Analysis of Naphthenic Acids (NAs) in Tissue: A derivatization based LC-MS/MS approach

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

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Overview:

- Introduction
- Method scope
- Method summary
- Challenges in NA analysis
- Method validation data
- Questions

Introduction:

- Naphthenic acids:
  - A complex mixture of organic acids that occur in crude oil and oil sand bitumen.
  - Extracted from oil sands during the caustic hot water or Steam Assisted Gravity Drainages (SAGD) production process.
  - Produced as waste product in aqueous phase of upgrading and refining processes.
  - Chemical raw material produced through refining process

Naphthenic Acids – Intro and Analyte Selection

Definition

- A complex mixture of saturated, cyclic and non-cyclic carboxylic acids described by the formula $C_nH_{2n+z}O_2$,

Where, n=# of C atoms, z ≤ 0, represents hydrogen deficiency

Occurrence

- Naturally occurring in crude oils and oil sand bitumen
- Extracted from oil sands during the caustic hot water production process
- Z = 0, Z = -2 have contributions (fatty acids) that are naturally occurring – important compositional information for assessing changes in Naphthenic acid concentrations.

Project purpose

- Develop a sensitive method that is:
  - able to sufficiently characterize NAs enabling source characterization and fingerprinting in field samples, including differentiation of Z=0 and Z=2 from NA totals
  - is useful for comparative data evaluation for spatial and temporal changes in occurrence
  - Overcome the false positive effects associated with GC-MS and FTIR

Naphthenic Acids - Isomer Groups – What to Measure?

Naphthenic Acids

- Over 200, 000 possible compounds, not practical to measure all compounds routinely
- Prevalence and toxicity of individual NA compounds not well developed
- Very limited specific compound standards and surrogates, technical Merichem standards used

Use of Isomer Groups

- Provide wide variety of information on NA isomer groups to monitor spatial and temporal concentrations - occurrence and change in space and over time
- Identify prevalent and important isomer groups for further study

Application to Tissue

- May demonstrate change in NA concentration / pattern over space and time
- Specific biota may have unique isomer groups for use as biomarkers

Naphthenic Acid Isomer Group Selection (Highlighted) – AXYS Aqueous Method MLA-077 / Solid Method MLA 091

% peak area for various naphthenic acid isomer groups in a surface water* sample

n	z=-12	z=-10	z=-8	z=-6	z=-4	z=-2	z=-0	Total
9	n/a	n/a	n/a	n/a	n/a	0	0.13	0.13
10	n/a	n/a	n/a	n/a	0	0	0	0
11	n/a	n/a	n/a	n/a	0.41	0.10	0	0.51
12	n/a	n/a	n/a	0.62	1.50	0.28	0	2.40
13	n/a	n/a	n/a	2.13	3.33	0.53	0.02	6.01
14	n/a	n/a	0.68	5.25	5.07	0.77	0.04	11.8
15	1.28	1.09	1.86	7.59	5.48	0.90	0.05	18.3
16	2.58	1.54	2.70	7.47	4.45	0.68	0.06	19.5
17	3.52	1.70	2.60	4.92	2.77	0.46	0.05	16.0
18	3.43	1.59	1.80	2.68	1.47	0.32	0.02	11.3
19	2.32	1.10	1.15	1.42	0.81	0.15	0	6.95
20	1.85	0.71	0.54	0.60	0.35	0.06	0	4.11
21	0.97	0.39	0.24	0.29	0.15	0.02	0	2.06
22	0.43	0.14	0.08	0.05	0.02	0	0	0.72
23	0.17	0.03	0.02	0.01	0	0	0	0.23

n/a = not applicable. * Surface water suspected of contamination from Oil sands.

Naphthenic Acid Isomer Group Selection – Specific to Tissue Type Analyzed AXYS Tissue Method MLA- 092

