# Theoretical understanding and method development for high performance liquid chromatography-mass spectrometry for environmental analysis

by

Caley Brooke Craven

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## Abstract

Method development is a fundamental key step for effective and efficient analytical separations and analyses. Of particular importance is method development for high performance liquid chromatography-mass spectrometry (HPLC-MS). Specifically, environmental analysis applications require dedicated method development to help solve the complex questions associated with any environmental sample. To effectively make methods for environmental analysis, a firm understanding of HPLC and MS is needed. **Chapter 1** includes a review on the theory of the instrumentation and methodology used in this thesis.

In **Chapter 2**, the selectivity and retention changes caused by mobile phase on four classes of hydrophilic interaction liquid chromatography (HILIC) columns were investigated. The effect of $Na^+Cl^-$, $Na^+ClO_4^-$, $Na^+PF_6^-$, and $Na^+CF_3CO_2^-$ mobile-phase additives were explored for neutral, cationic, anionic, and zwitterionic analytes under HILIC conditions of 70–90% acetonitrile. These electrolytes altered the retention and selectivity on silica, zwitterionic and diol columns. Neutral analytes were unaffected. Cationic and anionic analytes increase and decrease in retention, respectively, by the following order of electrolytes: $Na^+PF_6^- \approx Na^+ClO_4^- > Na^+CF_3CO_2^- > Na^+Cl^-$. Altering the buffer cation ($Li^+$, $Na^+$, $K^+$) caused small but statistically significant changes in retention. The accumulation of chaotropic ions at the ACN/water interface disrupt both the water layer and other ionic interactions, causing the changes seen in retention and selectivity.

In **Chapter 3**, the effect of mobile phase preparation in HILIC were examined. Three different eluent preparation methods were examined. Due to the contraction of solvent upon mixing, different eluent preparation methods result in significant differences in the retention in

HILIC. Additionally, differences in the buffer counterions used affected retention in HILIC, but to a less degree than the mobile phase preparation method.

In the latter half of the thesis, I moved from fundamental studies of HILIC to practical applications of HPLC-MS to show the importance of analytical method development for authentic environmental samples. **Chapter 4** provides a critical review of literature on cannabis and its associated pesticides used to aid its growth. This reviewed the history of cannabis and its current prevalence. Additionally, **Chapter 4** reviews the current analytical challenges and method development for the detection of pesticides in cannabis. This particularly focuses on current guidelines set by Health Canada to ensure the quality, safety, and efficacy of cannabis and cannabis products.

In **Chapter 5**, a high performance liquid chromatography mass spectrometry method was developed for the determination of pesticides in cannabis growing facilities. This method involved a simple wipe-sampling method with HPLC-MS/MS and was able to determine 82 of the 96 pesticides regulated by Health Canada. This method's applicability was demonstrated in two separate cannabis growing facilities, a certified and non-certified site. A total of 41 pesticides were detected at the non-certified site while only 6 were observed at the certified site.

In **Chapter 6**, a preliminary study is provided to highlight current gaps in method development in environmental analyses. This study examines the use of HILIC-MS/MS with water analysis to show challenges in method development and application. Proposed future work revolves around minimizing or overcoming these challenges and gaps. This thesis highlights the importance of thorough and sensitive analytical methodologies to answer important environmental analysis questions.

## Preface

This thesis is based on manuscripts that have been published in or are in preparation for submission for publication. All manuscripts included in this thesis were majorly written by Caley B. Craven, with critical comments provided by Charles A. Lucy and Xing-Fang Li. Contributions of any other authors are described below.

**Chapter 1: Introduction**

This chapter was written by Caley B. Craven with critical comments by Charles A. Lucy and Xing-Fang Li.

**Chapter 2: Effect of electrolytes on retention and selectivity in hydrophilic interaction liquid chromatography**

This chapter was published as Caley B. Craven, Cole W. Joyce, and Charles A. Lucy, Effect of nature of electrolytes on retention and selectivity in hydrophilic interaction liquid chromatography, *Journal of Chromatography A*, **2019**, *1584*, 80-86. Preliminary studies were performed by Cole Joyce. All final experiments within the manuscript and the writing of the manuscript were done by Caley B. Craven. Charles A. Lucy provided critical comments and assisted with the writing of the manuscript.

**Chapter 3: Eluent Preparation for Hydrophilic Interaction Liquid Chromatography**

This chapter was published as Charles A. Lucy, Caley B. Craven, Claudia Seidl, and Dwight Stoll (column editor), Eluent Preparation for HILIC Separations, Part I: Solvent Volumes and Buffer Counterions, *LCGC North America*, **2018**, *36*, 18-24. Reprinted in LCGC Europe **2018**, *31*, 22-27. Conception of the study and all its experiment were by Caley B. Craven. The original manuscript was written with Charles A. Lucy and Dwight Stoll, but was reformatted and written for this thesis by Caley B. Craven with critical comments provided by Charles A. Lucy.

**Chapter 4: Pesticides in cannabis: analytical and environmental challenges and opportunities**

This chapter was published as Caley B. Craven, Nicholas Wawryk, Ping Jiang, Di Wu, and Xing-Fang Li. Pesticides and trace elements in cannabis: Analytical and environmental challenges and opportunities, *J. Environ. Sci.*, **2019**, *85*, 82–93. For the paper, Caley B. Craven had primary responsibility for pesticides and Nicholas Wawryk was responsible for trace elements. The majority of writing was a collaboration of Caley B. Craven and Nicholas Wawryk. The chapter focuses solely on pesticides and was written by Caley B. Craven with critical comments provided by Charles A. Lucy and Xing-Fang Li.

**Chapter 5: Determination of eighty-two pesticides and application to screening pesticides in cannabis growing facilities**

This chapter was published Caley B. Craven, Afsoon Birjandi, Brigitte Simons, Ping Jiang, and Xing-Fang Li. Determination of eighty-two pesticides and application to screening pesticides in cannabis growing facilities, *J. Environ. Sci.*, **2021**, *104*, 11–16. The majority of experiments were done by Caley B. Craven. Samples were collected by Afsoon Pajand Birjandi and Ping Jiang (collaborators). As well, Afsoon Pajand Birjandi ran the collected samples on the 5600 mass spectrometer. All data was analyzed by Caley B. Craven. The chapter was written by Caley B. Craven with critical comments provided by Charles A. Lucy and Xing-Fang Li.

**Chapter 6: Conclusion and Future Directions**

Preliminary development of methods for amino acid analysis in authentic source water was performed by Di Wu. Further adjustment, application, and critical evaluation of the preliminary methods was done by Caley B. Craven. This chapter was written by Caley B. Craven with

critical comments by Charles A. Lucy and Xing-Fang Li. Implementation of the improved methods was performed by others in spring 2021, and is not included in this thesis.

**Other publications that arose during the time of my PhD studies, but are not included in this thesis are:**

Garza, L., Jones, M., Craven, C. B., Lucy, C. A., & Davis, E. J. 3D printing lifts the lid on the black box instruments. Analytical and Bioanalytical Chemistry, accepted September 20th, 2021.

Tang, Y.,* Craven, C. B.,* Wawryk, N., Qiu, J., Li, F., & Li, X.F. Advances in mass spectrometry-based omics analysis of trace organics in water. TrAC Trends in Analytical Chemistry, 2020, 128, 115918.

Liu, Z. S., Craven, C. B., Huang, G., Jiang, P., Wu, D., & Li, X. F. (2019). Stable isotopic labeling and nontarget identification of nanogram/liter amino contaminants in water. Analytical Chemistry, 91 (20), 13213-13221. doi:10.1021/acs.analchem.9b03642

Qiu, J., Zhang, Y., Craven, C. B., Liu, Z., Gao, Y., & Li, X. F. (2021). Nontargeted identification of an N-heterocyclic compound in source water and wastewater as a precursor of multiple nitrosamines. Environmental Science & Technology, 55 (1), 385-392. doi:10.1021/acs.est.0c06109

Wawryk, N.,* Craven, C. B.,* Blackstock, L. K., & Li, X. F. (2021) New methods for identification of disinfection byproducts of toxicological relevance: Progress and future directions. Journal of Environmental Sciences, 99, 151-159. doi:10.1016.j.jes.2020.06.020

Craven, C. B,* Blackstock, L. K.,* Xie, J., Yuan, C., Li, J., & Li, X. F. (2021) Analytical discovery of water disinfection byproducts of toxicological relevance: highlighting halobenzoquinones. Canadian Journal of Chemistry, Just-IN access. doi: 10.1139/cjc-2021-0036

My role in the Garza et al. paper was assisting in checking the 3D prints on different printers and providing edits and comments on the 3D print designs and manuscript. My role in the Tang, Craven, et al. paper was equally shared with my co-author in writing the manuscript. My role in the Lui et al. paper was providing some assistance in data analysis, and a larger role in writing the manuscript. My role in the Qui et al. paper was providing some assistance in data analysis, and a larger role in writing the manuscript. My role in the Wawryk, Craven, et al. review was equally shared with my co-author in writing of the manuscript. My role in the Craven, Blackstock, et al. paper was equally shared with my co-author in writing of the manuscript.

*"What you do makes a difference, and you have to decide what kind of difference you want to make"*

*- Jane Goodall*

*"There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after"*

*- J.R.R. Tolkien, The Hobbit*

## Acknowledgments

To express the gratitude and thanks I have for every person that has helped or supported me along this journey would be an arduous task. There are many I would love to personally name, but just know that even if your name isn't listed here, you have my deepest gratitude.
First, I would like to thank my two supervisors Dr. Charles A. Lucy and Dr. Xing-Fang Li. Without their constant support and wisdom, I would not have been able to achieve what I have during this Ph.D. journey. They have provided me with so many opportunities to learn and better myself, while helping me build my confidence and knowledge into the Scientist, and person, I am today. It was truly a blessing to be able to learn from the both of you.
I also would like to thank all the members of the Departments of Chemistry and of Analytical & Environmental Toxicology, that have made this journey so much better with their continued support and kindness. I would like to especially thank Katerina Carastathis and Anita Weiler for their constant support. As well as amazing collaborators like Brigitte Simons, Afsoon Pajand Birjandi, Ping Jiang, Claudia Seidl, and Dwight Stoll. Additionally, I would also like to thank the members of my research group, who have been there to support and guide me. Without the warm, caring, and enjoyable environment created by you all, I would not have enjoyed this time as much as I did. I especially thank Dr. Lindsay Jmaiff-Blackstock, Nicholas Wawryk, Teresa Kumblathan and Dr. Birget Moe. You have provided me with an incredible working environment and the best support system during the ups and downs we all inevitably face throughout our degree. I couldn't have asked for better lab mates.

Finally, I would like to thank the people directly outside of my research environment. While my research and progress in my Ph.D. journey have been the focus of my life for the last few years, I would not have been able to keep that focus if it weren't for my friends and family

supporting me. They have showered me with unconditional love and support, while forcing me to take breaks, step back, and enjoy life along the way. This body of work would not have been achievable without all of you, and this is as much your success as mine. I truly hope you can be proud of everything over the last few years. To my Mom and Dad, Shannon and Richard, who are my biggest supporters and always there, I don't have the words to truly express what I feel towards you both. To my siblings, Chad and Cody, you are my rocks who always keep me centered. And finally, to my friends Alexis, Beatrice, Brittany, Chelsea, Jordan, Nicole, Taylor, and so many more, you gave me the courage to keep going and filled this journey with so much joy. To everyone, thank you so much for they joy, laughter, frustrations, and memories we have made, I can never tell you all how much this has meant to me.

## Contents

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## List of Symbols and Abbreviations

| | |
|---|---|
| A | Absorbance |
| AA | Amino acids |
| ACN | Acetonitrile |
| AIDS | Acquired immunodeficiency syndrome |
| APCI | Atmospheric pressure chemical ionization |
| APHL | American Public Health Laboratories |
| APPI | Atmospheric pressure photoionization |
| b | Pathlength |
| BA | Benzoic acid |
| BC | British Columbia |
| BS | Benzene sulfonic acid |
| BTBA | Benzyltripropyl ammonium chloride |
| BTEA | Benzyltriethyl ammonium chloride |
| BTMA | Benzyltrimethyl ammonium chloride |
| C | Concentration |
| CE | Capillary electrophoresis |
| CE | Collision energy |
| CI | Chemical ionization |
| CID | Collision induced dissociation |
| $C_{MP}$ | Concentration in the mobile phase |
| $C_{SP}$ | Concentration in the stationary phase |

| | |
|---|---|
| CXP | Cell exit potential |
| Cyt | Cytosine |
| DBDI | Dielectric barrier discharge ionization |
| DC | Direct current |
| DP | Declustering potential |
| dSPE | dispersive solid phase extraction |
| EI | Electron impact |
| EIC | Extracted ion chromatogram |
| EPA | United States Environmental Protection Agency |
| ERLIC | Electrostatic repulsion liquid chromatography |
| ESI | Electrospray ionization |
| FA | Formic acid |
| GC | Gas chromatography |
| HC | Health Canada |
| HILIC | Hydrophilic interaction liquid chromatography |
| HPLC | High performance liquid chromatography |
| K | Equilibrium constant |
| $k$ | Retention factor |
| L | Column length |
| LC | Liquid chromatography |
| LLE | Liquid-liquid extraction |
| LOD | Limit of detection |
| LOQ | Limit of quantification |

| | |
|---|---|
| m/z | mass-to-charge |
| MAL | Maximum allowable levels |
| MeOH | Methanol |
| MP | Mobile phase |
| MRL | Maximum residual levels |
| MRM | Multiple reaction monitoring |
| MS | Mass spectrometry |
| MS/MS | Tandem mass spectrometry |
| ND | Not determined |
| NPLC | Normal phase liquid chromatography |
| pH | -log $A_{H+}$ |
| ${}_{w}^{w}$pH | pH measured in the aqueous phase |
| Phe | Phenylalanine |
| ppb | parts per billion |
| ppm | parts per million |
| ppt | parts per trillion |
| q | Collision cell |
| Q | Quadrupole mass analyzer |
| QA/QC | Quality assurance/quality control |
| QTRAP | Quadrupole ion trap |
| QuEChERS | Quick, easy, cheap, effective, rugged, and safe |
| $R^2$ | Coefficient of determination |
| RF | Radio frequency |

| | |
|---|---|
| RPLC | Reversed phase liquid chromatography |
| SD | Standard deviation |
| SP | Stationary phase |
| SPE | Solid phase extraction |
| SPME | Solid phase microextraction |
| SRM | Selected reaction monitoring |
| $t_0$ | Dead time |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| TOF | Time of flight |
| $t_R$ | Retention time |
| Trp | Tryptophan |
| Tyr | Tyrosine |
| $u_x$ | Linear velocity |
| UN | United Nations |
| Ura | Uracil |
| US | United States |
| USA | United States of America |
| UV | Ultraviolet |
| WHO | World Health Organization |
| ZIC | Zwitterionic |
| $\alpha$ | Selectivity |
| $\varepsilon$ | Molar absorptivity |

| | |
|---|---|
| %RSD | % Relative standard deviation |
| 4-Aba | 4-aminobenzoic acid |

# Chapter 1

# Introduction

## 1.1 Motivation

Method development is a fundamental key step for effective and efficient analytical separations and analyses. Of particular importance is method development for high performance liquid chromatography-mass spectrometry (HPLC-MS). Specifically, environmental analysis applications require dedicated method development to help solve the complex questions associated with any environmental sample. To effectively make methods for environmental analysis, a firm understanding of HPLC and MS is needed.

Liquid chromatography (LC) was initially developed in the early 1900s to separate compounds based on their polarity.[1, 2] This type of separation was termed Normal Phase Liquid Chromatography (NPLC). Later, Reversed Phase LC (RPLC) was developed to separate compounds based on their hydrophobicity. However, both NPLC and RPLC struggle to separate samples that are either highly polar or only soluble in water. This issue was resolved in 1990 when Hydrophilic Interaction Liquid Chromatography (HILIC) was developed.[3] Similar to NPLC, HILIC can separate polar compounds. But unlike NPLC, HILIC uses water and acetonitrile as a mobile phase, which is more similar to RPLC.[3-7] While these modes of chromatography provide a range of separation capabilities, further development into mobile phase modifiers has been done to expand the range of separation each mode can achieve.[6, 8-21]

Understanding of core components of mass spectrometry is necessary to optimize the method. This is particularly important for trace analytes in complex sample matrices typically seen in environmental analysis.[22-27] A thorough understanding of the main components and what each one is responsible for is needed. This allows for minute adjustments that can drastically

alter the sensitivity of a mass spectrometer. These details will be discussed in the following sections.

## 1.2 Introduction to Chromatography

As an analytical chemist, we are constantly trying to solve a question. The most important question we face is what the chemical composition of a sample is, and the quantification of each component. To do this, we analytical chemists have a variety of tools to breakdown and analyze these problems. One tool, which has been a major focus for me, is liquid chromatography (LC). Liquid chromatography's origin comes from the work of Russian botanist Mikhail Tswett, who wanted to separate the pigments of plants. As such, the name chromatography comes from the Greek word *chroma* (color) and *graphien* (to write).[1, 28] Since then, chromatography has continued to advance and develop as a tool. The turning point was in the 1940's when A. J. P. Martin developed this simple chromatography process into paper-based LC, thin-layer LC, and partition chromatography. Martin and Richard Synge received the 1952 Nobel prize for their development of Partition Chromatography. LC later developed into High Pressure Liquid Chromatography (HPLC) in the 1960's when J.C. Giddings published an article talking about LC conditions with high pressure. Csaba Horvath is generally credited with developing the first HPLC instruments.[29] Since then, the field of HPLC separations has grown at a rapid pace. With this exuberant growth came an understanding of basic principles involved in HPLC separations.

## 1.3 Theory of Liquid Chromatography

A chromatographic separation is based on equilibration between the mobile phase and stationary phase. Dependent on the mode of liquid chromatography employed, the mechanism and parameters involved can be different. The following sections address the general mechanism, and those specific to RPLC and HILIC separations.

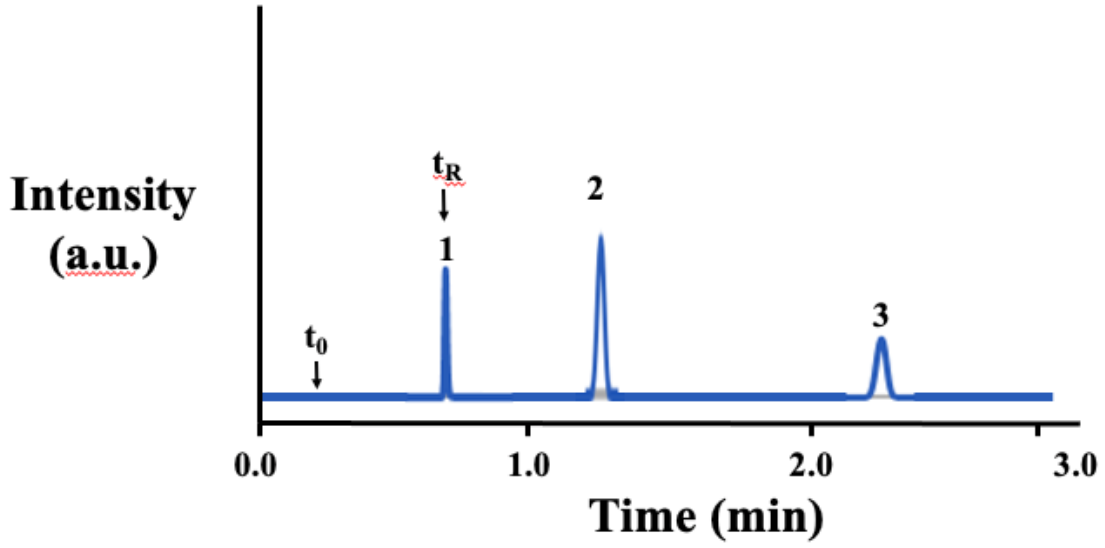


**Figure 1-1. Simple chromatogram depicting dead time ($t_0$) and retention time ($t_R$).** This chromatogram represents an isocratic separation.

### 1.3.1 Retention and Selectivity[30, 31]

Time in a chromatogram is recorded from when a sample is injected into the system. If an analyte is completely unretained, it will have no interaction with the stationary phase and be eluted at the dead time. Thus, the dead time ($t_0$) is the time required for an unretained compound to elute from a column, as shown in **Figure 1-1**. In other words, if a separation is run at constant

velocity $u_x$ (cm/min), and the column length is L (cm), then the time it takes for the mobile phase or an unretained compound to pass through the system is:

$$t_0 = L / u_x \quad (1\text{-}1)$$

The time required for an analyte to elute from the column is its retention time, $t_R$, as shown in **Figure 1-1**. The retention factor $k$ is the ratio of the moles of analyte in the stationary phase (SP) to that in the mobile phase (MP). The quantity of analyte in each phase is equal to the concentration in each phase ($C_{SP}$ or $C_{MP}$) multiplied by the volume of that phase. This relationship yields:

$$k = C_{SP} V_{SP} / C_{MP} V_{MP} = \beta K \quad (1\text{-}2)$$

This equation can be replaced the by phase ratio ($\beta$), which is the ratio of volume of the SP ($V_{SP}$) to MP ($V_{MP}$), and $K$, the equilibrium constant. $K$ represents the distribution of the concentration of analyte between the SP and MP. Retention factor can also be described as the ratio of time the analyte spends in the SP ($t_R - t_0$) to the time spent in the MP ($t_0$):

$$k = (t_R - t_0)/ t_0 \quad (1\text{-}3)$$

Selectivity ($\alpha$) is the relative retention factor $k$ of two adjacent analytes in a separation. That is, in **Figure 1-2** there are 5 analytes. The selectivity can be expressed between any two analyte peaks. The retention factor of the later eluted analyte is always divided by the retention factor of the earlier eluting. For example, selectivity of analyte 5 to 4 would give $\alpha > 1$ and represented by:

$$\alpha = k_5 / k_4 \quad (1\text{-}4)$$

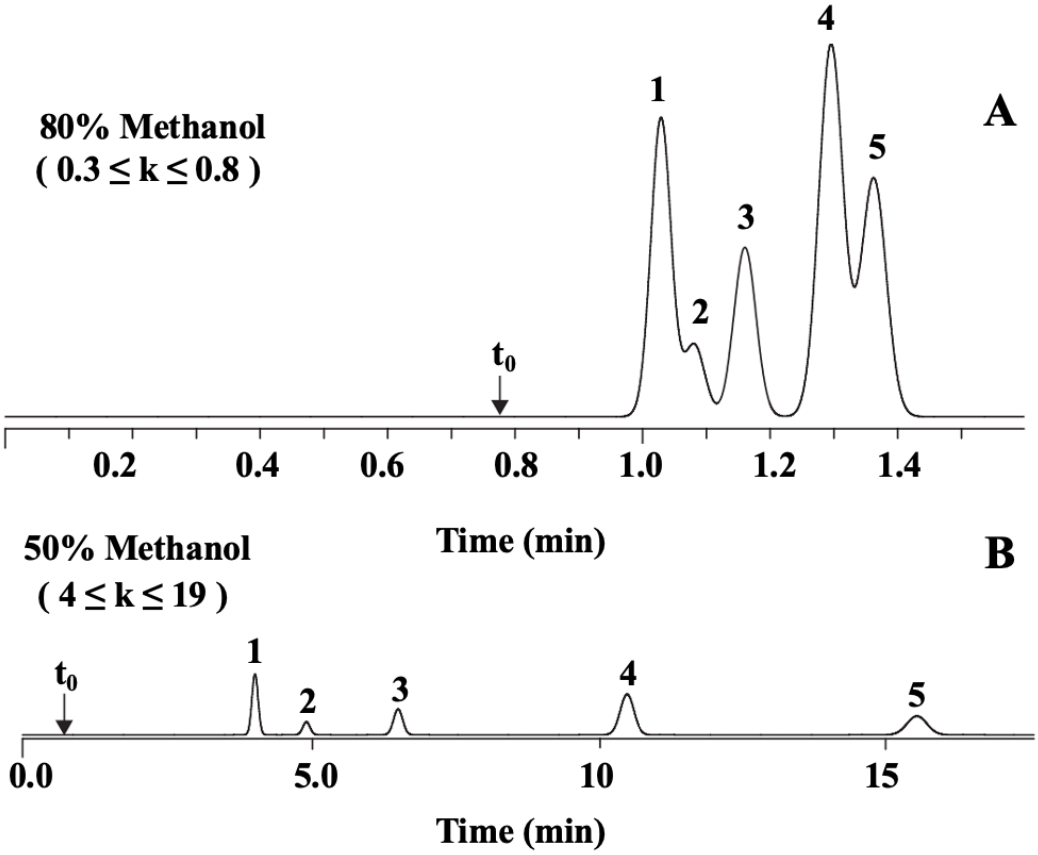


**Figure 1-2. Representative reversed phase separation at two different mobile phase compositions; A) 80% methanol, B) 50% methanol.** Sample: 1, monolinuron; 2, metobromuron; 3, diuron; 4, propazine; 5, chloroxuron. Conditions: 150 × 4.6 mm, 5 μm particles, C18 column; methanol/water mobile phase; 2.0 mL/min; ambient temperature. Figure adapted from Figure 2.6 of reference 31.[31]

### 1.3.2 Liquid Chromatography Modes

Modern Liquid Chromatography has a wide range of applications due to the availability of different LC modes. Typical HPLC stationary phases consist of 1.5–5 µm porous silica particles, whose surface has been covalently functionalized with a bonded phase (**Figure 1-3**). The liquid chromatography mode is dictated by the characteristics (e.g., polarity) of the bonded phase. The following sections focus on the use and mechanism of Reversed Phase (RPLC) and Hydrophilic Interaction Liquid Chromatography (HILIC). The main difference in these modes can be explained by the chemical properties of the solid phase and mobile phase.

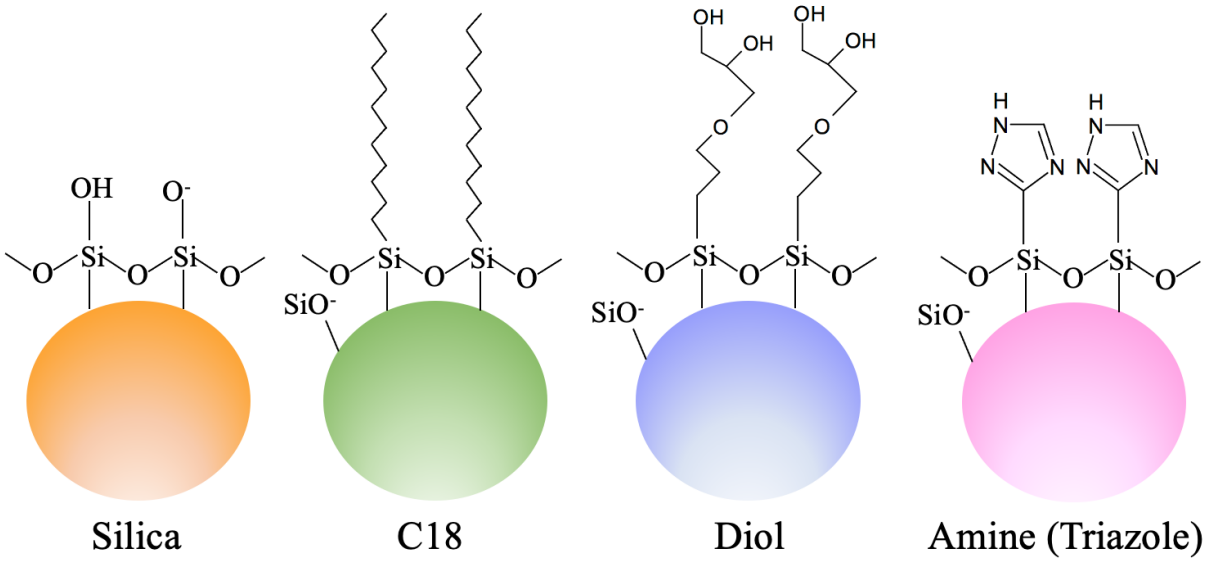


**Figure 1-3. Simple representation of HPLC stationary phases.**

#### 1.3.2.1 Reversed Phase (RP)

Reversed phase separations use a nonpolar stationary phase, such as the C18 phase shown in **Figure 1-3**, and a more polar mobile phase. The term "reversed phase" comes from the "reversal" of normal phase; coined as "normal" phase from first uses having a polar stationary phase and non-polar mobile phase. In reversed phase, compounds that have a low polarity are more strongly retained on the stationary phase, while compounds with a high polarity interact weakly with the stationary phase, and so are weakly retained. Common bonded phases, like C18 (**Figure 1-3**), are often considered to be a liquid phase into which analytes dissolve. Thus, compounds *partition* between the two liquid phases. The degree to which a compound partitions into each phase determines its retention. If a polar compound is considered, it will predominantly spend time, or partition, into the polar mobile phase, and thus elute quickly. Whereas a nonpolar compound will be retained longer, as it predominantly spends time, or partitions, into the nonpolar stationary phase.

The degree that an analyte partitions into the stationary phase is altered by changing the polarity of the mobile phase. The mobile phase is a mixture of two miscible solvents, typically water and either methanol or acetonitrile. Water is polar and so a weak mobile phase in RPLC, whereas methanol and acetonitrile are less polar and thus a stronger mobile phase component. We can increase or decrease the strength, or polarity, of the mobile phase by adjusting the ratio of water to organic modifier. Decreasing the percent methanol in the mobile phase, increases the partitioning of a hydrophobic analyte into the stationary phase, resulting in longer elution times. **Figure 1-2** illustrates this behaviour for the separation of 5 nonpolar compounds. The retention of the compounds at 80% methanol (**Figure 1-2 a**) have a maximum $k$ of 0.8 and at 50% methanol have a maximum $k$ of 19 (**Figure 1-2 b**). As the mobile was adjusted from 80% to 50%

methanol, the strength of the mobile phase was decreased, i.e., its polarity increased. Due to the decreased strength of the mobile phase, compounds partitioned more into the stationary phase, and were thus retained longer.

Retention and selectivity in reversed phase can also be altered by the type of stationary phase used. Reversed phase stationary phases typically use a silica backbone with nonpolar groups covalently bonded to the silica surface, as shown in **Figure 1-3**. Differences in the types of interactions that each bonded phase and the underlying silica provide alters the strength of the interaction with the stationary phases. These interactions include hydrogen-bonding, dipole-dipole interactions, London dispersion forces, etc. Choosing a stationary phase based on the type of compounds being examined improve method development and the ability to separate compounds effectively.

#### 1.3.2.2 Hydrophilic Interaction Liquid Chromatography (HILIC)

HILIC uses a polar stationary phase and a slightly less polar mobile phase.; the opposite of reversed phase. **Figure 1-4** shows that there is a complete reversal of retention order of the same 5 compounds between reversed phase and HILIC. A HILIC mobile phase consists of water and organic modifier, typically 70-95% acetonitrile. The term Hydrophilic Interaction Liquid Chromatography (HILIC) was first reported by Andy Alpert in the early 1990s.[3] In the 2000s HILIC rapidly increased in popularity, and has become a staple in chromatographic separations.[4-7, 32-36] The use of water in the mobile phase causes a water layer to form on the surface of the stationary phase.

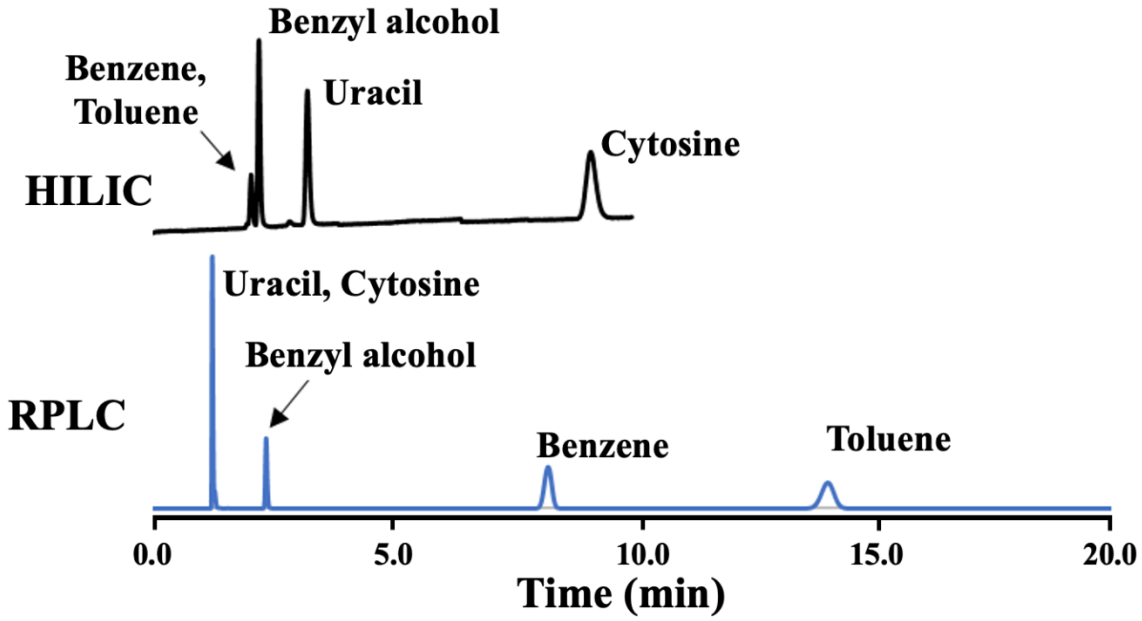


**Figure 1-4. Comparison of retention selectivity for a simple solutes separated by RPLC and HILIC**. HILIC conditions: Zorbax HILIC Plus (3.5 µm silica, 150 mm x 4.6 mm i.d.); 30 ºC; 0.5 mL/min 90:10 ACN:5 mM ammonium acetate (pH 6.8) (premixed volumes, Method A discussed in Chapter 3). Reversed Phase conditions: Zorbax SB-C18 (3.5 µm, 150 mm x 4.6 mm i.d.); 30 °C; 1.0 mL/min.; 40:60 ACN:water (premixed volumes).

No single type of interaction is solely responsible for the retention and selectivity in HILIC. **Figure 1-5** shows the main interactions responsible for retention in HILIC. First, retention arises from the partitioning of compounds into the aqueous water layer that forms on the surface of the polar stationary phase. Highly polar compounds, or hydrophilic analytes, have strong interactions with the water layer. These hydrophilic compounds partition strongly into the water layer and are thus strongly retained. Second, compounds can undergo electrostatic interactions. These electrostatic interactions can involve electrostatic repulsion or ion exchange,

and can be controlled when salts or buffers are added to the mobile phase. Thirdly, other secondary interactions with the stationary phase can occur. In **Figure 1-5** these are referred to as adsorption/secondary interactions, and hydrogen bonding, dipole-dipole interactions, etc.

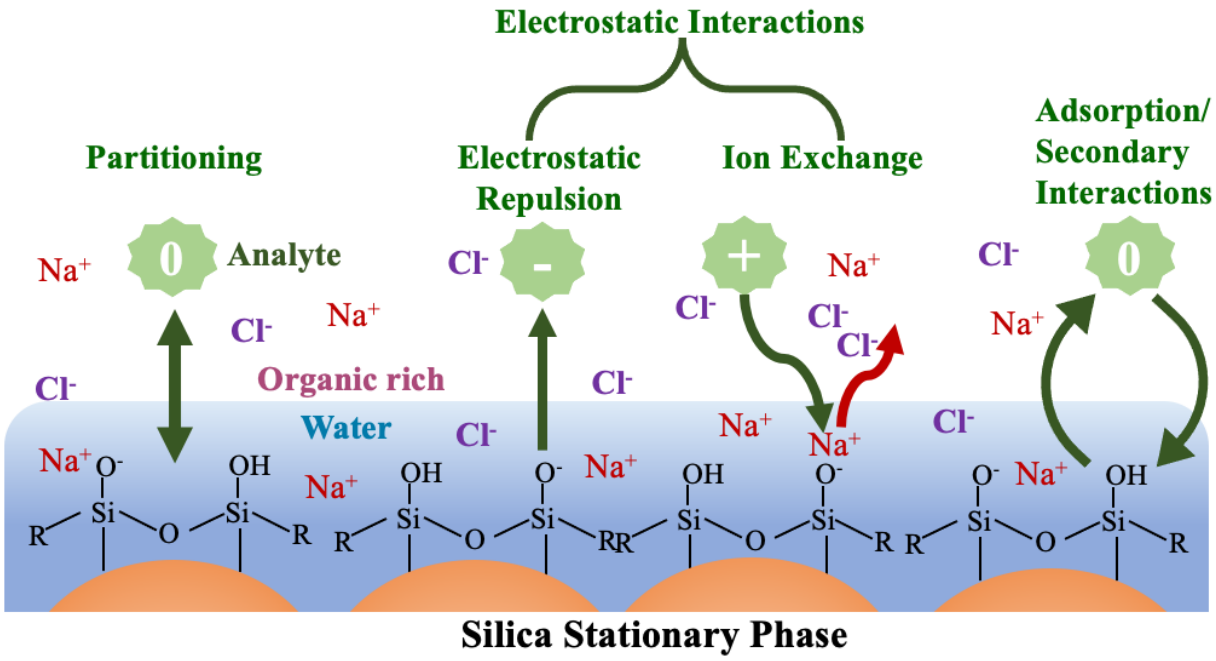


**Figure 1-5. Representation of HILIC retention mechanisms.** Shown with $Na^+Cl^-$ to facilitate showing electrostatic interactions.

As discussed, retention in HILIC is affected by adsorption and/or other secondary interactions. The exact type and strength of these interactions are determined by the type of column used. HILIC columns are typically bare silica, or silica with diol, amide, zwitterionic, or amine bonded phases. The stationary phase surface determines the propensity to form a water layer and the secondary interactions, and thus largely dictates selectivity.[8, 32, 34, 35, 37, 38] But finding the right column chemistry can be a matter of trial and error, and is expensive if many types of columns have to be explored. More commonly, variations in mobile phase conditions

are used to alter the retentive interactions of a separation. These conditions include pH[20, 39-42], buffer type and concentration[14, 39, 41, 43], ternary mobile phase[6, 41, 44], temperature[39, 41, 45, 46], and acetonitrile composition[16, 32, 41, 43, 47]. These factors can influence the water layer thickness, electrostatic interactions, and secondary interactions. **Chapter 2** of this thesis explores ionic additives to alter HILIC selectivity.

### 1.3.3 HPLC Instrumentation

The work horse of a separation is the instrumentation involved. In the following sections, the components of an HPLC are introduced.

#### 1.3.3.1 HPLC Components

While there are slight variations in arrangement, the base components of an HPLC are the same. These components include solvent reservoirs (which contain the mobile phase), autosampler, injector, pump, column, degasser, detector, and data system (typically a computer). These components and their order in a simple HPLC are shown in **Figure 1-6**.

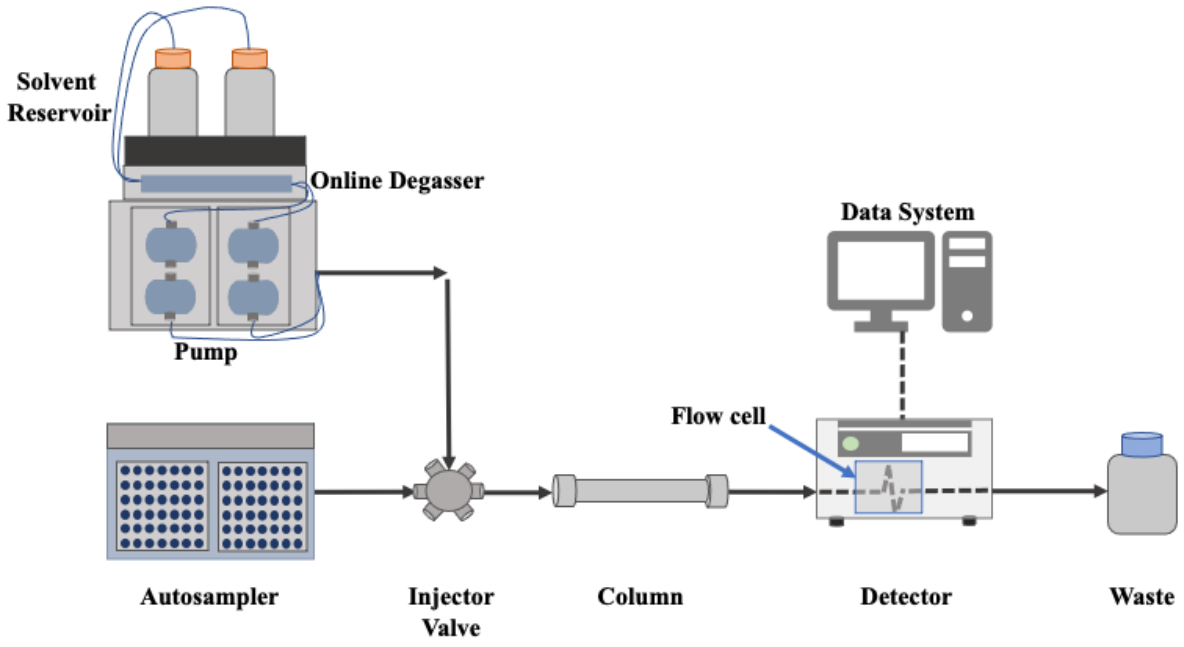


**Figure 1-6. HPLC schematic.**

The solvent reservoirs are where eluent for the mobile phase is kept. Wide-bore tubing (e.g., 1.6 mm ID) directly connects the reservoirs to the inlet of the pumps. The pumps draw the eluent, or solvent, from the reservoir and through the tubing, where it pushes the eluent through the remainder of the components. Modern pumps contain two pulseless pumps that push the eluent through the system at a specified flow rate. Each pump has two pump heads that work together to push eluent through the system such that a pulseless flow is achieved. Each pump is responsible for one of the two solvents that make up the eluent. The eluent composition is determined by the flow rate of each pump head relative to each other. The flow rate from a given pump may remain constant for isocratic separations (mobile phase composition is static) or may be altered based on specified steps for a gradient separation (mobile phase composition is dynamic). Modern HPLCs have an accuracy of ±1% of flow rates from approximately 0.05 – 10 mL/min. This accuracy is important when considering previous discussion on the effect of

mobile phase composition on retention factor. In most modern HPLCs, a degasser removes solvated gases, such as $O_2$ and $N_2$, that may be present in the mobile phase which may otherwise cause air bubbles in the pump head, column, or detector. Air bubbles in the pump head can lead to cavitation of the pump where no liquid is being pumped. Additionally, air bubbles can alter the absorbance reading and even cause enough change to obscure the entire separation (for UV detectors).

Following the degasser, eluent enters the injection valve. The valve connects the autosampler to the flow path of the eluent allowing for sample introduction. The autosampler can inject a range in volumes, approximately 2-100 µL, but common injection volumes range around 10-20 µL. The eluent carries sample from the injector to the column. Column dimensions can range greatly, with common characteristics from 30 to 300 mm in length, internal diameters of 2.1 to 4.6 mm, and particle sizes from 1.5 to 5 µm. From the column, eluent enters the detector. The two detectors used in this thesis were a UV-Vis and mass spectrometer. Both are discussed in further detail below. Eluent then exits the detector to waste. A data management system, typically a computer, is connected to the detector to record detector response.

