



Rapid Characterization of Naphthenic Acids Using High Resolution Accurate Mass MS and MS/MS with SelexION™ Differential Mobility Separation

John V. Headley¹, Kerry M. Peru¹, Takeo Sakuma², Paul Winkler², Matthew R. Noestheden² and J. Larry Campbell²

¹Environment Canada, Water Science and Technology Directorate, Saskatoon, Saskatchewan, (Canada) and

²AB SCIEX, Concord, Ontario (Canada)

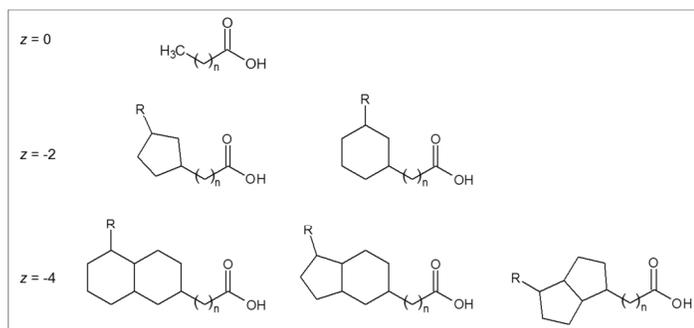
Overview

This study demonstrates the rapid gas-phase analysis of naphthenic acids using the AB SCIEX TripleTOF[®] 5600+ system with SelexION™ differential mobility separation. The assets of this workflow include:

1. The rapid characterization (< 2 min) of naphthenic acids in complex samples.
2. Utilizing SelexION™ to isolate individual naphthenic acids on-demand (including isomers and isobars) for in-depth structural analysis.
3. Accounting for background ions using their SelexION™ mobility.

Introduction

Naphthenic acids (NA) from oil sands process-affected water (OSPW) have been the subject of numerous mass spectrometry-based environmental studies.^{1, 2}



The classical definition of these compounds ($C_nH_{2n+z}O_2$, where z is an even negative integer representing hydrogen deficiency) has recently been expanded to the naphthenic acid fraction component (NAFC), which includes unsaturated and aromatic



NA derivatives, increased oxygen content and compounds containing nitrogen and/or sulfur.¹⁻³

NAFCs are of particular concern in northern Alberta, Canada, where the caustic extraction of bitumen from surface mineable oil sands produces large volumes of OSPW.⁴

A common workflow for NAFC analysis involves direct infusion into an ultrahigh resolution mass spectrometer. A resolving power >100,000 is critical to obtaining useful NAFC profiles with this approach. While such instruments can provide elemental compositions, additional steps are required to interrogate molecular structure. To overcome this, chromatography is often utilized. Unfortunately, the high complexity of OSPW extracts may necessitate relatively long run times, multiple sample handling steps, or multi-dimensional chromatography. Moreover, traditional chromatography is an inherently serial process, with limited time available to perform deeper structural interrogation of individual analytes.

The AB SCIEX TripleTOF[®] 5600+ high resolution accurate mass system coupled with SelexION[™] differential mobility spectrometry presents a unique workflow for NAFC analysis.⁵

The combination of gas-phase separation and the ability to access analytes on-demand via direct-infusion resolves NAFCs from OSPW extracts rapidly (< 2 min), generating complex and insightful datasets. Such rapid, information rich methods will become important as regulatory guidelines for NAFC analyses are implemented and testing laboratories have to accommodate increased demand.

Experimental

A technical NA mix was obtained from the Merichem Company (Houston, TX) and an OSPW extract was obtained by extracting a sample from an industrial location in the Athabasca River Basin, Alberta, Canada.⁶

Analyses were conducted using the AB SCIEX TripleTOF[®] 5600+ system with SelexION[™] or the AB SCIEX QTRAP[®] 5500 system with SelexION[™]. The operating parameters are listed in Table 1.