Table 3. % peak area for various naphthenic acid isomer groups in a surface water sample, isomer groups quantified in tissue (fish, frogs) highlighted

n	z=-12	z=-10	z=-8	z=-6	z=-4	z=-2	z=-0	Total
9	n/a	n/a	n/a	n/a	n/a	0	0.13	0.13
10	n/a	n/a	n/a	n/a	0	0	0	0
11	n/a	n/a	n/a	n/a	0.41	0.10	0	0.51
12	n/a	n/a	n/a	0.62	1.50	0.28	0	2.40
13	n/a	n/a	n/a	2.13	3.33	0.53	0.02	6.01
14	n/a	n/a	0.68	5.25	5.07	0.77	0.04	11.8
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20	1.85	0.71	0.54	0.60	0.35	0.06	0	4.11
21	0.97	0.39	0.24	0.29	0.15	0.02	0	2.06
22	0.43	0.14	0.08	0.05	0.02	0	0	0.72
23	0.17	0.03	0.02	0.01	0	0	0	0.23

n/a = not applicable

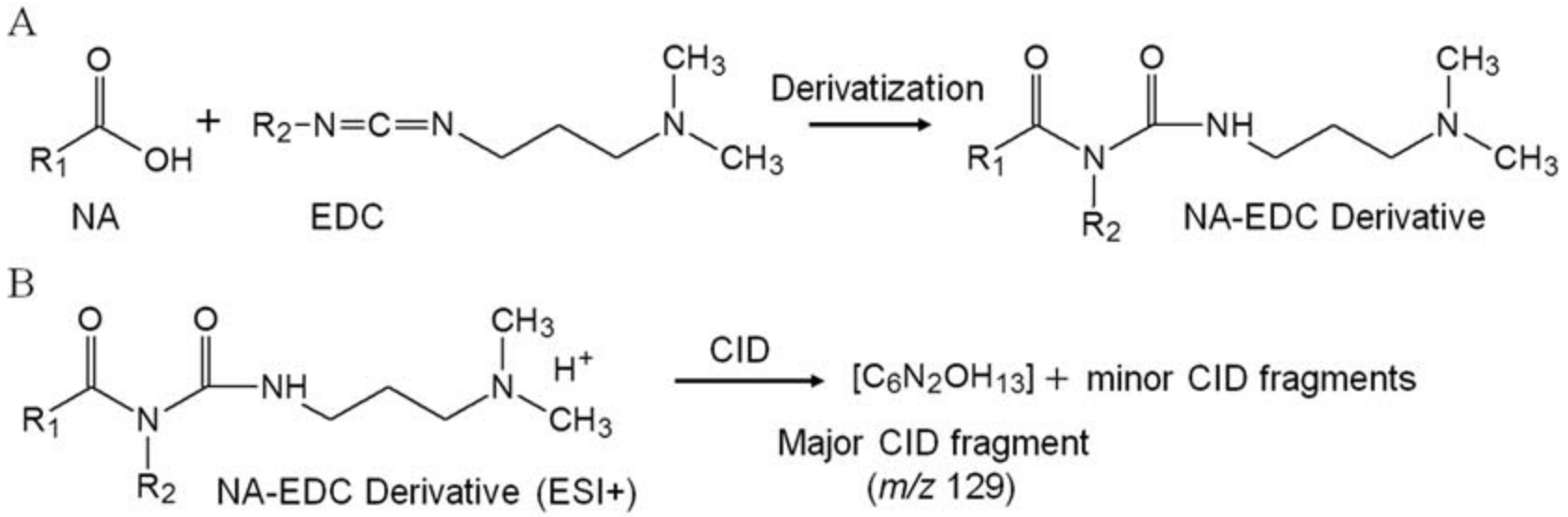
Tissue method scope:

Marker NA isomers
n=12,Z=-6
n=12,Z=-4
n=12,Z=-2
n=12,Z= 0
n=13,Z=-6
n=13,Z=-4
n=13,Z=-2
n=13,Z= 0
n=14,Z=-8
n=14,Z=-6
n=14, Z=-4
n=14, Z=-2
n=14, Z=0
n=15, Z=-12
n=15, Z=-10
n=15, Z=-8
n=15, Z=-6
n=15, Z=-4
n=15, Z=-2

- Target analytes
  - $C_nH_{2n+z}O_2$ where, n = # of 'C' atoms and Z = hydrogen deficiency
- Matrices:
  - Tissues
- Applicability:
  - For spatial and temporal trends as well as fingerprinting applications
- Quantification:
  - Pyrenebutyric acid equivalents
- Method detection limits
  - 12.5 ng/g, wet, for a 1 g tissue.

