Catalogue of Analytical Methods for Naphthenic Acids Related to Oil Sands Operations

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Oil Sands Research and Information Network

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- **Industry** with ready access to an integrated view of research that will help them make and execute reclamation plans – a view that crosses disciplines and organizational boundaries

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DISCLAIMER

The mention of names of individual instruments and/or methodologies is not to be taken as an endorsement of the instrument or technology by OSRIN, the University of Alberta, the Northern Alberta Institute of Technology, or Alberta Environment and Sustainable Resource Development.

REPORT SUMMARY

The purpose of this report is to identify challenges in analyzing naphthenic acids (NAs) associated with oil sands process water (OSPW). Naphthenic acids are present naturally in oil sands bitumen and have the classical formula $C_nH_{2n+Z}O_2$. Within this formula n represents the carbon number and Z is an even, negative integer corresponding to hydrogen deficiency mainly due to ring formation in the structure. Thus the absolute value of Z divided by 2 gives the number of the rings in the compounds. A Z-value of 0 means acyclic acids, which are believed to be highly branched rather than linear natural fatty acids. A Z-value of -2 represents monocyclic NAs; -4 represents bicyclic and so on. The Z-value may also include unsaturation in the chemical structure. The generality of the formula allows for a vast array of isomers for each value of n and Z. The challenge in analyzing NAs from OSPW is that microbial activity alters the structure of classical naphthenic acids creating a large number of compounds that are labeled as naphthenic acids but differ from the $C_nH_{2n+Z}O_2$ general formula. This increased number of compounds elevates the demands on the analytical methods used to characterize these compounds obtained from OSPW. In this report, issues affecting both qualitative and quantitative data from a variety of analytical methods will be reviewed to generate an awareness of the challenges faced by laboratories conducting NA determinations. The report also highlights the issues of naming these compounds "naphthenic acids" since many of the compounds being extracted from OSPW do not conform to the classical NA formula.

The method chosen has a significant effect on the interpretation of the analytical data. Analytical results are dependent on sampling, extraction and clean-up techniques. The report examines various approaches used to prepare samples for analysis based on the following themes: sampling tools and techniques, sample preservation and transport, extraction, and clean-up methods. There are numerous analytical instruments currently being used in the analysis of NAs. Within the field of spectroscopy Fourier Transform Infrared Spectroscopy (FTIR) has been used and is often considered the reference method for quantitative assessment of NAs in OSPW. Both, UV-Vis and fluorescence spectroscopy, and more recently Synchronous Fluorescence Spectroscopy (SFS) have been applied to studies of NAs in OSPW. Each of these methods are limited in the information that can be provided, however, they have value in assessing the types and possible sources of NAs being evaluated in a sample.

Major advancements in the analysis of NAs are being accomplished using the power of chromatography to attain a partial separation of thousands of compounds found in a NA extract and mass spectrometry (MS) for their detection. Early methods of analysis using unit mass resolution MS have created problems in properly assessing NAs present in OSPW. This has led to the overestimation of NA concentrations in OSPW. Similar problems have been encountered with FTIR. Misclassification and identification of false positives has been another issue plaguing early adopters of these analytical methods. Fortunately, new analytical tools are being developed which enable high resolution mass spectrometry (HRMS) to be performed enabling these errors in classification to be partially rectified. Although many efforts have been made in the development of analytical methods, no rugged routine method that can separate, identify, and quantify the individual components of NA mixtures has been achieved to date. This review will

provide an overview of methods currently used for the analysis of NA class of compounds including sampling, sample preservation, sample transport, extraction and clean-up, analytical techniques, and future needs, with a major focus on NAs from OSPW.

No method currently exists that is capable of identifying all isomers of NAs. Without this capability it is impossible to clearly assess the toxicity of individual "naphthenic acids" encountered in OSPW. Additionally it makes it difficult to fully understand the potential for biodegradation and remediation of NAs in fluid tailings or their long term impacts in the reclaimed landscape.

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We are most grateful to the laboratories that participated in the survey and provided information that was anonymously incorporated into the survey results.

1 INTRODUCTION

As conventional oil sources become depleted, much attention has been directed to non-conventional and heavy oils, such as oil sands[1]. The presence of oil sands in northeastern Alberta has been documented since the 1780s. It is estimated that over 1.7 trillion barrels of crude bitumen are contained in the Athabasca Basin, making it the third largest deposit of crude oil (MacLean 1998) and likely the largest accumulation of biodegraded oil in the world (Hunt 1979). In 2010, Alberta produced about 1.6 million barrels per day (bbl/d) of total crude bitumen, of which 1,468,900 bbl/d was marketable oil sands production, consisting of bitumen and synthetic crude oil. This represented about 72% of Alberta's and 52% of Canada's total crude oil and equivalent production (Energy Resources Conservation Board 2010).

Currently, there are two process technologies to recover bitumen from the deposit. The first is an in-situ technology to recover bitumen that is too deep to mine; currently this is accomplished through a number of technologies including steam assisted gravity drainage (SAGD), cyclic steam simulation (CSS), steam flood, in-situ combustion and cold heavy oil production with sand (ChOPS), etc. SAGD is currently prevalent. It involves the drilling of parallel horizontal wells into the oil sands deposit. Steam is injected into the upper horizontal well and released into the deposit to reduce the viscosity of bitumen. The fluid bitumen drains to the lower horizontal well by gravity where the bitumen and condensed water emulsion are collected and piped to the processing plant for further separation and treatment (Butler 2001). The majority of the water recovered from the SAGD process is recycled to generate new steam.

The second technology is surface or open pit mining of the raw ore in the shallow oil sand deposits. With a depth restriction of 75 m, mining operations can account for only 18% of the total recoverable bitumen (Alberta Department of Energy 2005). Bitumen extraction from mining operations uses modified versions of the Clark Hot Water Flotation and Extraction Process. In this process, oil sands are mixed with caustic hot water at 40 to 85°C allowing the bitumen to be separated from the matrix as a froth. Using this extraction method, about 80% to 90% of the bitumen can be recovered depending on ore quality, and the technology applied. Every 1 m^3 of mined oil sand requires approximately 3 m^3 of water and produces on average 4 m^3 of waste (Clemente et al. 2003). The tailings slurry consists mainly of solids (sand and clays), pore water, recycled water, organics, process additives and residual bitumen. Current practice by the oil sands companies is to release extraction wastes to external tailing ponds (Fine Tailings Fundamentals Consortium 1995b). Consequently, all process-affected waters and fluid tailings are contained on-site for an indeterminate time until they are successfully remediated and reclaimed. As an example, the volume of impounded process water at Syncrude's Lease 17/22 was approaching 1 billion m^3 in 2004 and the industry-wide tailings volumes will continue to increase due to the rapid expansion of the oil sands industry (Allen 2008). One of the major reclamation plans for fine tailings is a wet landscape approach (Gulley and MacKinnon 1993,

[1] Oil sands comprise quartz sands, silt, clay, organics and pore water, with bitumen trapped between the pore spaces of the sand (Fine Tailings Fundamentals Consortium 1995a, National Energy Board 2000)

List and Lord 1997). With this approach, the fine tailings would be transferred into an abandoned mined-out pit, over which a layer of water would be placed, establishing a water-cap over the fine tailings base, thus creating end pit lakes (Boerger et al. 1992).

Oil sand process water (OSPW) is mildly caustic and saline. It contains organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) and a complex mixture of naturally occurring aliphatic and alicyclic carboxylic acids known as naphthenic acids (NAs). Concentration of NAs in fresh OSPW normally exceeds 50 mg L^{-1} and may reach up to 120 mg L^{-1} in tailings ponds. The toxicity of fluid tailings has largely been attributed to NAs (Scott et al. 2005). However, other contaminants such as BTEX, ammonia and polyphenols can also exhibit toxic effects (Allen 2008). Though it is well known that NAs experience biodegradation in tailings pond to some extent, decades of storage in tailings ponds under various conditions have not proven effective at decreasing NAs to below 20 mg L^{-1} (Quagraine et al. 2005). Owing to the inherent persistence of NAs, complete remediation of OSPW has yet to be achieved.

The heightened interest in developing methods for the analysis of NAs arises from several sources. For example, the composition of NAs is helpful in identification of oil source maturation (Headley et al. 2002b, Meredith et al. 2000) and in fingerprinting fuel spills in the environment (Rostad and Hostettler 2007). The overall chemical and physical properties of extracted NAs may also vary between tailings ponds (Clemente et al. 2003a, Headley and McMartin 2004) which can lead to forensic applications. NAs and naphthenate salts can also be found in applications such as wood preservation (Brient 1998), defoaming in jet fuels, and as flame retardants in fabric (Brient et al. 1995). NAs are also known for their corrosive effects on oil refinery facilities (Kane and Cayard 1999). Additionally, the need to assess their toxicity to a variety of organisms (Clemente and Fedorak 2005), including their fate, transport, and degradation has heightened the need to determine NA concentrations in water, soil, sediment, plants and aquatic life at levels appropriate for regulatory and monitoring purposes. Improved characterization of the components of NAs is necessary since the corrosive and toxic effects are often structure-specific. Thus a more complete characterization of the class of compounds known as NAs will aid in the development of treatment methods for the detoxification of OSPW.

However, the myriad of different NAs present in OSPW presents a tremendous challenge in terms of both characterization and quantification. Although many efforts have been made in the development of analytical methods, no rugged routine method that can separate, identify, and quantify the individual components of NA mixtures has been achieved to date.

This review will provide an overview of methods used for the analysis of NA class of compounds including sampling, sample preservation, sample transport, extraction and clean-up, analytical techniques, and future needs with a major focus on NAs from OSPW. NAs extracted from petroleum, heavy oils or bitumen are different from those extracted from OSPW because they haven't experienced biodegradation. The review mainly focuses on the analysis and characterization of NAs found in the environment. The ultimate goals of this review are:

- to share information that will provide a common platform needed in research and development of suitable methods for the analysis of NAs,

- to facilitate future discussion related to the challenges in developing and achieving regional reclamation targets, and
- to inform policy and regulation.

2 NAPHTHENIC ACIDS OVERVIEW

2.1 Chemistry

IUPAC (International Union of Pure and Applied Chemistry) defines NAs as *acids, chiefly monocarboxylic, derived from naphthenes*. Naphthenes are primarily cycloalkanes especially cyclopentane, cyclohexane and their alkyl derivatives (McNaught and Wilkinson 1997). This definition recognizes NAs as a family of carboxylic acid surfactants composed predominantly of alkyl-substituted cycloaliphatic carboxylic acids with smaller amounts of acyclic aliphatic (paraffinic or fatty) acids. The rings may be fused or bridged. The carboxyl group is usually bonded or attached to a side chain rather than directly to a cycloaliphatic ring (Dzidic et al. 1988, Fan 1991). It is widely accepted that the complex compounds are represented by the general formula $C_nH_{2n+Z}O_2$ (Dzidic et al. 1988, Fan 1991), where n represents the carbon number and Z is an even, negative integer corresponding to hydrogen deficiency mainly due to ring formation in the structure. Thus the absolute value of Z divided by 2 gives the number of the rings in the compounds. A Z-value of 0 means acyclic acids, which are believed to be highly branched (Rudzinski et al. 2002) rather than linear natural fatty acids. A Z-value of -2 represents monocyclic or mono-unsaturated NAs; -4 represents bicyclic and so on. The Z-value may also include unsaturation in the chemical structure. The generality of the formula allows for a vast array of isomers for each value of n and Z. Figure 1 shows structural examples of what has been termed “classical NAs” by Grewer et al. (2010).

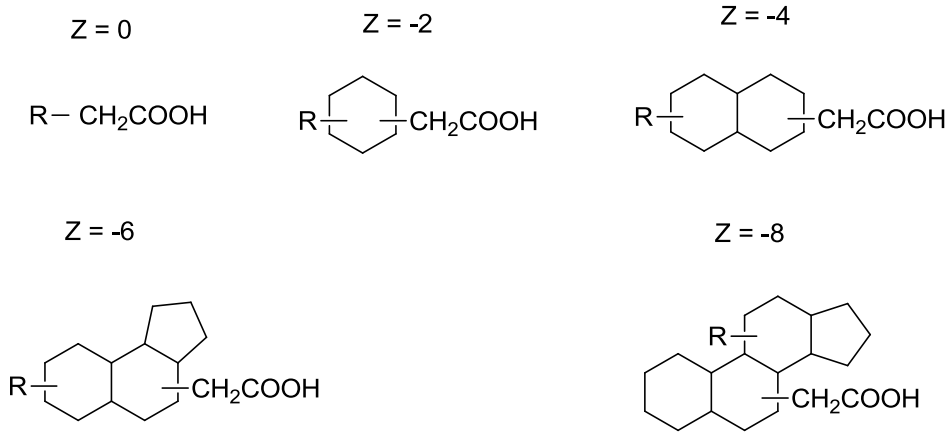


Figure 1. Examples of the classical structures of NAs.

Unfortunately the classical definition of NAs by IUPAC, is obsolete and obscure, when it relates to OSPW. Although most analyses and characterizations still focus on the classical NAs, it has been observed that >50% of the compounds in the extracts of OSPW are not classical NAs (Grewer et al. 2010). Thus the definition of "NAs" is in fact under hot debate.

For example, "NAs" extracted from OSPW often contain more than two oxygen atoms clearly showing deviation from the classical NA formula. Hydroxyl groups and more than one carboxylic group are examples of structures that contribute to the increased oxygen content in the "NA" extracts. Thus the formula $C_nH_{2n+Z}O_x$ where x= 2 to 5 has been found in NA mixtures from the oil sands area and OSPW (Barrow et al. 2009, Grewer et al. 2010, Han et al. 2009). In the responses to an NA Analysis Survey (section 6) of industry, conducted by NAIT, some indicated that the values for "x" in the NA formula could be as high as "7" showing a considerable deviation from the classical NA formula. Grewer et al. (2010) suggested that the term "naphthenic acids", which has been used for more than 25 years, should be replaced with the term "oil sands tailings water acid-extractable organics (OSTWAEO)". Classical and "oxy-NAs" as suggested by Lee (1940) when oxygen exceeds more than x=2 are components of OSTWAEO. Grewer et al. (2010) believe this term "OSTWAEO" will not be as misleading as the currently used term, "naphthenic acids".

Further deviations from the classical NA formula exist due to the presence of other heteroatoms such as sulphur and nitrogen in the acid extractable organics found in OSPW (Grewer et al. 2010, Headley et al. 2009a). NAIT's industry survey results suggest multiple sulphur and nitrogen atoms exist including variation in the number of oxygen atoms associated with these heteroatoms.

The acid extractable organics from OSPW also contain compounds showing aromaticity. These are considered to be aromatic acids (Kavanagh et al. 2009). In their responses to NAIT's survey some labs also indicated that polyaromatic hydrocarbons were also being detected in the extracts.

NAs common to OSPW are chemically stable and non-volatile compounds (Clemente and Fedorak 2004). As expected the polarity and non-volatility changes with molecular weight causing the NAs to exhibit a variety of chemical and physical properties (Brient et al. 1995, Clemente et al. 2003a, CONRAD Environmental Aquatics Technical Advisory Group 1998, Headley et al. 2002a, Herman et al. 1993).

2.2 Sources

2.2.1 *Raw Ore and Crude Oils*

NAs are present naturally in crude oils (Seifert and Teeter 1969, Tissot and Welte 1978). They comprise part of the petroleum acids whose concentration varies from undetectable to 3% by weight depending on the source of oil (Lochte and Litman 1955). Typically, oil sands crude oils contain NAs up to 4% by weight (Barrow et al. 2010). Also, the concentrations and the composition of the NAs vary with oil sands ore (Clemente et al. 2003a). For example, the NA

concentration in oil sands raw ore samples from Syncrude Canada Ltd. was about 200 mg per kg of ore (Syncrude Canada Ltd. 2000).

2.2.2 *Aqueous Presence*

NAs are useful in the extraction of bitumen from the oil sands because they are natural surfactants released during the Clark Hot Water Process that encourage bitumen liberation from the sand grains (Masliyah et al. 2004). Under current practice, oil sands operators store all the process waters and tailings on site. NAs separated from bitumen during the extraction process dissolve in alkaline solution and accumulate with other waste products in the fluid tailings ponds. The concentration of NAs in fluid tailings ranges from 20 to 120 mg L^{-1} (Holowenko et al. 2002). The high concentration of NAs in the oil sands tailings ponds pose a major concern, since the OSPW will ultimately need to be discharged to the environment.

NAs are also present in surface water and groundwater. They are found to occur naturally in some surface waters that are in contact with the oil sands deposits in northeastern Alberta. The concentrations of NAs in surface water taken at various locations along the Athabasca River were in the range of 0.1 to 0.9 mg L^{-1} (Schramm et al. 2000). Near-surface aquifer water has been found to contain 2 to 5 mg L^{-1} NAs, which reflect natural contact with oil sands (CONRAD Environmental Aquatics Technical Advisory Group 1998). NAs have also been found in natural groundwater with concentrations <4 mg L^{-1} and in basal and limestone aquifers at concentrations >55 mg L^{-1} (CONRAD Environmental Aquatics Technical Advisory Group 1998).

2.2.3 *Coal*

Scott et al. (2009) have suggested that a potential source of NAs in groundwater is coal. In their study, water from two domestic wells near coal deposits was extracted and analyzed by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (EI-FTICR-MS). The results unequivocally confirmed the presence of classical NAs with two oxygen atoms and other organic acids containing three, four, and five oxygen atoms. The reported NA concentrations using FTIR in these two wells were 1 mg L^{-1} and 0.3 mg L^{-1}, respectively. Furthermore, leachates from distilled water percolated through three different crushed coals were shown to contain various organic acids, including NAs with concentrations reported at 0.7, 0.2 and 0.4 mg L^{-1}.

2.3 Environmental Issues

NAs are toxic to aquatic algae and other micro-organisms. NA molecules possess hydrophilic and hydrophobic functional groups which allow these molecules to penetrate into cell membranes and disrupt cellular function, eventually resulting in cell death (Frank et al. 2008). NAs in fresh fluid tailings can cause an acutely toxic effect (MacKinnon and Boerger 1986, United States Environmental Protection Agency 1984) to aquatic organisms (LC50 <10% v/v for rainbow trout) and to mammals (oral LC50 =3.0g/kg body weight). Studies referenced by Herman et al. (1994) show that acute toxicity of OSPW by natural processes is reduced within one year while the removal of chronic toxicity requires 2 to 3 years. More recent studies

(MacKinnon 2004) show that the degradation of NAs in isolated tailings pond water occur at a rate of 16% per year over the first 5 years (from 130 to 24 mg L^{-1}), but further degradation of NAs beyond 5 years become negligible. However, the toxicity using Microtox® IC20 was shown to decline, reaching 100 vol% at 10 years. The degradation and detoxification rates have been shown by Han et al. (2009) to be related to structure. The most rapidly degraded NAs are the least cyclic (Z = 0 and Z = -2); whereas some of the more complex NAs can have half-lives in the order of 12.3 to 13.6 years. Thus, toxic effects do not relate to the NA concentration directly but are more a function of content and complexity of NAs (Brient et al. 1995, CONRAD Environmental Aquatics Technical Advisory Group 1998, Lai et al. 1996). Rogers et al. (2002b) state that the most toxic components in oil sands deposits and tailings pond water are NAs of low molecular weight generated during the bitumen extraction process. Unfortunately, it is not well established which specific NAs are the most toxic due mainly to the presence of hundreds of these compounds in oil sands. Although the acutely toxic fraction of NAs can degrade naturally in experimental pits and wetlands, the lengthy residence time required makes it impractical for a direct environmental discharge of water. Additionally, since NAs of high molecular weight are resistant to biodegradation, they can persist in reclaimed environments and pose a potential chronic toxicity risk.

