separations

MDPI

Review

Pros and Cons of Separation, Fractionation and Cleanup for Enhancement of the Quantitative Analysis of Bitumen-Derived Organics in Process-Affected Waters—A Review

Ralph Hindle [1,2], John Headley [3] and Douglas G. Muench [1,*]

[1] Department of Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada; ralph.hindle@ucalgary.ca
[2] Vogon Laboratory Services, Cochrane, AB T4C OA3, Canada
[3] Environment and Climate Change Canada, Watershed Hydrology and Ecology Research Division, National Hydrology Research Center, 11 Innovation Blvd., Saskatoon, SK S7N 3H5, Canada; john.headley@ec.gc.ca
* Correspondence: dmuench@ucalgary.ca

Abstract: Oil sands process-affected water (OSPW) contains a diverse mixture of inorganic and organic compounds. Naphthenic acids (NAs) are a subset of the organic naphthenic acid fraction compounds (NAFCs) and are a major contributor of toxicity to aquatic species. Thousands of unique chemical formulae are measured in OSPW by accurate mass spectrometry and high-resolution mass spectrometry (MS) analysis of NAFCs. As no commercial reference standard is available to cover the range of compounds present in NAFCs, quantitation may best be referred to as "semi-quantitative" and is based on the responses of one or more model compounds. Negative mode electrospray ionization (ESI-) is often used for NAFC measurement but is prone to ion suppression in complex matrices. This review discusses aspects of off-line sample preparation techniques and liquid chromatography (LC) separations to help reduce ion suppression effects and improve the comparability of both inter-laboratory and intra-laboratory results. Alternative approaches to the analytical parameters discussed include extraction solvents, salt content of samples, extraction pH, off-line sample cleanup, on-line LC chromatography, calibration standards, MS ionization modes, NAFC compound classes, MS mass resolution, and the use of internal standards.

Keywords: naphthenic acids; oil sands process-affected water; chromatography; high-resolution mass spectrometry; sample cleanup; semi-quantitation

Citation: Hindle, R.; Headley, J.; Muench, D.G. Pros and Cons of Separation, Fractionation and Cleanup for Enhancement of the Quantitative Analysis of Bitumen-Derived Organics in Process-Affected Waters—A Review. *Separations* **2023**, *10*, 583. https://doi.org/10.3390/separations10120583

Academic Editor: Anastasios Zouboulis

Received: 29 July 2023
Revised: 30 October 2023
Accepted: 13 November 2023
Published: 24 November 2023

1. Introduction

The oil sands in Canada's northern Alberta region represent the third-largest proven oil reserve in the world. Oil sands production is expected to grow from 2.91 million barrels per day (bpd) in 2018 to 4.25 million bpd by 2035 [1]. Mining and processing of oil sands involves a hot water bitumen extraction process that results in the production of large volumes of oil sands process-affected water (OSPW) that contains a mixture of organic and inorganic compounds. These compounds include salts, trace metals, BTEX (benzene, toluene, ethylbenzene, and xylene), polyaromatic hydrocarbons (PAHs), and naphthenic acids (NAs) [2,3]. NAs have been identified as a main contributor of OSPW toxicity [4–7]. The molecular speciation of NAs using accurate mass spectrometry and high-resolution mass spectrometry is required to generate and confirm chemical formulae to effectively characterize the organic compounds and monitor their removal during remediation experiments. Additionally, off-line sample cleanup and liquid chromatography (LC) separations can help reduce variable ion suppression and improve quantitative evaluations of OSPW.

There are three common classifications used to describe the organic chemical species in OSPW. The first are the classical NAs (cNAs) that have the general molecular formula

$C_nH_{2n+z}O_2$ where "n" is the number of carbon atoms and "z" is either zero or a negative even integer representing the hydrogen deficiency resulting from saturated alicyclic ring structures (Figure 1) [8]. Each value of z = −2 represents an additional ring or double bond and is referred to as a double bond equivalent (DBE). The double bond found in the carboxylate moiety is included in references to DBE, but it is excluded when referencing the z-value of NAs. The second classification group is NAs that include O_3, O_4 and higher oxygenated species, and are referred to as oxy-NAs. While cNAs are the most abundant species in freshly produced OSPW, relatively high abundances of O_3 and O_4 species may also be present in OSPW and may be temporal indicators of transformations. Naphthenic acid fraction compounds (NAFCs) are the third general classification group and is a broad, all-inclusive definition of OSPW organics. NAFCs are the acid extractable organics that include cNAs, oxy-NAs, and compounds with aromatic rings, unsaturated groups, nitrogen, and/or sulphur atoms [9]. Common chemical species identified in NAFCs include SO_2, SO_3 and various NO-containing compounds.

Effective OSPW detoxification strategies [10–15] rely on a thorough understanding of the organic compounds that contribute to toxicity [5,7]. Mass spectrometry (MS) is the most common method of detection for organic compounds in OSPW, with the simplest and least expensive approach being unit mass resolution quadrupole MS. Quadrupoles with unit mass resolution have limited applications due to low resolution. For example, such instruments cannot differentiate between the masses at 32 amu arising from either two oxygens (2 × 16 amu = 32 amu) and a single sulfur (32 amu) in a given ion. The complexity of OSPW components can be better determined using high-resolution accurate mass (HRAM) instruments. Fourier transform ion cyclotron resonance (FT-ICR), Orbitrap, and time-of-flight (TOF) mass spectrometers are capable of mass accuracies of four or more decimal points. This resolution is sufficient to determine or confirm molecular formulae in the mass range that is typical of NAs and able to speciate elemental composition (e.g., O_2 = 31.9898; S = 31.9721).

A previous review article [16] evaluated eight areas for consideration in designing a "semi-quantitation" approach to MS analysis of NAFCs in OSPW (Table 1). Semi-quantitative methods do not have authentic reference standards available for all target compounds. Therefore, they use the response factors of structurally similar compounds to estimate concentrations. In this review, we revisit these eight areas to highlight the pros and cons of the critical parameters for NAFC MS analysis, with a focus on chromatographic clean up and separations prior to mass spectrometry detection of OSPW and environmental samples. We also expand the discussion to ten key parameters, including separation effects of salt content, the benefits of sample clean up, and a chromatography step prior to MS. Optimization and control of these parameters can lead to robust methods of analysis, while seemingly reasonable but variable settings can lead to irreproducible and ambiguous results. A good example of conflicting results was reported previously, where OSPW extracts were prepared, split, and identical samples were distributed to two laboratories. Each laboratory analyzed the extracts by measuring ions under either acidic or basic conditions [17]. Large differences were evident for the O_2 class depending on whether acidic or basic conditions were employed, regardless of the instrument used by the two laboratories (Figure 2). Of note, the most toxic O_2 class would be considered a minor component if only acidic conditions were used. In contrast, separation under basic conditions indicated that the O_2 class is by far the most abundant NAFC. While the observed differences can be explained by pH effects on separation of ions (discussed later in this review), the apparent results can lead to confusion about the levels of principal toxicants in the OSPW. Such discrepancies can pose difficulties to end-users of the data for toxicity assessment.

Table 1. Factors affecting analytical results of classical NA quantitation methods (annotations refer to original publications) [16].

No.	Factor	Conclusions
1	Definition of total NAs	Use the classical definition of NAs
2	Extraction phase, pH, temperature	Liquid–liquid extraction at pH 2 and room temperature with DCM as organic phase, or use ENV + SPE
3	Use of surrogate standards	Use isotopically labelled model compounds as surrogate standards
4	Minimum resolving power of instrument	50,000 at m/z 200, acknowledging that potential interferences contribute to method uncertainty
5	Use of derivatization	Do not utilize derivatization
6	Polarity and mode of ionization	Negative-ion-mode ESI
7	Suitable calibration standard and internal standard	Use commercially available Merichem NA mixture and at least one isotopically labelled internal standard
8	Use of on-line or off-line fractionation of sample	Employ on-line chromatography prior to MS detection

