Centre for Energy and Environmental Sustainability
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APPLIED RESEARCH | NAIT

Discussion Notes for Naphthenic Acids Measurement Workshop

Date: July 29, 2025

Contents

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Workshop Objective

This was an invitation-only workshop with the primary objective of compiling comprehensive and authoritative information on the available measurement methods for naphthenic acids, with a focus on their respective advantages and disadvantages, associated costs, required equipment, and necessary operator training.

Key Notes of Discussions

1. What are NAFCs and why do we care about them?

- Definition: "Naphthenic acids (NAs) are a complex class of thousands of naturally occurring aliphatic and alicyclic carboxylic acids found in oil sands bitumen and in wastewater generated from bitumen processing." (Kovalchik et al. 2017).
- Classic NAs vs NAs in OSPW
    - IUPAC definition (classic NAs): chiefly monocarboxylic, derived from naphthenes. CnH2n+zO2 where n is the carbon number, Z is an even, negative integer corresponding to hydrogen deficiency due to ring formation.
- NAs in OSPW
    - In some sources of OSPW, >50% of the compounds in the extracts of OSPW are not classic NAs (Grewer et al. 2010).
    - $C_nH_{2n+z}O_x$ where x = 2 – 7
    - Other heteroatoms such as sulfur and nitrogen in the compounds
    - Aromatic acids were detected in NAs extracted from OSPW.
    - NAs common to OSPW are chemical stable and non-volatile compounds (Kavanagh et al. 2009).
    - Suggested definition: oil sands tailings water acid-extractable organics (Grewer, et al. 2010)

2. What are the methods used to measure NAFCs

- A list of methods (Chen et al., 2024)
    - UPLC-TOF-MS; HPLC-QqQ-MS, GC-Orbitrap-MS, UPLC-TWIM-TOF-MS, GCxGC-TOF-MS, FT-ICR MS, GC-EIMS, FITR, Microchip electrophoresis, Triple quadruple MS, GC-FT-ICR MS, HPLC-Orbitrap MS
- Calibration standards used for NAFC measurement
    - Seven chemicals have been used across labs: Merichem NA, Sigma-Aldrich NA mix, Acros NA (CAS 133820-45), TCL, Fluka NA, Kodak mix, and a Fischer mix.
        - Fluka NA was discontinued.
        - Merichem NA was derived from refinery-based NAs, and varies by batch. It is not a certified standard with concentrations defined for each component (its exact composition is unknown).

- Sigma-Aldrich NA mix is composed primarily of fatty acids, and solubility in water is a known challenge.
- Acros currently is used by some labs including BV.

o The standard chemical should be applicable to Alberta oil sands and have a reliable supply.

3. Sample preparation – importance and key notes

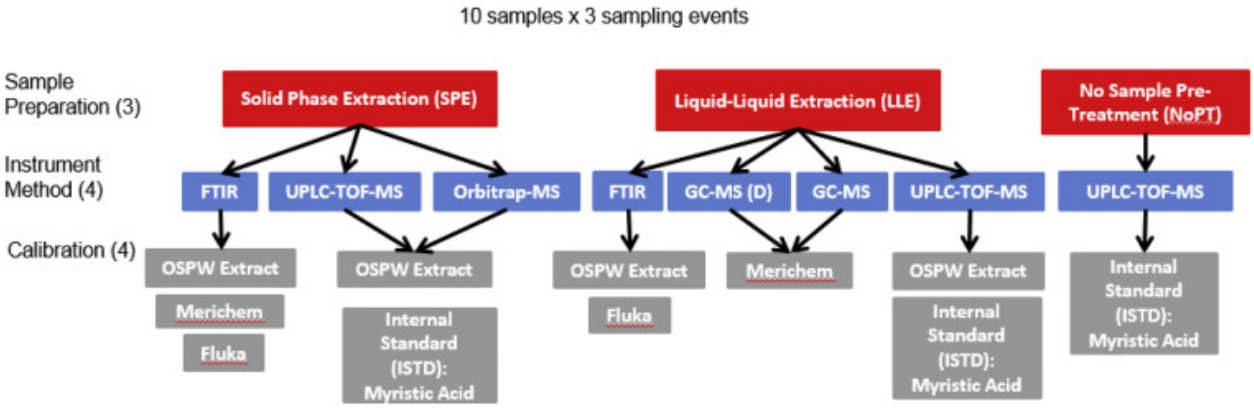

Figure 1 - Sample preparation overview provided by Hughes et al. (2017).

- Menti results showed that sample preparation including extraction of NAs from water and preparing extract for analysis (i.e. separation method) are the most critical step to obtain a good, reliable result.
- Solid phase extract (SPE) vs. liquid-liquid extraction (LLE)
  - Menti results showed that **SPE was recommended compared to LLE.**

ASPECT	SPE	LLE
SOLVENT USE	Ethanol, methanol, PEG-ethanol; compatible with LC-MS and bioassays	DCM (toxic and less compatible with downstream biological testing)
REPEATABILITY	High, especially with automated systems	Low
TARGET COMPOUNDS	Depends on sorbent: ENV+ for aromatics and acids, C18 for non-polar organics	Broad range, but less selective; may miss polar or aromatic compounds
SAMPLE VOLUME	Flexible; scalable from mL to L with cartridge size	Challenging with large volumes (e.g., 10 L); labor-intensive
AUTOMATION	Possible with systems like Dionex Autotrace	Rare; mostly manual
EQUIPMENT NEEDED	Cartridges, vacuum manifold, pump, nitrogen evaporator, Teflon tubing	Separatory funnels, solvents, drying equipment
SETUP COST	High upfront (Autotrace ~$88K–$100K)	Low; basic glassware and solvents