2.4 Analytical Issues

Total acid number (TAN), using either potentiometric titration (ASTM D664) or color-indicator titration methods (ASTM D974), are reported in terms of mg KOH/g oil sample (Brient et al. 1995, Drews 1998, Slavcheva et al. 1999). TAN values are generally considered to be a measure of all acidic components, including NAs and sulphur compounds (Fuhr et al. 2007). TAN values have been used to assess the potential for corrosion problems in a crude oil refinery. Values of TAN in the range of 0.1 to 3.5 mg KOH/g are common but can be as high as 10 for particular hydrocarbon fractions. The basis for the increase in corrosive effects with crude oil TAN is thought to be due to the availability of the carboxylic acid group to form metal complexes.

Fuhr et al. (2007) modified the ASTM D664 method, which included alteration of sample handling procedures, and analyzed two bitumen samples recovered from a SAGD operation. They found that the TAN results using this altered procedure represented only the NA content. Although these results suggested a direct relationship to NA content, TAN cannot provide detailed information about the composition of the complex mixture of NAs and structures.

Many analytical methods have been developed to characterize NAs, however, all methods tend to be semi-quantitative, and lack the ability to identify individual isomers in the extract of OSPW. Ideally, a robust and accurate analytical method is needed to meet the major challenges indicated below:

- quantitation of the total concentration of NAs in a sample,
- characterization of the structures of the compounds in the complex, poorly defined mixtures obtained using various sampling protocols,

- determination of the concentration of each individual NA and other components found in the mixture obtained following a sampling protocol, and
- assessment of the toxicity of each of the components found in the extracts.

3 SAMPLING

3.1 Sampling Tools and Techniques

There is no standard technique or set of tools to sample OSPW from oil sand fluid tailings ponds. A common practice is to ensure there is no headspace in the container. For example, Rogers et al. (2002a) collected tailings water samples from the upper, clarified tailings zone (0 to 3 m depth from the water surface) of Syncrude's primary holding pond, Mildred Lake Settling Basin (MLSB) in 20 L carboys made of PVC. The carboy was filled to the brim to keep the air space to a minimum.

Holowenko et al. (2000) used sterile 4 L glass bottles to collect samples at various depths from three Syncrude Canada Ltd. lease ponds, over two summers, to study methanogenesis in the fine tailings zone. The bottle was plugged with a size 5 or 6 rubber stopper equipped with an eye hook screw attached to a string. When the bottle was lowered to the desired depth in the pond, the string was pulled to dislodge the rubber stopper, allowing tailings sample from that depth to fill into the bottle. Though the bottle could not be re-sealed under the water surface, it was assumed that the chance for tailings from other depths entering the bottle as it ascended to the surface was very low due to the small size of the bottle mouth.

NAIT's NAs Analysis Survey of labs involved in NA analysis contains additional information on sampling protocols (section 6). From the published literature, only grab sampling methods are reported. Grab samples enable an estimate of NA concentrations at a given point in time and location. The concentration can be assessed since the sample volumes containing the NAs are known. However, in the NAIT survey, integrated (passive) sampling is also reported by a few labs. One of the labs indicated integrated (passive) sampling from tailings pond, surface water and ground water with POCIS (Polar Organic Chemical Integrative Sampler) of the pharmaceutical type over a period of more than 28 days. The advantage of using an integrated sampling method over the grab sampling method is that levels and types of NAs can be assessed over the long term rather than at a specific moment in time. The primary purpose of POCIS is to measure biological uptake. Unfortunately, calculation of NA concentration is more difficult with integrated sampling, since it is hard to assess the volume of water that the levels of captured NAs reflect.

3.2 Tailings Sample Preservation and Transport

There is no defined protocol for tailings pond water sample transport and preservation in the literature. The recalcitrance of NAs makes preservation less vital than for samples of leached metals or volatile organic compounds (VOCs) in water.

A fairly universal practice is to store tailings samples or any extracts from these tailings samples at 4ºC (Bataineh et al. 2006, Frank et al. 2006, Han et al. 2009, Rogers et al. 2002a, Young et al. 2007). Other options include: storing the extracted NAs at -20ºC (Merlin et al. 2007); or even using a refrigerated truck (2 to 5ºC) during collection and transport of the tailings pond water samples to the laboratory, followed by storage at 4°C until use (Rogers et al. 2002a).

A variety of storage containers are used. Frank et al. (2006) stored extracted NAs in amber glass bottles to limit exposure to light, while Rogers et al. (2002a) stored extracts in the dark. Since refrigeration is usually in the dark it is likely that almost all sample storage is in the dark. Results of the NAIT survey revealed that both glass and plastic containers, clear and amber, are used. Only one lab uses stainless steel containers for storage. Low temperature and darkness are the most popular conditions for sample storage. Both glass and plastic containers have their advantages and disadvantages. Plastic containers may result in the leaching of phthalates into the sample (Rogers et al. 2002a). The contamination by phthalates can distort the FTIR spectral pattern. Adsorption is also an issue with plastic bottles and potentially with Teflon. Rogers et al. (2002a) strongly suggested non-plastic lab ware.

Rogers et al. (2002a) have shown that NAs are stable when stored at 4ºC. Analysis of the stored samples over a period of 0, 4, 8, and 10 months showed that the NA concentrations were not significantly different from the initial concentration.

3.3 Naphthenic Acid Extraction Methods

Sample preparation prior to analysis depends on the analytical method employed. It can include simple steps such as solid removal and dilution, or it can undergo a series of tedious extraction and clean-up procedures with or without surrogate or internal standard addition. Surrogates added before sample preparation may be used for both quantification and percent recovery for result correction. The surrogates are usually deuterated compounds when using mass spectrometry detection so that they can be easily differentiated from the actual NAs extracted from the sample.

The solubility of NAs in water is highly pH dependent and reflects the fact that the pK_a of NAs are between 5 and 6. The NAs precipitate out at pH lower than 2, have a solubility less than 50 mg L^{-1} at pH 7 in water (Headley and McMartin 2004) and dissolve readily at an alkaline pH. NAs completely dissolve in organic solvents, such as dichloromethane and ethyl acetate. These properties play a key role in liquid-liquid extraction and clean-up procedures. The most commonly used solvent for extraction is dichloromethane; however, alternative extraction solvents are used. Common clean-up procedures consist of removing suspended particulates in the samples and then removing undesired organic compounds such as humic-like organics and polycyclic aromatic hydrocarbons. The number of repetitive extractions performed using liquid-liquid extraction (LLE) and the ratio of solvent to sample are other parameters in the extraction procedure that are chosen but are not consistent between labs.

Janfada et al. (2006) found that sorption of NAs on soils could significantly affect the levels of NAs in water. Preferential sorption occurred with components in the carbon number range of approximately 13 to 17. Enriched organic soils appear to cause higher level of sorption. This

suggests that removal of particulates in the clean-up stage could cause a loss of NAs from the total sample collected.

Generally, liquid-liquid extraction of NAs includes the following five steps but not necessarily in the order listed below:

- solids removal,
- NAs extraction,
- undesired organic compounds removal,
- NAs recovery/reconstitution, and
- concentrate sample if needed.

Solid phase extraction is mostly used to extract and clean-up NAs from heavy oils or crude oils (de Campos et al. 2006, Jones et al. 2001). Recently, this technique has been applied to extract NAs from aqueous samples. Generally, solid phase extraction includes the following steps:

- activation of the resin or conditioning of the cartridge,
- adsorption of NAs, desorption of non-acids components, and
- recovery of the NAs from the cartridge and possibly a concentration step.

Several labs report that automation of solid phase extraction can greatly improve precision and accuracy of the analytical method.

3.3.1 *Liquid-Liquid Extraction Method (LLE)*

3.3.1.1 Extraction Method Developed by Syncrude

Although there is no specified standard method for extracting NAs from tailings samples, the extraction method developed by Syncrude in 1995 (Jivraj et al. 1995) is dominant. In the Syncrude extraction method, the tailings sample is centrifuged or filtered through a 0.45 μm Millipore filter to remove suspended solids. The filtrate is then acidified with H_2SO_4 to a pH of 2 to 2.5 to precipitate out the NAs. The precipitated organic acids are extracted with dichloromethane twice at a 1:2 solvent to water ratio. The dichloromethane extracts are combined together and the solvent is evaporated overnight to dryness. The residue is reconstituted in alkaline water and subjected to ultrafiltration to separate NAs (molecular weights of <1,000, in general) from other organic acids which tend to have higher molecular weights and can skew analytical results.

3.3.1.2 Bulk Extraction Method by Rogers et al. (2002a)

The Syncrude extraction method is best suited to procuring small amounts of NAs for analytical purposes. For bulk extraction, the centrifugation/filtration step in Syncrude extraction method is impractical for a large water sample. The Rogers et al. (2002a) bulk extraction method use gravity settling of the suspended solids from non-acidified tailings samples (3 days). The water

is decanted, acidified to pH 2.5, and then NAs are extracted with dichloromethane at a 1:2 solvent to water ratio. Rotary evaporation is used to recover and recycle the solvent. The organic extract was reconstituted using 0.1N sodium hydroxide (pH 13). The pH was then reduced to 10 to produce insoluble "organic acids" which were removed by filtration using a 0.45µm glass fiber microfilter. The filtrate was then subjected to a 1,000 MW cutoff ultrafiltration to help remove additional organic acids from NAs. The extraction efficiency of NAs was reported to be 85%. Although gravity settling does not provide complete clarification compared with centrifugation or filtration, the modified procedure is simpler and welcomed by other researchers (Barrow et al. 2010, Janfada et al. 2006) when dealing with a large sample size.

3.3.1.3 Bulk Extraction Method by Frank et al. (2006)

Frank et al. (2006) modified the extraction procedure to optimize the preparation of larger samples and to help purify NAs. The collected tailings water was first acidified with H_2SO_4 to pH 2 and allowed to gravity settle for up to 98 h. The overlaying water was siphoned off and centrifuged to further separate the precipitated acids from the supernatant. The organic acids were reconstituted into an alkaline solution using 0.1 N NaOH to pH 12 and centrifugation was applied again. To ensure that all the organic acids were dissolved in the alkaline solution, the settled solids were washed three times after centrifugation. Instead of undergoing ultrafiltration, the reconstituted organic solution was vacuum filtered by passing through a column filled with DEAE-cellulose to remove the majority of undesirable humic-like material. The color of the column eluate changed from dark-brown to golden-yellow. The eluate was further extracted with dichloromethane (three times) to remove neutral organic compounds like polycyclic aromatic hydrocarbons. Though the extraction efficiency was only about 41.2%, the replacement of ultrafiltration with DEAE-cellulose column reduced the clean-up time significantly.

3.3.1.4 Extraction Method Developed by Lo et al. (2003) and Grewer et al. (2010)

Lo. et al. (2003) and Grewer et al. (2010) increased the pH of the tailings sample to between 10.5 to 12 with NaOH as the first step and then removed suspended particulates by centrifugation. The supernatant was extracted with dichloromethane to remove basic and neutral organic compounds. The aqueous phase was then adjusted to pH 2 to precipitate NAs for organic extraction with dichloromethane. Finally, the dichloromethane extracts and the precipitate were combined and filtered through anhydrous Na_2SO_4 into a round-bottom flask and evaporated to dryness.

3.3.1.5 Extraction Method Developed by Bataineh et al. (2006)

Bataineh et al. (2006) adjusted the tailings water to pH 11 using 2M NaOH as the first step and centrifugation was applied to remove suspended materials. The supernatant was recovered by using H_2SO_4 to lower pH to <2 and was extracted three times with ethyl acetate containing 2% acetic acid by volume. The extracts were combined together and washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Rotary evaporation was applied to concentrate the

sample. The residue was transferred to a small vial in ethyl acetate and taken to dryness under a gentle stream of nitrogen.

3.3.2 *Solid Phase Extraction Method (SPE)*

Headley et al. (2002a) developed a fast and robust solid phase extraction procedure for surface water samples. The Isolute ENV+ (a divinyl benzene supported sorbent) cartridge was conditioned with methanol and water followed by the addition of the sample which had been acidified to pH of 3 using formic acid. After the addition of the sample the cartridge was rinsed with Milli-Q water and subsequently dried under vacuum. The sorbed NAs on the cartridge were eluted using acetonitrile and the extract evaporated using dry nitrogen gas.

Bataineh et al. (2006) centrifuged the samples for 20 min (15,000 g) and then adjusted the pH to 3 using formic acid. Solid phase extraction (Oasis HLB sorbent) cartridges were conditioned sequentially with ethyl acetate, methanol and 0.1% formic acid prior to the addition of the acidified sample to the cartridge at a rate of 2 mL/min. Distilled water was used to rinse off all the water solution and the cartridges were dried under vacuum. The NAs were eluted with ethyl acetate. The extract was then dried by evaporating ethyl acetate under nitrogen at 35°C.

Mediaas et al. (2003) reported a method developed for Statoil to selectively isolate carboxylic acids from crude oils, distillates and other organic solvents. A sugar-based QAE Sephadex A-25 ion exchange resin (acid-IER) was used. The hydrophilic acid-IER is more selective towards carboxylic acids than hydrophobic IER. The acid-IER exhibited excellent isolation efficiency and selectivity when used to recover carboxylic acids from crude oils and its distillates. Acid recovery from the distillates, the residue, and the crude oil are reported to be between 95 to 100 mol%.

4 ANALYTICAL METHODS

4.1 Common Issues Associated with the Analysis of Naphthenic Acids

4.1.1 *Choice of Calibration Standards*

Both qualitative and quantitative analysis procedures are ideally carried out using calibration standards which are identical to the components being analyzed. Unfortunately, when analyzing NAs, there are too many compounds for the analytical method to adequately separate individual components, even with the most selective chromatographic methods. Additionally, pure standards for many of the NA compounds cannot be obtained. For this reason all the analytical methods generate semi-quantitative data, since a true response factor for each analytical component in the extracts cannot be determined. The options used for calibration of detector response are outlined below.

4.1.1.1 Calibration Using a Commercial Mixture of Naphthenic Acids Standards

A common standard used for calibration is commercial mixtures of NAs (such as Merichem and Kodak). The commercial mixture of NAs is a relatively pure (4% to 8% impurities) source of

NAs. The NAs are recovered from petroleum distillates (between 200 and 370 °C) using caustic extraction, followed by an ethanol extraction to remove unsaponifiable material. This purified extract is then acidified to return the NAs to their protonated form (Brient et al. 1995). However, the commercial NAs may not be ideal calibration standards for quantifying NAs in OSPW. Grewer et al. (2010) have shown that using commercial NAs to represent real NAs from OSPW and fresh surface water (rivers and lakes) is oversimplified since the isomer groups represented by these reference materials differs from OSPW. They also noted that the composition of NAs differ among the suppliers of the commercial NA mixtures.

Firstly, elemental analysis of commercial NAs show a low content of the heteroatoms sulfur (S) and nitrogen (N) compared to NA extracts from OSPW. The concentrations of these elements also vary with the source of the commercial NAs. For example, Merichem and Acros NAs do not contain S and N while Kodak NAs contain both S and N.

Secondly, a different distribution of oxy-NA is found between commercial NAs and OSPW (Grewer et al. 2010). Electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) analysis showed that the commercial NAs are dominated by x=2 series; the classical NA structure ($C_nH_{2n+z}O_2$). A considerable variation in the type of oxy-NAs ($C_nH_{2n+z}O_x$) is also found between suppliers. For example, x=3, 4 and 5 series of oxy-NAs were detected in Merichem and Kodak samples, but Acros sample only had x=4 series of NAs. Unlike commercial NAs, OSPW and fresh water samples have a greater quantity of the oxy-NAs (x=3, 4 and 5) than the x=2 NAs.

Thirdly, commercial NAs and the acids extracted from tailings water show different distributions of the relative abundances of $C_nH_{2n+z}O_2$ compounds for various Z values. For example, Merichem NAs have an even distribution of the Z=0 to -4 NAs; Kodak NAs have Z=-2 and -4 as the most abundant acids; and Acros NAs showed acids with Z=0. NAs extracted from tailings pond water from Syncrude and Suncor show a relatively low content of Z=0 acids in with more acids in Z=-2 to -6, while acids extracted from fresh water samples showed acids with Z=0.

All these findings suggest that commercial NAs may not represent the complexity of real NAs extracted from fresh water and OSPW samples. Also the variation shown in commercial NAs provided by different suppliers cannot be ignored. Logically, the choice of commercial NAs will influence the estimation of NAs concentrations in the test sample (Scott et al. 2005).

4.1.1.2 Calibration Using an Extract of OSPW

An alternative to purchasing a commercial NA mixture is to prepare a mixture of NAs by extracting OSPW (Martin et al. 2008). Although the researchers found that calibration plots were nearly identical for direct injection ESI-MS, there were significant differences in the calibration plots for HPLC ESI-HRMS when using the Merichem commercial and extracted OSPW NAs. The response factor for the refined Merichem standards was significantly greater than the OSPW NAs. Some of the difference is attributed to the fact that the high resolution mass spectrometry (HRMS) is highly selective due to better mass resolution compared to ESI-MS with only unit-resolution MS. This would make ESI-MS responsive to more of the non-NA components found in the OSPW NAs extract. Additionally, OSPW NAs are more cyclic and

extensively branched than the Merichem standards making the latter more hydrophobic and thus favoring migration to the surface of the electrospray droplets and therefore enhancing the response signal for the Merichem standards. This agrees with other studies (Cech and Enke 2000, Cech et al. 2001, Tang and Smith 2001, Wu et al. 2004, Zhou and Cook 2001), which found that hydrophobic analytes with higher affinity for the surface of ESI droplets demonstrate a higher response.

4.1.1.3 Calibration Based on a Single Component

In the NAIT survey some labs indicated a preference for using a single commercial naphthenic acid compound as a calibration standard; for example, 1-pyrenebutyric acid. This approach simplifies quantitation, reporting and data interpretation compared to the calibration methods discussed above.

4.1.2 *Misclassification and False Positives*

It will be evident from this report that a considerable difference in the individual isomer group assignments will arise depending on the analytical method. Particularly the results obtained using FTIR and unit-resolution mass spectrometry lead to overestimation of NA concentrations and erroneous isomer group assignments for OSPW. An example of misclassification is a C22+ cluster observed with GC-MS but not with high resolution mass spectrometry (HPLC QTOF-MS). The erroneous assignment originated from the double derivatization of hydroxylated NAs ($C_nH_{2n+Z}O_3$) resulting in two *tert*-butyldimethylsilyl derivatives rather than one predicted from the classical NA formula. Martin et al. (2008), when comparing unit-resolution MS with HRMS, found that direct injection ESI-MS resulted in many abundant ions with higher carbon number (>C20) and lower carbon number (< C10) and Z = 0 and Z = -2 NAs than with HRMS methods.