Method summary:

Derivatization and fragmentation



Extraction and cleanup

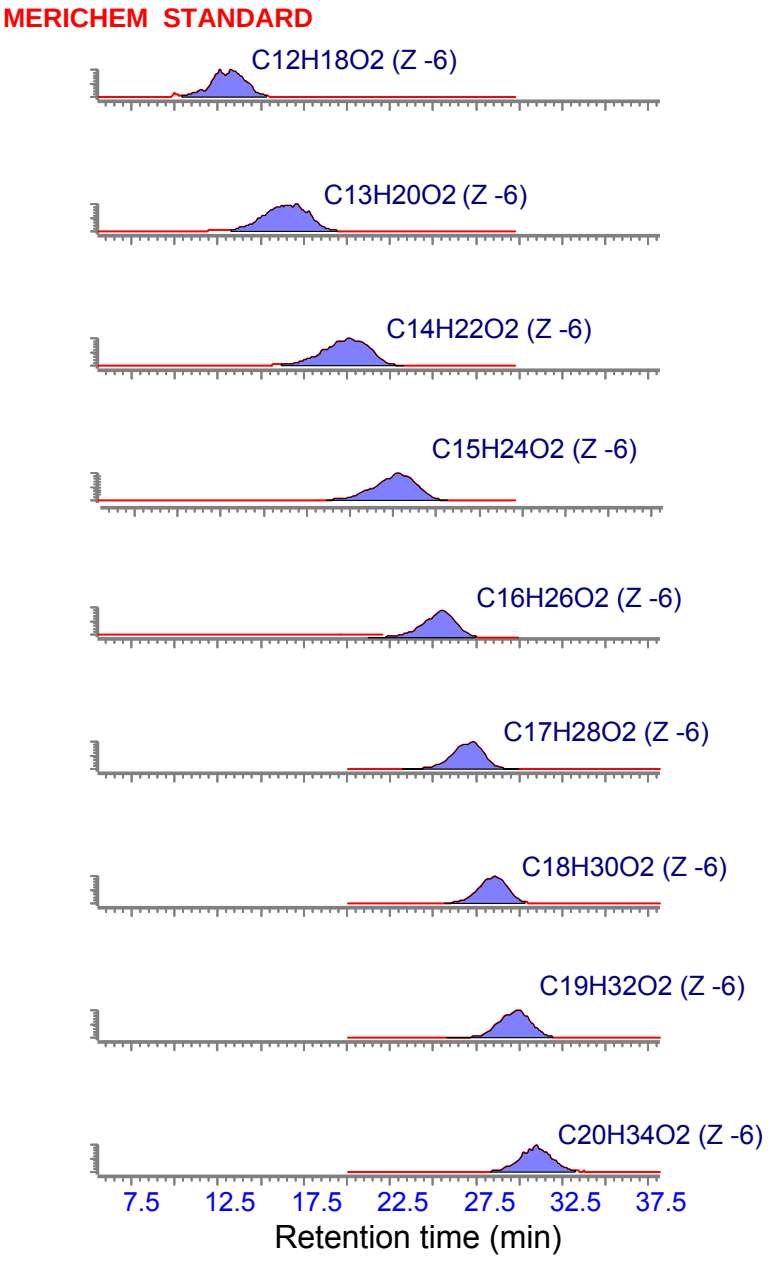
- Shaker table extraction with $CHCl_3$:MeOH:H_3O^+
- Extract cleanup uses SAX (2 g, 40µm)

Ref: Young et al. 2007. Chemosphere 68: 518–527.

