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Characterization and pattern recognition of oil–sand naphthenic acids using comprehensive two-dimensional gas chromatography/ time-of-flight mass spectrometry

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Abstract

Oil–sand naphthenic acids (NAs) are organic wastes produced during the oil–sand digestion and extraction processes and are very difficult to separate and analyze as individual components due to their complex compositions. A comprehensive two-dimensional gas chromatography/time of flight mass spectrometry (GC × GC/TOF–MS) system was applied for the characterization of two commercial mixtures of naphthenic acids (Fluka and Acros) and a naphthenic acid sample extracted from the Syncrude tailings. Contour plots of chromatographic distributions of different *Z* homologous series of the Fluka, Acros and Syncrude NAs were constructed using fragment ions that were characteristic of the NA's molecular structures. Well-ordered patterns were observed for NAs of $Z = 0$ and -2 which corresponded to acyclic acids and monocyclic acids, respectively. For NAs of $Z = -4$, -6, and -8, specific zones were observed which would allow the pattern recognition of these NAs obtained from different origins. As expected, gas chromatographic retention times increase with the number of the carbons and the number of rings in the molecules. Little signal was obtained for NAs with *Z* numbers of -10, or lower. Deconvoluted mass spectra of various NA isomers were derived from the reconstructed GC × GC chromatogram, permitting detailed structural elucidations for NAs in the future. The current study demonstrated that the combination of GC × GC and the TOF–MS is a powerful to identify origins of the NAs in an effective manner. GC × GC/TOF–MS alone, however, may not be enough to characterize each individual isomer in a complex mixture such as NAs. The use of mass deconvolution software followed by library search have thus become necessary to separate and study the mass spectrum of each individual NA component, allowing a detailed identification of the toxic components within the NAs mixture.

Keywords: Oil–sand naphthenic acids (NAs); Gas chromatography (GC); Comprehensive two-dimensional gas chromatography (GC × GC); Time-of-flight (TOF); Mass spectrometry (MS); Molecular weight (MW)

1. Introduction

Located in north-eastern Alberta, Canada, the oil–sand refining industry of the Athabasca Basin annually produces 120 million barrels of crude oil. The majority of the refining processes utilize the Clark caustic hot water extraction, which calls for the digestion of oil–sand ore by warm water and the conditioning agent NaOH. The digesting process separates bitumen from the sand along with vast quantities of tailing water containing clay, sand, organic compounds of high polarity and molecular weight. The tailing water is known to be toxic and is therefore contained in large settling ponds on site [1]. The primary toxic components of tailings pond water have been identified as oil–sand naphthenic acids (NAs), a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids that dissolve in water with a neutral or alkaline pH [2–4]. NAs are toxic to aquatic organisms including bacterial populations, phytoplankton, *Daphnia magna*, fish, and mammals [1,3,5–7] and show

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some endocrine disrupting characteristics. The ability to characterize NAs would allow the determination of origins of NAs as well as specific chemical properties related to NAs. Therefore, allow a more accurate study on the toxicity of individual NAs and the development of an effective abatement strategy and follow up environmental remedial activities to alleviate the possible effect of NAs to the environment.

NAs are a complex mixture with a general formula of $C_nH_{2n+z}O_2$ (Z is a zero or a negative even number that defines a specific homologous series), with molecular weights <500, and primarily a 5 or 6-carbon ring structure [3,8]. Toxicological properties of NAs are linked to their molecular structures and polarity, and numerous efforts have been focused on the development of characterization methods for NAs. These include the use of Fourier transform infrared spectrometry [9], nuclear magnetic resonance [10], fast ion/atom bombardment mass spectrometry [11], chemical ionization mass spectrometry [12], electrospray ionization mass spectrometry [13], and gas chromatography mass spectrometry (GC/MS) [14,15]. However, the structural complexity of NAs in concomitant with their similar chemical and physical properties, determination of individual NA components remains a challenge.

The advent of comprehensive two-dimensional gas chromatography (GC × GC) in the early 1990's [16] offered an unprecedented degree of improvement in chromatographic peak capacity that may be needed for the analysis of complex environmental samples [17,18]. Using a time-of-flight mass spectrometer (TOF–MS) as a detector allows the acquisition of up to 500 mass spectra/s, permits the accurate profiling of each GC × GC peak. Combining this unique ability with mass spectral deconvolution software, one could obtain accurate mass spectrum of each individual component for identification purposes. In view of the complex composition of NAs and the possible solution offered by the GC × GC/TOF–MS technology, we have critically evaluated the possibility of using a GC × GC/TOF–MS system for the purpose of source characterization and remediation of principal toxic NAs in the environment. Using two commercially available model NA mixtures and one NA mixture extracted from Syncrude tailings, GC × GC/TOF–MS protocols were developed and demonstrated for the characterization and differentiation of these three NA mixtures in an effective manner. Taking advantage of the GC × GC in chromatographic peak capacity, TOF–MS in rapid mass spectral data acquisition and the use of deconvolution software, we demonstrate that detailed structural information of NAs can be derived for individual NAs. This unique ability offers the possible identification of principal toxic components of NA mixtures in the future.

