A novel derivatization-based liquid chromatography tandem mass spectrometry method for quantitative characterization of naphthenic acid isomer profiles in environmental waters

Million B. Woudneh a, *, M. Coreen Hamilton a, Jonathan P. Benskin a, Guanghui Wang a, Preston McEachern b, 1, John R. Cosgrove a

a AXYS Analytical Services Ltd., 2045 Mills Road West, Sidney, British Columbia V8L 5X2, Canada
b Alberta Environment, 5637-81 Ave., Edmonton, Alberta T6G 0X6, Canada

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A method for quantitative characterization of naphthenic acid (NA) isomer groups by carbon number and extent of cyclization was developed and validated with water samples from northern Alberta. Following solid phase extraction, NAs undergo derivatization with N-(3-dimethylaminopropyl)-N’-ethy carbodiimide (EDC) allowing detection by positive electrospray ionization tandem mass spectrometry (+ESI)-MS/MS. NA-EDC derivatives produce a common product ion by MS/MS, regardless of structure of the starting NA. Thus, approximately constant relative response factors (RRFs) were assumed for the various isomer groups that elute at a given point in the elution gradient (supported by calculated RRFs for individual model NAs, facilitating quantification using a single standard (1-pyrenebutyric acid)). To reduce the impact of major background fatty acids on NA data, the method employed an optimized liquid chromatography method that separated straight chain (Z=0) analytes from other NAs. Method validation was performed at two spiking levels (7.72 µg and 38.6 µg total refined Merichem per 500 mL of reagent water) and good accuracy (mean recoveries of 82.4 ± 2.5% and 93.0 ± 2.6%, respectively; range ~50–130%) and precision (<17% RSD) were achieved at both spiking levels for all 60 NA isomer groups. The method also performed well in an independent method comparison study in which method accuracy values of 107%, 120%, and 121% were obtained for 2 spiked reagent waters (1 mg/L and 50 mg/L NAs) and spiked Athabasca River water (0.035 mg/L NAs), respectively. Application of the method to samples from northern Alberta revealed that NA concentrations decreased in the order: process water (52.8 mg/L) > tailings pond water (30.6 mg/L) > well water (0.086 mg/L) > surface water (0.007 mg/L), and that samples were distinguishable by NA isomer profile using Principal components analysis.

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1. Introduction

Among the numerous challenges facing Alberta’s rapidly expanding oil sands industry is the containment and remediation of oil sands process water and distinction between ambient and anthropogenic environmental chemical occurrence. Process water is a by-product of the extraction of oil from bitumen and is stored on site in large tailings ponds. Naphthenic acids (NAs), which occur naturally in the environment (in particular in oil deposits) and tend to concentrate during the extraction process, are among the major toxic constituents of this water [1]. NAs have the general formula C nH 2n+2O Z where ‘Z’ is zero or a negative integer representing the hydrogen deficit due to cyclization or the presence of double bonds and ‘n’ is the number of carbon atoms [2,3]. Monitoring for NA contamination in water presents a particular challenge due to the complexity of these substances and also due to the difficulty in distinguishing naturally-occurring NA contamination (i.e. in the ambient environment) from that arising due to industry operations.

Several analytical approaches have been developed over the past decade for characterizing NAs in environmental samples [4–9] yet accurate and quantitative analysis remains a challenge, even using state-of-the art instrumentation. Scott et al. [6] compared gas chromatography–low resolution mass spectrometry (GC–LRMS) and Fourier transform infrared (FTIR) spectroscopy and found much lower detection limits associated with the former method (0.01 mg/L versus 1 mg/L, respectively), while the latter method...
tended to overestimate NA concentrations. A comparison of high performance liquid chromatography–high resolution mass spectrometry (HPLC–HRMS) and GC–LRMS-based methods revealed considerable classification of some NAs by the low resolution method due to double derivatization of hydroxylated NAs (confirmed by GC–HRMS) [4]. Martin et al. [10] subsequently compared direct injection electrospray ionization mass spectrometry (ESI–MS) and HPLC–HRMS and found that while both methods produced very similar characterization of a pure commercial NA mixture, neither method produced consistent values with those obtained by FTIR. Overall, the authors concluded that both the MS-based methods could only be considered semi-quantitative at best.