#### 1.3.3.2 HPLC UV-VIS Detector

A common HPLC detector is an ultraviolet (UV)-Visible detector. In this thesis, a UV-visible detector is used for online analysis in Chapters 2 and 3. When eluent passes through a UV-Vis detector, it passes through the flow cell for an absorbance reading, as shown in **Figure 1-6**. Typical flow cells range from 2-15 µL volume size, with a path length of 0.6-1 cm. A UV-Vis detector can monitor either a single, or multiple, wavelengths of light. In this thesis, monochromatic light is used. As eluent passes through the flow cell, its absorbance is dependent

on the concentration of the compound present, the molar absorptivity of the compound at that wavelength, and the path length of the flow cell. Beer's law relates the absorbance (A) to concentration (C), as follows:

$$A = \varepsilon bC \tag{1-5}$$

where ε is the molar absorptivity and *b* is the path length of the flow cell. A compound's molar absorptivity is dependent on the structure of the compound and the wavelength of light. For instance, compounds that contain conjugated π-bonds absorb ultraviolet or visible light. In this thesis, the UV-detector in Chapters 2 and 3 contains a deuterium lamp and a monochromator with which allows selection of a narrow bandwidth (e.g., 254 nm) of light. Photodiode array (PDA) detectors are capable of measuring multiple wavelengths of light simultaneously. This process is better suited to untargeted analysis where unknowns may be identified by their differing spectra or in applications where peak purity must be validated (e.g., pharmaceutical analysis).

## 1.4 Introduction to Mass Spectrometry

As stated in Section 1.2, analytical chemists are trying to solve important questions like what the chemical composition of something is, and exactly how much of it is present. An additional important tool at our disposal are sensitive and accurate detectors, such as Mass Spectrometers (MS). Mass spectrometry was first developed to measure the masses of atoms and then slowly became more prominent in the 1940s for its use in measuring hydrocarbon abundance. Later, in the 1960s scientists discovered how compounds fragment inside the MS and began realizing the range of other uses this could be applicable for, which included proteomics,

metabolomics, drug testing, and many others.[48-50]

## 1.5 Theory of Mass Spectrometry

### 1.5.1 Mass spectrometry instrumentation

A mass spectrometer measures the mass-to-charge ratio (m/z) of ions in a vacuum. A mass spectrometer is composed of five main components: sample introduction, ionization source, mass analyzer, ion detector, and data system, as shown in **Figure 1-7**. The sample introduction is where a solid, gaseous, or in this thesis a liquid sample is introduced into the ionization source. In the ionization source, the sample is bombarded, typically with electrons or chemicals, to produce ions. Ionization may be hard or soft, dependent on the ionization method used. Soft ionization typically leaves the molecules unfragmented, or intact, whereas hard ionization produces fragmentation of the molecule being ionized. All ions produced are then carried to the mass analyzer, which separates the ions based on their m/z. The way these ions are separated depends on the type of mass analyzer and can use either magnetic or electric fields. The abundance of each m/z is recorded by the channel electron multiplier (CEM), and the output is a mass spectrum where the intensity is plotted versus m/z.[30, 51, 52] These spectra can be characteristic of the molecule, or fragment of a molecule, and can be used for identification and quantification.

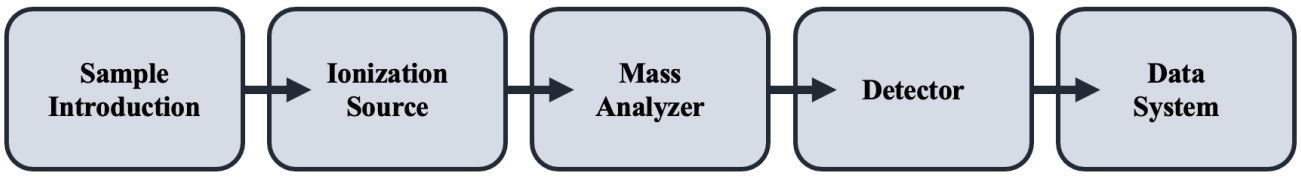


**Figure 1-7. Mass spectrometer schematic of 5 main components.**

Of the five components discussed above, the ionization source and mass analyzer are key components in dictating the type of information obtained and what samples can be analyzed. The ionization source dictates what kinds of analytes can be ionized, from small to large molecules or nonpolar, less polar, or polar molecules. The mass analyzer separates the ions. However, the way they are separated, and how accurately that separation is done, is dictated by the type of mass analyzer used. The types of ionization source and mass analyzer used in this thesis are discussed in further detail below.

#### 1.5.1.1 Ionization sources

The ionization source dictates the type of molecules that can be ionized. Common ionization sources for liquid chromatography include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). ESI covers a large molecular weight range (up to100,000) and works well for polar molecules. However, ESI is limited when considering nonpolar molecules. In this instance, both APCI and APPI can cover nonpolar, or less polar, molecules that ESI cannot. Additionally, APCI and APPI cover a lower range of molecular weights than ESI (20~1,000).[51-53] In this thesis, ESI was used and will be the focus of further discussion.

ESI produces ions at atmospheric pressure by using a high electric field to form an electrospray. ESI has no localized heating and little to no extra internal energy is imparted to the ions, and so is amenable to non-volatile and thermally-labile compounds.[51] Due to this, molecular ions are stable and typically do not fragment, which is why ESI is known as a soft ionization method. The electrospray process is shown in **Figure 1-8**. Electrospray is produced by

the introduction of liquid at flow rates of ~5-1000 µL/min through a 0.1 mm ID metal capillary. A high voltage of ~2000 to 4000 V is applied to the metal capillary. If the voltage is positive (known as positive mode) as shown in **Figure 1-8**, positive analytes move to the surface of the liquid where a Taylor cone forms. This Taylor cone becomes unstable and proceeds into a fine filament of liquid, which results in the formation of relatively large droplets. These droplets continuously undergo solvent evaporation to produce smaller and highly charged droplets. As smaller and highly charged droplets are formed, they reach the Rayleigh limit, where the surface tension of the formed droplet is equal to the Coulombic repulsion of the surface charge. When Rayleigh limit is exceeded, fragmentation of the droplets occurs to produce multiple smaller droplets. This process of solvent evaporation and fragmentation repeats to produce smaller and smaller charged droplets which eventually lead to the formation of gaseous phase ions.[30, 51]

As shown in **Figure 1-8,** ESI has both positive and negative ions present. The charge applied across the metal capillary, either negative or positive, determines if ESI runs in positive or negative mode. **Figure 1-8** also shows a drying gas that assists evaporation of the droplets. Adjusting this gas flow can alter the sensitivity of certain types of compounds or adjust the amount of ion that enters the MS inlet. Other factors in the ESI to consider when developing a method are needle height/distance, temperature, flow rate, entrance potential (voltage used to push ions into MS), and eluent (from HPLC). These factors can affect the efficiency of ionization, and therefore affect the sensitivity of a method.[54, 55]

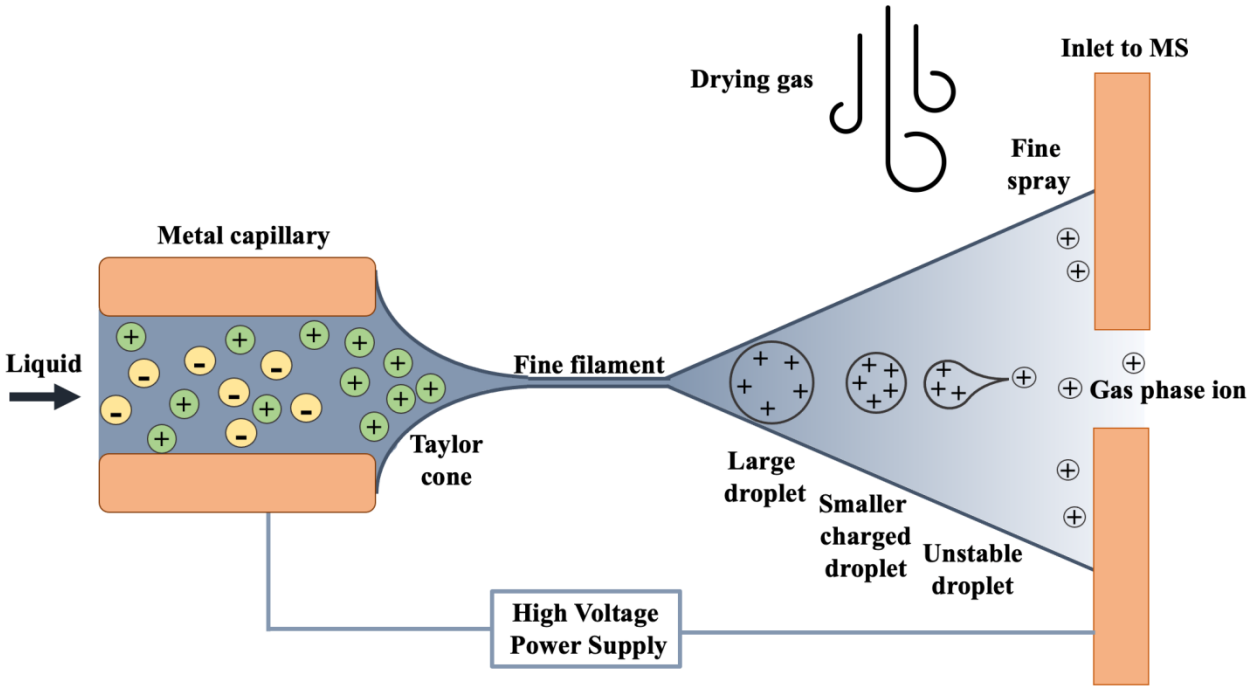


**Figure 1-8. Schematic of Electrospray Ionization.** Figure adapted from Figure 22-17 in reference 30.[30]

### 1.5.1.2 Mass Analyzer

Following ionization, ions are separated by their m/z with a mass analyzer. In this thesis, a triple quadrupole was used, which is made up of three quadrupoles. A quadrupole consists of four parallel rods arranged around a central axis. As shown in **Figure 1-9**, static (DC) and alternating (AC) voltages are applied to each rod. The same absolute potential is applied to each rod, but neighbouring rods have opposing charges. The applied voltages create a fluctuating electric field between the rods. Since we control the voltage applied, we can control the trajectories of ions down the length of the rods. The stability of trajectories are dependent on the ion's mass-to-charge ratio: targeted m/z have a stable trajectory, whereas unselected ions have unstable trajectories and crash out of the electric field, as shown in **Figure 1-9**. A quadrupole has

a mass range up to 4000 m/z, resolving power of 1000-4000, with a mass measurement accuracy of 0.1 Da, and 1-10 spectra/s scan speed.

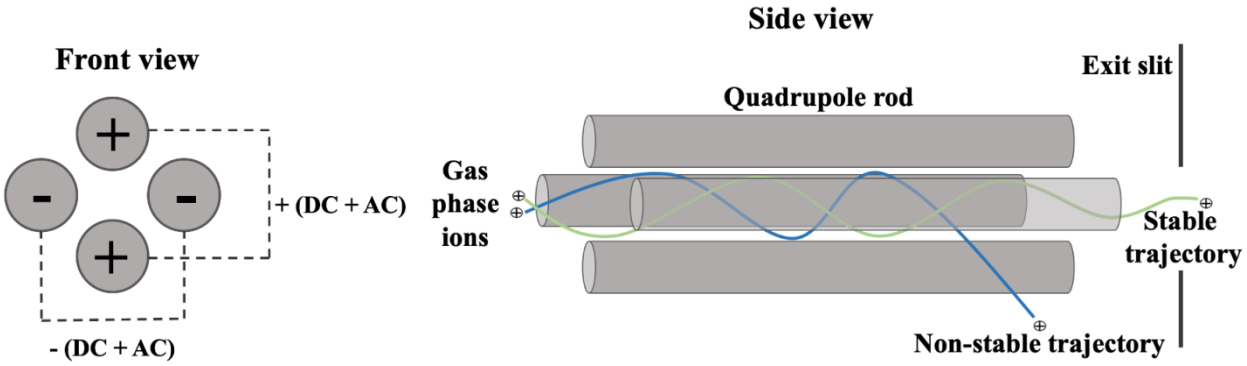


**Figure 1-9**. **Schematic of a quadrupole mass analyzer.** Left hand image shows the front-end view and the alternating voltages applied. Right hand view shows the side views and stable and unstable trajectory through the quadrupole.

A triple quadrupole or MS/MS is a tandem MS made of three quadrupoles. The first and third are traditional quadrupoles, while the second quadrupole is typically an RF-only quadrupole, which acts as a collision cell. **Figure 1-10** illustrates the triple quadrupole used in this thesis. The first quadrupole, Q1, acts as a mass filter to isolate the parent molecule, which is unfragmented. Followed is the collision cell, q2, which is used to fragment the parent molecule/mass using collision-induced dissociation (CID). The parent molecule collides with an inert gas, such as nitrogen, to produce fragments within the collision cell. The fragments, or products, are then filtered by the third quadrupole, Q3, such that only selected m/z's or ranges of m/z can pass through.

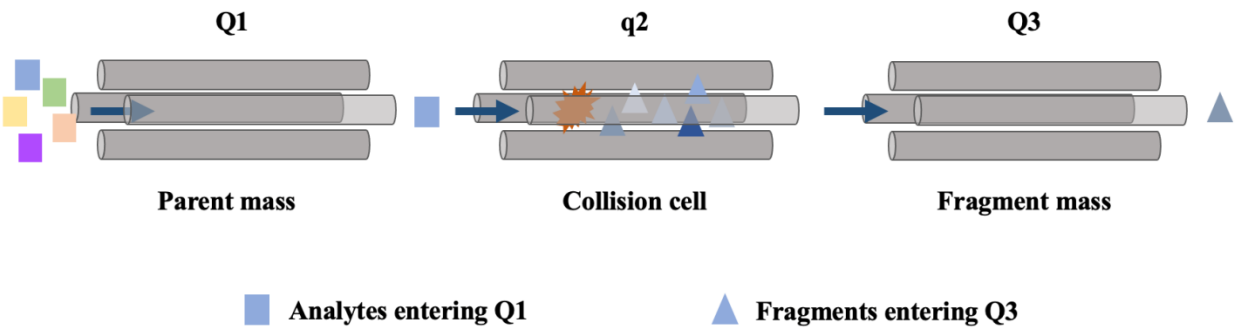


**Figure 1-10. Schematic of a triple quadrupole mass analyzer.**

The triple quadrupole can be used for quantification, or to obtain some structural information. It provides advantages over the single quad by providing greater selectivity, less chemical noise, and further types of information dependent on different scan modes. Different modes can be used to acquire this information, such as product ion scan, parent ion scan, neutral ion scan, and multiple reaction monitoring (MRM). Product ion scan is when a precursor, or parent mass, is selected for, allowed to fragment, and all resulting fragments are recorded. The precursor ion scan is opposite to this, such that a product mass is selected for in Q3 and all parent masses are scanned in Q1. The neutral scan allows all parent m/z through Q1, but scans the fragment m/z with Q3 for a specific loss in m/z that is related to the loss of a neutral fragment common to a particular class of compounds. In MRM both the Q1 and Q3 monitor specific parent and related fragment masses. This thesis uses the MRM mode to identify and sensitively quantify analytes.

## 1.6 Rationale and Scope of Thesis

Method development is a core process in any application to efficiently, and accurately, research that given topic. To do this, a thorough understanding of instrumentation and the theory

is needed. Mobile phase additives and modifiers can change the separation and the retention of analytes. The addition of electrolytes to HILIC could induce set changes in a separation. If so, a single column in HILIC could be used for a wider range of applications. Therefore, it is important to understand the effects of low concentration electrolytes in HILIC and their effect on the separation mechanism. This led to the study described in **Chapter 2**.

**Chapter 2** research objectives:

- Understand the complex separation and retention mechanism in HILIC
- Understand how the addition of electrolytes can affect the separation and retention of analytes on various columns.
- Show how a small mobile phase additive can be used for method development and reduce the need of different columns

Other changes in mobile composition can affect the separation. This can be more pronounced in the complex separation mechanisms of HILIC or RPLC. If the addition of electrolytes can alter the retention and selectivity of a separation, then other small changes could also have a drastic effect. This could influence reproducing methods from literature or even lab-to-lab method transfers. Small changes to how mobile phase is made could make significantly noticeable changes. This lead to the study in **Chapter 3**.

**Chapter 3** research objectives:

- Determine how small changes in mobile phase preparation affect reproducibility
- Show the importance of clear, concise, and detailed method protocols, such that methods can be reproduced person-to-person and lab-to-lab

Theoretical understanding and method development are important skills to hone before their use in an application. Once mastered, they can be used for application in a variety of samples. Such samples can range from environmental, industrial, biomedical, and many more. Of importance to me is the ability to understand how methods could be applied to environmental samples. One topical example that came up was the use of pesticides in the recently legalized cannabis market in Canada. More specifically, how these pesticides could accumulate in the environment, or growing facilities, of where cannabis is being produced. To prepare for studies of pesticide residues in growing facilities, **Chapter 4** reviews both cannabis and the analysis of pesticides in and around cannabis. **Chapter 5** describes the method development for a simple and effective method to determine if these pesticides were present in cannabis growing facilities.

**Chapter 4** objectives:

- To briefly review the history of cannabis up to its legalization in Canada
- To review how pesticides can accumulate in and around cannabis
- To review the analytical methods involved in studying pesticides and cannabis

**Chapter 5** research objectives:

- Develop a simple method for determining the presence of >80 pesticides in cannabis growing facilities
- Determine if pesticides remained on common surfaces in the cannabis growing facilities

The outcomes of my research show the need for a thorough theoretical understanding of method development. With this, it can assist in complicated applications when applied to environmental questions. **Chapter 6** provides a preliminary study of future work when using HILIC to analyze authentic water samples. This future work shows the challenges of authentic

samples, and the further complexities different sample types can bring. This work shows the importance of theoretical understanding to assist in method development for environmental applications. This understanding can assist in making more robust, efficient, and sensitive methods when asking important analytical chemistry questions.

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## Chapter 2

## Effect of nature of electrolytes on retention and selectivity in hydrophilic interaction liquid chromatography*

### 2.1 Introduction

Since the term hydrophilic interaction liquid chromatography (HILIC) was coined by Alpert in 1990, HILIC has become a staple in chromatographic separations.[1] HILIC's popularity is due to its ability to separate highly-polar compounds[2, 3] which are poorly retained by reversed-phase columns. HILIC retention arises from partitioning of analytes into an aqueous water layer that forms on the surface of the polar stationary phase, in addition to adsorption, ion exchange and/or other secondary interactions with the stationary phase surface.[2-5] HILIC columns may be grouped as silica, diol, amide, zwitterionic, or amine based on their surface functionality.[5-7] Selectivity in HILIC is largely dictated by the column. However, altering selectivity by changing columns is an expensive and largely unpredictable approach.

HILIC retention is also affected by a variety mobile phase conditions, such as pH,[8-12] buffer type and concentration,[10, 11, 13, 14] use of ternary mobile phases,[10, 15, 16] temperature,[10, 11, 17, 18] and acetonitrile composition.[2, 10, 11, 13, 19, 20] These factors affect water layer thickness, ionic interactions, and other secondary interactions. Adjusting these conditions alter both the retention and selectivity.

Recently, diethylamine has been used as an ion-pairing agent to uniquely alter retention of anionic solutes.[21-23] Additionally, McCalley has shown some unique retention and selectivity

*Craven, C. B.; Joyce, C. W.; Lucy, C. A., Effect of nature of electrolytes on retention and selectivity in hydrophilic interaction liquid chromatography. *Journal of Chromatogry A* **2019,** *1584*, 80-86.

changes upon addition of trifluoroacetic acid (TFA) to low pH mobile phases.[9] Alpert showed that retention of cationic versus neutral compounds can be uniquely altered by the addition of salts from 0-30 mM by altering ionic interactions.[24] These past studies focused on the effect of electrolyte concentration and pH, rather than on the nature of the electrolyte which is the focus of this chapter. A mechanistic reasoning for the behavior of the electrolytes is also provided.

In this chapter, electrolyte type is demonstrated to be a powerful and predictable means of altering retention and selectivity in HILIC. Electrolytes were initially chosen based on their known ability and strength to ion pair in reversed phase liquid chromatography.[25-27] We believed that these electrolytes could alter the retention and selectivity uniquely in HILIC, since mobile phase additives in other modes has been used to uniquely alter the retention and selectivity of a separation.[9, 21-24] In the course of this research we realized that the key electrolyte characteristic was its position in the Hofmeister series. The Hofmeister series has been correlated with many solution properties, including salting in/out, enzyme activities, ion pairing, and ion exchange. Of particular importance, the propensity for ions to accumulate at an interface follows the Hofmeister series:[25, 28]

$$Cl^- < TFA^- < ClO_4^- < PF_6^- \quad (2\text{-}1)$$

with ions such as $Cl^-$ being referred to as *kosmotropic* and ions high in the Hofmeister series such as $ClO_4^-$ being *chaotropic*.

This chapter examines the effect of the nature of these electrolytes as mobile phase additives on retention and selectivity of neutral, anionic and cationic solutes on HILIC columns. Four separate classes of HILIC columns (zwitterionic, silica, diol, and amine) are investigated.

## 2.2 Materials and Methods

### 2.2.1 Apparatus

All separations were performed on a Varian ProStar HPLC (Varian, Palo Alto, CA, USA) consisting of: a ProStar dual-pump system model 210 with a total flow of 0.5 mL/min; a ProStar Autosampler model 410 fit with a 10 μL loop; an Eppendorf CH-30 column heater (Alltech, Deerfield, IL, USA) with a TC-50 (Alltech, Deerfield, IL, USA) temperature control unit set at either 25 ºC or ambient, as indicated; and a Knauer Smartline 2500 UV Detector (Knauer-ASI, Franklin, MA, USA) set at 254 nm connected to a 2 μL flow cell with fiber optic cables. The detector time constant was 0.1 s.

### 2.2.2 Chemicals and reagents.

All solutions were prepared with Nanopure water (Barnstead, Dubuque, IA, USA). Cytosine, uracil, benzene sulfonic acid (BS), benzoic acid (BA), 4-aminobenzoic acid, phenylalanine, tryptophan, tyrosine, sodium hexafluorophosphate ($PF_6^-$), sodium trifluoroacetate ($TFA^-$), lithium perchlorate ($ClO_4^-$), potassium perchlorate, benzyltrimethyl ammonium chloride ($BTMA^+$), benzyltriethyl ammonium chloride, benzylpropyl ammonium chloride, and HPLC-grade acetonitrile (ACN) were from Sigma-Aldrich (St. Louis, MO, USA). Toluene was from Fisher Scientific (Fair Lawn, NJ, USA), ammonium acetate and hydrochloric acid from Fluka Analytical (St. Louis, MO, USA), sodium chloride from EMD Chemicals (Darmstadt, Germany), and sodium perchlorate from Anachemia (Montreal, QC, Canada).

**Table 2-1. Characteristics of the stationary phases used in this study.**

| # | Brand Name | Manu-facturer | Support | Function-ality | Particle Size (µm) | Pore size (Å) | Surface area ($m^2/g$) | Column length (mm) | Column diameter (mm) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | ZIC-HILIC | Merck | Silica | Polymeric sulfoalkyl-betaine zwitter-ionic | 5 | 100 | 180 | 150 | 4.6 |
| **2** | Zorbax HILIC Plus | Agilent | Silica | Underi-vatized | 3.5 | 95 | 160 | 100 | 4.6 |
| **3** | Cosmosil HILIC | Nacalai | Silica | Triazole | 5 | 120 | 300 | 150 | 4.6 |
| **4** | Fortis HILIC Diol | Fortis Technol | Silica | Alkyl diol | 3 | 100 | 380 | 100 | 4.6 |

### 2.2.3 Tested columns, electrolytes, and test probes

**Table 2-1** summarizes the characteristics of the columns studied. Analyte retention factors ($k$) were calculated as the average of 3–4 injections of standards prepared in buffered mobile phase. Peaks were identified by individually running each analyte separately under the same conditions as mixtures. Test probes were chosen to demonstrate the behavior of specific charge states. Cytosine and uracil are model neutral analytes, amino acids for model zwitterionic, $BTMA^+$, benzyltriethyl ammonium, benzyltripropyl ammonium for cations, and $BS^-$, $BA^-$ and aminobenzoate for anions. Toluene was used as the unretained dead time marker ($t_0$).[4, 9, 29] The electrolytes were chosen for their range of Hofmeister character, and their known ion-pairing behavior in reversed phase liquid chromatography.[25, 27, 30] The counter ion was sodium, unless indicated otherwise.

### 2.2.4 Chromatographic conditions

Mobile phases consisted of 70:30, 80:20, 85:15 or 90:10 ACN:aqueous buffer. There were slight variations in the preparation of mobile phase between the experiments, but the method was constant within each set of separations. Differences in mobile phase composition and preparation can affect the retention of a separation (**Chapter 3**).[31] Therefore, the mobile phase preparation is explicitly detailed. First, experiments studying a variety of electrolytes at varying concentrations used premixed mobile phases prepared by buffer addition to the aqueous phase and pH adjustment with HCl, addition of electrolyte, and finally mixing of measured volume of ACN to volume of aqueous solution. Second, the %ACN experiments were performed using a dual-pump system. The aqueous phase was buffer, $_{w}^{w}$pH adjusted with HCl, electrolyte added, and diluted to final volume with deionized water. All remaining experiments were run with pre-mixed solvents at v/v, aqueous phase was buffered and $_{w}^{w}$pH adjusted with HCl, electrolyte added, and diluted to volume with deionized water. The appropriate volume of ACN was measured with a graduated cylinder and the two volumes were then combined. All solvents were filtered (0.2 μm) prior to use. All concentrations stated hereafter refer to the final concentration in the combined aqueous/organic phase. The buffer for all runs was 5.0 mM ammonium acetate at $_{w}^{w}$pH 6.8, adjusted with HCl. The pH electrode used was an YSI True Lab pH 1110 pH meter and YSI True Line Electrode (YSI-Inc. Yellow Springs, OH, USA). The % ACN quoted represents the total volume relative to the total amount including aqueous, buffer, and organic volumes.

## 2.3 Results and Discussion

The objective of this chapter is to explore the effect of the nature of the electrolyte (specifically $Na^+ClO_4^-$, $Na^+TFA$, $Na^+PF_6^-$, and $Na^+Cl^-$) on HILIC retention and selectivity of cationic and anionic analytes. $ClO_4^-$, $PF_6^-$ and TFA are chaotropic electrolytes, and $Cl^-$ is a kosmotropic electrolyte and used as a control. Chaotropic electrolytes are ions that decrease the structuring of water and are often referred to as "disorder-makers". Whereas kosmotropic electrolytes would be called "order-makers".[32] These terms originate from the Hofmeister series, which orders ions based on their ability to stabilize or destabilize proteins and membranes.[32] Chaotropic electrolytes accumulate within regions of disorder in a solvent system, such as at the mobile phase/water layer interface. As will be shown, this tendency to accumulate at the surface of the water layer enhances the ion exchange and ionic screening effects of the mobile phase electrolyte. As these electrolytes alter the HILIC separation through both ionic screening and ion exchange, the separation can be altered in a predictable manner based on the electrolyte's placement in the Hofmeister series.

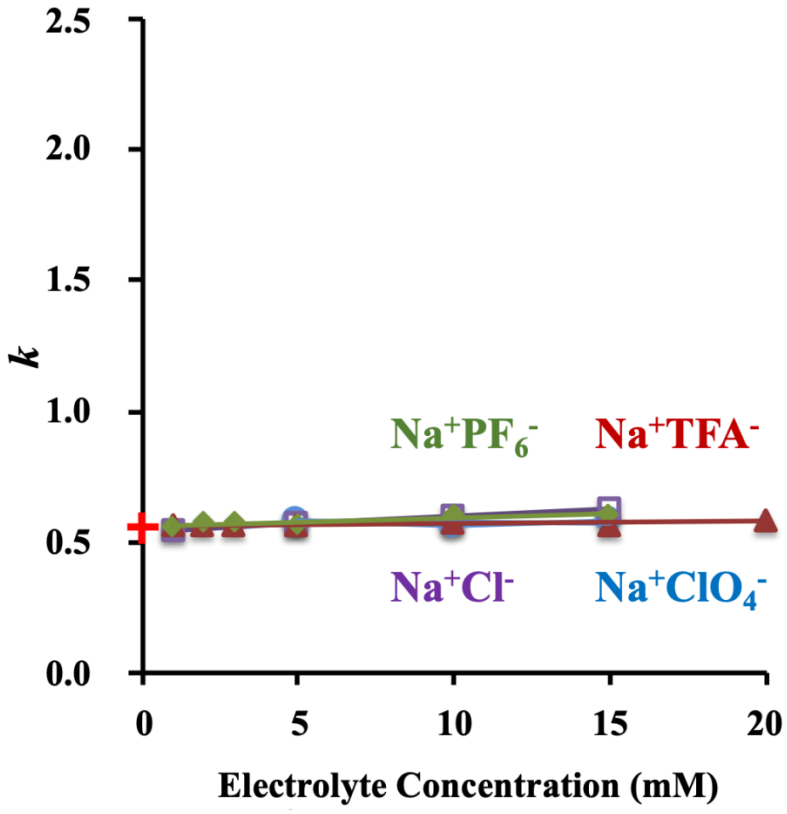


**Figure 2-1. Effect of formal concentration of $Na^+$ on retention of uracil.** Conditions: ZIC-HILIC (5 μm); 0.5 mL/min of 80% ACN with 5 mM ammonium acetate buffer at pH 6.8; ambient temperature; 10 μL loop injection of 0.25 mM uracil; 254 nm. The red cross on the y-axis indicates uracil retention with no added electrolyte (buffer only). Electrolyte addition had similar minimal effect for other neutrals such as cytosine. Size of data points are larger than the associated uncertainty. Analyte concentrations: $BS^-$ 1 mM, $BA^-$ 1 mM, $BTMA^+$ 1 mM, Cytosine 0.5 mM, Uracil 0.25 mM.

### 2.3.1 Non-specific Effects of Electrolyte Addition

Neutrals uracil and cytosine were included in the analyte set to reveal any changes to the retentivity of the water layer upon the addition of 1–20 mM electrolyte. No significant change in *k* was observed for uracil, as shown in **Figure 2-1** or cytosine (data not shown) upon electrolyte addition. Thus, addition of low concentrations (≤ 20 mM) of electrolytes do not cause a substantial change in the retentivity of the water layer. This is consistent with previous studies that show addition ≤ 20 mM buffer caused only small increases to the retentivity of the water layer, with modest change in retention for neutrals relative to that for ionic analytes.[4, 10, 20, 33] Similarly, Alpert recently showed that addition of 1-30 mM electrolyte (kosmotropic $SO_4^{2-}$ and chaotropic $ClO_4^-$) resulted in no significant changes in retention of neutrals compared to that for ionic analytes on a PolyHydroxyEthyl A column.[24] More substantial changes in retention of neutrals occur at higher electrolyte concentrations.[24] With regard to our study, the key observation from **Figure 2-1** is that changes in retention of neutrals are modest relative to the changes observed for ionic analytes.

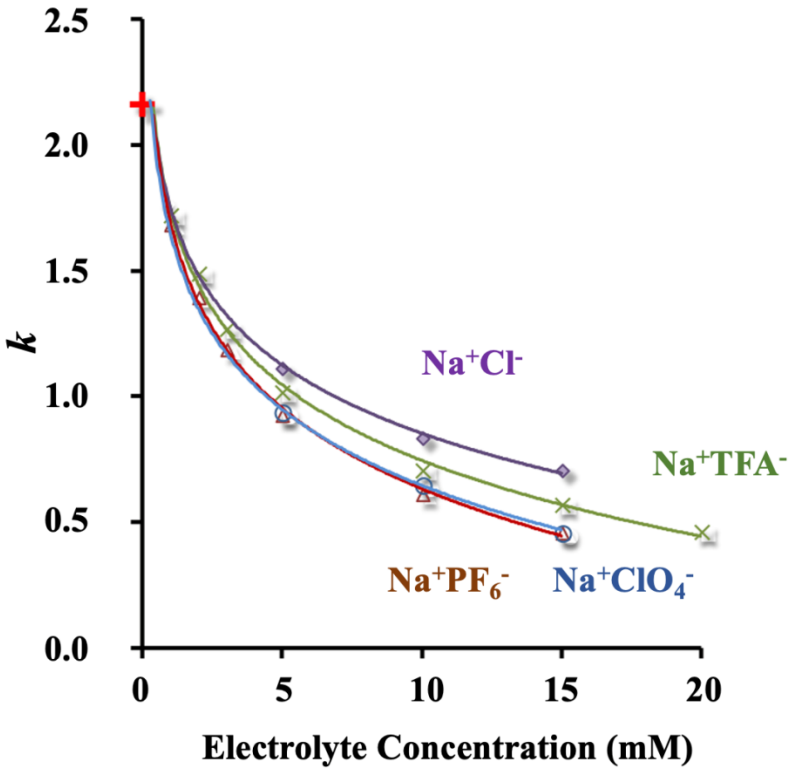


**Figure 2-2. Effect of formal concentration of $Na^+$ on retention of benzyltrimethyl ammonium ($BTMA^+$).** Ambient conditions for temperature. The red cross on the y-axis indicates uracil retention with no added electrolyte (buffer only). Lines are guides for the eye. See Figure 2-1 for conditions.

### 2.3.2 Effect of Electrolyte Addition on Cation Retention

BTMA decreases in retention on a ZIC-HILIC column with increasing $Na^+$ concentration, as shown in **Figure 2-2**. Zwitterionic columns exhibit weak cation exchange character.[5, 8, 34] The exchange of two monovalent cations, $A^+$ and $E^+$, competing for cation exchange sites is represented by:

$$A^+_m + E^+_s \leftrightharpoons A^+_s + E^+_m \quad (2\text{-}2)$$

for which the equilibrium constant is the selectivity coefficient $K_{A,E}$. For low concentrations of analyte—where the isotherm is linear—the retention factor for a monovalent analyte $A^+$ is:

$$k_A = \frac{K_{A,E}}{[E^+]_m} \cdot \frac{Q_{col}}{V_m} \tag{2-3}$$

where $Q_{col}$ is the cation exchange capacity and $V_m$ the dead volume of the column. The logarithmic form of **Equation 2-3** is the Linear Solvent Strength Model governing retention for a monovalent ion with a monovalent eluent.[35-37]

$$\log k_A = \log K_{A,E} + \log(Q_{col}) + \log\left(\frac{1}{V_m}\right) - \log[E_m^+] \tag{2-4}$$

For a given column, analyte and eluent, many terms in **Equation 2-4** are constant, simplifying the equation to:

$$\log k \approx \text{const} - \frac{x}{y} \log[E^{y+}] \tag{2-5}$$

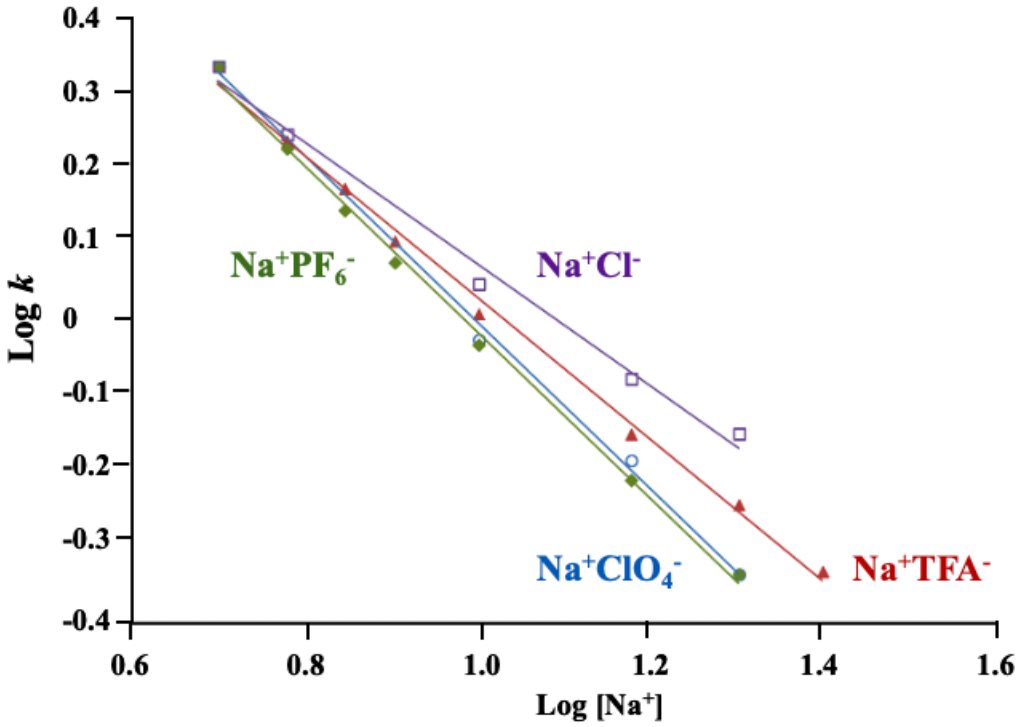


**Figure 2-3. Effect of formal concentration of $Na^+$ on retention of $BTMA^+$.** Conditions: ZIC-HILIC (5 µm); 0.5 mL/min of 80% ACN with 5 mM ammonium acetate buffer at pH 6.8; ambient temperature; 10 µL loop injection of 1 mM $BTMA^+$; 254 nm. Size of data points are larger than uncertainty. The log [$Na^+$] is reflective of the total concentration of sodium including the 5 mM from that of the buffer.

**Figure 2-3** shows the effect of the formal $Na^+$ concentration on retention of cationic $BTMA^+$ on a zwitterionic (ZIC-HILIC) column. All electrolytes exhibit the behavior predicted by Equation 2-5 ($R^2 > 0.99$). **Table 2-2** summarizes the regression parameters. However, contrary to **Equation 2-5**, the slopes in **Figure 2-3** depend on the identity of the counter-ion, ranging from –1.10 ($Na^+ClO_4^-$) to –0.80 ($Na^+Cl^-$) with the order $Na^+ClO_4^- \cong Na^+PF_6^- > Na^+TFA^- > Na^+Cl^-$. The nearness of the slopes in **Figure 2-3** to the theoretical value of –1 indicates that

cation exchange is occurring. However, the influence of the counter-ion indicates that additional effects are occurring under HILIC conditions.

**Table 2-2. Regression statistics for log *k* vs log [buffer + electrolyte] plots.**[a]

| **Electrolyte** | | **$Na^+Cl^-$** | | **$Na^+TFA^-$** | | **$Na^+PF_6^-$** | | **$Na^+ClO_4^-$** |
|---|---|---|---|---|---|---|---|---|
| | | **Regression Statistics** | | **Regression Statistics** | | **Regression Statistics** | | **Regression Statistics** |
| | **R Square** | 0.991 | **R Square** | 0.996 | **R Square** | 0.997 | **R Square** | 0.997 |
| | **Standard Error** | 0.022 | **Standard Error** | 0.017 | **Standard Error** | 0.015 | **Standard Error** | 0.018 |
| | **Observations** | 5 | **Observations** | 8 | **Observations** | 7 | **Observations** | 4 |
| | **Coefficients** | **Standard Error** | **Coefficients** | **Standard Error** | **Coefficients** | **Standard Error** | **Coefficients** | **Standard Error** |
| **Intercept** | 0.878 | 0.044 | 0.964 | 0.026 | 1.081 | 0.027 | 1.095 | 0.043 |
| **Slope** | -0.804 | 0.044 | -0.935 | 0.025 | -1.098 | 0.028 | -1.099 | 0.041 |

*a.* Conditions: ZIC-HILIC (5 μm); 0.5 mL/min of 80% ACN with 5 mM ammonium acetate buffer at pH 6.8; ambient temperature; 10 μL loop injection of 0.25 mM uracil; 254 nm. See Figure 2-3 for plots.

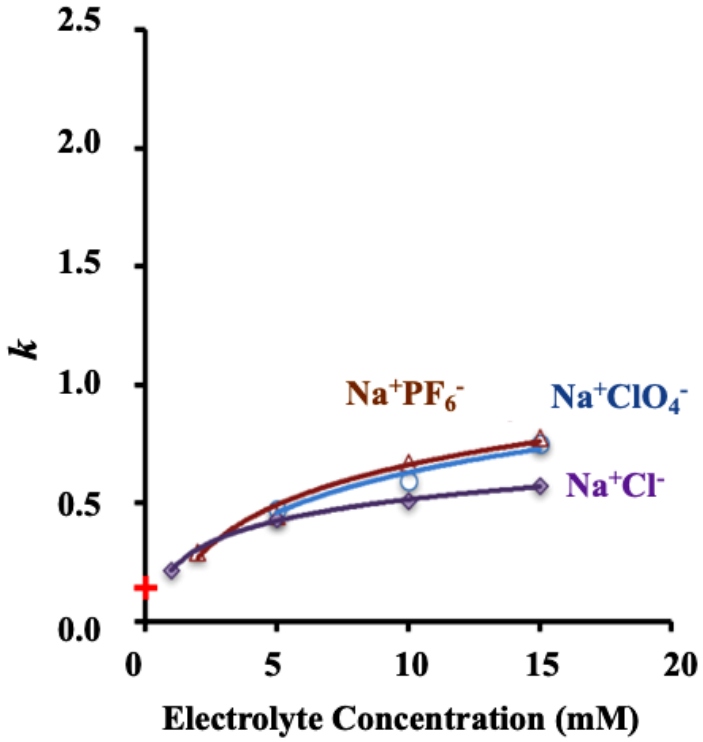


**Figure 2-4. Effect of formal concentration of electrolytes on retention of benzene sulfonate ($BS^-$).** Chloride shows the effect of ionic strength alone. Conditions as in Figure 2-1. $BS^-$ concentration was 1 mM. Size of data points are larger than associated uncertainty. Lines are guides for the eye. Representative separations with 10 mM $Na^+Cl^-$ and $Na^+ClO_4^-$ are shown in Figure 2-10, and with 10 mM $Na^+PF_6^-$ in Figure 2-5.