Table 1. Typical SelexION[™] and TripleTOF[®] operating parameters

Parameter	Value
Infusion rate	15 μ L/min
IonSpray voltage	-4500 V
Source temperature	100°C
Gas 1	20 psi
Gas 2	10 psi
Modifier composition	1.5% (v/v)
SelexION [™] temperature	150°C
Separation Voltage (SV)	4000 V _{pp}
Compensation Voltage (CoV)	-17 to +20 V (0.25 V steps)
TOF m/z range	50 - 2000
TOF accumulation time	250 msec
Declustering Potential (DP)	-100 V
MS/MS Collision Energy (CE)	-35 to -50 V

For the SelexION[™] experiments two operational modes were employed:

1. Separation voltage was held at an optimum value while the compensation voltage was scanned; and
2. At each CoV increment, MS or MS/MS spectra were recorded.

Data were plotted as ionograms (Signal Intensity versus CoV).

Data acquisition was carried out using Analyst[®] TF software and data was interrogated using PeakView[®] software version 2.0 and an accurate mass database (8,127 entries) with the following parameters: C₅₋₂₀, z₀₋₁₆, O₂₋₈, N₀₋₂, S₀₋₂. Results were confined to \pm 10 ppm of the database masses and were compared to suitable blanks.

Results and Discussion

SelexION[™] mobility functions by transmitting ions between two planar electrodes, across which a high-voltage rf asymmetric waveform is applied (Figure 1).

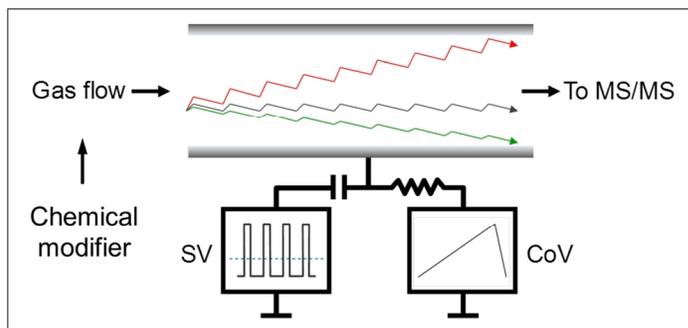


Figure 1. Schematic of the SelexION[™] mobility cell

The difference between the ion's mobility during the high- and low-field portions of the waveform determines their SelexION[™] mobility. Chemical effects are also critical to ion separation using SelexION[™], as the addition of volatile modifiers like methanol to the transport gas can alter ion mobility. To mitigate the complexity of NAFC analysis, a series of chemical modifiers were added to the transport gas and the resulting SelexION[™] peak capacities were evaluated. Since each ion was transmitted with a full-width half maximum of \sim 2.5 V, the greater the spread in total CoV space covered by all of the NAFC ions, the greater the peak capacity of the SelexION[™] separation. Among the modifiers examined, methanol yielded the greatest total spread in CoV (Figure 2).