2. Experimental

2.1. Chemicals

Two commercially available naphthenic acids standards were purchased from Acros Organics (Fisher Scientific, Edmonton AB) and Fluka, Riedel-de-Haen (Sigma-Aldrich, Oakville ON.). The Syncrude naphthenic acids sodium salt, a NA mixture extracted from the Syncrude tailing pond water, was a generous gift from the Toxicology Centre, University of Saskatchewan (Saskatoon, SK).

2.2. Methylation

To improve their chromatographic properties, NAs were derivatized to their respective methyl esters using boron trifluoride–methanol (BF_3–Methanol, Supelco, Oakville, ON) as a methylation reagent. For the Acros and Fluka NAs, this was done by adding 2-mL of BF_3–Methanol into a 5 mL reaction vial containing about 8 mg of the free acids. The vials were sealed with a Teflon lined cap and thoroughly mixed. The mixture was heated to 60 °C for 30 min and cooled to room temperature. 1-mL of MilliQ® water and 1-mL of hexane were added to the vial. The vial was sealed and shaken for 3–5 min to ensure that NA esters partitioned into the hexane phase. Upon the formation of two clear layers, the hexane layer was transferred to an auto-sampler vial using Pasteur pipette and subsequently analyzed by GC × GC/TOF–MS.

The Syncrude NAs were received as a sodium salt in an aqueous solution (pH 10). The approximate concentration was 8500 mg/L by weight of sodium salts. For the experiment, Syncrude NAs were first extracted from 1 mL of the aqueous solution by two consecutive, 2-mL dichloromethane extractions under a pH value of approximately <2 adjusted by HCl. The combined extracts (4-mL dichloromethane solution) were then brought to dryness using a gentle stream of nitrogen, methylated using the BF_3–Methanol derivatizing agent for 30 min. The NA esters was reconstructed by adding 1-mL of MilliQ® water and 1-mL of hexane into the vial, the vial was sealed, shaken for 3–5 min to ensure the NA esters partitioned into the hexane phase. Upon the formation of two clear layers, the hexane layer was transferred to an autosampler vial using Pasteur pipette and subsequently analyzed by GC × GC/TOF–MS. Assuming a 100% reaction and extraction efficiency, the concentration of methylated NAs sample was estimated at about 8 mg/mL and 8.5 mg/mL for the two commercially available NAs and Syncrude NAs, respectively.

2.3. Instrumentation

All GC and GC × GC experiments were performed on an Agilent 6890 GC (Agilent Technologies, Mississauga, Ontario, Canada) that was interfaced to a LECO Pegasus TOF–MS system (LECO Corp., St. Joseph, MI, USA). The Agilent GC was modified with a secondary oven and a dual-stage jet modulator. For the GC × GC/TOF–MS experiments, heated nitrogen was used for hot jets while cold nitrogen gas and liquid nitrogen were used for the first and the second cold jets, respectively. The primary GC column was a 40 m × 0.18 mm × 0.18 μ, VB-5 unimolecular column (5%-Phenyl-methylpolysiloxane equivalent, Gig Harbor, WA) and

the secondary column used was a 2 m × 0.1 mm × 0.1 μ VB-210 unimolecular column (Vici, (50%-Trifluoropropyl)-methylpolysiloxane equivalent, Gig Harbor, WA). The carrier gas used was He with the electronic pressure control set at a constant flow rate of 0.6 mL/min. Using a split-splitless injector, the injection volume was optimized at 3 μL for column loading and sensitivity. This was done by injecting 1–3 μL of methylated NAs into the first column; resulting estimated total column loadings of NAs at about 8, 16, and 24 μg, respectively. The initial temperature was 80 °C for the main oven, held for 1.5 min, ramped to 310 °C at 10 °C/min, and was held for an additional 5.5 min to complete the analysis. The secondary oven tracked the main oven temperature with a 10 °C offset. Modulation time was 3 s, with a 0.8 s hot pulse and 0.7 s cold pulse in each stage. Modulation temperature, the temperature at which the trapped analytes were heated and injected into the second column, was 20 °C above the main oven. Temperatures for the ion source, the injector and the transfer line were set at 210, 250 and 250 °C, respectively. Mass spectra were acquired in electron ionisation (EI) mode from 60 to 500 amu using an acquisition rate of 150 spectra per second.