Although this discrepancy between unit-resolution MS and HRMS is minimized when analyzing purified commercial NA mixtures, it is very evident that the modifications to the structure of NAs that occur in OSPW will lead to misclassification and false positives if methods based on HRMS are not being used and the classical NA formula is being applied.

4.1.3 *Reporting Format*

Information received in correspondence related to the NAIT survey indicates differences in the reporting of NAs. There are basically three approaches to reporting quantitative data:

- A total NA number representing a single number for all isomer groups,
- Concentration values for individual isomer groups of NAs. An example would be 60 isomer groups for n = 12 to n = 21 with a concentration value provided for each isomer group, and
- Percentage of the total NAs in each isomer group.

Certainly the latter reporting formats provide much greater qualitative and quantitative information about the NAs in the sample.

4.2 Quantitative Analysis of Naphthenic Acids Using Spectroscopy Methods

4.2.1 *Fourier-Transform Infrared Spectroscopy (FTIR)*

The FTIR spectroscopy method developed by Syncrude Canada Ltd. (Jivraj et al. 1995) has become a standard to quantify NAs in oils and in tailings samples and is used extensively by researchers. Following sample preparation the acids are analyzed by FTIR and the absorbances of the monomeric and dimeric forms of carboxylic groups are measured. The sum of the absorbances at the characteristic peaks is compared with the calibration curve, usually obtained by commercially available NAs with known concentrations under the same analytic method, to quantify the concentration of NAs in the water sample. NAs exist mainly as dimers in their liquid or solid states. Hydrogen bonding occurs between adjacent carboxylic groups. In a cast film FTIR analysis of the Merichem NAs, the dimeric C=O bond shows a single and sharp infrared photon absorbance near wavelength about 5,880 nm, or a wavenumber of 1,700 cm^{-1}. When diluted in dichloromethane, the dimeric form is in equilibrium with the monomeric form. The monomeric C=O bond absorbs photons at 1,743 cm^{-1} while the dimer absorbs at 1,704 cm^{-1} (Scott et al. 2008). Typically, the detection limit of NAs by FTIR is a few tenths of a milligram per litre (Clemente and Fedorak 2005) to one milligram per litre (Scott et al. 2008), depending on the volume of original aqueous sample and the extent the dichloromethane extract is concentrated before FTIR analysis.

FTIR often overestimates NA concentrations (Grewer et al. 2010, Yen et al. 2004), especially in surface water samples (Scott et al. 2008). The issue is caused by the nature of the FTIR method and the sample preparation:

- FTIR quantifies NA concentration in response to the absorbance of carboxylic groups. Thus, FTIR cannot identify the difference between classical naphthenic acids and the variety of non-classical NAs found in OSPW;
- The calibration curve is often obtained from commercial NAs, assuming that the commercial NAs can represent the real NAs extracted from tailings samples.

4.2.2 *Synchronous Fluorescence Spectroscopy (SFS)*

Fluorescence spectroscopy is a spectrochemical method of analysis that involves using a beam of light (usually ultraviolet light) to excite the electrons in molecules of the analyte and causes them to emit light (typically, but not necessarily, visible light). In a typical experiment, emission spectra (i.e., the different wavelengths of fluorescent light emitted by the analyte) are measured using a monochromator while holding the excitation light at a constant wavelength. An excitation spectrum is the opposite, whereby the emission light is held at a constant wavelength, and the excitation wavelength is varied. Synchronous fluorescence techniques have been developed depending on the scanning rates of the emission and the excitation monochromators and the manner in which the monochromators are synchronized. In constant-wavelength SFS, a constant wavelength difference is maintained between the excitation and emission monochromators.

Synchronous fluorescence spectroscopy (SFS) is reported to be a simple and fast way to monitor the presence of NAs present in ground and surface waters (Kavanagh et al. 2009). Water samples were collected from Syncrude's West In-pit settling basin and selected reference sites within the oil sands region, such as Gregoire Lake (south of Fort McMurray, Alberta), Poplar Creek Reservoir and Beaver Creek Reservoir (north of Fort McMurray). NAs were extracted from the water samples using the method described by Frank et al. (2006) and evaluated by SFS. The samples were scanned in a 1 cm quartz cuvette with PTFE stopper at 20°C. The wavelength offset between excitation and emission monochromators was set at 18 nm and synchronous fluorescence spectra were collected in the 250 to 400 nm excitation wavelength range. The excitation and emission monochromator slit widths were set at 5 nm with scan speed at 50 nm min^{-1} and resolution at 0.5 nm. The spectra were blank corrected with either Milli-Q water or 0.05 M $NaHCO_3$ (pH 8.3 for NA extract). NA extracts from OSPW and the commercial Fluka NAs showed similar fluorescence profiles with peaks at 280 nm and 320 nm. Although a decrease in SFS emission intensity was observed over a pH range from 4.3 to 10.5, no significant shift in the various fluorescent peaks for Fluka acids were found. Spectra for NA extract from surface water collected from reference sites showed only weak intensity for peaks at 282.5 nm and no peaks at 320 nm. The weak intensity at 282.5 nm was considered to coincide with the background level of NAs in surface waters in this region but the absence of the 320 nm peak suggested that the NAs did not originate with OSPW. The concentration of NAs exhibited a positive correlation with the spectra intensities both at 282 and 320 nm although it was not a quantitative measurement of NAs. Individual fluorescent spectra of toluene, naphthalene, quinoline, fluorene, phenanthrene and anthracene showed different fluorescent signature from those observed in the NA extract from OSPW, excluding the possibility that the fluorescent signature of NAs originated from these components. Since fluorescence spectra are indicative of aromatics, Kavanagh et al. (2009) concluded that the unique fluorescent peaks at 320 to 340 nm are caused by the presence of aromatic compounds closely associated with classic "NAs".

4.2.3 *UV-Vis Absorption and Fluorescence Emission Spectrophotometry*

Mohamed et al. (2008) reported that UV-Vis absorption and fluorescence emission spectrophotometry are potentially inexpensive and fast methods for screening of oil sands NAs, and for the semi-quantification of NA concentrations in OSPW. There are components in the NA complex that have various levels of unsaturation and aromaticity and contain functional groups that can absorb UV-Vis radiation and also generate an intense fluorescence emission. UV-Vis absorbance calibrations were prepared using a wavelength of 263 nm with standards adjusted to a pH of either 9 or 5. Good linear calibration curves were obtained at both pH 9 (R^2=0.997 with a mass extinction coefficient of 0.005 $L \cdot mg^{-1} \cdot cm^{-1}$) and 5 ($R^2$=0.997 with a mass extinction coefficient of 0.0063 $L \cdot mg^{-1} \cdot cm^{-1}$) in the range of 1 to 100 mg L^{-1}. The fluorescence emission spectra showed the intensity of the maxima varied with excitation wavelengths for a given concentration, indicating the presence of multiple fluorescent components in the NA mixture. Again, good linear calibration curves were obtained in the range of 1 to 100 mg L^{-1} at excitation wavelength of 346 nm using either fluorescence emission peak area (R^2=0.983) or

maximum quantum emission intensity of fluorescence against concentration (R^2=0.985). Good agreement was obtained for the quantitative estimation of NAs when comparing either UV-Vis absorbance and fluorescence emission spectrophotometry and direct injection ESI-MS.

4.3 Chromatographic Methods in Naphthenic Acids Determinations

4.3.1 *The Purpose of Chromatography*

Chromatography is a separation technique for sample mixtures. Components are separated on the basis of differences in the manner in which components interact with a stationary phase contained in a column as they are carried forward by a mobile phase. Thus the mechanism of separation is most commonly based on a differential partitioning of components in the sample mixture between the mobile and stationary phase. Both gas chromatography (GC) and liquid chromatography (LC) are powerful analytical tools, and are extensively used in many laboratories to separate components making it easier to identify and quantify components of sample mixtures. In GC, the sample to be analyzed is vaporized and carried forward by a gas mobile phase (such as helium and hydrogen), while in LC the sample is dissolved and carried forward by a liquid mobile phase (any miscible combination of water or various organic liquids, such as methanol and acetonitrile). The components of the mixture are separated as they progress along the column and elute out of the column at different retention times.

Recently, separation of NA components has been enhanced by using comprehensive 2-dimensional GC and LC techniques (GC x GC or LC x LC). The tremendous increase in the selectivity of components being analyzed is achieved by using two columns of different polarity. Components eluting from the first column are retained and then transferred to the second column in as small as 3 s intervals. Components that may be unresolved on the first column can be separated on the second column.

Both gas chromatography (GC) and liquid chromatography (LC) aid in the separation of NAs into isomer groups. These separation techniques are usually coupled with mass spectrometry (MS) for detection but other detection methods have been used. The purposes of chromatography are:

- to achieve at least partial separation of NA components into isomer groups,
- to reduce sample matrix effects, and
- to improve analytical sensitivity.

Several of the published methods for NAs which use chromatographic methods alter the structure of the NAs before performing the analysis. This process is called derivatization. It is the derivatized NAs that are injected and undergo the process of separation.

4.3.2 *Derivatization*

NAs contain polar carboxylic acid groups, which exhibit powerful hydrogen bonding forces and dramatically increase the boiling point of these compounds. Boiling points of NAs are reported

to between 250 and 350°C (Headley and McMartin 2004). Derivatization is a necessary step prior to GC analysis to:

- turn the thermally labile carboxylic acid group of NAs into a more thermally stable group,
- enhance volatility of NAs at a lower temperature, and
- help reduce the extent of molecular fragmentation during ionization when using MS.

Derivatization of NAs is not technically necessary for LC analysis but can:

- aid in the chromatographic separation of NA components,
- enhance detection of the ionized components when using mass spectrometry, and
- aid in the MS/MS specificity of the detection method.

Although derivatization procedures have advantages they also create concerns:

- derivatization steps are laborious and time-consuming,
- care has to be taken to avoid hydrolysis back to the parent acids,
- derivatization of all the components in naphthenic acids with one derivatizing reagent may be incomplete, inefficient, and inconsistent, affecting identification of NAs,
- since no individual NA can be available, the response factor of 1 is used for each component of NAs regardless of molecular weight, structure, or the origin of the NAs,
- components such as hydroxylated NAs or dicarboxylic acids found in the OSPW extracts can lead to false-positives and misclassification of NAs, and
- underivatized components will elute at different retention times and will have different masses when using MS detection, which could contribute to false positives.

4.4 Mass Spectrometry (MS) Detection Methods

MS is now a very common analytical tool in laboratories. MS works by ionizing chemical compounds to generate charged molecules or molecule fragments, and measures the mass-to-charge ratios to characterize the elemental compositions and the chemical structures of molecules. MS has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, determining the structure of a compound by observing its fragmentation, and quantifying the amount of a compound in a sample. MS instruments consist of three modules:

- an ion source which generates charged molecules or fragments,
- a mass analyzer which sorts the ions on the basis of mass and,
- an ion detector.

MS is the most common detector used in NA analysis, and can be used alone using direct injection or infusion methods or coupled with chromatography.

Ionization of the components being analyzed is necessary. Soft ionization techniques, such as electrospray ionization (ESI), chemical ionization (CI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI) cause minimal fragmentation of the parent compound and thus enhance detection and reduce the difficulty in classifying the compounds in complex mixtures. The hard ionization techniques, such as electron impact ionization (EI) and fast atom bombardment (FAB) fragment the parent compound allowing elucidation of structure based on fragment mass and abundance using a library search. Explanation of the performance of various ionization sources for NAs is covered in Section 4.4.1.

Mass analyzers separate the ions according to their mass-to-charge (m/z) ratio. A common term used to differentiate mass analyzers is the resolution (R) or resolving power of the mass analyzer. Resolution is defined as the ability of the mass spectrometer to discriminate the signals in the mass spectrum of ions with similar mass to charge ratio (m/z). This is calculated using the equation: $R = m/\Delta m$, where m = the nominal mass of an ion and Δm is the difference between the exact masses of the ions being compared. Unit-resolution MS would mean that you can separate each mass from the next integer mass. Thus m/z = 100 could be distinguished from m/z = 101. Unit-resolution MS methods have been extensively used primarily due to the broad availability and ease of operation/maintenance of the instruments. Unit-resolution MS is used for quadrupole and ion trap mass analyzers. However, low resolution methods are prone to false-positives and misclassification of NAs in OSPW samples (Bataineh et al. 2006, Martin et al. 2008).

High and ultrahigh-resolution MS (HRMS) methods can help identify specific NA structures and can better track microbial degradation of those structures (Han et al. 2008) since a more accurate mass assignment can be made. Examples of mass analyzers falling into this designation are the magnetic sector (R = 10,000), QTOF (R = 40,000), Orbitrap (R = 10,000 to 100,000 depending on scan rate) and FTICR (R = 1,000,000 or even higher in narrowband mode).

Another term used to distinguish the performance of mass spectrometers is mass accuracy, a measurement of how well the observed m/n correlates with the "true value". It is particularly important for complex mixtures because many possible elemental compositions could be assigned to a nominal, integer m/z value. Mass accuracy is often expressed in the units of parts per million (ppm). It is calculated using the following equation:

$$\text{Mass accuracy} = \frac{\left(m_{observed} - m_{theory}\right)}{m_{theory}} \times 1{,}000{,}000$$

where $m_{observed}$ is the m/z of the peak of interest in the mass spectrum obtained and m_{theory} the theoretical m/z expected for the species. Mass accuracy for unit resolution MS is about hundreds of ppm (for example, 200 ppm for single quadrupole MS) and can be several ppm for HRMS (for example, 2 ppm for FTICR-MS).

4.4.1 *Ionization Techniques Applied to the Analysis of Naphthenic Acids*

4.4.1.1 Electron Impact Mass Spectrometry (EI-MS)

Electron impact ionization or electron ionization is a hard ionization technique that occurs under high vacuum. It is widely used in mass spectrometry, particularly for gases and volatile organic molecules. This method employs energetic electrons (usually 70 eV) to interact with gas phase atoms or molecules to produce ions. The de Broglie wavelength[2] of the electrons matches the length of typical bonds in organic molecules (about 0.14 nm) and energy transfer to organic analyte molecules is maximized, leading to the strongest possible ionization and fragmentation.

Although pure compounds yield distinct fragmentation patterns that can be identified easily, it is problematic when using this method to characterize a complex mixture like NAs. This hard ionization technique imparts more energy than necessary and leads to extensive fragmentation. When the components entering this ion source are not pure it makes it difficult to differentiate the secondary fragment ions from the primary molecular ions. Thus, derivatives of NAs which can yield few fragments or a soft ionization source are preferred. Additionally, if the fragmentation is too extensive the sensitivity of detection can be reduced.

Holowenko et al. (2002) employed GC-MS with electron impact ionization to characterize nine water samples derived from oil sands extraction processes. For each sample, a valley between groups of NAs with carbon numbers <21 and carbon numbers >21 was found in the three-dimensional bar graphs based on the abundance of NAs to the corresponding carbon number and Z families. The group of NAs with carbon numbers 22 to 33 in Z families 0 to -12 was singled out and defined as "C22+ cluster". This was a useful means of comparing composition distribution in NAs from various OSPW and with varying degrees of acute toxicity. Their study suggested that the decrease in acute toxicity of OSPW was most likely caused by the enrichment in the abundance of C22+ cluster of NAs relative to the abundance NAs with smaller carbon numbers. The increase in C22+ cluster in NAs accompanied a decrease in the total concentration of NAs in OSPW that was associated with the selective removal of NAs with carbon numbers of ≤ 21. Headley et al. (2009a) noted that unit-resolution MS was not providing correct interpretation of the compounds being formed. Rather than an increase in C22+ there was actually an increase in concentration of the oxy-NAs, which increased the mass but when using the classical NA formula resulted in a misclassification of the NAs that were present in the sample (Bataineh at al. 2006, Clemente and Fedorak 2004).

4.4.1.2 Chemical Ionization Mass Spectrometry (CI-MS)

Chemical ionization is a lower energy process compared with electron impact ionization and also occurs under high vacuum. The lower energy yields less fragmentation, and usually a simpler spectrum. Electrons entering the ion source will preferentially ionize the reagent gas (most commonly used reagent gases are methane, ammonia and isobutane) which are present in excess

[2] See http://en.wikipedia.org/wiki/Matter_wave

compared to the analyte. Ions are produced through the collision between the analyte and the ions of the reagent gas. Positive and negative ions of the gas phase analyte are formed by the reaction with ions of the reagent gas. Almost all neutral compounds can form positive ions through these reactions. However, for negative chemical ionization, the analyte must be capable of producing a negative ion by electron capture ionization. Because not all components will form negative ions, this chemical ionization technique provides a certain degree of selectivity that is not available with other, more universal ionization techniques like EI. Negative chemical ionization can be used for the analysis of compounds containing acidic groups or electronegative elements.

Dzidic et al. (1988) developed a method based on negative ion chemical ionization mass spectrometry using fluoride ions produced from NF_3 as the reagent gas in the characterization of NAs in California crude oils and refinery wastewaters. NAs in the presence of other compounds, such as hydrocarbons, can be selectively ionized through an acid-base reaction in the gas phase where the base (F^-) reacts with the acid (RCOOH) to form the base $RCOO^-$ and the acid HF as shown below:

$$F^- + RCOOH \rightarrow RCOO^- + HF$$

The spectra exhibit only the single $RCOO^-$ carboxylate ions and nonacidic compounds such as hydrocarbons cannot be ionized by F^- ions. Thus, the spectrum is simplified.

Lu et al. (2004) developed a method based on positive CI mass spectrometry to characterize NAs in crude oils. NAs were isolated from the crude oil using an anion exchange resin. Following the removal of neutral, basic, and weak acid compounds, NAs were eluted with methanol containing formic acid from the anion exchange resin. The recovered NAs were converted to methyl esters using a methanol-boron trifluoride solution held at room temperature for 12 h. A direct injection was made and CI using methane as the reagent gas caused the formation of characteristic $(M+15)^+$ ions. The extent of molecular fragmentation was decreased greatly and strong base peaks representing the un-fragmented NA constituents could be obtained. Molecular weight and structural information for NA components were obtained from the prominent $(M+15)^+$ ion peaks. Hsu et al. (2000) characterized NAs in crude oil by mass spectrometry using chemical ionization with several different reagent gases; methane, ammonia and isobutane. Both negative- and positive-ion modes were applied. For the negative-ion CI mode, the reagent gases methane and isobutane generated near-zero-energy thermal electrons which resulted in resonance electron capture or dissociative electron capture. However, electron capture is more efficient for certain polycyclic aromatic hydrocarbons than for alicyclic NAs. Using ammonia as the reagent gas, CI generated a very basic NH_2^- that can abstract not only a proton from an acid but also a proton from the benzylic position of aralkyl compounds. Thus, negative ion CI using these three gases is not selective for the ionization of NAs alone.