LEARNING CURVE	Moderate to high; method tuning required	Low to moderate; but labor-intensive
USE CASE	Ideal for consistent, high-throughput, selective extraction	Suitable for simple, low-cost, broad-spectrum extraction
SELECTIVITY	Sorbent-dependent; can isolate specific fractions (e.g., aliphatic acids)	Less selective; depends on solvent polarity and partitioning behavior

- Filtration vs. centrifugation

ASPECT	FILTRATION	CENTRIFUGATION
PORE SIZE / CUTOFF	Defined by filter (0.2 or 0.45 µm)	Depends on RCF and time, typically removes particles down to ~0.2 µm
MATERIAL IMPACT	Filter material can adsorb analytes	
ANALYTE LOSS RISK	Higher, especially for amphiphilic compounds like NAs	Lower, but NAs may still adsorb to particles in sediment
CONSISTENCY	Can vary with sample turbidity and filter choice	More consistent if RCF, time, and temperature are standardized
COLUMN PROTECTION	Effective if properly filtered	Proven effective
BEST USE CASE	When defining dissolved fraction or removing emulsions	When minimizing analytes loss and ensuring reproducibility

4. Characterization Techniques:

Mass Spec Methods vs. FTIR

ASPECT	MASS SPEC	FTIR
RESOLUTION	High resolution (e.g., Orbitrap, QTOF) enables detailed profiling but not exact structures	Measures all C=O bonds; less specific, more general signal, less directly correlated to toxicity
DATA RICHNESS	Extremely data-rich; supports retrospective analysis	Simpler output; less data complexity
SAMPLE PREP SENSITIVITY	Highly sensitive to extraction method and instrument conditions	Also sensitive, but ostensibly less affected by minor prep variations (note: this may be not true due to NAIT unpublished study)
ROUTINE MONITORING SUITABILITY	Not ideal for routine use due to cost and complexity	More feasible for routine screening
STANDARDIZATION CHALLENGES	Difficult due to instrument variability and response factors	Easier to standardize, but still affected by sample composition

DETECTION LIMITS	Labs claims low limits (e.g., 0.2 µg/L, but often report "<0.2" due to visibility issues	Moderate limits; less prone to overstatement
RESPONSE FACTOR VARIABILITY	Varies with compound structure, ion source (ESI vs APCI), and mobile phase conditions	Measures functional groups; less affected by individual compound variability
INTER-LAB CONSISTENCY	Poor without standardization; same extract yields different results across labs	Better consistency if sample prep is controlled
USE CASE	Best for research, problem investigation, and retrospective analysis	Best for routine monitoring and performance-based testing
COST AND ACCESSIBILITY	Expensive; requires specialized equipment and expertise	Lower cost; widely available in environmental labs
CORRELATION ACROSS METHODS	Orbitrap and FTIR can correlate if sample prep is consistent	FTIR results vary with sample composition; not always comparable
AVOIDING DERIVATIZATION	GC-MS not recommended due to need for derivatization	

Key Takeaways and Next Steps

- **Scalability is essential**: with thousands of samples processed annually, we need a method that is scalable for high-throughput screening, followed by more complex analysis on selected subsets.
    - Need to focus next discussion on the high throughput screening methods and review what data is available on how these methods trend with the high-resolution data.
- **Standardization is challenging but necessary**: a universal method may not be perfect, but consistency across labs is critical to ensure reliable comparisons and trend detection.
    - First step is to obtain standard samples and calibration compounds that can be used to validate methods. Ideally this is an extract that is derived from oil sands water that can be used going forward. Is there a lab capable of creating such an extract?
- **Accuracy and error margins matter**: especially for long-term planning and closure strategies, acceptable levels of precision must be clearly defined and validated.
    - Ideally ECCC/Government publish the data used/methodologies used in defining these acceptable precision levels.
- **Tiered monitoring approach**: routine monitoring should rely on simple, fast methods to detect changes. When anomalies are observed, more advanced techniques should be applied to investigate further.

- **Consistency across operators and labs**: Measurement trends must remain reliable regardless of who performs the analysis or where it is conducted. Current inconsistencies hinder validation and trust.
    - Next discussions should highlight how to ensure consistent data between labs or some form of standard for comparison to build trust.
- **Research needs high resolution data:** FTIR data is not sufficiently detailed to be able to help understand mechanisms of toxicity or whether water treatment is effective at this stage. High resolution mass spec data is required for industrial samples over time to establish relevant baselines and enable understanding of mechanism.
    - Recommend that industry perform Orbitrap or Q-TOF analysis on a select baseline subset of river water and pond/tailings streams to complement high throughput screening. Water treatment tests should confirm potential "best performers" with high resolution data.

