DEVELOPMENT OF SUPERCRITICAL CO_2-BASED LIFE-DETECTION INSTRUMENTATION FOR ANALYSIS OF TRACE BIOMARKERS

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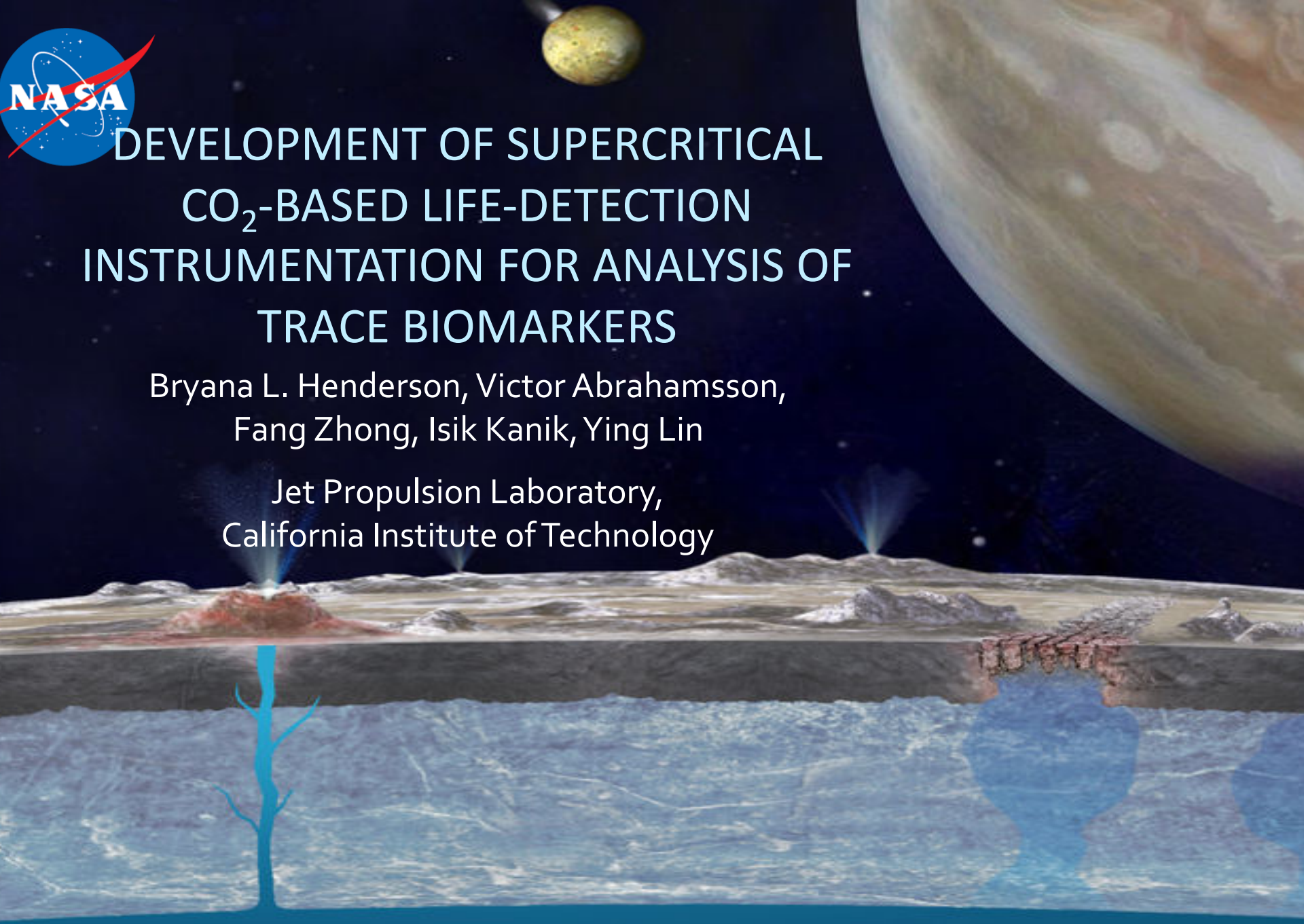

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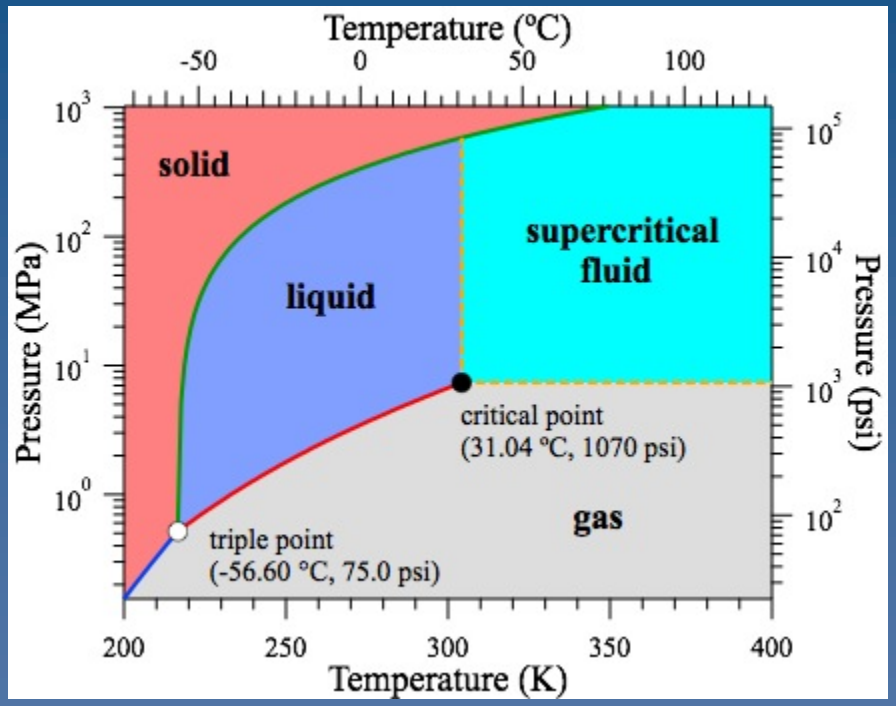
06/25/2019

Existing Risks in Organic Detection Instrumentation for Planetary Applications

- Contamination, degradation and Planetary Protection concerns:
  - Commonly-used methods like pyrolysis and GC-MS use high temperatures that cause degradation
  - Native salts cause interference in analysis and degradation reactions
  - GC-MS requires derivatization reagents if molecules aren't volatile enough or stable enough
  - Non-pyrolysis extractions and separations require organic solvent – and waste must be securely controlled
- Laser desorption (as used in MOMA's LD-MS) mitigates many of these issues -- no derivitization, no perchlorate interference -- but laser ionization can induce fragmentation