In the positive CI mode, the reagent gas methane yielded excess amounts of fragment ions while ammonia CI generated both protonated and ammonium-adduct molecular ions leading to an overall lower sensitivity. Isobutane CI produced the best CI spectra in which NAs yielded primarily protonated molecular ions with minimal fragmentation.

4.4.1.3 Electrospray Ionization Mass Spectrometry (ESI-MS)

Electrospray ionization (ESI) is a preferred technique for ionization of small amounts of large and/or labile molecules. The ESI source operates at atmospheric pressure. The mobile phase containing the ionized analyte is dispersed into a fine aerosol from a small tube in the presence of a strong electric field and a flow of warm nitrogen to assist evaporation. The droplets formed evaporate in a region maintained at a vacuum causing the charge to increase on the droplets which eventually lead to the loss of mobile phase allowing the analyte ion to enter the mass analyzer. Typical solvents used for electrospray ionization are prepared by mixing water with volatile organic compounds (e.g., methanol, acetonitrile) and other reagents to generate positive or negative analyte ion. The most obvious feature of an ESI spectrum is that the ions carry multiple charges which reduce their mass-to-charge ratio compared to a singly charged species. This is especially useful in producing ions from macromolecules because it overcomes the propensity of these molecules to fragment when ionized. Both positive and negative-ion spectra can be obtained. It has become one of the preferred MS methods for studying NAs since nonpolar contaminants do not form ions readily. A concern with this ionization method for OSPW is the high concentrations of inorganic salts which can overload the MS system with charged ions, block the electrospray probe and thus harm the MS instrument (Wang and Kasperski 2010). Dilution is not an ideal solution to reducing the high salt effects since this reduces the sensitivity of detection. Numerous methods of removing salts are available but will not be a focus of this review (Wilson and Konermann 2005).

Rogers et al (2002a) characterized NAs from Athabasca oil sands tailings pond water using negative ion ESI-MS. Their study revealed a high degree of compositional heterogeneity, both within and amongst z-series. The molecular weight profiles showed that compounds with molecular weight between 260 and 320 having a single ring and with carbon numbers in the range of 17 to 19 exhibited the highest overall concentration. One, two, three, four-ringed and acyclic compounds made up nearly identical proportions (~ 20%) of NAs. This differs from other reports (St. John et al. 1998) in which one and two-ringed NAs fractions dominated (> 70%). The most prominent impurities were identified to be biphenyls, naphthalenes, phenanthrene and anthracene using the bulk extraction protocol they developed. But the contamination levels were low ($\leq$13µg L^{-1}) even in a concentrated solution of NAs (8,549 mg L^{-1}).

Lo et al. (2003) examined the effect of concentration and instrument settings on apparent congener composition for both model NAs and an authentic NA sample from tailings pond water. They also used negative ion ESI-MS without chromatographic separation. NAs from tailings pond water were extracted by liquid-liquid extraction with dichloromethane. The extract was diluted with 1:1 v/v solution of 2-propanol and water containing 1% isopropylamine. High cone and extractor voltages gave undesirable collision-induced dissociation and increased the relative proportion of the signal at high m/z. The apparent congener distribution was quite dependent on the cone and extractor voltages. Under optimal conditions, model carboxylic acids, both alone and in mixture, showed a linear response in the concentration range from 5 µM to 100 µM with calibration sensitivities varying by a factor of 2. Minimal interactions were

observed when the model compounds were examined in mixtures. Linear response of major ions in tailings pond water-derived NAs was observed when the total concentration was <125 μg L^{-1}. Similar calibration sensitivities among congeners allowed the use of standard addition method to determine, at least semi-quantitatively, the concentration of NA congeners in tailings samples. The study reported that average concentration was 4.4 μM for congeners with $n \leq 20$ and 0.27 μM for congeners with $21 \leq n \leq 41$. ESI-MS analysis of tailings pond water-derived NAs showed that Z=-4 family was the most abundant and $12 < n < 17$ contributed >75% of the blank-corrected signal when all z families were aggregated. NAs showed apparent congener distributions quantitatively similar to those observed previously by GC EI-MS analysis of *tert*-butyldimethylsilyl derivatives of NAs extracted from oil sands (Holowenko et al. 2002). The distribution of congeners within a "Z" family usually peaked near C15.

Headley et al. (2002a) employed negative electrospray ionization mass spectrometry to determine dissolved NAs in natural waters. Water samples collected from several northern Alberta rivers near crude oil refineries were extracted using a solid phase extraction procedure utilizing a cross linked polystyrene-based polymer with acetonitrile elution. The extract was dried with nitrogen gas flow and ESI-MS analysis was performed on the extract with no further cleanup and derivatization steps. Recovery of Fluka Chemicals NAs was highly pH dependent, with 100% recovery at pH 3.0, but dropping to only 66% and 51% recoveries as increasing pH values to 7 and 9, respectively. Based on this study, all the samples were acidified to pH 3 with formic acid prior to SPE to maximize the recovery of NAs. The representative calibration curves were constructed using the area summation of the five major ions present in the full scan mass spectra. The five ions monitored were: m/z 205, 223, 237, 251 and 265. No correction was needed for calibration since all peaks were base-lined in the collection of the continuum mass spectra at the resolution employed. Linear calibration curves (r^2=0.9998) were obtained from analysis of both Fluka and Syncrude mixtures with NA concentration of 10 through 100 mg L^{-1} and a detection limit of 0.01 mg L^{-1}. The ESI-MS method proved to be a simple procedure for the quantitative analysis of NAs. However, the application of this procedure is limited to natural waters with relatively low to moderate concentration of other dissolved organic compounds (2 to 5 mg L^{-1}) due to the interferences from co-extractives such as humic and fulvic acids.

4.4.1.4 Atmospheric Pressure Chemical Ionization Mass Spectrometry (APCI-MS)

Atmospheric pressure chemical ionization (APCI) is an analogous ionization technique to chemical ionization but occurs at atmospheric pressure. Typically, the vapourized mobile phase functions as the reagent gas to ionize the sample. The mobile phase containing analyte is introduced into a pneumatic nebulizer, heated to a relatively high temperature (above 400°C), and sprayed using high nitrogen flow rates without diverting the larger fraction of volume to waste. The entire aerosol cloud is then subjected to a corona discharge to create ions. Because the solvent and analyte molecules are converted to a gaseous state before reaching the corona discharge pin, the APCI technique allows a nonpolar instead of a polar solvent to be used as a mobile phase solution. The spectra yielded are predominant in molecular and adduct ions with very little fragmentation. The general source set-up shares a strong resemblance to ESI and is most commonly used in conjunction with HPLC or other separation techniques. APCI is not as

"soft" an ionization technique as ESI and is commonly used to analyze smaller, thermally stable polar and non-polar compounds.

Hsu et al. (2000) characterized NAs in crude oil using APCI in both the negative and positive ion modes. Acetonitrile was found to be the best solvent and mobile phase for analyzing NAs using APCI in the negative ion mode. Under the APCI conditions, acetonitrile is deprotonated to form the acetonitrile anion, CH_2CN^-, which abstracts a proton from NAs to yield deprotonated molecular ions $[M-H]^{1-}$ with minimum fragmentation and high sensitivity. The negative ion mode was found to be the most selective and sensitive to the NAs than the positive ion mode.

Rudzinski et al. (2002) characterized a number of standard acids (cyclohexane carboxylic acid, 1-adamantane carboxylic acid, 1,2,3,4-tetrahydro-2-naphthoic acid, 5β-cholanic acid, and pyrene butyric acid) and two commercial NA mixtures (Fluka NAs and P&B NAs) by using APCI-MS in both positive and negative modes. The negative ion APCI-MS yielded a predominant $[M-H]^{1-}$ peak for all acids except 5β-cholanic acid, which produced the $[M-H+2H_2O]^{1-}$ peak; while the positive ion APCI-MS yielded very weak $[M+H]^+$ peaks. Overall, the negative ion APCI-MS appears to be the more preferable ionization mode for obtaining ring type and carbon number distribution of NA mixtures. Characterization of these standard acids and commercial acid mixtures was also carried out on ESI-MS (both negative and positive modes) and a similar conclusion was obtained: negative mode ESI-MS was more suitable than positive mode. Comparison between negative ion ESI-MS and negative ion APCI-MS found that negative ion ESI-MS gave stronger signal intensity than negative APCI-MS. This is different from the result of Hsu et al. (2000) who reported one order of magnitude higher sensitivity for APCI than for ESI-MS. Rudzinski et al. (2002) attributed this to the differences in solvent composition – the addition of 0.5% NH_3 to the (50:50) CH_3CN:CH_3OH solution enhanced the signal intensity.

Lo et al. (2006) pointed out that APCI-MS gave a wider range of quantitation than ESI-MS, but its detection limit was poorer and the model compounds showed greater variation in calibration sensitivity. Six reference carboxylic acids (cyclohexane carboxylic acid, cyclohexane butyric acid, decanoic acid, 1-pyrene butyric acid, abietic acid, 5β-cholanic acid) were detected under APCI negative conditions over the concentration range of 5μM to 2 mM. The response was linear from 25 μM to at least 2 mM whereas ESI-MS (Lo et al. 2003) showed a linear range of detection from 5 to 100 μM. The response of APCI-MS for major ions of NAs extracted from tailings pond water was linear from 25 μg/mL to 1 mg/mL with R^2>0.9946 for all major peaks, whereas the response of ESI-MS (Lo et al. 2003) was linear only below 125 μg/mL. However, the calibration sensitivity of the six reference carboxylic acids varied by a factor of 5 to 6 under APCI negative conditions compared to only a factor of 2 under ESI negative conditions (Lo et al. 2003). This difference of calibration sensitivity is due to the mechanism of analyte ionization. The ionization efficiency of APCI-MS is mainly determined by the gas phase proton affinities of analyte and solvent, whereas ESI-MS is affected by the matrix and analyte surface activity. Lo et al. (2006) further investigated the influence of cone and extractor voltage on the analysis sensitivity. The apparent distribution of NAs extracted from tailings pond water showed a suppressed signal in the Z=-4 and -6 families at higher (cone voltage = 40 V, extractor voltage = 25 V) or lower (cone voltage = 5 V, extractor voltage = 5 V) cone and extractor

voltages compared with the "standard" APCI-MS cone voltage of 25 V and extractor voltage of 8 V.

4.4.1.5 Atmospheric Pressure Photoionization Mass Spectrometry (APPI-MS)

Atmospheric pressure photoionization (APPI) is an atmospheric-pressure ionization technique. In this technique, UV light photons are used to ionize sample molecules. The technique works well with non-polar or low-polarity compounds not efficiently ionized by other ionization sources. The sample (analyte) is mixed with a solvent (such as acetonitrile and methanol) and then vaporized with the help of a nebulizing gas such as nitrogen before entering an ionization chamber at atmospheric pressure, where the mixture of solvent and sample molecules is exposed to ultraviolet light from a krypton lamp. The photons emitted from this lamp have a specific energy level that is sufficient enough to ionize the target molecules but not high enough to ionize components of the mobile phase such as acetonitrile and water. This selective ionization helps ensure that only the analyte molecules of interest proceed to the mass spectrometer to be measured. To increase the percentage of analyte molecules that are ionized, sometimes a third compound called a dopant (such as toluene, benzene, acetone) is added to increase the ionization potential.

Barrow et al. (2010) used FTICR-MS in conjunction with ESI and APPI, in both positive and negative ion modes, to characterize oil sands process water. The solvent system for ESI was acetonitrile/methanol (1:1) with no acids or bases being added to aid protonation/deprotonation. The solvent system for APPI was acetonitrile/toluene (1:1) with toluene assisting the ionization process through an efficient absorption of the photons from the krypton lamp and its subsequent action as a charge carrier. Overall, a greater number of peaks were observed in the positive ion mode than in the negative ion mode. Additionally, more peaks were observed using APPI than using ESI. Comparison between the negative ion and positive ion mode APPI data sets provided insights into the presence of species consisting of carbon, hydrogen, and a sole nitrogen or sulfur atom. Positive ion mode APPI data showed more variety of species with greater relative intensity than negative ion mode APPI. Due to the possibility of forming both protonated ions and radical ions, APPI is more amenable than ESI to access sulfur-containing species or hydrocarbons without a heteroatom. The study showed that the predominant ions observed by APPI in positive mode are protonated ions rather than radical ions for both nitrogen- and sulfur-containing species. The results from both ESI and APPI showed the predominant components of the oil sands process water sample were oxygenated species in addition to classical NAs. Though the profiles were similar with respect to carbon number and hydrogen deficiencies, NAs of the classical O_2 compound class with higher hydrogen deficiencies showed higher relative intensity when APPI (negative ion mode) was used than when ESI (negative ion mode) was used. The authors attributed this to the inherent characteristics of the APPI technique, which is more suitable for the study of polycyclic aromatic compounds than the ESI technique, and to the use of toluene, which could prove to be a more suitable solvent for naphthenoaromatic compounds.

4.4.1.6 Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)

High-field asymmetric waveform ion mobility spectrometry (FAIMS) is a new technique that can continuously separate ions in the gas phase at atmospheric pressure. This is not an ionization source but is placed before the mass analyzer to improve the resolution of the ions generated by the ion source. The basis of ion separation is dependent on the ratio of the high-field and low-field mobility of the ion. High and low electric field conditions are generated by the application of the asymmetric waveform to an inner cylindrical electrode. During each cycle of the wave form, the ions of the mixture entering into the space between two concentric cylindrical electrodes and experience a shorter duration of high electric field when moving toward one electrode, and a longer duration of low electric field when moving toward the opposite electrode. The difference in relative ion mobility in high and low fields results in a constant drift of the ion toward one of the cylindrical electrodes. A small dc voltage (compensation voltage – CV) is applied to the inner electrode to stop the drift of an ion toward either electrode. Ions can be separated and transmitted through FAIMS to the mass analyzer by controlling and applying an appropriate CV. The separation properties of FAIMS depend more on the ion structure (including ion dipole moment, ion polarizability, ion rigidity and shape) than on the ion size.

Recently, ESI-FAIMS was combined with quadrupole, time-of-flight, tandem mass spectrometry (TOF-MS/MS) to characterize commercial and naturally occurring NAs mixtures (Gabryelski and Froese 2003). With ESI-FAIMS-MS, it is possible to analyze NAs from different sources directly without extensive sample preparation. Commercial NAs (Kodak and Merichem) were simply diluted to 1 mg L^{-1} with methanol containing 0.05% (v/v) ammonium hydroxide prior to analysis without further clean-up. The tailings pond water was diluted 50 times with methanol (containing 0.1% ammonia) prior to analysis without sample preparation or a pre-concentration step. Diluted samples were pumped into the ESI source and analyzed directly by ESI-FAIMS-MS. This method is able to distinguish compositional differences between samples, relying not only on the mass assignment but also due to the effect of the CV on the distribution of isomers. Although larger NA ions were transmitted through FAIMS at larger CV, NAs with the same mass were detected at different CVs showing a large structural variety of NAs. The mass analyzer chosen to generate the mass spectrum affects analysis times. For example, ESI-FAIMS-TOF allows the mass spectrum to be generated in 3 min instead of 40 min required on the ESI-FAIMS-quadrupole. This technique appears to be especially useful for the characterization of fragile ions (m/z 253 and m/z 373 ions) that cannot be detected by other methods. Tandem mass spectrometry of NA ions separated by FAIMS provides more substantial information about the structure of NAs than other available methods.

4.4.2 *Unit Resolution Mass Spectrometry*

The most common unit-resolution mass analyzer used for NAs is the quadrupole (Q). In the review of NA methods that follows, the designation MS is almost always the quadrupole. The quadrupole is sometimes referred to as a mass selective detector since it allows only one mass at a time to reach the detector. Only a mass that has a stable trajectory between four rods in the mass analyzer will reach the detector. All other masses lower and higher than this will have

unstable trajectories and will be filtered out. In the SCAN mode of operation, a mass range is scanned to see the abundance of each mass that has been formed in the ion source. Selective Ion Monitoring (SIM) is used when information is known about the compounds being analyzed, allowing only a specified m/z to reach the detector even though numerous ions may have been generated in the ion source.

At times a triple quadrupole (QqQ), also known as tandem mass spectrometry or multistage mass spectrometry (MS/MS), is used to improve qualitative performance of the detector and to increase sensitivity. Q_1 and Q_3 are again used as mass filters with q_2 acting as a collision cell to fragment the ions released from Q_1 and which are analyzed in Q_3. Although improvements in sensitivity and identifications of components being analyzed improve with this approach the mass resolution is still unit-resolution.

High resolution mass spectrometry (HRMS) is discussed in section 4.4.3.

4.4.2.1 Unit-Resolution MS of MTBSTFA Derivatized Naphthenic Acids Using GC

St. John et al. (1998) developed a method, that can be performed easily on any bench-top GC-MS system under normal operating conditions, using N-methyl-N-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) to derivatize NAs to their *tert*-butyldimethylsilyl (*t*-BDMS) esters. Since the derivative is very stable, the extent of molecular fragmentation is greatly decreased compared with other esters of the acids (e.g., methyl and trisilyl esters). During the derivatization process, the *tert*-BDMS group adds 114 to the molecular mass of the acids for each proton exchange. Although molecular ions and secondary fragmentation ions are both present, fragmentation is predominantly directed toward the $[M+57]^{1+}$ ion, corresponding to the $Si(CH_3)_2$ derivatives resulting from the loss of C_4H_9 from *t*-BDMS. An example of fragmentation pathway leading to predominant *t*-BDMS derivatives of NAs is shown in Figure 2. EI-MS of the derivatized NAs presented data similar to Fluoride Ion Chemical Ionization-MS (Dzidic et al. 1988). Since the derivatives are not extensively fragmented, GC-MS analysis yields characteristic ions from which structural information can be extracted to classify the individual components eluting within the unresolved hump and provide percent composition of the NA components based on Z and carbon numbers. Because this simple technique requires little specialized training of the operator and the instrumentation is readily available in many labs, it has been widely used by many researchers (e.g., Clemente and Fedorak 2004, Merlin et al. 2007, Young et al. 2010).

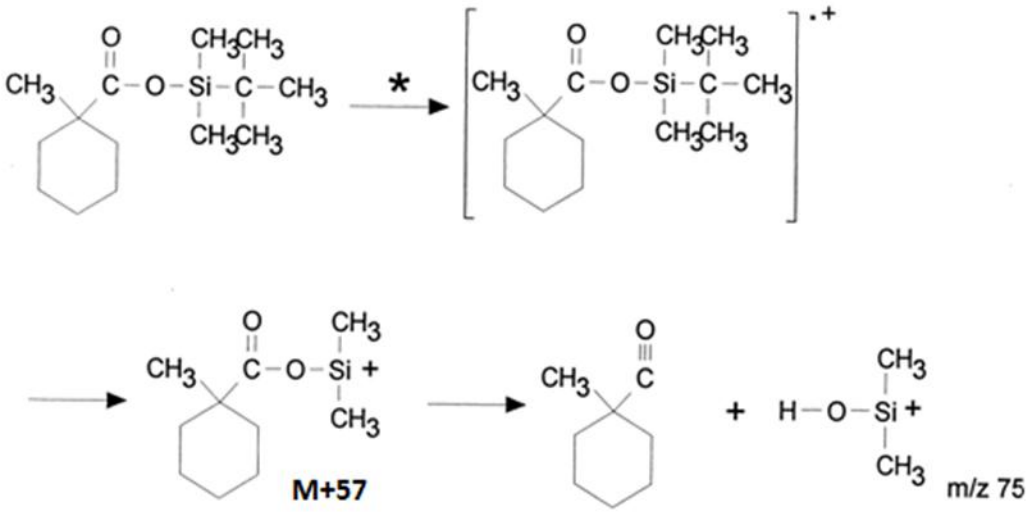


Figure 2. Fragmentation pathways leading to the predominant *t*-BDMS derivatives of NAs. After St. John et al. 1998.

