1
3-hydroxymandelic acid	12.33	**402.10068**	**404.10746**	0.2
alpha-aspartyl-lysine	12.58	**364.62477**	**366.63133**	-0.4
Isoleucine	12.62	**365.15118**	**367.15780**	-0.3
Pipecolic acid	12.73	**363.13701**	**365.14322**	-1.4
Cystathionine	12.74	**345.09219**	**347.09873**	-0.5
Leucine	12.87	**365.15119**	**367.15783**	-0.2
5-hydroxylysine	12.93	**315.10849**	**317.11512**	-0.2
Aspartame	13.13	**528.17991**	**530.18672**	0.2
Methyl-aminoisobutyric acid	13.19	**351.13732**	**353.14352**	-1.4
Phenylethanolamine	13.20	**371.14085**	**373.14709**	-1.3
4-hydroxy-3-methoxyphenyllactic acid	13.23	**446.12410**	**448.13036**	-1.0
Cystine	13.24	**354.06846**	**356.07518**	0.0
Norleucine	13.31	**365.15105**	**367.15794**	0.5
Hydroxyphenyllactic acid	13.60	**416.11543**	**418.12205**	-0.2
5-hydroxyindole-3-acetic acid (5-HIAA)	14.17	**425.11666**	**427.12282**	-1.3

Phenylpropanolamine	14.33	**385.15607**	**387.16266**	-0.3
Dimethylamine	14.41	**279.11563**	**281.12238**	0.2
6-hydroxynicotinic acid	14.50	**373.08565**	**375.09214**	-0.6
Homocystine	14.78	**368.08594**	**370.09254**	-0.3
Salicylic acid	14.82	**372.09018**	**374.09695**	0.1
2,3-diamino-proprionic acid	14.85	**286.08749**	**288.09399**	-0.7
2,4-diaminobutyric acid	14.86	**293.09489**	**295.10134**	-0.9
Ornithine	15.36	**300.10340**	**302.11005**	-0.2
Acetaminophen/4-acetamidophenol	15.45	**385.11931**	**387.12650**	1.2
5-methoxysalicylic acid	15.49	**402.09900**	**404.10560**	-0.3
Phe-Phe	15.50	**546.20564**	**548.21231**	-0.1
Homovanillic	15.55	**416.11368**	**418.12055**	0.4
ortho-hydroxyphenylacetic acid	15.59	**386.10354**	**388.10927**	-1.8
Methyl-phenylalanine	15.66	**413.15034**	**415.15663**	-1.0
2-pyrocatechuic acid	15.70	**388.08508**	**390.09143**	-0.9
5-methoxytryptamine	15.71	**424.16596**	**426.17263**	-0.1
Leu-Phe	15.79	**512.22023**	**514.22687**	-0.1
Syringic acid	15.79	**432.10865**	**434.11493**	-1.0
Homocarnosine	15.88	**354.11975**	**356.12628**	-0.5
3-hydroxyphenylacetic acid	15.89	**386.10339**	**388.11004**	-0.1
p-hydroxyphenylacetic acid	15.99	**386.10347**	**388.11003**	-0.4
3-cresotinic acid	16.04	**386.10582**	**388.11269**	0.4
Carnosine	16.09	**347.11189**	**349.11857**	-0.1
Gentisic acid	16.31	**388.08521**	**390.09180**	-0.3
Lysine	16.41	**307.11096**	**309.11760**	-0.2
Vanillic acid	16.43	**402.09853**	**404.10490**	-0.8
3-hydroxybenzoic acid	16.43	**372.09043**	**374.09738**	0.63
4-thialysine	16.44	**316.08963**	**318.09593**	-1.3
Isoferulic acid	16.46	**428.11354**	**430.12021**	-0.1
Aniline	16.65	**327.11508**	**329.12143**	-1.1
4-hydroxybenzoic acid	16.65	**372.08834**	**374.09480**	-0.7
Histidine	16.99	**311.59317**	**313.59974**	-0.4
Desaminotyrosine	17.02	**400.11928**	**402.12565**	-0.8
3-hydroxyanthranilic acid	17.05	**387.10104**	**389.10775**	0.0
Tryptamine	17.21	**394.15639**	**396.16264**	-1.2
Benzylamine	17.21	**341.13041**	**343.13672**	-1.2
Naringin	17.31	**524.14802**	**526.15451**	-0.4
m-coumaric acid	17.33	**398.10359**	**400.11036**	0.2
trans-ferulic acid	17.37	**428.11336**	**430.11982**	-0.6
Ephedrine	17.59	**399.17129**	**401.17769**	-0.8
6-dimethylamine purine	18.00	**397.14412**	**399.15083**	0.0
2-aminooctanoic acid	18.03	**393.18198**	**395.18846**	-0.6