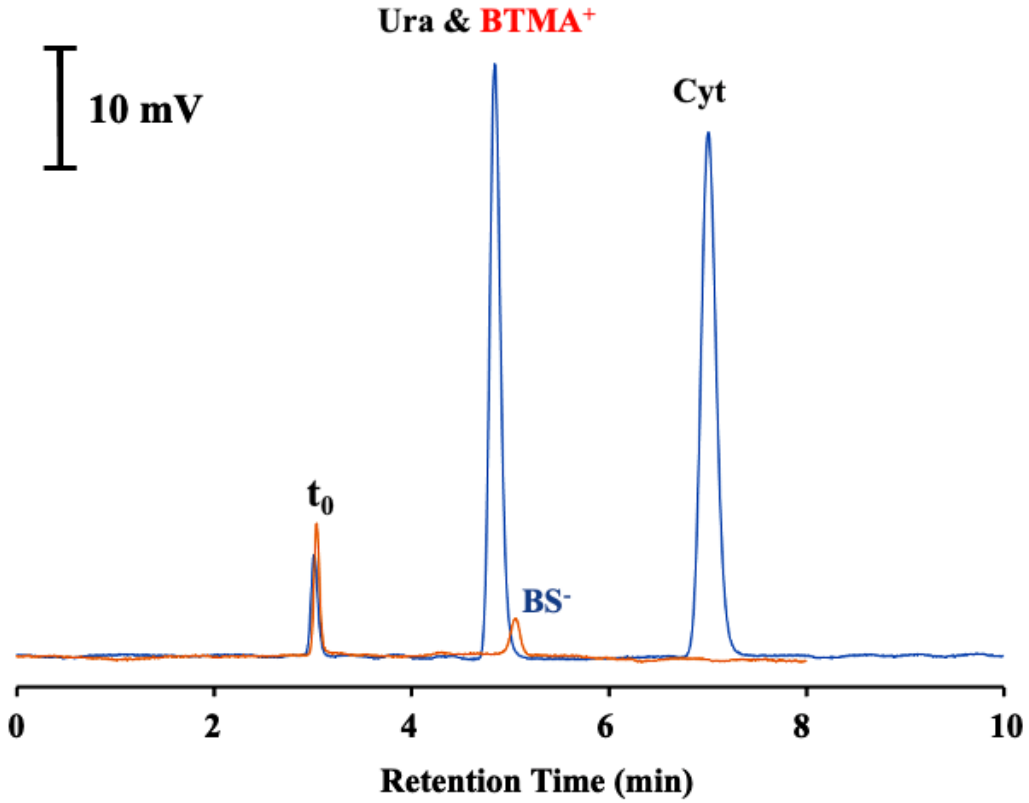


**Figure 2-5. Effect of 10 mM $Na^+PF_6^-$ on retention and selectivity.** Conditions: ZIC-HILIC (5 μm); 0.5 mL/min of 80% ACN with 5 mM ammonium acetate buffer at pH 6.8; ambient temperature; 10 μL loop injection of 0.25 mM uracil; 254 nm. Analyte concentrations: BS 1 mM, BTMA 1 mM, Cytosine 0.5 mM, Uracil 0.25 mM.

### 2.3.3 Effect of Electrolyte Addition on Anion Retention

Anionic benzene sulfonate ($BS^-$) increased in retention on ZIC-HILIC upon addition of electrolytes to the mobile phase (**Figure 2-4**), consistent with past comparable studies.[33, 34] As noted above, the ZIC-HILIC column has weak cation exchange character,[5, 34] meaning that the column possesses a net negative charge—presumably due to silanols. The anionic nature of the ZIC-HILIC column would result in $BS^-$ experiencing electrostatic repulsion from the surface, and thus diminished retention.[24, 38, 39] Increasing electrolyte concentration decreases this

electrostatic repulsion, and thus increases retention for $BS^-$, as has been seen in Electrostatic Repulsion Liquid Chromatography (ERLIC).[39] Other studies have shown that mobile phase additives can increase retention of analytes with same charge as that of the column.[9, 33]

**Figure 2-4** also shows that addition of $Na^+ClO_4^-$, $Na^+PF_6^-$, $Na^+TFA^-$, and $Na^+Cl^-$ differ in their ability to increase $BS^-$ retention ($Na^+ClO_4^- \cong Na^+PF_6^- > Na^+TFA > Na^+Cl^-$), which is the same order as observed for cation exchange in **Figure 2-2** and **2-3**, which follows the ranking of these chaotropic electrolytes in the Hofmeister series. Similar behavior is observed for analytes on other columns (see **Section 2.3.7**).

### 2.3.4 Hofmeister Effect on Retention

It can be hypothesized that the tendency of ions higher in the Hofmeister series to accumulate at interfaces[28] accounts for the differences in the decrease of BTMA's retention (**Figure 2-3** and **Figure 2-2**) and increase in $BS^-$ retention (**Figure 2-4**). Chaotropic electrolytes (*i.e.*, high in the Hofmeister series) such as $\underline{Na^+}ClO_4^-$ and $\underline{Na^+}PF_6^-$ accumulate more in the interfacial region (such as the surface of the water layer). Thus, there is a greater localized ion concentration in the water layer interfacial region. Accumulation of $Na^+$ in the interface regions would increase the localized concentration of eluent cation ($Na^+$), which reduces cation exchange retention as per **Equation 2-5** resulting in decreased retention of $BTMA^+$.

For $BS^-$, the accumulation of chaotropic electrolyte at the water layer/ACN interface would result in greater localized ionic strength in the interfacial region, which would result in greater ionic screening for anionic analytes. Zwitterionic columns such as ZIC-HILIC have a net negative charge,[5, 8, 34] and so the absence of electrolyte $BS^-$ experiences electrostatic repulsion which reduces its retention relative to that of a pure HILIC partitioning mechanism. The

accumulation of chaotropic ions at the interface reduces this electrostatic repulsion to a greater extent than that expected based for the bulk electrolyte concentration (*i.e.*, that exhibited upon addition of $Na^+Cl^-$). Hence a greater increase in retention is observed upon addition of $Na^+ClO_4^-$ and $Na^+PF_6^-$ than for $Na^+Cl^-$.

TFA is intermediate in the Hofmeister series,[25] and so its effect on ion retention is less than $Na^+ClO_4^-$ and $Na^+PF_6$, and greater than $Na^+Cl^-$. This placement of TFA in the Hofmeister series is consistent with the increases in anion retention seen by McCalley upon addition of 0.1% trifluoroacetic acid to 95% ACN with 5 mM ammonium formate (pH 3).[9, 12, 40] McCalley suggested that the changes in retention are likely due to protonation of the silica.[9, 12] The results in this chapter, which are at neutral pH levels, suggest that more than stationary phase protonation is at play. We believe that the anionic electrolytes cause ionic screening resulting in decreased repulsion for $BS^-$, thus increasing the time spent in the stationary phase and increasing retention.

Columns with a net negative charge have a change in the elution order as the concentration of the electrolyte is increased or the electrolyte is changed. As chaotropic electrolytes higher in the Hofmeister series ($ClO_4^-$ and $PF_6^-$) are added to the mobile phase, cations would decrease in retention and anions would increase in retention more than electrolytes lower in the series ($Cl^-$) are added to the mobile phase. Additionally, as the concentration is increased the change in retention increases, as discussed earlier in this section.

### 2.3.5 Effect of the Cation in the Electrolyte

**Figure 2-6** shows small changes ($\Delta k < 0.12$) in retention and selectivity were observed on the ZIC-HILIC column when the cationic counterion ($Li^+$, $Na^+$, $K^+$) to the electrolyte ($ClO_4^-$) was changed. Efficiencies remained constant within 5% with all buffer cations. BTMA decreased in retention in order of $Li^+ClO_4^- < Na^+ClO_4^- < K^+ClO_4^-$. This order reflects the ability of these cations to compete with $BTMA^+$ for the cation exchange sites on the ZIC-HILIC column (*i.e.*, reduced $K_{A,E}$ for Equation 2).[5] Thus, the greater the affinity of the ion exchange site for the buffer cation, the lower the retention for $BTMA^+$.[14, 41] A similar trend was observed for $BTMA^+$ on silica, with greater retention changes observed due to silica's greater cation exchange character.[31]

For neutral and anionic analytes, statistically significantly changes in retention were observed upon changing the electrolyte cation, with strongest retention observed with $Na^+$ (**Figure 2-6**). This may be due to changes in the retentivity of the water layer or other competing factors. Regardless, it shows the lab-to-lab reproducibility of methods can be compromised by even small changes in composition or preparation of HILIC eluents.[31] These results highlight the importance of describing the composition of the mobile phase in its entirety.[31]

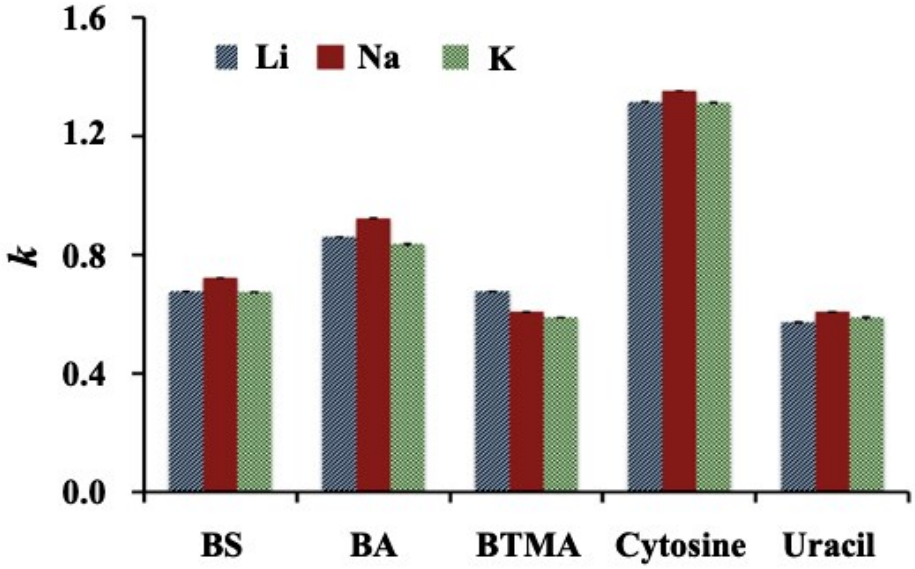


**Figure 2-6. Effect of changing the $ClO_4^-$ counterion on retention of analytes.** Electrolyte addition was 10 mM $ClO_4^-$ with $Na^+$, $Li^+$, or $K^+$ as the counterion. Temperature was 25 ◦C. Other conditions as in Figure 2-1. The error bars are ±1 standard deviation of triplicate measures. Analyte concentrations: BS 1 mM, BA 1 mM, BTMA 1 mM, Cytosine 0.5 mM, Uracil 0.25 mM. Representative chromatogram under these conditions is shown in Figure 2-10.

### 2.3.6 Effect of %ACN on Electrolyte Selectivity

**Figure 2-7** shows that changing the volume percent of acetonitrile (70–90%) in the presence of 10 mM electrolyte resulted in increased retention of neutrals (uracil and cytosine), as expected for a HILIC mechanism. Efficiencies were constant (within 5%) for all % ACN. With $Na^+Cl^-$ the cationic $BTMA^+$ shows much less change in retention than for the neutrals, indicating that cation exchange is an important contributor to its retention. In the presence of $Na^+Cl^-$ the retention of anionic $BS^-$ increases more rapidly than that of the neutrals. Upon changing the

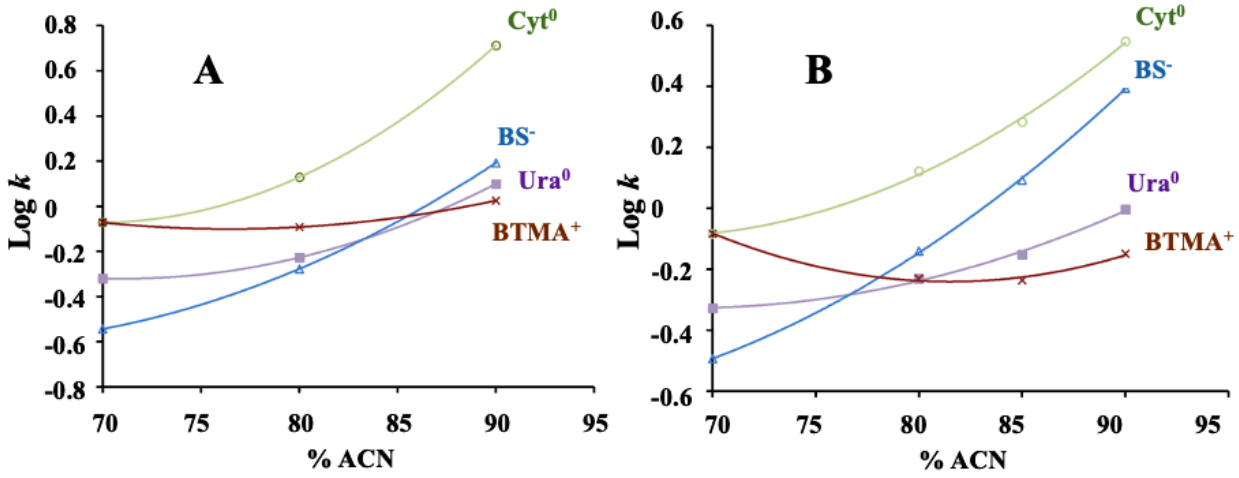


**Figure 2-7. Effect of changing the % ACN of eluents on the log retention factor of analytes.** Electrolyte addition was 10 mM final concentration for all trials. Other conditions as in Figure 2-1. Size of data points are larger than associated error. Lines are guides for the eye. Analyte concentrations: BS 1 mM, BA 1 mM, BTMA 1 mM, Cytosine 0.5 mM, Uracil 0.25 mM. A: 10 mM $Na^+Cl^-$ B: 10 mM $Na^+ClO_4^-$. Representative chromatograms under these conditions are shown in Figure 2-10 and Figure 2-9.

electrolyte from $Na^+Cl^-$ to $Na^+ClO_4^-$, the differences between the cation and anion retention behavior versus that of the neutrals becomes more extreme.

From a practical perspective, changes in elution occur based on the nature of the electrolyte. For instance, the uracil/$BS^-$ elution order changes at 83% ACN with 10 mM $Na^+Cl^-$ *vs*. 76.5% ACN with 10 mM $Na^+ClO_4^-$, while the corresponding uracil/$BTMA^+$ elution order changes occur at 87% and 80%, respectively.

As the % ACN is increased, the difference between the change in retention caused by $Na^+Cl^-$ and $Na^+ClO_4^-$ becomes more apparent. This is due to their differing tendencies to

accumulate at interfaces—$ClO_4^-$ being chaotropic will tend to accumulate at water layer/ACN interface, while $Cl^-$ being kosmotropic will remain more evenly distributed throughout the mobile phase. Consequently, for a given added electrolyte concentration there is a different localized concentration of $Na^+$ and $ClO_4^-$/ $Cl^-$ in the interfacial region due to the Hofmeister nature of the anion. Thus, addition of electrolytes to the mobile phase offers selectivity changes under a range of HILIC conditions. The trends on silica and diol columns, as shown in **Figure 2-8** and **Figure 2-9**, are similar to that on the zwitterionic column. Thus, stationary phase chemistry has little effect on the overall trend seen with addition of electrolytes at varying % ACN.

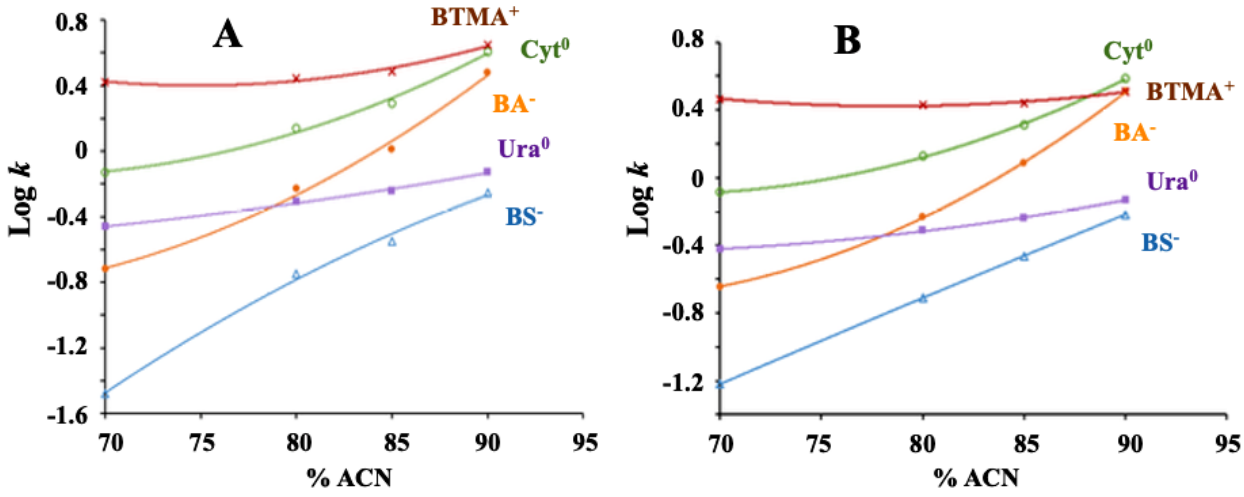


**Figure 2-8. Effect of changing the %ACN of eluents on the log retention factor of analytes. A) 10mM $Na^+Cl^-$ B) 10 mM $Na^+ClO_4^-$.** Electrolyte addition was 10 mM final concentration for all trials. Column was HILIC Plus (Silica). Other conditions as in Figure 2-1. Size of data points are larger than the associated uncertainty. Lines are guides for the eye. Analyte concentrations: BS 2 mM, BA 3 mM, BTMA 3 mM, Cytosine 0.5 mM, Uracil 0.2 mM.

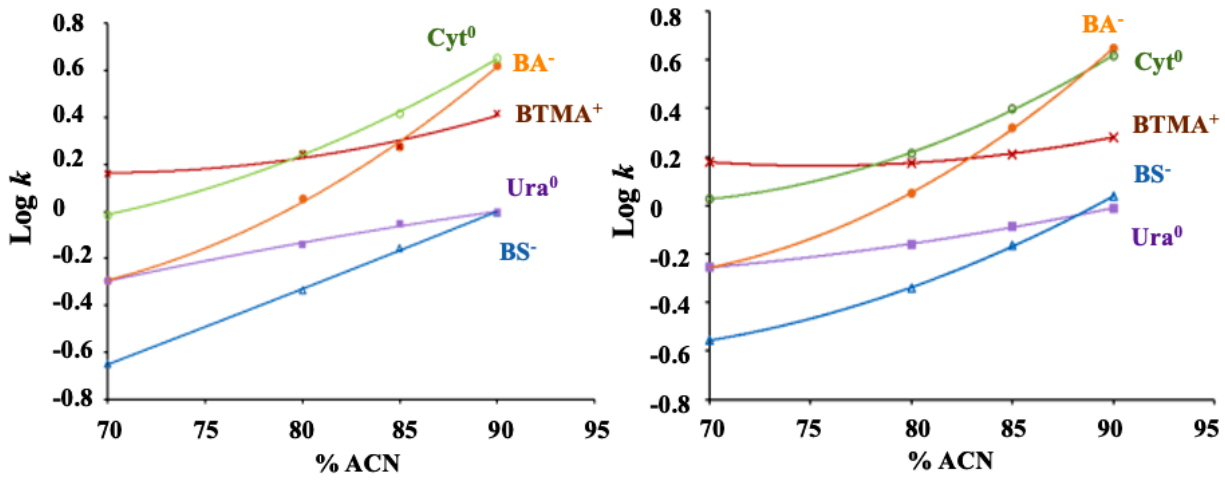


**Figure 2-9. Effect of changing the %ACN of eluents on the log retention factor of analytes. A) 10mM $Na^+Cl^-$ B) 10 mM $Na^+ClO_4^-$.** Electrolyte addition was 10 mM final concentration for all trials. Column was HILIC Diol. Other conditions as in Figure 2-1. Size of data points are larger than the associated uncertainty. Lines are guides for the eye. Analyte concentrations: BS 2 mM, BA 3 mM, BTMA 3 mM, Cytosine 0.5 mM, Uracil 0.2 mM.

### 2.3.7 Electrolytes on Other Types of HILIC Column

**Figure 2-10** show the effect of a 10 mM addition of kosmotropic sodium chloride ($Na^+Cl^-$) and chaotropic sodium perchlorate ($Na^+ClO_4^-$) on retention and selectivity on silica, diol and amine columns, respectively.

The effects of electrolytes with silica (**Figure 2-10A**) are similar to those on the ZIC-HILIC (**Figures 2-1**, **2-2**, **2-3**, and **2-4**). The retention of neutrals (uracil, cytosine) was unaffected by the nature of the electrolyte. Cationic analytes ($BTMA^+$) decreased in retention,

and anionic analytes ($BS^-$, $BA^-$) increased in retention. As with the ZIC-HILIC, the effect of addition of 10 mM $Na^+ClO_4^-$ was greater than 10 mM $Na^+Cl^-$. This is due to the electrolytes'

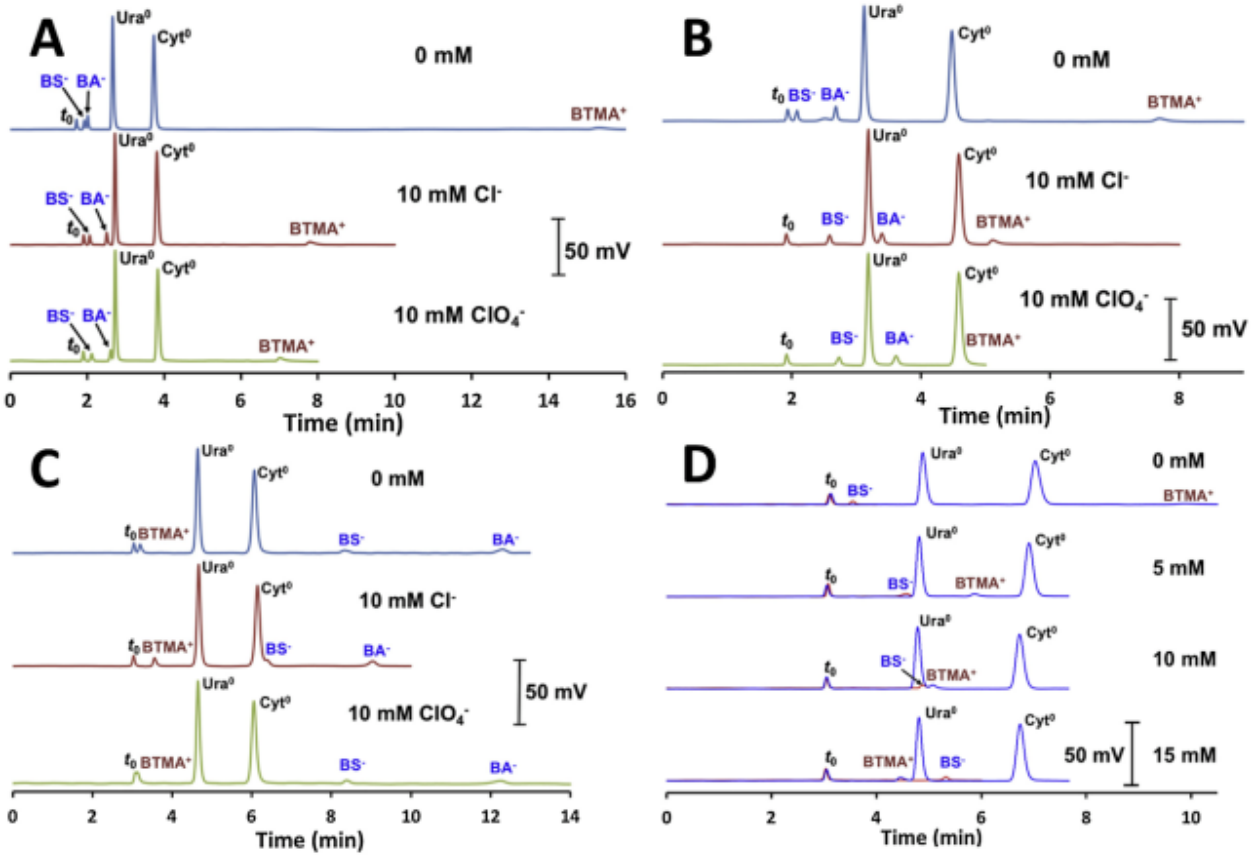


**Figure 2-10. Changes in retention on different classes of HILIC column upon addition of $Na^+Cl^-$ or $Na^+ClO_4^-$ to the mobile phase.** A) Silica (Zorbax HILIC Plus) column. Particle size was 3.5 µm and column temperature was 25 ˚C. B) Fortis HILIC Diol column. Particle size was 3.5 µm and column temperature was 25 ˚C. C) Cosmosil HILIC (triazole, similar results to aminopropyl column) column upon addition of $Na^+Cl^-$ or $Na^+ClO_4^-$ to the mobile phase. Particle size was 5 µm and column temperature was 25 ˚C. D) Selectivity changes on a ZIC-HILIC column upon addition of $Na^+ClO4^-$ to the mobile phase. For conditions for runs, please see Figure 2-3.

differing ability to accumulate at interfaces. Chaotropic $ClO_4^-$ has a greater localized ion concentration in the water layer interfacial region than kosmotropic $Cl^-$. To maintain charge balance there would be an accumulation of $Na^+$ in the interface regions along with the $ClO_4^-$ that would increase the localized concentration of eluent cation ($Na^+$), which reduces cation exchange retention (*i.e.*, decreased retention of $BTMA^+$). Greater localized ionic strength in the interfacial region with $ClO_4^-$, would result in greater ionic screening for anionic analytes and increased retention of $BS^-$ and $BA^-$.

**Figure 2-10B** shows the effect of addition of electrolytes on a diol column. Again, similar trends are observed, with comparable retention shifts to the ZIC-HILIC column (**Figures 2-1**, **2-2**, **2-3**, and **2-4**), but smaller changes than the silica column (**Figure 2-10A**). As discussed above, the electrolytes alter the retention based on their placement in the Hofmeister series and accumulation at the water later interface. The greater effect on the silica column is consistent with its stronger cation exchange character than the ZIC-HILIC or diol columns.[5]

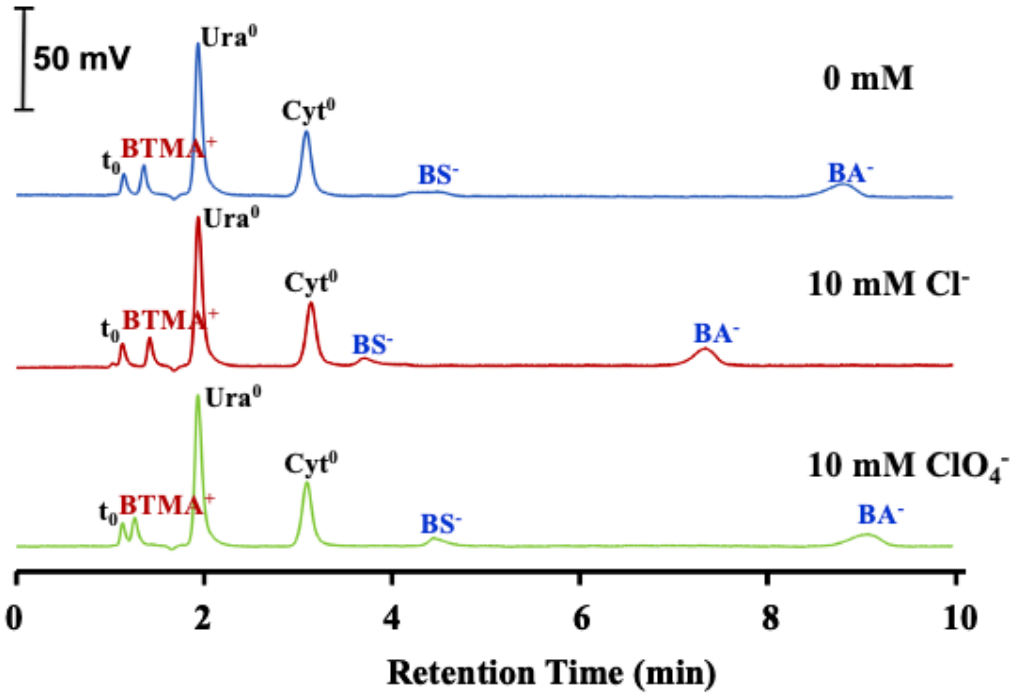


**Figure 2-11. Changes in retention on aminopropyl column upon addition of $Na^+Cl^-$ or $Na^+ClO_4^-$ to the mobile phase**. Particle size was 3.5 µm and column temperature was 25 °C.

Triazole (**Figure 2-10C**) and aminopropyl columns (**Figure 2-11**) possess anion exchange character under our experimental conditions. On these columns, addition of $Na^+Cl^-$ to the mobile phase caused a decrease in retention for anionic analytes and an increase in retention for the cationic $BTMA^+$, consistent with the previous results.[5, 6] Interestingly, addition of $Na^+ClO_4^-$ has very little effect on the separation relative to no electrolyte addition. Further research on the effect of electrolytes on HILIC retention on an amine column might elucidate the cause of this surprising behavior, and whether this has to do with the positively charged surface of the column. However, from a pragmatic perspective, no change in retention and selectivity has no benefit for method development, and so was not explored further.

### 2.3.8 Potential for Method Development

**Figures 2-3** and **2-4** demonstrate that addition of an electrolyte such as $Na^+ClO_4^-$ to the mobile phase causes more dramatic changes to retention of cationic and anionic analytes than does a comparable addition of $Na^+Cl^-$. This is due to the electrolytes' differing ability to accumulate at interfaces, such that there is a greater localized ion concentration in the water layer interfacial region with more chaotropic electrolytes. Accompanying accumulation of $Na^+$ with $ClO_4^-$ in the interface regions increases the localized concentration of eluent cation ($Na^+$), which reduces cation exchange retention and greater ionic screening for anionic analytes. **Figure 2-10D** illustrates the ability of perchlorate to dramatically alter the selectivity and retention of a separation. As little as a 5 mM change in $Na^+ClO_4^-$ concentration yields significant retention changes. As well, **Figure 2-10D** demonstrates the chromatographic behavior of analytes when ionic interactions contribute to HILIC retention. As neutrals remain almost unaffected, cationic ($BTMA^+$) and anionic analytes ($BS^-$) are altered such that the elution order is almost reversed (as discussed in **Section 3.2** and **3.3**). **Figure 2-12** demonstrates the ability to alter the selectivity and retention of a more complex sample.

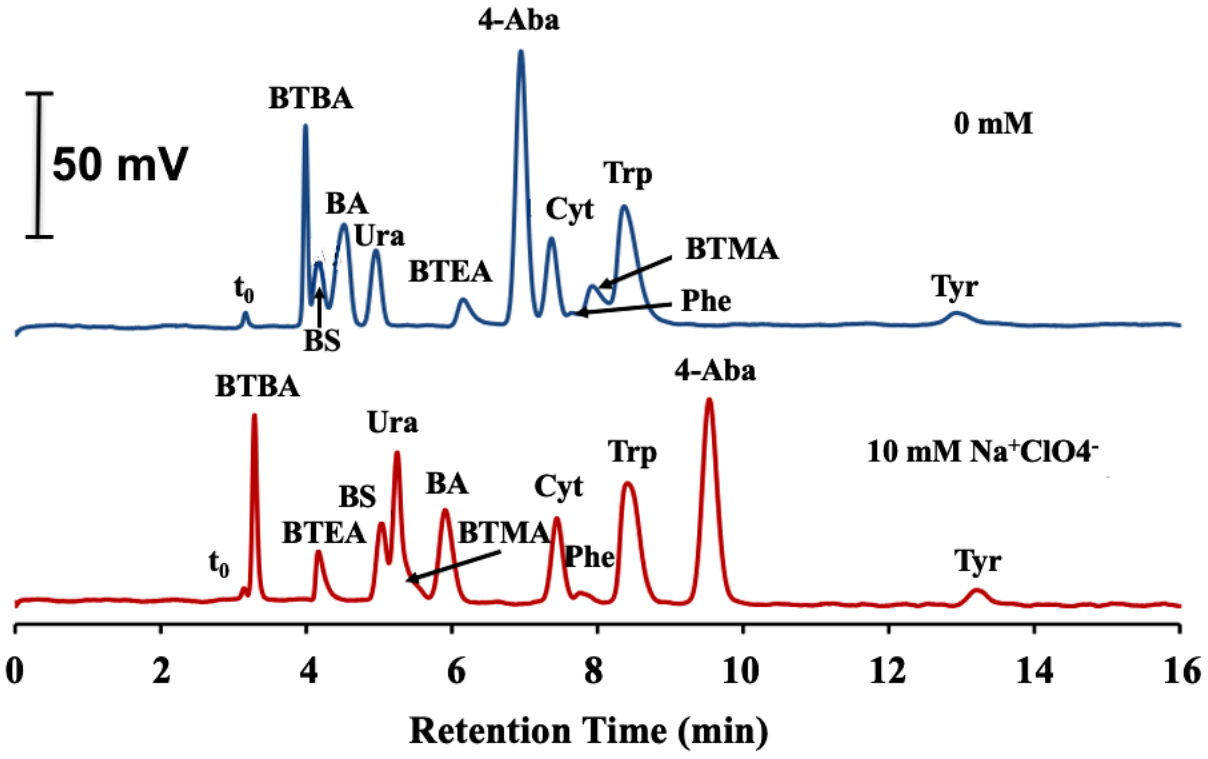


**Figure 2-12. Effect of formal concentration of $Na^+ClO_4^-$ and without on retention and selectivity.** Conditions: ZIC-HILIC (5 μm); 0.5 mL/min of 80% ACN with 5 mM ammonium acetate buffer at pH 6.8; ambient temperature; 10 μL loop injection; 254 nm. Analyte concentrations: BS 2 mM, BA 3 mM, BTMA 3 mM, Cytosine 0.5 mM, Uracil 0.2 mM, BTBA 2 mM, BTEA, 2 mM, 4-Aba, 1 mM, Trp 1.25 mM, Tyr, 1.5 mM, Phe 1.5 mM.

Addition of an electrolyte is an emerging tool in literature, which we have further refined for mobile phase tuning of retention and selectivity, that was previously dictated mainly by the column. Comparable illustrations of additives to the mobile phase are also beginning to appear in the HILIC literature.[9, 21-24] It should be noted that the low volatility of the majority of these electrolytes would result in loss in sensitivity and formation of complex adducts in LC-MS. Volatile electrolytes like TFA would still be a viable option.

## 2.4 Conclusions

The ability of four electrolytes to alter HILIC retention and selectivity on four classes of HILIC columns have been studied. The effect has been examined at varying additive concentration, % ACN, and varying the counter ion. The ability of the electrolyte to change retention of anionic and cationic analytes is in the order $Na^+Cl^- < Na^+TFA^- < Na^+ClO_4^- \approx Na^+PF_6^-$, which correlates with the electrolyte's ability to accumulate at interfaces (*i.e.*, the Hofmeister series). Overall, upon using electrolytes higher in the Hofmeister series, retention of neutrals remained unchanged, cation retention decreased, and anion retention increased on zwitterionic, silica and diol columns (summarized in **Table 2-3**). Cation exchange retention decreased more dramatically with chaotropic electrolytes containing anions better able to accumulate at interfaces ($PF_6^-$, $ClO_4^-$) than the kosmotropic electrolyte ($Cl^-$). Electrostatic repulsion would also decrease with enhanced localized concentration of the anionic chaotropic electrolytes ($PF_6^-$, $ClO_4^-$) that can better accumulate at interfaces, thus reducing the electrostatic repulsion and contributing to the increase retention of anionic analytes. Overall, the Hofmeister electrolytes dramatically alter the selectivity and retention of HILIC separations on zwitterionic, silica, and diol columns, providing an effective tool for altering selectivity and retention.

**Table 2-3. Summary of effects of different electrolytes on multiple classes of HILIC columns.[a]**

| Column functionality | Electrolyte | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NaCl | | NaTFA | | $NaClO_4$ | | $NaPF_6$ | |
| | Anion | Cation | Anion | Cation | Anion | Cation | Anion | Cation |
| Zwitterionic | + | - | + + | - - | + + + | - - - | + + + | - - - |
| Silica | + | - | + + | - - | + + + | - - - | + + + | - - - |
| Diol | + | - | + + | - - | + + + | - - - | + + + | - - - |
| Triazole | + | - | 0 | 0 | 0 | 0 | 0 | 0 |
| Aminopropyl | + | - | 0 | 0 | 0 | 0 | 0 | 0 |

Summary to show either increases or decreases in retention factor in a separation. Symbols: + increase in retention factor; - decrease in retention factor; 0 minimal or no change in retention factor; number of symbols represents the degree of change. Neutrals were not included as there was no statistically significant change in retention upon addition of electrolytes from 1-20 mM.

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## Chapter 3

## Eluent Preparation for Hydrophilic Interaction Liquid Chromatography*

### 3.1 Introduction

Hydrophilic interaction liquid chromatography (HILIC) is used in a variety of application areas, mainly as an alternative to reversed-phase liquid chromatography (RPLC).[1-3] HILIC is useful for highly polar compounds and has been reported in the literature to have a wide array of uses. For example, it has been used for metabolomics, proteomics, a range of environmental samples, as well as many clinical studies.[4-10] While increased use of HILIC is positive, I found that many descriptions of the experimental conditions reported in literature were incomplete. This makes it difficult for other researchers or analysts to accurately reproduce the results of that given study.

Whereas RPLC typically involves a relatively lipophilic stationary phase (for example, alkyl-modified silica) and relatively water-rich eluents (often more than 50% water), HILIC separations typically involve hydrophilic stationary phases (for example, bare or diol-modified silica) and organic solvent-rich eluents (often more than 80% ACN).[5, 7, 11-13] These differences can lead to radical differences in the retention of small molecules under the two conditions. **Figure 3-1** compares the separations of a simple mixture of five small molecules under RPLC and HILIC conditions. In RPLC, hydrophobic molecules such as benzene and toluene are well retained and separated, but polar molecules such as uracil and cytosine are unretained (uracil is a common dead time marker in RPLC). In contrast, benzene and toluene are unretained in HILIC (toluene is a

* Lucy, C.; Craven, C. B.; Seidl, C.; Stoll, D. R., LC Troubleshooting: Eluent Preparation for Hydrophilic Interaction Liquid Chromatography, Part I—Solvent Volumes and Buffer Counterions, *LC-GC North Am*erica, **2018**, *36*, 18–24. Reprinted in *LCGC Europe* **2018**, *31*, 22-27.

common dead time marker in HILIC),[3] while the polar molecules are well retained and separated.[1] Although most work involving HILIC to date has focused on separations of small molecules, recently there has been considerable interest in separations of large molecules such as proteins as well.[6] In this chapter, I focus on detailed aspects associated with preparation of eluents used for HILIC separations.

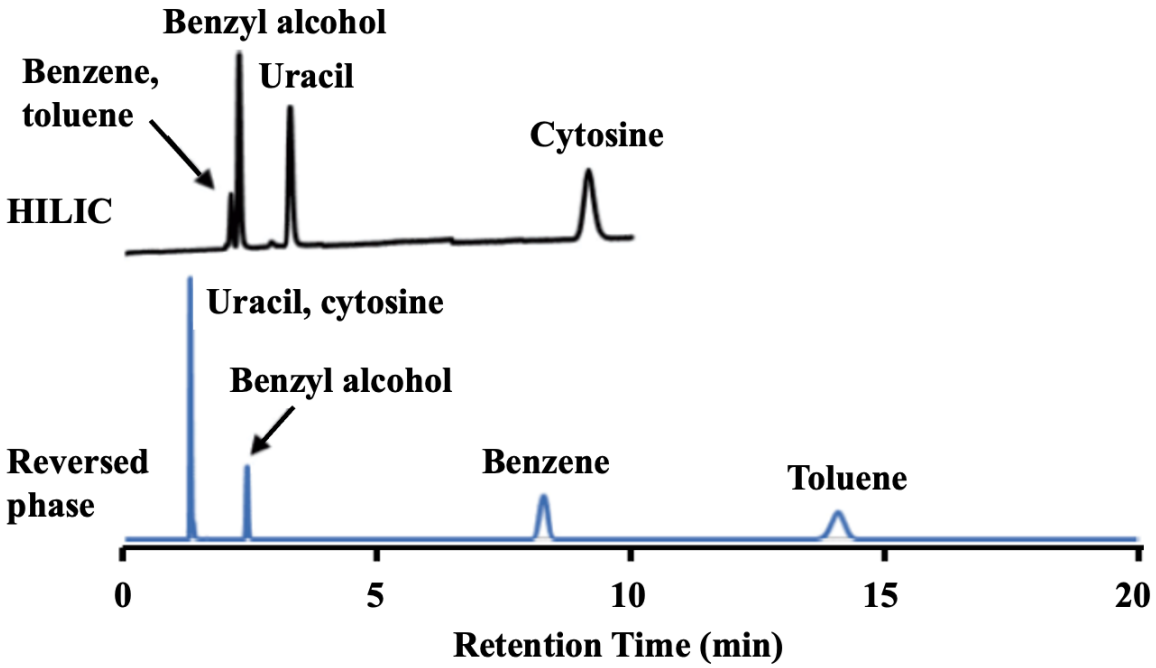


**Figure 3-1. Comparison of retention and selectivity for a set of simple small molecule probe solutes separated by reversed-phase LC and HILIC.** Reversed-phase LC conditions: 150 mm × 4.6 mm, 3.5-µm (silica) Zorbax SB-C18; mobile phase: 40:60 acetonitrile-water (premixed volumes, method A); temperature: 30 °C; flow rate: 1.0 mL/min. HILIC conditions: 150 mm × 4.6 mm, 3.5-µm (silica) Zorbax HILIC Plus; mobile phase: 90:10 acetonitrile-5 mM ammonium acetate (pH 6.8) (premixed volumes, method A); temperature: 30°C; flow rate: 0.5 mL/min.

Although there are some examples in the literature of very well-defined procedures for preparing eluents for HILIC separations,[14] it is far more common to see something like "*10 mM ammonium acetate in 90/10 ACN/buffer, at pH 6*". When the analyst goes to prepare this eluent in the laboratory, they quickly confront a number of practical questions. For the sake of this example, suppose we prepare 1 L of eluent.

- Is the concentration of ammonium acetate 10 millimoles in 1 L of the buffer, or 10 millimoles in 1 L of the aqueous/organic mixture?
- Was the pH adjusted/measured before or after the addition of the organic solvent to the buffer?
- Is the ratio of 90/10 on a volume (that is, v/v) or weight (that is, w/w) basis? And, if v/v, how exactly is this mixture prepared? (see **Section 3.3.1** for a list of possible approaches)

In this chapter, I tackle two aspects of eluent preparation for HILIC separations. First, I discuss some of the options for preparing the organic/aqueous solvent mixture and demonstrate that very different results arise from different preparation methods. Second, I demonstrate that the cation associated with an anionic buffering agent in the eluent can have a significant effect on retention.

## 3.2 Materials and Methods

### 3.2.1 Apparatus

All separations were performed on a Varian ProStar HPLC (Varian, Palo Alto, CA, USA) consisting of: a ProStar dual-pump system model 210 with a total flow of 0.5 mL/min; a ProStar Autosampler model 410 fit with a 10 μL loop; an Eppendorf CH-30 column heater (Alltech, Deerfield, IL, USA) with a TC-50 (Alltech, Deerfield, IL, USA) temperature control unit set at

either 30ºC, or as indicated; and a Knauer Smartline 2500 UV Detector (Knauer-ASI, Franklin, MA, USA) set at 254 nm connected to a 2 μL flow cell with fiber optic cables. The detector time constant was 0.1 s. The pH electrode used was an YSI True Lab pH 1110 pH meter and YSI True Line Electrode (YSI-Inc. Yellow Springs, OH, USA).

### 3.2.2 Chemicals and reagents

All solutions were prepared with Nanopure water (Barnstead, Dubuque, IA, USA). Cytosine, uracil, benzene sulfonic acid (BS), benzyltrimethyl ammonium chloride ($BTMA^+$), benzene, benzyl alcohol, and HPLC-grade acetonitrile (ACN) were from Sigma-Aldrich (St. Louis, MO, USA). Toluene was from Fisher Scientific (Fair Lawn, NJ, USA), ammonium acetate and hydrochloric acid from Fluka Analytical (St. Louis, MO, USA), sodium chloride, ammonium chloride, potassium chloride, and lithium chloride from EMD Chemicals (Darmstadt, Germany).