Evaluation of MTBSTFA as the derivatization reagent for quantification by GC-MS methods (Young et al. 2010) showed that the catalyzing agent 1% *t*-butyldimethyl-chlorosilane (*t*-BDMCS), usually contained or deliberately added in MTBSTFA to enhance the derivatization hindered by alcohols and amines, could be the major source for the large contamination peaks at 16.7 and 18.8 retention times in the SIM chromatograms. These contamination peaks could interfere with the integration of the NA hump on the chromatogram of unresolved NAs. It is ideal for amines present in the aqueous extract to be removed from the NAs during a clean-up step to avoid this problem. The hydroxylated NAs don't fit in the formula for classical NAs and are not targeted as part of this SIM GC-MS method. The study showed that the absence of *t*-BDMCS would not reduce the yield of derivatized carboxylic acids. Thus, the application of catalyzing agent *t*-BDMCS was not necessary. Grewer et al. (2010) quantified NAs in tailings water of Syncrude and Suncor and fresh water from the rivers or lakes in oil sand region with this GC-MS method, using MTBSTFA without 1% *t*-BDMCS, and compared with the results from FTIR method. The GC-MS method gave more than 25% lower estimation of the NAs than FTIR. Detection limit for the GC-MS method was reported to be 0.03 mg L^{-1}.

Clemente and Fedorak (2004) further evaluated the analyses of *tert*-butyldimethylsilyl derivatives of NAs by GC-EI MS. Six surrogate pure compounds (1-methyl-1-cyclohexanecarboxylic acid, *trans*-1,4-pentylcyclohexanecarboxylic acid, 2-hexyldecanoic acid, eicosanoic acid, dicyclohexylacetic acid, and 5β-cholanic acid) were derivatized with MTBSTFA and analyzed by GC-MS both individually and in mixtures. The analyses of these six derivatives individually by GC-MS showed that the loss of the *tert*-butyl group, to give the [naphthenate +dimethylsilyl]$^+$ ion, was always the major fragment with abundances in the range of 59% to 89%.

In a discussion of their GC-MS method, Clemente and Fedorak (2004) concluded that ion misassignment and overestimation of the abundance of lower-molecular-mass NAs was due to the presence of isotopes and ions generated as a result of an unexpected cleavage. When considering [naphthenate +dimethylsilyl]$^+$ ([M+57]$^+$) ions, the A+1 ion occurs when there is one ^{13}C atom or one ^{29}Si atom present and the A+2 ion occurs when there are two ^{13}C atoms, a combination of one ^{13}C and one ^{29}Si atom or one ^{30}Si atom present in the [M+57]$^+$ ions. A significant proportion of [M+57]$^+$ ions from each surrogate acid appear as A+1 ions because of the presence of isotopes such as ^{13}C and ^{29}Si. It was noted that the surrogate acids with larger number of carbons (16, 20 and 24) yielded [M+57]$^+$ ions with relative lower abundances than surrogate acids with smaller number of carbons (8, 12 and 14). The [M+99]$^+$ ions resulted from the loss of a methyl group from the *tert*-methyl group and [M+57]$^+$ ion containing heavy isotopes of C and/or Si (A+1 or A+2 ions). The occurrence of [M+99]$^+$, A+1, and A+2 ions reduces the relative abundance of the [M+57]$^+$ ions used to assign a compound to the corresponding carbon and Z number. This effect becomes more serious as molecular mass increases since the probability of ^{13}C being present increases with the number of carbon atoms. Corrections were made by taking account of the probability of the isotopes occurrence. No statistically significant difference in the distribution of ions was found after correction since the correction cannot include the effect of [M+99]$^+$ ions and the effect of missing A+1 ions. An important contribution of this work is the establishment of the matrix pattern for ion assignment (based on the [naphthenate +dimethylsilyl]$^+$ ion) to be NAs (carbon number range of 5 to 33 with Z=0 to -12 representing 0 to 6 rings) that can fit the empirical formula $C_nH_{2n}O_2$. The pattern excluded the ions which fit the empirical formula but are not NAs. This approach helps simplify the data analysis and decrease the ion misassignment.

Merlin et al. (2007) extracted NAs from water samples and derivatized with MTBSTFA (N-methyl-N-(*t*-butyldimethylsilyl)-trifluoroacetamide). The extract derivatives were analyzed by GC-MS using three ions (nominal m/z=265, 267 and 279). These three ions rarely occur naturally in fatty acids with 13 carbon atoms and two (C_{13}Z=-4) or three (C_{13}Z=-6) rings or with 14 carbon atoms with three (C_{14}Z=-6) rings, but are commonly found in NAs from various sources with relative abundances of 3% to 19%. Using GC-MS to detect $C_{15}H_{27}O_2Si^+$ (nominal m/z=267), within an unresolved hump that eluted between 16 and 21 min in the reconstructed ion chromatograms, is an excellent indicator of the presence of NAs at concentrations $\geq$10μg L^{-1}.

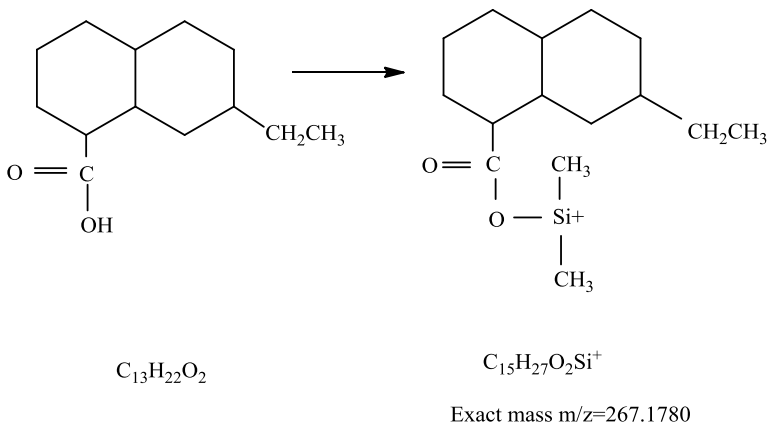


Figure 3. An example of a naphthenic acid that would yield *t*-butyldimethylsilyl ions when derivatized and analyzed by GC-MS.
Based on the nominal mass fragment of m/z=267 the formula assigned of the NA would be assigned as $C_{13}H_{22}O_2$.

4.4.2.2 Unit-Resolution MS of MTBSTFA Derivatized Naphthenic Acids in Non-Aqueous Samples

Young et al. (2007) used GC/MS in the SIM mode to detect NAs in fish that were exposed to commercial NAs or OSPW. Quantitation was done by integrating the area under the hump of m/z 267 between 16 and 21 min, against the area of the surrogate standard 9-fluorenecarboxylic acid. Despite the high background of naturally-occurring fatty acids in fish, monitoring specifically for the m/z=267 ion proved to be very selective for NAs. Scott et al. (2008) applied this GC-MS method with SIM to specifically quantify NAs in water samples and compared the results with those from FTIR. In general, GC-MS method gave lower estimation due to its higher specificity.

De Campos et al. (2006) investigated NAs in heavy gas oil from Marlim petroleum (Brazilian petroleum from Campos, Rio de Janeiro) by GC-MS with electron impact ionization. NAs were extracted through liquid-liquid extraction and further cleaned up by an ion exchange resin (Amberlyst A-27) before derivatization with MTBDMSTFA. A great variety of non-acid compounds were identified from the total ion chromatogram. These compounds were eluted at the beginning of the chromatogram and were composed mainly of aromatic hydrocarbons, sulfur and nitrogen compounds. The analysis of each acid fraction was accomplished by monitoring the ions of each carbon number and Z family of NAs. Associated with the poor resolution of components being chromatographed a high number of isomers for each base peak $[M+57]^+$ corresponding to the $C_nH_{2n+Z-1}O_2$-*t*-BDMS acids were observed. The numbers of isomers increased with carbon number as expected. For the Z=0 family, acids containing carbon number 7 to 29 were identified but the acids with carbon number 30 to 33 were absent, indicating the predominance of low-molecular weight acyclic saturated acids in these samples. NAs with one to four rings were also identified.

4.4.2.3 Unit-Resolution MS/MS Using HPLC

Smith and Rowland (2008) developed a high performance liquid chromatography/electrospray ionization multistage mass spectrometry (HPLC ESI-MS^n) method to characterize synthesized amide derivatives of NAs. This method was successfully applied to commercially available carboxylic acids, novel synthetic NAs, commercial NAs refined from crude oils, crude oil NAs and Athabasca oil sands NAs. Following SPE extraction, the NA mixture was derivatized to form NA amides by reaction with oxalyl chloride. The reaction mixture was extracted using ethyl acetate, dried with Na_2SO_4, and concentrated. The results showed that derivatization and analysis by HPLC ESI-MS of amide derivatives of NAs in the positive ion mode improved the detection response by two orders of magnitude compared to that of NAs in negative ion mode and allowed multistage MS experiments and more detailed fragmentation pathways for individual NAs to be established. Data-dependent mass spectrometry provided unique MS^2 spectra of individual amide derivatives of NAs, from which bi- to polycyclic acids containing ethanoate side chains, in addition to alkyl substituents, were confirmed to be widespread amongst the oil and oil sands NAs. However, the derivatization step is time-consuming and the efficiency of derivatization can become problematic for quantitative analysis of NAs.

To use the extensively available unit-resolution MS, Wang and Kasperski (2010) developed an HPLC-MS/MS method to characterize NAs by combining liquid chromatography with unit-resolution mass spectrometric detection. The advantage of this method over direct injection MS is that the chromatographic separation prior to mass spectrometry can minimize the occurrence of false positives caused by low resolution mass spectrometry and shed more light on the structural differentiation of NAs based on their characteristic HPLC retention times. Mixtures of six pure NAs were used as model compounds to optimize chromatographic separation and mass spectrometric response. To assist with the deprotonation of NAs, 10 mM ammonium acetate was added into the initial aqueous mobile phase. A one hour chromatographic run using gradient elution (70% water containing 10 mM ammonium acetate + 30% methanol 0 to 6 min, ramped to 100% methanol 6 to 50 min, and held for 10 min) gave the best resolved peaks. Most inorganic salts could be eluted from the HPLC column within the first 5 min of the run, meaning that pre-treatment of samples could be eliminated. The results showed that the retention times of NAs increased with increasing carbon number and decreasing degrees of cyclization (i.e., less negative Z), consistent with the results obtained from HPLC/HRMS (Bataineh et al. 2006). Calibration curves with the mixture of pure NAs or commercial Fluka NAs mixture showed good linearity, indicating the method is quantitative.

4.4.3 *High and Ultrahigh Resolution Mass Spectrometry*

4.4.3.1 Orbitrap

An Orbitrap is a type of mass spectrometer invented by Alexander Makarov. It consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode that form an electrostatic field with quadro-logarithmic potential distribution. In an Orbitrap, ions are injected tangentially into the electric field between the electrodes and trapped due to the balance obtained between their electrostatic attraction to the inner electrode and the centrifugal forces. Thus, ions of a

specific mass-to-charge ratio cycle around the central electrode in rings while at the same time oscillate along the axis of the central electrode. The frequency of these harmonic oscillations is independent of the ion velocity and is inversely proportional to the square root of the mass-to-charge ratio (m/z or m/q). By sensing the ion oscillation in a manner similar to that used in FTICR-MS, the trap can be used as a mass analyzer. Orbitraps have a high mass accuracy (1 to 2 ppm), a high resolving power (up to 200,000) and a high dynamic range (around 5,000). Since Orbitrap doesn't need radio frequency (RF) or a magnetic field like FTICR-MS, the instrument is more compact and free of maintenance.

A recent report (Headley et al. 2011) demonstrated that Orbitrap technology appears to be very promising as a quantitative and qualitative tool at high mass resolution to study NA contamination in plant tissue. NA standards prepared from OSPW were used to spike, prior to extraction, the tissue of alder saplings obtained from the Athabasca oil sands region. The analysis was conducted using a liquid chromatography coupled to a dual pressure linear ion trap Orbitrap mass spectrometer, equipped with ESI interface operated in the negative ion mode. Data were acquired in the full scan mode from m/z 80 to 600. To ensure the efficient deprotonation for negative ion ESI analysis, 10 µl of ammonium hydroxide (29%) was added to each 1 mL of the sample extract. Mass spectral peaks at m/z 195.139, 247.170 and 277.181were chosen to identify the NAs in the plant tissue based on the fact that these three ions were present in the NA standard and the spiked plant extract but were absent in the plant blank . The high mass resolution and accurate mass assignments obtained with the Orbitrap, facilitates the accurate determination of the molecular formula for different isomer groups of NAs. The diagnostic ions, m/z 195.139 and 247.170, correspond to classical NAs, with $C_{12}H_{19}O_2$ indicating Z number of -4 and $C_{16}H_{23}O_2$ indicating a Z number of -8. The third diagnostic ion, m/z 277.181, corresponds to oxy-NAs, with $C_{17}H_{25}O_3$. The mass spectra confirmed a sharp and well-resolved NA profile using a mass resolution of 100,000 compared with the unresolved hump obtained using typical medium mass resolution analyzer of 7,500. This requirement proved to be very important for the quantification of NAs in plant tissue. They suggested that a mass resolution of at least 10,000 is required to resolve oil sands acid-extractable organics from interferences in plant tissue while mass resolution of 30,000 is required to resolve acid-extractable OSPW components. Using an NA standard prepared from OSPW, the calibration plot using the diagnostic ion m/z 195.139 provided good linearity over the range from 1 to 100 mg/kg dried plant tissue with an R^2=0.9998.

4.4.3.2 Time of Flight (TOF)

Time-of-flight mass spectrometry (TOF-MS) is a method of mass spectrometry in which the mass-to-charge ratio of an ion is determined via the travelling time of the charged ion. It is based on the simple fact that ions with the same energy but different masses travel with different velocities. Ionized species starting from the same position at the same time are accelerated by a constant homogeneous electric field of known strength. This acceleration results in ions with the same charge having the same kinetic energy.

The velocities of ions travelling over a drift path indicate their masses:

$$t = \left(\frac{2md}{eE}\right)^{1/2} + L\left(\frac{m}{2eV_0}\right)^{1/2}$$

where m is the mass of particle, e is the electronic charge, E is the electrostatic field applied in the ion source, d is the length of accelerating region, L is the length of field-free region and V_0 is the accelerating potential. This means that lighter masses reach the detector before the heavier ones. The ability to sort the masses by the time needed to reach the detector means that a mass spectrum can be recorded. In a more sophisticated design, the TOF analyzer corrects for small differences in initial energy and angle to achieve high mass resolution. Combinations of linear drift paths and electrostatic sectors or ion mirrors enable mass resolutions above 10,000 to be achieved. The major advantage of this mass analyzer is the theoretically unlimited mass range and its very high scanning speed which enables ultrafast chromatographic methods to be used with this MS.

Comprehensive two dimensional gas chromatography (GC×GC) is a chromatographic method which best utilizes TOF-MS. GCxGC was developed as early as the 1990's (Liu and Philips 1991). It offers an unprecedented degree of improvement in chromatographic peak capacity that can be useful for the analysis of complex mixtures such as NAs. GC×GC combined with TOF-MS allows the acquisition of up to 500 mass spectra per second and permits the accurate profiling of each GC×GC peak.

Hao et al. (2005) developed GC×GCTOF-MS protocols for characterization and differentiation of two commercial NAs (Fluka and Acros) and an NA sample extracted from Syncrude tailings. The commercial NAs and the tailings NAs were derivatized with boron triflouride-methanol to form methyl esters. The GC instrument was modified with a secondary oven and a dual-stage jet modulator. Naphthenic acid samples were partly separated by the first column. The modulator continuously concentrated the eluents from the first column and injected them into the second column every 3 sec for further separation. This configuration of GC×GC analysis eliminates the possibility of column overloading while giving the highest selectivity and sensitivity possible for NA analysis. The software can automatically combine modulated peaks for one component according to its retention times and unique mass spectrum. Contour plots of chromatographic distributions of different Z homologous series of the Fluka, Acros and Syncrude NAs were constructed using fragment ions that were characteristic of NA molecular structure. As expected, retention time increased with the numbers of carbons and the numbers of rings. Well-ordered patterns were observed for NAs of Z=0 and Z=-2 series. The contour plot shows that low MW components corresponding to Z=0 and Z=-2 homologues of NAs decreases significantly from Fluka to Acros and was the lowest for Syncrude NAs. However, the pattern of high MW components corresponding to Z=0 and Z=-2 homologues are very similar among these three NA sources. If distinctive patterns of NAs from different sources can be shown it may be possible to use pattern recognition to identify the origin of NA contamination, an important feature for forensic applications.

Rowland et al. (2011a) also applied GC×GC coupled with TOF-MS to study NAs extracted from OSPW. They found that hundreds of the components are sufficiently well resolved by GC×GC TOF-MS method to enable identification of individual isomers. Potentially interpretable and library-searchable mass spectra could be obtained. NAs were derivatized by refluxing with BF_3-methanol to form methyl esters of the NAs. GC×GC TOF-MS analysis was conducted in positive ion electron ionization mode and calibrated with perfluorotributylamine. The scan speed was 50 Hz and the resolution of the mass spectrometer was 1,000 at mass 1,000. Samples were injected into the GC and the temperature was two-stage programmed to optimize the chromatographic separation. The mass spectra clearly showed that NAs from oil sands process water comprised mainly C_{11-19} bi- to penta-cyclic acids. These authors also found that NAs from OSPW contain an extensive series of diamondoid tricyclic acids. Structures of adamantane-1-carboxylic acid and 3-ethyl-adamantane-1-carboxylic acid (Figure 4) were reported with some other tentative identification of methyl, dimethyl, and ethyladamantane carboxylic acids and adamantane ethanoic acids isomers. Rowland et al. (2011b,c) used the same GC×GC TOF-MS method to further identify individual tetra- and pentacyclic NAs in oil sands process water and individual acids in commercial NA samples. They suggested the diamondoid adamantane acids were the result of biotransformation of the corresponding alkyl-adamantane hydrocarbons. Using the same method, they identified numerous pentacyclic NAs as diamantane (Figure 5) and alkyldiamantane acids. Tentative structures for some of the tetracyclic acids formed by ring-opening of alkyldiamantanes were suggested. The study of a commercial NA originally refined from petroleum confirmed that oil sand process water and refined petroleum contain very different distributions of acids, although some of the diamondoid acids were detectable in both. This provides a basis for future studies of the petroleum geochemistry, toxicities and environmental impacts of the acids. GC×GC TOF-MS has been shown to be a powerful tool in elucidating the complex distributions of NAs in oil sands process water and in facilitating the identification of the source of NAs in contaminated surface waters, a potentially important feature when analyzing forensic samples.