One-dimensional GC/TOF–MS analysis used the same two column configuration, the GC operated with the modulator turned off and the TOF–MS acquiring the data at a rate of 20 spectra per second. All other parameters were kept identical to the GC × GC/TOF–MS analysis.

Data acquisition and processing were done by proprietary ChromoTOF software of the Pegasus 4D platform. The software has auto peak-find, deconvolution, and peak-combination functions, and can automatically perform a full spectral library search to identify the peaks. The peak width and signal-to-noise ratio were set at 3 s and 20:1, respectively, for data processing. The software determined the unique mass of each component and mathematically deconvoluted and derived the mass spectra of the overlapping compounds. The main library used for spectral search was the NIST EI mass spectrum database.

3. Results and discussion

With a general formula of $C_nH_{2n+z}O_2$, NAs could take the form of a saturated chain ($Z = 0$), a monocyclic ($Z = -2$), a bi-cyclic ($Z = -4$), a tri-cyclic ($Z = -6$), tetra-cyclic ($Z = -8$) structure, and so on [3,4,19]. Example structures of NAs with Z values from 0 to −6 are displayed in Fig. 1. Upon methylation, the H-atom of the −COOH was substituted by a $-CH_3$ to form an ester, $-COOCH_3$, producing a series of stable derivatives of NAs in the form of $C_nH_{2n+z-1}O_2CH_3$. The mass difference between the free acid and its methylated derivative was 14. Therefore, using the general formula $C_nH_{2n+z-1}O_2CH_3$ of methylated NAs, Z values from 0 to −8, and number of carbons in NAs (n) from 2 to 32, the possible molecular weights (MWs) of methylated NAs could be calculated and used in the interpretation of mass spectral data as well as to determine the homologous series (Z values) and carbon number (n) for NAs of different origins.

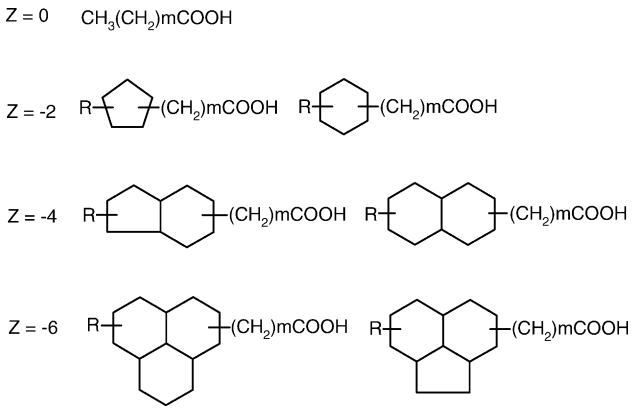

Fig. 1. Example structures of naphthenic acids for various Z-families. R represents an alkyl group, m represents the length of the alkyl chain, and z series correspond to different ring numbers in the acids.

3.1. Reconstructed GC × GC/TOF–MS ion chromatograms and contour plots

Previous GC/MS studies of NA derivatives of methyl [14] or *tert*-butyldimethylsilyl [15] showed re-constructed total ion chromatograms (TIC) of an unresolved "hump" [3,4,15,21], a common feature for petroleum environmental samples [20]. Prior to the GC × GC analysis, we attempted to use one-dimensional GC/TOF–MS to optimize the separation of NAs with the two column configuration. The GC/TOF–MS total ion chromatograms of NAs were very similar to previously published results with an unresolved hump. There was not enough separation to unambiguously identify NAs from Fluka, Acros, and Syncrude origins.

We also observed from the experiment that the GC columns would be overloaded with an injection volume of 3 μL but not when the injection volume was 2 μL. We therefore estimated that the two column configuration would have a column loading about 16–24 μg total in the one-dimensional mode. In the GC × GC mode we had an analysis time of 30 min. The 3-s GC × GC modulation time used suggests that the modulator continuously concentrated analytes eluted from the first column and injected them into the second column every 3 s. The result of the GC × GC analysis is therefore, from the outset, the combination of 20 injections/minute or about >500 individual injections into the second column and followed by a 3 s GC analysis. This inherent nature in the GC × GC analysis would eliminate the possibility of column overloading while giving the highest sensitivity possible for the analysis.