### 3.2.3 Chromatographic Conditions

Analyte retention factors ($k$) were calculated as the average of 3–4 injections of standards prepared in buffered mobile phase. Peaks were identified by running each analyte separately under the same conditions as the mixtures. All solvents were filtered (0.2 μm) prior to use. All concentrations stated hereafter refer to the final concentration in the combined aqueous/organic phase. The buffer for all runs was 5.0 mM ammonium acetate at ${}_w^wpH$ 6.8 (that is, measured in the water phase using a pH meter calibrated with pure aqueous standards), adjusted with HCl. The % ACN quoted represents the total volume relative to the total amount including aqueous,

buffer, and organic volumes. The column used was a Zorbax HILIC Plus (3.5 µm silica, 150 mm x 4.6 mm i.d.) kept at 30 ºC, with flow rate set to 0.5 mL/min.

## 3.3 Results and Discussion

### 3.3.1 Preparing the Organic/Aqueous Solvent Mixture

In his *LC Troubleshooting* column, John Dolan addressed preparation of organic/aqueous solvents mixtures from the point of view of RPLC separations (**Table 3-1**),[15] and as part of a discussion of how modern LC pumps work.[16] I will review the aspects of those discussions that are most relevant here, and extend the ideas to the impact on HILIC separations.

Using the 90/10 organic/aqueous example for the eluent, we can hypothesize a few ways to prepare the mixture.

A. Transfer 900 mL ACN and 100 mL aqueous buffer to a solvent bottle. These portions could be measured using graduated cylinders, or gravimetrically (that is, by mass instead of volume).
B. Transfer 900 mL of ACN to a 1-L volumetric flask; fill to mark using aqueous buffer.
C. Transfer 100 mL of aqueous buffer to a 1-L volumetric flask; fill to mark using ACN.

**Table 3-1. Effect of mobile phase preparation technique on toluene retention, from reference 15.[15]**

| Volume Fraction Methanol | Retention time (min) | Retention factor (***k***) | Prep method |
|---|---|---|---|
| **0.70** | 3.40 | 1.34 | A |
| **0.60** | 5.40 | 2.72 | A |
| **0.50** | 9.08 | 5.16 | A |
| **[0.62]*** | 4.82 | 2.32 | C (MeOH to fill) |
| **[0.58]**** | 5.87 | 3.05 | B ($H_2O$ to fill) |

* 40 mL of water brought to 100 mL with methanol. Volume fraction estimated from *k*.

** 60 mL of methanol brought to 100 mL with water, calculated from *k*.

Approaches A-C assume that the mixture will be prepared in a bottle, and then delivered to the HPLC column using a single channel of the pumping system. We must also consider a case where the preparation of the eluent is done by the pump itself.

D. Bottles of ACN and the aqueous buffer are set up on the HPLC instrument, and the pump is set to deliver an eluent that is 90/10 organic/aqueous.

One would assume that preparing the mobile phase by either A, B, or C would produce the same solution. However, these three approaches do not produce similar solutions. This is because of the so-called 'volume of mixing' associated with mixing different liquids, shown in **Figure 3-2**.

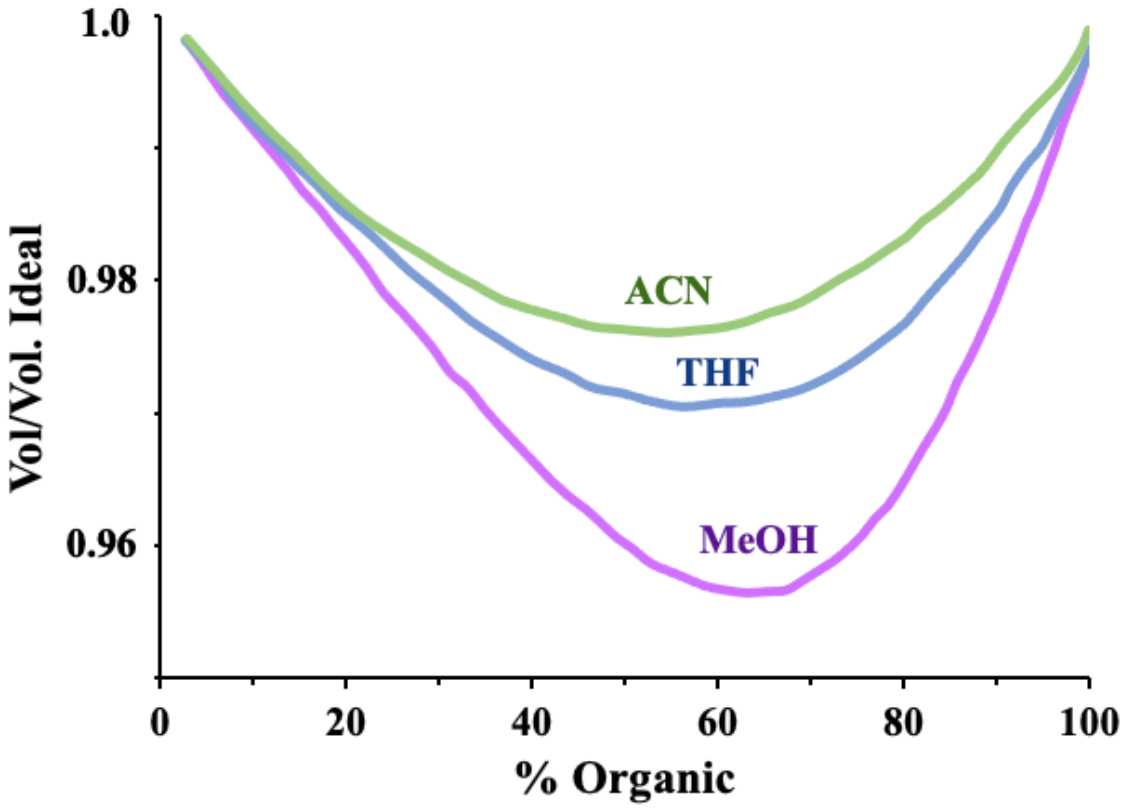


**Figure 3-2. Volume change of solutions when water is mixed with acetonitrile, tetrahydrofuran (THF), or methanol.** Volume relative to the ideal volume if there were no volume change on mixing. Based on data from reference 16.[16]

This has been pointed out previously by Dolan,[15, 16] and others.[17] In the specific case of mixing ACN with water, the volume of the mixture is always less than the sum of the volumes of

the constituent parts. For example, if we mix 500 mL of water with 500 mL of ACN, we will not have 1000 mL of solution in our bottle–it will be less than 1000 mL (about 20 mL at room temperature). **Figure 3-3** shows the percent loss in total volume as a function of increasing content of ACN in the mixture.

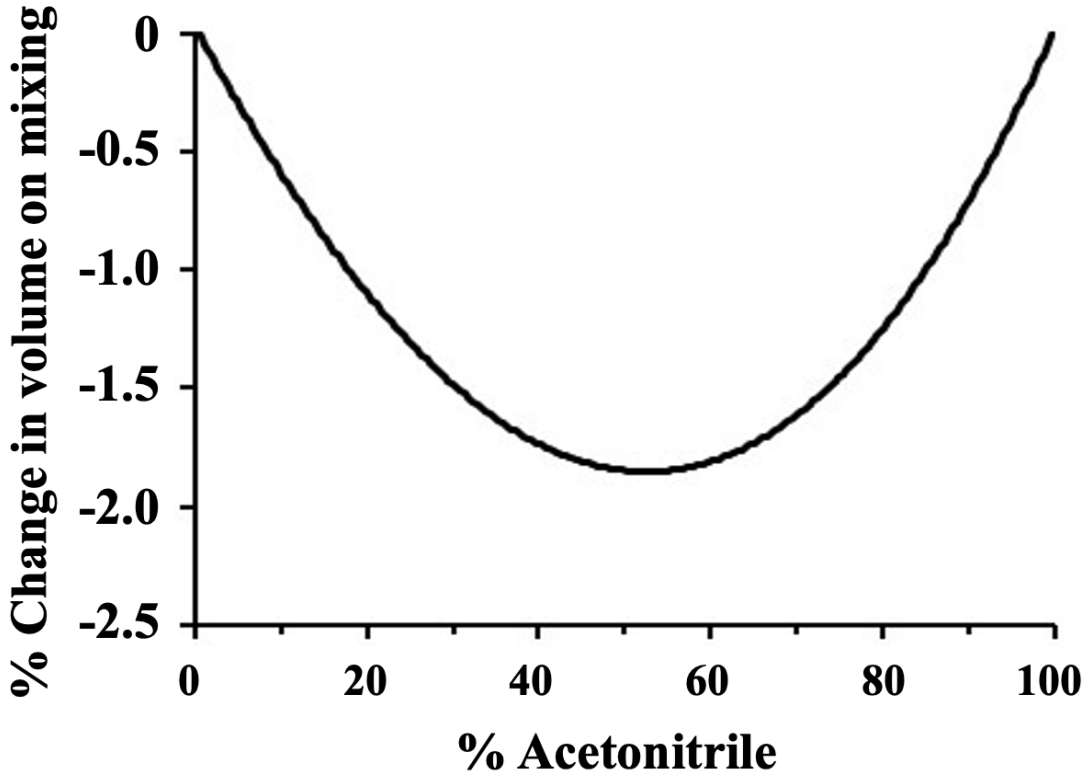


**Figure 3-3. Percent change in total volume for mixtures of acetonitrile (ACN) and water.** Based on data from ref 17.[17]

Due to the contraction in volume when mixing, we must consider the various methods for preparing the ACN/water mobile phase listed above. In Method A we mix pre-measured volumes of ACN and water. The error in our %ACN in these mobile phases would be 0%. If we used

Method B, where we fill the flask to the mark with aqueous buffer, the final %ACN would be lower than we intended. Whereas making up the final volume with ACN (Method C) would result in a higher %ACN than intended. The impact of these differences can be seen in **Table 3-2** that contains experimental retention data for two neutral analytes (uracil and cytosine) and a cationic analyte (benzyltrimethyl ammonium, BTMA) obtained under HILIC conditions.

**Table 3-2. Effect of mobile phase preparation technique on polar analyte retention**

| | | Retention Factor | | |
|---|---|---|---|---|
| **Nominal composition** | **Prep Method** | **Uracil** | **Cytosine** | **BTMA** |
| **80% ACN** | A | 0.408 | 1.24 | 7.42 |
| | B (water to fill) | 0.387 | 1.08 | 7.06 |
| | C (ACN to fill) | 0.408 | 1.25 | 7.59 |
| **85% ACN** | A | 0.454 | 1.75 | 9.08 |
| | B (water to fill) | 0.424 | 1.44 | 8.13 |
| | C (ACN to fill) | 0.459 | 1.81 | 9.47 |
| **90% ACN** | A | 0.578 | 3.44 | 16.4 |
| | B (water to fill) | 0.531 | 2.71 | 12.9 |
| | C (ACN to fill) | 0.584 | 3.57 | 16.4 |

Conditions: Zorbax HILIC Plus (silica) column at 30 ºC. Aqueous buffer was 5 mM ammonium acetate at pH 6.8.

For all of the HILIC mobile phases studied in this chapter, preparing a mobile phase using Method C (filling to mark with ACN) resulted in slightly higher retention than expected (that is, compared to Method A), whereas making the mobile phase to the mark with aqueous buffer (Method B) resulted in substantially lower retention.

The moral of the story is that the exact manner in which ACN and water are mixed must be described in full detail for that procedure to be reproducible in another laboratory, or in the same laboratory by a different analyst.

### 3.3.2 Counterions Matter

HILIC uses mobile phases with a high fraction of ACN, and so buffer solubility is a factor to consider. The solubility of buffers is lower in ACN/water mixtures than in methanol/water mixtures, and the solubility follows the trend that ammonium salts tend to be most soluble, whereas sodium salts tend to be the least soluble of the commonly used salts ($NH_4^+ > K^+ > Na^+$).[18] Hence the popularity of buffers involving ammonium salts in HILIC work is not just due to the volatility of these buffers—making them suitable for use with mass spectrometric detectors—but also due to their solubility.

But let's consider other impacts of the buffer counterion. HILIC retention is largely due to the partitioning of polar analytes into the water layer that forms on the surface of polar stationary phases (**Section 1.3.2.2)**. However, other interactions such as ion exchange and hydrogen bonding also contribute to the retention and selectivity of HILIC phases.[19, 20] A bare silica HILIC column will retain analytes based on both partitioning into the water layer and also ionic interactions with deprotonated silanols ($-SiO^-$) on the silica surface. **Figure 3-4** shows the impact of the addition of 5 mM of different chloride salts to a 5 mM ammonium acetate (pH 6.8) buffer on retention for

neutral (uracil, cytosine), anionic (benzene sulfonate, BS) and cationic (benzyltrimethyl ammonium, BTMA) analytes, both in absolute (panel A), and relative terms (panel B).

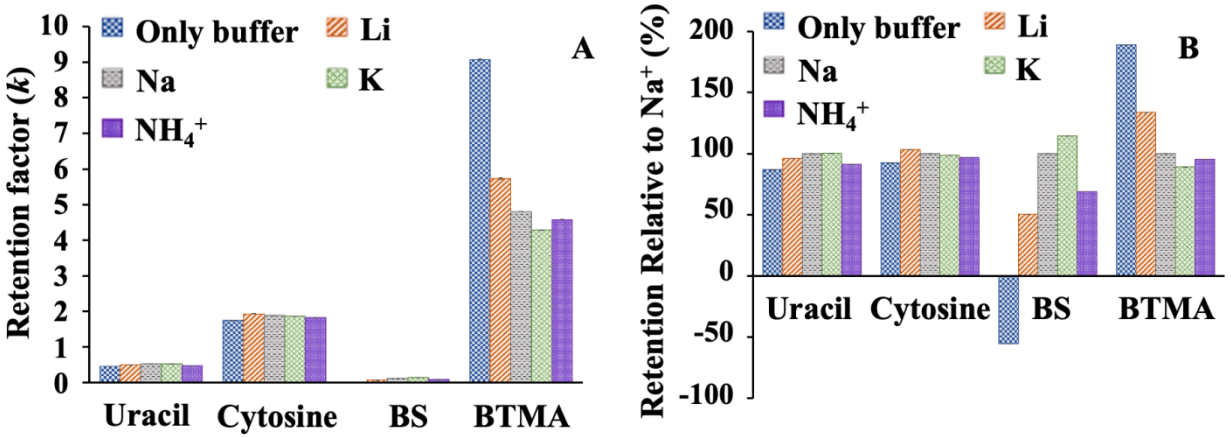


**Figure 3-4. Retention changes due to addition of 5 mM $NH_4Cl$, LiCl, NaCl or KCl to an 85% ACN / 15% 5 mM ammonium acetate (Method A) mobile phase.** Conditions: Zorbax HILIC Plus (silica) column at 30 °C.

For neutral uracil and cytosine, the identity of the buffer cation causes a small but persistent shift in retention factors. For ionic analytes the impact is much greater. The anionic benzene sulfonate (BS) experiences electrostatic repulsion from the anionic silica surface at pH 6.8 at which most surface silanols are deprotonated and negatively charged.[11] Increasing the electrolyte concentration screens the electrostatic repulsion, allowing retention of the anionic analyte to increase. The magnitude of the change in retention follows the trend $Li^+ < NH_4^+ < Na^+ < K^+$. Cationic analytes such as $BTMA^+$ undergo cation exchange with the silica surface. Addition of salt to the mobile phase provides cations that compete for the silanol exchange sites. So salt addition reduces $BTMA^+$ retention in a manner that mirrors the cation exchange selectivity— that is, $Li^+ < Na^+ < NH_4^+ < K^+$.

## 3.4 Conclusions

The volume contraction that occurs when acetonitrile and water mix means that *how* a mobile phase is prepared affects the actual composition of the mixture in percent terms. This in turn can affect the observed retention and selectivity observed under HILIC conditions. Descriptions of experimental procedures for HILIC separations should describe in detail the manner in which the two solvents are mixed. One important aspect of this that is usually not described, involves indicating how the mixture is brought to a fixed final volume (for example, Methods B and C described above), or if the measured volumes are combined to produce some nominal final volume (that is, Method A above). The counterion present in the aqueous buffer also matters, particularly for HILIC separations of charge analytes.

A final bit of discussion is to beware of assumptions. At the beginning of the work we did to produce the data presented here, we favored Method A (where the volume of each solvent is pre-measured) because it mimics how most HPLC pumps mix two separate solvents (Method D). However, when a 15-year old HPLC was allowed to do that, we observed much higher retention than that observed with method A. Of course, this suggests that something is not quite right with the pump (for example, one channel is not delivering solvent at the expected rate).

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## Chapter 4

## Pesticides in Cannabis: Analytical and Environmental Challenges and Opportunities[*]

### 4.1 Introduction

Cannabis is a psychoactive drug from the two commonly known strains Cannabis *sativa* and Cannabis *indica,* as well as hybrid strains of the two.[1, 2] **Figure 4-1** shows the Cannabis plant consisting of a well-developed root system, stem, stalk, fan leaves, and bud consisting of sugar leaves, pistils, and trichomes. Different components of the cannabis plant contain varying concentrations of the active compounds, i.e., cannabinoids. The leaves and bud are the most commonly used parts of the plant. The bud is commonly dried and used for smoking. Additionally, cannabis is processed to produce a variety of products in different forms, as detailed in **Table 4-1**.

[*] A version of this chapter has been published as Caley B. Craven, Nicholas Wawryk, Ping Jiang, Di Wu, and Xing-Fang Li. Pesticides and trace elements in cannabis: Analytical and environmental challenges and opportunities, *J. Environ. Sci.*, **2019**, *85*, 82–93

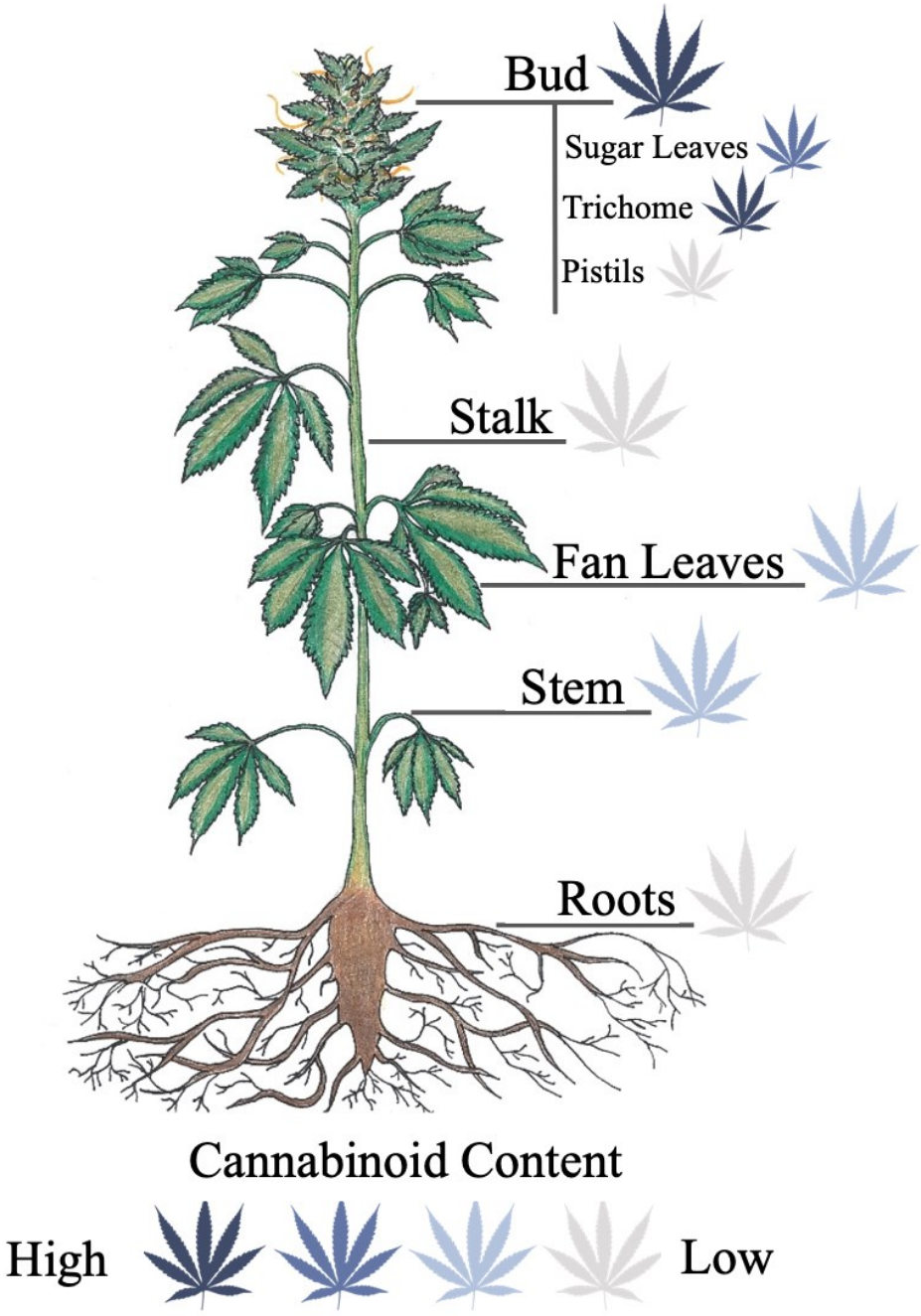



**Figure 4-1. Schematic of cannabis plant.**

**Table 4-1. Different forms of Cannabis products and their mode of use.** Adapted from Government of Canada at Health, Drug and health products, Drugs and medication, Cannabis web page.

| Form | Description | Made from | Mode of use |
|---|---|---|---|
| Fresh or dried herbal material | Natural plant component, is not altered or changed – used in its natural fresh or dried form – also called marijuana. | Mainly uses the leaves and flowers of the plant | Inhalation |
| Cannabis oil | Cannabis extract that is dissolved in a carrier oil (like olive oil or grapeseed oil). This can be used to make other forms. | Mainly uses the leaves and flowers of the plant | Inhalation, ingestion, absorption |
| Chemically concentrated extracts | Highly concentrated cannabis extract dissolved in petroleum-based solvent (i.e., butane). Most concentrated forms are shatter, butter, and wax. | Mainly uses the leaves and flowers of the plant | Inhalation, ingestion |
| Physically concentrated extracts | Loose trichomes or pressed resin from the cannabis plant | Whole plant | Inhalation, ingestion, absorption |
| Tinctures/sprays | Cannabis extract dissolved in a solvent (usually alcohol). Can be used to make other products (i.e., edibles) | Mainly uses the leaves and flowers of the plant | Ingestion, absorption |
| Edibles | Food and drinks containing extracts of cannabis (can be made from other forms) | Depends | Ingestion |
| Creams/Salves/Liniments | Cannabis extract preparation (can use other forms or prepare) prepared with alcohol, oil, or wax and then applied to the skin | Depends | Absorption |

Cannabis and related products have been widely used for medicinal, recreational, and industrial purposes.[1-5] Medicinal use of cannabis has been dated back to the end of the Bronze Age.[3] Since then, cannabis has been used to treat epilepsy or symptoms from multiple sclerosis, stimulate appetite or suppress nausea for cancer or AIDS patients, achieve analgesia, and relieve chronic headaches and migraines.[4-7] Cannabis also helps some people relax and reduce anxiety. Additional industrial applications use the fibrous material of cannabis for bioplastics, clothes, and ropes.[1-5] While cannabis has beneficial functions, health risks are of great concern. Regular use or abuse of cannabis may increase the risk of developing respiratory disease, psychosis, motor vehicle collisions, and low birth weight offspring. Underage users of cannabis are of particular concern because of potential damage to brain development, addiction, and lack of focus and motivation over time.[8-15]

Because of the positive and negative health effects of cannabis, controversy has existed over its legality throughout history. Legal controversy of cannabis arose in the early 1800s after cannabis started to spread globally. Since then, prohibition of cannabis use was implemented. In the mid-20$^{th}$ century, many countries began switching from banning cannabis completely to legalizing medicinal use of cannabis or allowing medical use of cannabis within strict guidelines. Over the past 10 years, a few countries including Uruguay, Canada, and several states (e.g., Oregon and California) in the USA have legalized recreational cannabis use.[15-17]

The 2017 World Drug Report estimated that 183 million of the world's population use cannabis annually.[18] The global legal cannabis market is expected to reach 147 billion USD by the end of 2025.[19] With the growing demand for cannabis, safety of cannabis products has become urgent. In the countries (e.g., USA and Canada) that have legalized cannabis, regulatory agencies have set strict guidelines to ensure the quality, safety, and efficacy of cannabis

products. These guidelines regulate similar groups of contaminants, but may vary in the specific compounds and their concentration action limits. In general, quality control of cannabis products includes its testing for pesticides, heavy metals, mycotoxins, and microbes. As well, cannabis oil is occasionally tested for residual solvents. All of the above contaminants require monitoring because they can be introduced to cannabis products in the growth and/or processing stages of the plant. Consumers may be exposed to these contaminants when consuming cannabis products. Toxicological effects of pesticides, heavy metals, mycotoxins and pathogenic microbes are well documented in the literature, and include carcinogenicity, neurotoxicity, and teratogenicity, among many others.[2, 20-31]

Consumers exposed to these contaminants through consumption of cannabis products may experience short and long term adverse health effects. In addition to ensuring consumer safety, proper testing and quality assurance/quality control (QA/QC) of cannabis and related products protects economic interest for industry. When QA/QC is assured from the growing stage to the final products, producers can eliminate recalls, seizure, or fines and meet legal requirements. It is important for growers to perform QA/QC throughout the growing stages, as this provides predictive markers for contaminant levels in their final product. Thus, this allows some risk removal of economic loss by not investing into products that would have otherwise failed to meet criteria.

To comply with regulations and to provide safe cannabis products, many licensed testing laboratories have been established. Currently, licensed laboratories have developed multiple advanced analytical methods for testing contaminants in cannabis. Additionally, analytical instrumentation developers and venders have dedicated great efforts into helping customers develop application notes for the analysis of cannabis. An open access magazine *Cannabis*

*Science and Technology* has been launched as a platform for exchanging ideas and problem-solving on analytical testing and quality control of cannabis. To date, publications on current analytical development for testing of contaminants in cannabis are limited in peer-reviewed scientific journals. This **Chapter** reviews the current analytical methods commonly used for testing pesticides in Cannabis.

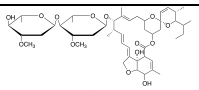
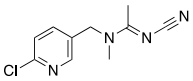
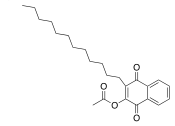
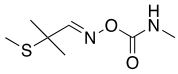
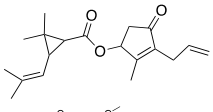
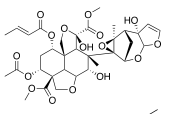
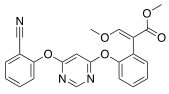
### 4.2 Pesticides

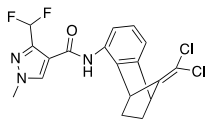
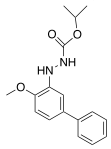
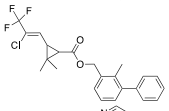
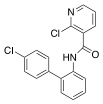
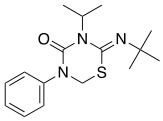
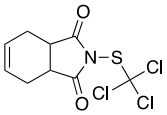
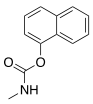
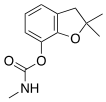
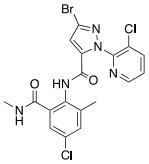
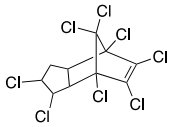
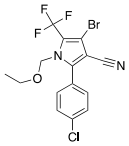
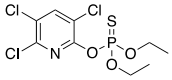
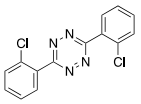
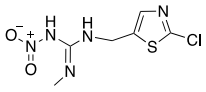
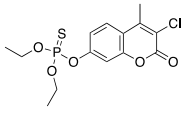
Pesticides can accumulate in cannabis and its related products throughout the growth and processing stages. Pesticides are typically sprayed during cannabis cultivation and their residues may either stay, or be enriched, in different cannabis products during processing. Consumer exposure to residual pesticides has become an important issue because of potential adverse health effects. Several pesticides have shown carcinogenic and mutagenic effects in humans and could be lethal when overdosed.[2, 20-22, 30, 31] Pesticides may also transform during cannabis processing. While this has not been studied in cannabis, there are many examples of pesticide transformation in environment and food matrices.[32, 33] Pesticide transformation could add further complexity and importance when analyzing cannabis for pesticide residues.

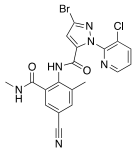
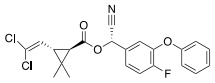
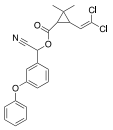
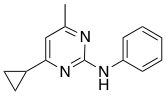
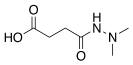
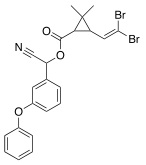
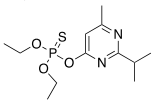
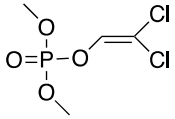
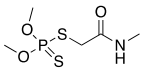
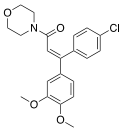
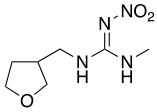
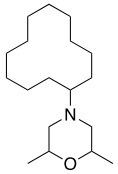
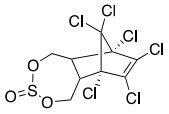
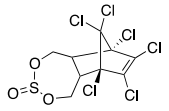
Regulations on pesticides differ from country to country and state to state. For example, in the USA, pesticide regulations in cannabis are specific to each state, while the American Public Health Laboratories (APHL) recommends limits for common pesticides used across the country in medical cannabis.[34] As shown in **Table 4-2**, Oregon mandates the testing of 59 pesticides, while the California pesticides list includes 58 from the Oregon list and has 8 additional pesticides which are not on the Oregon pesticide list. To date, Canada has set wider and stricter controls of pesticides in cannabis products compared to that in the USA. In Canada,

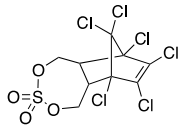
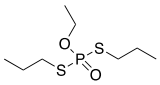
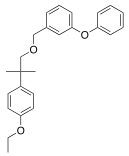
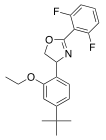
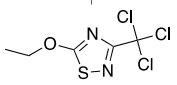
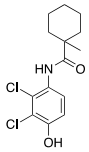
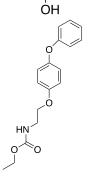
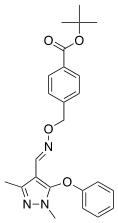
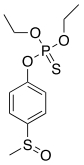
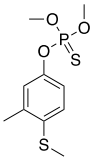
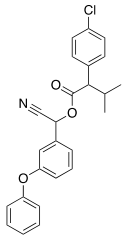
since the legalization of recreational use of cannabis on October 17th, 2018, it is mandatory to test 96 pesticide-active ingredients for cannabis products before any lot is released. The maximum residual levels (MRL) or action limits also vary regionally. Oregon sets action limits of 0.2-1.0 µg/g for all cannabis products. Whereas in Canada, depending on the form of the products, the limits of quantification (LOQ) of pesticide active ingredients are 0.01-1.5 µg/g, 0.02-1.0 µg/g, and 0.01-1.5 µg/g for fresh cannabis plants, dry flowers, and cannabis oils, respectively.

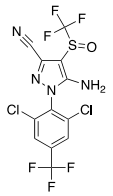
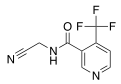
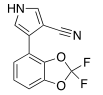
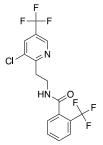
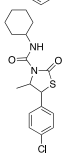
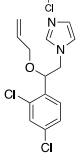
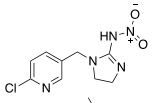
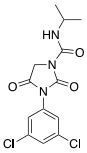
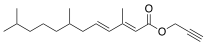
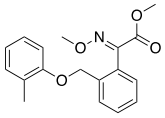
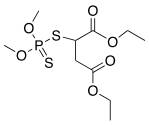
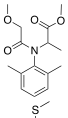
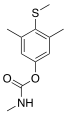
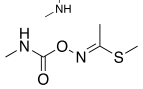
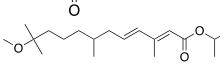
**Table 4-2. Structure, limits of quantification, and common analytical testing methods of pesticides or active ingredients listed in Health Canada Guidelines.**

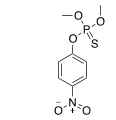
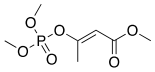
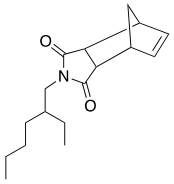
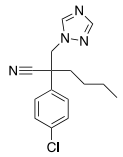
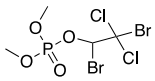
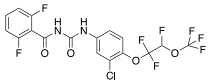
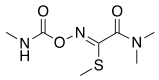
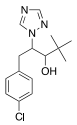
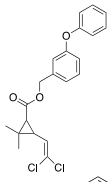
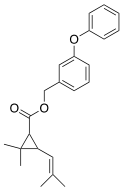
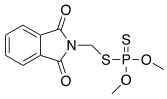
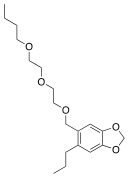
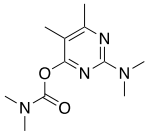
| Active Ingredient | Structure | Limits of Quantification, Health Canada, Canada - µg/g | | | Action limits, California, USA - µg/g | | Action limits, Oregon, USA - µg/g | Amenability (GC/LC/GC& LC) |
|---|---|---|---|---|---|---|---|---|
| | | Fresh cannabis /plants | Dried cannabis | Cannabis oil | Inhalable Cannabis Goods | Other Cannabis Goods | Cannabis | |
| Abamectin |  | 0.25 | 0.1 | 0.25 | 0.10 | 0.30 | 0.5 | LC |
| Acephate |  | 0.05 | 0.020 | 0.050 | 0.10 | 5.0 | 0.4 | LC or GC |
| Acetamiprid |  | 0.050 | 0.10 | 0.050 | 0.1 | 5.0 | 0.2 | LC or GC |
| Acequinocyl |  | 0.05 | 0.03 | * | 0.10 | 4.0 | 2.0 | LC |
| Aldicarb |  | 0.50 | 1.0 | 0.50 | 0.10 | 0.10 | 0.4 | LC or GC |
| Allethrin |  | 0.10 | 0.20 | 0.10 | | | | LC or GC |
| Azadirachtin |  | 0.50 | 1.0 | 0.50 | | | | LC |
| Azoxystrobin |  | 0.010 | 0.020 | 0.010 | 0.10 | 40.0 | 0.2 | LC or GC |

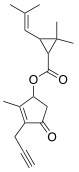
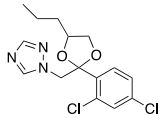
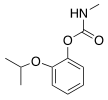
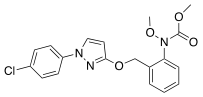
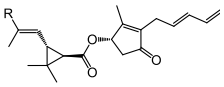
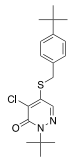
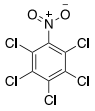
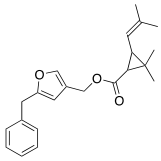
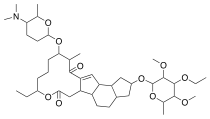
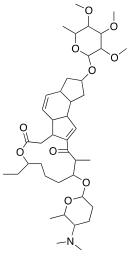
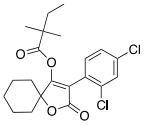
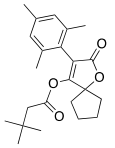
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Benzovindiflupyr |  | 0.010 | 0.020 | 0.010 | | | | LC or GC |
| Bifenazate |  | 0.05 | 0.020 | 0.010 | 0.10 | 5.0 | 0.2 | LC or GC |
| Bifenthrin |  | 0.10 | 1 | * | 3.0 | 0.50 | 0.2 | LC or GC |
| Boscalid |  | 0.010 | 0.020 | 0.010 | 0.10 | 10.0 | 0.4 | LC or GC |
| Buprofezin |  | 0.010 | 0.020 | * | | | | LC or GC |
| Captan |  | | | | 0.7 | 5.0 | | GC or LC with APCI |
| Carbaryl |  | 0.025 | 0.050 | 0.025 | 0.5 | 0.5 | 0.2 | LC or GC |
| Carbofuran |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Chlorantraniliprole |  | 0.01 | 0.02 | * | 10.0 | 40.0 | 0.2 | LC |
| Chlordane |  | | | | 0.10 | 0.10 | | GC or LC with APCI |
| Chlorphenapyr |  | 0.10 | 0.05 | 1.5 | 0.10 | 0.10 | 1.0 | GC or LC with APCI |
| Chlorpyrifos |  | 0.010 | 0.04 | 0.50 | 0.10 | 0.10 | 0.2 | GC or LC with APCI |
| Clofentezine |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.50 | 0.2 | LC |
| Clothianidin |  | 0.025 | 0.050 | 0.025 | | | | LC |
| Coumaphos |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | | LC or GC |

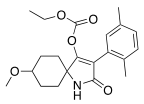
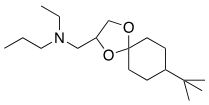
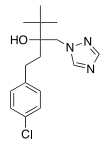
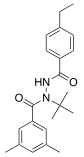
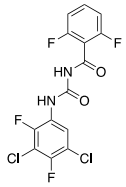
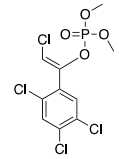
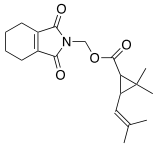
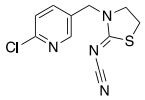
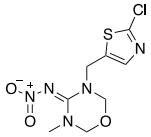
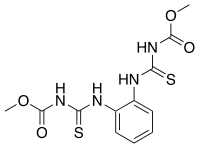
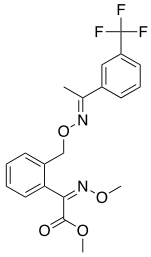
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cyantranilipole |  | 0.010 | 0.02 | 0.010 | | | | LC |
| Cyfluthrin |  | 1 | 0.2 | * | 2.0 | 1.0 | 1.0 | LC or GC |
| Cypermethrin |  | 1 | 0.3 | * | 1.0 | 1.0 | 1.0 | LC or GC |
| Cyprodinil |  | 0.25 | 0.25 | 0.010 | | | | LC or GC |
| Daminozide |  | 0.05 | 0.1 | * | 0.10 | 0.10 | 1.0 | LC |
| Deltamethrin |  | 1 | 0.5 | * | | | | LC or GC |
| Diazinon |  | 0.010 | 0.02 | * | 0.10 | 0.2 | 0.2 | LC or GC |
| Dichlorvos |  | 0.050 | 0.10 | 0.050 | 0.10 | 0.10 | 1.0 | GC or LC with APCI |
| Dimethoate |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Dimethomorph |  | 0.05 | 0.05 | * | 2.0 | 20.0 | | LC or GC |
| Dinotefuran |  | 0.050 | 0.10 | 0.050 | | | | LC |
| Dodemorph |  | 0.050 | 0.05 | * | | | | LC |
| Endosulfan-alpha |  | 0.10 | 0.2 | 2.5 | | | | GC or LC with APCI |
| Endosulfan-beta |  | 0.50 | 0.05 | 2.5 | | | | GC or LC with APCI |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Endosulfan sulfate |  | 0.50 | 0.05 | 2.5 | | | | GC or LC with APCI |
| Ethoprophos |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Etofenprox |  | 0.01 | 0.05 | * | 0.10 | 0.10 | 0.4 | LC or GC |
| Etoxazole |  | 0.010 | 0.020 | * | 0.10 | 1.5 | 0.4 | LC or GC |
| Etridiazol |  | 0.010 | * | 0.15 | | | | GC |
| Fenhexamid |  | | | | 0.10 | 10.0 | | LC or GC |
| Fenoxycarb |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Fenpyroximate |  | 0.02 | 0.020 | * | 0.10 | 2.0 | 0.4 | LC or GC |
| Fensulfothion |  | 0.010 | 0.020 | 0.010 | | | | GC |
| Fenthion |  | 0.010 | 0.02 | 0.010 | | | | LC or GC |
| Fenvalerate |  | 0.1 | 0.1 | * | | | | LC or GC |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fipronil |  | 0.010 | 0.060 | 0.010 | 0.10 | 0.10 | 0.4 | LC or GC |
| Flonicamid |  | 0.025 | 0.050 | 0.025 | 0.10 | 2.0 | 1.0 | LC |
| Fludioxonil |  | 0.010 | 0.020 | 0.010 | 0.10 | 30.0 | 0.4 | LC or GC |
| Fluopyram |  | 0.010 | 0.020 | 0.010 | | | | LC or GC |
| Hexythiazox |  | 0.01 | 0.01 | * | 0.10 | 2.0 | 1.0 | LC or GC |
| Imazalil |  | 0.01 | 0.05 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Imidacloprid |  | 0.010 | 0.020 | 0.010 | 5.0 | 3.0 | 0.4 | LC |
| Iprodione |  | 0.50 | 1.0 | 0.50 | | | | LC or GC |
| Kinoprene |  | 0.050 | 0.5 | 1.25 | | | | LC or GC |
| Kresoxim-methyl |  | 0.010 | 0.02 | 0.15 | 0.10 | 1.0 | 0.4 | LC or GC |
| Malathion |  | 0.010 | 0.020 | 0.010 | 0.50 | 5.0 | 0.2 | LC or GC |
| Metalaxyl |  | 0.010 | 0.020 | 0.010 | 2.0 | 15.0 | 0.2 | LC or GC |
| Methiocarb |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Methomyl |  | 0.05 | 0.050 | 0.025 | 1.0 | 0.10 | 0.4 | LC |
| Methoprene |  | 1.0 | 2.0 | * | | | | LC or GC |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Methyl parathion |  | * | * | * | 0.10 | 0.10 | 0.2 | LC or GC |
| Mevinphos |  | 0.025 | 0.050 | 0.025 | 0.10 | 0.10 | | LC or GC |
| MGK-264 |  | 0.05 | 0.05 | * | | | 0.2 | GC |
| Myclobutanil |  | 0.010 | 0.020 | 0.010 | 0.1 | 9.0 | 0.2 | LC or GC |
| Naled |  | 0.2 | 0.1 | * | 0.10 | 0.50 | 0.5 | LC or GC |
| Novaluron |  | 0.025 | 0.050 | 0.025 | | | | LC or GC |
| Oxamyl |  | 1.5 | 3.0 | 1.5 | 0.50 | 0.20 | 1.0 | LC |
| Paclobutrazol |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.4 | LC or GC |
| Permethrin |  | 0.50 | 0.5 | * | 0.50 | 20.0 | 0.2 | GC or LC with APCI |
| Phenothrin |  | 0.025 | 0.050 | * | | | | GC or LC with APCI |
| Phosmet |  | 0.01 | 0.02 | * | 0.10 | 0.20 | 0.2 | LC or GC |
| Piperonyl butoxide |  | 0.25 | 0.2 | 1.25 | 3.0 | 8.0 | 2.0 | LC or GC |
| Pirimicarb |  | 0.010 | 0.020 | 0.010 | | | | LC or GC |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Prallethrin |  | 0.05 | 0.05 | * | 0.10 | 0.40 | 0.2 | LC or GC |
| Propiconazole |  | 0.010 | 0.1 | * | 0.10 | 20.0 | 0.4 | LC or GC |
| Propoxur |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC or GC |
| Pyraclostrobin |  | 0.010 | 0.020 | 0.010 | | | | LC |
| Pyrethrin I (R = $CH_3$)<br>Pyrethrin II (R = $COOCH_3$) |  | 0.025 | 0.050 | * | 0.50 | 1.0 | 1.0 | LC or GC |
| Pyridaben |  | 0.025 | 0.050 | 0.020 | 0.10 | 3.0 | 0.2 | LC or GC |
| Quintozene |  | 0.010 | 0.02 | * | 0.10 | 0.20 | | GC or LC with APCI |
| Resmethrin |  | 0.02 | 0.10 | 0.050 | | | | LC or GC |
| Spinetoram |  | 0.01 | 0.02 | 0.010 | 0.10 | 3.0 | | LC |
| Spinosad |  | 0.01 | 0.1 | 0.010 | 0.10 | 3.0 | 0.2 | LC |
| Spirodiclofen |  | 0.25 | 0.25 | * | | | | LC or GC |
| Spiromesifen |  | 0.05 | 3.0 | * | 0.10 | 12.0 | 0.2 | LC or GC |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Spirotetramat |  | 0.1 | 0.020 | 0.010 | 0.10 | 13.0 | 0.2 | LC |
| Spiroxamine |  | 0.01 | 0.1 | * | 0.10 | | 0.4 | LC or GC |
| Tebuconazole |  | 0.01 | 0.05 | 0.010 | 0.10 | 2.0 | 0.4 | LC or GC |
| Tebufenozide |  | 0.010 | 0.020 | 0.010 | | | | LC or GC |
| Teflubenzuron |  | 0.025 | 0.050 | 0.025 | | | | LC |
| Tetrachlorvinphos |  | 0.010 | 0.020 | 0.010 | | | | GC or LC with APCI |
| Tetramethrin |  | 0.050 | 0.10 | * | | | | GC |
| Thiacloprid |  | 0.010 | 0.020 | 0.010 | 0.10 | 0.10 | 0.2 | LC |
| Thiamethoxam |  | 0.010 | 0.020 | 0.010 | 5.0 | 4.5 | 0.2 | LC |
| Thiophanate-methyl |  | 0.03 | 0.050 | * | | | | LC |
| Trifloxystrobin |  | 0.010 | 0.020 | 0.010 | 0.10 | 30.0 | 0.2 | LC or GC |

* Data not available.
Levels reported as last checked July 2021.