Unique Advantages of $scCO_2$ Extraction

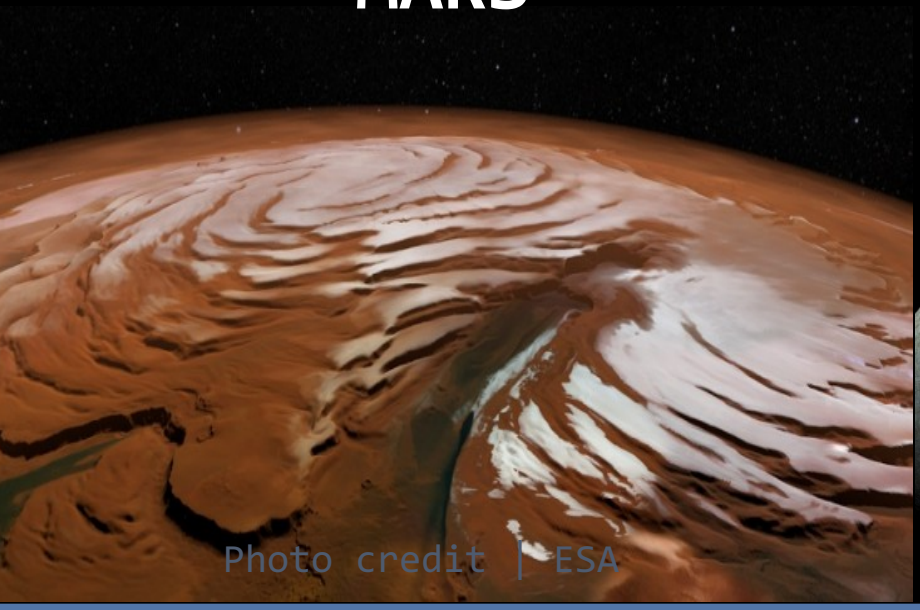


- Supercritical CO_2 ($scCO_2$) has the viscosity, permeability, and diffusivity of a gas but the solvating power of a liquid
- No organic solvents needed, and no derivatization

- CO_2 and H_2O are both inert and "green", and can typically be vented to atmosphere without risk of organic contamination of the target body (reducing planetary protection risks)

Inherent risks: sample site variability (ice, brines, salt, regolith); samples of low concentration and high complexity

MARS



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EUROPA

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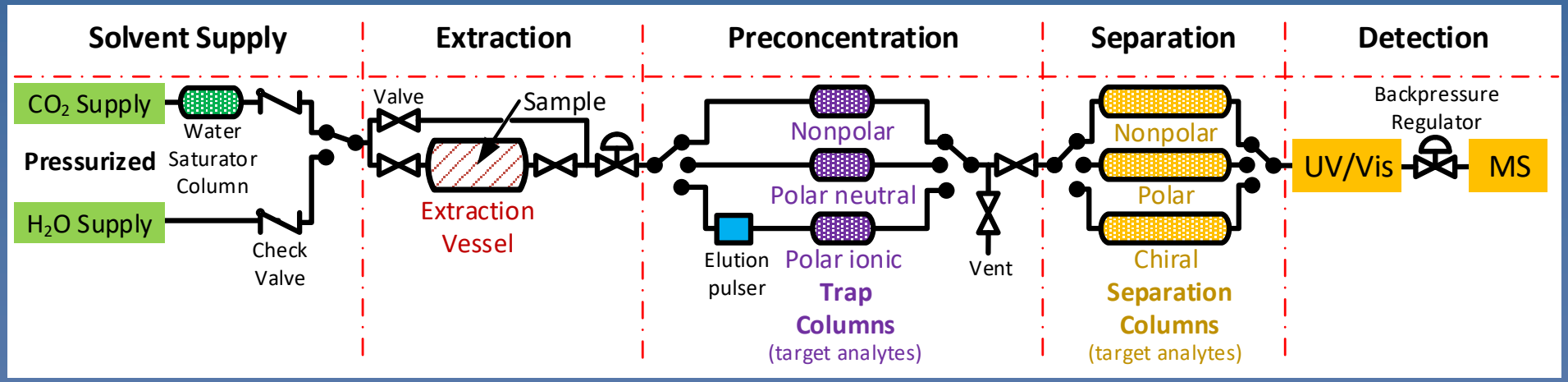
Any organic extraction instrument should be able to extract *a wide variety of species from a wide variety of materials* -- regardless of site location (salt content, water content, material type, etc.)

Our plan: develop an instrument to mitigate existing organic detection challenges for planetary exploration.

Challenges for Organic Detection in the Solar System		Technology Proposed to Mitigate These Challenges	Status of Improvements
1	Need to extract from *aqueous* and/or mixed/unknown materials	Use a ***support matrix*** for extraction from aqueous or mixed samples	Implemented
2	Native organics can be very *dilute (ppb or ppt)*	Use a trap to ***preconcentrate*** them into a small "plug" for analysis	Implemented
3	Mixtures of organics can be very *complex*	Develop an end-to-end system with supercritical CO2 chromatography to ***separate*** these species for analysis	Implemented
4	Salt can lead to *reduced sensitivity* in a mass spectrometer	***Minimize salt transfer to the detector by using a trap*** to attract polar molecules. [scCO2 extractions are unaffected by salt.]	In Progress

Extraction of complex organics from aqueous samples for Ocean Worlds applications

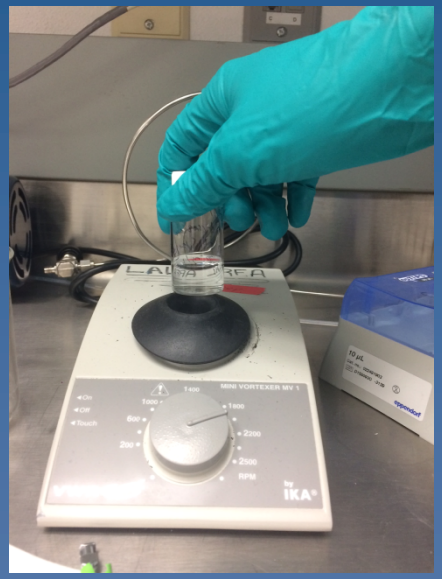
- Sample (aqueous, solid or mixed) is manually placed in extraction vessel (sample carousel could be used in future)
- After sample is placed in vessel, everything is on-line and automated using benchtop equipment
- Chemical analysis with CO_2 and water is done <u>sequentially</u>
- Supercritical CO_2 is used for nonpolar analytes; water is used for polar analytes