Recommended literature

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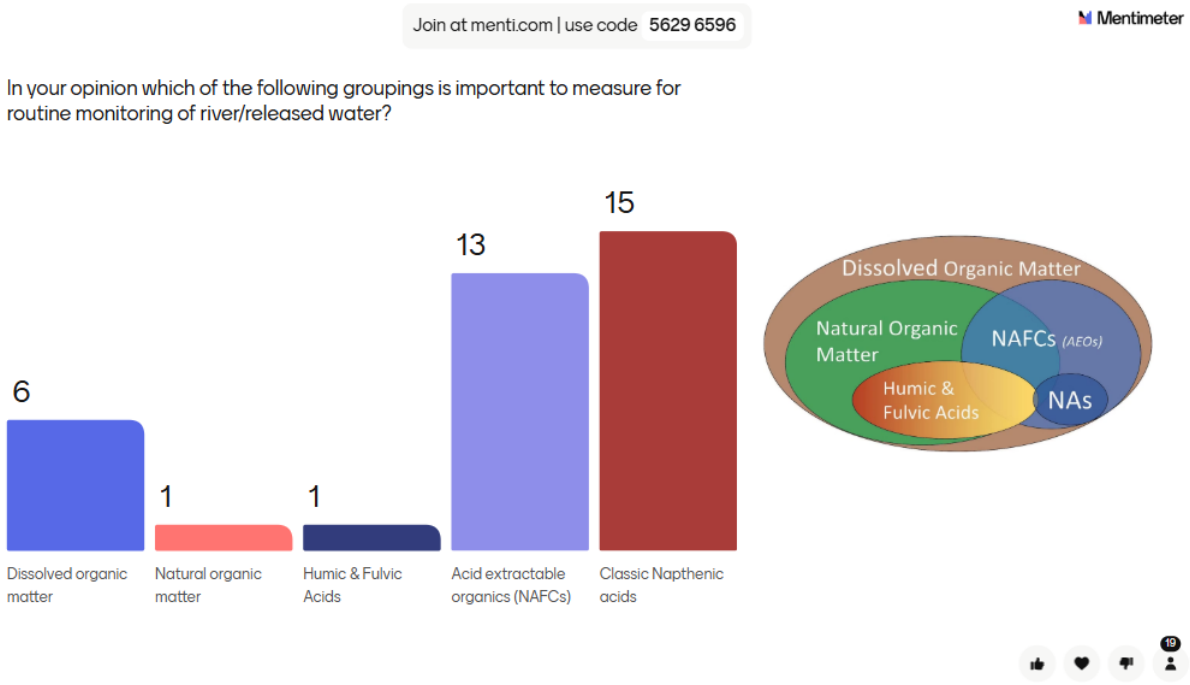
- Rui Qin, Dustin Lillico, Zuo Tong How, Rongfu Huang, Miodrag Belosevic, James Stafford, Mohamed Gamal El-Din. Separation of oil sands process water organics and inorganics and examination of their acute toxicity using standard in-vitro bioassays. Science of The Total Environment, Volume 695, 10 December 2019, 133532. https://doi.org/10.1016/j.scitotenv.2019.07.338.
- Kyle D. Duncan, Larissa C. Richards, Joseph Monaghan, Monique C. Simair, Chukwuemeka Ajaero, Kerry M. Peru, Vanessa Friesen, Dena W. McMartin, John V. Headley, Chris G. Gill, Erik T. Krogh. Direct analysis of naphthenic acids in constructed wetland samples by condensed phase membrane introduction mass spectrometry. Science of The Total Environment. Volume 716, 10 May 2020, 137063. https://doi.org/10.1016/j.scitotenv.2020.137063
- Remy GavardHugh. JonesDiana Catalina Palacio LozanoMary J. ThomasDavid RossellSimon E. F. SpencerMark P. Barrow. KairosMS: A New Solution for the Processing of Hyphenated Ultrahigh Resolution Mass Spectrometry Data. Anal. Chem. 2020, 92, 5, 3775–3786. https://doi.org/10.1021/acs.analchem.9b05113
- Ralph Hindle, John Headley and Douglas G. Muench. Pros and Cons of Separation, Fractionation and Cleanup for Enhancement of the Quantitative Analysis of Bitumen-Derived Organics in Process-Affected Waters—A Review. Separations 2023, 10(12), 583; https://doi.org/10.3390/separations10120583

Appendix

Menti Results

Responses to "What are you hoping to get out of this workshop?":