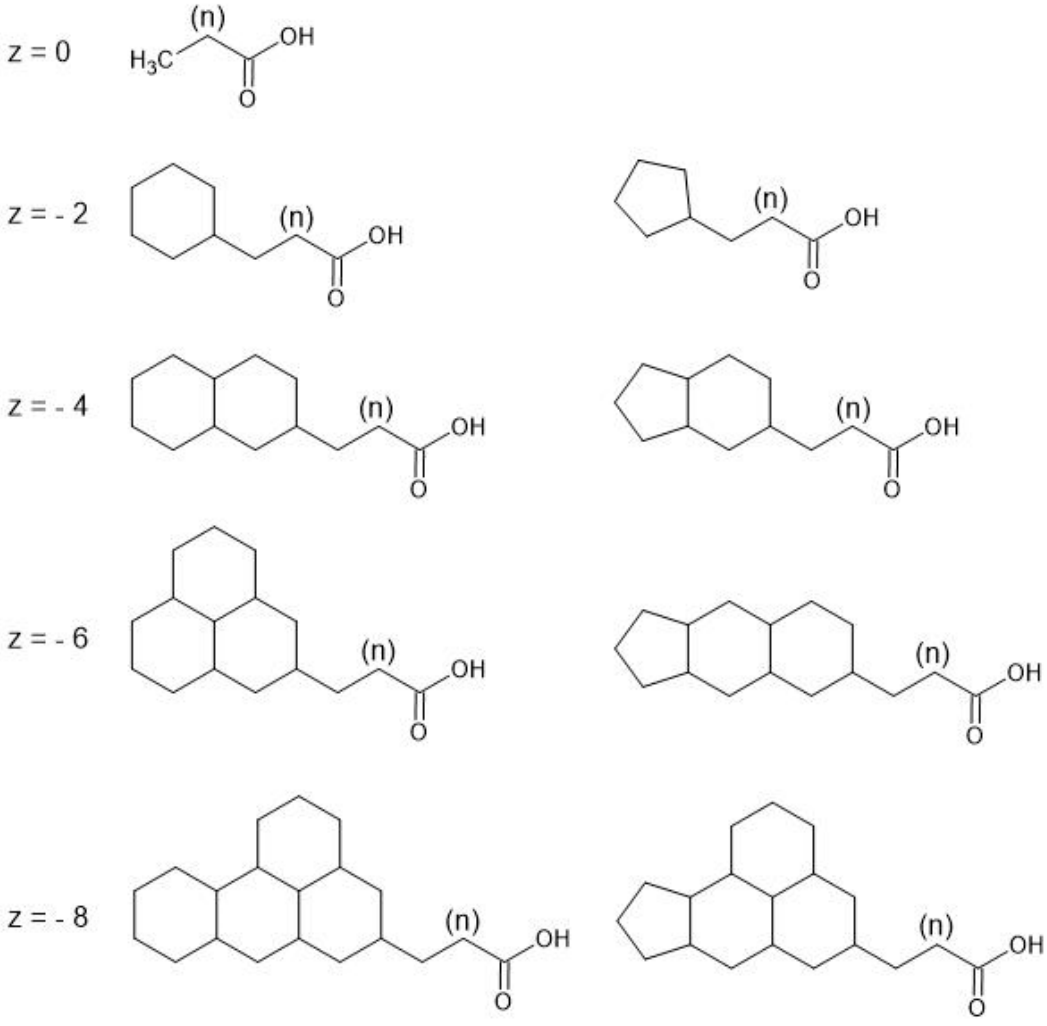

Figure 1. Examples of classical mono-carboxylic naphthenic acid (cNA) structures [18].

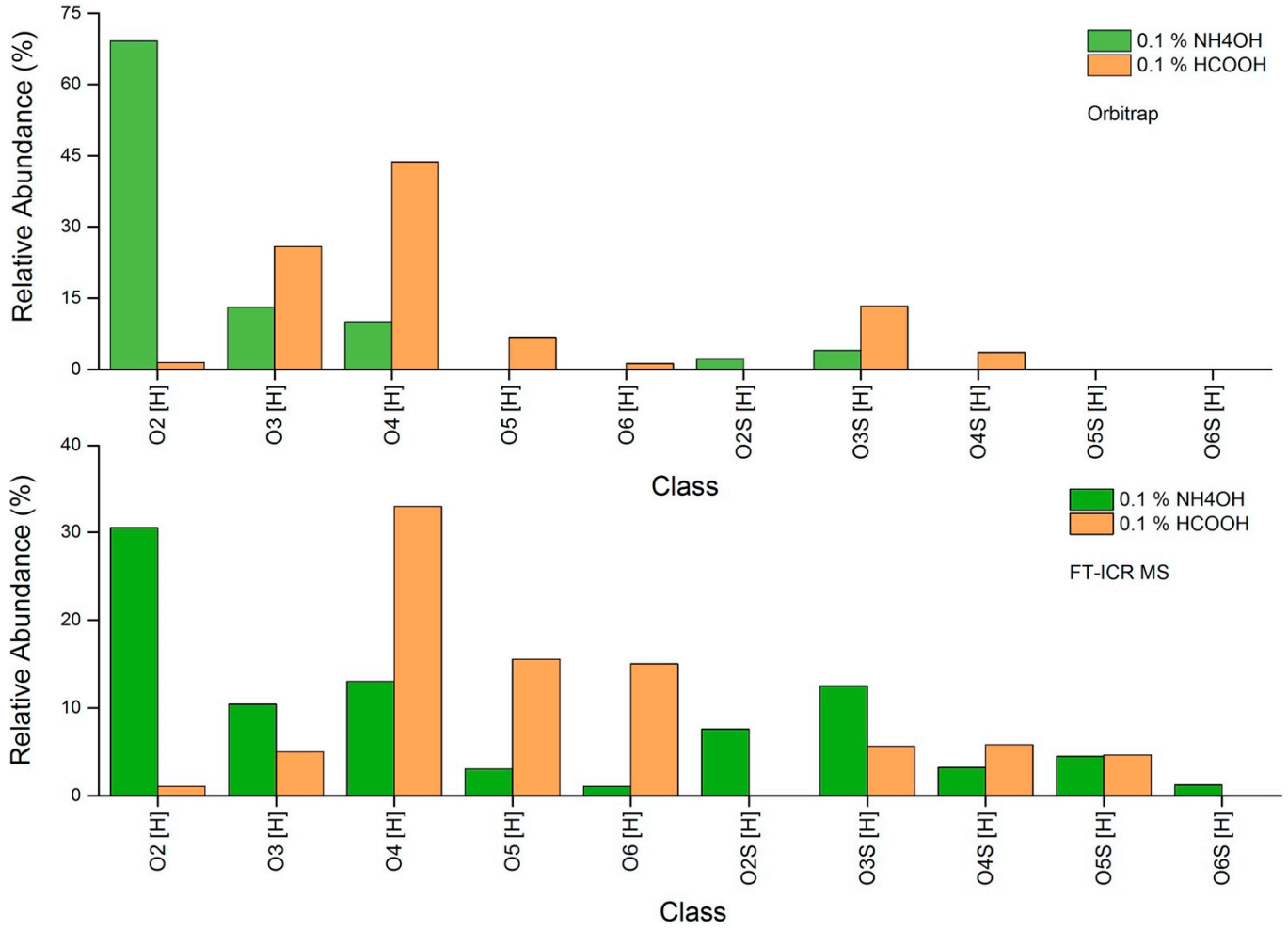

Figure 2. O_2 DBE plots from ESI- Orbitrap (**top**) and FT-ICR MS (**bottom**) data using acidic (brown) and basic (green) conditions [17].

2. Critical Analytical Parameters

2.1. Choice of Extraction Solvent

The solvent used for NAFC extraction from water should be based on the chemical properties of target compounds. For example, in the case of relatively non-polar PAHs, lipophilic solvents that are immiscible with water are used. Cyclohexane and dichloromethane are common choices for neutral compounds and are directly amenable to GC analysis following a drying step to remove residual water, typically by passing the solvent through a column of anhydrous sodium sulfate. NAs have surfactant properties, with hydrophobic ring structures and carbon side chains, as well as hydrophilic carboxylic acid groups. Extraction and concentration of NAFCs from OSPW is carried out to lower detection limits, much like that performed for PAH analysis but with additional consideration of aqueous pH that takes advantage of the ionic and neutral forms of the acids. Use of high pH (between 10 and 12) during partitioning will ionize NAs for their retention in the water phase, while non-polar compounds are extracted into the organic solvent and discarded. Lowering of pH to ~2 will then neutralize NAFCs, so they partition into the organic phase and are dried, and concentrated.

The choice of extraction solvent can also align with the solvent used to prepare the sample for MS analysis. For example, a procedure was reported where an NAFC extract concentrate was solvent-adjusted to prepare a series of fractions for nanospray infusion into the ESI source (Figure 3) [18]. The NAFC solution was acidified to pH 2.5, then the neutral NAFCs were extracted into dichloromethane (DCM), concentrated, and the solvent

was exchanged into acetonitrile. The acetonitrile extract was then amended with five solvents used separately or in various combinations: Milli-Q water (9.0 polarity index, PI)/acetonitrile (ACN; 5.8 PI); ACN; methanol (MeOH; 5.1 PI)/ACN; DCM (3.1 PI)/ACN; and 1-octanol (<3.9 PI)/ACN. Ammonia solution (35% v/v in water) was added to each solution to improve deprotonation of NAs in the MS source. Of the five solvents, water/ACN, ACN, and MeOH/ACN were similar in their respective spectra. The DCM/ACN and 1-octanol/ACN solvents, however, showed marked differences in intensity and molecular weight distribution compared to the other three, particularly when plotted as carbon number vs. z-values and broken out as monocarboxylate O_2 vs. dicarboxylate O_4 species (Figure 4, ACN and MeOH/ACN not shown). The authors attributed the difference in NA characterization as an effect of greater solubility of higher-molecular-weight acids in the lipophilic solvents DCM and 1-octanol. Researchers using a more polar solvent system would, therefore, reach a very different conclusion about the NAFC characterization of the same OSPW sample.

More recent work has shown the effects of the apparent pH of the transfer solvent (mobile phase), where identical OSPW extracts were analyzed by Orbitrap and FT-ICR-MS in different laboratories using both acidic and basic mobile phases on each instrument [17]. In this particular application, neither the Orbitrap (flow injection analysis) nor the FT-ICR-MS (infusion) used chromatographic separation before ionization in the source of the mass spectrometer. A flow injection experiment would result in a mixture of the mobile phase and the solvent in the sample vial when the sample reaches the source within seconds, but infusion contains the vial solvent alone. These solvent differences would result in a different apparent pH in the source and therefore different spectra. On-line chromatography will diffuse the sample vial solvent to a greater extent by the time the sample arrives at the source, with the apparent pH being that of the mobile phase alone.