Using GC × GC/TOF–MS and operating parameters described in the Experimental section, methylated Fluka NAs sample was first analyzed and used as an example to explain typical results can be expected from a GC × GC/TOF–MS experiment. A typical GC × GC/TOF–MS selected ion chromatogram (SIC) in one-dimensional time axis is shown in

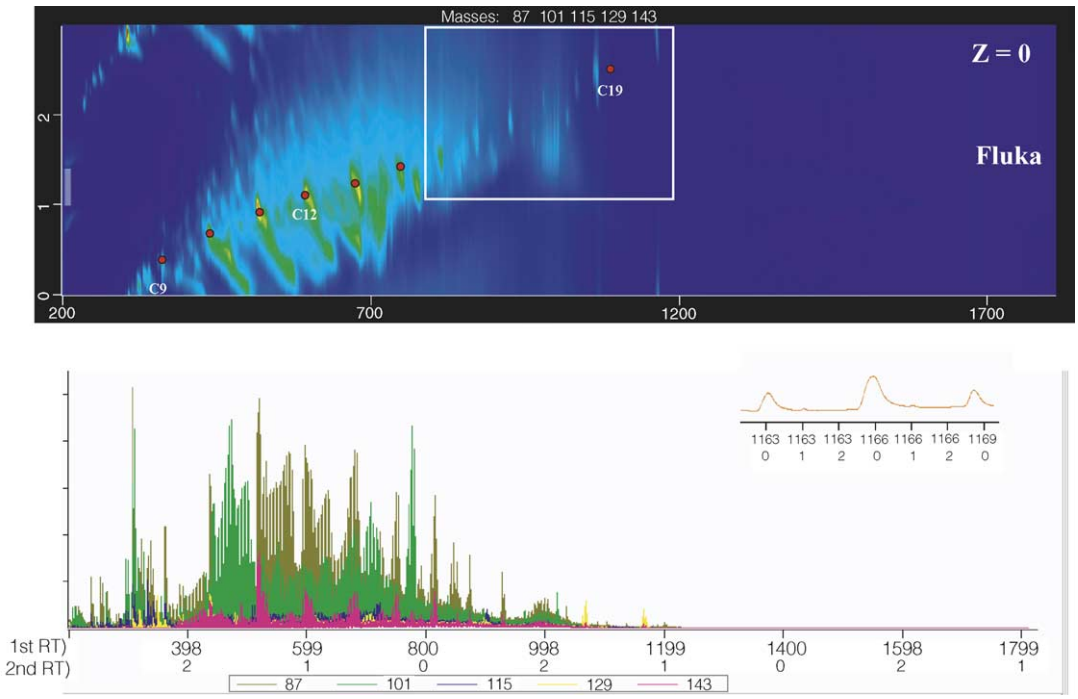

Fig. 2. Comprehensive GC × GC/TOF–MS summed ion chromatogram of Fluka $Z = 0$ NA homolog. Upper trace is the 3-dimensional contour plot, and the lower trace is the reconstructed chromatogram in one time axial. Carbon numbers of several peaks in the main pattern are labelled. The inset shows an enlarged part of lower trace to demonstrate 3-s modulation segments.

Fig. 2 (lower trace). Analytes from the first column were divided into 3-s segments by the modulator and were introduced into the second column for the secondary separation. The contour plot of the same GC × GC experiment in two-dimensional time axis is shown in Fig. 2, upper plot. A hue/saturation/value (HSV) color palette was used in the contour plot with which a color gradient ranging from low to high represented the intensities of the corresponding ions analyzed. Because of the GC × GC modulation and, depending on the FWHH of each individual component, one primary chromatographic peak could appear in two or more different secondary chromatograms (inset, Fig. 2, shows the primary (1st RT) and the secondary (2nd RT) retention time in s). The software automatically combines modulated peaks for one component according to its retention time and unique mass spectrum. The SIC shown in Fig. 2 were from several possible fragment ions from remote-charge fragmentation of saturated chain acids, *m*/*z*'s 87, 101, 115, 129, and 143 (*m*/*z* 73 was excluded because of background noise) of $Z = 0$ NA homolog. As NAs are a mixture of many individual compounds and as expected, there is minimal information available from the SIC. From the contour plot, however, one can see a clear main pattern from RT 300 to 1100 s along with several minor, but characteristic patterns of the Fluka NAs GC × GC contour plot. The carbon numbers, *n*, were determined, according to the molecular ions in the mass spectra of derivatized acids, and were labelled using closed circles for C9, C12, and C19 NAs. As will be discussed in the following sections, NAs from different origins are expected to show different patterns according to their specific compositions, carbon numbers and types of molecules. Therefore, the possibility of using patterns generated by the GC × GC/TOF–MS contour plots to characterize NAs.