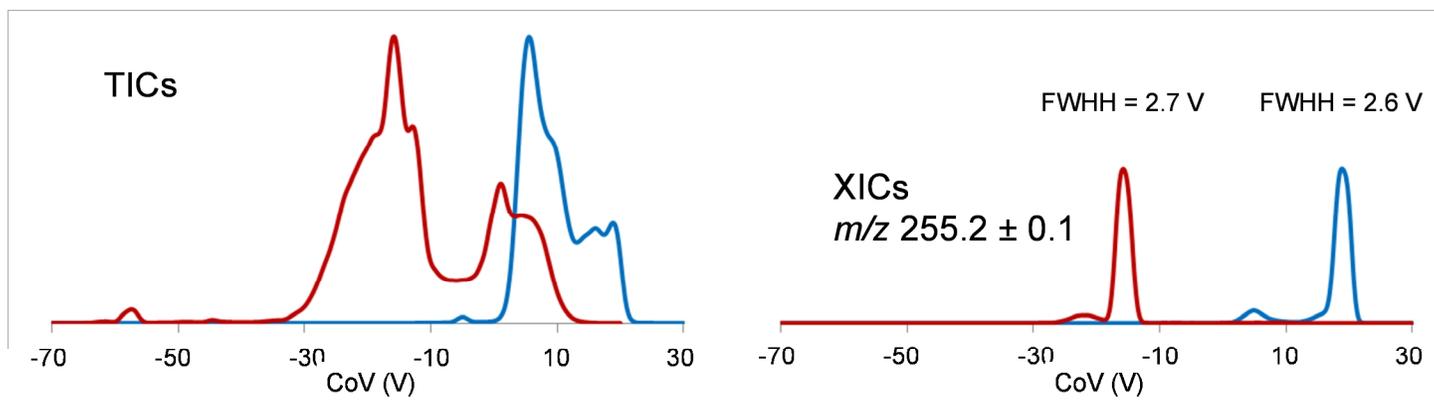


Figure 2. SelexION™ behavior of an OSPW extract with (red) and without (blue) methanol added in the N₂ carrier gas. Extracted ion chromatograms are shown as an example of typical peak widths (right). Signal intensity was normalized for ease of data presentation. FWHM – full width at half maximum height.