The inaccruacies surrounding quantitative analysis of NAs in environmental samples arise in part because the exact structure of NAs in environmental samples has not yet been fully elucidated [11,12]. As a result, standards used for quantification (typically commercial formulations) may be inconsistent with the analytes being quantified, leading to inaccuracies and poor intercomparison of data. Furthermore, commercially available NA products tend to contain impurities which are not traditionally defined as NAs [13]. In fact, as little as ~20% of the peaks in an NA standard mixture were recently identified as fitting the traditional NA formula [14]. In other instances, methods have been calibrated using the response generated from one NA isomer which was then used to determine the total NA concentration in a sample [6,9]. This presents a problem for mass-spectrometric based detection due to differences in response factors and ionization efficiencies between the various NA isomers, as well as the presence of co-eluting isobaric substances which may be mistakenly quantified as NAs. In addition, the relative proportions of the various NA isomer groups cannot be assumed to be constant in all samples. Overall, these issues are expected to produce considerable variability between methods and laboratories and will make comparisons difficult.

In the present work, we hypothesized that analytical accuracy may be significantly improved using a derivatization–LC–MS/MS based approach, whereby derivatized NAs undergo collision-induced dissociation to produce a single common product ion (regardless of the structure of the parent NA), which is then quantified using a single, purified standard. Several NA-derivatization procedures exist, however most are designed to improve volatility and facilitate GC-based analysis [15,16]. For LC-based applications, Clemente et al. [2], and Yen et al. [8] reported the use of 2-nitrophenylhydrazine derivatives which were detected using a UV–vis diode array detector; however, detection limits were quite high using this approach (15 and 5 mg/L, respectively). Subsequently, Smith and Rowland [17] also developed a derivatization method for NAs designed for analysis by LC–MS/MS. In that work, NAs were reacted with oxalyl chloride to form acyl chlorides, which were subsequently converted to NA-amide derivatives with ammonium hydroxide. While this method was highly effective for structural elucidation of individual NAs, the use of amide derivatives resulted in unique product ions depending on the starting NA, making it difficult to apply to routine analysis of samples in which the structure of the parent NA is unknown [17].

In the present work, we examined the derivatization of NAs using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC). The resulting derivatives were subjected to LC separation followed by +ESI MS/MS to produce a single product ion (regardless of the structure of the starting NA), which could be quantified using a single standard. In addition to quantitative capabilities, we sought to develop a method incorporating sub-ng/L detection limits appropriate to the concentrations of NAs in the ambient environment, as well as the ability to separate various NA isomer groups to enable fingerprinting and source characterization. The optimized method was subjected to external validation through independent inter-method comparison.

2. Experimental methods

2.1. Standards and reagents

Refined Merichem NA mix was a gift from Dr. P. Fedorak (University of Alberta, Edmonton, AB, Canada). 1-Pyrenebutyric acid (PYB) (97%), cyclohexanecarboxylic acid (≥98%), cyclohexanebutyric acid (99%), decanoic acid (DA) (>98%), β-hydroxybutyric acid (>98%) were all obtained from Sigma–Aldrich, Oakville, ON, Canada. Abietic acid (92%) was obtained from Alfa Aesar, Ward Hill, MA, USA. The isotopically-labeled compounds [1H19-decanoic acid (98.8%), [2H35- eicosanoic acid and [2H35-heptadecanoic acid were obtained from CDN Isotopes (Pointe-Claire, QC, Canada), while [2H33-dodecanoic acid (98%), [2H37-tetradecanoic acid (98%), [2H31-hexadecanoic acid (98%) and [13C3]-atrazine were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). All standard solutions were prepared in methanol (MeOH).