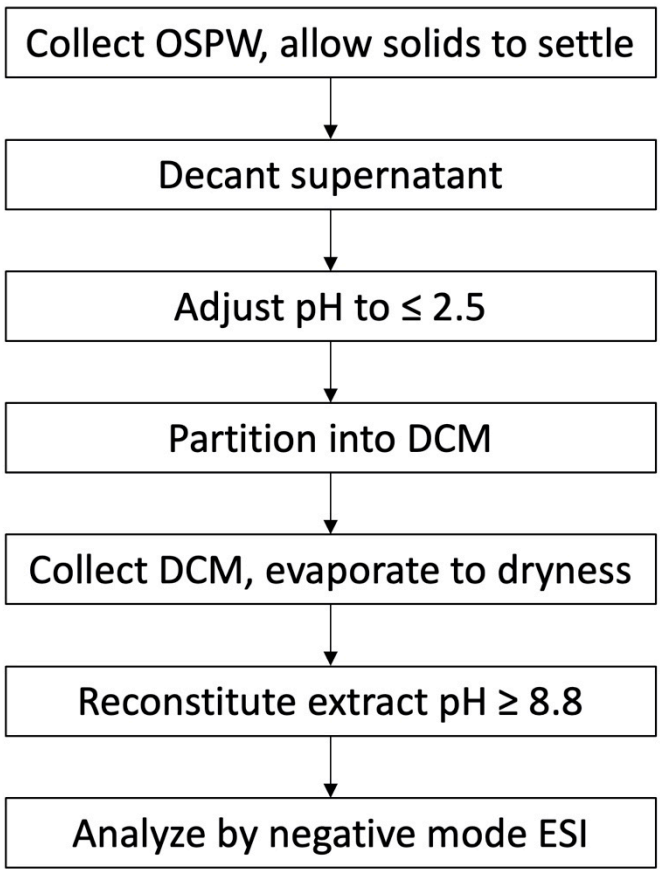

Figure 3. Summary of liquid–liquid extraction of NAFCs from OPSW.

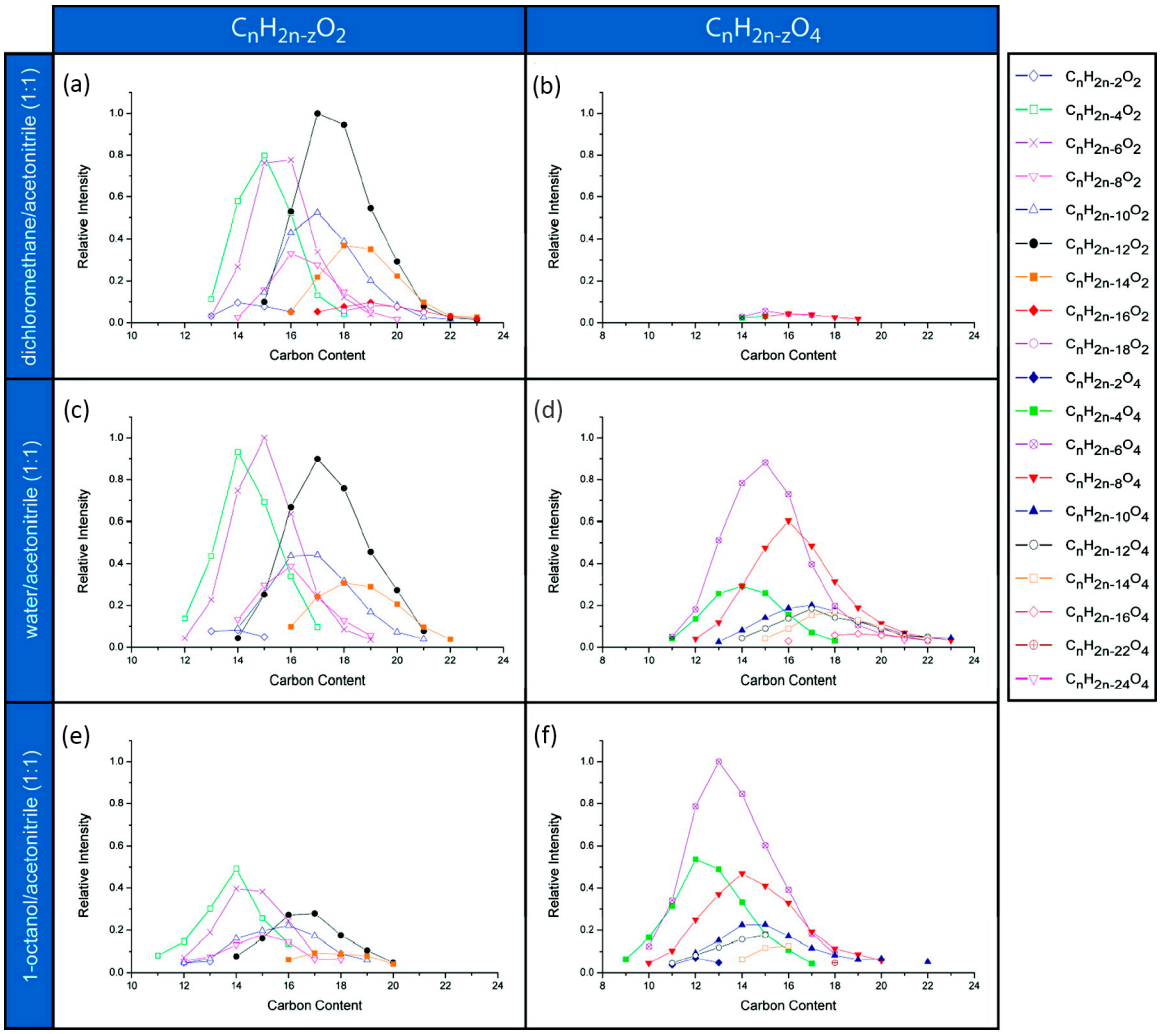

Figure 4. Effect of solvent on FT-ICR MS abundances of z-series NAs for DCM/acetonitrile, Milli-Q water/acetonitrile, and 1-octanol/acetonitrile for O_2-NAs (**a**,**c**,**e**) and O_4-NAs (**b**,**d**,**f**) [19].

Syncrude researchers describe their routine method for NAFC analysis using DCM extraction and FT-IR detection based on the carbonyl stretching bands at ~1740 cm^{-1} and ~1703 cm^{-1} for the monomers and dimers, respectively [20]. Extraction efficiency compared to DCM was 36% for tetrachloroethylene (TCE) and 49% for hexane. The higher extraction rate for DCM was attributed to its higher polarity. This was further borne out by extracting OSPW with a mixture of 1:1 DCM:TCE. When measured gravimetrically, the extract contained twice as much material as a DCM extraction alone, but FT-IR analysis showed that the concentration of NAFC was the same. Therefore, DCM alone is sufficient to extract the acidic components while not extracting less polar compounds.

2.2. Salt Content of the Sample

OSPWs may contain high levels of natural salts from some oil sands formations or from high levels of water reuse in the bitumen extraction process. Salting-out effects in MS analysis have been noted for crude oil acids in sea water, and the same may be true in OSPW with high salt content [21]. Salinity values over 2800 mg/L have been reported in OSPW samples [22], and these concentrations may affect the partitioning behaviour and separation of NAFCs by the formation of ion-pair complexes between the salt cations and NA anions.

A comparison of NAFC extracts in a DCM/ACN solvent mixture showed significant spectral differences when a saturated salt solution was added [21]. Intensities were en-

hanced for shorter carbon chain acids with lower z-values for $C_nH_{2n-z}O_2$, $C_nH_{2n-z}O_3$, and $C_nH_{2n-z}O_4$ species but suppressed for longer chain, higher z-value acids. The authors attributed this decrease in response to salting-out effects. High sodium content can lead to the formation of anionic sodium dimers [23], with masses corresponding to $[2M\text{-}2H + Na]^-$, where M is the mass of the neutral compound. The mass range would be approximately double the expected value for the $[M\text{-}H]^-$ anion monomer. The acids forming the dimer may not be the same species, which further complicates spectral evaluation. A possible remedy to these sodium-bridged dimers would be to reduce the sodium in the source of the MS when the acids are present by injecting the sample onto an LC column rather than infusing the sample directly into the source. Sodium ions would be unretained on the column and directed to waste. Alternatively, off-line extraction of NAFCs from water can be carried out using solid phase extraction (SPE) by adjusting the pH to <3 for SPE loading, removing salts with the subsequent wash step, followed by elution of the NAs with organic solvent [24]. Sodium adducts in positive mode ESI can be reduced by adding ammonium to the mobile phase, or by decreasing source temperatures. The same may be true in negative mode. After dimer formation in the source, increasing the TOF fragmentor voltage may also be used to fragment dimers into their monomers. Care must be exercised when using this technique as it can lead to unwanted fragmentation of ions, reducing the abundance of precursor ions.

2.3. pH of Extraction

Bitumen-derived NAFCs possess a wide range of polarities and pKa values for neutral and ionic compounds, respectively. As a result, the choice of extraction solvent may skew the analytical data and could result in selective fractionation or separation of NAs based on solvent polarity and pH. Extractions from a Fluka NA standard containing predominantly O_2 species showed that maximum NA solubility was achieved at pH 7 [24], with a 37% increase in signal intensity from pH 7 to 9, but with the same MS spectrum profile. The authors attributed this to a dependence on pH for total solubility, correlating the effect to refining scrubbing processes. A pH-dependent OSPW extraction study investigated the influence of pH on O_2, O_3, and O_4 compounds using LC/TOF [25]. In this study, the pH of the OSPW (pH 8.5) was adjusted to pH 12.4 and extracted with cyclohexane to remove non-polar compounds. The OSPW was then adjusted to pH 10 and re-extracted with DCM, and the extract was dried with sodium sulfate and weighed. Further extraction cycles were performed by lowering the pH in the water sample by 1 unit in each cycle, down to pH 2. The total mass of O_2, O_3, and O_4 NAs was 23.5%, 6.6%, and 3.5%, respectively, and these NAs were preferentially extracted in pH ranges of 3–5, 5–7, and 6–8. A mathematical model was developed to estimate the pK_a values of the oxy-NAs to help understand their distribution in wastewater treatments. This revealed that, as oxygen numbers increased in the O_2, O_3, and O_4 series, pKa also increased (4.2, 6.2, and 7.7, respectively). Increases in pK_a indicate that the O_3 NAs are hydroxylated, and the O_4 NAs are more likely to be di-hydroxylated rather than di-carboxylic acids, which otherwise would have resulted in lower pK_a values (e.g., propanoic acid pK_a = 4.87; propanedioic acid pK_{a1} = 2.87).