3.2. Source characterization of NAs

NAs from different sources have been reported to display different chemical compositions [4]. The ability to characterize NAs as well as to determine their origin would be a valuable addition for environmental remedial purposes. We used the same characteristic fragment ions of NAs with $Z = 0$ (*m*/*z*'s 87, 101, 115, 129, and 143) and $Z = -2$ (*m*/*z*'s 127, 141, 155, 169, 183, 197, 211, 225 and 239) to reconstruct summed ion chromatograms for NAs of Fluka, Acros and Syncrude origins and created three contour plots for $Z = 0$ and $Z = -2$ homolog of the three different mixtures of NAs. The contour plots are shown in Fig. 3a–c; and Fig. 3d–f for $Z = 0$ and $Z = -2$ NA homologs, respectively. From Fig. 3, we noted that low MW components corresponding to $Z = -2$ and $Z = 0$ homolog of NAs decrease significantly from Fluka to Acros and can hardly be observed in the Syncrude. The Syncrude NAs show the simplest low MW composition for this series of NAs.

We also note that patterns of the high MW components corresponding to the $Z = 0$ and $Z = -2$ homolog of NAs are very similar in the Fluka, Acors and Syncrude NAs and remain almost unchanged in these NAs (Fig. 3). The distinctive patterns of these NAs show in the low MW region provided

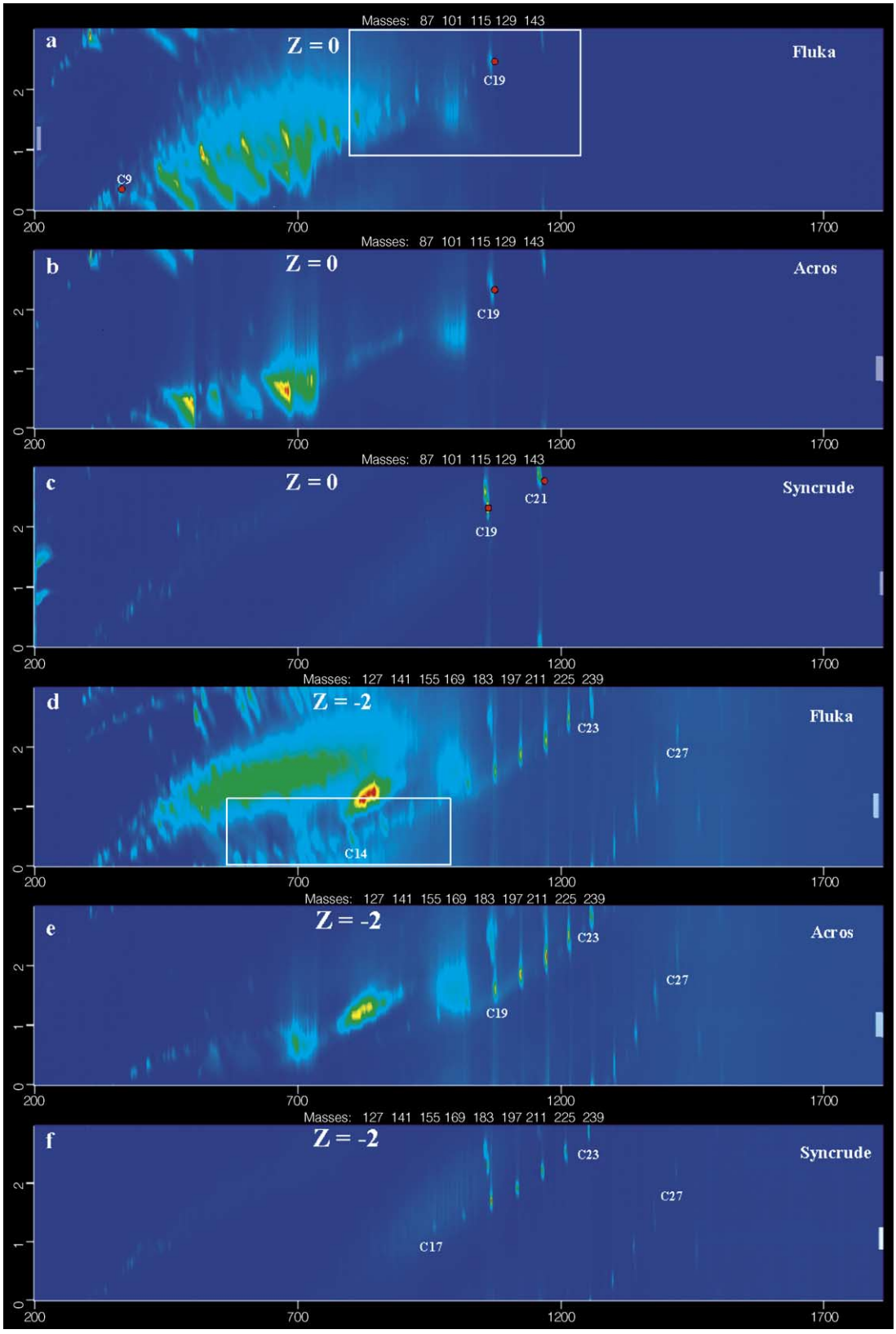

Fig. 3. Contour plots of $Z = 0$ homolog obtained from methylated Fluka (a), Acros (b) and Syncrude (c) NAs. Contour plots of $Z = -2$ homolog obtained from methylated Fluka (d), Acros (e) and Syncrude (f) NAs.