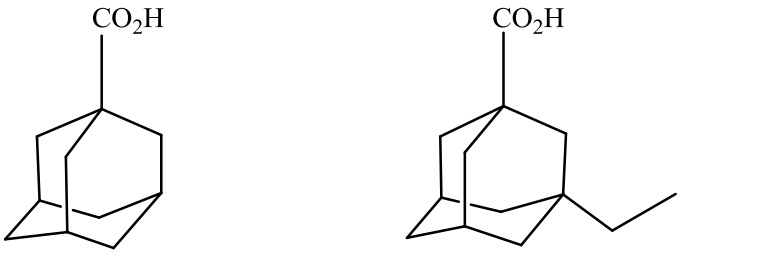
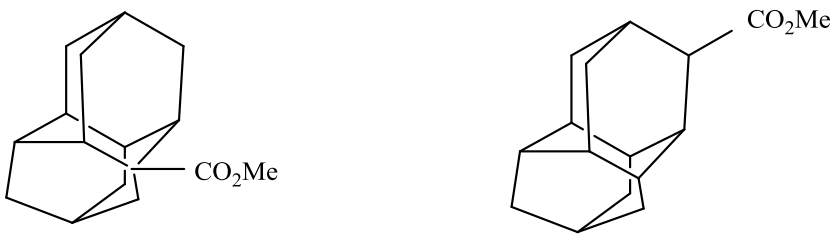


Figure 4. Structures of two individual NAs were identified by Rowland et al. (2011a) by GC×GC TOF-MS.



diamantane-1-carboxylic acid (methyl ester) diamantane-3-carboxylic acid (methyl ester)

Figure 5. Structures of methyl esters of pentacyclic acids positively identified in OSPW NAs by comparison of the spectra and GC×GC retention times with reference acids (methyl esters).
After Rowland et al. 2011c.

4.4.3.3 Quadrupole Time of Flight (QTOF)

QTOF is a hybrid mass analyzer that is more correctly represented with the acronym QqTOF. This is very similar in design to the triple quadrupole MS, discussed previously, with the exception that Q_3 has been replaced by a TOF mass analyzer. The first quadrupole, Q is the mass resolving quadrupole and can be used for the isolation of precursor ions. The q component refers to a radiofrequency (rf)-only cell which with a sufficiently high pressure of several millitorr to effect collisional focusing. The focusing of the collision induced fragment ions and possibly parent ions is particularly important for the TOF analyzer to achieve optimal mass resolution (Chernushevich et al. 2001). When chromatography is used with QTOF it is generally HPLC.

Headley et al. (2009a) made a comparison of low, high (~10,000), and ultrahigh-resolution (~400,000) mass spectrometry as a common tool for investigating the complex organic compound mixtures in OSPW. The study found that dissipation of oil sands NA isomer groups was masked using low resolution (1,000) ESI mass spectrometry by components such as fatty acids and heteroatomic species containing oxygen (O_3, O_4, O_5, O_6, and O_7), sulfur, and nitrogen. These heteroatomic species could not be resolved by low resolution ESI-MS, leading to misclassification. High-resolution QTOF-MS and ultrahigh resolution FTICR-MS with negative ESI ionization both provided evidence for the selective dissipation of components in oil sands process water. With ultrahigh mass accuracy and resolution of FTICR-MS, 95% of all observed species could be assigned by molecular formula providing information of the complete heteroatom content ($N_nO_oS_s$) and C_nH_{2n+Z} assignments.

A reversed-phase capillary HPLC QTOF-MS method (Bataineh et al. 2006) was developed to characterize the complex NAs in tailings water from the clarified zone of Syncrude West In Pit and their microbial transformation. NAs were extracted from tailings water by SPE method and

dried by nitrogen. The extracts obtained from 100 mL tailings water were dissolved in 60% methanol containing 0.1% formic acid and brought to a final volume of 1 mL for analysis of the NAs using HPLC QTOF-MS without a derivatization step. Chromatographic separation by HPLC prior to QTOF-MS gave significant quantitative and qualitative advantages over direct infusion MS analysis. The response linearity over the range of 100 to 2,500 µg/mL was much better for the calibration curves based on HPLC peak areas of model compounds ($r^2 > 0.98$) than those constructed by direct infusion based on peak intensity (r^2 between 0.74 and 0.88). Analytical sensitivity (slope of calibration curves) was increased between 40 to 350 fold using HPLC compared to direct infusion. Both HPLC retention times and QTOF-MS detection gave far more qualitative information than that from direct infusion. In general, chromatographic retention times were influenced by carbon number, cyclization (i.e., Z-series), and degree of alkyl branching. Increasing carbon number resulted in increased retention time, whereas increased cyclization (i.e., more negative Z) and degree of alkyl branching within each isomer class resulted in decreased retention time. The dynamic range of this method over 0.1 to 100 mg L^{-1} gave $r^2 > 0.99$ for individual isomer classes, indicating that this method could be used quantitatively. In an interlaboratory study, data from established GC-MS techniques were compared to data produced by HPLC/QTOF-MS for Refined Merichem NAs and tailings water. For Refined Merichem NAs, the profile produced by HPLC QTOF-MS was similar to the profile produced by GC-MS. The most obvious difference was the absence of NAs below n=7 by HPLC QTOF-MS, whereas GC-MS detected ions at n=5, 6, and 7 in the Z=0 series. However, more substantial differences were observed when comparing profiles of tailings water by these two methods. The most intense masses by HPLC QTOF-MS were for Z=-4 (36%) and Z=-6 (36%), whereas most abundant masses by GC-MS were for Z=0 (21%), Z=-6 (20%), Z=-4 (17%), and Z=-2 (16%). Many abundant ions at lower (<C9) and higher (>C22) carbon numbers, including the prominent "C22+ cluster", were observed by GC-MS and also proved by other unit-resolution infusion techniques (Frank et al. 2006, Hao et al. 2005, Holowenko et al. 2002, Lo et al. 2006) regardless of sample source, but were absent by HPLC/QTOF-MS. Bataineh et al. (2006) believed that NAs appearing in the "C22+ cluster" observed by GC-MS were artifacts caused by double derivatization of hydroxylated NAs. Thus, they suggest that unit resolution MS methods should only be used for profiling purified commercial NAs and HRMS techniques are more suitable for characterization of more complex mixtures like tailings water and background environmental samples.

Moreover, another advantage of HPLC QTOF-MS over unit-mass spectrometry methods was its capability to detect the oxidized products in the same chromatographic run (Frank et al. 2006, Hao et al. 2005, Holowenko et al. 2002, Lo et al. 2006). Biodegradation of Refined Merichem and NAs from tailings water over 28-days was studied by HPLC QTOF-MS. Depletion of the least alkyl substituted NAs were observed in the commercial mixture but there was no noticeable degradation for any isomer class in the tailings water incubations. This confirms that NAs in tailings water are more recalcitrant to degradation, suggesting that tailings water microorganisms preferentially deplete the least alkyl-substituted fraction. The structure-related biodegradability of NA was also confirmed in subsequent studies using the HPLC QTOF-MS method (Han et al. 2008, 2009).

Martin et al. (2008) conducted an inter-laboratory comparison of ESI-MS and HPLC ESI-QTOF-MS analytical methods for NA mixtures obtained from both environmental water systems and commercial sources. Both methods produced similar NA profiles for commercial Refined Merichem NA mixtures. However for the NAs in tailings water, the profiles produced by the two methods were very different, ESI-MS reported a relatively large portion of very small ($<C_{10}$) and very large ($>C_{20}$) NAs in the OSPW whereas HPLC ESI-QTOF-MS reported a very small percentage of these fractions. Between C_{10} and C_{20}, Z=0 series were almost absent by HPLC ESI-QTOF-MS while direct ESI-MS reported nearly 15% contribution. Additionally, the Z=-2 series accounted for only 3.7% contribution when analyzed by HPLC ESI-QTOF-MS, but 19% by direct injection ESI-MS. Overall, profiles of NAs from tailings water (collected from an unnamed oil sands extraction operation) produced by direct injection ESI-MS were very similar to those produced by unit-resolution GC-MS. Martin et al. (2008) concluded that unit-resolution mass spectrometry resulted in substantial false-positives and misclassifications when used to characterize environmental mixtures of NAs in aqueous samples. For quantification, both methods provided a response that correlated with the concentrations determined by FTIR method, thus could give a semi-quantitative estimations.

4.4.3.4 Fourier Transform Ion Cyclotron Resonance (FTICR)

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), also known as Fourier transform mass spectrometry (FT-MS), is a type of mass analyzer (or mass spectrometer) with high resolution and high accuracy for determining the mass-to-charge ratio of ions based on the cyclotron frequency of the ions in a fixed magnetic field. It differs significantly from other mass spectrometry techniques in that the ions are not detected by hitting a detector. The masses are resolved only by the cyclotron (rotational) frequency produced by each ion as it rotates in a magnetic field and all ions are detected simultaneously over some given period of time. In the basic FTICR-MS instrument, the ions are generated in the source (as usual) and then pass through a series of pumping stages at elevated vacuum. The ions enter the cell (ion trap) located inside a spatially uniform static superconducting high field magnet (typically 4.7 to 13 Tesla) cooled by liquid helium and liquid nitrogen. Pressures are in the range of 10^{-10} to 10^{-11} mBar with temperatures close to absolute zero. When the ions pass into the magnetic field they are bent into a circular motion in a plane perpendicular to the field by the Lorentz Force and are trapped in a Penning trap (a magnetic field with electric trapping plates) where they are excited to a larger cyclotron radius by an oscillating electric field perpendicular to the magnetic field. In their larger orbits, each group coalesces into a packet. The oscillations continue as the packets follow a spiral-like path, reaching a maximum radius close to the detector plates and the signal is detected as an image current on a pair of plates. The resulting signal is called a free induction decay (FID), transient or interferogram that consists of a superposition of sine waves. The useful signal is extracted from this data by performing a Fourier transform to give a mass spectrum. FTICR is useful in dealing with complex mixtures since the resolution (narrow peak width) allows the signals of two ions of similar mass to charge (m/z) to be detected as distinct ions. The high resolving power of the FTICR is extremely useful when dealing with large molecules which

contain a distribution of isotopes. FTICR is relatively new; however, the machines are bulky and very expensive.

Barrow et al. (2004) demonstrated that FTICR-MS utilizing high magnetic fields are inherently capable of ultra-high resolution and mass accuracy, affording unequivocal ion assignment. Two commercial NAs (Fluka and Acros) and one tailings sample from Syncrude were characterized using negative mode nanospray ionization FTICR-MS. The relative average mass errors for Acros, Fluka and Syncrude tailings sample were 0.57, 0.52 and 1.14 ppm, respectively. The total percentage of signals with a mass accuracy of 1 ppm or better for Acros, Fluka and Syncrude tailings sample were reported to be 87.32%, 93.11% and 76.12%, respectively. FTICR-MS can assign ions to molecular formulae unequivocally with ultra-high resolution of 1,000,000. High field FTICR-MS can reach resolutions of hundreds of thousands in "broadband mode". Barrow et al. (2004) confirmed that the Syncrude tailings sample was more complex than the commercial Acros and Fluka samples. For Syncrude tailings sample, $C_nH_{2n-12}O_2$ family (six rings) was surprisingly intense with a maximum being located at 18 carbon atoms though $C_nH_{2n-4}O_2$ (two ring) and $C_nH_{2n-6}O_2$ (three ring) families were the most intense. While for Acros and Fluka NAs, $C_nH_{2n-0}O_2$ and $C_nH_{2n-4}O_2$ were the most intense families, respectively.

Headley and Peru (2007) investigated the significant influence of solvents using negative ion ESI-FTICR-MS to characterize NAs present in natural waters. ESI-FTICR-MS exhibited similar mass spectra when using the acetonitrile (only), methanol/acetonitrile (1:1), and Milli-Q water/acetonitrile (1:1) solvent systems. For most runs, the distribution of molecular weights was similar with a number-average molecular weight of ~260 and a predominance of NAs in the range of m/z ~180 to 300, indicating these polar solvents were well suited for the polar fraction of oil sands NAs. When two less-polar solvent systems were used, the mass spectra exhibited a smaller number peaks for a dichloromethane/acetonitrile (1:1) system and a larger number of peaks for a 1-octanol/acetonitrile (1:1) system. Using the results obtained from Milli-Q water/acetonitrile as a benchmark, it showed that the $C_nH_{2n+z}O_2$ species are relatively less intense in a 1-octanol/acetonitrile system while $C_nH_{2n+z}O_4$ species are relatively less intense in a dichloromethane/acetonitrile system. This difference was attributed to the selective solubilities of some components. 1-octanol/acetonitrile shows apparently higher solubility of some components having molecular weights above 300 amu and the apparent enrichment of z=-6 series for dicarboxylic or $C_nH_{2n+z}O_4$ NAs may be due in part to differences in polarity and of the NAs. These findings imply that the more soluble components in 1-octanol are expected to be more bioavailable for ingestion and less persistent in tailings, oil sands sludges, and groundwater. Thus the different solubilities of various NAs in organic solvents with different polarities may reflect the preferential availability for uptake by biota in aquatic systems and could be used as a guide in the isolation of principal toxic components of NAs. This information may be very useful when applied to the remediation of oil sands contaminated soils and groundwater.

4.5 Other Detection Methods

4.5.1 *Flame Ionization Detection (FID)*

Jones et al. (2001) extracted NAs from North Sea Oil (provided by Enterprise Oil PLC, U.K.) using a strong anion exchange (SAX) quaternary amine solid phase extraction ion exchange column and derivatized the NAs to esters either by BF_3 in methanol or by diazomethane. The derivatized sample extract was further cleaned up by using a silica SPE column (IST Technologies). The column was conditioned with hexane (5 to 10 mL). The derivatized sample extract was loaded on the column and the interferences were eluted with hexane washing (using minimum amount, 4 mL). The carboxylic acid methyl esters were eluted with hexane/dichloromethane and concentrated by rotary evaporation. The esters were subject to GC analysis using the methyl ester of 1-phenyl-1-cyclohexanecarboxylic acid as the internal standard. 5β-cholanic acid was used as surrogate standards and the recoveries of the surrogate standards were taken into account in the measurement. The GC analysis of the carboxylic acid methyl esters was performed on a gas chromatograph equipped with a cold on-column injector and a flame ionization detector (FID). Acids fractions were quantified by measurement of the area of the unresolved hump between 5 and 80 min above the baseline of a blank (dichloromethane) against the area of an internal standard.

Herman et al. (1994) employed GC analysis to investigate the biodegradation of NAs. The NAs were extracted from oil sands tailings samples by liquid-liquid extraction procedure. The acid extracts were evaporated under nitrogen stream to remove the residual organic solvent and then mixed with methanol and concentrated H_2SO_4. The mixture was refluxed to derivatize the carboxylic acids by a procedure adapted from Fedorak and Westlake (1983). After cooling, water was added into the reaction mixture and the methyl esters were extracted into hexanes (pesticide grade) and dried over Na_2SO_4. Routine analyses of derivatized extracts were performed using a gas chromatograph with a FID. Methyl ester of stearic acid was used as the internal standard. GC analysis yielded an unresolved hump. Integration of the unresolved hump area was divided by the area of the internal standard.

4.5.2 *UV-Vis Diode Array Detection*

Clemente et al. (2003b) developed an HPLC method to quantify NAs so that the removal of NAs from aerobic laboratory cultures could be followed. The NA samples were derivatized with 2-nitrophenylhydrazide (2-NPH) in the presence of 1-ethyl-3(3-dimethylaminopropyl) carbondiimide hydrochloride (1-EDC·HCl). The derivatized sample was then injected into an HPLC for analysis. The UV-visible diode array detector was set at 400 nm (with a band width of 10 nm), and the reference wavelength of 510 nm (with bandwidth of 60 nm). Like the GC analysis mentioned above (Herman et al. 1994, Jones et al. 2001), HPLC also yielded an unresolved hump. The quantitation was made by integrating the area under the unresolved hump between 2.9 min and 6.0 min and comparing with the area under the humps of known concentrations of derivatized commercial preparations. High background area of the reagent blanks limits the ability to measure low concentration of NAs. The reported detection limit was 15 mg L^{-1}.

Yen et al. (2004) modified the derivatization step by altering concentrations and proportions of the 2-NPH and 1-EDC·HCL reagents and the volumes of aqueous sample to improve the sensitivity of the modified HPLC method. A modification of the mobile phase for gradient elution reduced the baseline disturbance and decreased the background area of the reagent blanks. The detection limit of the modified method was reported to be 5 to ~10 mg L^{-1}. For comparison, 58 water samples were analyzed by both FTIR method and modified HPLC methods with Kodak acids used for calibration. In general, there was good agreement between the two methods, with FTIR method giving slightly higher concentrations in many cases.

5 DATA PRESENTATION METHODS

Since NAs are such a complex mixture, the analysis of one of these mixtures can yield a huge amount of data. Many different methods are used to present the analysis in the literature. The most common methods used to present data by MS analysis are summarized below.

5.1 Mass Spectrum

Some papers simply present the mass spectrum with relative abundance on the y-axis and m/z values on x-axis (Figure 6)(Frank at al. 2006, Grewer et al. 2010, Headley and Peru 2007, Scott et al. 2009, Smith and Rowland 2008, St. John et al. 1998).

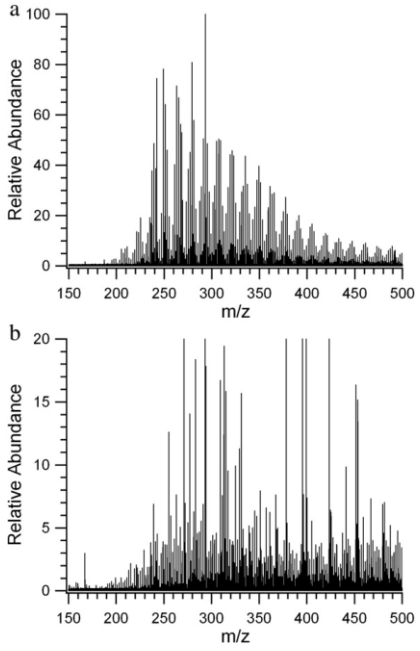


Figure 6. An example of NAs mass spectrum.
ESI-FTICR-MS analyses of well water #13 (a) and water percolated through horseshoe Canyon sub-bituminous coal (b) (Scott et al. 2009)

5.2 Data Presented In Tables

The table is designed with carbon numbers in the first column and Z number in the following columns. Percentage of NAs or relative intensive of each m/z value in the cell corresponds to the appropriate carbon and Z numbers (Lo et al. 2003, St. John et al. 1998).