In an ideal situation, the MS detector response for a given number of ions would be the same regardless of the mass-to-charge (m/z) value. In that case, any compound that was fully ionized could be used as a reference and its response factor determined directly when using a known concentration. Comparative analysis [17] provides insight into relative response factors between transfer solvents in the mobile phase. Table 2 shows a 111-fold increase in the response for biphenyl-4-carboxylic acid (B4CA) with ammonium hydroxide added to pH 8.91 compared to formic acid to pH 3.52. The authors confirmed that the formic acid pH, expected to be ~2.7, was the result of a blend of formic acid and OSPW, which had a pH of 8.1. The percent ionization changed rapidly with pH such that a weak acid will effectively be fully neutralized (99.85%) or fully ionized at 2 pH units below and above its pK_a, respectively.

Table 2. Spectrum abundances by FT-ICR-MS (ESI-) on model NAs with acidic and basic pH conditions [17].

Compound	Additive	Intensity	Apparent pH
Biphenyl-4-carboxylic acid	0.1% NH_4OH	5.34×10^{10}	8.91
$C_{13}H_{10}O_2$	0.1% HCOOH	4.79×10^{8}	3.52
Anthraquinone-2-carboxylic acid	0.1% NH_4OH	5.46×10^{10}	9.19
$C_{15}H_8O_4$	0.1% HCOOH	3.31×10^{9}	3.68
Trimesic acid	0.1% NH_4OH	2.81×10^{10}	8.75
$C_9H_6O_6$	0.1% HCOOH	1.86×10^{10}	3.59

This change in ionization is described by the Henderson–Hasselbalch equation relating pH to the dissociation of weak acids (Equation (1)), where [HA] is the concentration of the neutral acid and [A−] is the concentration of the anion. When solution pH equals the pK_a, an acid is 50% ionized.

$$\mathrm{pKa} - \mathrm{pH} = \log 10 \frac{[\mathrm{HA}]}{[\mathrm{A}-]} \quad (1)$$

Equation (1). Henderson–Hasselbalch equation describing the dissociation of weak acids in solution.

Because the acidic mobile phase pH (3.52) is below the pK_a of B4CA (4.19), B4CA will be 17.6% ionized in solution but fully ionized at a basic pH of 8.91. The dependence on solution pH indicates that ions already present in solution may be more readily detected than non-ionized neutral molecules which only ionize in the ESI source. The dissociation rule is further supported by the relative intensities of anthraquinone-2-carboxylic acid (A2CA), which has a pK_a of 3.42. This pK_a value is closer to the acidic mobile phase pH (3.68) and is more highly ionized in solution (59.7%) and, therefore, gives a higher response, as did trimesic acid (Table 1).

2.4. Sample Cleanup or No Cleanup

Sample cleanup has many advantages over direct analysis, but comes at the cost of increased time, expense, potential analyte losses, and waste generation. Cleanup is usually required for complex and 'dirty' matrices such as food, soil, petroleum, and biological matrices to separate matrix co-extractives from target compounds. The sample cleanup also serves the purpose of analyte concentration and can include a solvent exchange step suitable to the instrumental technique of choice. For water samples, cleanup may not be necessary if the instrument has sufficient sensitivity to meet detection limit requirements and if the samples are relatively clean (e.g., drinking water, groundwater, and surface water). Complex samples such as OSPW can also be analyzed without prior cleanup by using LC separation before the MS, or by using HRAM MS instruments that can differentiate the masses of target ions from the background. However, ion suppression may occur in these samples and can bias results. The presence of high salt concentrations in OSPW may lead to sodium-bridged dimers and can complicate data analysis. The choice ultimately comes down to having an injectable sample or extract that contains all the compounds of interest at sufficient concentrations to measure with minimum interference and low ion suppression. Comparison of samples with and without cleanup is an important step in method development and is particularly true for the analysis of the thousands of organic compounds present in OSPW [23,26,27].

Selectivity in OSPW cleanup is generally carried out using one of two extraction techniques. Liquid–liquid extraction is a simple procedure where a strong base is added to the samples to ionize the weakly acidic NAFCs and keep analytes dissolved in the aqueous phase while neutrals are partitioned into DCM and discarded, removing much of the dissolved organic content (DOC) [23]. The water is then acidified to pH~1.5 using H_2SO_4 and re-partitioned to extract the NAFCs into fresh DCM, then dried and concentrated by

evaporation. The neutralized acids are reconstituted into a suitable solvent such as MeOH or ACN: water for MS analysis. Neutral compounds that are not ionized in either extraction procedure will be extracted into the final DCM phase based on their K_{ow} coefficients, whereas salts remain in the aqueous waste fraction.

The second cleanup procedure uses SPE (described above) to selectively remove acids from samples using neutral sorbents such as divinyl benzene (DVB) [24]. A study that used a highly selective, mixed-mode polymer with additional anion exchange properties for acidic compounds (Oasis® MAX) [28] required no acidification of the OSPW. Also, for the MAX column cleanup procedure, the organic compounds were separated into two fractions, first using MeOH to elute the non-acidic compounds followed by acidified MeOH to elute neutralized NAs. Methanol alone eluted 10% of the DOC from the total MAX recoveries. The highest DOC recovery (95.4%) was achieved when using universal sorbent divinylbenzene hydrophilic–lipophilic balanced (DVB-HLB) cartridges and MeOH elution. The MAX sorbent only recovered 53.5%, an indication that only half of the organic compounds were acidic.

2.5. On-Line Chromatography vs. Injection or Infusion

Injection or infusion of samples directly into the source of an LC/MS is a fast and convenient method for qualitative characterization of OSPW. Injection or infusion is used more frequently with FT-ICR MS compared to other instrumental methods for two main reasons. First, the ultrahigh-resolution accurate mass feature allows for mass separation from interfering compounds. Second, the acquisition rate of some instruments may be too slow to produce high-resolution spectra when using LC separations [29]. A downside to infusion can be the presence of high salt levels that result in ion suppression and the formation of salt-bridged dimers, both of which can be reduced by using on-line chromatography (LC/MS).

NAs are classified as weak acids, with pKa values calculated to be 3.5, 4.8, and 6.8 for O_2, O_3, and O_4 species, respectively [25]. Under acidic conditions (pH < 1.5), the acids will be present in their neutral form and become more hydrophobic. Condensed phase-membrane introduction mass spectrometry (CP-MIMS) used this property of NAs to calculate the pK_a of many compounds of environmental concern, including pharmaceuticals, algal toxin biomarkers, and naphthenic acids [30]. The technique uses a semipermeable polydimethylsiloxane membrane probe immersed into pH-controlled solution. Neutral hydrophobic compounds permeate the membrane and infuse into the MS source with a methanol carrier, leaving salts and other ionic interferences behind. This approach works for both individual compounds in solution at parts-per-billion concentrations as well as complex mixtures such as OSPW. The CP-MIMS technique was extended to monitor the compositional changes in OSPW in a constructed wetland treatment system (CWTS), using unit mass quadrupole MS. The analysis took~15 min per sample and ion suppression effects were corrected through the use of lauric acid-d_2 ISTD infused into the methanol stream [31]. Because LC separation is not required, the technique is proposed as an approach to implement in situ monitoring of treatment systems with relatively inexpensive MS systems.

An ISO/IEC 17025-validated method for cNAs using reversed-phase LC separation and TOF analysis showed excellent linearity (R^2 = 0.9996) based on average response factors for all detected O_2 acids [32]. Many researchers have used reversed-phase chromatography to provide additional cleanup for samples, separation from salts that are concentrated in OSPW, and to increase resolution by compound separation over time [9,28,33,34]. When using reversed phase LC, the mobile phase is typically acidic to neutralize the NAs, allowing retention on the LC column. Negative polarity ionization takes place in the source of the MS. Mobile phase modifiers such as ammonium acetate or ammonium hydroxide can lead to ionization in solution, enhancing ESI signals, although column retention can decrease as a result. Hydrophobic interaction liquid chromatography (HILIC) can be used as an alternative separation technique, where anionic NAs in a high organic mobile phase are

selectively retained, reversing elution order, and providing the benefits of ion formation before entering the source, enhancing the response (Hindle et al., unpublished). HILIC requires longer equilibration times between injections and is sensitive to method parameters such as solvent strength in the vial and injection volume (Figure 5).