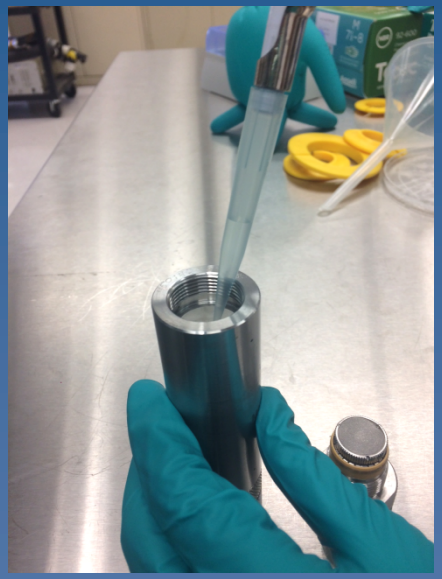
1. Enabling Extraction from Aqueous Samples

We incorporate diatomaceous earth (mainly silica, cleaned and QA tested for bioanalytical applications) for “supported liquid extraction” of the aqueous (or mixed aqueous) samples. Our support material can hold an aqueous sample of nearly twice its weight.

Fill 10 mL extraction column with sorbent material (approx. 4 g)



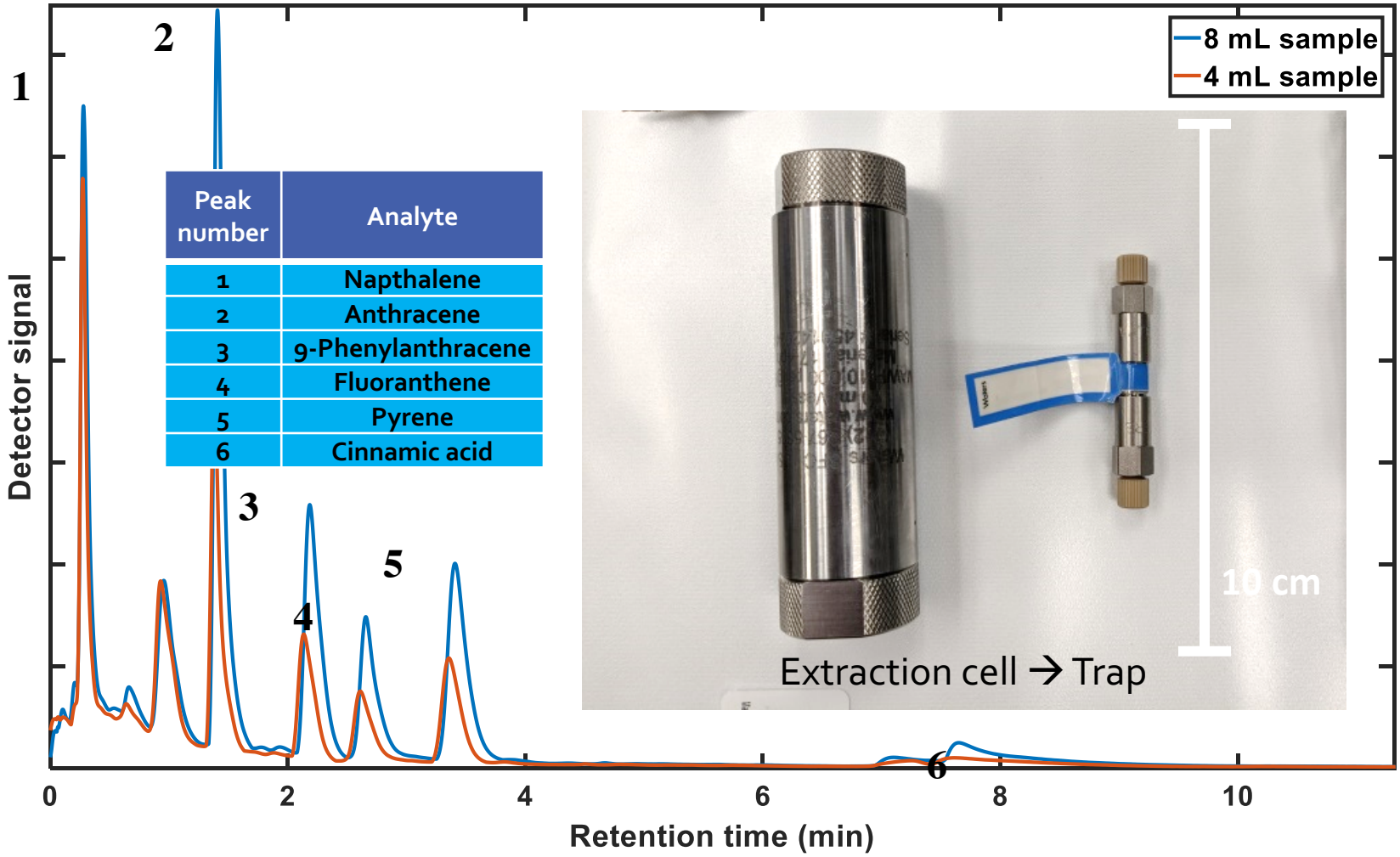
Use a mixture of standards to spike the aqueous samples



Load column with 4 mL spiked water.