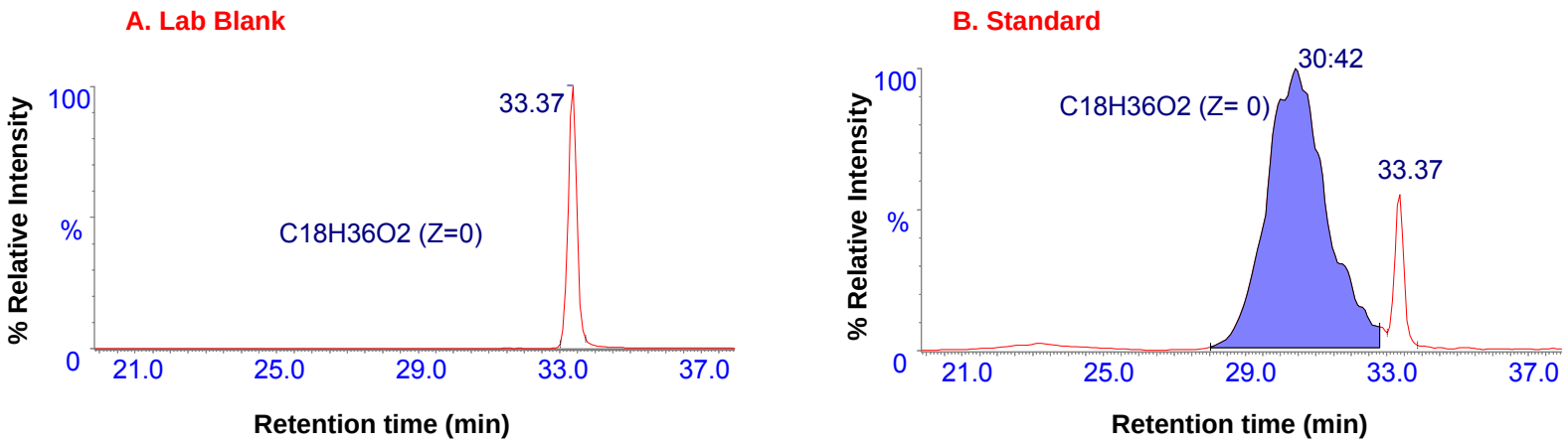
Chromatographic separation

LC Column : Xterra C18, 10.0 cm, 2.1 mm i.d., 3.5 µm

Mobile Phase: 0.1% formate buffer and methanol



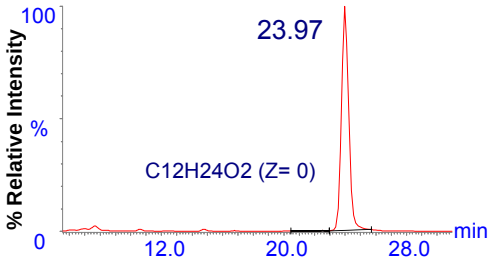
Laboratory background : Fatty acids



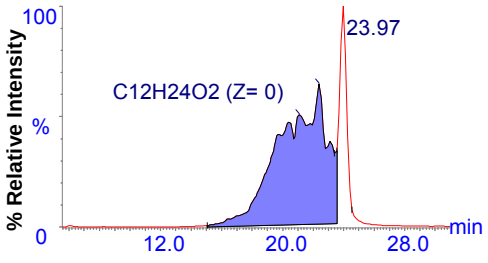
✓ By convention, straight chain Z=0 NA isomer is excluded.