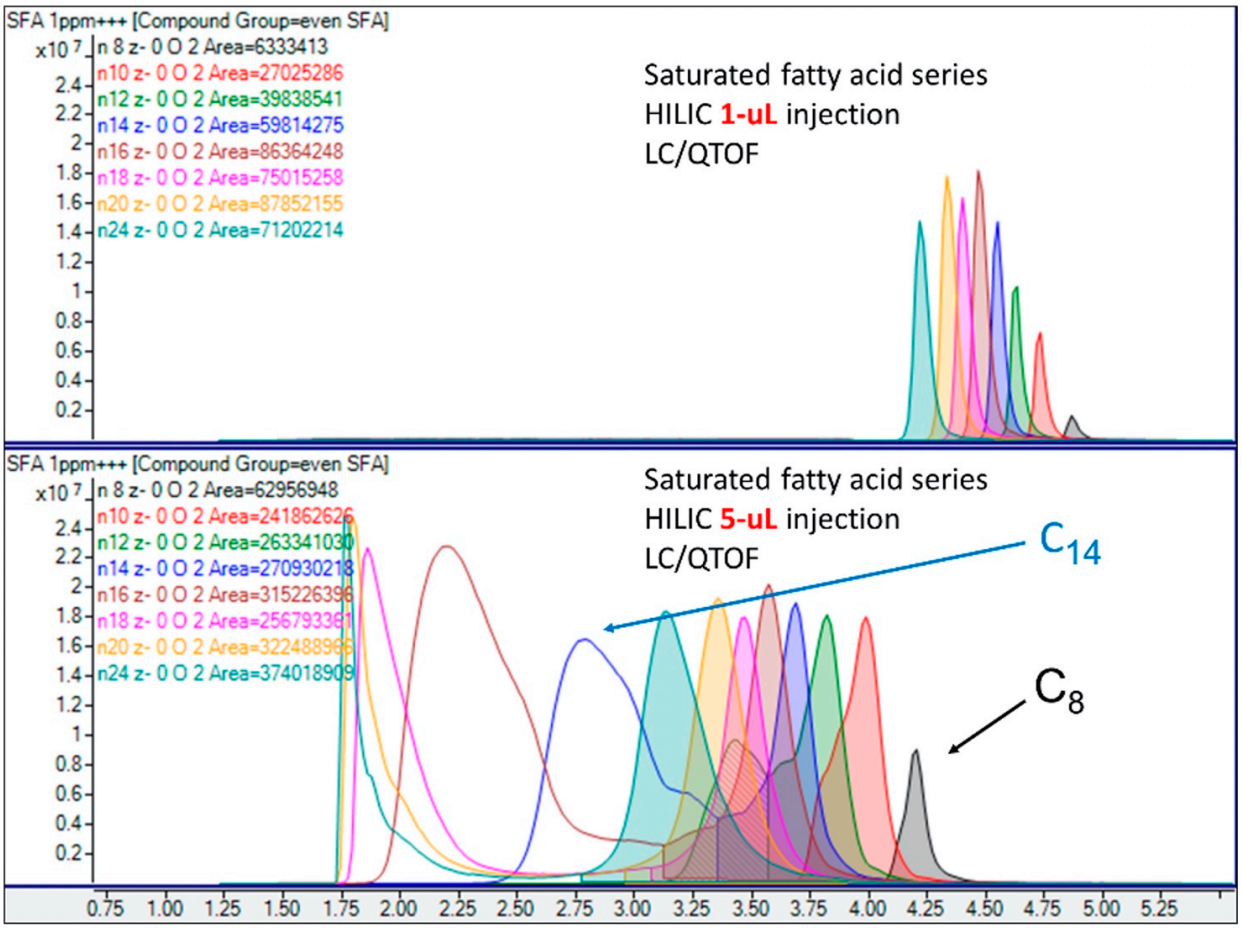

Figure 5. Effects on chromatography on fatty acid separation (C_8 to C_{24}) resulting from reduced injection volume on HILIC LC column [35].

2.6. Calibration Standards

Quantitative analytical methods use authentic reference standards of high purity for each compound to be measured. For example, the Canadian Water Quality Guidelines list twelve PAHs that require monitoring for the protection of aquatic life [36]. PAHs are well defined, limited in number, and commercially available. Many isotopically labelled analogs are also available that can be used as internal standard (ISTDs). The analysis is straightforward: a series of calibration standards is prepared at various concentrations, the response versus concentration is plotted for each compound, and sample concentrations are calculated from the generated calibration curves.

Measuring the concentrations of NAs in OSPW is complex. Approximately 3000 elemental compositions have been found in OSPW samples from the oil sands in northern Alberta using LC with Orbitrap HRAM/MS, including $O_{1\text{-}6}$, $NO_{1\text{-}4}$, $SO_{1\text{-}4}$, NO_2S, N, and S [27]. Each chemical formula can further be expanded into a large number of combinatorial structural isomers [37] which do not separate well chromatographically. Commercial mixtures of calibration standards of this complexity are not available, and NA mixtures that are available are not representative of the wide variety of OSPWs. Model compounds representing various chemical classifications may provide a means for semi-quantitative analysis only.

The 'Naphthenic Acids Strategies Workshop' (Saskatoon, Canada, 24–25 November 2011) was held with the goal of reviewing the different routine methods used by laboratories for measuring, classifying, and quantitating NAFCs. Researchers from Canadian provincial and federal governments, universities (including one from the United Kingdom), and commercial laboratories were invited. The forum was also used to gain consensus on chemical classification terms for describing what was being measured, i.e., classical naphthenic acids (cNAs) and the naphthenic acid fraction compounds (NAFCs). Each lab gave a presentation outlining their acquisition approach and quantitation method and the findings were published [9]. Most of the review was concerned with the qualitative characterization of NAFCs as the first step in defining what was being measured.

The following observation was made in the workshop review and is still true today, twelve years later: "The need for the development of target analyses using authentic standards is evident as quantification of NAFC expressed as total NAs is currently at best, semi-quantitative" [9]. No OSPW-derived reference standard has yet been produced for general availability. The consensus from the workshop was that the NAFC fraction from OSPW was too complex for a single technique, and that a working group should be formed to develop protocols for standardized analyses, with interlaboratory studies designed to show conformance of results.

In studying the impact of temperature, pH, and salinity changes on partitioning of NAs [22], 55 model NAs were selected based on their identification by other researchers [27,38], including compounds from the following types: thiophene, indane, tetralin, cyclohexane, and adamantane. A comprehensive list of compounds such as this that spans a variety of elemental compositions may provide representative response factors that differ due to chemical structures. However, as noted earlier, OSPW from different sources contains different compounds. A selection of 39 model NAs showed up to 26-fold differences in response factors using LC/TOF [32], indicating that a one-size-fits-all approach to reference standards is problematic. The use of a commercially available Merichem NA mixture was recommended for semi-quantitation of classical NAs, due to the predominance of O_2 acids.

2.7. Ionization Mode for Detection of the Analytes

Selection of the mass spectrometer ion source parameters is based on the chemical nature of the targeted NAFCs for any particular experiment. NAFCs and sulfonic acids in OSPW are typically analyzed with negative polarity due to the ready formation of anionic precursor ions, while many nitrogen-containing compounds can be preferentially ionized with positive polarity due to the basic nitrogen within the molecule. In some cases, more than half of the NAFCs extracted from OSPW at pH 1.5 are not classified as naphthenic acids [23]. Thus, multiple ionization techniques are needed for full NAFC characterization.

Three common ionization sources are available to many analytical laboratories: (1) electrospray (ESI); (2) atmospheric pressure chemical ionization (APCI); and (3) atmospheric pressure photoionization (APPI). Polar compounds such as carboxylic acids and amines are typically ionized using ESI, where the compounds are ionized either in the mobile phase due to selective pH, or within the ion source, often in the presence of mobile phase additives to enhance the ionization process. Non-polar organics such as ketones and aldehydes can be analyzed by APCI [38], where a charge is induced on protic solvent molecules such as methanol, which then transfer the charge as a reagent gas to the analytes. APPI can be used for the analysis of non-polar aromatic compounds such as PAHs by using photoionization to directly ionize compounds when their ionization potential is lower than the energy of the APPI lamp [39,40]. When the ionization potential of compounds is higher than the lamp, either the solvent or added dopant molecules (e.g., acetone or toluene) can be ionized, and this is followed by charge transfer to the target compounds. Both APCI and APPI can produce radicals as well as charged adducts [38].

To demonstrate the need for additional ionization techniques and polarity, Barrow et al. compared both positive and negative polarities using ESI and APPI sources (Figure 6) [26]. The negative ion mode showed that most ions are in the 190–450 amu range, while the

positive ion mode included ions up to 700 amu. The distribution within those ranges also changed along with the number of peaks. A greater number of peaks was observed in the positive-ion mode than negative-ion mode, and by APPI than ESI. The APPI positive-ion mode showed approximately 19,000 peaks.