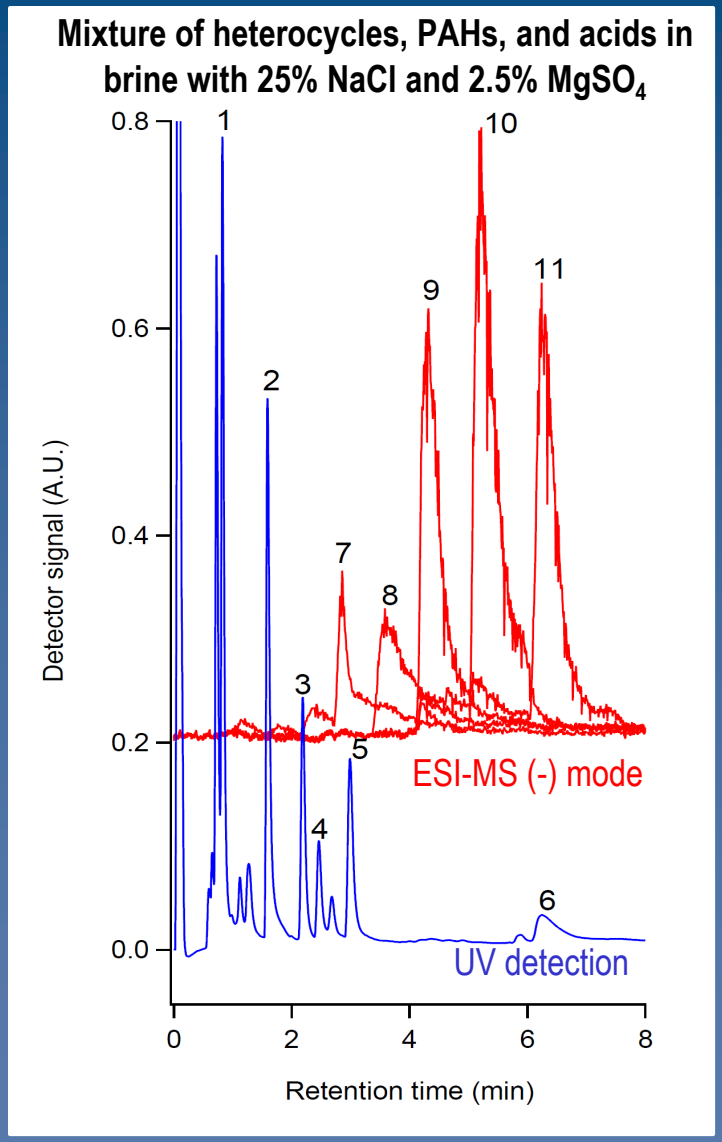
Perform supported extraction
- 10 min equilibration/ static extraction
- 30 min dynamic extraction

2. Improved Sensitivity by Preconcentration



Peak number	Analyte
1	Napthalene
2	Anthracene
3	9-Phenylanthracene
4	Fluoranthene
5	Pyrene
6	Cinnamic acid

3. Separation of Organics: End-to-End Demonstration



Peak number	Analyte	LOD (in ppb)	Recovery from brines with 27.5% salt (=measured amt./doped amt.)	RSD retention time
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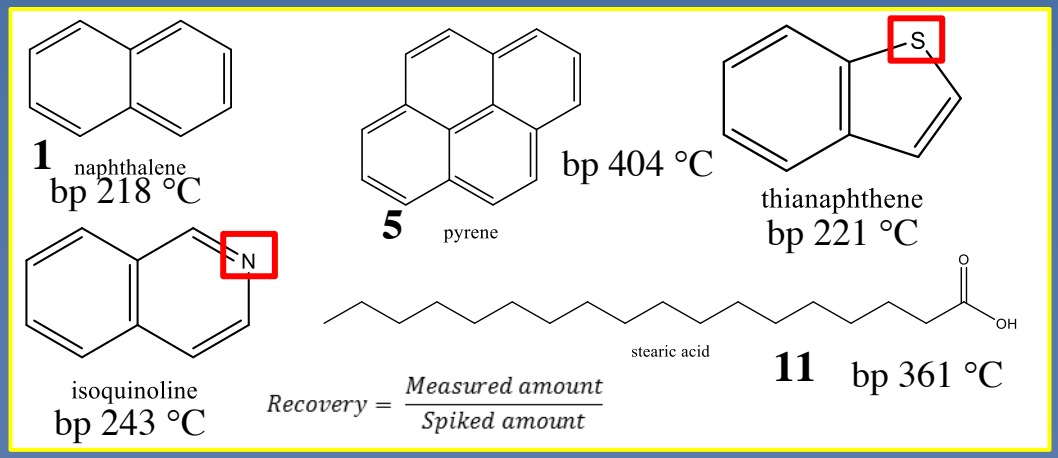
6	Cinnamic acid	3 ppb	92% (10 ppb)	0.8%
	Thianaphthene	3 ppb	52% (7.5 ppb)	2%
	Dibenzothiophene	0.6 ppb	71% (3 ppb)	1%
	Indole	1 ppb	73% (5 ppb)	2%
7	Capric acid (C10)	40 ppb*	100% (100 ppb)	1%
8	Lauric acid (C12)	30 ppb*	109% (100 ppb)	1%
9	Myristic acid (C14)	20 ppb*	90% (50 ppb)	0.8%
10	Palmitic acid (C16)	20 ppb*	105% (50 ppb)	1%
11	Stearic acid (C18)	15 ppb*	68% (50 ppb)	1%
	Isoquinoline	0.02 ppb	60% (0.05 ppb)	2%
	Acridine	0.03 ppb	90% (0.05 ppb)	2%

* analytes are not ideal for ESI-MS detection



$$Recovery = \frac{Measured\ amount}{Spiked\ amount}$$

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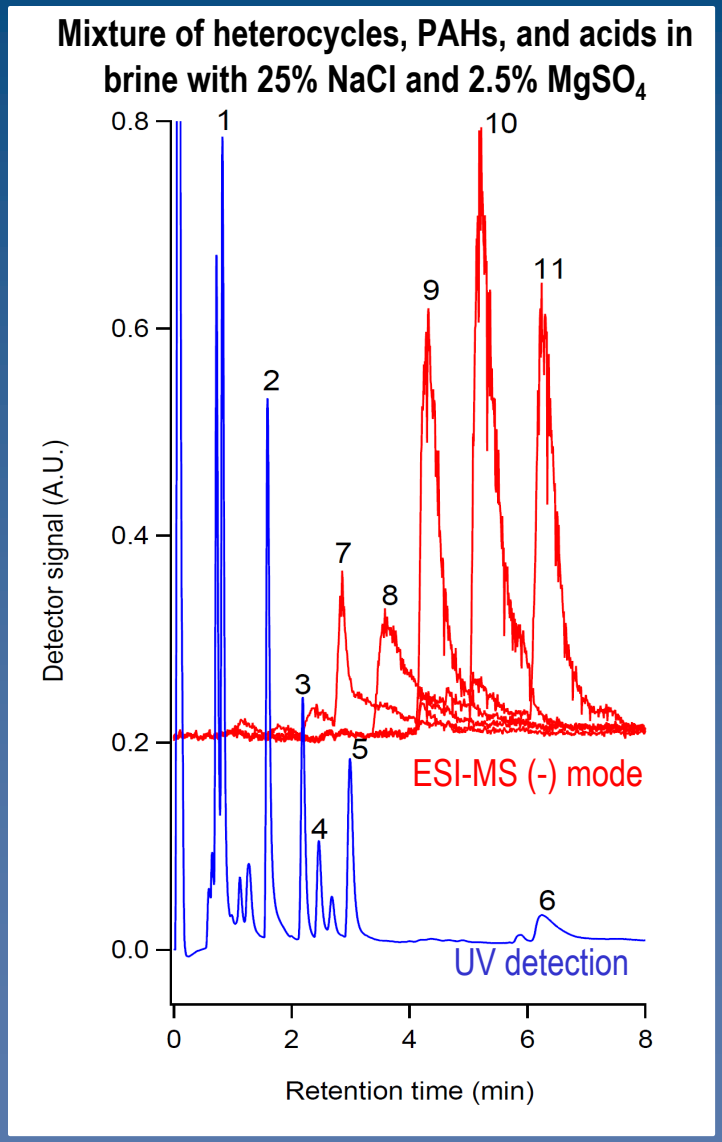