The derivatization reagent, N-(3-dimethylaminopropyl)-N′- ethylcarbodiimide hydrochloride (EDC) was obtained from Sigma–Aldrich (Oakville, ON, Canada) and pyridine (99.9%) was obtained from Fisher Chemicals (Aurora, ON, Canada). All solvents used were Trace grade. The derivatization reagent was prepared by dissolving 480 mg of EDC reagent in 10 mL of ethanol with 10 mL of 3% pyridine in ethanol (v/v).

2.2. Sample collection and preparation

Well water, surface water, tailings pond water and process water samples were collected from sites in northern Alberta. Grab samples were collected using 4L amber glass bottles and stored at −4 °C prior to extraction and analysis. The extraction method utilized 1–20 mL of process water, tailings pond water and well water and 500 mL of surface water.

Aquiferous samples were shaken and filtered through an glass fiber filter (161 grade, 1.1 μm, 42.52 mm diameter; Ahlstrom, Helsinki, Finland) using a Millipore vacuum filtration apparatus (EMD Millipore, Billerica, MA, USA). A portion of the filtered sample (2–500 mL) was spiked with 50 ng each of the isotopically-labeled standards in Table S1 (SI). Sample volumes less than 500 mL were diluted to 500 mL with reagent water prior to extraction and adjusted to pH 5–7 (when outside this range) with conc. ammonium hydroxide or hydrochloric acid as necessary. Oasis HLB cartridges (1 g = 20cc; Waters, Milford, MA, USA) were conditioned with 3 × 20 mL of methanol and equilibrated with 1 × 20 mL of reagent water. The aqueous sample was then loaded onto the SPE cartridge at a rate of 5–10 mL/min. After loading, the cartridge was washed with 10 mL of reagent water and allowed to dry under vacuum for 10 min. NAs were eluted with 60 mL of MeOH, which was reduced to 1 mL by rotary evaporation and transferred to a 15 mL centrifuge tube. The extract was further reduced to 500 μL under a gentle stream of nitrogen and a water bath temperature of 40 °C in preparation for derivatization.

2.3. Extract derivatization

To increase instrument sensitivity and enable tandem mass spectrometric (MS/MS) detection, we tested a modified version of the derivatization reaction employed by Yen et al. [8] and Miwa et al. [18]. In those studies, carboxylic acids were reacted with EDC and 2-nitrophenylhydrazine hydrochloride (2-NPH HCl) to form carboxylic acid hydrazides which were amenable to photometric detection. However, in our experience, this reaction resulted in the formation of multiple products, including NA–NPH and NA–EDC. To reduce byproduct formation and increase sensitivity under (+) ESI MS/MS conditions, 2-NPH HCl was not used and the reaction was allowed to proceed to the formation of the acylurea.

derivative (NA—EDC). In brief, 80 µL of 0.25 mM EDC was added to the extract which was then heated in a water bath at 60 °C. After 20 min, the extract was removed and 40 µL of 140 mM KOH in 80:20 MeOH:water was added and heated for a further 15 min at 60 °C. The microvial was then cooled in a water bath (−10 °C). The resulting extract was spiked with 50 ng of 13C3-atrazine recovery standard and adjusted to a final volume of 1 mL prior to analysis by LC–MS/MS. The recovery standard, 13C3-atrazine, was used to monitor instrument performance and quantitatively surrogacy recovery values.

2.4. Instrumental analysis and quantification approach

Analysis was performed by LC–MS/MS using a Waters 2795 HPLC connected via an electrospray interface to a Waters Quattro Ultima tandem mass spectrometer (Micromass, Manchester, UK) operated in positive ion electrospray multiple reaction monitoring (MRM) mode. Details of optimized liquid chromatographic, mass spectrometric and calibration conditions are provided in the SI. Precursor and product ions selected for the derivatized NAs and model NAs are summarized in Tables S1 and S2 (SI). Calibration equations and coefficient of determinations for each isomer group are provided in Tables S3 and S4 (SI).