- Learn the pros and cons for various techniques
- Would like to get ideas about state of the art for NAs
- Current state of the field as we pivot our analysis from qualitative to quantitative.
- A path forward; there is a great deal of uncertainty right now, but enough knowledge around the subject that there is a feasible path forward.
- Understand the NA testing landscape, the value of each option and validate Biosensor as a consideration.
- Find out who is doing NA analysis and get best practices
- Information regarding the most recent state of naphthenic acid analysis and current challenges being faced in NA analysis.
- Education on various techniques. Pro's/Con's. Rapid methods available for real-time analysis?
- To proselytize on the value of high-res mass spec
- Understanding limitations of different techniques and understand what's the minimum standard can be developed so that we are comparing similar variables
- I'm curious to see what other groups are primarily using for their naphthenic acid analyses, both qualitative and quantitative. What standards are most used?
- Insights on breadth of MS approaches and extraction procedures for NAFC
- Which standards are being used for Quant?
- To increase awareness of a new NA biosensor technology we are developing, and to meet more people working in the NA detection and remediation, at all levels.
- I'd like to learn what methods are used to determine NAs and which instruments are used.
- Overview of frequently used methods, accuracy/resolution of each, cost and commerciality of each, comparability between methods, linkage to toxicity, approaches to compare historically published data
- understand the different perspectives from government, academic, commercial labs; perhaps some consensus on key factors for a successful standardized method that is viable for wide adoption
- To make sure there is enough understanding out there for the intent from NAs measurement (direct and indirect) and how they relate to reclamation.
- What is the proper standards for NAs quantification by mass spectrometry? A proper and consistent standard is crucial for accurate NAs quantification.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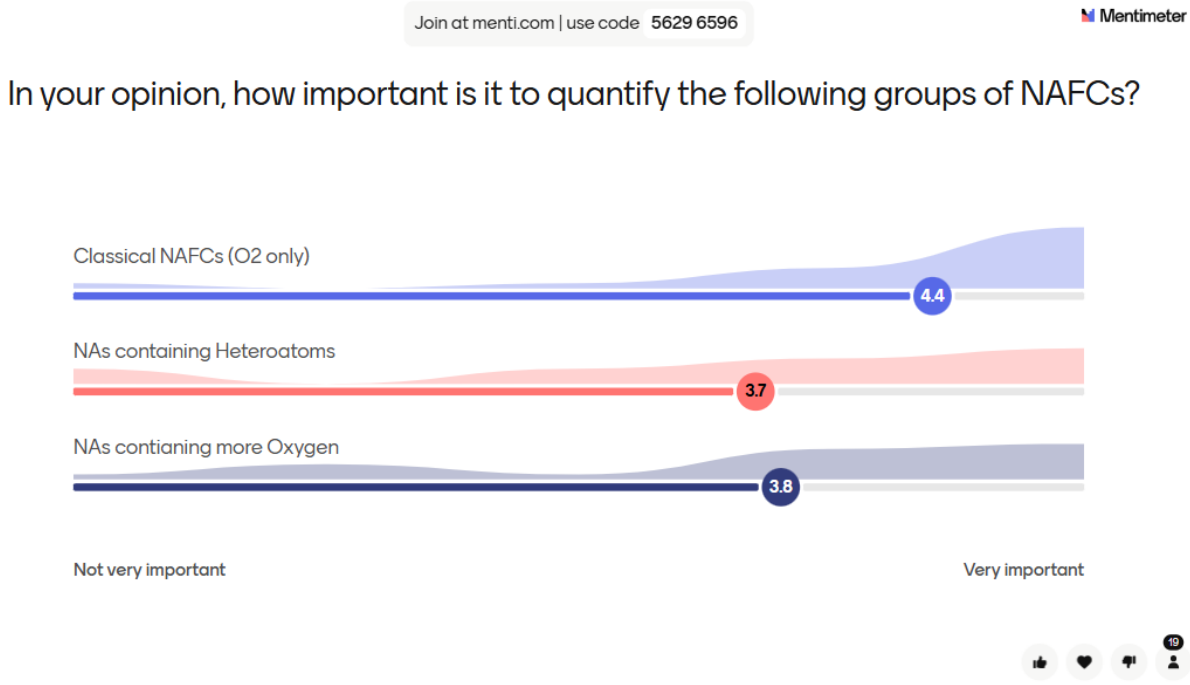
Mentimeter
In your opinion which of the following groupings is important to measure for routine monitoring of river/released water?
6
1
1
13
15
Dissolved organic matter
Natural organic matter
Humic & Fulvic Acids
Acid extractable organics (NAFCs)
Classic Napthenic acids
Dissolved Organic Matter
Natural Organic Matter
NAFCs (AEOs)
Humic & Fulvic Acids
NAs
19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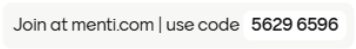
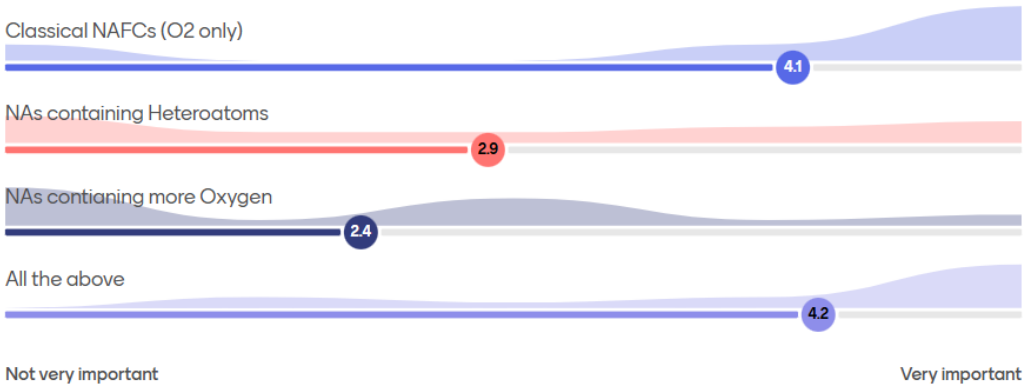
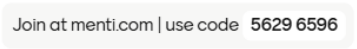
Mentimeter
In your opinion, how important is it to quantify the following groups of NAFCs?
Classical NAFCs (O2 only)
4.4
NAs containing Heteroatoms
3.7
NAs contianing more Oxygen
3.8
Not very important
Very important
19