Development of analytical methods for determination of pesticides in cannabis and cannabis related products is challenging. First, cannabis plants are highly variable in their active compounds and are processed using different protocols to produce diverse products (**Table 4-1**). Cannabis products consist of complex chemicals in vastly different compositions. Analysis of cannabis products must overcome severe matrix effects, requiring a practical sample extraction and cleanup technique. Second, analytical methods must be sensitive, selective, and accurate for quantification of pesticides in cannabis products to comply with regulations. Third, the 96 pesticides regulated by Health Canada have large variation in their physical and chemical properties making it difficult, but desirable, to develop a high throughput method for all 96 pesticides. For the QA/QC of cannabis products, analytical testing of pesticides begins at cultivation and continues all the way to the final product. This testing is a massive amount of analytical work. The first hurdle is the sample preparation, including extraction and cleanup prior to instrumental analysis.

## 4.3 Sample Preparation for Pesticides Testing

Sample preparation is a key step to determine pesticides in cannabis and related products at the sub-μg/g levels necessary to meet legal requirements (**Table 4-2**). **Figure 4-2** presents a general schematic on sample preparation for pesticide analysis in cannabis. Sample homogenization is the first step in cannabis sample preparation. Fresh or dried cannabis flowers are ground (automatic or manual grinder) to produce a homogenous powder or paste. Oil products only require mixing with organic solvents (commonly acetonitrile (ACN)), followed by vortexing, to obtain a homogenized solution.

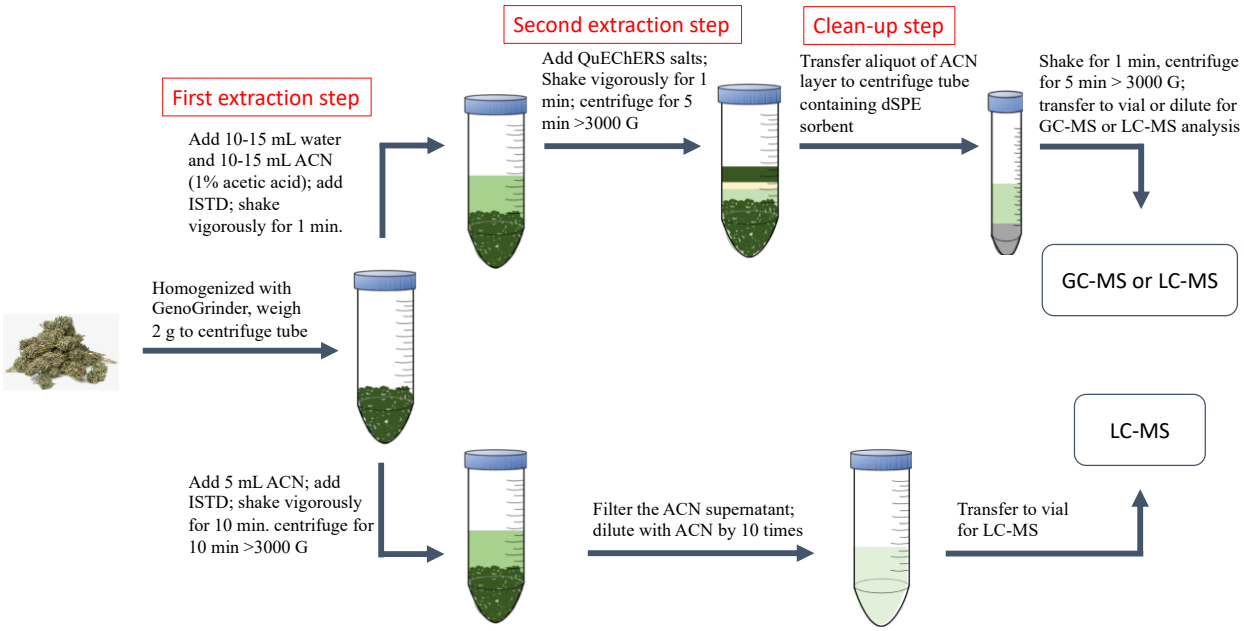



**Figure 4-2. Major steps in QuEChERS (top) and acetonitrile extraction process (bottom).**

Note: ISTD represent internal standard; QuEChERS salts: $MgSO_4$ (4 g), NaCl (1 g), $Na_3$Citrate dihydrate (1 g), and $Na_2$HCitrate sesquihydrate (0.5 g) for every 10 mL of water; dSPE sorbent: PSA (25 mg) and $MgSO_4$ (150 mg) for every 1 mL of aliquot.

After homogenization, various extraction methods have been used to cleanup and concentrate pesticides for quantitative analysis. These methods include liquid-liquid-extraction (LLE),[35] solid phase microextraction (SPME),[36, 37] and QuEChERS (quick, easy, cheap, effective, rugged, and safe).[38-41] Among these methods, QuEChERS is recommended in European EN 15662 method[42] for pesticide analysis in foods of plant origins and has been modified and widely used for cannabis testing.[39, 40, 43-46]

**Figure 4-2** displays a simplified standard procedure for the QuEChERS method in EN15662. First, the homogenized samples are suspended in water, then extracted by liquid-

liquid extraction with organic solvent (typically ACN). Dependent on what pesticides are being targeted, acids such as formic, acetic, or citric acid may be added to further improve extraction.[40, 43-45] This is followed by addition of QuEChERS salts (i.e., $MgSO_4$, NaCl, and citrate buffer) to the solution. These salts help absorb the water in solution, as well as induce the phase separation between ACN and water, which further enhances the extraction efficiency. Finally, a cleanup step with dispersive solid phase extraction (dSPE) is performed. The dSPE sorbent is composed of $MgSO_4$ or $CaCl_2$ with primary/secondary amines (PSA) and graphitized carbon black (GCB). The sorbent removes proteins, lipids, and chlorophyll from solution. Many vendors, as well as regulatory agencies (e.g. European EN 15662[42]), recommend QuEChERS or modified QuEChERS for the extraction of pesticides. Simple modification of the QuEChERS protocols can improve the extraction for cannabis analysis. A recent study examined extraction efficiency by altering the QuEChERS buffer (acetate or citrate buffer) during the extraction step or by altering the sorbent during the dSPE cleanup ($MgSO_4$ or $CaCl_2$). When all other conditions are the same, switching from acetic buffer to citrate buffer extracted nine more pesticides. Without changing other conditions, the use of $MgSO_4$ in the dSPE step enabled detection of six more pesticides compared to the use of $CaCl_2$. Overall, the method using citrate buffer for extraction and $MgSO_4$ during dSPE provided the best performance–which is 46 of the 61 pesticides that have been approved for agriculture use in Uruguay can be detected in seized cannabis products with a recovery of 70–120%.[43]

Though QuEChERS is widely used, it does encounter some challenges for pesticide analysis. These methods cannot remove hydrophobic interfering compounds, such as cannabinoids and terpenes, as these are also extracted into the organic solvent. As well, QuEChERS has poor (< 10%) recovery for polar pesticides, such as daminozide. During the

dSPE step, many acidic pesticides can bind to the basic adsorbent PSA, resulting in a low recovery.[47] Alternatively, liquid-liquid extraction methods using acetonitrile have been developed for pesticide analysis (Bottom flowchart in **Figure 4-2**). In this method, the homogenized sample is both suspended and extracted with pure ACN (instead of an ACN/$H_2O$ mixture). No $H_2O$ is added so that polar pesticides such as daminozide can be extracted. Due to the solubility of pesticides in ACN, pesticides are easily extracted from the homogenized samples. After extraction, the sample is centrifuged, and the supernatant is then diluted ten times to reduce matrix effects. With this simple ACN-based extraction method, the 66 pesticides regulated by California were recovered with a range of 70-120% and with an RSD of < 20%.[35] Acetonitrile extraction is simple, fast, and compatible with liquid chromatography mass spectrometry (LC-MS) analysis.

Solid phase micro extraction (SPME) has been reported for the extraction of nine frequently observed pesticides. A SPME with polydimethylsiloxane (PDMS) fiber was used for a 5-minute extraction of the headspace above cannabis plant. This fiber was then directly inserted into the GC injection port and desorbed at 280 °C. This SPME procedure obtained a recovery of 96-105% with an RSD of < 13% for the nine pesticides.[37] SPME is rapid, simple, and dramatically reduces the use of organic solvent as no other sample preparation is required for headspace analysis other than sample homogenization. This method is useful for the small numbers of pesticides analyzed. SPME warrants further development to analyze a larger number of pesticides in cannabis to meet both the Canadian and US guidelines.

## 4.4 Qualitative and Quantitative Analysis of Pesticides in Cannabis

Pesticides vary significantly in their polarity and other chemical properties. Both gas chromatography (GC) and liquid chromatography (LC) with mass spectrometry (MS) techniques are used for pesticide analysis. GC-MS is the best choice for volatile pesticides, while LC-MS is preferred for ionic and polar pesticides.

### 4.4.1 Gas Chromatography Mass Spectrometry

Volatile and highly hydrophobic pesticides are often analyzed using GC-MS or GC-MS/MS, as described in **Table 4-2**. During GC separation, pesticides are resolved on (5% phenyl)-methylpolysiloxane capillary column with helium as the carrier gas.[36, 37, 40] New phases with trademark of ZB-multiple residue-1 have been reported to provide higher resolution for pesticides, but the chemical property of this phase is unknown.[48] For trace analysis, samples prepared by QuEChERS or SPME are injected using splitless mode and are ionized using electron impact (EI). For GC-MS detection of pesticides, quadrupole mass selective analyzer in selected ion monitoring (SRM) mode is used. For each pesticide, one ion is selected for quantification and one or two extra ions are monitored for identification. Alternatively, GC has also been combined with MS/MS on a triple quadrupole mass analyzer in multiple reaction monitoring (MRM) mode for pesticide analysis in cannabis.[36, 37] GC-MS with SRM mode provides high sensitivity. For example, combined with headspace-SPME, GC-MS with SRM mode enabled quick screening of nine chlorinated pesticides in cannabis with limits of detection (LOD) of 0.014-0.83 μg/g.[36] Also, GC separations can provide high peak capacity and resolution. GC-MS with SRM detected up to 100 pesticides in seized cannabis leaves and

flowers with LOQ of 0.15 µg/g.[40] Approximately 30% of the 100 pesticides analyzed in this study can be found in the Health Canada watch list.[40]

GC-MS is a sensitive and selective method for hydrophobic and volatile pesticides, but is limited in the analysis of polar, high molecular weight, or thermally labile compounds. Derivatization prior to GC-MS could be performed, but this increases the sample preparation time, as well as complicating sample matrices. Furthermore, the injection port of the GC instrument is susceptible to contamination from complex cannabis sample matrices, which requires extra cleanup of the sample or demands frequent instrument maintenance. Thus, for current pesticide testing in cannabis, GC-MS often complements LC-MS for full coverage of the list mandated by regulatory agencies. It is commonly accepted that GC-MS, or GC-MS/MS, works well for analysis of several pesticides in the Oregon list, including chlorfenapyr, cyfluthrin, naled, MGK-264, permethrin, pyrethrin I, and pyrethrin II.

### 4.4.2 Liquid Chromatography with Mass Spectrometry

LC-MS is a powerful technique for analysis of polar and ionic pesticides. A large number of regulated pesticides are polar, labile, and can be easily ionized using electrospray ionization (ESI). Therefore, LC-ESI-MS/MS is better suited for analysis of these pesticides that GC/MS cannot easily do. For trace analysis, LC is necessary to separate pesticides from each other and from the complex cannabis matrix to reduce interference with ESI-MS detection. A large number of pesticides are soluble in aqueous-organic mixtures, and extracts of samples are often in acetonitrile or an acetonitrile/water mixture. Therefore, separation of pesticides in these extracts can be achieved using reverse phase LC (RPLC) with C18 or biphenyl columns. The injection volume should be kept small, at 3-10 µL, to avoid the distortion of peak shape caused by the

high percentage of acetonitrile in the sample extracts.[49] When the concentrations of pesticides in samples are high, simple dilution of the extracts can reduce matrix effects and improve peak shapes.

A majority of the pesticides found in the Health Canada, California, or Oregon lists yield protonated ions $[M+H]^+$ in positive mode ESI. MRM using triple quadrupole or quadrupole-ion trap MS instruments provide high sensitivity and selectivity. The identification of pesticides is based on matching the retention time, using two or more parent-fragment ion pairs of each compound, and comparing the ratio between the signals of each ion-pairs with those of standards. Other tips on improving sensitivity of pesticides are also useful. For example, some pesticides have significantly higher sensitivity using ammonium adducts $[M+NH_4]^+$ (e.g., acequinocyl), thus detection of $[M+NH_4]^+$ can reduce the limit of detection for pesticides containing a -COOH group.

With QuEChERS or acetonitrile extraction, LC-MS/MS methods provides LOQs of 0.01-0.2 μg/g for the dry cannabis flower. This is adequate to meet the requirements of most regulations.[40, 43] An LC-MS/MS method that is capable of detecting the 59 pesticides on the Oregon list has been reported extensively in application notes by different instrumentation vendors.[50, 51] However, other regions such as California and Canada regulate additional pesticides that are not in the Oregon list. New challenges emerge for the quantification of pesticides in cannabis using the LC-MS/MS method. Specifically, the California list of 66 pesticides includes eight chlorinated pesticides that are not amenable with ESI, making analysis with LC-ESI-MS/MS difficult.

To overcome this problem, atmospheric pressure chemical ionization (APCI) has been used to analyze these low polarity pesticides. For example, chlorinated pesticides including

chlordane, chlorfenapyr, and pentachloronitrobenzene are rarely detectable using LC-ESI-MS/MS, while LC-APCI-MS/MS can achieve LOQ of 0.05, 0.05, and 0.01µg/g, respectively. These LOQs are comparable to those of GC-MS, and are in some cases 10 times lower than the action limits set by California. By performing two separate runs using LC-ESI-MS/MS and LC-APCI-MS/MS, the 66 pesticides on the California list can be quantified in cannabis flowers with an accuracy of 70-120%.[35] These two ionization methods can be done in parallel on the same MS instrument.[35, 52]

Other ionization techniques such as dielectric barrier discharge ionization (DBDI) have also been reported. DBDI ionizes samples in a plasma with a partially ionized gas (like argon) that contains free electrons to reduce the cationization phenomena and reduce formation of Na adducts while providing similar sensitivity to ESI.[53] However, using DBDI for pesticides analysis requires further development for negatively charged pesticides.

Currently, Health Canada mandates the testing of 96 pesticides with lower action limits compared to other regulations. This presents additional challenges for pesticide analysis. It is desirable to use a single method for analysis of the full set of 96 pesticides. This singular method should provide comparable performance to the LC-MS/MS and GC-MS/MS methods to improve sample throughput as well as reducing the cost of testing for regulatory agencies, consumers, and industry. Development of such a method is discussed in **Chapter 5**.

### 4.4.3 Other Methods

Other methods have also been used for analysis of pesticides in cannabis. LC-UV was used for quantification of paraquat with an LOD of 2 μg/g.[54] Paraquat is one of the most widely used herbicides in cannabis. LC-UV is relatively easy to perform with an acceptable LOD for this compound.[54] However, such a method is not suitable for multiresidue pesticide analysis. In another example, a 3-minute capillary electrophoresis (CE)-UV method was developed to determine herbicides paraquat, glyphosate, and aminomethyl-phosphonic acid in cannabis. The CE-UV method is fast, but the LOQ was 5-10 μg/g for targeted analytes, which does not meet regulatory action limits.[55] GC coupling with infrared spectroscopy has also been used to detect paraquat and its pyrolysis products at specific temperatures in cannabis. However, this method does not provide a sufficiently low LOD and would be difficult to identify multiple pesticides due to its low specificity.[56]

In summary, LC-MS/MS and GC-MS/MS provide complementary analysis of pesticides in cannabis to meet regulatory demands.[57] LC-MS/MS is capable of analyzing the full list of pesticides from California and Oregon's lists; however, this requires advanced MS instrumentation such as a dual ionization source and an intricate method for analysis. For several pesticides, such as the chlorinated pesticides and herbicides, GC is a much simpler option while still having great sensitivity and selectivity. Both peer reviewed articles and technical notes have acknowledged the benefits of using both GC-MS and LC-MS for the analysis of pesticides. GC-MS/MS and LC-MS/MS methods have been developed for the screening of 100 and 50 pesticides, respectively, in seized cannabis plants.[40] Similarly, 144 medical cannabis samples were screened using both GC-MS/MS and LC-MS/MS methods with GC targeting 19 pesticides and LC targeting 73 pesticides.[58] Integration of the GC-MS/MS and LC-MS/MS information

enables the establishment of a database containing MS information for over 100 pesticides. The database is highly beneficial to laboratories for testing pesticides in cannabis and for growers to produce safe cannabis products.

## 4.5 Perspective

The use of cannabis is dramatically increasing due to legalization in many states and countries. The safety of cannabis products and protection of consumers from adverse health effects become an urgent responsibility for both regulatory agencies and cannabis industry. To ensure the quality, safety, and efficacy of cannabis products, a number of states and countries have established various guidelines to control the chemical and biological contaminants, including pesticides, toxic elements, mycotoxins, pathogens, as well as residual solvents in regard to cannabis oil. Therefore, there is a great need for the development of rapid and reliable analytical methods for the detection of these contaminants in cannabis products as well as in growing environments. However, development of analytical methods for these contaminants in cannabis products confronts several challenges, such as complying with various guidelines, large varieties in cannabis products, simultaneous detection of multiple contaminants, and trace-levels of some contaminants. Currently, the combination of LC-MS and GC-MS is used to cover the full spectrum of regulated pesticide residues. Although a great deal of effort has been devoted to efficient extraction of contaminants from cannabis products, it is still desirable to develop fast, and effective extraction methods that are applicable to various contaminants and cannabis products. Additionally, rapid, simple, and sensitive analytical methods are required for further onsite detection and quality control. **Chapter 5** examines the development and application of a fast and reliable method for determining pesticides and its application to screen pesticides in

cannabis growing facilities. With increasing use of cannabis, its impact on the environment and human health has become an important area of research.[30, 59-61]

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## Chapter 5

## Determination of eighty-two pesticides and application to screening pesticides in cannabis growing facilities*

### 5.1 Introduction

Cannabis has been used historically for a variety of purposes, the most common being medicinal and recreational.[1-4] Medicinal benefits are primarily pain management and appetite stimulation, while the psychoactive components of cannabis have been used recreationally. Following the illegalization of other drugs like opioids in the 1800 – 1900s cannabis became a banned, or controlled, substance in many regions of the world. Nevertheless, the diverse medicinal properties of cannabis, like the seeds being used as a laxative, meant its use prevailed in several countries.[4-8] In recent years, many regions and nations have decriminalized or legalized cannabis use. Notably, cannabis use became legal in Canada in October 2018.

Increasing legalization and decriminalization of cannabis has dramatically increased recreational use over the last decade. While global numbers of cannabis users have remained stable, from 2007 to 2017 the number of recurring users increased by about 70% in the United States and daily users more than doubled.[9, 10] In 2018, it was estimated that there were 188 million global cannabis users, placing it as the most widely used recreational drug in the world.[10] Numerous reports have indicated high prevalence of pesticides on illegal crops or medicinal crops, leading to strict requirements for quality assurance and quality control (QA/QC) of cannabis products to ensure the health and safety of consumers.[11-13] New regulations in Canada

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stipulate that all active ingredients and contaminants present in the final product need to be reported and must be below maximum allowable levels for product release. Regulated contaminants of concern include pesticides, heavy metals, microbials, and in some regions, residual solvents.[14-17] Health Canada has released a watch list of 96 pesticides with a maximum allowable level in the range of 0.01 – 1 μg/g for each class of cannabis product: fresh plant, dried plant, or oil.[15, 16] The maximum allowable level is the highest level at which a pesticide can detected at, and if above this is illegible for sale. However, because of the speed at which legalization occurred, the released regulatory values are in-complete. **Table 4-2*** shows that a few pesticides are missing some, or all, of the maximum allowable level for each product type.[15, 16]

Similar to the control of pesticides in food products, the control of pesticides in production of cannabis products is regulated under the Pest Control Act of Canada.[18] All materials used from seeds to the final products must be certified and documented. The strict QA/QC for pesticide residues is necessary because of their potential adverse health effects.[17, 19-21] These include carcinogenicity, mutagenicity, and other toxic effects, some of which may be lethal at sufficient exposure.[13, 20-26] Additionally, some pesticides are persistent pollutants and can remain in the environment long-term.[21, 27, 28] While the requirements for cannabis products are clear, there are no regulations or guidelines for acute or chronic occupational exposure within the growing facilities. Research on the presence of pesticides in cannabis growing facilities and growers' exposure to pesticides is scarce. This chapter investigates the occurrence of pesticides

* Table 4-2 can be found on page 86.

in one certified and one non-certified cannabis growing facility (which was under the certification process).

To assess pesticide occurrence in cannabis growing facilities, a new high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method was developed for the analysis of 82 of the 96 pesticides listed by Health Canada. A simple sampling method for monitoring pesticides was also developed for these facilities to easily be reproduced at each facility. The wipe-sampling procedure was used to investigate the presence of pesticides. The non-certified site had protocols and guidelines still under review and was awaiting their license at the time of sampling. The certified facility has a license to grow cannabis and has established proper protocols and guidelines for all workers.

## 5.2 Materials and Methods

### 5.2.1 Chemicals and Materials

Formic acid (FA) and syringe filters (PVDF, 0.22 μm) were from Sigma-Aldrich (St. Louis, MO, USA). Optima water, methanol (MeOH), acetonitrile (ACN), and pesticide standards (listed in **Table 5-5**) were from Fisher Scientific (Fair Lawn, NJ, USA). Internal standards were from Toronto Research Chemicals (TRC; North York, ON, Canada). 50 mL Polypropylene centrifuge tubes were from Fisher Scientific (Fair Lawn, NJ, USA).

### 5.2.2 HPLC-MS/MS Analysis of Pesticides

The HPLC-MS/MS method was first developed and validated with standards at the University of Alberta using a Shimadzu (Kyoto, Japan) HPLC system with a 5500 Sciex MS system (Sciex, Concord, ON, USA). A Restek biphenyl column (100 mm x 3.0 mm, 4.5 μm

particle size; Bellefonte, PA, USA) was used for the separation and kept at 40 °C. The mobile phase included solvent A consisting of water containing 0.1% FA and 5 mmol/L ammonium formate, and solvent B containing MeOH with 0.1% FA and 5% water (mobile phases prepared *V/V,* as described as method A in Chapter 3). The flow rate was 0.8 mL/min. The HPLC system was equipped with an autosampler and the injection volume was 3 µL. The optimized gradient elution is detailed in **Tables 5-1 and 5-2**. The optimized method was used to determine limit of detection (LOD) and extraction efficiency from the Kimwipes in the University of Alberta laboratory.

**Table 5-1**. **Optimized gradient for the HPLC-MS/MS method with the Sciex 5500 MS system in the university laboratory**.[a]

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.8 | 95 | 5 |
| 2 | 0.8 | 95 | 5 |
| 4 | 0.8 | 50 | 50 |
| 7.5 | 0.8 | 25 | 75 |
| 11 | 0.8 | 14 | 86 |
| 15 | 0.8 | 0 | 100 |

a. Flow rate was 0.8 mL/min and column temperature was 40 ºC.

**Table 5-2. MS valve position for the HPLC-MS/MS method in the university lab.**

| Time | Valve | Position |
|---|---|---|
| 0 | A | To Waste |
| 0.8 | B | To MS |
| 12 | A | To Waste |

The validated method was transferred to the Molecular Science Co. (MSC) mobile laboratory equipped with a Shimadzu (Kyoto, Japan) HPLC system with a 6500+ Sciex MS system (Sciex, Concord, ON, USA). The same type of column, Restek biphenyl column (100

mm x 3.0 mm, 4.5 µm particle size; Bellefonte, PA, USA), and the same composition of the mobile phase was used for the analysis. Injection volume was 3 µL. To increase the sample throughput, we increased the flow rate to 1.0 mL/min and the column temperature to 50 °C. The gradient program was re-optimized and is described in **Tables 5-5 and 5-6**. The method was re-validated by analysis of the standards to provide consistent results with those from the university laboratory. All samples collected from the non-certified and certified site were analyzed using the HPLC-MS/MS (Sciex 6500+) method in the MSC mobile laboratory.

**Table 5-3**. **Optimized gradient for HPLC-MS/MS method with Sciex 6500+ MS system in the MSC mobile laboratory.**[a]

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0.0 | 1.0 | 95 | 5 |
| 0.5 | 1.0 | 95 | 5 |
| 2.0 | 1.0 | 50 | 50 |
| 3.8 | 1.0 | 30 | 70 |
| 11.0 | 1.0 | 14 | 86 |
| 11.1 | 1.0 | 0 | 100 |
| 12.5 | 1.0 | 0 | 100 |
| 12.51 | 1.0 | 95 | 5 |
| 14.0 | 1.0 | 95 | 5 (stop) |

a. Flow rate was 1.0 mL/min and column temperature was at 50 °C. This method was used for analysis of all the samples collected from growing facilities.

**Table 5-4. MS valve positions used for the HPLC-MS/MS method in the MSC mobile lab.**

| Time (min) | Valve | Position |
|---|---|---|
| 0.0 | A | To Waste |
| 0.6 | B | To MS |
| 15.0 | A | To Waste |

Multiple reaction monitoring (MRM) mode was used to detect and quantitate pesticides and internal standards for both methods. The MRM transitions for individual pesticides and the selected internal standards are listed in **Table 5-5**. Specific mass spectrometry parameters are in **Table 5-6**. Confirmation and identification of pesticides in the samples were based on the match of retention time, detection of both MRM transitions, and their ion ratios to those of the commercial standards. The declustering potential (DP), collision energy (CE), and cell exit potential (CXP) were optimized for each transition. The DP is a voltage applied prior to Q1 to assist in stopping ions from clustering together as they enter the MS. This would have already been applied to the ions entering Q1 shown in **Figure 1-10**. The CE is applied in q2 to impart energy to the ions so that they have appropriate kinetic energy to produce the desired fragments. The CXP is applied to the end of q2 to accelerate and focus the ions out of q2 and into Q3.

**Table 5-5**. **Optimized analyte mass spectrometry parameters.** T1, T2, and T3 stands for transition 1, 2, and 3. All pesticides listed were analyzed in positive mode.

| ID | Q1 | Q3 | Retention time (min) | DP (V) | CE (V) | CXP (V) |
|---|---|---|---|---|---|---|
| Azadirachin-$NH_4$ -T1 | 738.4 | 703.3 | 7.6 | 55 | 11 | 20 |
| Azadirachin-$NH_4$ -T2 | 738.4 | 685.4 | 7.6 | 70 | 17 | 20 |
| Prallethrin -T1 | 301.4 | 133.1 | 9.6 | 76 | 16 | 12 |
| Prallethrin -T2 | 301.4 | 105.0 | 9.6 | 75 | 27 | 11 |
| Prallethrin -T3 | 301.4 | 123.0 | 9.6 | 78 | 21 | 14 |
| Pyrethrin I -T1 | 329.3 | 169.3 | 10.9 | 75 | 14 | 16 |
| Pyrethrin I -T2 | 329.3 | 142.9 | 10.9 | 96 | 23 | 17 |
| Pyrethrin I -T3 | 329.3 | 161.2 | 10.9 | 87 | 12 | 15 |
| Spinetoram -T1 | 748.6 | 142.2 | 10.6 | 100 | 38 | 16 |
| Spinetoram -T2 | 748.6 | 98.0 | 10.6 | 108 | 92 | 12 |
| Spirodiclofen -T1 | 411.3 | 313.0 | 11.4 | 150 | 15 | 9 |
| Spirodiclofen -T2 | 411.3 | 71.0 | 11.4 | 150 | 21 | 8 |
| Acephate -T1 | 184.0 | 142.9 | 2.7 | 90 | 12.5 | 12 |
| Acephate -T2 | 184.0 | 48.9 | 2.7 | 90 | 26 | 8 |
| Acephate -T3 | 184.0 | 125.0 | 2.7 | 90 | 24 | 16 |
| Dichlovos -T1 | 220.9 | 109.0 | 6.2 | 118 | 23 | 13 |
| Dichlovos -T2 | 220.9 | 127.2 | 6.2 | 120 | 23 | 11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ethoprophos -T1 | 242.9 | 173.1 | 8.2 | 103 | 19 | 15 |
| Ethoprophos -T2 | 242.9 | 215.1 | 8.2 | 100 | 15 | 20 |
| Ethoprophos -T3 | 242.9 | 131.1 | 8.2 | 90 | 28 | 15 |
| Fensulfothion -T1 | 309.1 | 157.1 | 7.8 | 137 | 32 | 16 |
| Fensulfothion -T2 | 309.1 | 235.0 | 7.8 | 140 | 30 | 20 |
| Fensulfothion -T3 | 309.1 | 253.1 | 7.8 | 140 | 24 | 21 |
| Tetrachlorvinphos -T1 | 367.0 | 217 | 9.1 | 133 | 19 | 13 |
| Tetrachlorvinphos -T2 | 367.0 | 206.2 | 9.1 | 134 | 54 | 6 |
| Tetrachlorvinphos -T3 | 367.0 | 240.9 | 9.1 | 140 | 26 | 24 |
| Naled -T1 | 378.9 | 127.0 | 7.3 | 120 | 16.5 | 12 |
| Naled -T2 | 378.9 | 144.8 | 7.3 | 106 | 17 | 17 |
| Naled -T3 | 378.9 | 252.6 | 7.3 | 130 | 24.5 | 21 |
| Dimethoate -T1 | 230.1 | 124.9 | 5.4 | 70 | 28 | 17 |
| Dimethoate -T2 | 230.1 | 199.1 | 5.4 | 63 | 12 | 18 |
| Dimethoate -T3 | 230.1 | 171.2 | 5.4 | 73 | 19 | 15 |
| Aldicarb -T1 | 208.2 | 115.9 | 5.9 | 45 | 10 | 10 |
| Aldicarb -T2 | 208.2 | 89.0 | 5.9 | 47 | 22 | 10 |
| Buprofezin -T1 | 306.0 | 201.2 | 10.1 | 78 | 16 | 16 |
| Buprofezin -T2 | 306.0 | 116.2 | 10.1 | 65 | 22 | 10 |
| Clothianidin -T1 | 250.2 | 169.1 | 5.05 | 66 | 17.5 | 15 |
| Clothianidin -T2 | 250.2 | 131.9 | 5.05 | 63 | 22 | 12 |
| Fipronil -T1 | 437.1 | 367.9 | 7.5 | 175 | 24 | 10 |
| Fipronil -T2 | 437.1 | 289.9 | 7.5 | 170 | 38 | 25 |
| Flonicamid -T1 | 230.1 | 203.1 | 4.4 | 135 | 23.5 | 18 |
| Flonicamid -T2 | 230.1 | 97.9 | 4.4 | 133 | 52 | 13 |
| Flonicamid -T3 | 230.1 | 174.1 | 4.4 | 127 | 24.5 | 15 |
| Hexythiazox -T1 | 353.2 | 228 | 11.2 | 77 | 20 | 20 |
| Hexythiazox -T2 | 353.2 | 168.1 | 11.2 | 71 | 35 | 15 |
| Hexythiazox -T3 | 353.2 | 271.1 | 11.2 | 66 | 17.5 | 24 |
| Methiocarb -T1 | 226.2 | 169.1 | 7.6 | 77 | 13 | 15 |
| Methiocarb -T2 | 226.2 | 121.1 | 7.6 | 80 | 25 | 11 |
| Methomyl -T1 | 163.1 | 105.9 | 4.9 | 62 | 14 | 12 |
| Methomyl -T2 | 163.1 | 87.9 | 4.9 | 52 | 13 | 9 |
| Oxamyl -T1 | 237.2 | 72.1 | 4.8 | 41 | 26 | 8 |
| Oxamyl -T2 | 237.2 | 90.0 | 4.8 | 59 | 10 | 11 |
| Pyridaben -T1 | 365.3 | 309.1 | 12.2 | 75 | 17 | 10 |
| Pyridaben -T2 | 365.3 | 147.1 | 12.2 | 74 | 31 | 15 |
| Thiacloprid-T1 | 253.2 | 126.0 | 6.8 | 118 | 28 | 15.5 |
| Thiacloprid -T2 | 253.2 | 186.0 | 6.8 | 111 | 19 | 17 |
| Thiamethoxam -T1 | 292.2 | 211.1 | 5.3 | 100 | 15.5 | 19 |
| Thiamethoxam -T2 | 292.2 | 181.3 | 5.3 | 100 | 30 | 19 |
| Thiamethoxam -T3 | 292.2 | 131.9 | 5.3 | 110 | 35 | 12 |
| Thiophanate-methyl -T1 | 343.2 | 311.0 | 6.9 | 99 | 15 | 9 |
| Thiophanate-methyl -T2 | 343.2 | 151.0 | 6.9 | 75 | 26 | 17 |
| Carbanyl -T1 | 202.2 | 145.0 | 6.8 | 110 | 15.3 | 17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Carbanyl -T2 | 202.2 | 127.0 | 6.8 | 80 | 40 | 10 |
| Carbofuran -T1 | 222.2 | 123.1 | 6.7 | 85 | 29.5 | 11 |
| Carbofuran -T2 | 222.2 | 165.3 | 6.7 | 100 | 16.4 | 15 |
| Chlorantraniliprole -T1 | 484.0 | 452.8 | 7.8 | 74 | 28 | 13 |
| Chlorantraniliprole -T2 | 484.0 | 285.9 | 7.8 | 70 | 19 | 8 |
| Clofentezine -T1 | 303.2 | 137.8 | 10.0 | 72 | 20.5 | 17 |
| Clofentezine -T2 | 303.2 | 101.7 | 10.0 | 61 | 56 | 12 |
| Cyprodinil -T1 | 225.9 | 108.0 | 8.65 | 175 | 35 | 14 |
| Cyprodinil -T2 | 225.9 | 93.1 | 8.65 | 150 | 46 | 12 |
| Cyprodinil -T3 | 225.9 | 118.1 | 8.65 | 180 | 40 | 11 |
| Dinotefuran -T1 | 203.2 | 129.2 | 4.1 | 69 | 16 | 12 |
| Dinotefuran -T2 | 203.2 | 112.9 | 4.1 | 50 | 14 | 14 |
| Fenoxycarb -T1 | 302.3 | 115.9 | 8.8 | 72 | 15 | 18 |
| Fenoxycarb -T2 | 302.3 | 88.2 | 8.81 | 65 | 26 | 8 |
| Imazalil -T1 | 297.1 | 159.1 | 7.07 | 148 | 29 | 13 |
| Imazalil -T2 | 297.1 | 141.2 | 7.07 | 139 | 47 | 15 |
| Myclobutanil -T1 | 289.3 | 124.9 | 7.94 | 92 | 46 | 12 |
| Myclobutanil -T2 | 289.3 | 70.0 | 7.94 | 107 | 23 | 8 |
| Novaluran -T1 | 493.2 | 158.0 | 8.9 | 123 | 24 | 15 |
| Novaluran -T2 | 493.2 | 141.0 | 8.9 | 160 | 67 | 16 |
| Paclobutrazole -T1 | 294.3 | 69.9 | 7.4 | 106 | 24 | 9 |
| Paclobutrazole -T2 | 294.3 | 125.0 | 7.4 | 111 | 52 | 10 |
| Propiconazole -T1 | 238.9 | 72.0 | 7.0 | 95 | 30 | 10 |
| Propiconazole -T2 | 238.9 | 182.1 | 7.0 | 108 | 20 | 16 |
| Propoxur -T1 | 210.2 | 168.2 | 6.45 | 62 | 10.5 | 16 |
| Propoxur -T2 | 210.2 | 110.8 | 6.45 | 71 | 19 | 14 |
| Pyraclostrobin -T1 | 388.3 | 194.1 | 10.4 | 80 | 16 | 18 |
| Pyraclostrobin -T2 | 388.3 | 163.1 | 10.4 | 76 | 32 | 15 |
| Pyraclostrobin -T3 | 388.3 | 296.1 | 10.4 | 70 | 17 | 8 |
| Tebuconazole -T1 | 308.3 | 70.0 | 8.3 | 117 | 26 | 8 |
| Tebuconazole -T2 | 308.3 | 125.2 | 8.3 | 103 | 54 | 11.5 |
| Tebutenozide -T1 | 353.3 | 297.1 | 8.45 | 62 | 10.5 | 9 |
| Tebutenozide -T2 | 353.3 | 132.9 | 8.45 | 60 | 24 | 16 |
| Teflubenzuron -T1 | 381.1 | 158.2 | 9.8 | 113 | 19.5 | 15 |
| Teflubenzuron -T2 | 381.1 | 141 | 9.8 | 134 | 48 | 13 |
| Bifennazate -T1 | 301.2 | 198.3 | 8.5 | 85 | 13 | 19 |
| Bifennazate -T2 | 301.2 | 170.2 | 8.5 | 80 | 26.5 | 12 |
| Spinosad A/D -T1 | 732.5 | 142.1 | 9.85 | 92 | 36 | 13 |
| Spinosad A/D -T2 | 732.5 | 98.3 | 9.85 | 107 | 91 | 8 |
| Spinosad A/D -T3 | 746.6 | 98.1 | 10.3 | 106 | 98.5 | 13 |
| Spinosad A/D -T4 | 746.6 | 189.1 | 10.3 | 90 | 41.5 | 14 |
| Spinosad A/D -T5 | 732.5 | 142.1 | 9.8 | 92 | 36 | 13 |
| Spinosad A/D -T6 | 732.5 | 98.3 | 9.8 | 107 | 91 | 8 |
| Spinosad A/D -T7 | 746.6 | 98.1 | 10.3 | 106 | 98.5 | 13 |
| Spinosad A/D -T8 | 746.6 | 189.1 | 10.3 | 90 | 41.5 | 14 |

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|---|---|---|---|---|---|---|
| Acetamiprid -T1 | 223.2 | 125.8 | 6.25 | 83 | 27 | 13 |
| Acetamiprid -T2 | 223.2 | 89.9 | 6.25 | 82 | 48 | 11 |
| Acetamiprid -T3 | 223.2 | 55.9 | 6.25 | 100 | 20 | 10 |
| Azoxystrobin -T1 | 404.2 | 372.3 | 9.3 | 92 | 19 | 10 |
| Azoxystrobin -T2 | 404.2 | 344.2 | 9.3 | 71 | 33 | 10 |
| Boscalid -T1 | 343.1 | 307.0 | 8.15 | 162 | 27 | 10 |
| Boscalid -T2 | 343.1 | 271.6 | 8.15 | 150 | 44 | 24 |
| Boscalid -T3 | 343.1 | 140.0 | 8.15 | 136 | 27 | 12 |
| Cyantanilipore -T1 | 475.0 | 444.1 | 7.5 | 70 | 24 | 12 |
| Cyantanilipore -T2 | 475.0 | 285.7 | 7.5 | 81 | 20 | 24 |
| Etoxazole -T1 | 360.2 | 140.8 | 11.0 | 132 | 38 | 13 |
| Etoxazole -T2 | 360.2 | 177.3 | 11.0 | 160 | 26 | 16 |
| Fenpyroximate -T1 | 422.3 | 107.3 | 12.0 | 103 | 73 | 10 |
| Fenpyroximate -T2 | 422.3 | 135.3 | 12.0 | 105 | 39 | 11 |
| Fenpyroximate -T3 | 422.3 | 366.1 | 12.0 | 107 | 23 | 11 |
| Flodioxonile -T1 | 266.1 | 229.1 | 7.3 | 61 | 19 | 7 |
| Flodioxonile -T2 | 266.1 | 157.9 | 7.3 | 60 | 48 | 21 |
| Fluopyram -T1 | 397.2 | 208.1 | 7.75 | 100 | 29 | 6 |
| Fluopyram -T2 | 397.2 | 173.1 | 7.75 | 90 | 37 | 15 |
| Imidacloprid -T1 | 256.2 | 209.2 | 5.9 | 77 | 21 | 6 |
| Imidacloprid -T2 | 256.2 | 175.2 | 5.9 | 76 | 28 | 15 |
| Kresoxim methyl -T1 | 314.1 | 115.9 | 9.4 | 85 | 19 | 10 |
| Kresoxim methyl -T2 | 314.1 | 222.2 | 9.4 | 87 | 19 | 20 |
| Kresoxim methyl -T3 | 314.1 | 132.0 | 9.4 | 65 | 22 | 11 |
| Methalaxyl -T1 | 280.2 | 219.9 | 7.7 | 65 | 18 | 20 |
| Methalaxyl -T2 | 280.2 | 191.9 | 7.7 | 81 | 24 | 15 |
| Piperomyl butoxide -T1 | 356.3 | 117.1 | 10.4 | 50 | 64 | 10 |
| Piperomyl butoxide -T2 | 356.3 | 119.2 | 10.4 | 42 | 49 | 10 |
| Spiromesifen -T1 | 371.2 | 255.1 | 11.0 | 98 | 30 | 21 |
| Spiromesifen -T2 | 371.2 | 273.0 | 11.0 | 92 | 15 | 8 |
| Spirotetramat -T1 | 374.2 | 329.9 | 8.9 | 82 | 20 | 9 |
| Spirotetramat -T2 | 374.2 | 302.3 | 8.9 | 91 | 21 | 9 |
| Spiroxamine -T1 | 298.3 | 144.3 | 7.3 | 102 | 26 | 17 |
| Spiroxamine -T2 | 298.3 | 100.1 | 7.3 | 168 | 45 | 13 |
| Trifloxystrobin -T1 | 409.2 | 185.9 | 10.1 | 56 | 23 | 18 |
| Trifloxystrobin -T2 | 409.2 | 206.3 | 10.1 | 63 | 18 | 6 |
| Methoprene -T1 | 328.2 | 279.3 | 10.85 | 44 | 11.5 | 8 |
| Methoprene -T2 | 328.2 | 191.3 | 10.85 | 50 | 20 | 18 |
| Benzovindiglupyr -T1 | 398.1 | 342.1 | 8.6 | 140 | 24 | 10 |
| Benzovindiglupyr -T2 | 398.1 | 321.9 | 8.6 | 140 | 32 | 9 |
| Dodemorph -T1 | 281.8 | 116.0 | 7.5 | 110 | 28 | 10 |
| Dodemorph -T2 | 281.8 | 98.0 | 7.5 | 116 | 36 | 9 |
| Allethrin -T1 | 303.3 | 135.0 | 10.0 | 102 | 16 | 16 |
| Allethrin -T2 | 303.3 | 169.2 | 10.0 | 84 | 11 | 5 |
| Allethrin -T3 | 303.3 | 93.0 | 10.0 | 95 | 17.5 | 11 |