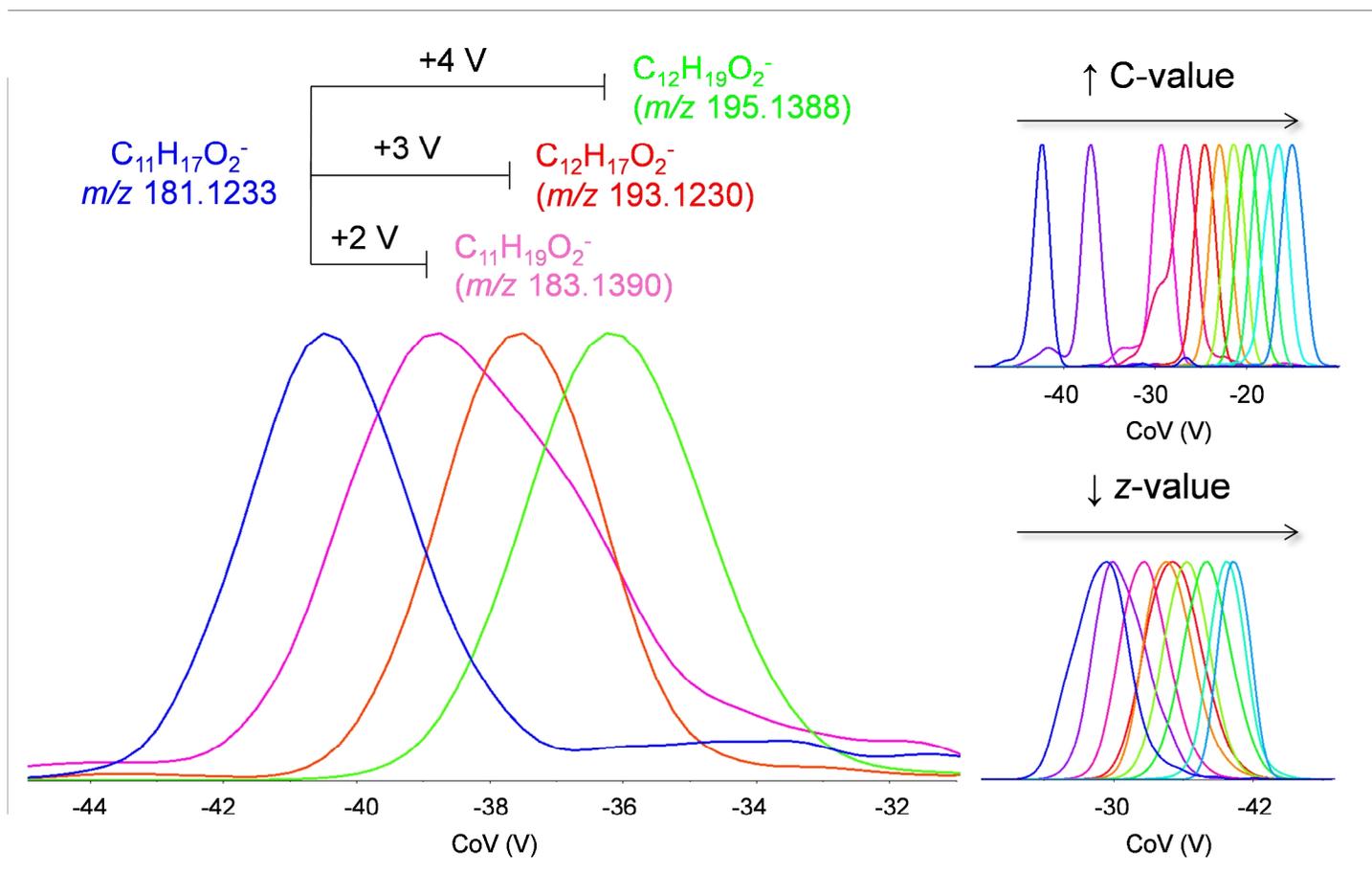


Figure 3. Thorough data analysis demonstrated that SelexION™ behavior was not simply correlated to m/z (left). Trends in CoV shifts for carbon number (top right) and z-series (bottom right) are consistent with reported LC behavior of similar samples. Data was normalized to 100% within each ionogram and is shown for an OSPW extract.

The SelexION™ separation of individual NAFC ions suggested a correlation between ion mobility and structure (Figure 3).

For example, m/z 181.1233 ($C_{11}H_{17}O_2^-$) was separated from the more saturated analogue at m/z 183.1390 ($C_{11}H_{19}O_2^-$, only two hydrogens added) by +2 V. However, this $C_{11}H_{17}O_2^-$ ion was only separated by +3 V from $C_{12}H_{17}O_2^-$ (an acid one carbon atom heavier) and by +4 V separated from $C_{12}H_{19}O_2^-$ (one CH_2 unit heavier).

Clearly, the more unsaturated analogues exhibited more negative CoVs, and the ring/double bond analogues displayed different mobility behavior than the linear chain extended analogues. The fact that such subtle structural differences result in the separation of closely related ions demonstrates the analytical potential of SelexION™-based workflows. In addition to the above observations, correlations between CoV and homologous carbon series and z-series emerged (Figure 3).

A Kendrick mass plot (plot of Kendrick mass defect as function of Kendrick mass) provides a high level means of assessing the composition of complex mixtures like OSPW extracts. In the Kendrick mass plot (Figure 4) all relevant mass peaks in a spectrum are normalized against methylene, such that a horizontal line represents a homologous carbon series for a particular compound class. Moreover, as the degree of unsaturation increases, so does the Kendrick mass defect. This translates to easy to assess trends. For instance, in Figure 4 the red lines indicate a series of simple acids (i.e., only O_2 compounds) with increasing z-values, while the green lines represent a homologous series of O_3 compounds at differing degrees of unsaturation. Finally, the blue lines demonstrate the observable trends in unsaturation for a given carbon number.

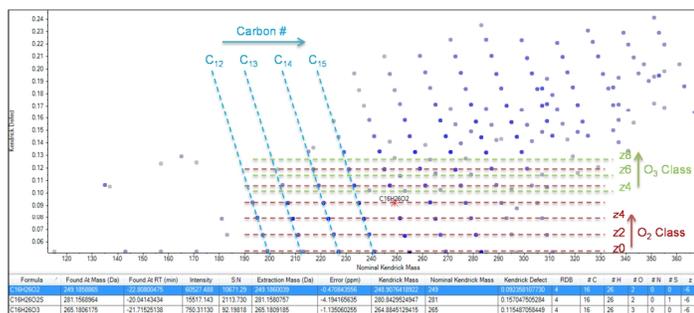


Figure 4. Kendrick mass plot of the OSPW extract generated in an add-in of PeakView™ software

Another interesting attribute of the SelexION™ separation of NAFCs was the gas-phase resolution of isobaric and isomeric

ions. For example, in the OSPW extract m/z 143.1080, corresponding to $C_8H_{15}O_2^-$ (1.7 ppm), was transmitted through the SelexION™ cell at two CoVs (Figure 5).