Intrinsic “interferences”: Fatty acids, Z=0

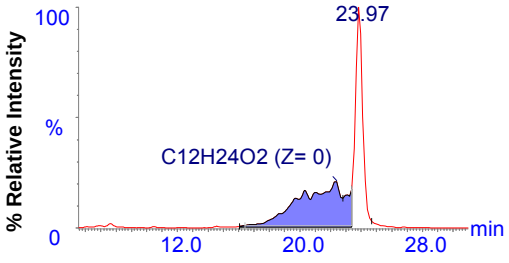
A. Un-spiked tissue extract



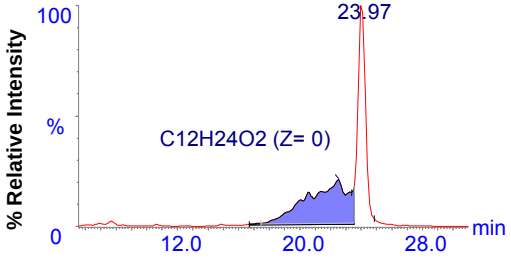
B. Merichem standard



C. Spiked tissue extract

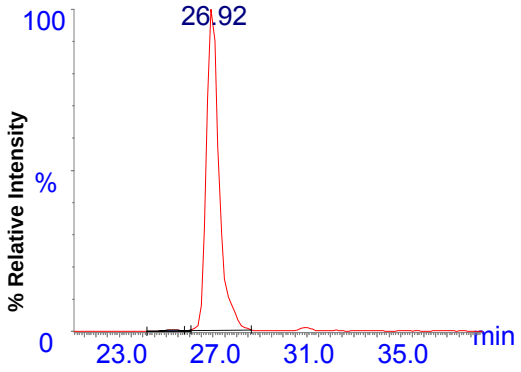


D. Spiked and extracted

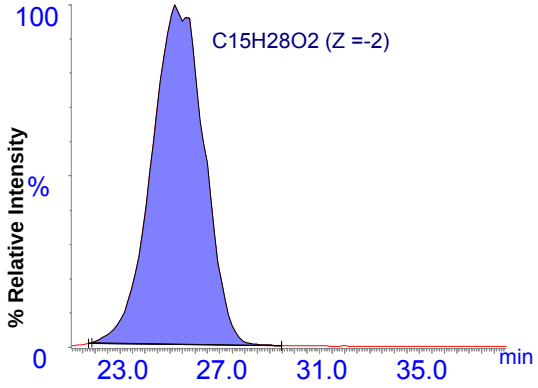


Intrinsic “interferences”: Fatty acids, Z=-2

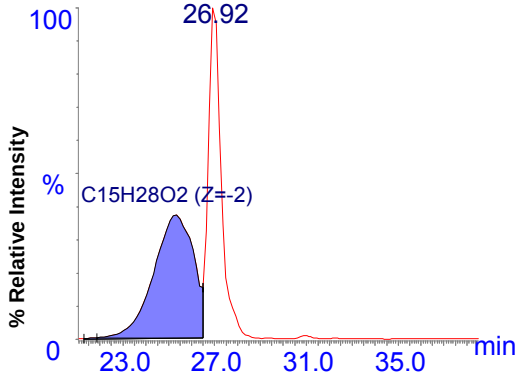
A. Un-spiked tissue extract



B. Merichem standard



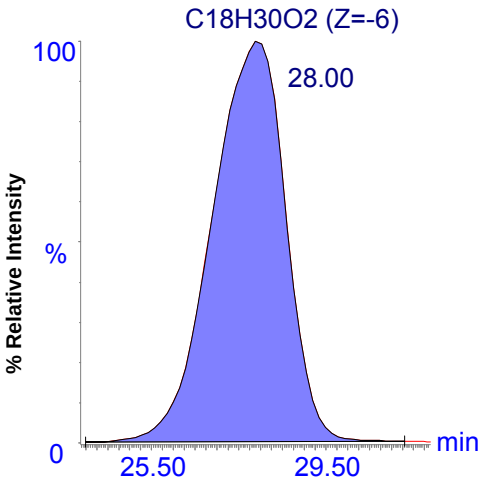
C. Spiked tissue extract



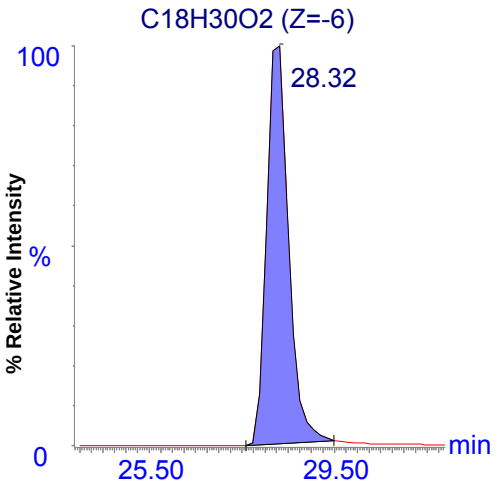
✓ By convention, straight chain Z-2 NA isomer is excluded.