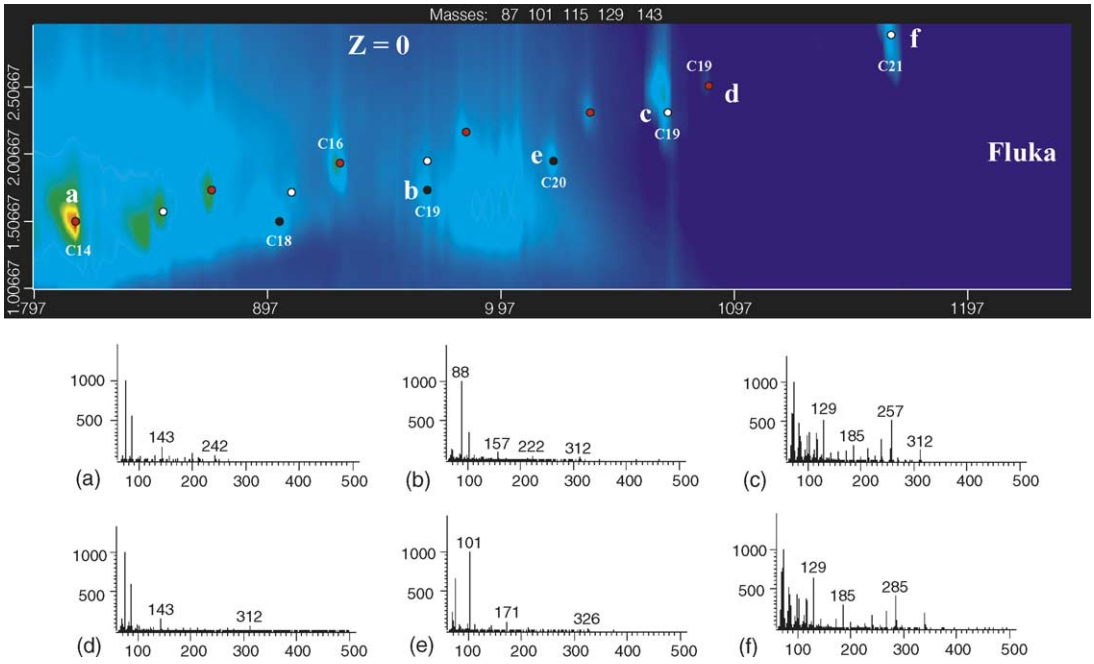

Fig. 4. Enlarged view of the square area shown in Fig. 3a (Fluka, $Z = 0$). Peaks of different patterns are labelled with dots and the carbon numbers are given. The lower traces are the mass spectra of peaks labelled "a", "b", "c", "d", "e" and "f".

by the GC × GC/TOF–MS and post data processing demonstrated the feasibility of using pattern recognition as an effective tool for determining the source of NAs of different origins. Patterns derived from the high MW NAs were very similar between the three NA mixtures studied and could not be used for the source characterization of NAs.

3.3. Characterization of individual component in NAs Mixture

Fig. 4 shows an enlarged plot of the square area in Fig. 2. Three major patterns were observed and were denoted by dots in different grey scales, with the carbon number labelled for several identified NA components. Components in each pattern are considered to have similar molecular structure but different carbon numbers. The retention time increases with increasing carbon number "*n*" in the molecule. The corresponding mass spectra obtained by using the mass deconvoltion software and a unique mass *m*/*z* 312 are provided as examples for the peaks labelled "a" to "f" in the chromatogram. Among them, peaks b, c, and d might be considered as NA isomers with 19 carbon atoms (MW 312 for methylated NAs). Due to the rather low elution temperature of peak b, we can assume the isomer "b" has a more branched structure than its isomers "c" and "d". This is supported by the boiling point of 3,3-dimethylbutyric acid and hexanoic acid, both are with a molecular formula of $C_6H_{12}O_2$ but a boiling point of 185 and 202 °C, respectively. One would expect the difference between the boiling points of $C_{19}H_{38}O_2$ isomers will be even more and would account for the separation in the GC × GC chromatogram. This unique ability of the GC × GC/TOF–MS provides a deconvoluted mass spectrum for each individual component in a complex mixture would allow the use of mass spectra to locate molecular isomers of different boiling points. This not only facilitates the identification of individual NAs and their isomers; but is also a useful tool for the determination of their acute toxicity.