Mentimeter

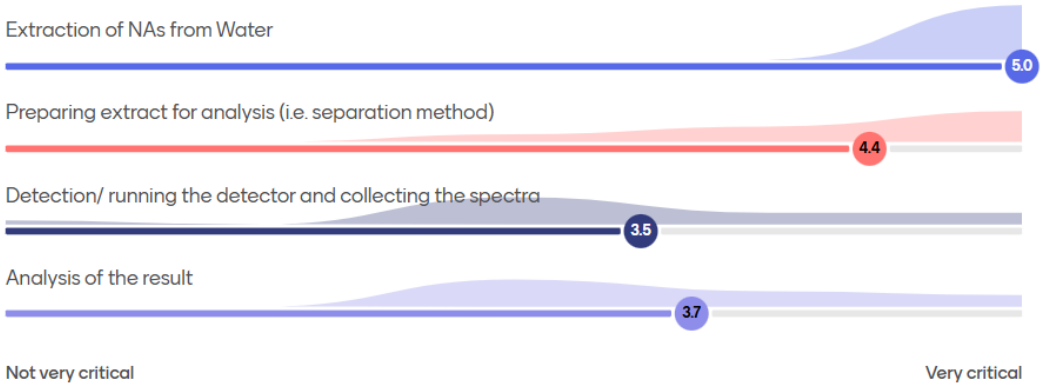
In your opinion, how important is it to quantify the following groups of NAFCs for the **purpose of routine monitoring**?

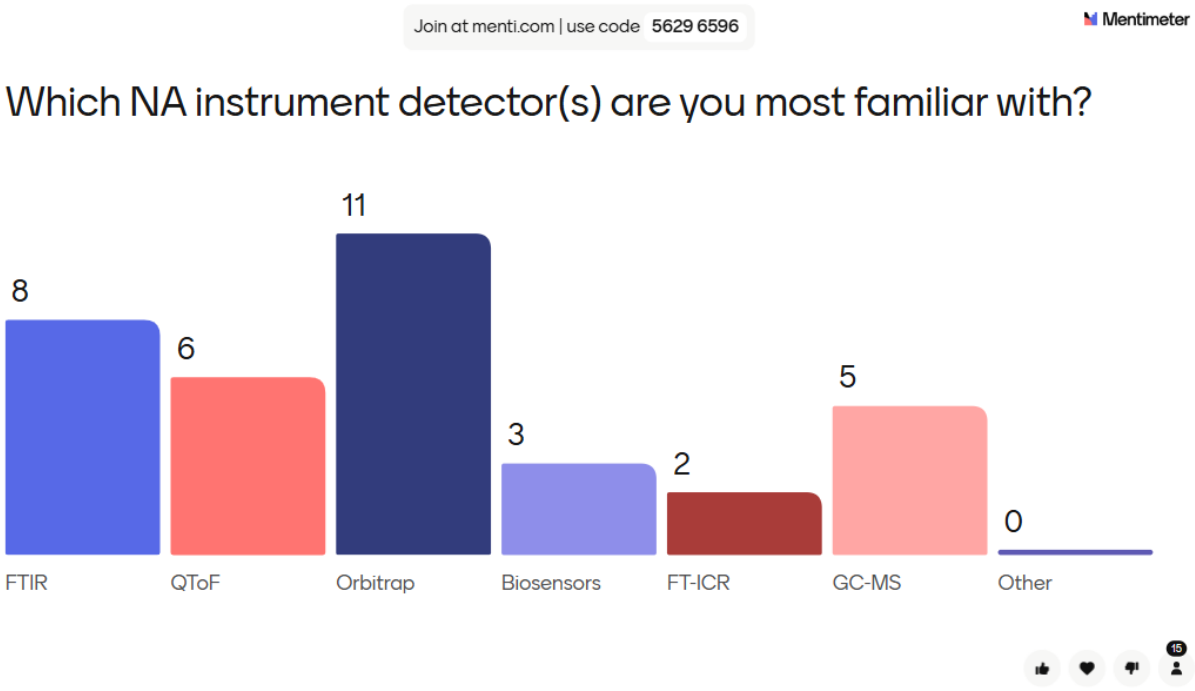




Mentimeter

Rate how critical each stage of the method is to a good, reliable result in your opinion:

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Mentimeter
Which NA instrument detector(s) are you most familiar with?
8
6
11
3
2
5
0
FTIR
QToF
Orbitrap
Biosensors
FT-ICR
GC-MS
Other
15