2.5. Fingerprint analysis of NA isomer profiles

Principal component analysis (PCA) [19,20] was performed on NA data using Applied Biosystems/MDS Analytical Technologies MarkerView Software (Carlsbad, CA) to examine differences and similarities among NA isomer profiles between samples. We included 5 reference samples which displayed 3 unique NA fingerprints from (a) non-industry impacted environments (i.e. pristine surface water, far from naturally occurring oil deposits or industry operations), (b) industrial activities from outside Alberta’s oil sands (refined Merichem NAs—a petroleum distillate from Texas, USA), (c) environments from within Alberta’s oil sands (tailings pond water, process water, and well water). Individual NA isomer group concentrations from the three categories of sites were normalized to a percentage of the total NA concentration in a given sample to minimize the impact of widely varying concentrations and reveal signature covariance. The PCA was performed using autoscale without weighting.

2.6. Quality control (QC)

Initial and ongoing QC measures were included in this study. Five replicates of 0.5 L reagent water samples were spiked at two levels (7.72 and 38.6 µg total refined Merichem), and analyzed by the optimized method for assessing initial demonstration of method accuracy and precision. The PYB equivalent amounts for each of the spiked isomer groups along with precision and recovery values are provided in Table S5 (SI). Recovery values of 82.4 ± 2.5% and 93.0 ± 2.6% (overall mean recovery ± standard error) were obtained for the first and second spiking levels respectively (range 50–130% for both spiking concentrations). Overall, percent recovery values did not appear to be dependent on ‘n’ or ‘Z’ number. However, notably low (<50%) and high (>130%) recovery values were observed for n = 14, Z = 0 and n = 21, Z = −10 isomer groups respectively. Percent relative standard deviations (%RSD) were all below 17% indicating good method precision.

Refined Merichem (38.6 µg) was spiked to 0.5 L of reagent water and analyzed along with each batch of aqueous samples as an ongoing demonstration of analyte recovery. For measurement of laboratory background levels a 0.5 L reagent water sample was analyzed along with each batch of field samples. Analyses of duplicate samples were conducted with each batch of aqueous samples. Relative percent differences calculated against the mean were generally below 24%. In addition, samples were spiked with isotopically-labeled quantification standards ([2H15-decanoic acid and 2H31-hexadecanoic acid] and recovery values of these isotopically-labeled standards were used for additional quality control. To correct for any variation in instrument response the two surrogates were selected so that they elute close to the beginning ([2H31-decanoic acid, RT = 19.32 min) and end of the analytical run ([2H31-hexadecanoic acid, RT = 30.09 min). Instrument stability and extent of matrix suppression were assessed by monitoring area response of 13C3-atrazine as the instrumental recovery standard. When suppression of this standard is observed the extract is diluted and reanalyzed to bring the recovery values for 13C3-atrazine within 50–150%.

The lowest PYB calibration standard, 0.25 ng/mL, adjusted for the sample size and extract volume, was used as the method detection limit (MDL). To reflect variations in detection limits as a result of chromatographic noise from matrix co-extractives, sample specific detection limits (SDL) were also calculated. The higher of the MDL or SDL value was used as a detection qualifier for reporting field sample data, for details see section titled Example calculation in SI.

3. Results and discussion

3.1. Experimental approach

Owing to the lack of available standards, initial method development involved the use of six model NAs namely; cyclohexanecarboxylic acid, cyclohexanebutyric acid, decanoic acid, 1-pyrenebutyric acid, abietic acid, 5β-cholanic acid and deuterium labeled surrogates. Analysis of model NAs was initially investigated under (−) ESI MS/MS conditions, in which NAs produce strong [M−H]− precursor ions which undergo neutral loss of carbon dioxide by collision induced dissociation to form [M−H−CO2]− product ions. However, these product ions do not easily maintain negative charge and in general produce only a weak signal under ESI–MS/MS conditions. In a MS scan of a solution of commercial NAs it was possible to detect most precursor ions corresponding to [M−H]− matching the general formula C3H2n−2O2. However, due to the poor sensitivity of these ions and their associated product ions under MS/MS analysis, a derivatization approach was investigated.