Applying the same procedure to the Fluka NAs for $Z = -2$ homolog (boxed area in Fig. 3d), the characteristic fragment ions started at *m*/*z* 127, the smallest fragment ions to have the one ring structure. Two major patterns were found and are marked by the dots along with four example mass spectra of NAs. This is shown in Fig. 5. Note that mass spectra a and b as well as c and d are in fact two pairs of chemical isomers of NAs with unique *m*/*z*'s 226 and 254, respectively, are with very similar mass spectra but different boiling points, and are isolated via the mass deconvolution software, too.

GC × GC/TOF–MS analysis of NAs with more than one ring, i.e., $Z = -4$, -6, and -8 NA homolog, did not show well-resolved patterns as the $Z = 0$ and -2 homolog. Specific bright zones were observed for $Z = -4$, -6 and -8 homolog in the GC × GC contour plots. Shown in Fig. 6 is a typical example of the contour distribution (upper plot) of $Z = -4$ homolog of Fluka NAs with the brightest portion of the contour displayed below. The carbon numbers of several major peaks have been identified and labelled. However, co-elution of the NAs was obvious as indicated by the continuous HSV palette used by the software between the peaks. Chromatogram contour plots of $Z = -6$ and -8 homolog were similar to $Z = -4$ homolog with unresolved zones.

The featureless pattern from retention times 1200 to 1700 s in Fig. 6 is due to the wrap-around phenomenon. This pat-

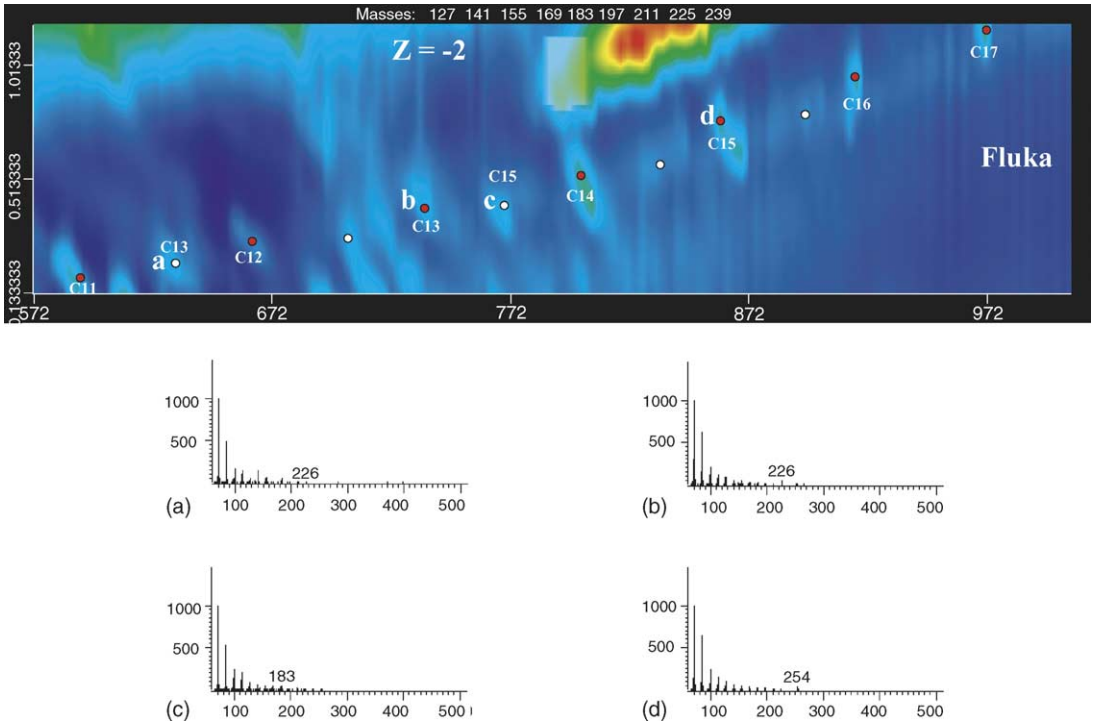

Fig. 5. Enlarged view of the square area shown in Fig. 3d (Fluka, $Z = -2$). Peaks of different patterns are labelled with dots and the carbon numbers are given. The lower traces are the mass spectra of peaks labelled "a", "b", "c", "d", "e" and "f".

tern arose when high boiling-point, unresolved compounds appear at the early stage of the next modulation period of the GC × GC experiment and, as a result, are broken into two or more consecutive chromatograms in the GC × GC analysis. This pattern (the bright zones) would shift to the upper right direction with the increase of ring numbers in the molecules. Also, the intensity of the zone decreased with the increase of ring numbers in the NAs which indicated less abundance of multi-ring NAs. Little signal was observed for Z values less than −8.