5.3 2-D Chart: Relative Intensity Versus Carbon Number

Data are reported with relative intensity versus carbon number (Figure 7)(Barrow et al. 2003, 2010, Headley and Peru 2007). This yields a curve for each Z number. Other similar methods include percentage of NAs versus carbon number (Hsu et al. 2000, Lo et al. 2006) and normalized signal intensity versus molecular weight (Rogers et al. 2002a), all yielding curves for each Z number.

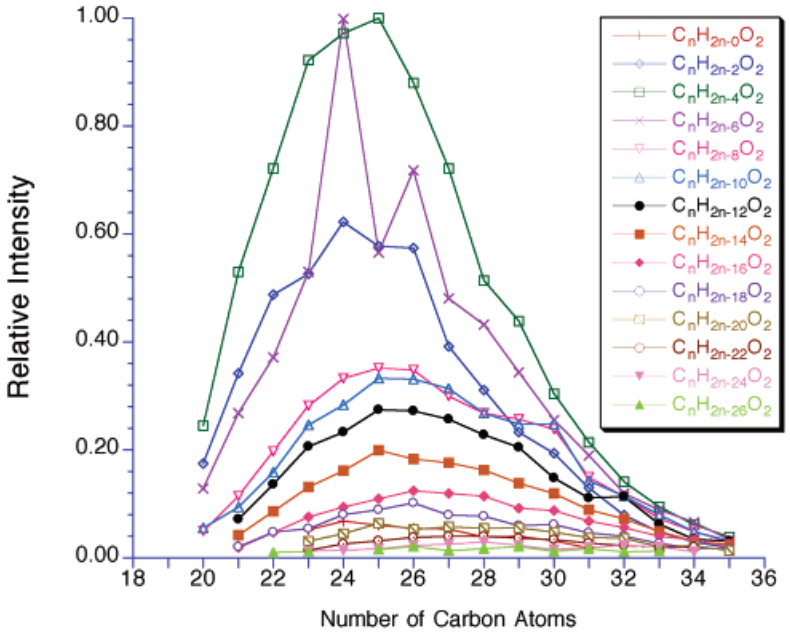


Figure 7. An example of 2-D chart percentage of NAs versus carbon number. Plot of the different naphthenic acid families observed in the oil field B spectrum (Barrow et al. 2003).

5.4 Pie Chart

Pie charts are used to present the percent distribution of each Z series to the total NA response (Martin et al. 2008, Rogers et al. 2002a).

5.5 3-D Plot

Recently, 3-D plots with carbon numbers on the X-axis, Z number on the Y-axis and percentage of total response on the Z-axis have been extensively used (Bataineh et al. 2006, Clemente and Fedorak 2004, Clemente et al. 2003a, Han et al. 2008, 2009, Holowenko et al. 2002, Lo et al.

2003, 2006, Martin et al. 2008, Scott et al. 2005). The sum of all bars is 100%. This 3-D plot provides good visual exhibition and helps to spot the differences among mixtures conveniently (Figure 8).

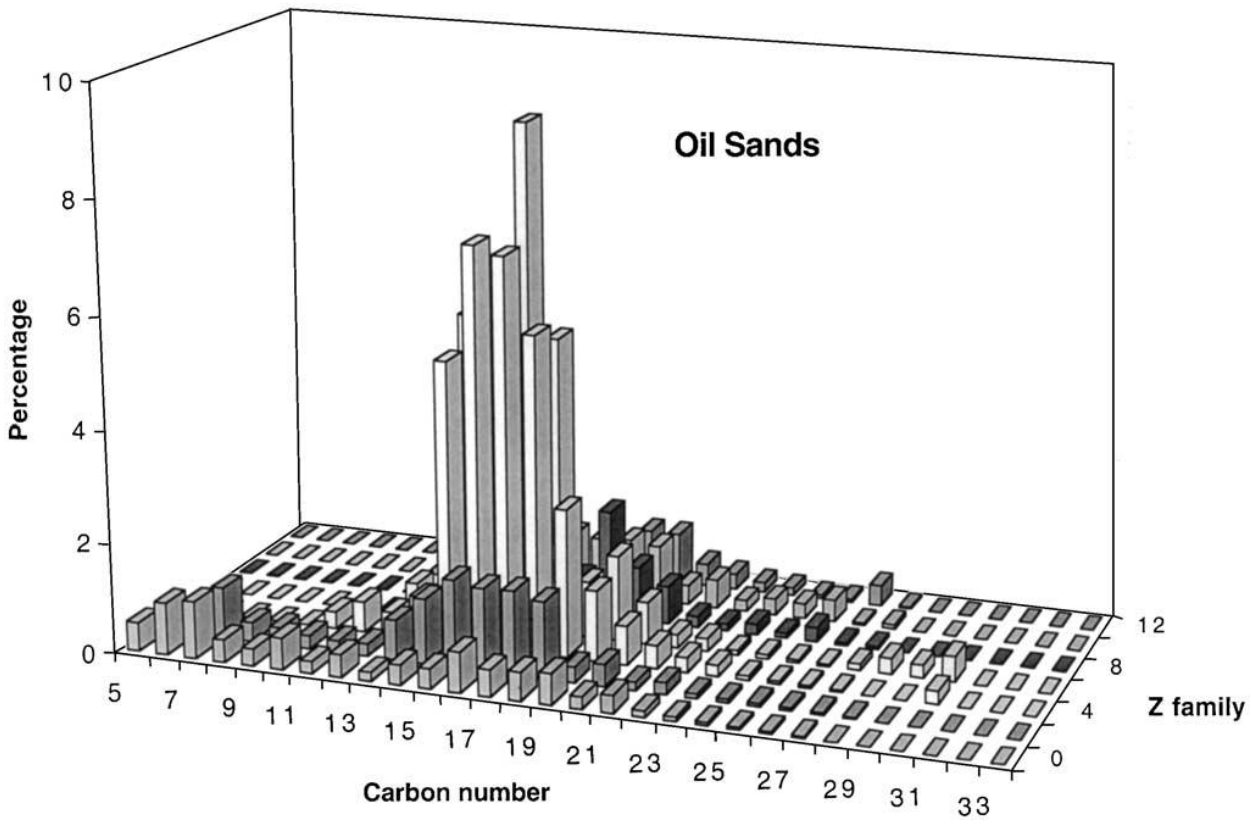


Figure 8. An example of 3-D plot. "Distribution carbon numbers and Z families of NAs in the complex NAs mixture extracted and derivatized from oil sand ore.
The bars represent the percentage (by number of ions) of NAs in the mixture that account for a given carbon number of a given Z family (corresponding to a specific m/z value from GC-MS analysis). The sum of all the bars equals to 100% (Holowenko et al. 2002).

Other methods are used when high and ultrahigh resolution MS is employed to give more details about the structure of complex NAs.

5.6 2D-Double-Bond Equivalent (DBE) Versus Carbon Number

DBEs versus carbon numbers is used to present the relative abundance of each NA within the mixture (Figure 9)(Headley et al. 2009a). DBE for $C_nH_hN_nO_oS_s$ is calculated using the following equation:

$$DBE = c - \frac{h}{2} + \frac{n}{2} + 1$$

It should be noted that information may be lost where different families of compounds overlaps in terms of DBE since divalent atoms, such as sulfur and oxygen, do not influence the DBE value. Higher DBE range potentially demonstrates the presence of naphthenoaromatic species. It is more broadly used when characterizing NAs in oils (Barrow et al. 2009, Mapolelo et al. 2011, Smith et al. 2008).

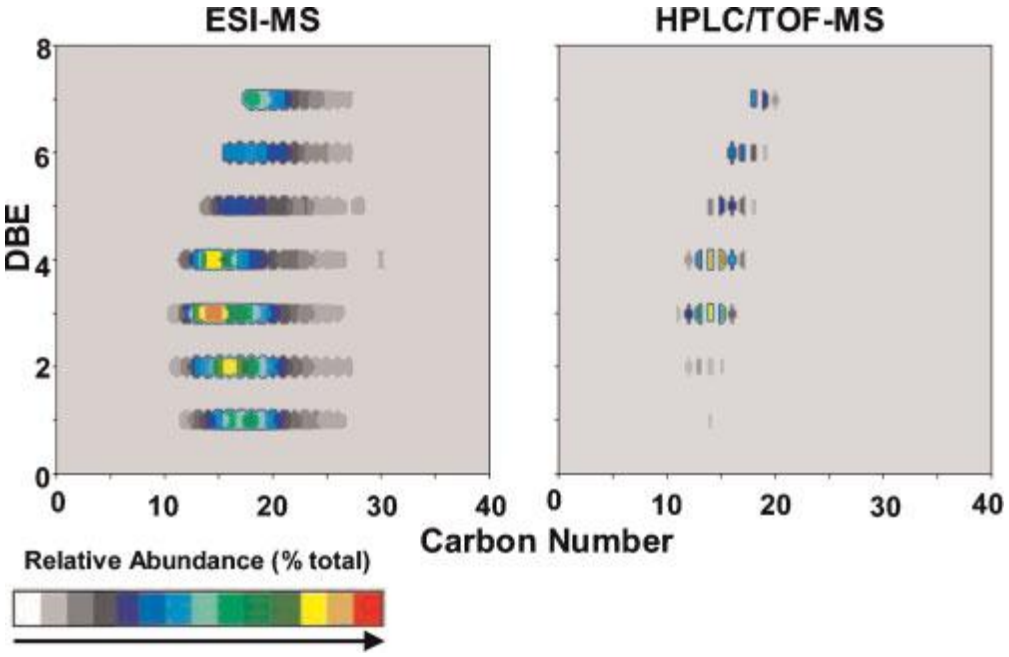


Figure 9. An example of 2D double-bond equivalent (DBEs) versus carbon number. NA profile for oil sands processed water from Athabasca oil sand region, obtained by low-resolution ESI-MS and high-resolution HPLC QTOF-MS (Headley et al. 2009a). This figure is available in color online at www.interscience.wiley.com/journal/rcm.

5.7 2D-Kendrick Mass Defect Versus Nominal Kendrick Mass

Kendrick (1963) introduced an alternative mass scale in order to easily classify families of compound within complex mixtures. The IUPAC mass scale defines ^{12}C as having a mass of exactly 12 Da. The Kendrick mass scale defines CH_2 as having a mass of 14.00000 Da instead

of IUPAC value of 14.01565Da. To convert an IUPAC mass to the Kendrick mass scale, use the following equations:

$$\text{Kendrick mass} = \text{IUPAC mass} \times \frac{14.00000}{14.01565}$$

$$\text{Kendrick mass defect} = \text{nominal Kendrick mass} - \text{exact Kendrick mass}$$

where exact Kendrick mass is the value obtained from the conversion equation while the nominal Kendrick mass is the Kendrick mass of the compound rounded to the nearest integer.

Barrow et al. (2010) presented analysis data in a plot of Kendrick mass defect versus nominal Kendrick mass with the relative intensity in a color scale for the mass spectra and showed clearly that the positive mode in both ESI and APPI gave a greater number of species, where the heteroatom content and Z-homologues are constant and lie along horizontal lines with adjacent points differing by one CH_2 unit.

5.8 Heat Map

A heat map is a graphical representation of data where the values taken by a variable in a two-dimensional table are represented as colors. Relative intensity is given in a color scale. Barrow et al. (2010) presented their data in heat maps for single nitrogen- and sulfur-containing, hydrocarbon, O_3S-containing, NO-containing, and NO_2-containing species observed using the APPI source in both positive-ion and negative-ion modes. Carbon numbers are displayed on the X-axis and species with different Z-homologues are displayed on the Y-axis. Barrow et al. (2009) also used heat map to present their data for Athabasca oil sands sample. The color scaled map helps data visualization and makes it easy to identify the most abundant species.

5.9 2D-Van Krevelen Diagram

The diagram is plotted as hydrogen/carbon (H/C molar ratio) versus oxygen/carbon (O/C molar ratio). Bataineh et al. (2006) employed van Krevelen diagrams to show parent NA compounds and their oxidized products in Syncrude tailings water. The 2D-Van Krevelen diagram graphically separated NA congeners into their respective Z-series (top to bottom) and arranged each homologous Z-series by increasing carbon number (from right to left). The hydroxylated species can be plotted on the same axes based on their H/C and O/C ratio, only shifting horizontally to the right from their precursor. Barrow et al. (2009) also used 2D-van Krevelen diagrams to present the NAs from Athabasca oil sands samples.

Other methods, such as 3D-contour plot (Hao et al. 2005), principal component analysis (Barrow et al. 2009, Grewer et al. 2010) and t-test (Clemente et al. 2003a) were used to provide useful information.

6 NAPHTHENIC ACIDS ANALYSIS WORKSHOP AND SURVEY

A workshop on analytical strategies for NAs was organized by Environment Canada and held in Saskatoon, Saskatchewan on November 24 to 25, 2011 to meet the high demands for communication among different laboratories about new developments in analytical methods for NAs. The workshop invited NA researchers from around the world to present their latest research. In the workshop inter-laboratory comparisons and issues were discussed and communicated.

Using the contact information from this meeting a survey form regarding NA analysis was prepared by NAIT to cover as many factors as possible that might influence the qualitative and quantitative analytic results. This included sampling, sample storage conditions, sample clean-up, NA extraction procedure, sample preparation before analysis, to detailed conditions and techniques for a variety of analytical methods. The form was in a fillable Adobe format with checkbox, drop-down lists, click to type text, and buttons to facilitate access to the appropriate sections of the survey.

The NAs Analysis Survey was circulated among all the attendees of the workshop (48 in total including 18 from 3 federal government organizations, 16 from 8 universities (including 2 attendees from 2 universities outside of Canada), 10 from 6 commercial labs, 2 from 2 provincial government organizations, and 2 from others (organization/company information not collected)) starting on January 7, 2012. There were eleven completed survey forms submitted by March 12, 2012. The eleven responses cover almost all the types of organizations/companies attending the workshop – 3 out of 3 federal governments, 1 out of 2 provincial governments, 5 out of 8 universities (including 1 from outside of Canada), and 2 out of 6 commercial labs.

Summary of the survey is based on eleven completed responses. Detailed information on the organizations/companies is not disclosed due to confidentiality. Only general information is selectively summarized. The objectives of this survey were:

- to provide a snapshot of the variety of labs currently conducting NA analysis and research,
- to provide information about the variety of NA analytical methods currently performed in different labs,
- to help policy regulators relate the complexity of the NAs found in OSPW and the challenges this presents to all labs engaged in the development of suitable analytical procedures,
- to accurately determine the unique characteristics of acid extractable NAs found in OSPW, and
- to facilitate future discussion on collaborative efforts to assess the utility of the methods used in various labs to analyze NAs.

6.1 Laboratory Information

The eleven completed responses include three different federal government labs, one provincial government lab, five university labs (2 different labs from one university and 3 labs from three other universities), and two commercial labs. No college/technical institute is reported in the survey. Among the eleven responses, only two labs, one commercial lab and one federal government lab, are accredited for NA determination.

The number of samples processed annually in a particular lab depends on the type of laboratory and the type of clients it serves/works for:

- University labs, working with clients from research labs, government, industrial clients, and consultants for industrial clients, process 30 to about 100 samples annually,
- Federal government labs, working with clients from industry, government, research labs, or monitoring organizations depending on the lab, report analyzing 200 to 350 samples annually,
- The provincial government lab, working with clients from industry, consultant, government, and monitoring organization, reports to analyze about 250 samples annually,
- Commercial labs, covering almost all the client types from industry, consultant, government, research lab and monitoring organization, have the ability to process hundreds to more than 20,000 samples annually.

The analytical cost for each sample ranges from C$150 to C$400 depending on the lab and also on the sample preparation and requested final reports.

6.2 Sampling Information

The group(s) responsible for sampling may include in-house labs, third-party Company or industrial clients, depending on the type of clients the lab works/serves for.

One response indicates that it does not do sampling and sample preparation (sample has to be ready for analysis). The other ten responses indicate the sample sources from tailings pond, nine among them indicate the sample source from surface water, eight among them indicate sample source from groundwater. Three labs indicate biological samples and only one lab indicates soil samples. One lab indicates that the method to analyze soil and tissue samples is under development and may be available later this year.

Among the ten responses, all labs indicate grab sampling method irrespective of the sample sources (tailings pond, surface water or groundwater). To store grab samples, eight labs use glass containers (2 labs use only clear glass containers, 5 labs use only amber containers, and 1 lab uses both clear and amber containers) and three labs use plastic containers (2 labs use only clear plastic containers and 1 lab uses both clear and amber containers). One lab chooses to use stainless steel containers but didn't disclose the seal type of the containers.

One lab indicates integrated (passive) sampling method from tailings pond, surface water and groundwater with POCIS (polar organic chemical integrative sampler) of the pharmaceutical type for more than 28 days (exposure time is specified by the client). The same lab also indicates forensic sampling methods are used. Another lab indicates passive sampling method from surface water and groundwater but didn't disclose any more information.

Eight labs provided detailed information about sample storage conditions. Seven labs store the aqueous samples at about 4°C and only one lab at ambient temperature. For the passive samples, the lab stores them at -20°C. All of the eight labs store the samples without organic solvent addition in containers sealed with screw caps. All labs store the samples at pH equal to or higher than 7.5 (2 labs at pH 7.5, 1 lab at pH 8, 1 lab at pH 8.5, 1 lab at pH 9, and 3 labs at pH $\geq$ 10.5). Five of the eight labs indicate that samples are stored without pH adjustment and the other three labs use ammonium hydroxide to adjust pH (1 lab also includes phosphoric acid for pH adjustment). One lab stores samples from groundwater at pH 6.5 to 7.5 (without pH adjustment).

Three labs report the maximum sample storage period before extraction and analysis is less than 2 weeks, two labs report less than 1 month, and one lab reports less than 6 months. The other two labs didn't answer.

6.3 Sample Preparation Prior to Analysis

For sample preparation prior to analysis, seven labs report that NA extraction and/or clean up procedures are used while the other four labs report that no sample preparation is done except for a possible dilution step. One lab indicates that no sampling and sample preparation (sample has to be ready for analysis) is done and recommends that clients follow the procedures used in Dr. Fedorak's lab[3], i.e., a double liquid/liquid extraction procedure.

6.4 NA Extraction Procedures

NA extraction procedures are tedious, including many factors and steps – such as surrogate/internal standard addition, solids treatment, clean-up treatment (prior to or post NA recovery), and extraction method (liquid-liquid extraction or solid phase extraction).

Three labs did not respond to the questions related to surrogate/internal standard addition. Three labs indicate that no surrogate/internal standard is added. Five labs indicate internal standard is added and agree with the definition of internal standard: a compound added after sample preparation and used in the analytical method to determine the response ratio for calibration plot. Four labs indicate surrogate standard is added and agree with the definition of surrogate standard: a compound never found in the sample but added before sample preparation to assess percentage of recovery. But only two of these four labs indicate that the percentage of recovery data is used to correct for the actual NAs concentration in the sample.