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|---|---|---|---|---|---|---|
| Bifenthrovin -T1 | 440.3 | 181.2 | 12.3 | 66 | 21 | 14.5 |
| Bifenthrovin -T2 | 440.3 | 166.0 | 12.3 | 60 | 57 | 11 |
| Cypermethrin -T1 | 416.2 | 191.2 | 12.05 | 170 | 16 | 16 |
| Cypermethrin -T2 | 416.2 | 127.2 | 12.05 | 175 | 39 | 15 |
| Deltamethrin- $NH_4$ -T1 | 523.1 | 280.8 | 12.4 | 80 | 21 | 33 |
| Deltamethrin- $NH_4$ -T2 | 523.1 | 181.3 | 12.4 | 97 | 52 | 11 |
| Deltamethrin- $NH_4$ -T3 | 523.1 | 208.3 | 12.4 | 80 | 25 | 18 |
| Etofenprox -T1 | 394.4 | 177.3 | 12.7 | 77 | 19 | 16 |
| Etofenprox -T2 | 394.4 | 107.1 | 12.7 | 70 | 57 | 12 |
| Etofenprox -T3 | 394.4 | 359.2 | 12.7 | 60 | 15 | 10 |
| Permethrin -T1 | 408.3 | 183.2 | 12.3 | 75 | 26 | 17 |
| Permethrin -T2 | 408.3 | 355.0 | 12.3 | 63 | 11 | 10 |
| Permethrin -T3 | 351.3 | 183.1 | 12.1 | 77 | 28 | 16 |
| Permethrin -T4 | 351.3 | 156 | 12.1 | 105 | 39 | 13.5 |
| Permethrin -T5 | 351.3 | 249.2 | 12.1 | 102 | 26 | 9 |
| Resmethrin -T1 | 339.3 | 171.1 | 11.7 | 135 | 20 | 21 |
| Resmethrin -T2 | 339.3 | 143.1 | 11.7 | 119 | 33 | 19 |
| Resmethrin -T3 | 339.3 | 120.9 | 11.7 | 156 | 31 | 16 |
| Tetramethrin -T1 | 332.3 | 164.2 | 11.6 | 140 | 30 | 5 |
| Tetramethrin -T2 | 332.3 | 135.2 | 11.6 | 140 | 23 | 12 |
| Chlorpyritos -T1 | 350.1 | 197.9 | 10.7 | 118 | 30 | 17 |
| Chlorpyritos -T2 | 350.1 | 153.0 | 10.7 | 77 | 18 | 12 |
| Coumaphos -T1 | 363.1 | 226.9 | 11.0 | 163 | 34 | 15 |
| Coumaphos -T2 | 363.1 | 211.0 | 11.0 | 160 | 38 | 6 |
| Diazinon -T1 | 305.0 | 169.2 | 8.9 | 137 | 31 | 15 |
| Diazinon -T2 | 305.0 | 153.1 | 8.9 | 117 | 29 | 15 |
| Fention -T1 | 279.2 | 168.9 | 9.4 | 110 | 23 | 14 |
| Fention -T2 | 279.2 | 247 | 9.4 | 122 | 17 | 7 |
| Malathion -T1 | 331.2 | 284.9 | 8.4 | 66 | 10 | 28 |
| Malathion -T2 | 331.2 | 127.1 | 8.4 | 94 | 17 | 17 |
| Methyl parathion -T1 | 281.2 | 171.0 | 9.4 | 117 | 23 | 17 |
| Methyl parathion -T2 | 281.2 | 104.9 | 9.4 | 113 | 32 | 11 |
| Mevinphos cis/trans -T1 | 225.1 | 193.1 | 5.85 | 62 | 9.5 | 16 |
| Mevinphos cis/trans -T2 | 225.1 | 127.1 | 5.85 | 60 | 20 | 11 |
| Mevinphos cis/trans -T3 | 225.1 | 193.1 | 5.5 | 62 | 9.5 | 16 |
| Mevinphos cis/trans -T4 | 225.1 | 127.1 | 5.5 | 60 | 20 | 11 |
| Phosmet -T1 | 318.2 | 160.0 | 8.8 | 96 | 18 | 14 |
| Phosmet -T2 | 318.2 | 132.7 | 8.8 | 67 | 50 | 16 |
| Acequinocyl- $NH_4$ -T1 | 402.3 | 343.3 | 13.2 | 50 | 17 | 10 |
| Acequinocyl- $NH_4$ -T2 | 402.3 | 189.0 | 13.2 | 50 | 39 | 20 |
| MGK-264-cis/tran -T1 | 276.4 | 210.1 | 9.15 | 90 | 19 | 27 |
| MGK-264-cis/trans -T2 | 276.4 | 98.1 | 9.15 | 97 | 31 | 12 |
| MGK-264-cis/tran -T3 | 276.4 | 210.1 | 9.5 | 90 | 19 | 27 |
| MGK-264-cis/trans -T4 | 276.4 | 98.1 | 9.5 | 97 | 31 | 12 |
| Pirimicarb -T1 | 239.1 | 182.1 | 7.0 | 78 | 23 | 13 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Pirimicarb -T2 | 239.1 | 72.1 | 7.0 | 61 | 37 | 11 |
| **Internal Standards** | | | | | | |
| Acetamiprid-$D_3$a -T1 | 226.1 | 126.1 | 6.25 | 74 | 27 | 4 |
| acetamiprid- $D_3$a -T2 | 226.1 | 90.0 | 6.25 | 74 | 45 | 8 |
| Boscalid- $D_4$a -T1 | 347.0 | 177.0 | 8.15 | 40 | 42 | 13 |
| Boscalid- $D_4$a -T2 | 347.0 | 217.0 | 8.15 | 107 | 49 | 7 |
| Boscalid- $D_4$a -T3 | 347.0 | 311.1 | 8.15 | 107 | 26 | 8 |
| Carbofuran- $D_3$a -T1 | 225.1 | 123.0 | 6.7 | 43 | 30 | 11 |
| Carbofuran- $D_3$a -T2 | 225.1 | 165.1 | 6.7 | 43 | 16 | 15 |
| Cyprodinil- $D_5$a -T1 | 231.1 | 109.1 | 8.6 | 70 | 36 | 10 |
| Cyprodinil- $D_5$a -T2 | 231.1 | 93.9 | 8.6 | 96 | 45 | 11 |
| Cyprodinil- $D_5$a -T3 | 231.1 | 118.0 | 8.6 | 100 | 45 | 10 |
| Deltamethrin- $D_3$a -T1 | 528.1 | 281.0 | 12.4 | 81 | 35 | 4 |
| Deltamethrin- $D_3$a -T2 | 528.1 | 304.1 | 12.4 | 76 | 24 | 8 |
| Dichlorvos- $D_6$a -T1 | 227.0 | 115.0 | 6.1 | 43 | 26 | 9 |
| Dichlorvos- $D_6$a -T2 | 227.0 | 133.0 | 6.1 | 57 | 25 | 14 |
| Dinotefuran- $D_3$a -T1 | 206.1 | 116.9 | 4.1 | 42 | 16 | 10 |
| Dinotefuran- $D_3$a -T2 | 206.1 | 132.0 | 4.1 | 42 | 16 | 10 |
| Dinotefuran- $D_3$a -T3 | 206.1 | 90.2 | 4.1 | 53 | 22 | 8 |
| Fenthion- $D_6$a -T1 | 285.1 | 250.0 | 9.3 | 41 | 17 | 7 |
| Fenthion- $D_6$a -T2 | 285.1 | 169 | 9.3 | 41 | 26 | 17 |
| Fipronil- $13C_2$-$15N_2$a -T1 | 441.0 | 371.6 | 7.5 | 128 | 24 | 10 |
| Fipronil- $13C_2$-$15N_2$a -T3 | 441.0 | 256.9 | 7.5 | 120 | 45 | 11 |
| Fludioxinil- $13C_3$a -T1 | 269.0 | 181.0 | 7.3 | 36 | 13 | 15 |
| Fludioxinil- $13C_3$a -T2 | 269.0 | 232.0 | 7.3 | 15 | 20 | 12 |
| Imazilil- $D_5$a -T1 | 302.0 | 159.0 | 7.1 | 62 | 32 | 15 |
| Imazilil- $D_5$a -T2 | 302.0 | 203.0 | 7.1 | 47 | 26 | 18 |
| Imazilil- $D_5$a -T3 | 302.0 | 255.0 | 7.1 | 46 | 26 | 22 |
| Imidacloprid- $D_4$a -T1 | 260.1 | 213.1 | 5.8 | 46 | 26 | 18 |
| Imidacloprid- $D_4$a -T2 | 260.1 | 179.2 | 5.8 | 42 | 26 | 15 |
| Kresoxim-methyl- $D_7$a -T1 | 321.1 | 206.0 | 9.4 | 67 | 17 | 4 |
| Kresoxim-methyl- $D_7$a -T2 | 321.1 | 116.0 | 9.4 | 30 | 13 | 8 |
| Malathion- $D_6$a -T1 | 337.1 | 127.1 | 8.35 | 52 | 17 | 9 |
| Malathion- $D_6$a -T2 | 337.1 | 291.0 | 8.35 | 51 | 11 | 24 |
| Malathion- $D_6$a -T3 | 337.1 | 99.0 | 8.35 | 40 | 33 | 9 |
| Metalaxyl- $D_6$a -T1 | 286.2 | 198.3 | 7.7 | 75 | 25 | 17 |
| Metalaxyl- $D_6$a -T2 | 286.2 | 226.0 | 7.7 | 52 | 17 | 21 |
| Methomyl- $D_3$a -T1 | 166.1 | 88.1 | 4.8 | 36 | 15 | 7 |
| Methomyl- $D_3$a -T2 | 166.1 | 106.0 | 4.8 | 45 | 13 | 12 |
| Myclobutanil- $D_9$a -T1 | 298.1 | 125.0 | 7.9 | 55 | 48 | 11 |
| Myclobutanil- $D_9$a -T2 | 298.1 | 71.0 | 7.9 | 59 | 22 | 8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Paclobutrazol- $D_4$a -T1 | 298.0 | 71.0 | 7.9 | 55 | 22 | 8 |
| Paclobutrazol- $D_4$a -T2 | 298.0 | 125.0 | 7.9 | 77 | 48 | 11 |
| Phosmet- $D_6$a -T1 | 324.1 | 160.3 | 8.8 | 43 | 17 | 12 |
| Phosmet- $D_6$a -T2 | 324.1 | 133.1 | 8.8 | 43 | 49 | 10 |
| Phosmet- $D_6$a -T3 | 324.1 | 265.0 | 8.8 | 73 | 18 | 19 |
| Piperonyl butoxide- $D_9$a -T1 | 365.0 | 177.1 | 10.3 | 56 | 18 | 15 |
| Piperonyl butoxide- $D_9$a -T2 | 365.0 | 119.0 | 10.3 | 49 | 51 | 11 |
| Pyridaben-$D_{13}$a -T1 | 378.1 | 160.1 | 12.0 | 50 | 33 | 13 |
| Pyridaben- $D_{13}$a -T2 | 378.1 | 322.1 | 12.0 | 42 | 17 | 10 |
| Thiamethoxam- $D_4$a -T1 | 296.0 | 215.1 | 5.3 | 74 | 17 | 4 |
| Thiamethoxam- $D_4$a -T2 | 296.0 | 149.1 | 5.3 | 37 | 27 | 13 |
| Thiamethoxam- $D_4$a -T3 | 296.0 | 132.0 | 5.3 | 60 | 32 | 13 |
| Trifloxystrobin- $D_6$a -T1 | 415.0 | 186.1 | 10.0 | 55 | 25 | 16 |
| Trifloxystrobin- $D_6$a -T2 | 415.0 | 212.1 | 10.0 | 60 | 19 | 16 |

**Table 5-6**. **Mass spectrometry parameters used for both 5500 and 6500+.**

| **Parameter** | **Value** |
|---|---|
| Curtain Gas | 30 A.U. |
| Collision Gas | Medium |
| Ion spray Voltage | 5500 V |
| Temperature | 650 °C |
| Ion Source Gas 1 | 40 A.U. |
| Ion Source Gas 2 | 30 A.U. |
| Scan Type | MRM |
| Polarity | Positive |
| MRM Window | 70 s |
| Target Scan Time | 0.5 s |
| Pause between scan times | 0.3 s |
| Entrance Potential | 10 V |

A.U. – Arbitrary Units

### 5.2.3 Control Experiments for Wipe Sampling Tests

First, two types of sampling surfaces were tested for extraction: a polypropylene and a glass surface, each approximately 930 $cm^2$. Each surface was washed four times with 5 mL each of MeOH followed by ACN, water, and finally MeOH. Surfaces were left to dry fully after each wash. The extraction procedures summarized in **Figure 5-1**. One mL of a 500 µg/L pesticide mix was prepared, with each pesticide at a final concentration of 500 µg/L, in methanol deposited onto the surface. This was done via a pipette, drop wise onto the surface as the surface was slowly moved to ensure full coverage. In total, there were twelve surfaces tested for recovery, 6 plastic and 6 glass. After drying, a Kimwipe (21 × 11 cm) was used to thoroughly wipe along the surface. The Kimwipe was then placed into a new sterile 50 mL-polypropylene (Falcon) centrifuge tube. Ten mL of MeOH was added to the tube to extract the pesticides from the Kimwipe. The tubes were sonicated (in a water bath) for 10 min. Liquid samples were collected from the tubes and filtered through a 0.22 µm PVDF 13 mm diameter filter unit (Merck Millipore, Burlington, MA, USA). A blank Kimwipe was extracted, and no pesticides were detected.

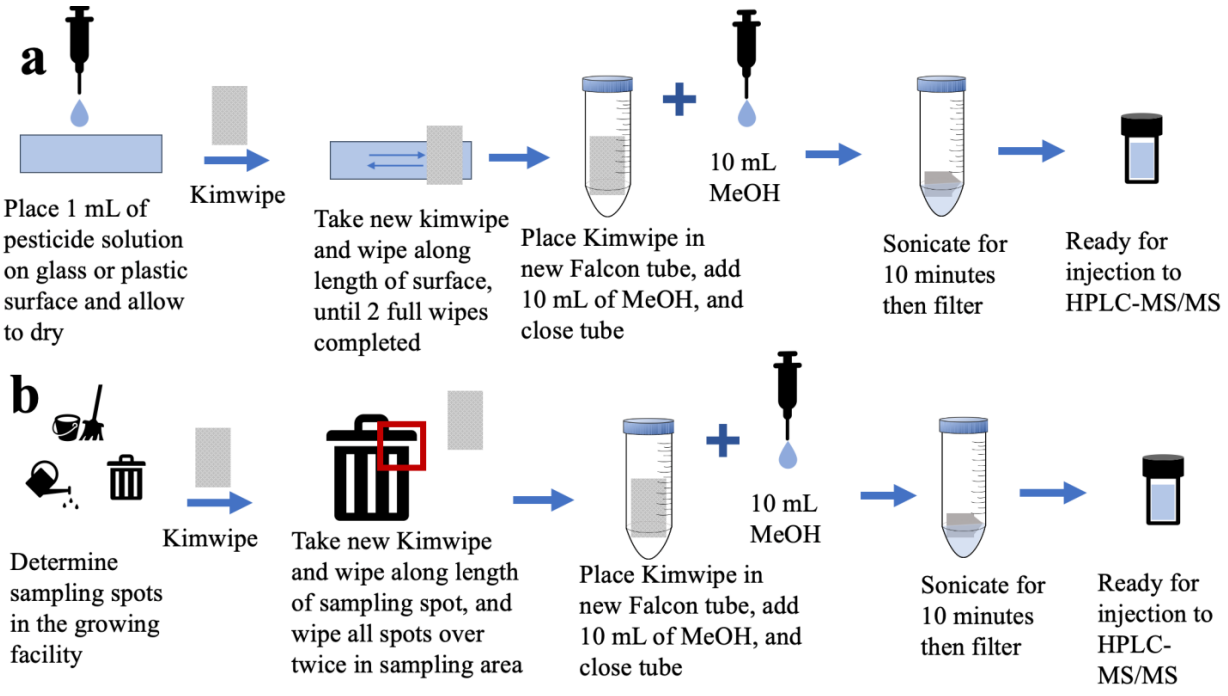



**Figure 5-1**. Schematic of sampling procedure. Top **(a)** shows the sampling validation procedure in the lab. Bottom (**b**) shows the sampling procedure in the growing facility. Falcon tubes are sterile 50 mL polypropylene tubes.

### 5.2.4 Sample Collection

Samples were collected from two different growing sites in British Columbia, Canada. Permission to access was granted through our collaboration with Molecular Science Co. These sites will be referred to as non-certified and certified. Samples were taken from common surfaces. Common surfaces included door handles, light switches, plant pots, metal stems for plant containment, and other similar surfaces. Inequal access to the two growing facilities resulted in differences in the number of samples and sample types obtained. As many surfaces were sampled as possible within the limited facility access and sampling time constraints at the non-certified site. The non-certified site had additional swabs taken from the water sprayer

nozzle and the water reservoir. At the certified site, permission was granted to collect samples without time restriction, therefore samples covered the entire facility. The certified site involved a greater number of individual swabs of apparatus such as the HVAC screen, dry room screen, twister trimmer, dry room, solvent wash, doors, and handles.

At both sites, all of the surfaces were sampled with a Kimwipe, which was then stored in a new, clean sterile 50 mL polypropylene centrifuge tube. **Figure 5-1b** shows a schematic of the sampling procedure is described. The area wiped was estimated for the swabs and values are reported in μg of pesticide/square centimeter of area wiped, as shown in **Table 5-7**.

**Table 5-7**. **Estimation of the square foot of sampling areas**.

| **Sample** | **Site** | **Estimated area of wipe ($cm^2$)** |
|---|---|---|
| Sprayer Nozzle | Non-certified | 930 |
| Room 2 | Non-certified | 23230 |
| Room 3 | Non-certified | 23230 |
| Room 4 | Non-certified | 23230 |
| Water Reservoir | Non-certified | 9290 |
| HVAC Screen | Certified | 1860 |
| Dry Room Screen | Certified | 1860 |
| Trimmer Room Cart | Certified | 14860 |
| Twister Trim | Certified | 4640 |
| Dry Room Cart | Certified | 14860 |
| Plastic Surface | Lab Test | 930 |
| Glass Surface | Lab Test | 930 |

### 5.2.5 Extraction

Stored Kimwipes were extracted using the same liquid extraction method described in **Section 5.2.3**. To extract pesticides, 10 mL of MeOH was added to each of the 50 mL-polypropylene tubes and spiked to 10 µg/L final concentration with each internal standard. The sample vials were thoroughly shaken (by hand) for 5 min. Samples were filtered through a 0.22 µm PVDF 13 mm diameter filter unit, as shown in **Figure 5-1b**.

## 5.3 Results and Discussion

### 5.3.1 HPLC-MS/MS Method

We developed a novel, accurate, sensitive, and fast HPLC-MS/MS method for the detection and quantitation of 82 of the 96 Health Canada pesticides using an optimized and scheduled MRM approach. **Figure 5-2** shows the separation of the pesticide standards at 50 µg/L. **Table 5-8** shows this method achieved an instrument limit of detection, in the range of 0.02–5 µg/L for all but six pesticides. Methyl parathion, permethrin, cypermethrin, MGK-264, azadirachin, and daminozide had LODs of 6, 9, 15, 20, 37, and 93 µg/L, respectively.

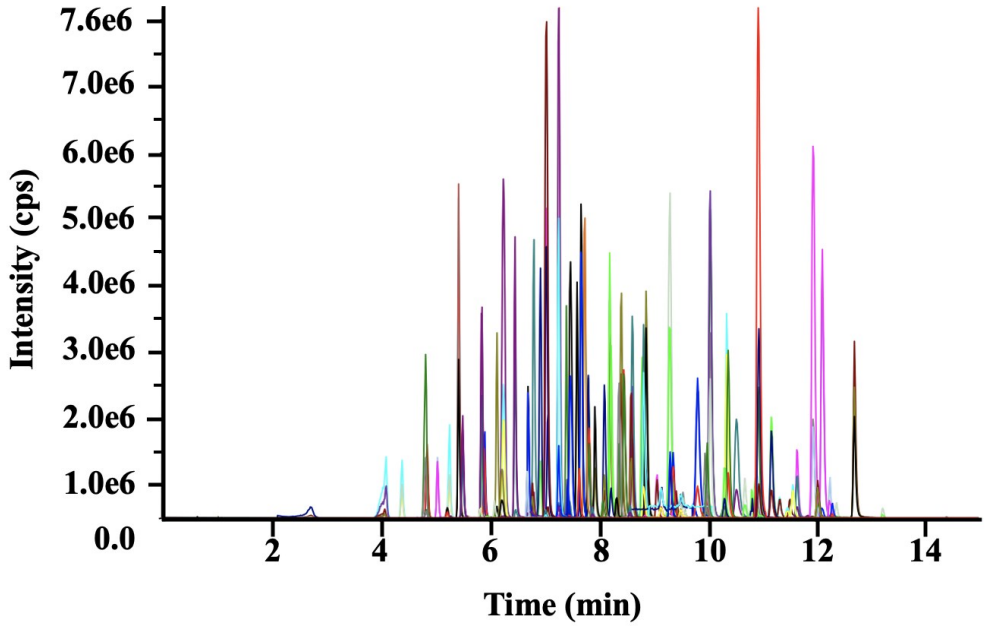



**Figure 5-2. HPLC-MS/MS chromatogram of the 82 out of the 96 standard pesticide mix**. Gradient separation performed with a Restek biphenyl column at 40 °C at the university laboratory. Retention times and mass spectrometer parameters for each pesticide are stated in **Table 5-5.**

**Table 5-8. Limit of detection (LOD) and limit of quantification (LOQ) of quantifiable pesticides.** The lowest calibration is the lowest calibrant that gave a meaningful signal. LOD was calculated as 3 times the standard deviation of the lowest calibration divided by the slope of the calibration curve. LODs/LOQs for a few pesticides were higher than the lowest calibration standard due to larger standard deviations associated with the lowest standard.

| | (µg /L) | | | |
|---|---|---|---|---|
| **Pesticides** | **Lowest Calibration** | **LOD** | **LOQ** | **S/N** |
| Acephate | 0.5 | 0.1 | 0.5 | 5.5 |
| Acetamiprid | 0.5 | 0.1 | 0.2 | 13.5 |
| Acequinocyl | 1.0 | 0.4 | 1.3 | 3 |
| Aldicarb | 0.5 | 0.1 | 0.4 | 24 |
| Allethrin | 2.5 | 0.7 | 2.2 | 6 |
| Azadirachtin | 20.0 | 37.5 | 125.0 | 3 |
| Azoxystrobin | 0.5 | 0.1 | 0.2 | 13 |
| Benzovindiflupyr | 0.5 | 0.2 | 0.7 | 10.5 |
| Bifenazate | 1.0 | 0.3 | 0.9 | 3 |
| Bifenthrin | 0.5 | 0.5 | 1.6 | 3 |
| Boscalid | 0.5 | 0.2 | 0.8 | 6.5 |
| Buprofezin | 0.5 | 0.1 | 0.2 | 26 |
| Carbaryl | 2.5 | 0.5 | 1.6 | 8 |
| Carbofuran | 0.5 | 0.2 | 0.6 | 6 |
| Chlorantraniliprole | 1.0 | 0.3 | 0.9 | 16 |
| Chlorpyrifos | 1.0 | 0.5 | 1.7 | 3 |

| | | | | |
|---|---|---|---|---|
| Clofentezine | 1.0 | 0.2 | 0.8 | 7 |
| Clothianidin | 0.5 | 1.9 | 6.5 | 4 |
| Coumaphos | 1.0 | 0.1 | 0.4 | 7 |
| Cyantranilipole | 2.5 | 1.0 | 3.2 | 12 |
| Cypermethrin | 20.0 | 15.5 | 51.7 | 6 |
| Cyprodinil | 0.5 | 0.2 | 0.5 | 9 |
| Daminozide | 50.0 | 92.9 | 309.8 | 10 |
| Deltamethrin | 5.0 | 1.3 | 4.2 | 3 |
| Diazinon | 0.5 | 0.1 | 0.2 | 13 |
| Dichlorvos | 2.5 | 0.2 | 0.7 | 10 |
| Dimethoate | 0.5 | 0.2 | 0.5 | 6.5 |
| Dimethomorph | 0.5 | 0.1 | 0.2 | 5 |
| Dinotefuran | 1.0 | 0.1 | 0.4 | 10 |
| Ethoprophos | 0.5 | 0.1 | 0.5 | 4 |
| Etofenprox | 0.5 | 0.1 | 0.3 | 55 |
| Etoxazole | 0.5 | 0.0 | 0.2 | 9 |
| Fenoxycarb | 0.5 | 0.2 | 0.6 | 12 |
| Fenpyroximate | 0.5 | 0.1 | 0.3 | 10 |
| Fensulfothion | 0.5 | 0.2 | 0.5 | 5 |
| Fenthion | 1.0 | 0.2 | 0.6 | 7 |
| Fipronil | 2.5 | 0.7 | 2.2 | 5.5 |
| Flonicamid | 2.5 | 0.3 | 1.1 | 6 |
| Fludioxonil | 1.0 | 1.1 | 3.8 | 7 |

| | | | | |
|---|---|---|---|---|
| Fluopyram | 0.5 | 0.2 | 0.7 | 11 |
| Hexythiazox | 0.5 | 0.2 | 0.6 | 7 |
| Imazalil | 2.5 | 0.3 | 1.1 | 8 |
| Imidacloprid | 1.0 | 1.1 | 3.5 | 3 |
| Kresoxim-methyl | 2.5 | 0.6 | 1.9 | 7 |
| Malathion | 0.5 | 0.2 | 0.7 | 12.5 |
| Metalaxyl | 0.5 | 0.1 | 0.4 | 14 |
| Methiocarb | 0.5 | 0.1 | 0.4 | 12 |
| Methomyl | 0.5 | 0.2 | 0.6 | 10 |
| Methoprene | 2.5 | 0.8 | 2.8 | 5 |
| Methyl parathion | 20.0 | 6.1 | 20.3 | 4 |
| Mevinphos | 0.5 | 0.0 | 0.7 | 4.5 |
| cis/trans | 0.5 | 0.2 | 0.7 | 8 |
| MGK-264 | 20.0 | 20.2 | 67.5 | 5 |
| Myclobutanil | 0.5 | 0.3 | 1.1 | 4 |
| Naled | 20.0 | 4.0 | 13.4 | 3 |
| Novaluron | 0.5 | 0.2 | 0.5 | 4 |
| Oxamyl | 0.5 | 0.1 | 0.3 | 24 |
| Paclobutrazol | 0.5 | 0.2 | 0.6 | 6 |
| Permethrin I | 5.0 | 9.0 | 29.8 | 3 |
| Permethrin II | 5.0 | 2.6 | 8.7 | 3 |
| Phosmet | 0.5 | 0.2 | 0.5 | 3 |
| Piperonyl butoxide | 0.5 | 0.4 | 1.3 | 5 |

| | | | | |
|---|---|---|---|---|
| Pirimicarb | 0.5 | 0.1 | 0.3 | 3 |
| Prallethrin | 5.0 | 4.9 | 16.3 | 7 |
| Propiconazole | 0.5 | 0.1 | 0.3 | 38 |
| Propoxur | 2.5 | 0.4 | 1.2 | 5 |
| Pyraclostrobin | 0.5 | 0.1 | 0.2 | 25 |
| Pyrethrin I | 0.5 | 0.4 | 1.3 | 7 |
| Pyridaben | 0.5 | 0.1 | 0.2 | 9 |
| Resmethrin | 2.5 | 0.3 | 0.9 | 9 |
| Spinetoram | 0.5 | 0.1 | 0.3 | 16 |
| Spinosad | 0.5 | 0.1 | 0.2 | 31 |
| Spinosad 2 | 1.0 | 0.6 | 1.8 | 3 |
| Spirodiclofen | 0.5 | 0.1 | 0.4 | 7 |
| Spiromesifen | 2.5 | 2.2 | 7.2 | 3 |
| Spirotetramat | 0.5 | 0.1 | 0.4 | 17 |
| Spiroxamine | 0.5 | 0.5 | 0.2 | 79 |
| Tebuconazole | 1.0 | 0.2 | 0.6 | 5 |
| Tebufenozide | 0.5 | 0.1 | 0.4 | 21 |
| Teflubenzuron | 2.5 | 1.1 | 3.6 | 4 |
| Tetrachlorvinphos | 0.5 | 0.2 | 0.7 | 5 |
| Tetramethrin | 1.0 | 0.7 | 2.4 | 5 |
| Thiacloprid | 0.5 | 0.1 | 0.4 | 3 |
| Thiamethoxam | 0.5 | 0.2 | 0.8 | 5 |
| Thiophanate-methyl | 2.5 | 1.6 | 5.4 | 4 |

| | | | | |
|---|---|---|---|---|
| Trifloxystrobin | 0.5 | 0.1 | 0.4 | 16 |

Of the remaining 14 pesticides on Health Canada's watch list, seven were detectable but not quantifiable using the LC-ESI-MS/MS method. The seven non-detectable pesticides were abamectin, endosulfan (alpha and beta) sulfate, etridiazole, fenvalerate, kenoprene, phenothrin, and quintozene (also known as pentachloronitrobenzene). These seven pesticides are not readily compatible with LC or ESI-MS, so they are not detected. However, other methods, including APCI-MS or GC-MS, have detected these seven pesticides.[15, 29, 30]

### 5.3.2 Swiping Sampling and Extraction

We evaluated the suitability of our method for environmental studies by screening two cannabis growing facilities that we got permission to access. Prior to our work, no study had investigated the occurrence of pesticides—or the potential exposure of workers—in cannabis growing facilities. To rapidly screen two cannabis growing facilities, we used a modified wipe sampling method based on the United States Pharmacopeia (USP) method for inspecting contamination of hazardous drugs in healthcare settings.[31] **Figure 5-1** shows the procedures of sampling and extraction schematically.

The sampling method was designed to be simple and sufficient for qualitatively inspecting facilities, as the USP method intended. **Figure 5-3** shows the estimated average recoveries from the simulated sampling method ranged from 1% to 126% on glass and plastic surfaces. The range of recoveries is due to the vast differences in the physicochemical properties

of the 82 pesticides. Further details on the simulated sampling method is described in **Figure 5-1** and **Table 5-9**.

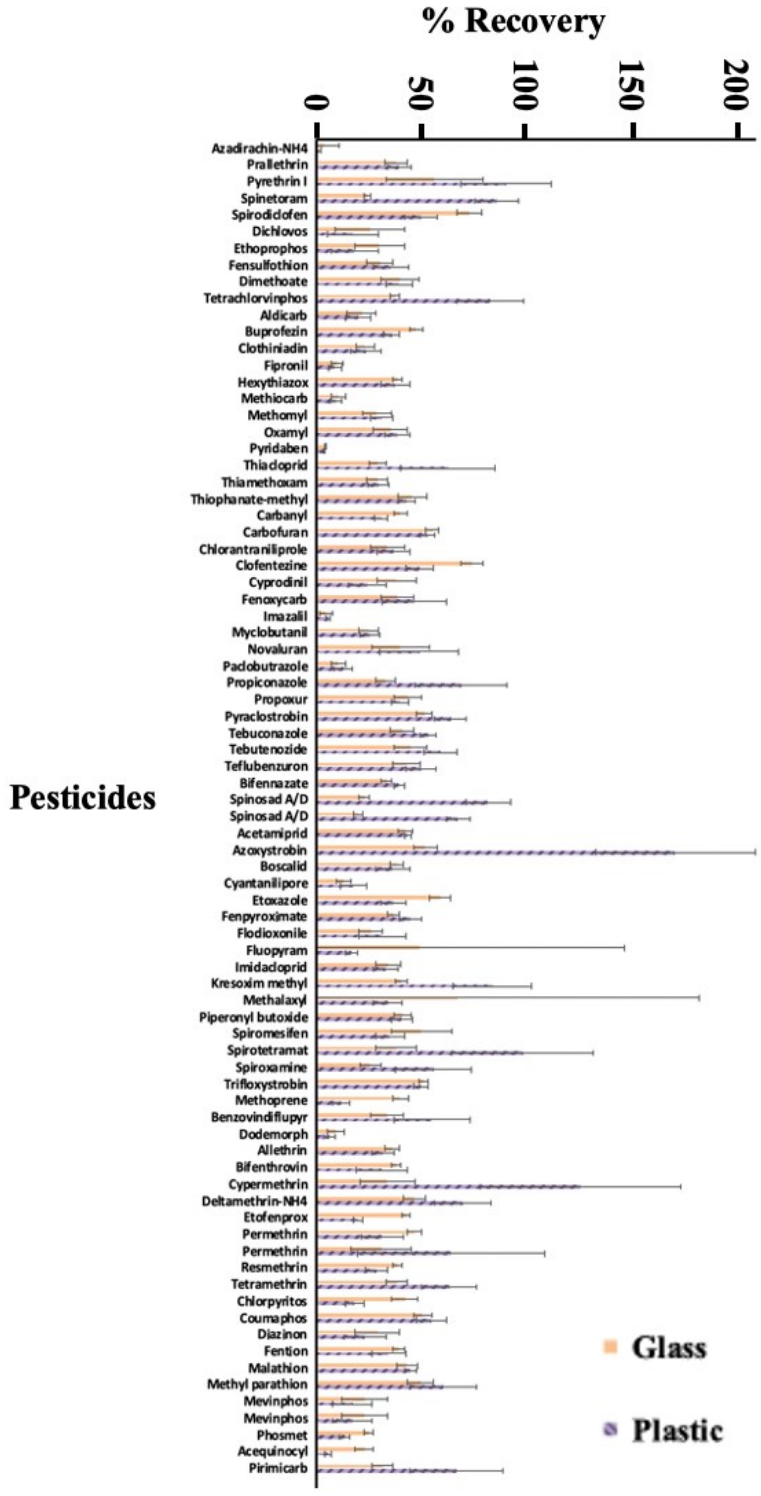
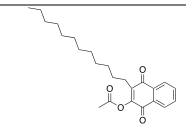
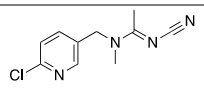
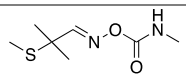
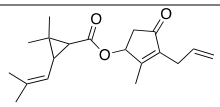
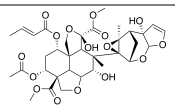
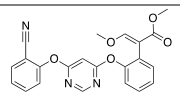
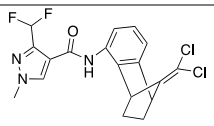
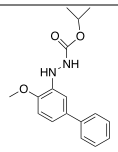
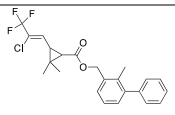
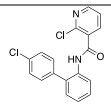
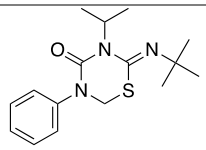
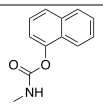
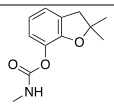
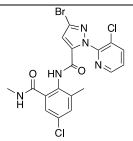
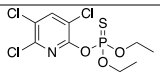
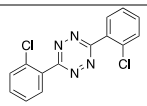
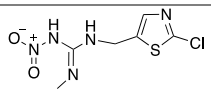
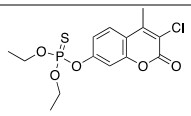
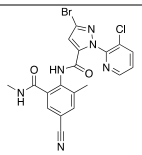
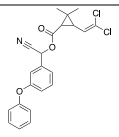
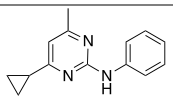
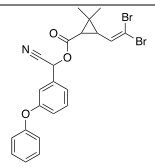
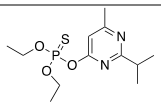
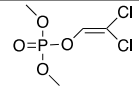
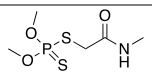
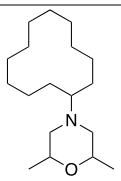
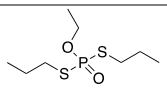
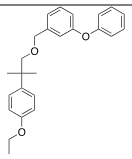
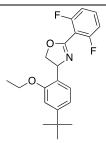
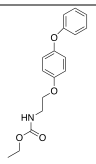
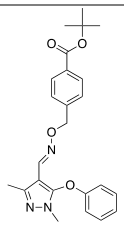



**Figure 5-3**. **Recovery (%) of the spiked standard pesticide mixture on either glass or plastic surfaces.** Error bars are the standard deviation of 6 replicates. Data also shown in **Table 5-9**.