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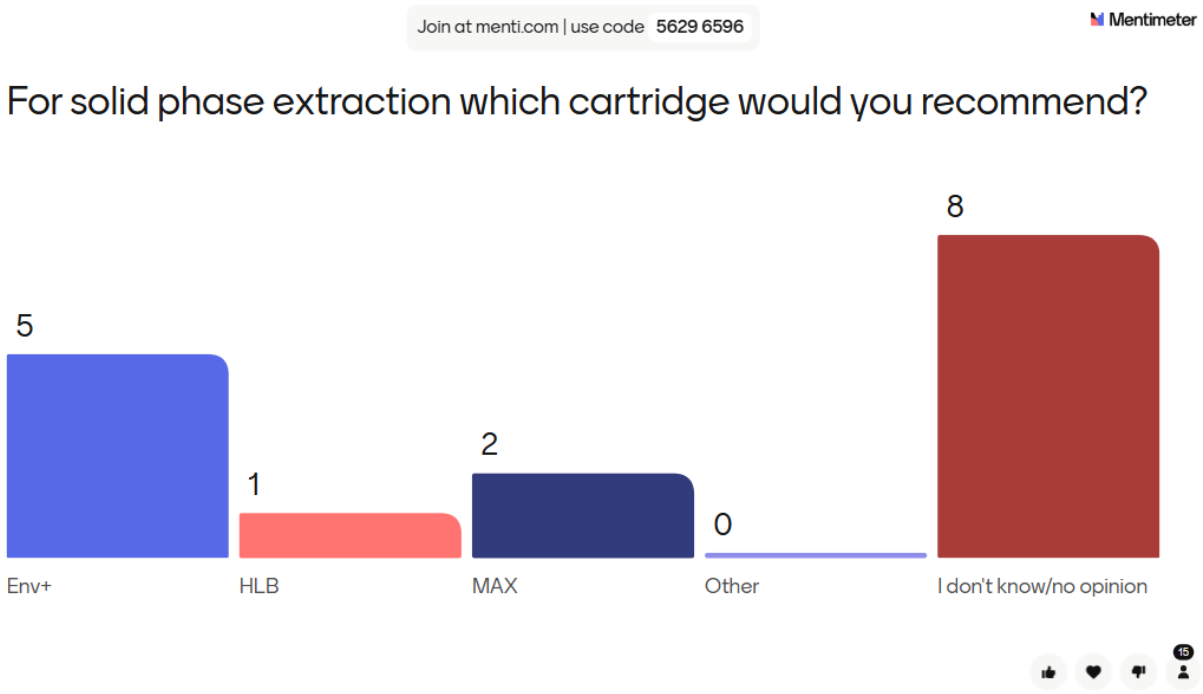
For liquid -liquid extraction which solvent would you recommend?
9
1
0
1
1
5
DCM
n-hexane
n-pentane
ethyl ether
Other
I don't know/no opinion
15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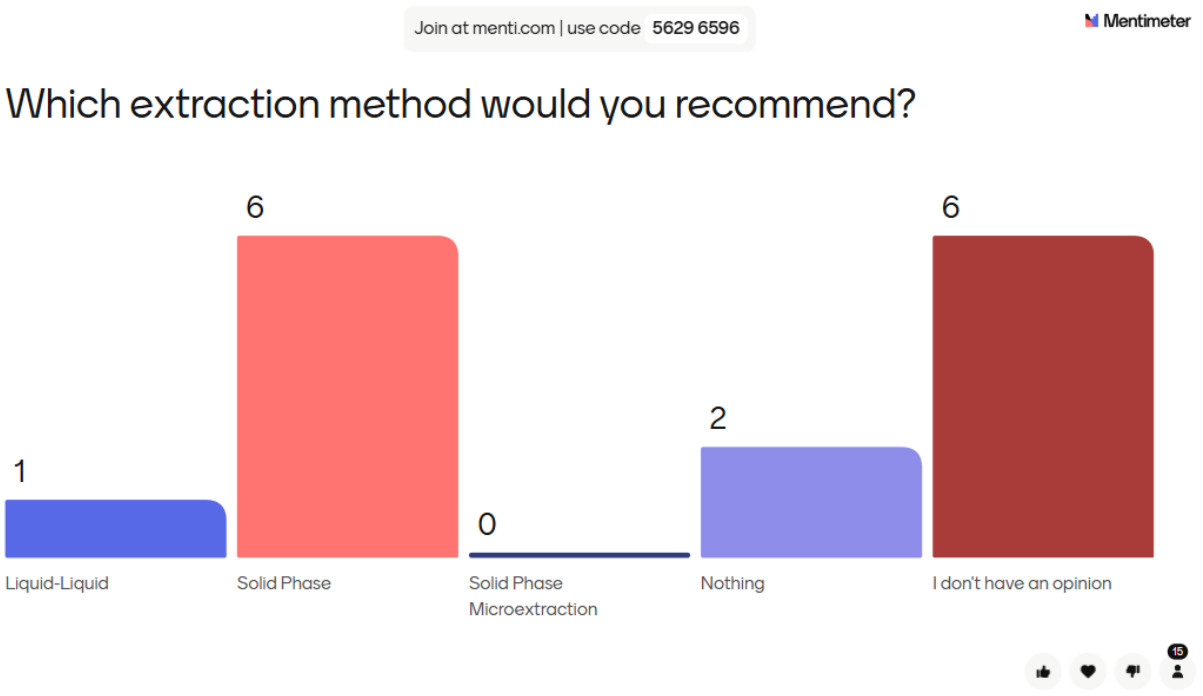
Mentimeter