Four labs report no sample preparation is done except for a possible dilution step. One lab didn't disclose any more information about clean-up procedures. All four labs indicate the solids in the

[3] Dr. P.M. Fedorak, University of Alberta.

sample are removed at acidic pH. One lab among them indicates centrifugation is used for solid removal. Three labs indicate that no clean-up treatment is done before NA recovery. The clean-up treatment after NA recovery varies with the labs, from on-line SPE (available if required by customer), to centrifugation at 5,000 rpm, to filtration using 0.2 µm syringe filter.

Among the other seven labs that report extraction and/or clean-up procedures are used, three labs indicate that the solids are removed without pH adjustment (1 lab uses centrifugation, 1 lab uses filtration with glass microfiber filter papers, and 1 lab uses both centrifugation and filtration with glass microfiber filter papers), two labs indicate removing the solids at acidic pH, one lab indicates removing the solids at an alkaline pH by centrifugation, and 1 lab indicates that no solids are removed. Among these seven labs, two labs indicate no clean-up treatment before NA recovery and the other three labs clean-up the sample before NA recovery using solvent extraction to remove base and/or neutral organic materials, one lab indicates precipitating NAs by acidification of the sample and discarding the supernatant and removing large molar mass components (e.g., 1,000 molar mass cutoff) by ultrafiltration of an alkaline sample, and one lab didn't disclose any information about clean-up treatment. Also, among these seven labs, three labs indicate no clean-up treatment after NA recovery and the other three labs clean-up the sample after NA recovery by SPE (1 lab uses SAX cartridges and one labs uses Oasis HLB cartridges), and 1 lab didn't disclose any information about clean-up treatment.

Among the eleven labs, four labs indicate no sample preparation is done except for the possible dilution step (dilute and shoot concept), one lab indicates no sample preparation (sample from customer has to be ready for the analysis and the double liquid/liquid extraction procedure used in Dr. Fedorak's lab is recommended), one lab indicates only clean-up but no extraction procedure. Among the five labs reporting NA extraction procedures, three labs indicate liquid-liquid extraction, one lab uses solid phase extraction exclusively, and one lab uses both liquid-liquid extraction and solid phase extraction.

Among the three labs conducting liquid-liquid extraction procedure, all indicate the sample pH is adjusted to very a low value (pH 2 for 2 labs and pH≤1.5 for 1 lab) before liquid-liquid extraction by using either hydrochloric acid (1 lab) or phosphoric acid (2 labs). Two labs use dichloromethane without acid addition to extract NAs at the sample volume to solvent volume ratio of 5 and repeat the extraction 3 times. The other lab uses ethyl acetate/DCM (acidified using a small organic acid) at the ratio of 4 to extract NAs at the sample volume to solvent volume ratio of 2 and extract only once. Two labs do not use drying agent and one lab uses acidified anhydrous sodium sulphate as the drying agent. As for the concentration factor achieved following solvent evaporation and reconstitution, one lab indicates 1/2,000, one lab indicates 1 to 10 depending on downstream analytical method used, and one lab indicates about 100 varying for water and tissue samples and based on expected NA concentration.

Although two labs indicate that a solid phase extraction procedure is used, one lab didn't disclose any detailed information except the concentration factor achieved following solvent evaporation and reconstitution. The concentration factor was up to 500 for grab samples and much larger for passive samples. The other lab gives more information. It indicates that the solid phase extraction method is not automated and cartridges used for extraction are from Waters and

Agilent. 5% methanol is used for conditioning the cartridge and the sample is added in at 0.5 to ~1 mL/min. 5% methanol is also used as wash solvent and the cartridge is not dried. Acetonitrile and 2-propanol are used as elution solvent. The concentration factor achieved following solvent evaporation and reconstitution is 1 to 5 depending on concentration.

6.5 General Chemical Formula Expected to be Found in Oil Sands Process Water Following Sample Preparation

The survey results show that the general formula for "classical" NAs, $C_nH_{2n+Z}O_2$, does not express the complexity of the compounds recovered in the acid extractables from OSPW.

Among the eleven respondents, only two labs indicate that they expect the compounds to follow the general "classical" chemical formula; the other nine labs expect more extensive expressions including all or part of $C_nH_{2n+Z}O_x$ (X=2 to 5), $C_nH_{2n+Z}SO_x$ (X=2 to 6), $C_nH_{2n+Z}NO_4$, polycyclic aromatic compounds (PACs), and aromatic acids. One lab expects compounds containing species of O_7, and multiple S and N. One lab indicates to expect "all neutral (pyrroles) to acidic compounds containing oxygen, sulphur, and/or nitrogen".

6.6 Data Presentation Methods Used

Due to the complexity of NAs and the varieties of analytical methods used, data presentation also shows multiple choices. One lab didn't disclose the data presentation method. Among the other responses, only one lab chooses one data presentation method – 3-D plots with carbon numbers on the X-axis, Z number on the Y-axis, and percentage of total NAs response on the Z-axis. Other labs use more than one data presentation method.

Among different presentation methods, the 3-D plot, principal component analysis, and 2-D bar (amount % versus compound class) are the most used methods (8 out of 10 labs), followed by 2-D charts (relative intensity versus carbon content: 7 out of 10 labs), and van Krevelen 2-D plots (H/C molar ratio versus O/C molar ratio) and Kendrick mass defect versus nominal Kendrick mass 2-D plots (5 out of 10 labs for both 2-D plots). One lab uses double bond equivalent versus carbon number, and another lab uses other methods from an in-house software package.

An unfortunate omission in the survey was the manner in which the quantitative results are reported by the lab. Fortunately one of the labs indicated this omission and stressed the importance of reporting the results as either total NAs or as the concentration or percentage of the individual isomer groups found within the total NAs. The manner in which the concentration of NAs is expressed would be an important consideration if results from different labs are compared.

6.7 Analytical Methods Used

Analytical methods for NAs can be classified into spectroscopy (FTIR and SFS), chromatography (gas chromatography and liquid chromatography), and mass spectrometry (used alone or with chromatography). Among the eleven responses, liquid chromatography (LC) with mass spectrometry is the most used analytical method (6 out of 11 labs), followed by gas chromatography (GC) with mass spectrometry (2 out of 11 labs), mass spectrometry without

chromatography (2 out of 11 labs), Fourier transform infrared spectroscopy (FTIR, 1 out of 11 labs), and synchronous fluorescence spectroscopy (SFS, 1 out of 11 labs). Mass spectrometry is used as a detector for all the labs; other detectors including infrared spectroscopy and fluorescence spectroscopy are reported.

6.7.1 *Fourier Transform Infrared Spectroscopy (FTIR)*

FTIR method is a standard method to analyze NAs in industry. The lab reporting using FTIR dissolves NAs in dichloromethane and monitors the peaks at wave numbers of 1,743 and 1,706. In calibration, the responses of both wave lengths are summed and both peak height and peak area are used. A commercial mixture of NAs is used as a calibration standard and a laboratory blank is analyzed. The detection limit is reported to be 10 mg L^{-1} and the linear dynamic range approximately 10 to 300 mg L^{-1}. The lab indicates the potential for false positive and misclassification because non-classical NAs will be detected with this method.

6.7.2 *Synchronous Fluorescence Spectroscopy (SFS)*

The lab using SFS, prepares samples by dissolving NAs in water (HPLC grade) and then analyzing for NAs by monitoring the peaks at wave lengths of 282.5 and 320 nm. In preparing the calibration plots, a positive correlation is observed between the peak intensities (peak height) with NA concentrations. Both field blanks and laboratory blanks are analyzed. The detection limit is reported to be 1 mg L^{-1} total NAs (aromatic organic acids included) and the linear dynamic range is approximately 1 to 20 mg L^{-1} total NAs (aromatic organic acids included). The lab indicates the potential for false positive and misclassification because non-classical NAs, such as aromatic acids within oil sand organic acid mixtures, will be detected with this method.

Further comments from this lab indicate that this is currently not a true quantitative method because differences in the concentration of aromatic acids present within NA mixtures will impact fluorescence. The detection limit and linear dynamic range are calculated using a purified mixture of NAs isolated from "fresh" OSPW, using the methodology described by Frank et al. (2006).

6.7.3 *Gas Chromatography*

Two labs use conventional gas chromatography (with mass spectrometry) as the analytical method, but no lab uses a comprehensive 2-D GC×GC method. Both labs indicate the achievement of partial separation of NA components to improve qualitative analysis being one of the major purposes of chromatography. One lab indicates chromatography can help improve analytical sensitivity, the other indicates chromatography can help reduce sample matrix effects. Both labs use dichloromethane to inject NAs and choose MTBSTFA as the derivatizing reagent. Both labs believe that derivatization can help to enhance volatility of NAs and increase thermal stability of the derivatives, and to reduce the extent of molecular fragmentation during ionization when using mass spectrometry. One lab also believes that derivatization can improve analytical selectivity. The major concern identified by both labs with derivatization of extracts from OSPW is that components such as hydroxylated NAs or dicarboxylic acids in the OSPW can

lead to false positives and misclassification of NAs. One lab is also concerned that derivatization may be incomplete, affecting identification of NAs.

Detailed chromatographic conditions for analysis are not disclosed here due to confidentiality. Readers can refer to related literature or contact the labs. The conditions from both labs are quite similar.

6.7.4 *Liquid Chromatography*

Six labs use conventional liquid chromatography (with mass spectrometry) as the analytical method, but no lab uses comprehensive 2-D LC×LC method. Five labs indicate the achievement of partial separation of NAs components to improve qualitative analysis being one of the major purposes of chromatography. Other purposes of chromatography include: reducing sample matrix effects (4 out of 5 labs); improving analytical sensitivity (3 out of 5 labs); creating fragments for MS/MS detection (1 out of 5 labs). One lab indicates chromatography can help enhance the specificity of analytical method – "isomers are not individually resolved (by chromatography) but (it) permits quantification of each isomer group for each n and z combination." Only one lab employs a derivatization procedure because it can aid in the chromatographic separation of components, enhance detection of ionized component, and help in the MS/MS specificity of detection method. However, this lab didn't disclose the reagent used for derivatization.

Detailed chromatographic conditions for analysis are not disclosed here due to confidentiality. Readers can refer to related literature or contact the lab.

6.7.5 *Mass Spectrometry*

Mass spectrometry is widely used as a detector either alone or with chromatography (10 out of 11 labs).

Among the six labs that employ an LC-MS analytical method, electrospray ionization (ESI), a soft ionization technique, is the most widely used technique (two labs use ESI in negative ionization mode, one lab uses ESI in positive ionization mode, and two lab use ESI in both positive and negative ionization mode). One lab indicates using atmospheric pressure photoionization (APPI) in both positive and negative mode, and one lab mentions that APCI and APPI can be available upon request as well as LC-QqQ and GC-QqQ (EI/CI). Only one lab employs electron impact ionization technique (EI), a hard ionization technique, in negative ionization mode. Selection of mass spectrometry is very broad from low to unit mass resolution (quadrupole, Q-trap, or QqQ), to high to ultrahigh mass resolution (Orbitrap, QTOF, or FTICR).

Two labs employ MS alone for NA analysis. One uses ESI ionization technique in negative ionization mode with FTICR (ultrahigh mass resolution) as the mass detector. The other lab uses ESI, APCI and APPI ionization techniques in both positive and negative modes. FTICR (ultrahigh mass resolution), linear ion trap-FTICR/Orbitrap, and MALDI (Matrix Assisted Laser Desorption Ionization) are available as mass spectrometry methods for this lab.

The two labs that employ a GC-MS analytical method use electron ionization technique, one in positive ionization mode and the other in negative ionization mode. Quadrupole and ion trap are used as mass spectrometry (low to unit mass resolution). One lab mentions that FTICR (ultrahigh mass resolution) is used for a few samples though not as a routine analytical method.

Four labs add an internal standard after sample preparation but prior to the analytical procedure to determine the response ratio for calibration plots. However, the internal standards used vary with labs, including picloram and 2,4-Dichlorophenoxyacetic acid herbicides, 9-fluorene carboxylic acid, cholic acid, and $^{13}C_3$ atrazine

Five labs use commercial mixture of NAs (e.g., Merichem or Kodak) as the calibration standard (the lab using $^{13}C_3$ atrazine as internal standard uses 1-pyrene butyric acid as the quantification reference), one lab uses an in-house laboratory standard (prepared by using selected NA compounds), and one lab uses extract from a given oil sands source (e.g., OSPW) as the calibration standard. One lab mentions the calibration is done by initial external followed by internal calibration. One lab specifies Agilent ESI mix as the calibration standard. Two labs indicate that the calibration plot is based on a mixture of selected compound standards.

Eight labs indicate that laboratory blanks are analyzed and four labs indicate that field blanks are also analyzed. One lab mentions that passive sampling medium blank, reagent blank and instrument blank are analyzed. The Z=0 compounds, if detected, are isolated and therefore eliminated by the method parameters.

The detection limit and linear dynamic range vary from lab to lab in values and units, as summarized in Table 1 (5 labs didn't respond to these questions).

Table 1. NAs analysis detection limit and linear dynamic range reported from different labs.

	Detection Limit	Linear Dynamic Range
Lab 1	0.5 ppm	0.5 to 500 ppm
Lab 2	1 mg L^{-1} total acids	1 to 100 mg L^{-1}
Lab 3	100 ppb	0.1 to 5 ppm
Lab 4	1 µg	1 to 500 µg
Lab 5	0.02 mg/L	
Lab 9	0.25 ng L^{-1} per each isomer group for passive sample; 0.5 ng L^{-1} per each isomer group for grab sample	Adjustment of sample size gives upper limit of 25 µg L^{-1} per each of the 60 isomer groups reported.

7 CONCLUSIONS

Analysis of NAs from various sources (oil sands ores, heavy oil and petroleum, OSPW and other environmental samples) is a formidable task. Many methods have been developed for the qualitative and quantitative analysis of these complex mixtures. Great advances have been made in the qualitative identification of NAs using ultrahigh resolution MS. However, no single analytical method exists to quantify all the components found in the acid extractable samples from OSPW referred to as NAs.

Clarification of what should be considered NAs is needed to help develop extraction and cleanup procedures so that consistency in the analytical results can be achieved among laboratories. This is needed since the classical NAs based on the general formula $C_nH_{2n+z}O_2$ constitute only part of the complex mixtures of compounds being recovered from OSPW.

Further research will be needed to address the following concerns raised by Headley et al. (2009b):

- development of fast and convenient methods to extract and clean-up NAs from environmental samples,
- development of more reliable quantitative methods that employ chromatography and suitable internal standards,
- exploration of representative model NAs as surrogates for complex NA mixtures, and
- development of congener-specific analysis of the principal toxic components.

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9 GLOSSARY

9.1 Terms

Adduct ion

An adduct ion is formed by the interaction of a precursor ion and a molecule. It contains all of the constituent atoms of the precursor ion as well as additional atoms or molecules. Adduct ions are often formed in a mass spectrometer ion source.

Analyte

The sample being analysed.

Deprotonation

The removal of a proton from a molecule, forming the conjugate base.

Deuterate

To treat or combine with deuterium, the heavy hydrogen (hydrogen^{-2}). The nucleus of deuterium contains one proton and one neutron, whereas the far more common hydrogen isotope (hydrogen^{-1}) has no neutron in the nucleus.

Dopant

A dopant is also called a doping agent. It is a compound added intentionally in a very small, controlled amount to the analyte to change its electrical properties and to increase the ionization potential.

Eluate

Eluate is the effluent emerging from a chromatographic bed/column when elution is carried out. It specifically includes both the mobile phase "carrier" and the analyte material passing through the column, while eluent is only the carrier.

Fourier transform

Fourier transform is a mathematical operation that expresses a mathematical function of time as a function of frequency.

Internal standard

A compound added before sample preparation and used in the analytical method to determine the response ratio for calibration plot.

Lorentz Force

The force acting on an electrically charged particle due to an external electromagnetic field.

Mass extinction coefficient

A parameter that defines how strongly a substance absorbs light at a given wavelength per mass density.

Naphthenic acids

IUPAC defines NAs as acids, chiefly monocarboxylic, derived from naphthenes. Naphthenes are primarily cycloalkanes especially cyclopentane, cyclohexane, and their alkyl derivatives.

Non-polar compound

A non-polar compound occurs where there is an equal sharing of electrons between two different atoms. Non-polar compounds include fats, oil and petro/gasoline.

Polar compound

A polar compound contains polar covalent bonds (i.e., electrons are not share equally between two bonding atoms). Polar compounds, including inorganic acids, bases, and salts, can ionize when dissolved or fused.

Protonation

The addition of a proton to an atom, molecule, or ion.

Surrogate standard

A compound never found in the sample but added before sample preparation to assess percentage of recovery.

9.2 Acronyms

Many of the method acronyms get combined in the report (e.g., GC-MS); the combinations are not repeated here.

APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photoionization
BTEX	Benzene, Toluene, Ethylbenzene, and Xylene
ChOPS	Cold-heavy Oil Production with Sand
CI	Chemical Ionization
CSS	Cyclic Steam Simulation
CV	Compensation Voltage
DBE	Double-Bond Equivalent
EI	Electron Impact Ionization
ESI	Electrospray Ionization
ESI-FTICR-MS	Electrospray Ionization-Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
FAB	Fast Atom Bombardment
FAIMS	Field Asymmetric Waveform Ion Mobility Spectrometry
FID	Flame Ionization Detector
FID	Free Induction Decay
FT	Fourier-Transform
FTIR	Fourier-Transform Infrared
GC	Gas Chromatography
GC-MS	Gas Chromatography - Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
ICR	Ion Cyclotron Resonance
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid Chromatography
LLE	Liquid-Liquid Extraction
MALDI	Matrix-Assisted Laser Desorption Ionization
MLSB	Mildred Lake Settling Basin

MS	Mass Spectrometry
MW	Molecular Weight
NA / NAs	Naphthenic Acid / Naphthenic Acids
NAIT	Northern Alberta Institute of Technology
OSPW	Oil Sands Process Water
OSRIN	Oil Sands Research and Information Network
OSTWAEO	Oil Sands Tailings Water Acid-Extractable Organics
POCIS	Polar Organic Chemical Integrative Sampler
Q	Quadropole
R	Resolution
SAGD	Steam Assisted Gravity Drainage
SAX	Strong Anion Exchanger
SEE	School of Energy and the Environment
SFS	Synchronous Fluorescence Spectroscopy
SPE	Solid Phase Extraction
TAN	Total Acid Number
TOF	Time of Flight
UV	Ultraviolet
VOC	Volatile Organic Carbon

9.3 Chemicals

BDMS	Butyldimethylsilyl
EDC	Ethyl-3(3-dimethylaminopropyl) carbodiimide
MTBSTFA	N-methyl-N-(*tert.*-butyldimethlsilyl) trifluoroacetamide
N	Nitrogen
NPH	Nitrophenylhydrazide
S	Sulphur

10 LIST OF OSRIN REPORTS

OSRIN reports are available on the University of Alberta's Education & Research Archive at https://era.library.ualberta.ca/public/view/community/uuid:81b7dcc7-78f7-4adf-a703-6688b82090f5. The Technical Report (TR) series documents results of OSRIN funded projects. The Staff Reports series represent work done by OSRIN staff.

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