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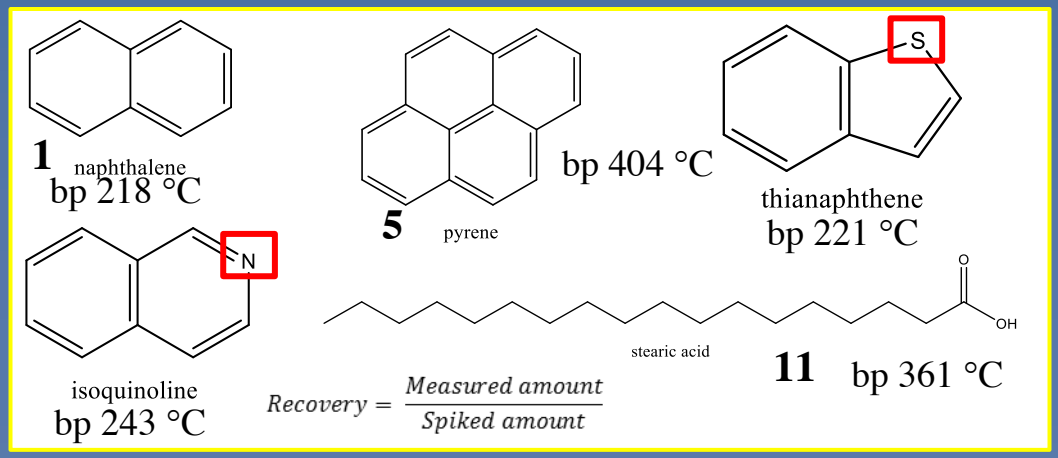

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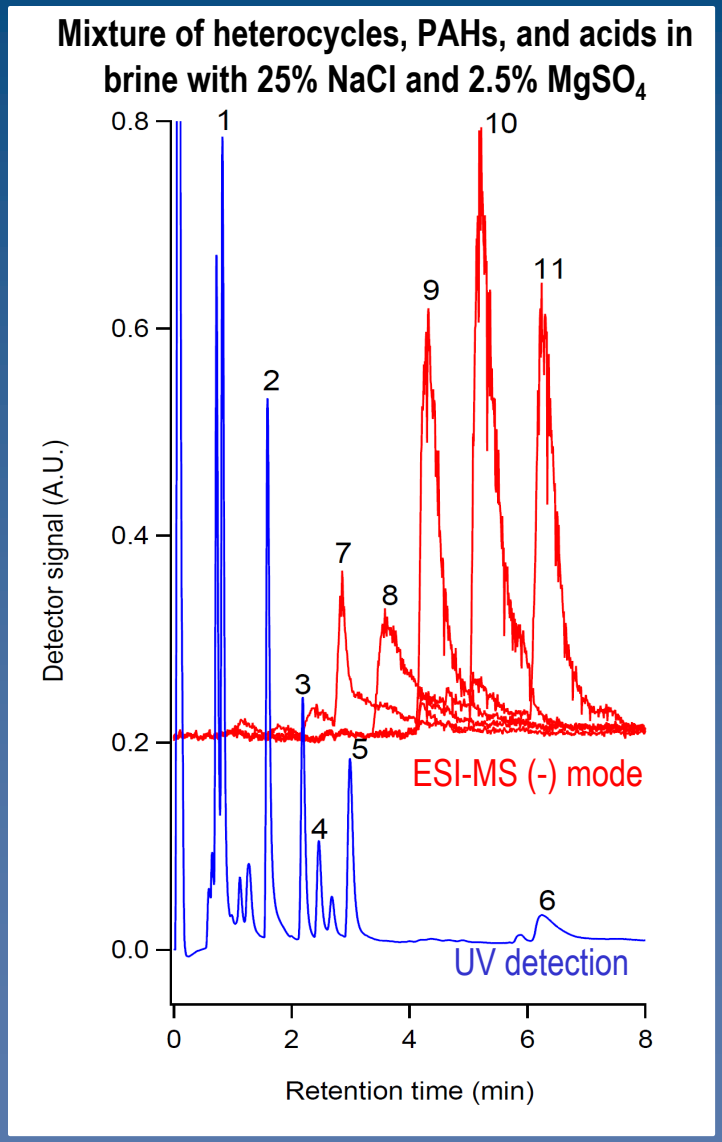

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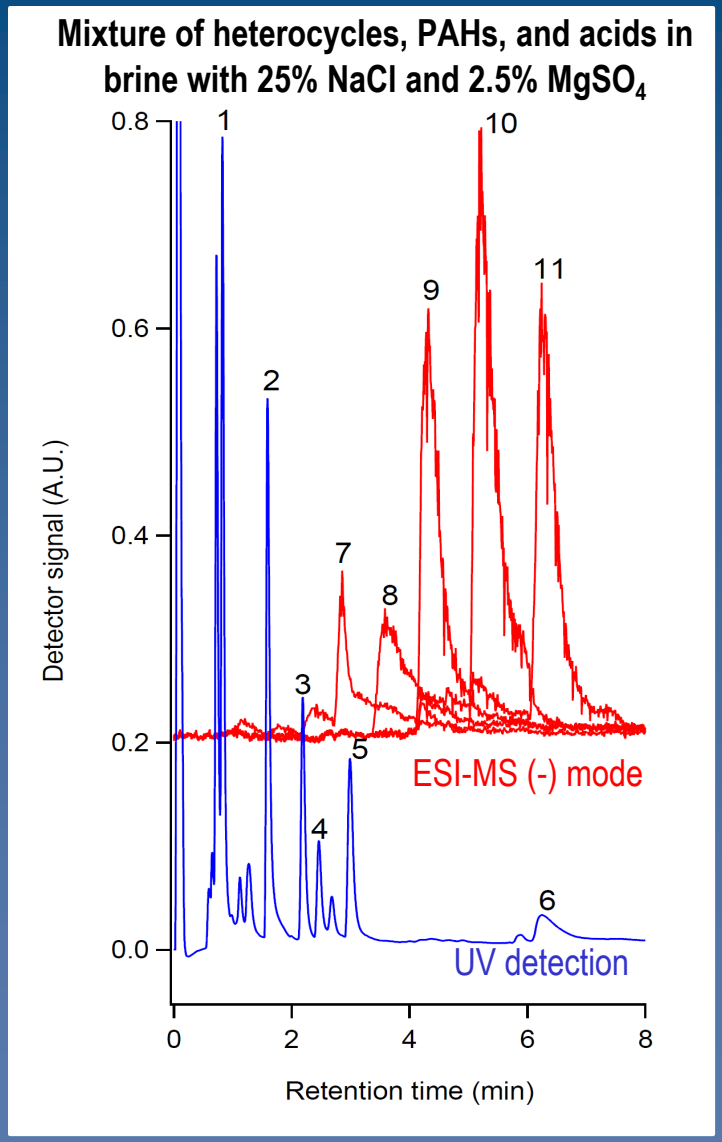
For comparison: the SAM instrument's recovery for pyrene from quartz is 10% (same as for pyrene in an empty oven with no matrix); Stalport et al. 2012