Intrinsic “interferences”: Fatty acids, Z<-2

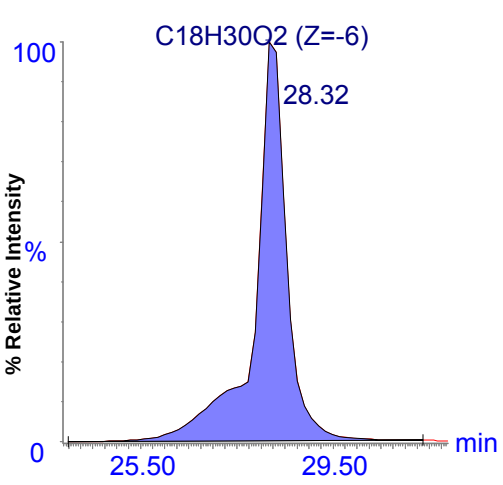
A. Merichem standard



B. gamma-Linolenic acid

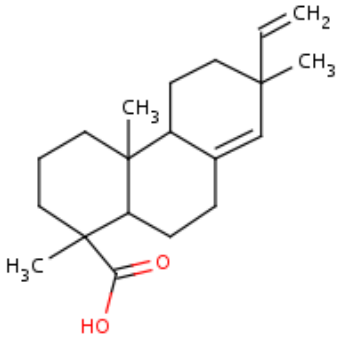


C. Mixed Merichem and linoleic acid

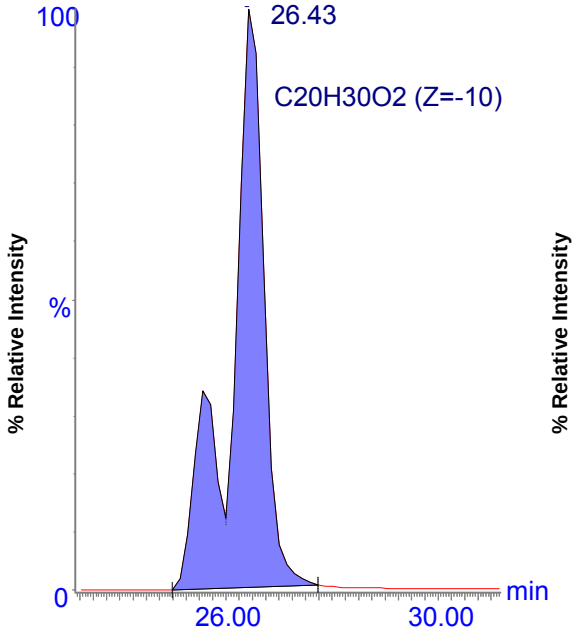


✓ Fatty acid isomers with Z<-2 cannot be separated from NAs with the same formula.

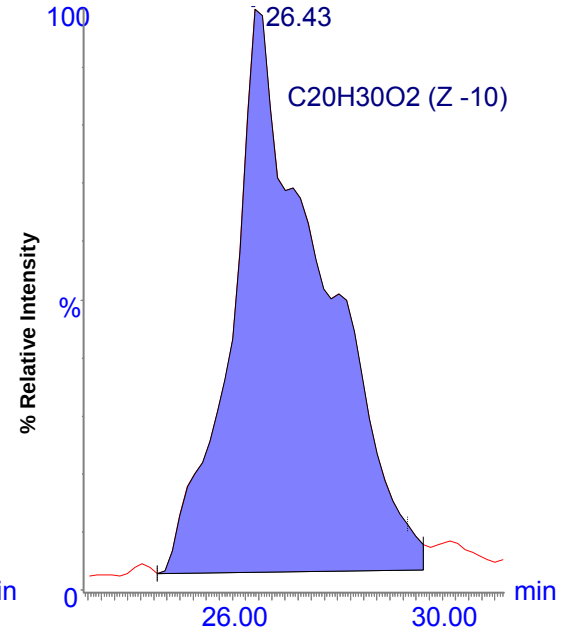
Intrinsic “interferences”: Resin acids



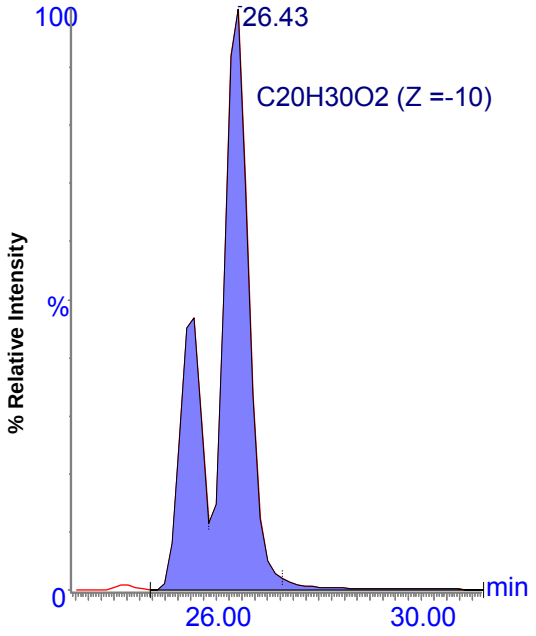
A. Pimaric acid standard



B. Merichem standard



C. Merichem + Pimaric acid standard



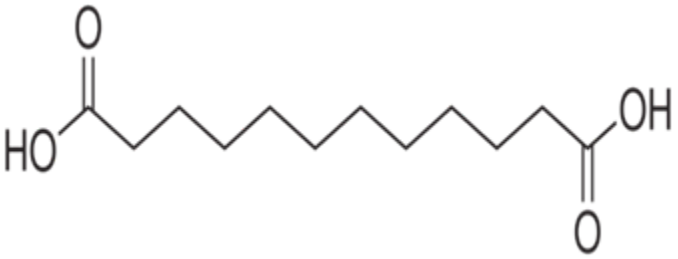
✓ Resin acids present interference for n=20, Z=-10 and -8 NA isomer groups.