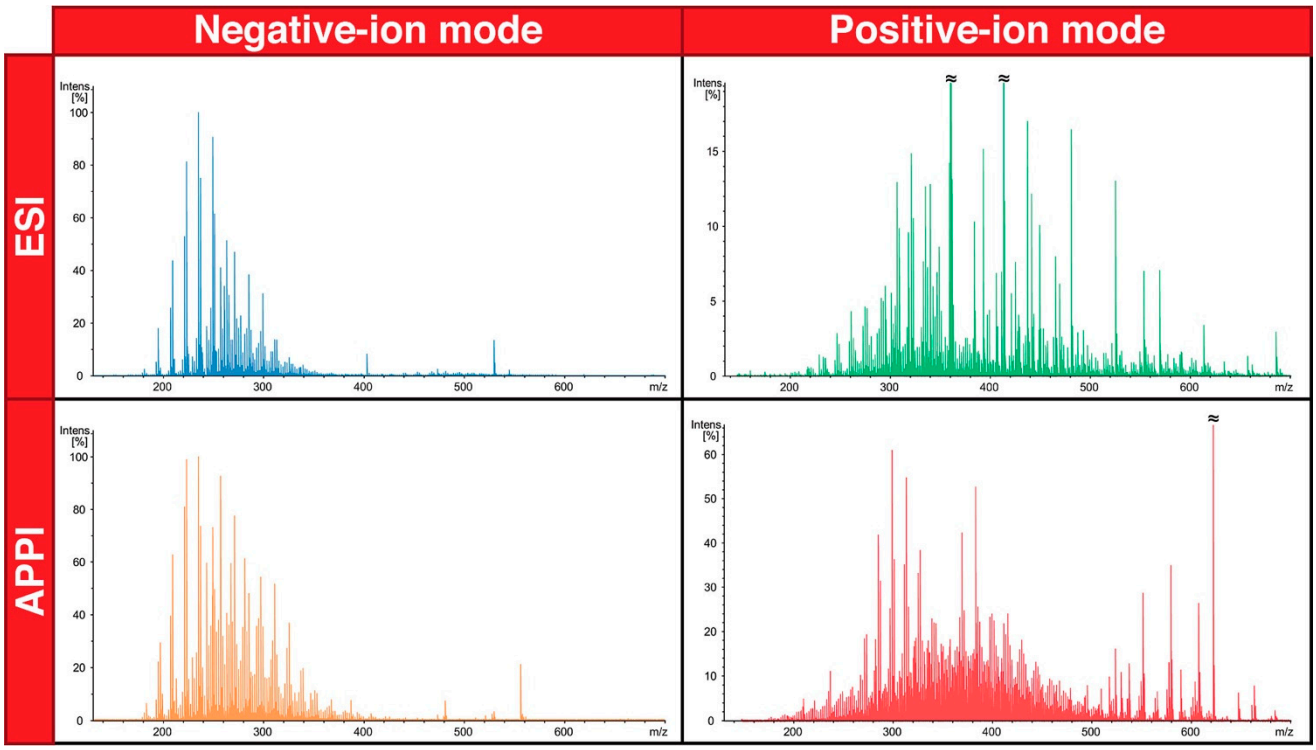

Figure 6. Comparison of negative- and positive-ion FT-ICR spectra of OSPW with ESI and APPI ionization [26].

Some overlap and distinct differences exist in the ionization efficiencies of compounds using different ionization methods and polarities. Pereira et al. showed that compounds with the same empirical formula eluted with different chromatographic retention times in positive and negative mode ESI [27], indicating the presence of different compounds. Using ESI+, 162 and 85 compounds were identified in OSPW produced from surface mined and in situ extracted bitumen, respectively, that were not detected by ESI-. The O_2^+ species were determined to be dihydroxy, diketo, or ketohydroxy groups based on MS^2 and MS^3 fragmentation and similar behaviour of model compounds. The authors noted that some sex hormones, such as testosterone, androstenedione, and 5-androstenediol, belong to these chemical classes and that OSPW has endocrine-disrupting effects. ESI does not ionize these functional groups in either positive or negative polarities. Thus, the analysis of OSPW for possible toxic compounds should include additional ionization techniques. Matrix effects, especially ion suppression, are a well-known phenomenon of ESI. Ion suppression with reverse-phase chromatography is most evident in samples containing high salts and other hydrophilic compounds. LC separations can help reduce salt-induced ion suppression compared to infusion by retaining organic compounds on the column while the non-retained salts are directed to waste. However, suppression effects are often evident due to other co-eluting compounds. APCI and APPI are less prone to ion suppression than ESI.

2.8. Naphthenic Acids vs. Acid Extractable Organics

Ultrahigh-resolution electrospray ionization FT-ICR MS was used to characterize various OSPWs that contained between 1 and 7% sulfur, as determined by elemental analysis [23]. MS spectra contained up to 1880 peaks, with over 99% of the ions between

145 and 600 amu. Of these, less than 20% were determined to be cNAs, and less than half of the total abundance was due to cNAs plus oxy-NAs, indicating that >50% of the extracted material was not naphthenic acids. The authors, therefore, proposed replacing the term "naphthenic acids" with "oil sands tailings water acid-extractable organics (OSTWAEO)" which has subsequently been replaced by the simpler phrase acid-extractable organics (AEO) or NAFCs [9].

2.9. Mass Resolution of the Mass Spectrometer Instrument

Mass resolution is defined as the mass of an ion divided by the mass width at half height of the spectral peak (Equation (2)) and is a measure of how well two similar masses are separated in a spectrum. Accurate mass instruments such as Orbitraps and TOFs provide much higher mass accuracies than unit mass quadrupoles due to their higher mass resolutions.

$$R = m/\Delta m \quad (2)$$

Equation (2) is a formula for determining mass resolution of a mass spectrometer, where m is the mass of an ion, and Δm is the mass width at half height.

Mass resolution for TOF instruments using the formula in Equation (2) is defined when two peaks have equal height and width. When the peak height ratio is 100:1, the resolution may need to be 10× higher than calculated, since the mass at lower abundance will otherwise be included in the base of the more abundant ion [29]. High-resolution accurate mass (HRAM) instruments such as FT-ICR, TOF, and Orbitrap are, therefore, better suited to chemical characterization of NAFCs than unit mass quadrupoles. Their relatively high cost of purchase, maintenance, and operation preclude them from use in many labs. They are, however, required for elemental speciation and separation of OSPW into compound classes due to their ability to produce accurate mass assignments to the fourth or fifth decimal place. Mass accuracy at this level is required for unambiguous assignment of chemical formulae to unknown compounds. Correct formula assignments typically require mass accuracies of ±5 ppm when restricting the formula to C, H, O, N, and S in the molecules. This forward calculation between two chemical formulae provides straightforward mass error calculations. However, since mass spectrometers measure accurate masses, the calculation must be carried out in reverse, i.e., start with an accurate mass and use it to determine the chemical formula. Constraints for the type and number of elements that are included in the calculation are used to limit the number of possible formulae. For example, if the mass of an ion was measured to be 281.1763 (mass error −1.7 ppm for $C_{16}H_{26}O_4$), there are six possible formulae when C, H, O, N, and S are allowed, with mass error up to ±10 ppm, (Table 3). Using an MS with mass accuracy known to be ±5 ppm, only the top three formulae would be possible. The third ion formula ($C_{17}H_{21}N_4$) would not be forbidden but is unlikely to be an anion. If mass accuracy was known to be ±2 ppm, neither of the first two formulae could be dismissed but the third would be eliminated. Adding an LC separation to the analysis and using model compounds with known formulae and retention times may separate isobaric compounds (i.e., compounds with the same unit mass) and add more confidence to otherwise ambiguous compound assignments.

Table 3. Mass accuracies of chemical formulae of ions within 10 ppm of m/z measured at 281.1763 and the mass resolution that would be required to separate that chemical species from the observed mass.

Ion Formula	*m/z* (Calc)	Diff (ppm)	Resolution
$C_9H_{25}N_6O_2S$	281.1765	0.8	1,406,000
$C_{16}H_{25}O_4$	281.1758	−1.7	562,000
$C_{17}H_{21}N_4$	281.1772	3.1	312,000
$C_{11}H_{27}N_3O_3S$	281.1779	5.5	176,000
$C_{14}H_{23}N_3O_3$	281.1745	−6.4	156,000
$C_{19}H_{23}N\,O$	281.1785	7.8	128,000

2.10. Use of Internal vs. External Standard

Internal standards (ISTDs) are compounds of similar chemical structure to the target compounds that are not present in the sample matrix. Preferred ISTDs are isotopically labelled analogs of target compounds that contain isotopes such as ^{2}H (D, deuterium), ^{13}C, ^{15}N, and/or ^{17}O. ISTDs can correct for a multitude of instrumental variances and their use increases the robustness of analytical methods. They are often used to correct for ion suppression in LC/MS applications, where co-eluting compounds may suppress the response of target analytes relative to their response in pure solvent and are very effective when each target has its corresponding ISTD analog. However, in the case of complex mixtures such as OSPW, this is not possible. Figure 7 illustrates that using a single ISTD may only correct for ion suppression within a small portion of a chromatographic run (R. Hindle, Vogon Labs, unpublished data). The Total Ion Chromatogram (TIC) is the sum of all MS responses at a given time. The spectrum captured at 4.12 min, the apex of the TIC, is much more complex than the spectrum at 6.85 min, where myristic acid-d27 elutes. As greater ion suppression is assumed at the earlier retention time, the single ISTD does not make a proportional correction for all components. A single ISTD may be more useful as a measure of instrument performance [33] between injections within a batch and over a longer period of time [41].