3. Separation of Organics: End-to-End Demonstration



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A reliable retention time (i.e. an analyte always appearing at the same time in the chromatogram) is extremely important for identification of unknowns in complex samples.
Also important : multiple dimensions of selectivity (chromatography PLUS mass spectrometry)

3. Separation of Organics: End-to-End Demonstration

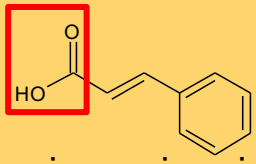
So far, we have successfully analyzed:

- Astrobiologically relevant species
- Other species as needed to strategically test the limits of our instrument (high and low volatility, different functional groups, etc.)

We have successfully recovered analytes (DGs and β-carotene) that cannot be analyzed through existing state-of-the-art GC-MS instruments. We extract even large, complex species while using much lower temperatures.

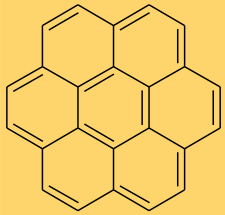
Free fatty acids (FFAs)

palmitic acid

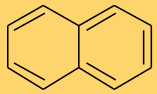


cinnamic acid

Polycyclic aromatic hydrocarbons (PAHs)

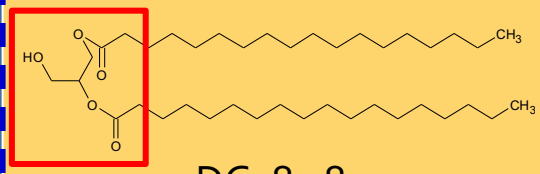


coronene



naphthalene

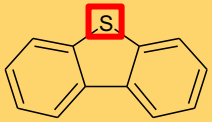
Acylglycerols



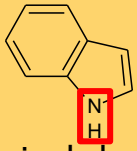
DG18:18

Polycyclic aromatic compounds (PACs)

Neutral

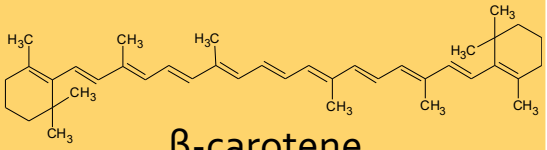


dibenzothiophene



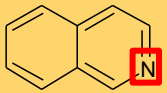
indole

Pigments



β-carotene

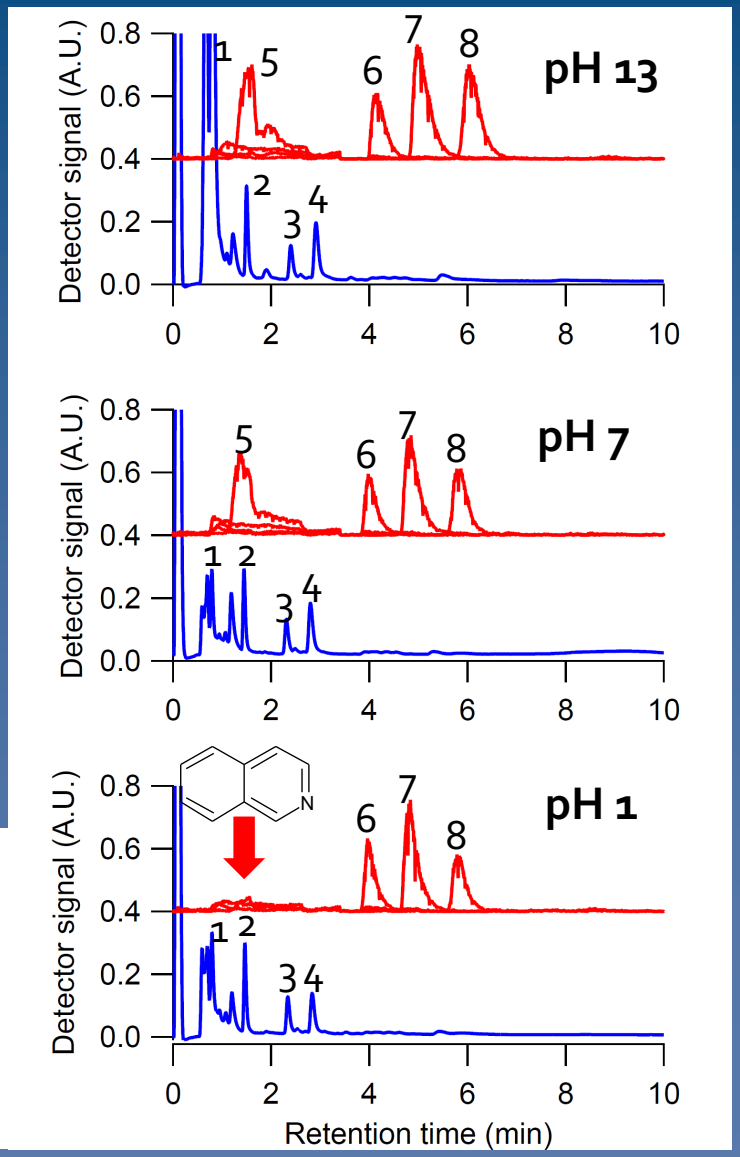
Basic



isoquinoline

Demonstration: extreme pH

- The method performs well even at extreme pH (1 or 13) of aqueous samples with ppb levels of analytes
- However, pH does affect the charge of a few ionizable analytes (this was expected)
  - Trueness for these ionizable analytes is poor for bases at low pH due to added charge of the molecule



UV	MS
1. Naphthalene	5. Isoquinoline
2. Dibenzothiophene	6. Myristic acid
3. Fluoranthene	7. Palmitic acid
4. Pyrene	8. Stearic acid

Conclusions

Our end-to-end supercritical CO_2 extraction instrumentation improves upon current organic detection instrumentation by offering:

- Extraction from aqueous and/or solid materials at 40 °C -- no organic solvents, no derivatization needed
- Wide range of nonpolars detected at LODs of parts per billion to parts per trillion. Can run with a larger volume with preconcentration to improve sensitivity
- No salt effects with nonpolar extractions, currently working on polar trapping options to minimize salt transfer.
- Can be used with a variety of detector types, compatible with chiral analysis.