The separation of these two potential isomers was rapid (~3 sec) and due to the infusion-based sample analysis, MS/MS analysis of each m/z 143.1080 ion was easily performed by fixing the SelexION™ cell at the appropriate CoV value. The resulting MS/MS spectra yielded fragmentation patterns that were consistent with the presence of distinct isomeric species. Analysis of three authentic $C_8H_{16}O_2$ isomers revealed that the OSPW extract contained 2-ethylhexanoic acid and n-octanoic acid (Figure 5). Separation of such isomers by GC or LC generally requires minutes of elution time. Using SelexION™, these isomers were separated in seconds and could be analyzed on-demand.

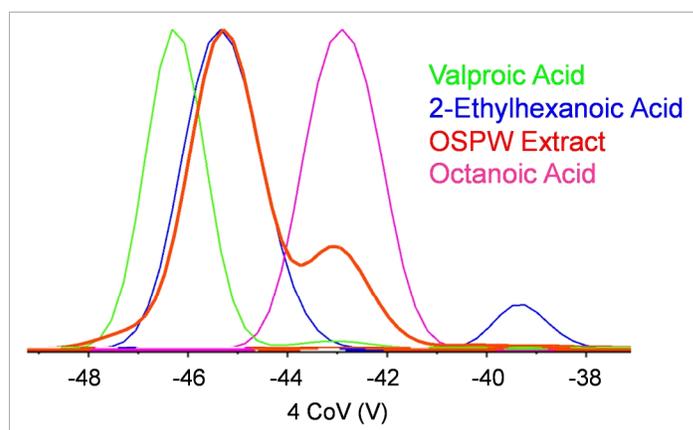


Figure 5. Apparent structural isomers of $C_8H_{16}O_2$ were identified in the OSPW extract (red). Subsequent analysis of valproic acid (green), 2-ethylhexanoic acid (blue) and octanoic acid (pink) standards showed that 2-ethylhexanoic acid and octanoic acid were present in the OSPW extract and partially resolved using SelexION™.

There were also numerous examples where SelexION™ separated isobaric species. In one case, palmitic acid ($C_{16}H_{31}O_2^-$), which is a known contaminant in laboratory environments, was observed at m/z 255.2329 (Figure 6).

Alongside the ionized palmitic acid was an isobar at m/z 255.1405. TOF-MS can easily resolve these ions, but further interrogation by MS/MS would be complicated without SelexION™ since both ion populations would be sampled by the quadrupole mass filter and a heavily convolved MS/MS spectrum would result. Like the $C_8H_{16}O_2$ isomer separation, selectively tuning the CoV facilitated the interrogation (MS and MS/MS) of each isobar in real-time.

The anion at m/z 255.2329 (CoV = -21 V) fragmented via loss of water (m/z 237.2234, 4.3 ppm) as the dominant product ion, which is consistent with palmitic acid. The MS/MS of m/z 255.1405 (CoV = -26 V) showed carbon dioxide loss (m/z 211.1520, 2.3 ppm) as the dominant product ion, suggesting a carboxylic acid (Figure 6). The elemental composition $C_{17}H_{19}O_2^-$ correlated with m/z 255.1405 (5.7 ppm).

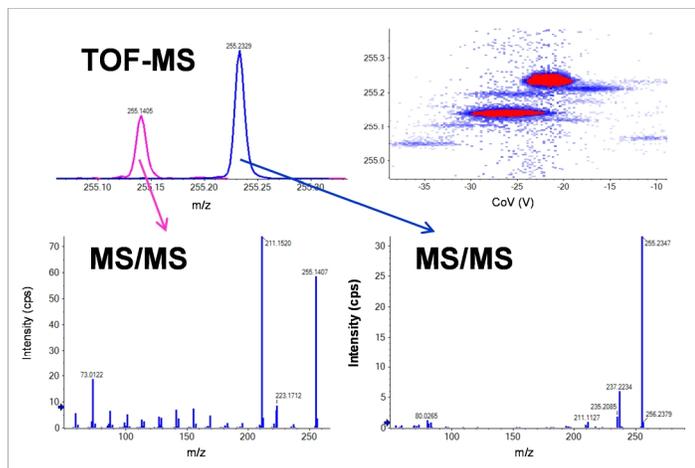


Figure 6. SelexION™ is capable of rapidly resolving isobars from an OSPW extract via mass resolution (top left) and ion mobility (top right). MS/MS analysis of m/z 255.2329 is consistent with palmitic acid (bottom right), while the m/z 255.1405 is consistent with another carboxylic acid (bottom left).