**Table 5-9. Percent recovery (%) of each pesticide from spiking standard pesticide mixture onto glass or plastic in laboratory setting after Kimwipe collection ($n$ = 6).**

| Analyte | Structure | Plastic | Uncert-ainty | Glass | Uncert-ainty | $t_R$ (min) |
|---|---|---|---|---|---|---|
| **Acequinocyl-$NH_4$** |  | 5.6 | 1.4 | 23.0 | 4.4 | 5.6 |
| **Acetamiprid** |  | 43.8 | 1.8 | 42.6 | 3.5 | 43.8 |
| **Aldicarb** |  | 20.2 | 6.0 | 21.8 | 7.0 | 20.2 |
| **Allethrin** |  | 31.8 | 5.2 | 36.2 | 3.5 | 31.8 |
| **Azadirachin-$NH_4$** |  | 1.3* | 0.7 | 3.7* | 7.5 | 1.3 |
| **Azoxystrobin** |  | 171.6 | 37.9 | 52.2 | 5.8 | 171.6 |
| **Benzovindiflupyr** |  | 55.3 | 18.3 | 33.8 | 7.6 | 55.3 |
| **Bifenazate** |  | 39.6 | 2.6 | 33.5 | 2.3 | 39.6 |
| **Bifenthrin** |  | 31.2 | 12.1 | 38.4 | 2.2 | 31.2 |
| **Boscalid** |  | 36.7 | 8.1 | 38.6 | 3.2 | 36.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Buprofezin** |  | 36.1 | 3.7 | 47.7 | 3.1 | 36.1 |
| **Carbaryl** |  | 31.1 | 3.1 | 40.2 | 3.2 | 31.1 |
| **Carbofuran** |  | 53.3 | 3.3 | 55.5 | 3.2 | 53.3 |
| **Chlorantraniliprole** |  | 37.1 | 7.9 | 34.1 | 8.1 | 37.1 |
| **Chlorpyrifos** |  | 18.4 | 4.5 | 42.5 | 6.3 | 18.4 |
| **Clofentezine** |  | 49.5 | 6.5 | 74.4 | 5.0 | 49.5 |
| **Clothianidin** |  | 23.9 | 7.3 | 23.5 | 4.1 | 23.9 |
| **Coumaphos** |  | 55.1 | 7.0 | 51.0 | 4.4 | 55.1 |
| **Cyantanilipore** |  | 17.9 | 6.4 | 13.2 | 3.5 | 17.9 |
| **Cypermethrin** |  | 126.3 | 47.8 | 34.0 | 13.1 | 126.3 |
| **Cyprodinil** |  | 24.5 | 8.9 | 38.5 | 9.1 | 24.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Deltamethrin-$NH_4$** |  | 70.1 | 13.2 | 47.0 | 5.5 | 70.1 |
| **Diazinon** |  | 23.5 | 9.9 | 29.3 | 10.5 | 23.5 |
| **Dichlorvos** |  | 17.5 | 12.0 | 25.7 | 16.4 | 17.5 |
| **Dimethoate** |  | 39.7 | 6.4 | 40.2 | 9.0 | 39.7 |
| **Dodemorph** |  | 6.4 | 2.6 | 9.3 | 3.9 | 6.4 |
| **Ethoprophos** |  | 18.5 | 11.1 | 30.3 | 11.7 | 18.5 |
| **Etofenprox** |  | 19.9 | 2.1 | 43.1 | 2.0 | 19.9 |
| **Etoxazole** |  | 36.9 | 5.7 | 59.4 | 5.0 | 36.9 |
| **Fenoxycarb** |  | 47.1 | 15.3 | 38.9 | 8.0 | 47.1 |
| **Fenpyroximate** |  | 45.2 | 5.3 | 37.1 | 2.7 | 45.2 |

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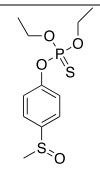
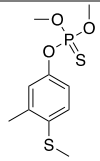
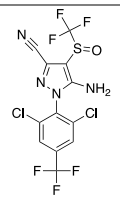
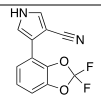
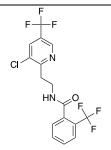
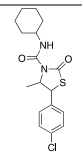
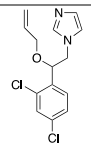
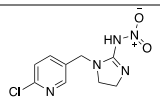
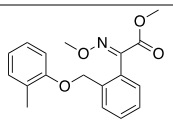
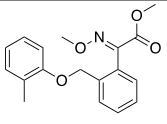
|---|---|---|---|---|---|---|
| **Fensulfothion** |  | 35.7 | 8.6 | 30.5 | 6.5 | 35.7 |
| **Fention** |  | 34.5 | 8.2 | 39.5 | 2.9 | 34.5 |
| **Fipronil** |  | 9.2 | 3.3 | 10.0 | 3.0 | 9.2 |
| **Fludioxonile** |  | 31.5 | 11.3 | 26.0 | 5.9 | 31.5 |
| **Fluopyram** |  | 16.9* | 2.8 | 49.5* | 97.5 | 16.9 |
| **Hexythiazox** |  | 37.7 | 6.8 | 39.1 | 2.1 | 37.7 |
| **Imazalil** |  | 5.5 | 1.0 | 4.8 | 2.9 | 5.5 |
| **Imidacloprid** |  | 33.4 | 5.9 | 34.4 | 6.1 | 33.4 |
| **Kresoxim methyl** |  | 84.1 | 18.7 | 40.8 | 2.8 | 84.1 |
| **Malathion** |  | 45.0 | 3.0 | 43.4 | 5.0 | 45.0 |

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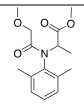
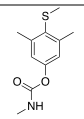
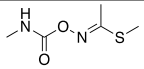
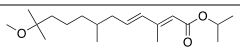
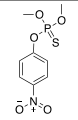
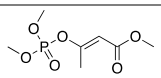
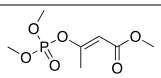
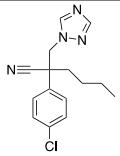
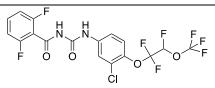
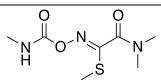
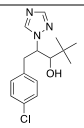
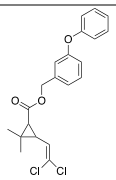
|---|---|---|---|---|---|---|
| **Methalaxyl** |  | 34.3* | 7.0 | 67.7* | 115.0 | 34.3 |
| **Methiocarb** |  | 9.7 | 2.9 | 10.9 | 3.5 | 9.7 |
| **Methomyl** |  | 31.5 | 5.4 | 28.9 | 6.9 | 31.5 |
| **Methoprene** |  | 12.0 | 4.1 | 40.2 | 3.7 | 12.0 |
| **Methyl parathion** |  | 60.6 | 15.8 | 49.8 | 6.3 | 60.6 |
| **Mevinphos-cis/trans** |  | 17.5 | 9.3 | 23.2 | 10.9 | 17.5 |
| **Mevinphos-cis/trans** |  | 17.5 | 9.3 | 23.2 | 10.9 | 17.5 |
| **Myclobutanil** |  | 25.8 | 4.6 | 25.1 | 4.8 | 25.8 |
| **Novaluran** |  | 49.2 | 18.7 | 40.3 | 13.5 | 49.2 |
| **Oxamyl** |  | 39.1 | 5.9 | 35.5 | 8.1 | 39.1 |
| **Paclobutrazole** |  | 13.0 | 4.4 | 10.9 | 3.3 | 13.0 |
| **Permethrin-cis/trans** |  | 31.6 | 9.8 | 47.0 | 3.5 | 31.6 |

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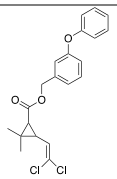
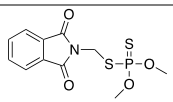
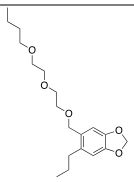
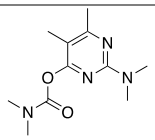
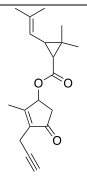
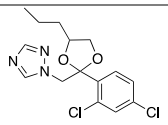
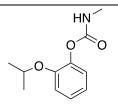
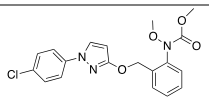
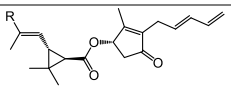
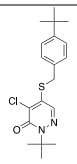
|---|---|---|---|---|---|---|
| **Permethrin-cis/trans** |  | 64.4 | 44.9 | 31.2 | 14.4 | 64.4 |
| **Phosmet** |  | 13.5 | 2.2 | 25.2 | 2.2 | 13.5 |
| **Piperomyl butoxide** |  | 41.0 | 5.0 | 41.4 | 3.9 | 41.0 |
| **Pirimicarb** |  | 67.0 | 22.1 | 31.8 | 4.9 | 67.0 |
| **Prallethrin** |  | 39.7 | 5.6 | 38.1 | 5.4 | 39.7 |
| **Propiconazole** |  | 69.2 | 21.9 | 33.2 | 4.9 | 69.2 |
| **Propoxur** |  | 40.2 | 3.9 | 43.7 | 6.6 | 40.2 |
| **Pyraclostrobin** |  | 64.2 | 7.4 | 51.7 | 3.8 | 64.2 |
| **Pyrethrin I** |  | 90.7 | 21.5 | 56.5* | 23.2 | 90.7 |
| **Pyridaben** |  | 4.0 | 0.4 | 4.7 | 0.3 | 4.0 |

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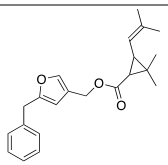
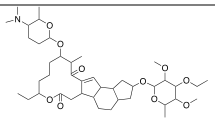
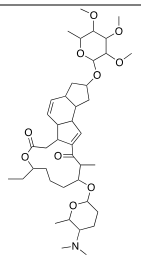
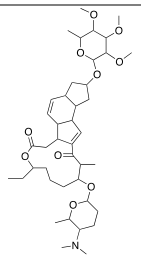
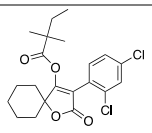
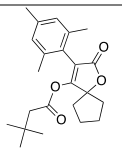
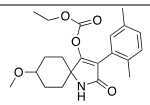
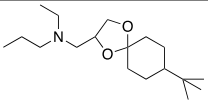
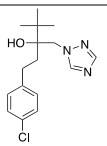
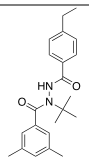
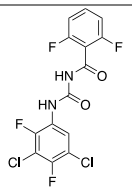
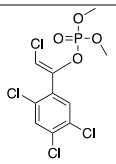
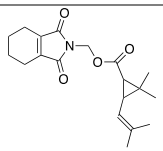
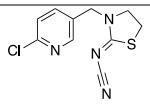
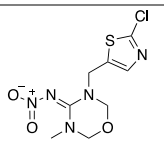
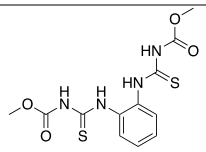
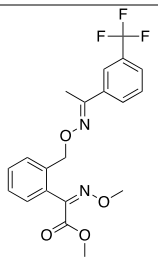
|---|---|---|---|---|---|---|
| **Resmethrin** |  | 28.6 | 5.3 | 39.0 | 2.1 | 28.6 |
| **Spinetoram** |  | 86.4 | 10.4 | 24.3 | 1.7 | 86.4 |
| **Spinosad A/D-cis/trans** |  | 82.1 | 10.7 | 23.1 | 2.5 | 82.1 |
| **Spinosad A/D-cis/trans** |  | 67.8 | 5.7 | 20.2 | 2.0 | 67.8 |
| **Spirodiclofen** |  | 50.2 | 7.8 | 73.4 | 5.9 | 50.2 |
| **Spiromesifen** |  | 35.4 | 6.7 | 50.2 | 14.3 | 35.4 |
| **Spirotetramat** |  | 98.7 | 33.8 | 38.5 | 9.6 | 98.7 |
| **Spiroxamine** |  | 56.1 | 18.2 | 25.8 | 5.0 | 56.1 |
| **Tebuconazole** |  | 53.6 | 3.9 | 41.1 | 5.8 | 53.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Tebufenozide** |  | 59.5 | 7.7 | 45.3 | 7.8 | 59.5 |
| **Teflubenzuron** |  | 50.0 | 7.3 | 43.1 | 6.5 | 50.0 |
| **Tetrachlorvinphos** |  | 83.2 | 16.2 | 37.7* | 2.3 | 83.2 |
| **Tetramethrin** |  | 63.8 | 13.1 | 38.7 | 5.0 | 63.8 |
| **Thiacloprid** |  | 63.1 | 22.6 | 29.6 | 3.9 | 63.1 |
| **Thiamethoxam** |  | 29.9 | 4.9 | 29.2 | 5.1 | 29.9 |
| **Thiophanate-methyl** |  | 43.4 | 4.0 | 45.8 | 6.9 | 43.4 |
| **Trifloxystrobin** |  | 50.0 | 3.6 | 51.6 | 2.2 | 50.0 |

* Recovery data had outlier removed by Q test.

### 5.3.3 Pesticides in Cannabis Growing Facilities

Two cannabis growing facilities at different stages of licensing, designated "non-certified" and "certified", were sampled with the wipe sampling method. Sampling at each location was dictated by the level of access provided by the facility, and each wipe was extracted in 10 mL MeOH. **Figure 5-4** shows the estimated amount of pesticides per square centimeter of area wiped at the non-certified site: 13 pesticides at $2.8e^{-5}$ – $9.0e^{-5}$ μg/cm$^2$ (**Figure 5-4a**) and 9 pesticides greater than $9.0e^{-5}$ μg/cm$^2$ (**Figure 5-4b**). Data for the pesticides detected at less than $2.8e^{-5}$ μg/cm$^2$ are shown in **Figures 5-5** and **5-6**. In the 10 mL extracts, Pyrethrin I and II were estimated at 0.01 and 0.1 μg/cm$^2$, respectively, which were orders of magnitude higher than any of the other detected pesticide.

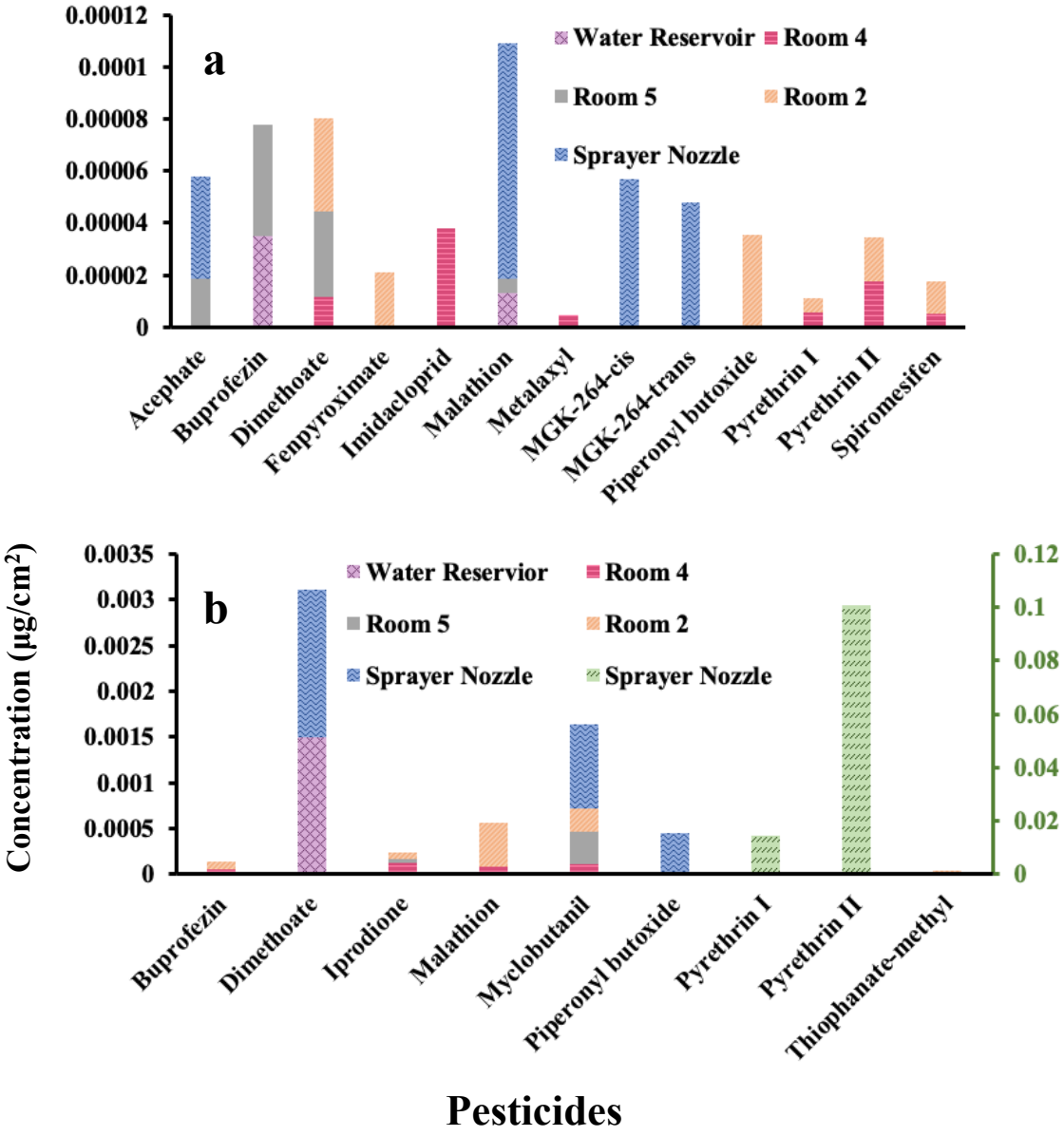



**Figure 5-4. Amount of high abundance pesticide per square centimeter detected at the non-certified growing facility.** (a) Shows the mid-range of pesticides from $2.8e^{-5}$ – $9.0e^{-5}$ µg/cm$^2$. (b) Shows the highest concentration pesticides detected, detected at greater than $9.0e^{-5}$ µg/cm$^2$. The secondary y-axis represents pyrethrin I and pyrethrin II, which were present at much higher concentrations than the other pesticides.

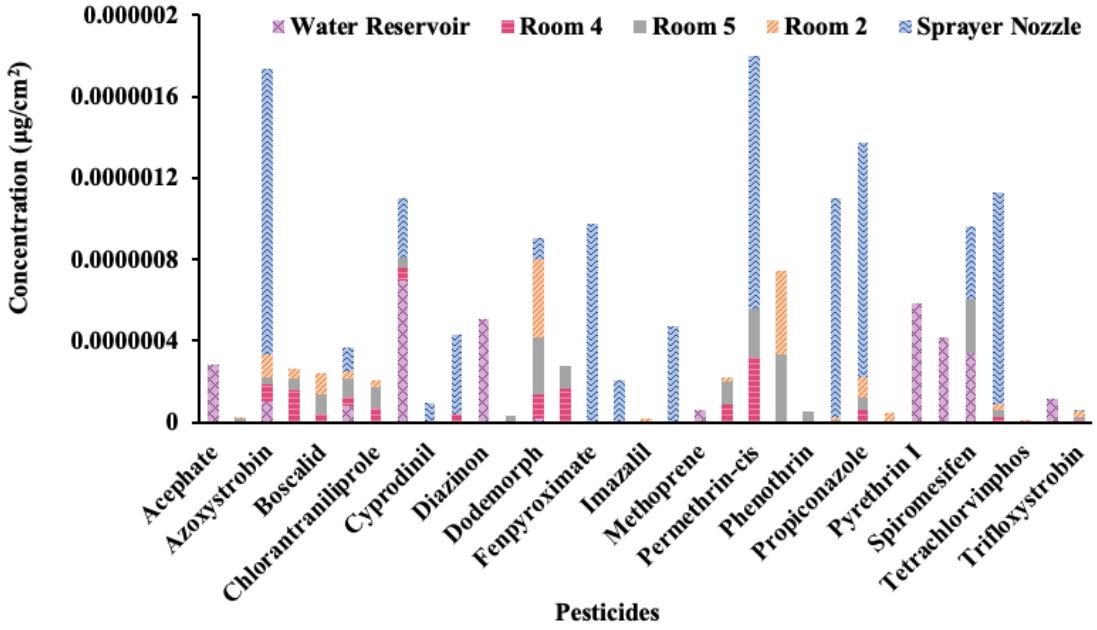



**Figure 5-5**. **Amount of mid abundance pesticide per square centimeter detected at the non-certified growing facility.** These are the lowest amount of pesticides detected below $1.4e^{-6}$ µg/cm$^2$.

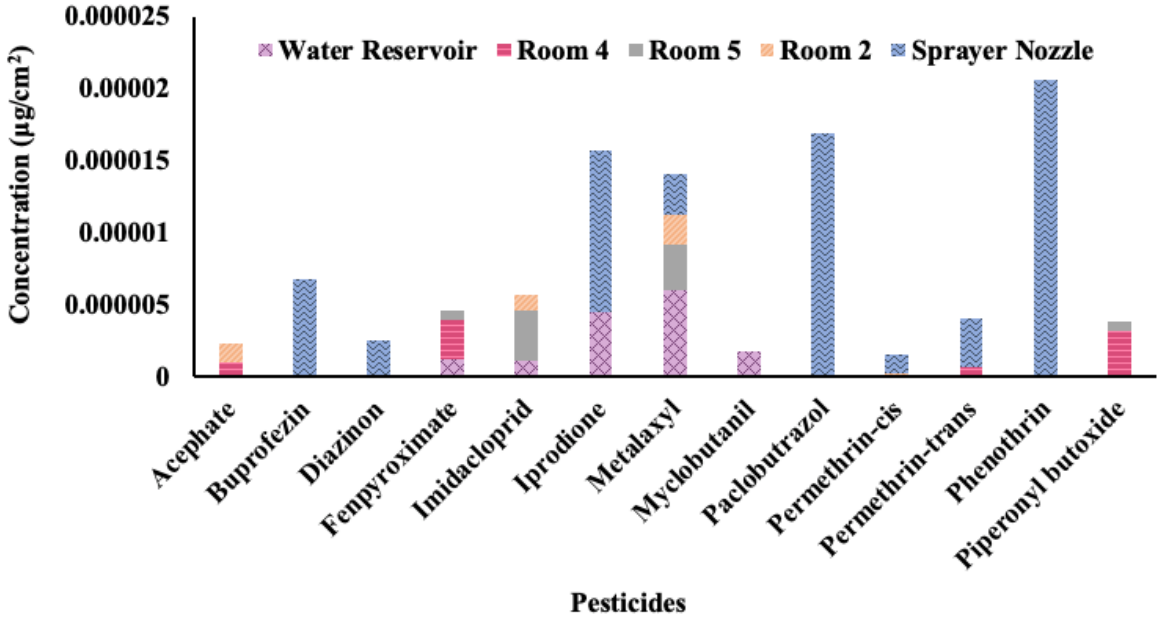



**Figure 5-6**. **Amount of low abundance pesticide per square centimeter detected at the non-certified growing facility.** These are the lowest amount of pesticides detected from $1.4e^{-6}$ µg/cm$^2$ - $2.8e^{-5}$ µg/cm$^2$.

**Figure 5-7** shows the estimated amount of pesticides per square centimeter detected throughout the certified site. A total of 6 pesticides were detected, all at estimated amounts below 0.0000002 μg/cm$^2$. This is in stark contrast to the non-certified site where 41 pesticides were detected at much higher concentrations. Although we only received access to screen two sites, the results show a difference in the occurrence of pesticides in the two growing facilities and potential workers' exposure. Therefore, it is needed to regularly screen the facilities to eliminate pesticides. The results indicate the need to screen the materials (e.g. soil, water, and nutrients) used to grow cannabis, as they can contain pesticides. It is necessary to establish strict protocols for QA/QC and personal safety throughout the facility, supported by the fact that the certified site has significantly less pesticides detected. Some protocols the certified site employed were controlled watering, limited spraying of growth and pest control agents, and thorough cleaning of various apparatus as well as the growing rooms was enforced and regularly performed. The non-certified site had incomplete or fewer protocols in place at the time of sampling. The simple wipe test developed for this study can help growers improve their protocols to ensure safe working conditions for workers in the facilities as well as better QA/QC for cannabis products. This is supported by the fact that similar methods for pharmaceutical production are used.[31] In fact, follow-up communication with the non-certified growing facility regarding these results helped the site to actively clean up and improve the protocols within the facility.

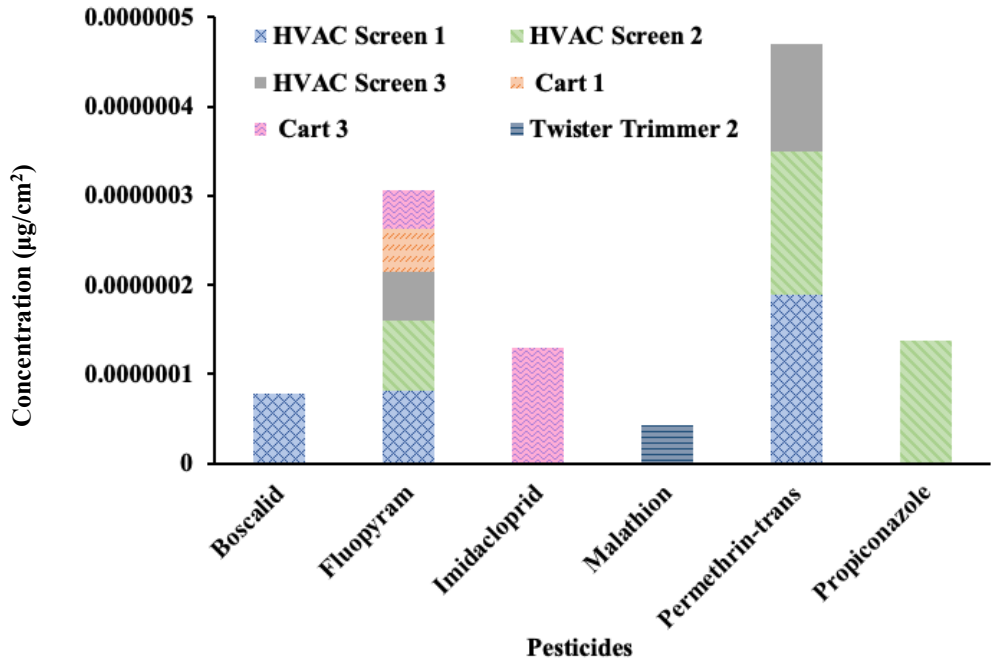



**Figure 5-7. Amount of pesticide per square centimeter detected at the certified growing facility.**

## 5.4 Conclusions

This study highlights the need for proper monitoring of pesticides in cannabis growing facilities, not just in the final consumer product. The simple wipe sampling procedure with HPLC-MS/MS analysis demonstrated the occurrence of Health Canada regulated pesticides at two different cannabis-growing facilities. This is the first study of its kind to highlight the need of routine monitoring of these pesticides in cannabis growing facilities. Such monitoring should help to minimize pesticides in final products, as well as potentially help reduce workers' occupational exposure. Ensuring a pesticide-free product and facility will be important to the financial outcome of the producers and health safety of the consumers and workers. The screening method should also be useful for monitoring pesticides in other produce and food production facilities.

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# Chapter 6

# Conclusions and Future Work

## 6.1. Conclusions

This thesis examined the theoretical aspects of high performance liquid chromatography separations, specifically, electrolytes and mobile phase composition in HILIC separations. I continued to apply these to develop and demonstrate high performance liquid chromatography and mass spectrometry methods to environmental applications. Examples included pesticides in cannabis growing facilities, as well as preliminary studies involving amino acids in water, which will be discussed later in this chapter.

### 6.1.1. Electrolytes in HILIC

**Chapter 2** studied the selectivity and retention changes on four classes of hydrophilic interaction liquid chromatography (HILIC) columns upon addition of 1-20 mM electrolyte. Four different electrolytes were tested: $Na^+Cl^-$, $Na^+ClO_4^-$, $Na^+PF_6^-$, and $Na^+CF_3CO_2^-$. These electrolytes were tested on neutral, cationic, anionic, and zwitterionic analytes under HILIC conditions (70-90% ACN). The electrolytes altered the retention and selectivity on silica, zwitterionic, and diol columns through ion exchange and ionic screening mechanisms. Neutral analytes were unaffected by addition of 1-20 mM electrolyte, indicating minimal change to the retentivity of the water layer. Cationic and anionic analytes increase and decreases in retention, respectively. The strength of electrolytes were as follows: $Na^+PF_6^- \approx Na^+ClO_4^- > Na^+CF_3CO_2^- > Na^+Cl^-$. Chaotropic electrolytes (e.g., $PF_6^-$ and $ClO_4^-$) accumulate at interfaces such as the mobile phase/adsorbed water layer interface, enhancing the ion exchange and ionic screening effects of

the mobile phase electrolyte. Altering the buffer cation ($Li^+$, $Na^+$, and $K^+$, all of which are kosmotropic) caused small but statistically significant changes in retention.

### 6.1.2. Mobile Phase Preparation

**Chapter 3** investigated the effect of mobile phase preparation and counterions on HILIC retention. Three different methods of mixing were investigated, assuming use of a graduated cylinder and volumetric flask: a.) measure both organic and water, b.) measure organic and fill to mark with organic, c.) measure water and fill to mark with organic. The volume contraction that occurs when acetonitrile and water mix means that *how* a mobile phase is prepared affects the actual composition of the mixture in percent terms. This in turn can affect the observed retention and selectivity observed under HILIC conditions. Descriptions of experimental procedures for HILIC separations should describe in detail the manner in which the two solvents are mixed. One important aspect of this, that is usually not described in the literature, is how the mixture is brought to a fixed final volume (for example, Methods B and C described in **Section 3.3.1**), or if the measured volumes are combined to produce some nominal final volume (Method A). The counterion (e.g., $Na^+$, $K^+$, $NH_4^+$) present in the aqueous buffer also matters, particularly for HILIC separations of charge analytes, such that the retention relative to the sodium ion changed could almost double for some charged analytes

### 6.1.3. Pesticides

**Chapter 4** provides background, current methods and technology, and future perspective on cannabis and pesticides. **Chapter 5** described the development of a HPLC-MS/MS method and its application to determination of residue pesticides in cannabis facilities. My method

involved wipe sampling, liquid chromatography separation, and tandem mass spectrometry, and enabled the determination of 82 pesticides out of the 96 regulated by Health Canada. To demonstrate application of the method, we sampled and measured pesticides in two cannabis growing facilities, representing a non-certified and a certified site. We detected 41 pesticides in surface wipe samples at the non-certified site and only 6 at the certified site. This study provided the first evidence showing pesticide occurrence on common surfaces in cannabis growing facilities and points to a need for routine monitoring and strict control of pesticide use in cannabis facilities.

## 6.2. Preliminary Study: Amino Acids in Water

### 6.2.1. Background

Water disinfection is essential to remove waterborne pathogens.[1] Natural organic matter (NOM) present in water can react with the disinfectants to form disinfection byproducts (DBPs), which may have potential adverse health effects.[2-8] Therefore prior to disinfection, water treatment plants (WTP) implement a variety of strategies to remove NOM to limit DBP formation.[9-15] However, current treatment technologies cannot remove all NOM. Water soluble and small NOM remaining in the source water may form unwanted odorous and/or toxic DBPs.[16-19] Spring run-off often introduces complex organic compounds, contributing to the problem. Therefore, it is necessary to identify markers of spring run-off to assist WTP's timely adjustment of treatment processes to assure high quality drinking water.[7, 8, 20, 21]

Spring run-off occurs when warmer weather and temperatures melt ice and snow that has deposited on the land during winter. This snow melt feeds into creeks and rivers that can directly join with the source water. Most important is run-off on or around farms that are rich in complex

organic materials and introduce additional NOM to the source water. Chemical markers that signal spring run-off are useful for timely adjustment of the WTP treatment processes. This preliminary study investigates the proposal that amino acids may serve as markers. Amino acids are ubiquitous in nature and are at high levels in the soil, especially surrounding plant life. Therefore, spring run-off should contain high levels of amino acids. While amino acids are not odorous themselves, it is suspected that amino acids react with chlorine or chloramine during disinfection to form DBPs that may be odorous.[16, 18]

Reversed phase liquid chromatography (RPLC)-MS/MS is not adequate to directly analyze amino acids in water by itself. Most common methods for trace compounds in water involve time-consuming steps using solid phase extraction (SPE) and/or derivatization.[22, 23] Preconcentration is required to enrich analytes in the sample while derivatization improves the separation by RPLC and ESI ionization efficiency. However, the need for preconcentration has been circumvented by the integration of large volume injections (LVI) with HPLC.[24-26] LVI is comparable to methods like SPE due to the large volumes injected with no loss of analyte in comparison to extraction techniques, like SPE, that have analyte loss.[24, 25] Additionally, RPLC has poor separation of small polar compounds. Thus, we use the unique separation capabilities of hydrophilic interaction liquid chromatography (HILIC) to avoid the need to derivatize amino acids. As shown in Chapter 2, HILIC preferentially retains small polar molecules. As well, its use of high organic solvent (~65-95%, typically acetonitrile) enhances ESI ionization efficiency, yielding better sensitivity.[27] HILIC has become a staple for separation of amino acids in many fields.[28-33]

The objective of this preliminary work was to develop a fast and simple LVI-HILIC-MS/MS method and determine the method's viability for monitoring amino acids in authentic

water samples. We applied this method to samples collected during the 2020 spring run-off. Comparatively, we developed a SPE-RPLC-MS/MS for monitoring of amino acids in the same samples. COVID-19 restrictions limited the work which could be done. But the preliminary results allow the benefits and limitations of each method to be compared, and recommendations for future work to be made.

### 6.2.2. Materials and Methods

#### 6.2.2.1. Chemicals and Materials

Formic acid (FA), ammonium formate (AF), and polyvinylidene difluoride (PVDF) syringe filters (0.22 μm and 045 μm) were purchased from Sigma-Aldrich (St. Louis, MO). Optima water, acetonitrile (ACN), and amino acid standards were from Fisher Scientific (Fair Lawn, NJ). Oasis MCX cartridges (6 cc, 150 mg) were purchased from Waters (Milford, MA).

#### 6.2.2.2. Sample Collection

The spring runoff samples in 2020 were collected and provided by EPCOR staff through collaboration. The samples included the source water from North Saskatchewan River at the water treatment plant (WTP) and upstream creeks. Source water samples were collected on March 5; April 8, 15, 17, 18, 22; and May 04. Additionally, four different creek water samples were collected on April 16; creek W, creek S, creek T, creek M. The creeks are located around farms and flow into the North Saskatchewan River, upstream of the WTP. All samples were collected in 4L amber glass bottles. Bottles were rinsed three times with sample before they were fully filled without headspace remaining. All samples were filtered using 0.45 μm PVDF filters and stored at 4 ºC prior to analysis. The WTP measured the following water quality parameters

throughout the pilot study: raw water turbidity, raw water colour, favour profile analysis (FPA) odour intensity, total organic nitrogen (TON), ammonia, and total Kjeldahl nitrogen (TKN). TKN is sum of the total organic and inorganic nitrogen present.

#### 6.2.2.3. Sample Preparation

##### 6.2.2.3.1. Sample preparation for HILIC

For the HILIC-MS/MS analysis, large volume injections (LVI) instead of SPE was used. Standard addition was used for quantification. Samples were prepared in triplicate with standard additions at 0, 0.01, 0.05, and 0.1 ppb final concentration. Samples were then filtered with 0.25 µm PVDF filters. **Figure 6-1** overviews the sample preparation steps for both RPLC and HILIC methods.

##### 6.2.2.3.2. Sample preparation for RPLC

For the RPLC-MS/MS analysis, the water samples were extracted using solid-phase extraction (SPE). Oasis MCX cation exchange cartridges (5 cc, 150 mg, Waters, Milford, MA) were first preconditioned with methanol (12 mL), followed by acidified water (12 mL, 0.25% FA, V/V). 250 mL Water samples were acidified with 0.25% FA then passed through the cartridge at ~1-2 mL/min. Following loading, the SPE cartridges were washed with acidified water (6 mL, 0.25% FA, V/V) and then eluted with ammonium hydroxide (10 mL, 5 wt%). The eluate was concentrated to 0.1 mL under a gentle stream of nitrogen. The samples were reconstituted to 1 mL with a 0.1% FA and methanol water solution (80:20 MeOH:$H_2O$) and filtered using 0.25 µm PVDF syringe filters. Samples were quantified using external calibration.

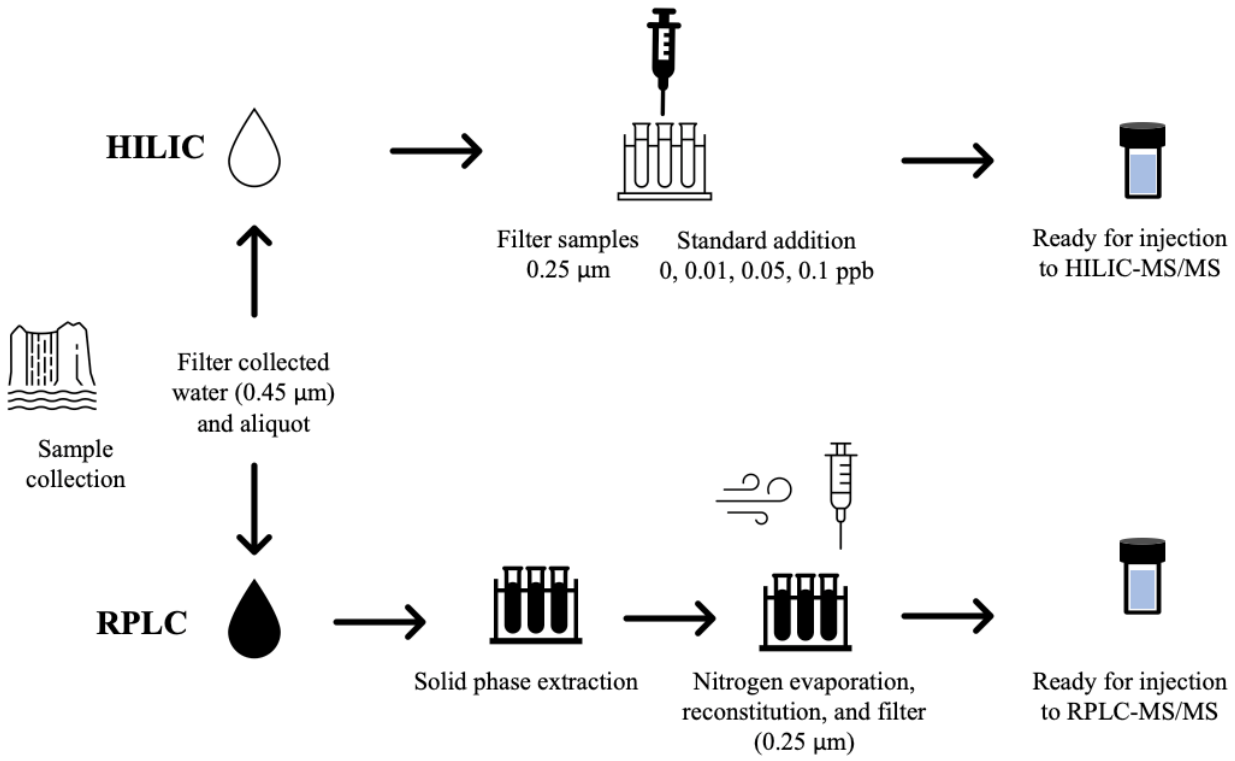



**Figure 6-1**. **Procedures of sample collection and preparation for the HILIC-MS/MS and RPLC-MS/MS analysis.** The top row shows the steps involved in the large volume-HILIC-MS/MS method. The bottom row shows the steps of the SPE-RPLC-MS/MS method.

#### 6.2.2.4. HPLC-MS/MS Parameters

##### 6.2.2.4.1. LVI-HILIC-MS/MS

An Agilent 1290 series HPLC system was used for HILIC analysis. The HILIC method used an InfinityLab Poroshell 120 HILIC column (1.9 µm × 100 mm x 2.1 mm ID) (Agilent, Santa Clara, CA). **Table 6-1** summarizes the gradient profile, re-equilibration conditions, and other parameters for the HILIC separation. Mobile phase A consisted of water with 0.1% FA and 10 mM ammonium formate, while mobile phase B consisted of acetonitrile with 0.1% FA and 5% water (made V/V, 0.45 µm filtered, and sonicated prior to use). Other parameters were 0.4

mL/min and column oven 40°C. A Sciex 5500 triple quad was used. A mix standard of 20 amino acids was used for method development. Amino acid standards were made up in a 95:5 ACN:$H_2O$ with 0.1% FA (starting mobile phase) at concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, and 5 ppb. The mass spectrometry parameters were optimized with 5 ppb amino acid standards (in starting mobile phase) first using direct injection, and then coupled with the HPLC. **Table 6-2** presents the optimized MS ionization parameters for the HILIC method, and **Table 6-3** shows the optimized MRM transitions and parameters for the HILIC-MS/MS method. Two transitions for each amino acid are selected which provides further confidence in identification. The ion ratio between the two transitions can help eliminate other compounds that may have similar m/z and fragmentation but that will not fragment at the same ratio. The LODs (S/N = 3) of the LVI-HILIC-MS/MS method are shown in **Table 6-4**. Under the conditions examined, cysteine was difficult to detect and had low sensitivity. Cysteine showed better sensitivity when the ion source temperature was lowered to 250 ºC, however this resulted in a loss of sensitivity for many other amino acids. For this preliminary study, we focused on achieving better sensitivity for the majority of amino acids rather than for just a single amino acid, cysteine.

**Table 6-1. HILIC gradient program and autosampler conditions.**

| HPLC Gradient | **Time (min)** | **Flow (µL/min)** | **%A** | **%B** |
|---|---|---|---|---|
| | 0 | 400 | 5 | 95 |
| | 1 | 400 | 5 | 95 |
| | 10 | 400 | 28 | 72 |
| | 14 | 400 | 28 | 72 |
| | 14.5 | 400 | 5 | 95 |
| | 30 | 400 | 5 | 95 |
| HPLC Autosampler | | | | |
| | Syringe size | 20 µL | | |
| | Injection volume* | 100 µL | | |
| | Draw speed | 100 µL/min | | |
| | Eject speed | 200 µL/min | | |
| | Needle level | 0 mm | | |
| | Autosampler Temperature | 8 ºC | | |
| | Column temperature | 40 ºC | | |

*Injector program was used for large volume injection. 5 replicate injections of 20 µL's of sample into injection loop before sample was injected.

**Table 6-2**. **Mass spectrometry parameters for HILIC method.**

| Mode | Positive | | |
|---|---|---|---|
| Mode | MRM | | |
| Curtain gas (AU)* | 30 | | |
| Collision gas | Medium | | |
| Ion spray voltage (V) | 4600 | | |
| Ion Source Temperature | 550 ºC | | |
| Ion source gas 1 (AU) | 50 | | |
| Ion source gas 2 (AU) | 40 | | |
| Entrance potential (V) | 10 | | |
| | | | |
| Integrated MS Valve | | | Time (min) |
| | A | To Waste | 0 |
| | B | To MS | 0.5 |
| | A | To Waste | 14 |

*AU = Arbitrary units

**Table 6-3. HILIC-MS/MS method optimized conditions: MRM transitions and parameters.**

| Amino Acids | Q1 | Q3 | Declustering Potential (V) | Collision Energy (V) | Cell Exit Potential (V) |
|---|---|---|---|---|---|
| Cysteine-1 | 122.3 | 59.3 | 25.0 | 25.5 | 6.7 |
| Cysteine-2 | 122.3 | 76.0 | 26.0 | 7.2 | 12.3 |
| Alanine-1 | 90.1 | 44.1 | 22.3 | 13.9 | 7.2 |
| Alanine-2 | 90.1 | 72.1 | 89.2 | 15.0 | 9.2 |
| Arginine-1 | 175.0 | 116.1 | 75.4 | 18.3 | 13.8 |
| Arginine-2 | 175.0 | 60.1 | 84.8 | 18.0 | 9.7 |
| Aspartic acid-1 | 134.3 | 74.0 | 22.0 | 19.9 | 9.7 |
| Aspartic acid-2 | 134.3 | 88.1 | 32.1 | 11.6 | 9.9 |
| Glutamic acid-1 | 148.8 | 47 | 50.3 | 39.8 | 6.8 |
| Glutamic acid-2 | 148.4 | 77 | 16.1 | 33.6 | 6.2 |
| Glycine-1 | 76.0 | 30.1 | 19.1 | 16.1 | 4.8 |
| Glycine-2 | 76.0 | 59.2 | 122.7 | 18.1 | 6.8 |
| Histidine-1 | 156.0 | 109.8 | 69.0 | 19.0 | 14.5 |
| Histidine-2 | 156.0 | 83.0 | 57.0 | 33.6 | 8.8 |
| Lysine-1 | 147.1 | 84.0 | 49.4 | 22.4 | 10.1 |
| Lysine-2 | 147.1 | 90.4 | 172.2 | 42.4 | 14.7 |
| Methionine-1 | 150.3 | 61 | 29.9 | 30.9 | 9.4 |
| Methionine-2 | 150.3 | 56.0 | 50.4 | 19.9 | 9.6 |
| Phenylalanine-1 | 166.4 | 120.4 | 25.8 | 18.9 | 11.1 |
| Phenylalanine-2 | 166.4 | 103 | 57.6 | 36.8 | 12.7 |
| Proline-1 | 116.4 | 70 | 40.9 | 21.1 | 14.1 |
| Proline-2 | 116.4 | 43 | 48.2 | 40.8 | 6.8 |
| Serine-1 | 106.0 | 60 | 22.2 | 15.5 | 9.9 |
| Serine-2 | 106 | 42 | 44.3 | 30.4 | 6.4 |
| Threonine-1 | 120.4 | 77 | 149 | 36.0 | 8.5 |
| Threonine-2 | 120.4 | 103.0 | 149 | 36.0 | 8.5 |
| Tyrosine-1 | 182.1 | 91.0 | 37.0 | 36.8 | 11.1 |
| Tyrosine-2 | 182.1 | 119.0 | 45.0 | 27.0 | 14.9 |
| Valine-1 | 118.2 | 55.1 | 36.9 | 29.3 | 9.0 |
| Valine-2 | 118.2 | 72.1 | 40.3 | 14.6 | 9.5 |
| Glutamine-1 | 147.0 | 67.1 | 207.8 | 19.3 | 15.3 |
| Glutamine-2 | 147.0 | 119.0 | 207.8 | 19.3 | 15.3 |
| Tryptophan-1 | 205.6 | 118.2 | 54.0 | 33.1 | 13.7 |
| Tryptophan-2 | 205.6 | 145.8 | 48.1 | 23.7 | 22.3 |
| Asparagine-1 | 133.2 | 118.1 | 178.7 | 27.9 | 14.6 |
| Asparagine-2 | 133.2 | 106.1 | 166.1 | 25.8 | 12.0 |
| Leucine-1 | 132.8 | 57.1 | 76.6 | 26.0 | 8.2 |
| Leucine-2 | 132.8 | 90.9 | 76.6 | 26.0 | 8.2 |
| Isoleucine-1 | 132.8 | 45 | 48.9 | 34.4 | 7.3 |
| Isoleucine-2 | 132.8 | 87.3 | 48.1 | 14.1 | 10.1 |

**Table 6-4 The lowest standard concentrations of the amino acids detected by HILIC-MS/MS method.** Estimated limit of detection (LOD) projected at S/N=3 for each amino acid.

| Amino Acid | Lowest Standard (ppb) | S/N | Estimated LOD (ppb) |
|---|---|---|---|
| Cysteine | N/A | N/A | >> 5 ppb |
| Alanine | 0.05 | 3 | 0.05 |
| Arginine | 0.01 | 23 | 0.0013 |
| Aspartic acid | 0.01 | 13 | 0.002 |
| Glutamic acid | 0.05 | 4 | 0.038 |
| Glycine | 0.1 | 3 | 0.1 |
| Histidine | 0.5 | 3 | 0.5 |
| Lysine | 0.01 | 12 | 0.0025 |
| Methionine | 0.01 | 24 | 0.0013 |
| Phenylalanine | 1 | 2.5 | 1 |
| Proline | 0.01 | 14 | 0.0021 |
| Serine | 0.01 | 6 | 0.005 |
| Threonine | 1 | 4 | 0.75 |
| Tyrosine | 0.01 | 4 | 0.0075 |
| Valine | 0.05 | 6 | 0.025 |
| Glutamine | 0.01 | 23 | 0.0013 |
| Tryptophan | 0.01 | 172 | 0.00017 |
| Asparagine | 0.01 | 18 | 0.0017 |
| Leucine | 1 | 5 | 0.6 |
| Isoleucine | 0.5 | 4 | 0.38 |

#### 6.2.2.4.2. SPE-RPLC-MS/MS

An Agilent 1290 series HPLC system with a Phenomenex Luna C18(2) column (3 μm, 2 mm ID × 100 mm, Agilent, Santa Clara, CA) was used for RPLC analysis. Gradient steps and other HPLC settings are shown in **Table 6-5**. Mobile phase B consisted of MeOH with 0.3% FA and 5% water, while mobile phase A was water and 0.3% FA (made V/V, 0.45 μm filtered, and sonicated prior to use). Other parameters were 0.15 mL/min flow rate and column temperature of

40 °C. SPE parameters are described in **Section 6.2.2.3.2**. A Sciex 5000 triple quad mass spectrometer was used for reversed phase analysis, as this was available. Mass spectrometry parameters (**Table 6-6**) were optimized with standards first using direct injection then coupled with the HPLC. Optimized MRM transitions and parameters are shown in **Table 6-7**. RPLC-MS/MS LODs are shown in **Table 6-8** with a minimum signal-to-noise of 3 (S/N = 3).