For solid phase extraction which cartridge would you recommend?
5
1
2
0
8
Env+
HLB
MAX
Other
I don't know/no opinion
15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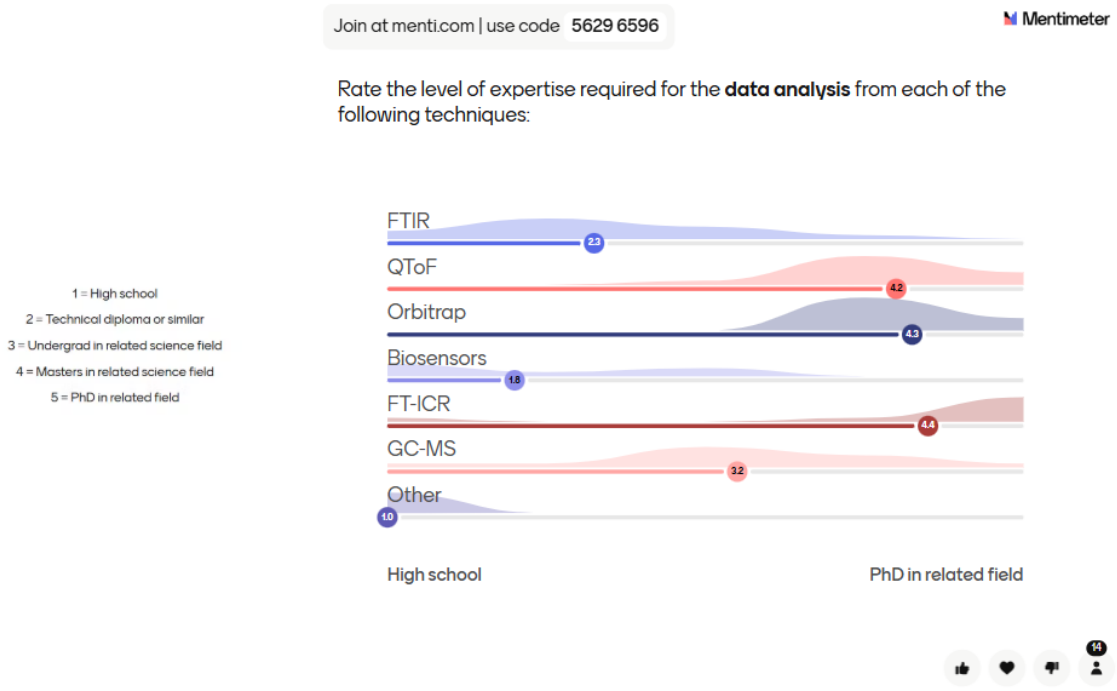
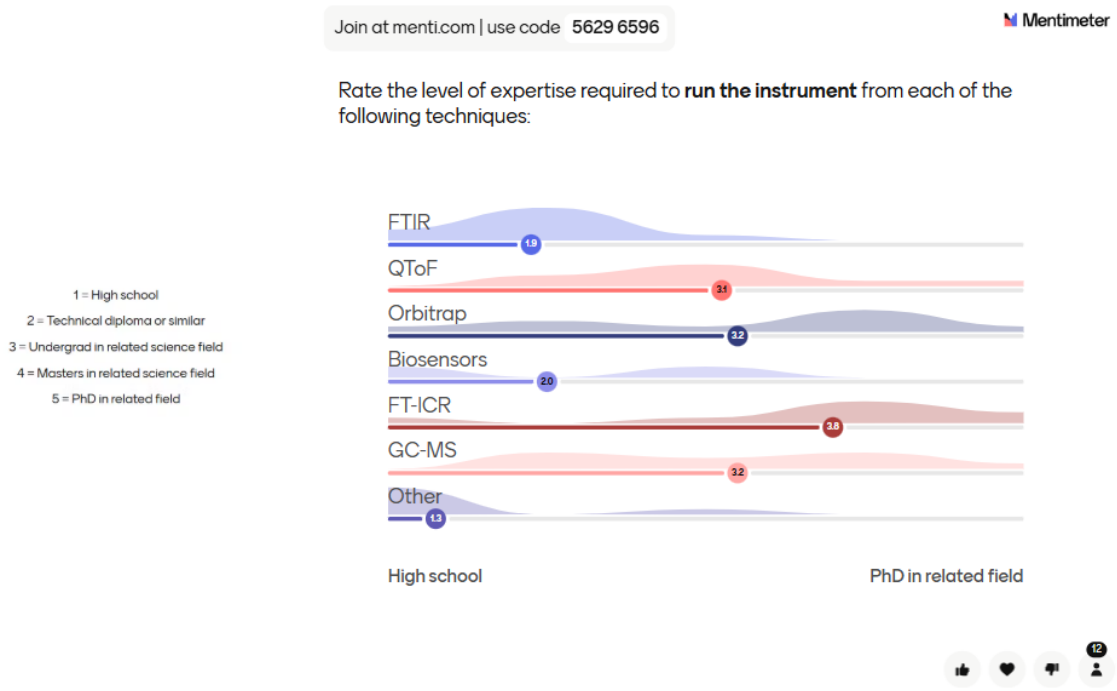
Which extraction method would you recommend?
1
6
0
2
6
Liquid-Liquid
Solid Phase
Solid Phase Microextraction
Nothing
I don't have an opinion
15