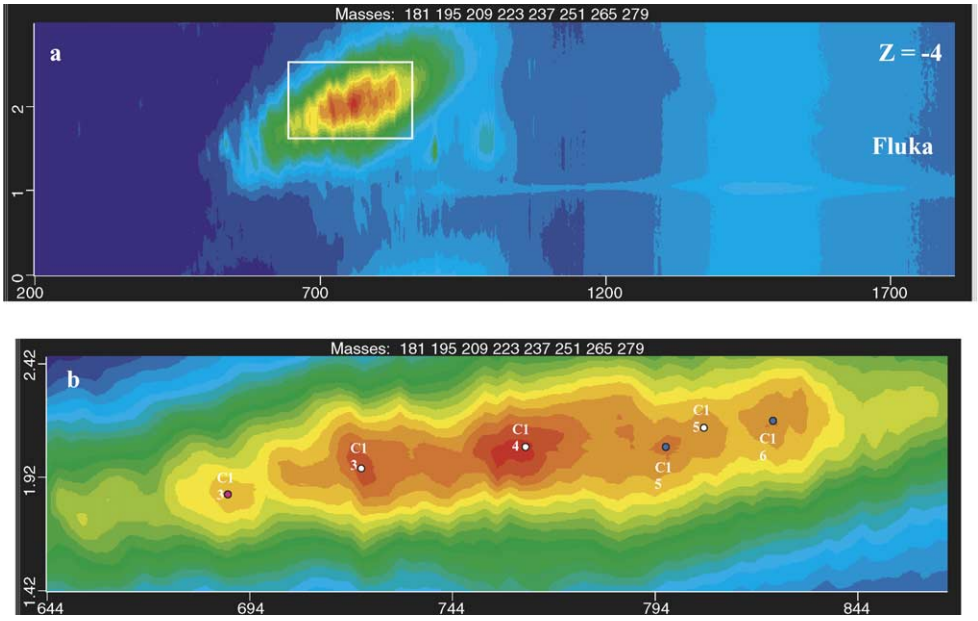

Fig. 6. Comprehensive GCxGC/TOF–MS summed ion chromatogram of Fluka $Z = -4$ NA homolog. Upper trace is the 3-dimensional contour plot, and the lower trace is the enlarged plot of circled area in upper trace. Carbon numbers of some peaks are labelled in lower trace.

3.4. Special features of Syncrude NAs

Of the three NAs studied, Syncrude NAs was extracted from a water sample collected from the Syncrude tailing pond. The summed ion chromatogram contour plots of Syncrude NAs shown in Fig. 3 indicated that Syncrude NAs had fewer of $Z = 0$ and -2 homolog than the Fluka and Acros NAs. For the $Z = 0$ Syncrude homolog, the abundant components included two isomers of C19 acids and two isomers of C21 acids. For the $Z = -2$ Syncrude homolog, observed was a C17 to C27 series of acids with similar structure and an unresolved zone for $Z = -4$ homolog. Intensities of the chromatographic peaks became weak and difficult to observe for multi-ring (>3 rings) acids. This was different from some previous GC/MS studies of the Syncrude NAs where NAs with $Z = -12$ (6 rings) have been reported according to the averaged mass spectrum across the GC hump. We contribute this discrepancy to the possible existence of chemical species with molecular weights equal to NAs and were co-eluted from the GC prior to MS analysis. For example, $C_{18}H_{36}O$ and $C_{18}H_{38}N$ would have the same MW (268) as methylated naphthenic acid $C_{16}H_{29}O_2CH_3$ ($n = 16$ and $Z = -2$) and, therefore, increase the composition ratio when analyzed by a typical GC/MS system with unit mass resolution.

4. Conclusions

The feasibility to classify NAs, according to their origins as well as the ability to provide detailed mass spectral information of individual NAs was demonstrated for the first time using GC × GC/TOF–MS technique. Specific patterns were observed for acyclic and monocyclic NAs from Fluka and Acros commercial mixtures and a Syncrude oilsand tailing pond water extract. Characteristic co-elution zones were also observed for NAs with two to four rings ($Z = -4$ and -8). The use of deconvolution software facilitated the extraction of electron ionisation mass spectra of resolved naphthenic acid isomers for the first time, granting a powerful tool for the toxicity study of NAs. Optimization of the GC separation parameters is expected to further extend the ability of GC × GC/TOF–MS to the homolog of $Z = -4$ and less. The use of gel permeation chromatography fractionation to simplify the GC × GC separation and thus, the identification of toxic components is currently underway and will be reported later.

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