**Table 6-5. RPLC gradient program and autosampler conditions.**

| **RPLC Gradient** | **Time (min)** | **Flow (µL/min)** | **%A** | **%B** |
|---|---|---|---|---|
| | 0 | 150 | 98 | 2 |
| | 1 | 150 | 98 | 2 |
| | 4.5 | 150 | 98 | 2 |
| | 9.0 | 150 | 5 | 95 |
| | 10 | 150 | 5 | 95 |
| | 11 | 150 | 98 | 2 |
| | 18 | 150 | 98 | 2 |
| | | | | |
| HPLC Autosampler | | | | |
| | Syringe size | 20 µL | | |
| | Injection volume | 20 µL | | |
| | Draw speed | 100 µL/min | | |
| | Eject speed | 200 µL/min | | |
| | Needle level | 0 mm | | |
| | Autosampler Temperature | 8 °C | | |
| | Column temperature | 40 °C | | |

**Table 6-6. Mass spectrometry parameters for RPLC method.**

| | | | |
|---|---|---|---|
| Mode | Positive | | |
| Mode | MRM | | |
| Curtain gas (AU) | 40 | | |
| Collision gas | Medium | | |
| Ion spray voltage (V) | 5500 | | |
| Temperature | 650 °C | | |
| Ion source gas 1 (AU) | 40 | | |
| Ion source gas 2 (AU) | 30 | | |
| Entrance potential (V) | 10 | | |
| | | | |

| MS Integrated Valve Switch | Time | Valve | |
|---|---|---|---|
| | 0 | A | To waste |
| | 0.4 | B | To MS |

**Table 6-7. RPLC MRM transitions and parameters.**

| Amino Acids | Q1 | Q3 | Declustering Potential (V) | Collision Energy (V) | Cell Exit Potential (V) |
|---|---|---|---|---|---|
| Cysteine-1 | 122.6 | 59.4 | 35.0 | 29.5 | 5.0 |
| Cysteine-2 | 122.6 | 42.1 | 33.0 | 59.7 | 18.0 |
| Alanine-1 | 89.9 | 44.1 | 25.0 | 20.3 | 18.0 |
| Alanine-2 | 89.9 | 42.0 | 62.0 | 51.5 | 18.0 |
| Arginine-1 | 175.3 | 116.1 | 85.0 | 23.7 | 12.0 |
| Arginine-2 | 175.3 | 158.0 | 86.0 | 18.3 | 23.0 |
| Aspartic acid-1 | 134.8 | 57.8 | 22.0 | 19.9 | 9.7 |
| Aspartic acid-2 | 134.8 | 47.0 | 32.1 | 11.6 | 9.9 |
| Glutamic acid-1 | 148.4 | 51.0 | 76.0 | 53.8 | 8.0 |
| Glutamic acid-2 | 148.4 | 87.9 | 54.0 | 38.6 | 15.0 |
| Glycine-1 | 75.9 | 30.0 | 26.0 | 28.9 | 12.0 |
| Glycine-2 | 75.9 | 58.9 | 120.0 | 19.5 | 24.0 |
| Histidine-1 | 156.3 | 110.0 | 63.0 | 23.2 | 10.0 |
| Histidine-2 | 156.3 | 83.0 | 56.0 | 34.5 | 37.0 |
| Lysine-1 | 147.0 | 130.1 | 55.0 | 18.0 | 13.0 |
| Lysine-2 | 147.0 | 84.0 | 53.0 | 25.8 | 12.0 |
| Methionine-1 | 150.3 | 56.0 | 43.0 | 25.4 | 23.0 |
| Methionine-2 | 150.3 | 104.0 | 42.0 | 17.7 | 10.0 |
| Phenylalanine-1 | 166.1 | 102.5 | 40.0 | 35.8 | 35.0 |
| Phenylalanine-2 | 166.1 | 94.6 | 40.8 | 40.0 | 12.0 |
| Proline-1 | 116.2 | 70.4 | 36.0 | 36.1 | 13.0 |
| Proline-2 | 116.2 | 43.0 | 40.0 | 43.1 | 18.0 |
| Serine-1 | 106.0 | 59.9 | 20.0 | 18.2 | 14.0 |
| Serine-2 | 106.0 | 96.0 | 191.0 | 13.8 | 10.0 |
| Threonine-1 | 120.0 | 103.0 | 128.0 | 26.4 | 20.0 |
| Threonine-2 | 120.0 | 92.0 | 74.0 | 24.5 | 11.0 |
| Tyrosine-1 | 182.1 | 91.8 | 26.0 | 55.4 | 12.0 |
| Tyrosine-2 | 182.1 | 77.4 | 124.0 | 52.0 | 6.0 |
| Valine-1 | 118.3 | 71.7 | 60.0 | 17.4 | 8.0 |
| Valine-2 | 118.3 | 55.0 | 43.0 | 27.8 | 21.0 |
| Glutamine-1 | 147.4 | 55.9 | 207.8 | 19.3 | 15.3 |
| Glutamine-2 | 147.4 | 83.6 | 14.5 | 20.6 | 15.8 |
| Tryptophan-1 | 205.3 | 91.7 | 30.0 | 68.2 | 10.0 |
| Tryptophan-2 | 205.3 | 115.0 | 152.0 | 60.0 | 8.0 |
| Asparagine-1 | 133.4 | 44.0 | 39.0 | 35.0 | 18.0 |

| | | | | | |
|---|---|---|---|---|---|
| Asparagine-2 | 133.4 | 73.9 | 105.0 | 35.0 | 34.0 |
| Leucine-1 | 132.8 | 39.0 | 76.6 | 26.0 | 8.2 |
| Leucine-2 | 132.8 | 58.0 | 133.8 | 21.1 | 8.8 |
| Isoleucine-1 | 132.8 | 57.8 | 48.9 | 34.4 | 7.3 |
| Isoleucine-2 | 132.8 | 60.9 | 68.1 | 14.1 | 10.1 |

**Table 6-8. The lowest concentrations of the amino acids detected by RPLC-MS/MS method**

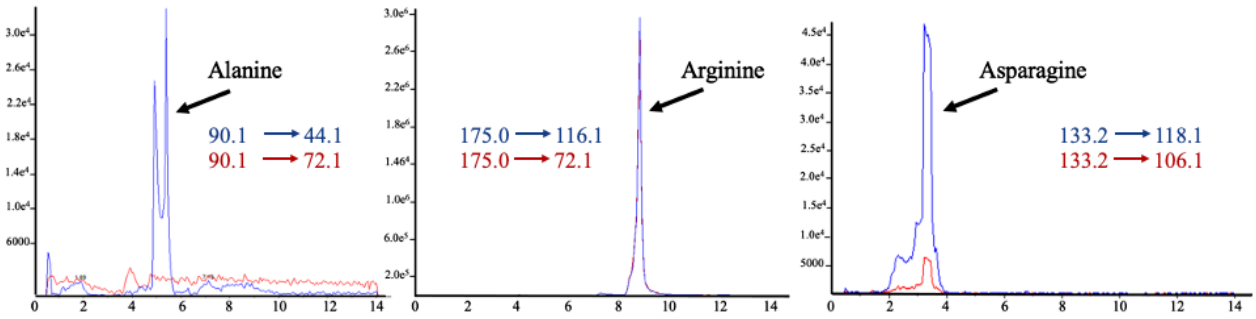
Estimated limit of detection (LOD) projected at S/N=3 for each amino acid.

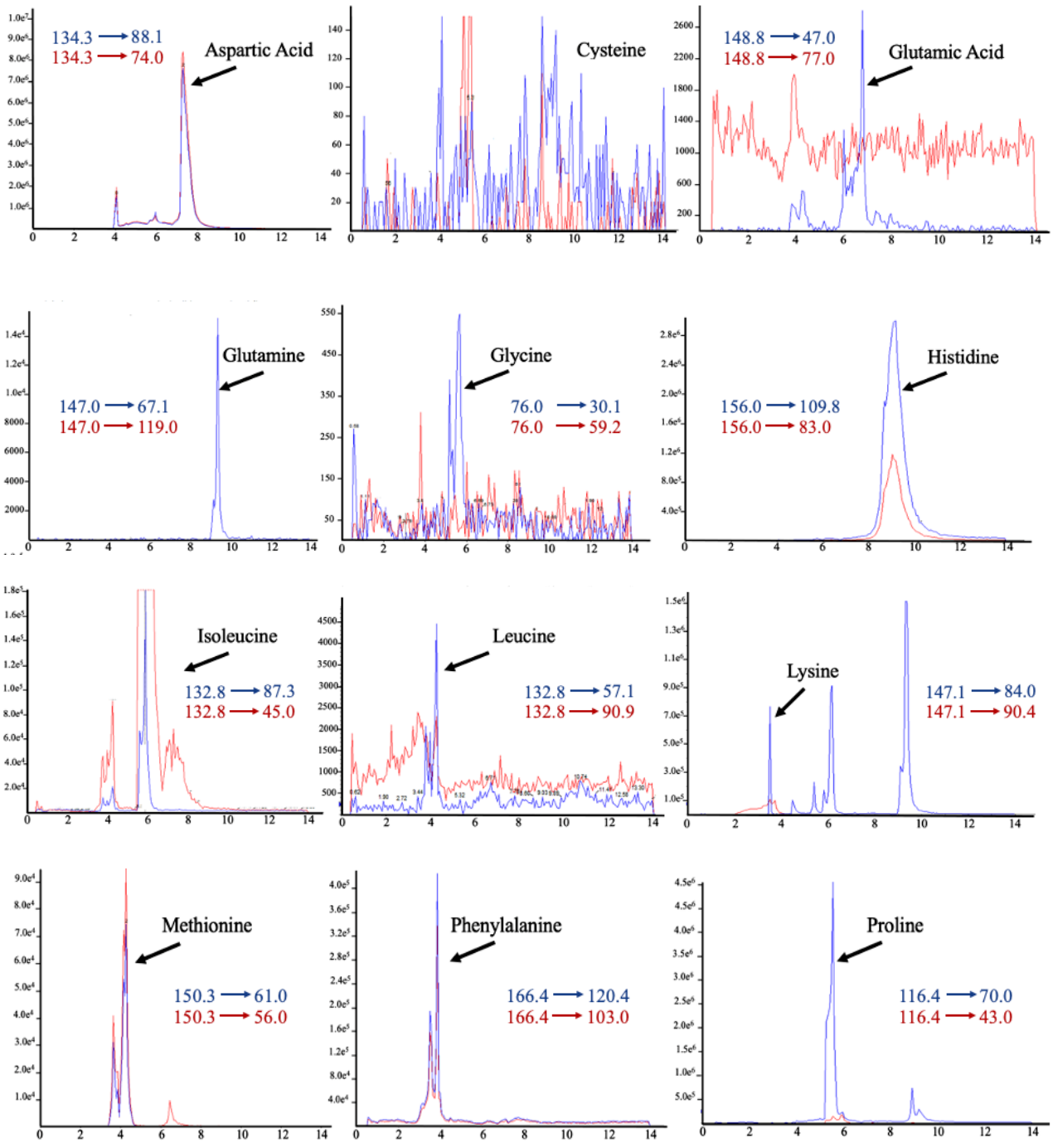
| Amino Acid | Lowest Standard (ppb) | S/N | Estimated LOD (ppb) |
|---|---|---|---|
| Cysteine | 0.1 | 46 | 0.0065 |
| Alanine | 0.01 | 2.4 | 0.010 |
| Arginine | 0.1 | 54 | 0.0055 |
| Aspartic acid | 0.01 | 27 | 0.0011 |
| Glutamic acid | 0.1 | 3 | 0.1 |
| Glycine | 0.01 | 3 | 0.01 |
| Histidine | 0.1 | 78 | 0.0038 |
| Lysine | 0.1 | 70 | 0.0043 |
| Methionine | 0.5 | 8 | 0.19 |
| Phenylalanine | 0.1 | 10 | 0.030 |
| Proline | 0.01 | 10 | 0.0030 |
| Serine | 0.01 | 3 | 0.01 |
| Threonine | 0.1 | 3 | 0.1 |
| Tyrosine | 0.01 | 27 | 0.0011 |
| Valine | 0.1 | 21 | 0.014 |
| Glutamine | 0.1 | 4 | 0.075 |
| Tryptophan | 0.01 | 675 | 0.000044 |
| Asparagine | 0.01 | 110 | 0.00027 |
| Leucine | 0.01 | 27 | 0.0011 |
| Isoleucine | 0.01 | 27 | 0.0011 |

### 6.2.3. Preliminary Results

#### 6.2.3.1. LVI-HILIC-MS/MS method

I finished optimization and development of a simple and fast LVI-HILIC-MS/MS method based on preliminary experiments done in the group. **Figure 6-2** shows the extracted ion chromatogram (EIC) of each amino acid and its two MRM transitions at 1 ppb. The concentration of 1 ppb was chosen based on a previous report stating that odour-causing chemicals in water were detected at an odour threshold of low ppb levels. Additionally, the odour-causing chemicals reported in the study were proposed to arise from amino acids during the water treatment process.[19] The HILIC-MS/MS method achieved limits of detection (LOD defined as S/N=3) in the range of 0.00017–0.75 ppb for the amino acids listed in **Table 6-4**, excluding cysteine. Under the optimized conditions, cysteine was undetectable at all standards run (up to 5 ppb). As discussed in **Section 6.2.2.4.1**, cysteine was better detected at lower ionization temperatures, but this resulted in a loss of sensitivity for other amino acids. Thus, we focused on the majority of amino acids.




134.3 → 88.1
134.3 → 74.0
Aspartic Acid
Cysteine
148.8 → 47.0
148.8 → 77.0
Glutamic Acid
Glutamine
147.0 → 67.1
147.0 → 119.0
Glycine
76.0 → 30.1
76.0 → 59.2
Histidine
156.0 → 109.8
156.0 → 83.0
Isoleucine
132.8 → 87.3
132.8 → 45.0
Leucine
132.8 → 57.1
132.8 → 90.9
Lysine
147.1 → 84.0
147.1 → 90.4
Methionine
150.3 → 61.0
150.3 → 56.0
Phenylalanine
166.4 → 120.4
166.4 → 103.0
Proline
116.4 → 70.0
116.4 → 43.0

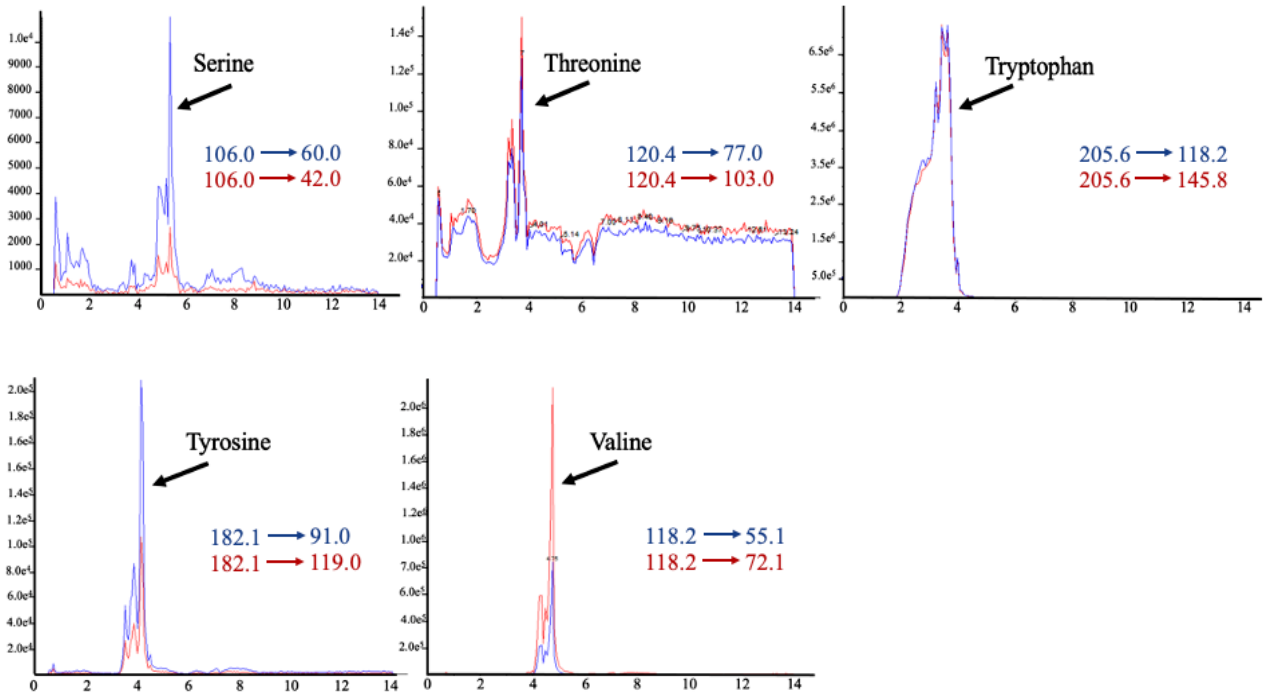



**Figure 6-2**. **LVI-HILIC-MS/MS chromatogram of amino acids at 1 ppb.** Individual EIC of each amino acid are shown. Each amino acid has two MRM transitions, labelled in blue and red with their corresponding MRM transition.

**Figure 6-2** shows some peaks other than those specific to the individual amino acid. The correct peak for each amino acid was identified based on the increase in peak intensity with a correlating increase in concentration of the specific amino acid. The noise and other peaks present could be due to several potential factors. These could include some degradation of amino acids, interference of other amino acids having close m/z values, and/or other impurities present. The mass resolution of the 5500 quadrupole is 1 Da. Thus some interference from other compounds can occur, especially when working at the lower range of m/z where many small molecules may interfere with the detection of the target compound. Additionally, the large

volume injection may cause some complexities in retention in HILIC, which will be discussed in further detail below. Further optimization will be necessary for future studies.

Because this was an exploratory study, I continued to test the performance of the LVI-HILIC-MS/MS method for rapid analysis of amino acids in authentic water samples collected during the 2020 spring run-off. **Figure 6-3** shows the levels of the amino acids detected by the LVI-HILIC-MS/MS method in each sample identified by its collection date. The more prevalent aminos acids are shown on the left while less frequently detected, aminos acids are on the right. During sampling from March 17 to May 4, a total of 15 amino acids were detected. The most prevalent amino acids were serine, alanine, histidine, and valine, which were at higher levels throughout the entire sampling period. This preliminary study showed the fast and simple method is promising for monitoring amino acids in authentic water samples during spring run-off. However, while the method was able to detect amino acids, I encountered multiple complications caused by the large volume injection, including the operation of the HILIC separation and mass spectrometer, and the limitations of this method during sample analysis. For example, authentic water samples did suffer from poor peak shape, retention time shifts, and large standard deviations (average standard deviation was between 10-20 ppt). I will discuss the limitations and future improvements needed for the LVI-HILIC-MS/MS method in **Section 6.2.5**, after comparing it to performance of the SPE-RPLC-MS/MS method for the same samples.

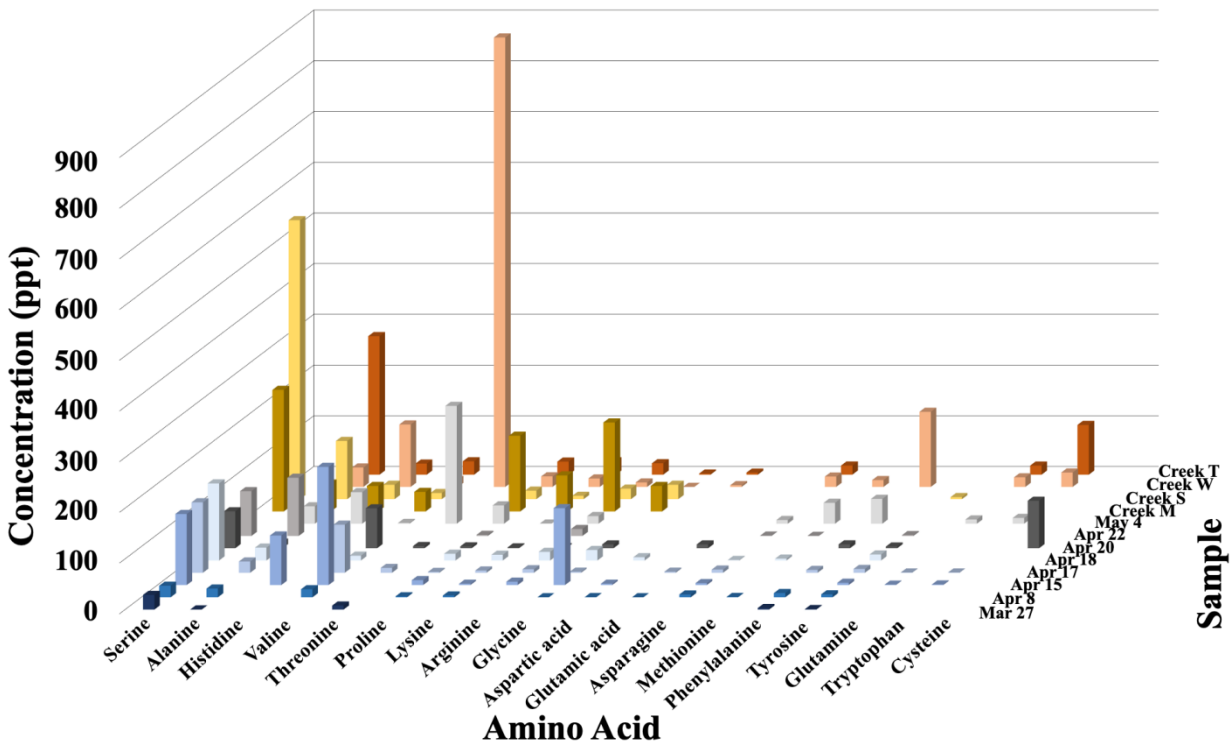
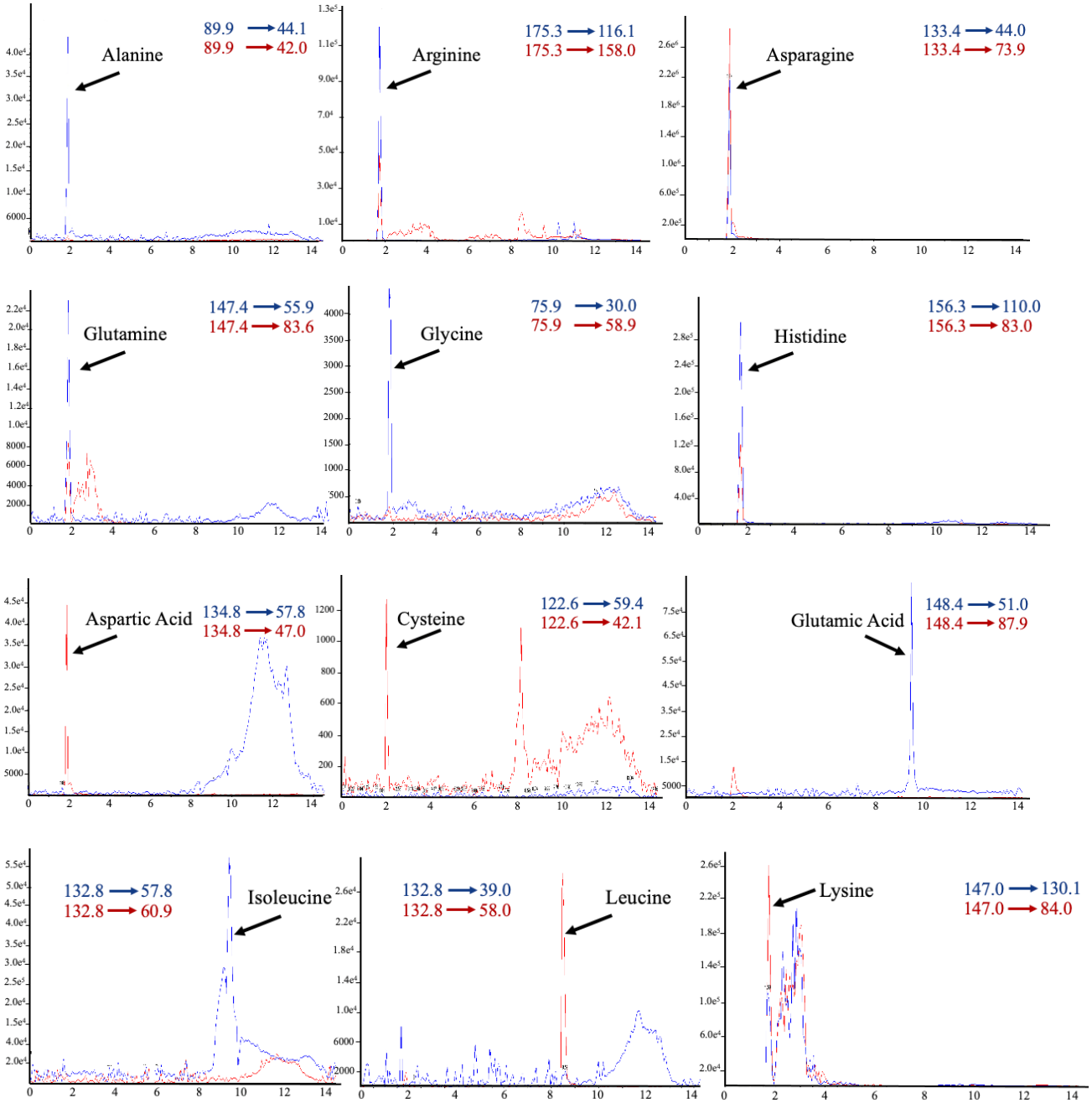



**Figure 6-3**. **Amino acids detected in authentic water samples collected during the 2020 Spring run-off using LVI-HILIC-MS/MS.**

### 6.2.3.2. SPE-RPLC-MS/MS

For comparison with LVI-MS/MS method, I quickly finished development of a SPE-RPLC-MS/MS method for analysis of amino acids in the same samples collected during the 2020 spring run-off. **Figure 6-4** shows the EIC for 1 ppb of each amino acid and its two MRM transitions for the SPE-RPLC-MS/MS method. The method achieved LOD (S/N=3) in the range of 0.00004-0.19 ppb. However, this method was unable to separate most of the 20 amino acids by chromatography, but was resolved by m/z. The method cannot differentiate the isomeric structures of leucine and isoleucine by chromatography and mass difference. Isoleucine and leucine provided poor peak shape and could not be determined in authentic water samples. **Figure 6-4** shows some chemical noise, or extra peaks, as seen in the HILIC separation in

**Figure 6-2.** As discussed in **Section 6.2.3**, the correct peak for each amino acid was identified based on the increase in peak intensity with a correlating increase in the concentration of the amino acid and matched. These extra peaks could be due to some degradation of amino acids, interference from other amino acids having close m/z values, and/or other impurities present.



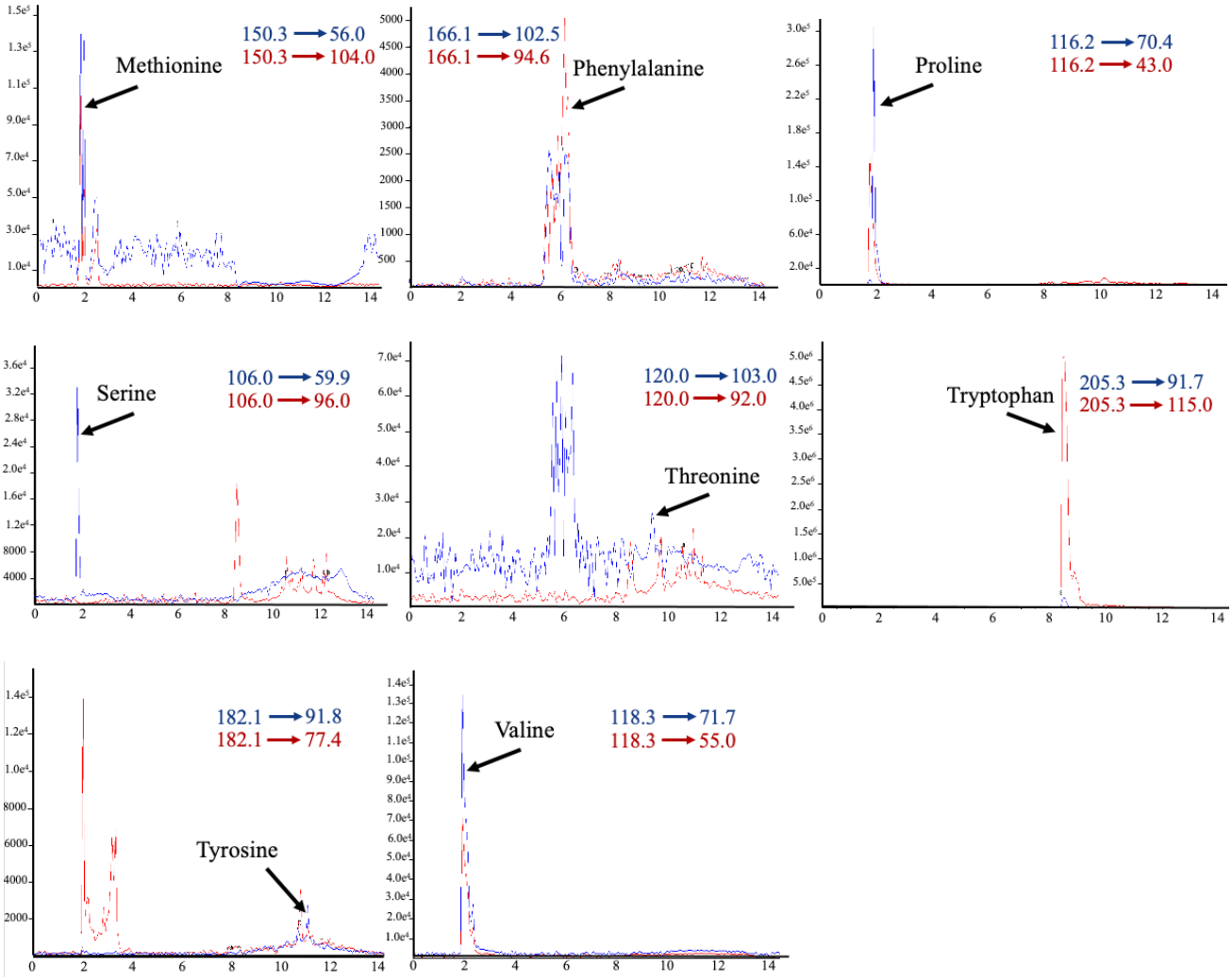



**Figure 6-4**. **SPE-RPLC-MS/MS chromatograms of the amino acids at 1 ppb.** Individual XIC of each amino acid are shown. Each amino acid has two MRM transitions, labelled in blue and red with their corresponding MRM transition.

RPLC-MS/MS methods used for water analysis, such as analysis of DBPs and potential precursors of DBPs, often require SPE.[20, 34, 35] SPE concentrates trace compounds in water prior to RPLC-MS/MS for separation and detection. Thus, SPE was also used in this method to preconcentrate the amino acids in the spring 2020 water run-off samples (in this preliminary work it was concentrated ~250 fold). The SPE-RPLC-MS/MS method detected 10 amino acids

in the samples collected across the sampling period, as shown in **Figure 6-5.** Little to no change in peak shape and retention time was shown. The RPLC method provided smaller standard deviations than HILIC **(**average standard deviation was between 0.01-4.6 ppt). The most prevalent amino acids detected using the RPLC method were threonine, tyrosine, phenylalanine, and glutamic acid. These amino acids were detected at higher levels in all samples collected during the sampling period. This method detected different amino acids compared to the HILIC-MS/MS method. The detection of different amino acids is likely due to SPE enrichment that favors compounds preferential to its sorbent. In this preliminary work, strong cation exchange was the basis for SPE extraction, thus strong cationic amino acids were favored. I will further discuss the limitations and differences between the two methods, and future work needed in **Section 6.2.5**.

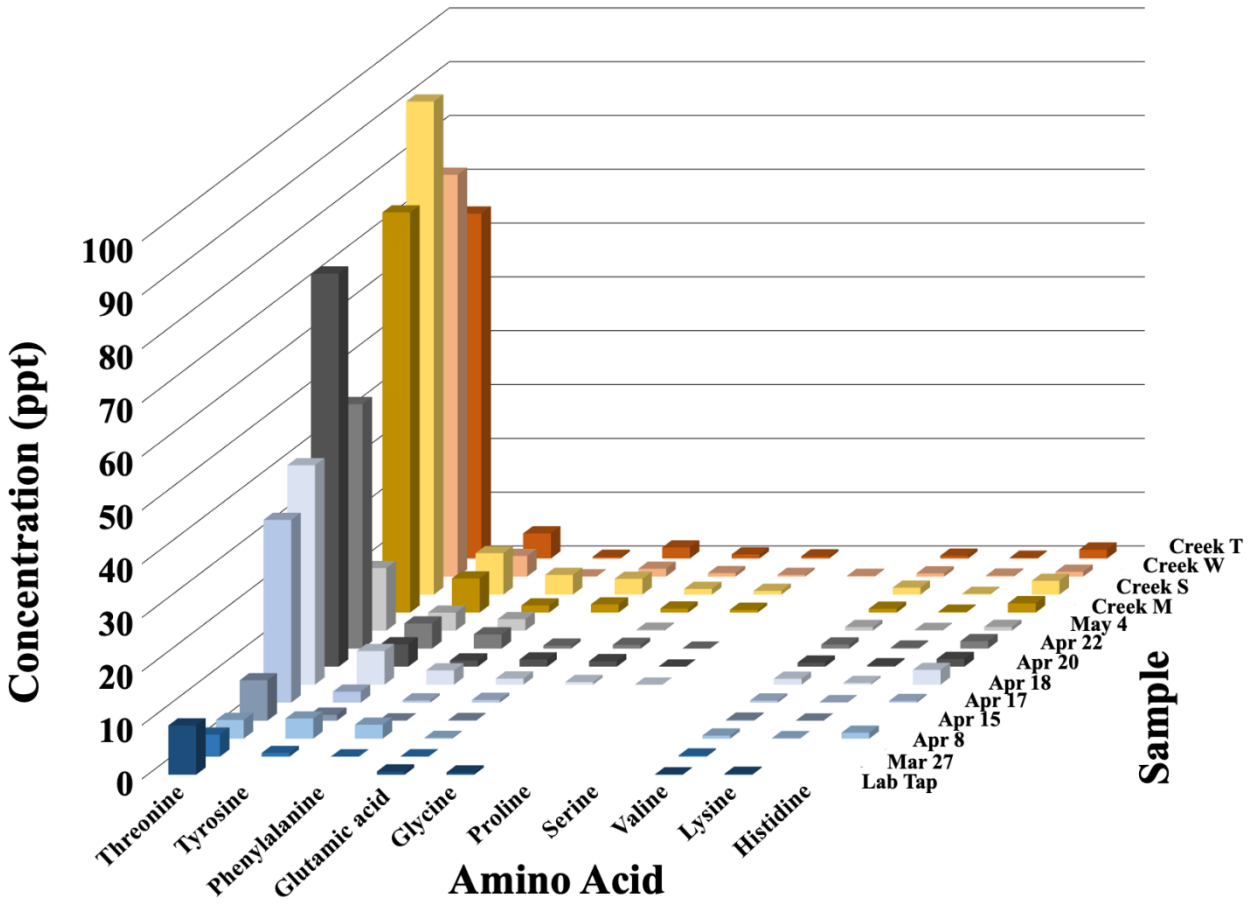



**Figure 6-5. Amino acids detected in authentic water samples collected during the 2020 Spring run-off using the SPE-RPLC-MS/MS method.**

### 6.2.4. Evaluation of the LVI-HILIC-MS/MS and SPE-RPLC-MS/MS methods

In this preliminary work, I finished optimization and development of a simple and fast LVI-HILIC-MS/MS method based on preliminary experiments done in the group to determine amino acids in source water. In addition, I quickly finished development of an SPE-RPLC-MS/MS method, which is the common approach used in water research. Each method was able to detect several amino acids in authentic water samples. However, each method had its own benefits and limitations which are discussed in terms of separation, matrix effects, column lifetime, effects on the MS performance, and overall time and cost of the method. HILIC retains

small and polar molecules, and was able to separate amino acids with gradient elution. Comparatively, RPLC provided little to no retention of the majority of the amino acids. Of the 20 amino acids, 12 eluted at the dead time of the RPLC separation. The remaining amino acids eluted later in the separation. The RPLC-MS/MS method relied on the mass selectivity to detect the majority of amino acids in this preliminary study. Derivatization of the amino acids or different columns could improve the separation.

Matrix effects also had a large role in the performance of the methods for analysis of authentic water samples. The HILIC method suffered from retention time shifts and poor peak shape. This is likely due to several factors: 1) one of HILICs major modes of retention is based on the aqueous water layer formed on the surface of the stationary phase (**Section 1.4.5**), thus changes to the amount of water in the column at a given time can significantly alter the separation, thus the large volume injections could cause retention shifts and can cause peak distortion; 2) large volume injections of 100% water can also cause shifts in retention time and affect peak shape and resolution by injecting sample in an eluent stronger than that of the mobile phase; 3) HILIC separation is complex and relies on more than one mode of separation (**Section 1.4.5.4**). The large volume injections may also contain compounds that interact with the other modes of separation, such as ions to affect ionic interactions, causing retention time shifts and potential changes in peak shape. Thus, it is possible that matrix effects could be more pronounced in HILICs complex retention mechanism with the large volume injections. HILIC suffered from large standard deviations of triplicate measurements of authentic water samples (average standard deviation was between 10-20 ppt). Comparatively, RPLC exhibited little to no change in both peak shape and retention with smaller standard deviations (average standard deviation was between 0.01-4.6 ppt). **Figure 6-6** shows the same water sample analyzed by both

the HILIC and RPLC method. The HILIC method exhibits a large background and broad peaks (**Figure 6-6 A**). In contrast, the RPLC separation in **Figure 6-6 B** has much a smaller background and better peak shape than the HILIC method.

Additionally, there was a difference in the column lifetime between the HILIC and RPLC method. The HILIC method had a significant decrease in the column lifetime, such that 3 columns were used over the course of the project (approximately 25 authentic samples on each). This could be due to the large volume injections of authentic water samples and the limited sample clean-up done in combination with HILICs lower column stability. Over time, complete loss in retention of the amino acids could be observed. New columns were required after retention was lost. We believe compounds in the column could permanently adsorb to the surface of the column, causing loss of retention. Loss of retention was not seen in the RPLC method, where only one column was needed, which could be due in combination to the greater stability of RPLC separations and the SPE clean-up of samples.

Finally, there is a difference in the cost and time required for each method. SPE is more time consuming and costly than LVI. This is because SPE requires a cartridge for each sample run, as well as lengthy extraction times (a 250 mL sample can take 4-6 hours). However, SPE does provide some sample clean-up which can help limit matrix effects and maintain the columns lifetime.

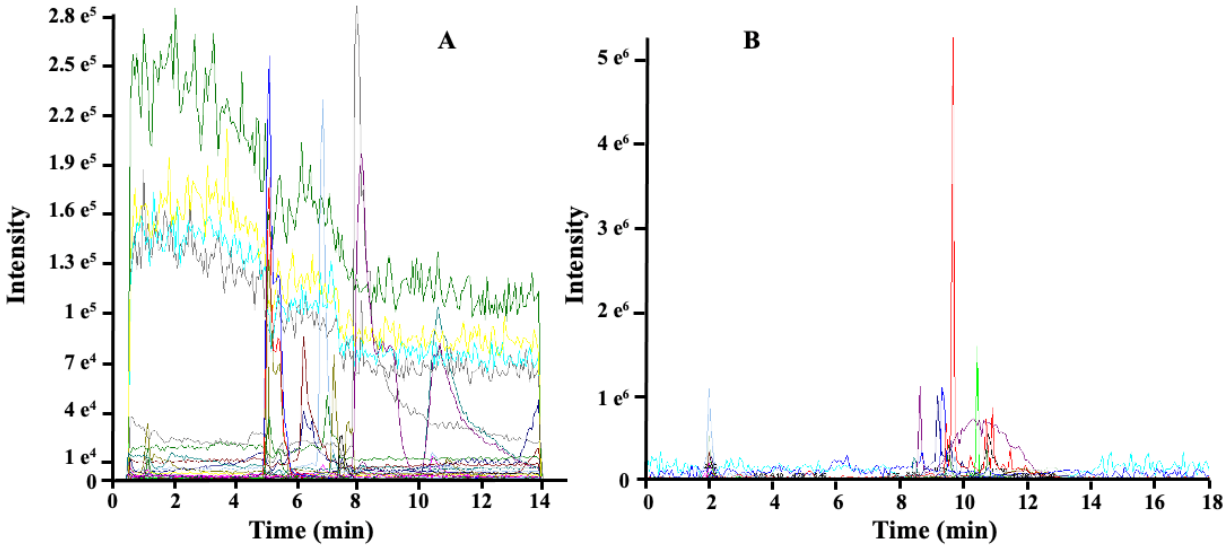



**Figure 6-6. Chromatograms of the same creek water sample collected in city 1, Creek W.** A) Separation of sample with 0.05 ppb standard addition final concentration by LVI-HILIC-MS/MS method, B) Separation by SPE-RPLC-MS/MS method.

## 6.3. Future work

This study showed the feasibility of coupling HILIC with tandem MS for the determination of polar analytes such as amino acids in water. It demonstrated the need for further development of the HILIC-MS method for rapid analysis of water samples. Future development should focus on how to reduce matrix effects, improve the separation of polar analytes, and extend the column lifetime. For example, the cation exchange SPE preconcentration may be used to concentrate trace analytes while reducing matrix effects in not only RPLC but HILIC as well. Combining SPE with HILIC may improve the reproducibility and LOD of the HILIC-MS/MS method, although it will increase analysis time and cost. Additionally, SPE can reduce/eliminate matrix effects on the separation and MS detection, which will increase the column lifetime and reduce damage to the MS ion source.

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*"For me, becoming isn't about arriving somewhere or achieving a certain aim. I see it instead as forward motion, a means of evolving, a way to reach continuously toward a better self. The journey doesn't end."*

***— Michelle Obama, Becoming***

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