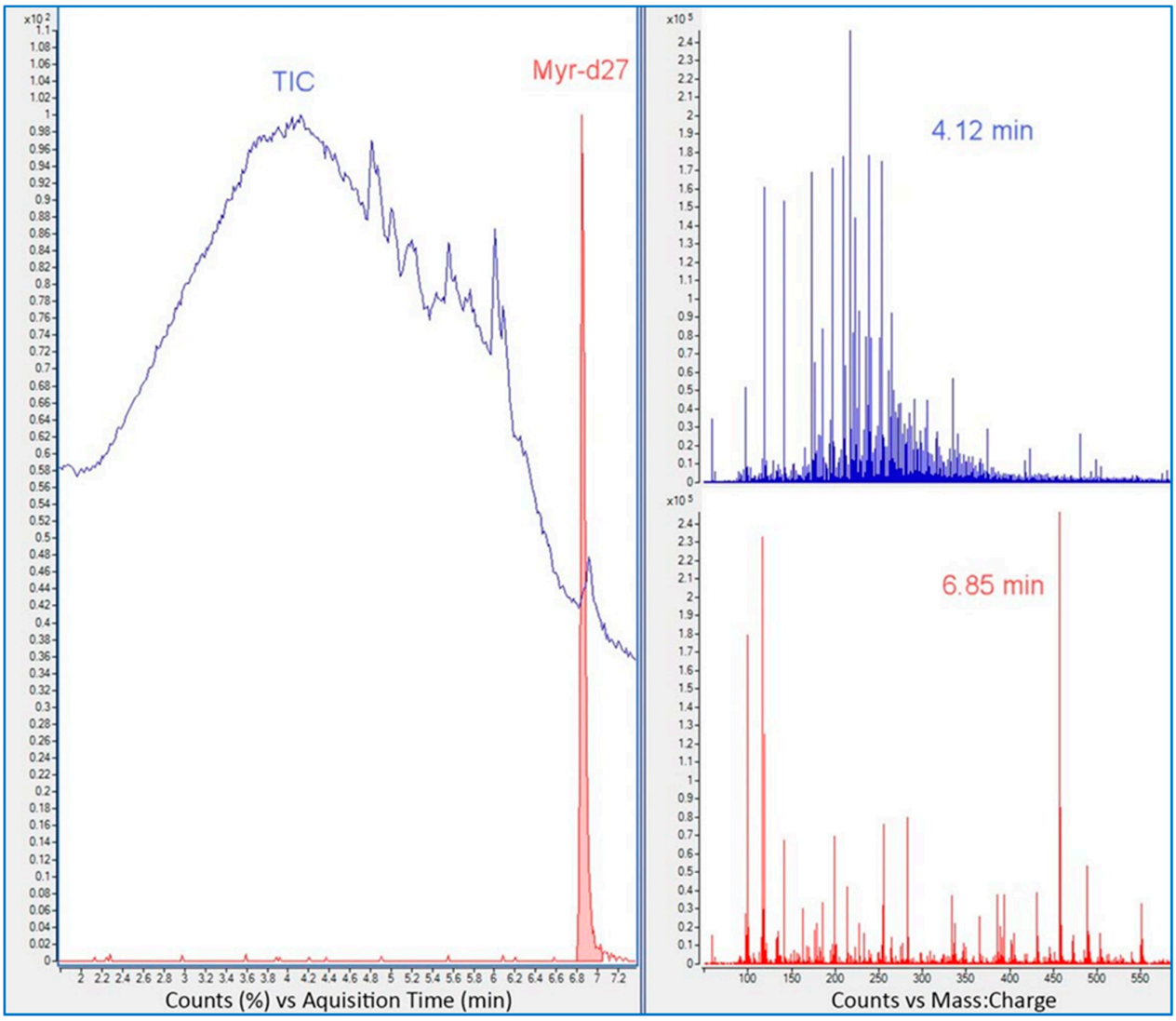

Figure 7. Total ion chromatogram (TIC; abundance = 6×10^{7}) of OSPW and myristic acid-d27 ISTD (abundance = 6×10^{4}) with corresponding spectra (both spectra at the same scale) at apex of peaks [42].

When comparing external standardization (ESTD) to ISTD within the same set of experiments, Huang et al. showed that ESTD required the use of liquid–liquid extraction (LLE) at high pH to remove the interfering matrix before quantitation [43]. However, this resulted in an underestimation of higher O_x compounds that partitioned into DCM. Addition of myristic acid-$^{13}C_1$ as an ISTD avoided the necessity of LLE cleanup prior to analysis and showed that either technique was suitable for cleaner matrices such as groundwater. For ESTD, calibration curves are prepared by analyzing a series of different concentrations of the target compounds, either in pure solvent, an analyte-free matrix (matrix blank) that has been taken through all the method steps, or a simulated matrix when no suitable matrix blank is available. Sample background interferences must be considered closely so that cleanup methods remove as many interferences as possible.

3. Conclusions and Recommendations

Ensuring consistency of data among laboratories has always been a cornerstone of effective analytical methods. The methods do not necessarily have to be prescriptive, where specified columns, mobile phases, or analytical instruments are mandatory for a given end use. Performance based methods allow a wider range of choices by individual laboratories while still providing confidence in the data as long as critical objectives such as compound specificity, sensitivity, and reproducibility are met. In the case of the complex mixtures of NAFCs, seemingly different data from the same extract can be obtained unless due care is given to key factors [17]. Although thousands of structures are present in OSPW, the focus should be the classical NAs as these components are implicated as primary toxicants [7]. However, further studies are warranted to determine the fractions within the classical NAs that are most bioavailable and toxic according to carbon number and double bond equivalence. Such studies are needed to help guide the development of NA guidelines for the protection of aquatic environments. In the case of labs providing classical NA data for regulatory purposes, the following recommendations are made for the listed categories that were detailed in this review paper:

i. Choice of extraction solvent: If using liquid–liquid extraction, adjust the aqueous pH to $\leq$1.5 and extract with DCM.
ii. Salt content of sample: Partition cNAs into a water-immiscible solvent such as DCM or use a wash step with SPE cleanup to avoid salt in the final extract. Use LC and discard the early eluant to waste before directing column eluant to the MS source.
iii. pH of extraction: Classical NAs have pK_a values that range from 3.5 to 6.8. When partitioning into the organic phase, use pH $\leq$ 1.5. When partitioning into the aqueous phase, use pH $\geq$ 8.8. A narrow range will reduce the extraction of additional unwanted compounds.
iv. Sample cleanup: Perform LLE or SPE cleanup prior to MS analysis to reduce interferences and minimize ion suppression.
v. On-line chromatography: Use LC separation to reduce interferences, minimize ion suppression, and add a time dimension to compound resolution.
vi. Calibration standards: Use Merichem® mixture or concentrated NAFC extract from OSPW in acetonitrile.
vii. Ionization mode: For cNAs, use electrospray negative-ion mode.
viii. NAs versus NAFCs: Classical NAs are a subset of NAFCs. Use method parameters to focus on recovering and measuring only cNAs.
ix. Mass resolution: The minimum resolution should be 25,000 when using an LC separation. The infusion should be 50,000 for previously characterized OSPW, and 100,000+ for unknowns.
x. Use of internal standard: use an ISTD to monitor instrument performance.