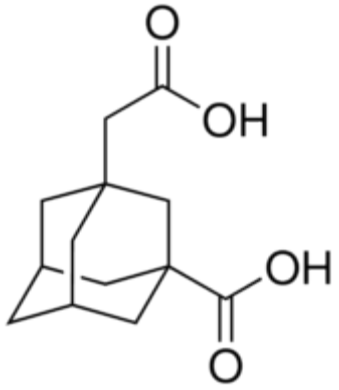
Potential marker NA isomer groups:

Isomer groups
n=12,Z=-6
n=12,Z=-4
n=12,Z=-2
n=12,Z= 0
n=13,Z=-6
n=13,Z=-4
n=13,Z=-2
n=13,Z= 0
n=14,Z=-8
n=14,Z=-6
n=14, Z=-4
n=14, Z=-2
n=14, Z=0
n=15, Z=-12
n=15, Z=-10
n=15, Z=-8
n=15, Z=-6
n=15, Z=-4
n=15, Z=-2

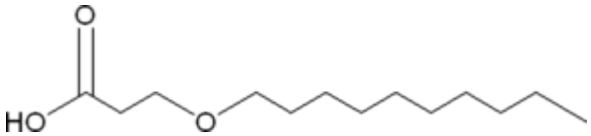
Method selectivity:

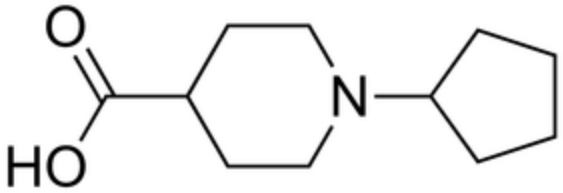
1. Dicarboxylic acids



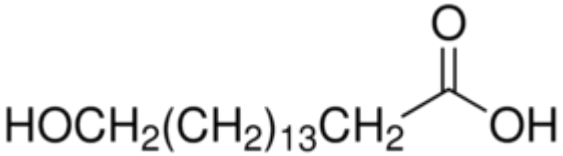


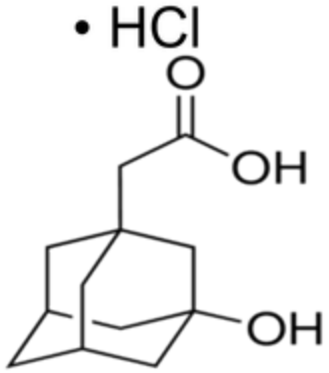
2. Hetero atomic acids





3. Hydroxy acids





Study design considerations:

- Control samples:
  - Samples that are known to NOT be impacted by a potential NA source
- A sample of suspected NA source:
  - e.g. Oil Sands Process Water (OSPW)
- Target / suspected impacted sample:
  - Samples of tissue from the target organism in the potentially affected area
- Statistical considerations
  - Good experimental design and number of replicates

Method applicability:

- Special and temporal change measurement
- For fingerprinting

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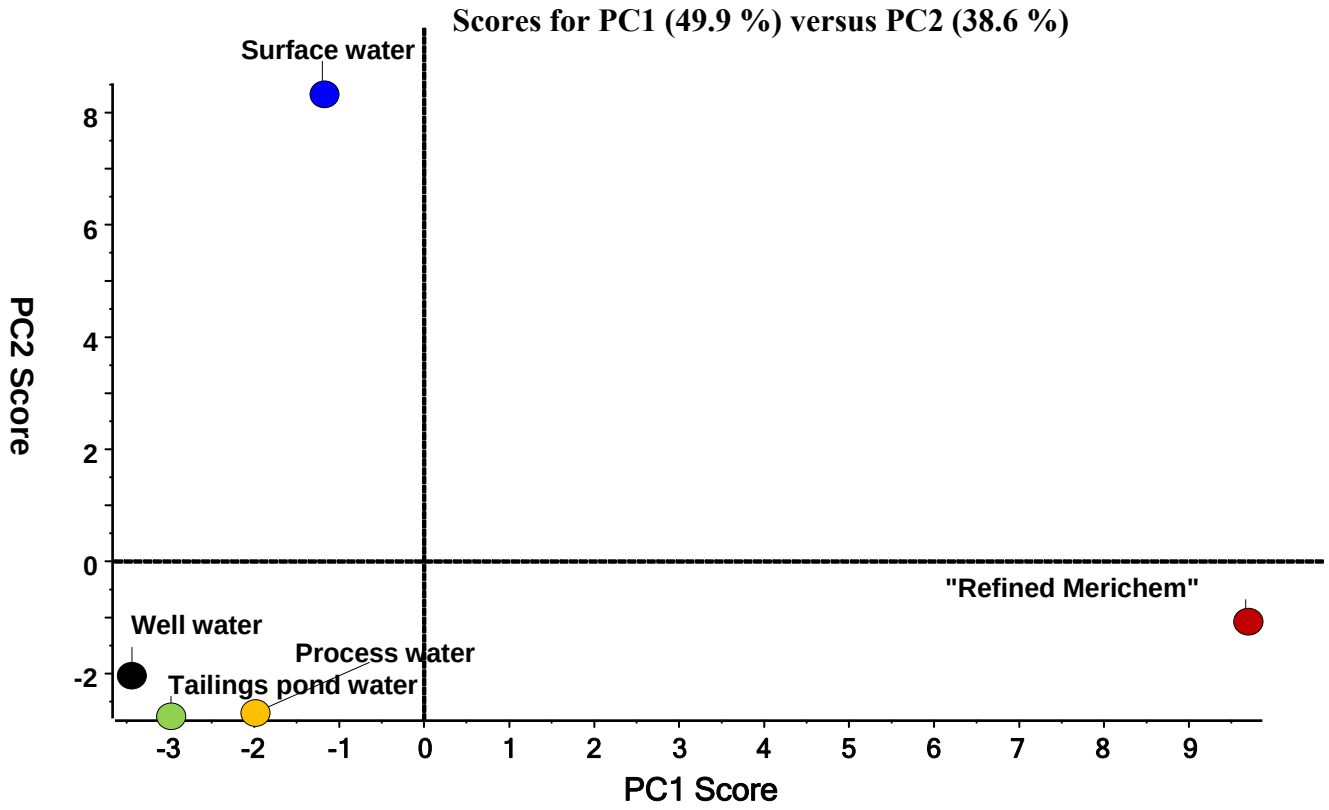
- Special and temporal change measurement
- For fingerprinting



Method validation data:

Isomer group	%rec. n=5, (RSD)
n=12,Z=-6	96 (8)
n=12,Z=-4	98 (8)
n=12,Z=-2	94 (8)
n=12,Z= 0	81 (13)
n=13,Z=-6	104 (7)
n=13,Z=-4	108 (7)
n=13,Z=-2	108 (8)
n=13,Z= 0	90 (9)
n=14,Z=-8	114 (12)
n=14,Z=-6	109 (7)
n=14, Z=-4	112 (6)
n=14, Z=-2	95 (4)
n=14, Z=0	87 (3)
n=15, Z=-12	206 (27)
n=15, Z=-10	125 (11)
n=15, Z=-8	126(5)
n=15, Z=-6	116 (6)
n=15, Z=-4	115 (5)
n=15, Z=-2	89 (3)

Quality control:

Surrogates	% rec. n=5
D15-Adamentane Carboxylic Acid	49.8
D9-Anthracene Carboxylic Acid	60.2
D4-Lithochlolic Acid	59.0
13C4 Mono-n-octyl phthalate	51.7
Derivatization controls	
D23-Dodecanoic Acid	78.3
D35-Octadecanoic Acid	N/A
Recovery standards	
13C3-Atrazine	102

Summary:

- A versatile derivatization and (+ESI) MS/MS fragmentation method was developed for monitoring NAs in tissue.
  - Enabled MRM, increased specificity, sensitivity and lowered detection limits.

- Tissue samples present intrinsic “interference” to analysis of fatty acids.
  - Chromatographic separation was employed to minimize the impact of endogenous fatty acids on NA data.

- Fingerprinting and source characterization have been shown to be useful as a fingerprinting tool.
  - Multivariate methods such as principal components analysis and discriminant analysis have been shown to group samples based on NA isomer group covariance (source).