3.2. Derivatization reaction and identification of precursor and product ions

We hypothesized that the reaction of NAs with a carbodiimide to form N-acylurea derivatives might improve the poor sensitivity and selectivity that was observed under (−)ESI conditions. In the presence of pyridine and under alkaline environments, carboxylic acids react with carbodiimides to form N-acylurea derivatives [21,22]. The efficacy of the derivatization reaction was tested using the selected model NA native and isotopically-labeled compounds. A MS scan under (+)ESI mode was conducted on the reaction mixtures of both the model compounds and the isotopically-labeled surrogate standards. An example MS scan for decanoic acid and 2H15-decanoic acid is presented in Fig. 1 (scans A and B respectively). The distinct reaction products containing masses that are equal to the sum of the mass of the native or isotopically-labeled decanoic acid and that of the derivatization reagent [DA + EDC + H]+ are circled on the MS spectra. Analysis of the derivatized extracts by (−)ESI MS mode revealed an absence of signal for decanoic acid and 2H15-decanoic acid indicating that the reaction went to completion. All native and isotopically labeled model compounds demonstrated similar behavior and formed analogous products. Investigation of
suitable precursor and product ions were conducted to enable MS/MS analysis.

The proposed derivatization and fragmentation patterns are shown in Fig. 2. Product ion scans of [DA + EDC + H]^+ revealed three major product ions for each of the native (m/z 212, 257 and 129) and isotopically-labeled (m/z 231, 276 and 129) compounds. The product ion pairs m/z 212/231 and m/z 257/276 contain the precursor DA (mass difference of 19), resulting from the fully deuterium-labeled molecule. The product ion scan also showed that one ion (m/z 129) was generated for both the native and deuterium-labeled compound. This pattern was observed for every model compound investigated (Table S2, SI). Based on the behavior of these model compounds, precursor/product ion transitions corresponding to the mass ions [M + EDC + H]^+ → m/z 129 were selected for analysis under (+)ESI MS/MS. Under these conditions, for the model compounds the signal produced by the m/z 129 product ion was up to 5000 times stronger than that produced by (-)ESI MS/MS conditions (Table S6, SI).

3.3. NA target isomer group selection

A three dimensional plot of the NA isomers profiles generated by this method is provided in Fig. 1. SI. In general, NA isomer profiles generated by this method for refined Merichem were consistent with those generated by ESI-FT-ICR [23] and HPLC–HRMS methods, [4,10] with n = 12–16 isomer groups accounting for dominant NAs by all methods. By Z' numbers, the dominant isomer groups followed the order Z' = 2 > 4 > 0 > 6 > 8 > 10 > 12 by all methods.

Target NAs for this method were selected based on their presence in impacted water and refined Merichem (Tables S7 and S8, SI). In addition, four isomer groups having masses equal to the isomer groups with, n = 15, Z' = 12, and 10; n = 16, Z' = 12, and n = 17, Z' = 12, but not meeting the classical definition for NAs have been included in this method (Table S7, SI). The relevance of these isomer groups is reflected in their detection in both the surface water and refined Merichem. Grewer et al. [14] argued that the classical definition of NA by the general formula C_nH_m2nZ'O_2 is not adequate as it does not cover NAs with multiple carboxylic acid functionalities and other o xo-NAs. These NA groups are not yet well characterized and were therefore not investigated in the present work.