In LC-MS analyses of NAFCs, interferences like palmitic acid, stearic acid, or dodecyl sulfate, often present as systematic contaminants, are ionized continuously throughout the chromatographic run. These ever-present background ions may deteriorate mass spectrometer performance. SelexION™ efficiently removes background ions by sequestering them in discrete CoV ranges, outside of which they will not appear in the analytical data. In this study, it was critical to obtain an accurate assessment of the potential interfering species present in the analytical samples to ensure that only NAFCs were reported from the database search.

The combination of SelexION™ differential mobility separation and detection using the AB SCIEX TripleTOF® 5600+ revealed 12 background compounds that were present, including saturated and monounsaturated fatty acids, several of which are known background ions in laboratory solvents and analytical instrumentation. One series of interest belonged to the O_3S compound class, with $z = -6$. The series ranged from C_{16} to C_{19} with mass accuracies of 0.5 to 3.0 ppm. MS/MS of these ions

after isolation with SelexION™ verified the presence of alkyl-substituted benzy sulfonates (Figure 7).

These detailed structural analyses were quickly and easily facilitated using SelexION™ by tuning the CoV to the appropriate value for each background ion. While this series exhibited relatively low responses, their inclusion in the Merichem and OSPW data would present a false indication of the compound classes present.

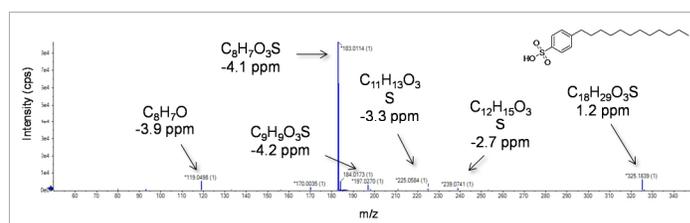


Figure 7. Example MS/MS analysis for the O_3S components identified in the ACN Blank, Merichem standard and OSPW extract. Proposed structure and detailed MS/MS assignments are shown for $m/z = 325.1839$, which corresponds to $C_{18}H_{30}O_3S$ within 1.2 ppm at the MS level and within 3.7 ppm at the MS/MS level. Values shown for product ion assignments are given with calculated mass errors. The product ions at m/z 183.0114 and m/z 119.0498 are characteristic of alkyl-substituted benzy sulfonates.⁷

All Merichem standard and OSPW extract database matches were compiled as a function of compound class and z -value. Results were broken down further based on total response and the number of homologues identified (Figure 8).

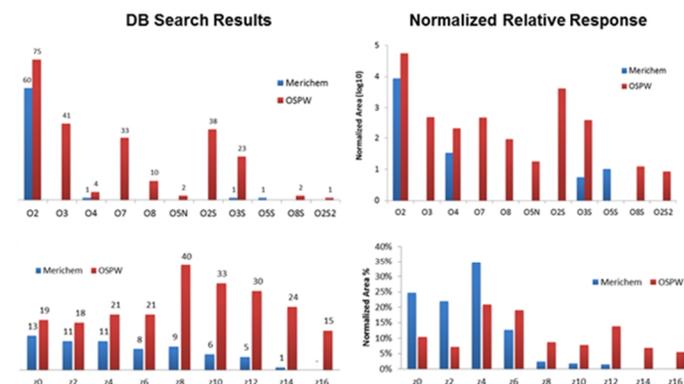


Figure 8. Evaluation of Merichem and OSPW samples as a function of the number of database-identified compounds grouped by class (left) and area response (right). Results are further broken down by compounds class (top) and z -value (bottom). Values shown above the homologue plots are the number of homologues identified during database (DB) searching. Due to the large differences in absolute response between compound classes this data was converted to a log10 scale.

Exact agreement between the distribution of ion classes observed in the current study and literature values was not expected given the heterogeneity of NAFCs.