Author Contributions: Writing—original draft preparation, R.H.; writing—review and editing. R.H., D.G.M. and J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Genome Canada through a Large Scale Applied Research Project (LSARP) grant (grant number 18207), in partnership with Genome Alberta and Genome Quebec.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Canadian Association of Petroleum Producers. *2019 Crude Oil Forecast, Markets and Transportation*; Canadian Association of Petroleum Producers: Calgary, AB, Canada, 2019; Volume 26.
2. Monaghan, J.; Richards, L.C.; Vandergrift, G.W.; Hounjet, L.J.; Stoyanov, S.R.; Gill, C.G.; Krogh, E.T. Direct mass spectrometric analysis of naphthenic acids and polycyclic aromatic hydrocarbons in waters impacted by diluted bitumen and conventional crude oil. *Sci. Total Environ.* **2021**, *765*, 144206. [CrossRef]
3. Allen, E.W. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J. Environ. Eng. Sci.* **2008**, *7*, 123–138. [CrossRef]
4. Clemente, J.S.; Fedorak, P.M. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* **2005**, *60*, 585–600. [CrossRef]
5. Morandi, G.D.; Wiseman, S.B.; Pereira, A.; Mankidy, R.; Gault, I.G.M.; Martin, J.W.; Giesy, J.P. Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. *Environ. Sci. Technol.* **2015**, *49*, 12395–12404. [CrossRef]
6. Scarlett, A.G.; West, C.E.; Jones, D.; Galloway, T.S.; Rowland, S.J. Predicted toxicity of naphthenic acids present in oil sands process-affected waters to a range of environmental and human endpoints. *Sci. Total Environ.* **2012**, *425*, 119–127. [CrossRef]
7. Hughes, S.A.; Mahaffey, A.; Shore, B.; Baker, J.; Kilgour, B.; Brown, C.; Peru, K.M.; Headley, J.V.; Bailey, H.C. Using ultrahigh-resolution mass spectrometry and toxicity identification techniques to characterize the toxicity of oil sands process-affected water: The case for classical naphthenic acids. *Environ. Toxicol. Chem.* **2017**, *36*, 3148–3157. [CrossRef]
8. Clemente, J.S.; Prasad, N.G.N.; MacKinnon, M.D.; Fedorak, P.M. A statistical comparison of naphthenic acids characterized by gas chromatography-mass spectrometry. *Chemosphere* **2003**, *50*, 1265–1274. [CrossRef]
9. Headley, J.V.; Peru, K.M.; Mohamed, M.H.; Frank, R.A.; Martin, J.W.; Hazewinkel, R.R.O.; Humphries, D.; Gurprasad, N.P.; Hewitt, L.M.; Muir, D.C.G.; et al. Chemical fingerprinting of naphthenic acids and oil sands process waters—A review of analytical methods for environmental samples. *J. Environ. Sci. Health Part A* **2013**, *48*, 1145–1163. [CrossRef]
10. Yue, S.; Ramsay, B.A.; Wang, J.; Ramsay, J.A. Biodegradation and detoxification of naphthenic acids in oil sands process affected waters. *Sci. Total Environ.* **2016**, *572*, 273–279. [CrossRef]
11. Folwell, B.D.; McGenity, T.J.; Whitby, C. Diamondoids are not forever: Microbial biotransformation of diamondoid carboxylic acids. *Microb. Biotechnol.* **2020**, *13*, 495–508. [CrossRef]
12. Cancelli, A.M.; Gobas, F.A.P.C. Treatment of naphthenic acids in oil sands process-affected waters with a surface flow treatment wetland: Mass removal, half-life, and toxicity-reduction. *Environ. Res.* **2022**, *213*, 113755. [CrossRef]
13. Ajaero, C.; Meulen IVander Simair, M.C.; Roux MLe Parrott, J.; Peru, K.M.; McMartin, D.W.; Headley, J.V. Developments in molecular level characterization of naphthenic acid fraction compounds degradation in a constructed wetland treatment system. *Environments* **2020**, *7*, 89. [CrossRef]
14. Leshuk, T.; Wong, T.; Linley, S.; Peru, K.M.; Headley, J.V.; Gu, F. Solar photocatalytic degradation of naphthenic acids in oil sands process-affected water. *Chemosphere* **2016**, *144*, 1854–1861. [CrossRef]
15. Quinlan, P.J.; Tam, K.C. Water treatment technologies for the remediation of naphthenic acids in oil sands process-affected water. *Chem. Eng. J.* **2015**, *279*, 696–714. [CrossRef]
16. Kovalchik, K.A.; MacLennan, M.S.; Peru, K.M.; Headley, J.V.; Chen, D.D.Y. Standard method design considerations for semi-quantification of total naphthenic acids in oil sands process affected water by mass spectrometry: A review. *Front. Chem. Sci. Eng.* **2017**, *11*, 497–507. [CrossRef]
17. Peru, K.M.; Thomas, M.J.; Palacio Lozano, D.C.; McMartin, D.W.; Headley, J.V.; Barrow, M.P. Characterization of oil sands naphthenic acids by negative-ion electrospray ionization mass spectrometry: Influence of acidic versus basic transfer solvent. *Chemosphere* **2019**, *222*, 1017–1024. [CrossRef]
18. Rogers, V.V.; Liber, K.; MacKinnon, M.D. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. *Chemosphere* **2002**, *48*, 519–527. [CrossRef]
19. Headley, J.V.; Peru, K.M.; Barrow, M.P.; Derrick, P.J. Characterization of naphthenic acids from Athabasca oil sands using electrospray ionization: The significant influence of solvents. *Anal. Chem.* **2007**, *79*, 6222–6229. [CrossRef]
20. Ripmeester, M.J.; Duford, D.A. Method for routine "naphthenic acids fraction compounds" determination in oil sands process-affected water by liquid-liquid extraction in dichloromethane and Fourier-Transform Infrared Spectroscopy. *Chemosphere* **2019**, *233*, 687–696. [CrossRef]
21. Headley, J.V.; Barrow, M.P.; Peru, K.; Derrick, P.J. Salting-out effects on the characterization of naphthenic acids from Athabasca oil sands using electrospray ionization. *J. Environ. Sci. Health Part A* **2011**, *46*, 844–854. [CrossRef]
22. Celsie, A.; Parnis, J.M.; Mackay, D. Impact of temperature, pH, and salinity changes on the physico-chemical properties of model naphthenic acids. *Chemosphere* **2016**, *146*, 40–50. [CrossRef]

23. Grewer, D.M.; Young, R.F.; Whittal, R.M.; Fedorak, P.M. Naphthenic acids and other acid-extractables in water samples from Alberta: What is being measured? *Sci. Total Environ.* **2010**, *408*, 5997–6010. [CrossRef]
24. Headley, J.V.; Peru, K.M.; McMartin, D.W.; Winkler, M. Determination of dissolved naphthenic acids in natural waters by using negative-ion electrospray mass spectrometry. *J. AOAC Int.* **2002**, *85*, 182–187. [CrossRef]
25. Huang, R.; Sun, N.; Chelme-Ayala, P.; McPhedran, K.N.; Changalov, M.; Gamal El-Din, M. Fractionation of oil sands-process affected water using pH-dependent extractions: A study of dissociation constants for naphthenic acids species. *Chemosphere* **2015**, *127*, 291–296. [CrossRef] [PubMed]
26. Barrow, M.P.; Witt, M.; Headley, J.V.; Peru, K.M. Athabasca oil sands process water: Characterization by atmospheric pressure photoionization and electrospray ionization Fourier Transform Ion Cyclotron Resonance mass spectrometry. *Anal. Chem.* **2010**, *82*, 3727–3735. [CrossRef]
27. Pereira, A.S.; Bhattacharjee, S.; Martin, J.W. Characterization of oil sands process-affected waters by liquid chromatography orbitrap mass spectrometry. *Environ. Sci. Technol.* **2013**, *47*, 5504–5513. [CrossRef] [PubMed]
28. Qin, R.; Lillico, D.; How, Z.T.; Huang, R.; Belosevic, M.; Stafford, J.; Gamal El-Din, M. Separation of oil sands process water organics and inorganics and examination of their acute toxicity using standard in-vitro bioassays. *Sci. Total Environ.* **2019**, *695*, 133532. [CrossRef]
29. Marshall, A.G.; Blakney, G.T.; Chen, T.; Kaiser, N.K.; McKenna, A.M.; Rodgers, R.P.; Ruddy, B.M.; Xian, F. Mass resolution and mass accuracy: How much is enough? *Mass Spectrom.* **2013**, *2*, S0009. [CrossRef]
30. Feehan, J.F.; Monaghan, J.; Gill, C.G.; Krogh, E.T. Direct measurement of acid dissociation constants of trace organic compounds at nanomolar levels in aqueous solution by condensed phase–membrane introduction mass spectrometry. *Environ. Toxicol. Chem.* **2019**, *38*, 1879–1889. [CrossRef]
31. Duncan, K.D.; Richards, L.C.; Monaghan, J.; Simair, M.C.; Ajaero, C.; Peru, K.M.; Friesen, V.; McMartin, D.W.; Headley, J.V.; Gill, C.G.; et al. Direct analysis of naphthenic acids in constructed wetland samples by condensed phase membrane introduction mass spectrometry. *Sci. Total Environ.* **2020**, *716*, 137063. [CrossRef] [PubMed]
32. Hindle, R.; Noestheden, M.; Peru, K.; Headley, J. Quantitative analysis of naphthenic acids in water by liquid chromatography–accurate mass time-of-flight mass spectrometry. *J. Chromatogr. A* **2013**, *1286*, 166–174. [CrossRef] [PubMed]
33. Brunswick, P.; Shang, D.; van Aggelen, G.; Hindle, R.; Hewitt, L.M.; Frank, R.A.; Haberl, M.; Kim, M. Trace analysis of total naphthenic acids in aqueous environmental matrices by liquid chromatography/mass spectrometry-quadrupole time of flight mass spectrometry direct injection. *J. Chromatogr. A* **2015**, *1405*, 49–71. [CrossRef]
34. Hughes, S.A.; Huang, R.; Mahaffey, A.; Chelme-Ayala, P.; Klamerth, N.; Meshref, M.N.A.; Ibrahim, M.D.; Brown, C.; Peru, K.M.; Headley, J.V.; et al. Comparison of methods for determination of total oil sands-derived naphthenic acids in water samples. *Chemosphere* **2017**, *187*, 376–384. [CrossRef] [PubMed]
35. Hindle, R.; (Vogon Laboratory Services, Cochrane, AB, Canada). Manuscript in preparation. 2023.
36. Canadian Council of Ministers of the Environment. *Canadian Water Quality Guidelines for the Protection of Aquatic Life: Ammonia*; Canadian Environmental Quality Guidelines; Canadian Council of Ministers of the Environment: Winnipeg, MB, Canada, 2010; Volume 8.
37. Kind, T.; Fiehn, O. Metabolomic database annotations via query of elemental compositions: Mass accuracy is insufficient even at less than 1 ppm. *BMC Bioinform.* **2006**, *7*, 234. [CrossRef]
38. Bowman, D.T.; Warren, L.A.; Slater, G.F. Isomer-specific monitoring of naphthenic acids at an oil sands pit lake by comprehensive two-dimensional gas chromatography–mass spectrometry. *Sci. Total Environ.* **2020**, *746*, 140985. [CrossRef] [PubMed]
39. McEwen, C.N.; Larsen, B.S. Ionization mechanisms related to negative ion APPI, APCI, and DART. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1518–1521. [CrossRef]
40. Raffaelli, A.; Saba, A. Atmospheric pressure photoionization mass spectrometry. *Mass Spectrom. Rev.* **2003**, *22*, 318–331. [CrossRef]
41. Brunswick, P.; Hewitt, L.M.; Frank, R.A.; Kim, M.; van Aggelen, G.; Shang, D. A traceable reference for direct comparative assessment of total naphthenic acid concentrations in commercial and acid extractable organic mixtures derived from oil sands process water. *J. Environ. Sci. Health Part A* **2017**, *52*, 274–280. [CrossRef]
42. Hindle, R.; (Vogon Laboratory Services, Cochrane, AB, Canada). Manuscript in preparation. 2023.
43. Huang, R.; Chen, Y.; Meshref, M.N.A.; Chelme-Ayala, P.; Dong, S.; Ibrahim, M.D.; Wang, C.; Klamerth, N.; Hughes, S.A.; Headley, J.V.; et al. Characterization and determination of naphthenic acids species in oil sands process-affected water and groundwater from oil sands development area of Alberta, Canada. *Water Res.* **2018**, *128*, 129–137. [CrossRef]