3.4. Chromatographic separation

Chromatographic separation of NAs was optimized using the masses of the various NA isomer groups and the selected MRM transitions corresponding to [C_nH_m2nZ'O_2 + H + EDC]^+ → m/z 129. Under the described LC gradient, NA isomer groups are separated by ‘n’ and ‘Z’ numbers. Examples of the chromatographic separation of NA isomers present in a standard solution and field sample extract are provided for Z' = 6 and n = 12–20 in Fig. 3. Similarly, example separation of n = 15 and Z' = 0 to 12 isomer groups is provided in Fig. S2 (SI). Typical retention times for the various isomer groups and the associated internal standards are provided in Table S1 (SI).

As can be seen from the chromatograms, for a given Z number, retention time increased with increasing n value. Similarly, for a given n number retention time increased with increasing Z value, i.e. for a fixed n value compounds with greater extent of cyclization eluted earlier than those with lesser extent of cyclization.

3.5. NA standards and quantification

Among the challenges associated with analysis of NAs by LC–MS/MS is that each NA in a given isomer group may undergo different ionization and produce different ions resulting in multiple response factors. In this study, the derivatization reaction that facilitated (+) ESI ionization on the EDC moiety of NA–EDC derivative followed by dissociation of each NA–EDC derivative at exactly the same point in the molecular structure. In addition, the product ion monitored for each molecule was identical for every NA compound. Taken together, the RRFs for different NAs from the same isomer group that elute at a given point in the elution gradient were assumed to be identical or very close to identical, greatly simplifying quantification. To support this assumption, RRFs for seven model compounds and all NA isomers covered by the current method were calculated and are provided in Tables S9–S11 (SI). Note that compounds that have comparable mass and elute close together have comparable RRFs.

Another challenge pertaining to quantitative analysis of NAs is the lack of suitable standards for spike/recovery experiments and marking retention times of the various isomer groups. In the present work this was overcome by characterizing a commercially available NA mixture (refined Merichem) in terms of equivalent concentrations of PYB. A PYB equivalent concentration of an NA isomer peak is a concentration of NAs that will produce a signal which is equal in strength to that produced by a PYB solution of the same concentration. The procedure used for characterization of refined Merichem is described in the SI. Any concentration that was reported in PYB equivalents could be converted to a refined Merichem solution equivalent by multiplying the numbers expressed in PYB equivalent by a factor of 0.38. This provides a reference point for data comparability with other studies where concentrations may have been reported using only refined Merichem as the quantitative standard.

3.6. Analyte identification and method specificity

A specific NA that is defined by a given ‘n’ and ‘Z’ number represents a complex mixture of compounds or isomers. As a result the NA isomers present elute over a relatively wider elution window of 2–5 min. In this work, the following criteria were used for positive identification of a given target compound(s): (1) target NA must be identified with a precursor and product ion with specific mass to charge ratios (Table S1, SI) and elute within ±0.2 min from the beginning and end (either side) of the window predicted from a standard solution of refined Merichem and analyzed at the beginning of the analysis run. (2) Peak responses were at least three times the background noise level. (3) For a given NA isomer group with a specific n number, peaks with a smaller Z number must elute after peaks with a larger Z number.

Overall, specificity was achieved for NAs defined by the general formula C_nH_m2nZ'O_2 through the combination of the following four factors: a derivatization reaction specific to carboxylic acids (i.e.
conversion of carboxylic acid functionality to N-acylurea derivative); LC separation of the derivatized extract components; selection of precursor mass specific to [NA + EDC + H]+; and selection of a product ion that is characteristic of the carboxylate EDC linkage.

3.7. Reaction byproduct

The reaction between carboxylic acids and carbodiimides has been reported to produce a less stable O-acylisourea derivative [22]. To determine the relative extent of the two products under the experimental conditions the method was validated under different initial concentrations of the NA mixture. Evaluation of data presented in Table S4 (SI) demonstrated that the formation of the N-acylurea derivative was linearly correlated to the concentration of starting NA. This confirmed that the ratio of the N-acylurea derivative to that of the O-acylisourea derivative was constant under the described experimental conditions and did not impact quantitative measurement.