4-hydroxy-3-methylbenzoic acid	18.16	**384.11751**	**390.13816**	1.3
Pyridoxamine	18.22	**318.10316**	**320.10995**	0.2
Pseudoephedrine	18.50	**399.17185**	**401.17803**	-1.3
5-hydroxyltryptophan	18.68	**344.10074**	**346.10749**	0.1
Estriol/17a-estradiol	18.70	**522.22638**	**524.23218**	-1.7
2-methylbenzyl amine	19.03	**355.14641**	**357.15243**	-1.9
1,3-diaminopropane	19.03	**271.10019**	**273.10678**	-0.4
Tyrosinamide	19.15	**324.10230**	**326.10866**	-1.1
1,2-diaminopropane	19.36	**271.10006**	**273.10679**	0.1
Umbelliferone	19.43	**396.09023**	**398.09697**	0.1
1,4-diaminobutane	19.59	**278.10788**	**280.11431**	-1.0
3,4-dihydroxymandelic acid	19.80	**326.07718**	**328.08405**	0.5
o-Tyrosine	20.04	**324.59533**	**326.60190**	-0.4
Metoprolol	20.08	**501.23800**	**503.24396**	-1.5
Thyroxine	20.20	**505.87677**	**506.87974**	-0.8

Cadaverine	20.21	**285.11524**	**287.12190**	-0.2
3-nitrotyrosine	20.29	**347.08814**	**349.09469**	-0.4
Tyrosine	20.30	**324.59412**	**326.60083**	0.0
Melatonin	20.38	**466.08352**	**468.09062**	0.8
Phenol	20.73	**328.09925**	**330.10558**	-1.1
Cysteamine	20.74	**310.07964**	**312.08612**	-0.7
3-chlorotyrosine	20.79	**341.57462**	**343.58132**	-0.1
4-nitrophenol	20.82	**373.08419**	**375.09053**	-1.0
16b-hydroxyestradiol	20.84	**522.22632**	**524.23206**	-1.9
Iodotyrosine	20.89	**387.54107**	**389.54752**	-0.7
Octopamine	20.91	**310.59679**	**312.60337**	-0.4
4,9-dioxa-1,12-dodecanediamine	21.11	**336.14904**	**338.15562**	-0.4
Protocatechuic acid	21.38	**311.07179**	**313.07852**	0.1
m-Cresol	21.44	**342.11615**	**344.12265**	-0.6
Gentisic acid	21.50	**311.07180**	**313.07861**	0.34
p-Cresol	21.51	**342.11492**	**344.12113**	-1.5
Serotonin	21.52	**322.10610**	**324.11268**	-0.4
o-Cresol	21.70	**342.11604**	**344.12262**	-0.4
3,4-dihydroxybenzeneacetic acid	21.75	**318.14326**	**319.14672**	0.3
Caffeic acid	21.75	**647.27906**	**649.28617**	0.6
Metanephrine	21.77	**332.60988**	**334.61650**	-0.3
4-aminophenol	21.84	**288.58517**	**290.59183**	-0.2
Propranolol	21.88	**493.21279**	**495.21868**	-1.7
Thyronine	21.88	**370.60667**	**372.61325**	-0.4
Piperazine	21.89	**277.10020**	**279.10662**	-1.0
Phenylephrine/Synephrine	21.93	**317.60446**	**319.61107**	-0.3
Phenylephrine/Synephrine	21.95	**317.60447**	**319.61131**	0.4
Tyramine	22.31	**302.59982**	**304.60640**	-0.4
Pyrocatechol	22.70	**577.14616**	**581.15958**	0.0
Diiodothyronine	22.76	**496.50585**	**498.51217**	-0.8
Homogentisic acid	22.78	**318.07981**	**320.08643**	-0.3
Spermidine	22.84	**423.16340**	**426.17322**	-0.6
Xanthurenic acid	22.89	**336.57761**	**338.58414**	-0.5
Estradiol	23.00	**506.23201**	**508.23797**	-1.5
2,4-dichlorophenol	23.10	**396.02103**	**398.02755**	-0.5
3-isopropylphenol	23.10	**370.14582**	**372.15228**	-0.7
4-isopropyl phenol	23.12	**370.14576**	**372.15220**	-0.7
Estrone	23.71	**504.21619**	**506.22223**	-1.3
4-methylcatechol	23.78	**591.16180**	**595.17531**	0.2
Norepinephrine	23.83	**435.12139**	**438.13130**	-0.4
Hydroquinone	23.99	**577.14618**	**581.15929**	-0.5
Thymol	24.02	**384.16127**	**386.16789**	-0.2
Deoxyepinephrine	24.20	**317.09020**	**319.09688**	-0.1
Desipramine	24.36	**500.23729**	**502.24422**	0.4
Naringenin	24.78	**486.61873**	**489.62858**	-0.4