What reference materials/standards do you use?

- Merichem
- Sigma Aldrich CAS # 1338-24-5
- Sigma NA mix
- NAFC from the Headley lab, SPE extract. I wouldn't use a Sigma Aldrich or Acros as standard, they are mostly fatty acids from what I read in the literature.
- Merichem, Sigma, Fisher, Fluka (discontinued), TCL. I ran calibration curves on each for FTIR and they are all different
- An OSPW-derived NAFC extract (800 L --> 1 L extracted by LLE) previously standardized against Merichem via FTIR
- Acros
- Internal standard (myristic acid-1-13C) for semi-quantification of NAs by QTOFMS.
- I like the "OSPW-derived…" approach
- Sigma
- we have compared Fluka and Acros as standard for FTIR analysis, the results are different
- CAS 1338-24-5

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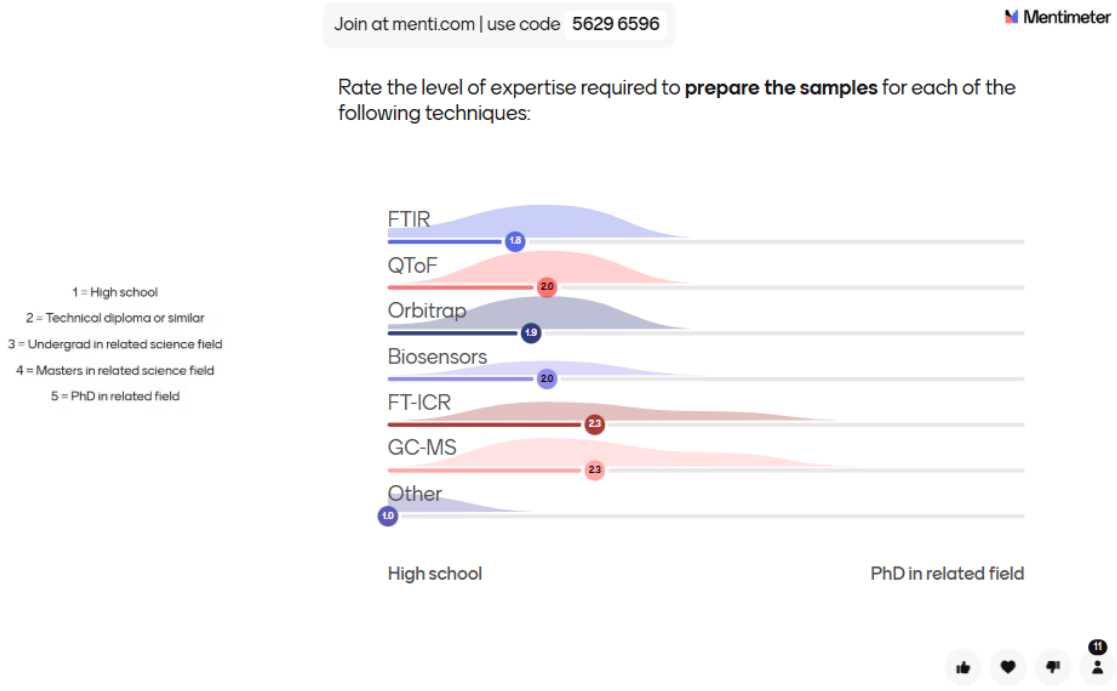
Mentimeter
Rate the level of expertise required to **run the instrument** from each of the following techniques:
1 = High school
2 = Technical diploma or similar
3 = Undergrad in related science field
4 = Masters in related science field
5 = PhD in related field
FTIR
1.9
QToF
3.1
Orbitrap
3.2
Biosensors
2.0
FT-ICR
3.8
GC-MS
3.2
Other
1.3
High school
PhD in related field
12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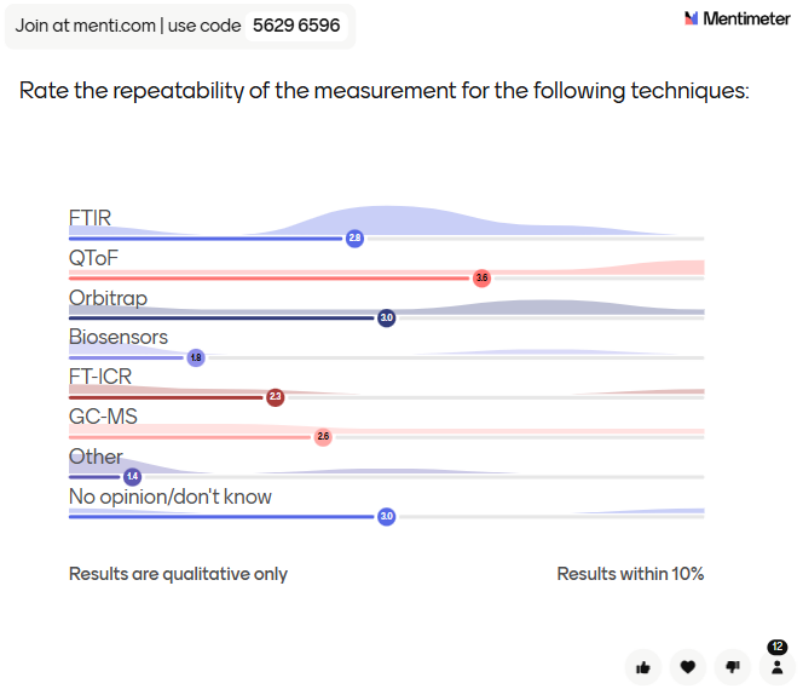
Mentimeter
Rate the level of expertise required to **prepare the samples** for each of the following techniques:
1 = High school
2 = Technical diploma or similar
3 = Undergrad in related science field
4 = Masters in related science field
5 = PhD in related field
FTIR
1.8
QToF
2.0
Orbitrap
1.9
Biosensors
2.0
FT-ICR
2.3
GC-MS
2.3
Other
1.0
High school
PhD in related field
11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Rate the repeatability of the measurement for the following techniques:
1 - Results are qualitative only (vary by >100%)
2 - Results vary by less than 100%
3 - Results vary within 50%
4 - Results within 25%
5 - Results within 10%
FTIR
QToF
Orbitrap
Biosensors
FT-ICR
GC-MS
Other
No opinion/don't know
Results are qualitative only
Results within 10%


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Mentimeter
What you think the detection limits are for each detector
FTIR
QToF
Orbitrap
Biosensors
FT-ICR
GC-MS
Other
No opinion/I don't know
g/L
ppb