3.8. NA laboratory background levels

Generally, NA laboratory background levels were below detection limits or were less than ten times the levels observed in field samples. Notable exceptions include background fatty acids which also meet the definition for NAs. Fatty acids are ubiquitous and are particularly difficult to completely remove from laboratory blanks. These compounds may heavily skew field measurements of NAs particularly for Z = 0 isomers. Chromatographic separation of straight chain isomers (i.e. Z = 0 isomers) from other isomers of the same Z-value was used to minimize the impact of fatty acid interferences. In the example provided in Fig. 4 separation of lauric acid (n = 12, Z = 0) from the other Z = 0 isomers for n = 12 is depicted both for a laboratory blank and a field sample. The signal for lauric acid, a common and ubiquitous fatty acid from multiple sources (such as detergents, any tissue, human contact, milk, oils etc.) is detected in laboratory blanks even after multistep solvent cleanup. This single NA isomer elutes later than its structural isomers (peak at 24.35 min in Fig. 4). As can be seen from the figure the propor-

![Diagram of the reaction between a naphthenic acid (NA) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) to form the NA-EDC derivative.](image)

**Fig. 2.** (A) Derivatization of a naphthenic acid (NA) with N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) to form the NA-EDC derivative. (B) Collision-induced dissociation (CID) of NA-EDC derivative under +ESI conditions resulting in the formation of the m/z 129 product ion. R1 = alkyl group, R2 = CH3CH2.

![Sample chromatograms for isomer group Z = −6, n = 12–20, for (A) a solution of refined Merichem (154 μg/mL) and (B) a field sample from northern Alberta.](image)

**Fig. 3.** Sample chromatograms for isomer group Z = −6, n = 12–20, for (A) a solution of refined Merichem (154 μg/mL) and (B) a field sample from northern Alberta.
tional contribution of this acid from the laboratory background is significant to the sample \( n = 12, Z = 0 \) isomer groups and is excluded from integration. Similar peaks are often observed for \( n = 13–19 \) and \( Z = 0 \) NAs. In this study the straight chain isomer peak was consistently excluded from the data for the specified isomer groups. The peaks for straight chain isomers were confirmed by a spiking study for selected saturated fatty acids; see Figs. S3 and S4 (SI).

3.9. Extract stability

Evaluations of the stability of the underivatized acid extracts and the NA–EDC derivatives were conducted. The NA–EDC derivative showed no significant degradation over 7 days. Stability of the underivatized NA extracts was studied over a period exceeding one year. For this experiment two sample extracts corresponding to two different surface water sampling locations in northern Alberta were analyzed over a period of 425 days. The sample extracts were placed in amber ampoules and stored in the dark under ambient conditions. Evaluation of the results over the study period showed no downward trend in concentration for all NAs detected in the extracts, indicating that sample extracts could be stored underivatized.

3.10. Field sampling results

The validated method was applied to ambient surface water (collected far from the oil sands region of Alberta), along with tailings pond water, process water, and well water (each impacted to varying degrees by natural and industrial sources associated with the oil sands). Each sample type was extracted and analyzed in duplicate and concentrations (in ng/L) of the various NA isomer groups are plotted in Fig. S5 (SI). For comparison, the concentrations of each of the NA isomer groups are also provided for a 0.039 mg/mL refined Merichem standard in the same figure. As might be expected the highest total concentration was detected in process water (52.8 mg/L), followed by tailings pond water (30.6 mg/L), well water (0.086 mg/L), and surface water (0.007 mg/L) samples. Overall NA profiles were similar between the process water, tailings pond water and well water samples. The surface water showed a marked difference in relative distribution pattern for \( n = 20 \) and \( Z = -10 \) and \(-8 \) likely due to the presence of resin and fatty acids that are not related to NAs by source. The possibility of these interferences was confirmed using spiking study employing a common resin acid isomer, pimaric acid, and a fatty acid with \( n = 20 \) and \( Z = -10 \) structure, eicosapentaenoic acid, see Figs. S6 and S7 (SI). Similarities between isomer profiles were compared further using PCA.