Next steps for this project:

- Refine the method for extraction of polar species (amino acids, nucleobases)
- Incorporate chiral chromatography for analysis of amino acid enantiomers.

Acknowledgements

Special thanks to Victor Abrahamsson, and the rest of the team.

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Backup Slides

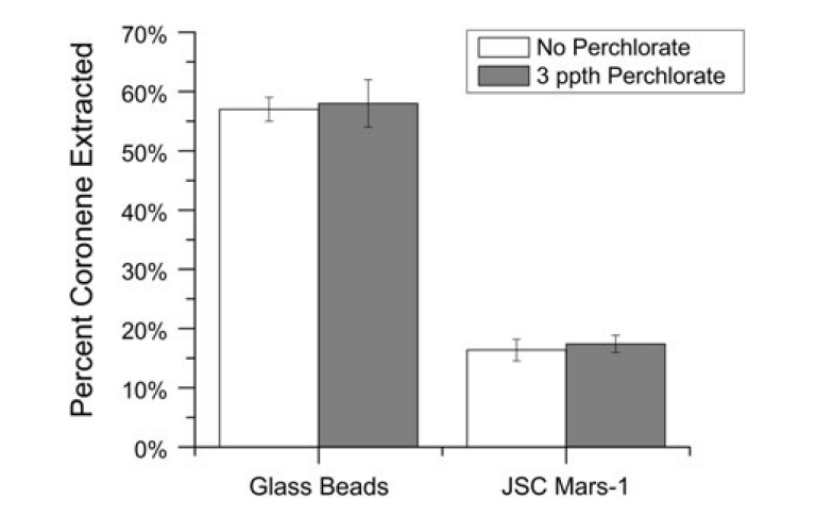
Organic Detection Instrumentation: Mars

- Viking and Curiosity have reported chlorinated species with gas chromatography – mass spectrometry (GC-MS). It is hypothesized that these are due to reactions with perchlorates at ppth levels that oxidize and destroy native organics at high pyrolysis/GC-MS temperatures
- SAM's GC-MS and evolved gas analyzer (EGA) have more recently discovered thiophenes, aromatics and aliphatics released at 500-820 °C, likely evolved species from degradation of larger molecular structures.

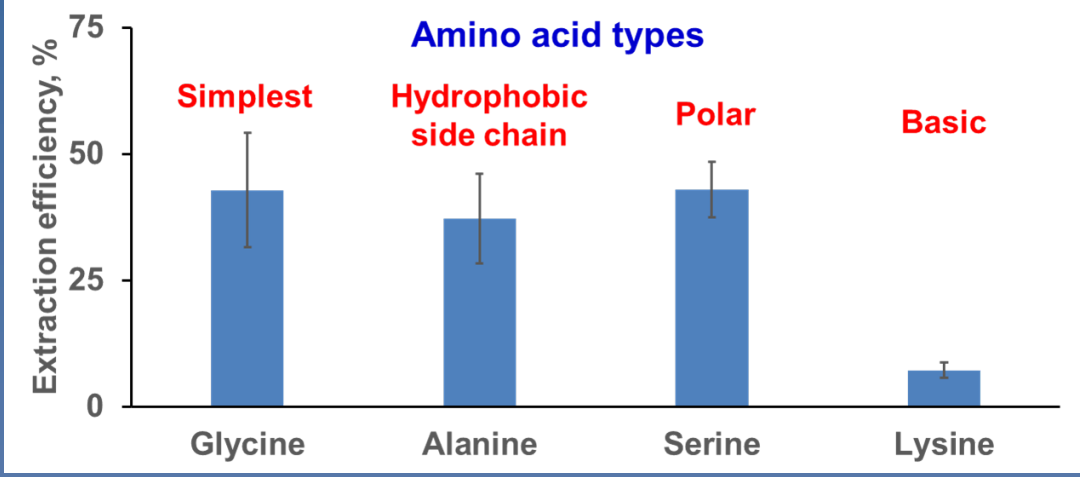
Eigenbrode, Jennifer L., et al. "Organic matter preserved in 3-billion-year-old mudstones at Gale crater, Mars." *Science* 360.6393 (2018): 1096-1101.

Extraction of Organics with Our Instrumentation

We've successfully extracted a wide range of nonpolar species, including PAHs, PANHs, fatty acids, and thiophene derivatives with pure $scCO_2$, **at low temperatures, with no organic solvents and with no salt interference.**



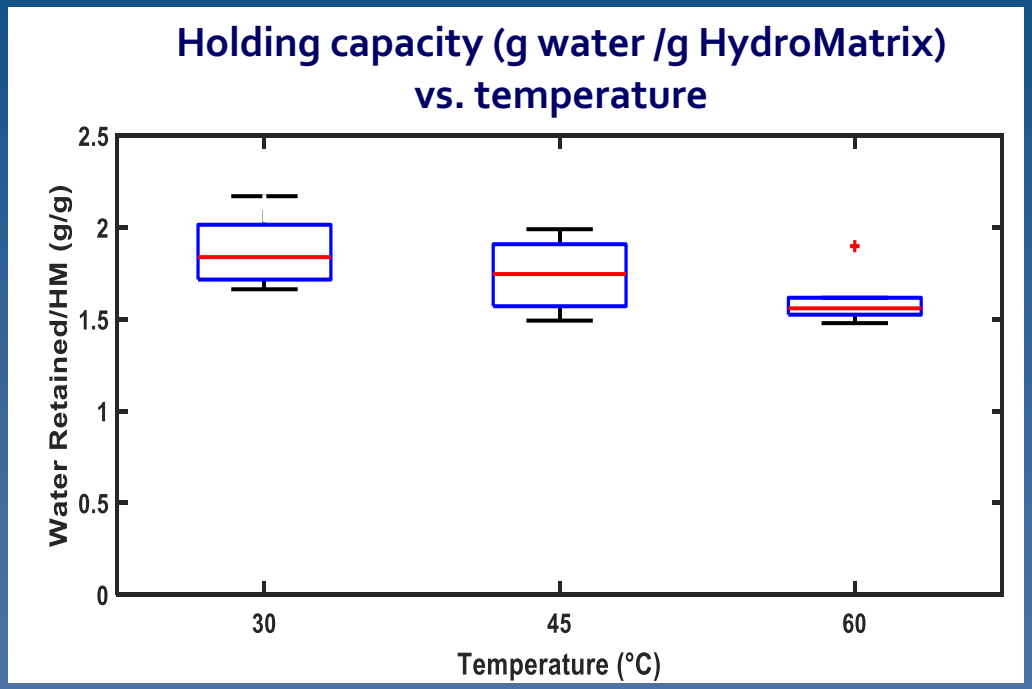
We've also found that polar species such as amino acids can be extracted from soil simulants by adding small amounts (<5%) of water during the $scCO_2$ extraction.