By area response (99%) and the number of identified homologues (97%), the Merichem standard consisted primarily of O₂ species. Analysis of the Merichem results by z-value showed a significant number (42%) of homologues z ≤ -6 (i.e., -8, -10, etc.). This result seemed contradictory to reported compositions, which state that the Merichem standard consists predominantly of compounds z ≥ -4.^{1,3} However, these compositions were all response comparisons, not an identification of the number of homologues present. The response data in the current study showed that those compounds z ≥ -4 accounted for 82% of the total area response, consistent with literature reports. The results by compound class (log₁₀ scale) supported this comparison, with the Merichem standard containing almost exclusively O₂ species and a small amount of higher oxygen content and heteroatom-containing species.

The OSPW extract showed a more widely distributed number of database matches across the compound classes and z-values evaluated (Figure 8). Interestingly, despite comprising 49% of the positive DB matches, O₃ to O₈ compounds only accounted for 2.8% of the total area response. Similar to the Merichem results, this suggests that such compounds either have low response factors and/or are present at low levels. Conversely, the z-value results for the OSPW extract showed a relatively even distribution across the number of database matches and the relative area responses of the different z-classes. These results are consistent with the demonstrated composition of OSPW extracts, which are known to contain higher oxygen content and increased unsaturation/polycyclic compounds due to natural weathering and metabolic processes.^{1,3}

A detailed breakdown of the compound classes (log₁₀ scale) by area response showed that the OSPW extract was composed of mostly O₂-species (86%), with significant amounts of O₂S (6.6%), O₃ (0.8%) and O₃S (0.6%) compounds (Figure 7). In addition, several potentially interesting compound classes (e.g., O₂NS, O₅NS, and O₄N₂S₂) were observed at very low abundances (<0.08% of total area).

Summary

The AB SCIEX TripleTOF[®] 5600+ system with SelexION[™] differential mobility separation generates complex and insightful datasets very quickly (< 2 min), including the resolution of isomers and isobars. The ability to scan the SelexION[™] cell to target specific analytes provides on-demand access to deeper structural interrogation. With its speed and lower resource cost compared to chromatographic alternatives, SelexION[™] represents a greener analytical technique that requires much less organic solvent or gas consumption.

Analysis of a technical Merichem standard and an OSPW extract demonstrated results that are consistent with literature values for similar samples. Owing to the unique separation mechanism of SelexION[™], structural isomers can be resolved and rapidly interrogated in real-time. Finally, compared to standard infusion-based NAFC analyses, SelexION[™] coupled to the TripleTOF[®] 5600+ should provide more accurate qualitative and quantitative results owing to the mitigation of background ions and deleterious space charge effects possible when directly infusing complex mixtures.

Acknowledgement

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References

- ¹ J.V. Headley et al.: 'Chemical fingerprinting of naphthenic acids and oil sands process waters—A review of analytical methods for environmental samples' *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 48 (2013) 1145-1163
- ² J.V. Headley, K.M. Peru, M.P. Barrow: 'Mass spectrometric characterization of naphthenic acids in environmental samples – a review' *Mass Spectrom. Rev.* 28 (2009) 121-134
- ³ D.M. Grewer et al. 'Naphthenic acids and other acid-extractables in water samples from Alberta: what is being measured?' *Sci. Total Environ.* 408 (2010) 5997-6010
- ⁴ X. Han, M.D. MacKinnon, J.W. Martin: 'Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS' *Chemosphere* 76 (2009) 63-70
- ⁵ M.R. Noestheden et al.: 'Rapid characterization of naphthenic acids using differential mobility spectrometry and mass spectrometry' *Environ. Sci. Tech.* 48 (2014) 10264-10272
- ⁶ V.V. Rogers, K. Liber, M.D. MacKinnon: 'Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water' *Chemosphere* 48 (2002) 519-527
- ⁷ U. Ceglarek et al.: 'Determination of linear alkylbenzenesulfonates in communal wastewater by means of solid phase microextraction coupled with API-MS and HPLC-FLD' *Fresenius J Anal Chem* 365 (1999) 674-681

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