3.11. Fingerprinting analysis of NA isomer profiles

Elucidating the source of NAs in water samples is challenging as these substances are naturally present in the ambient environment. The objective of the PCA was to investigate whether a diverse set of samples, all containing NAs at concentrations spanning 4 orders of magnitude, could be distinguished from one another by NA isomer profile. Fig. 5A, displays a scores plot of PC1 (accounting for 49.9% of the variability) versus PC2 (accounting for 38.6% of the variability) for the 5 water samples. Samples which group together on the scores plot indicate similar overall NA isomer patterns, while groupings on the loadings plot indicate individual NA isomers which display covariance. Three groupings can be observed from the scores plot: well water, tailings pond water, and process water were all negatively scored on PC1 and PC2, while pristine surface water was grouped independently and displayed a large positive score on PC2. Refined Merichem also grouped independently and displayed a large positive score on PC1. The separate grouping of refined Merichem from oil sands-impacted waters on the PCA is not surprising considering refined Merichem is a petroleum distillate from a process in Texas, USA. In contrast, pristine surface water was collected from a site far from industry operations and contains an NA signature indicative of the ambient environment. Similar groupings of samples by source of contamination using PCA have been previously reported [24] and [25].

Inspection of the loadings plot in Fig. 5B helps to explain the groupings observed on the scores plot. The large positive loading on PC1 for \( Z = 0 \), \( n = 12–18 \), \( Z = 0 \)–10, \( n = 12–20 \), and \( Z = -4 \), \( n = 12, 19–21 \) NAs appears to explain the large positive score of refined Merichem, while NAs such as \( Z = -10 \), \( n = 19–20, Z = -8 \), \( n = 18, 19, 20, Z = -6 \), \( n = 17, 19, 20 \) tend to describe the score of pristine surface water. On the other hand, the negative scoring on both PC1 and PC2 of industry impacted waters appears to be dictated specifically by \( Z = -10 \) and \( Z = -12 \) NAs.

3.12. Potential limitations of the method

In the current method the use of a unique derivatization specific to carboxylic acids, along with chromatography and tandem mass spectrometry limits the multitude of possible isobaric interferences to only compounds containing a single carboxylic acid functional group. Non-carboxylated isobaric interferences that co-elute are unlikely to produce the specific \( m/z \) 129 product ion following collision induced dissociation. Likewise, multiple carboxylate functional NAs will be altered at various sites on the molecule by the polar derivatization reagent, EDC, and are unlikely to present isobaric interferences to the current method. However, the existence of previously reported heteroatom-, and hydroxylated–NAs, [4,14,24,26,27] which will undergo derivatization and formation of the \( m/z \) 129 product ion, may represent possible interferences in cases where they co-elute and display isobaric precursor ions with traditional NAs. Confirmation of the presence or absence of co-eluting heteroatom or hydroxyl–NAs was not possible due to a lack of authentic standards. The future availability of standards will enable such validation.

In the absence of standards and in an effort to generate independent precision and accuracy data, the current method was used in an inter–method comparison [28] for samples of wide-ranging
4. Conclusions

In this work a method was developed for extraction and quantification of NAs from water samples using SPE followed by a derivatization-based LC (+)ESI–MS/MS approach. The utility of the method was demonstrated with real samples (oil sands process water, tailings pond water, surface water and well water) and validated through inter-method comparison across a range of purported concentrations. The three main advantages of the method include: (1) up to 3 orders of magnitude improvement in sensitivity compared to direct NA analysis by (−) ESI MS/MS; (2) a (+) ESI MS ionization that is based on an EDC moiety and a fragmentation pattern that produced single product ion regardless of the structure.
of the parent NA, resulting in approximately constant RRFs among various isomers in a given NA peak which facilitated quantification using a single standard; (3) an optimized chromatographic method which enabled separation of ubiquitous straight chain fatty acids from NAs, while separating the NAs by carbon number and extent of cyclization.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2013.03.040.

References