McCaig, Heather C., et al. "Supercritical Carbon Dioxide Extraction of Coronene in the Presence of Perchlorate for In Situ Chemical Analysis of Martian Regolith." *Astrobiology* 16.9 (2016): 703-714.
Menlyadiev, Marlen, et al. "Extraction of amino acids using supercritical carbon dioxide for in situ astrobiological applications." *International Journal of Astrobiology* (2018): 1-10.

1. Enabling Extraction from Aqueous Samples

- Our support material can hold an aqueous sample of nearly twice its weight.
- The holding capacity decreases slightly as temperature increases.



	Conditions evaluated	p-Value	Significant (α>0.05)
Pressure	15, 20, 25 MPa	0.200	No
Temp	30, 45, 60 °C	0.009	Yes
Amount CO_2	10, 15, 20 g	0.990	No
Time	5, 12.5, 20 min	0.167	No
No. of experiments	27 (fractional design of experiments)		

Left: Statistical results of factor screening. Temperature is the only variable with a significant influence ($p < 0.01$) on the amount of water retained by the support material. Variation in pressure, CO_2 amount, and extraction time did not show a significant influence ($p > 0.05$).

Evaluation of limitations based on 29 analytes

Property	Range evaluated	Limitations
logP (hydrophobicity)	2.1 – 16 (Isoquinoline – DG(18:18))	• No experimentally observed lower or upper limit
Boiling point (volatility)	218 – 662 °C (Naphthalene – DG(18:18))	• Lowest evaluated (218 °C): Good performance at trapping temperatures ≤25 °C • No experimentally observed upper limit yet
Functional groups	Carboxylic acid, hydroxyl, amine, phosphate, phosphocholine	• PAHs (large system of conjugated double bonds) that contain carboxylic acid or hydroxyl groups adsorb strongly to trap & chromatography column (requires methanol to desorb) • Ionic functional groups (phosphate & phosphocholine) are repelled by the CO_2 phase (but methanol clusters around these to enable analysis)
Analyte class	Polycyclic aromatic hydrocarbons, heterocycles (nitrogen and sulfur), carboxylic acids, pigments, free fatty acids, diacylglycerides, phospholipids	• See above.

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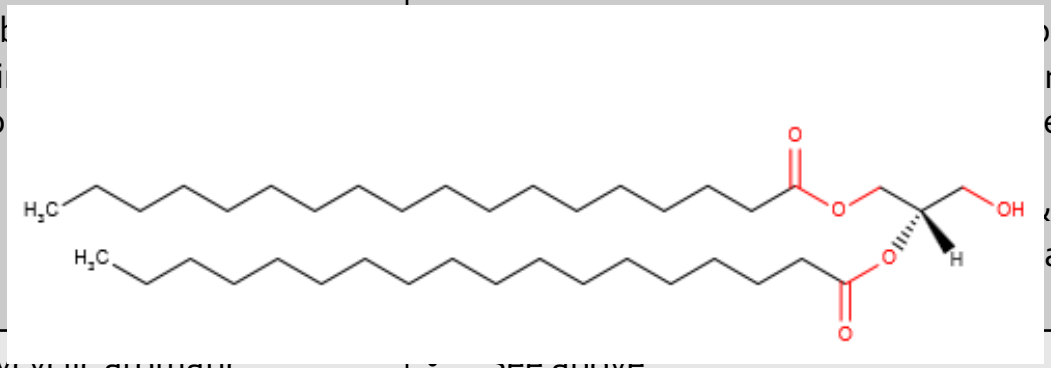
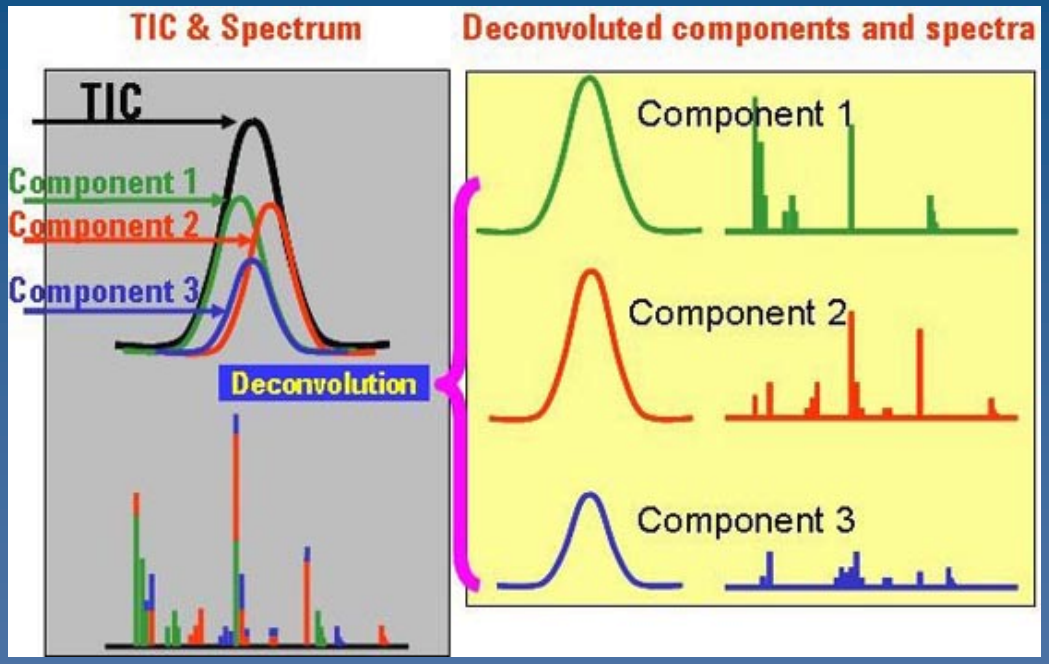
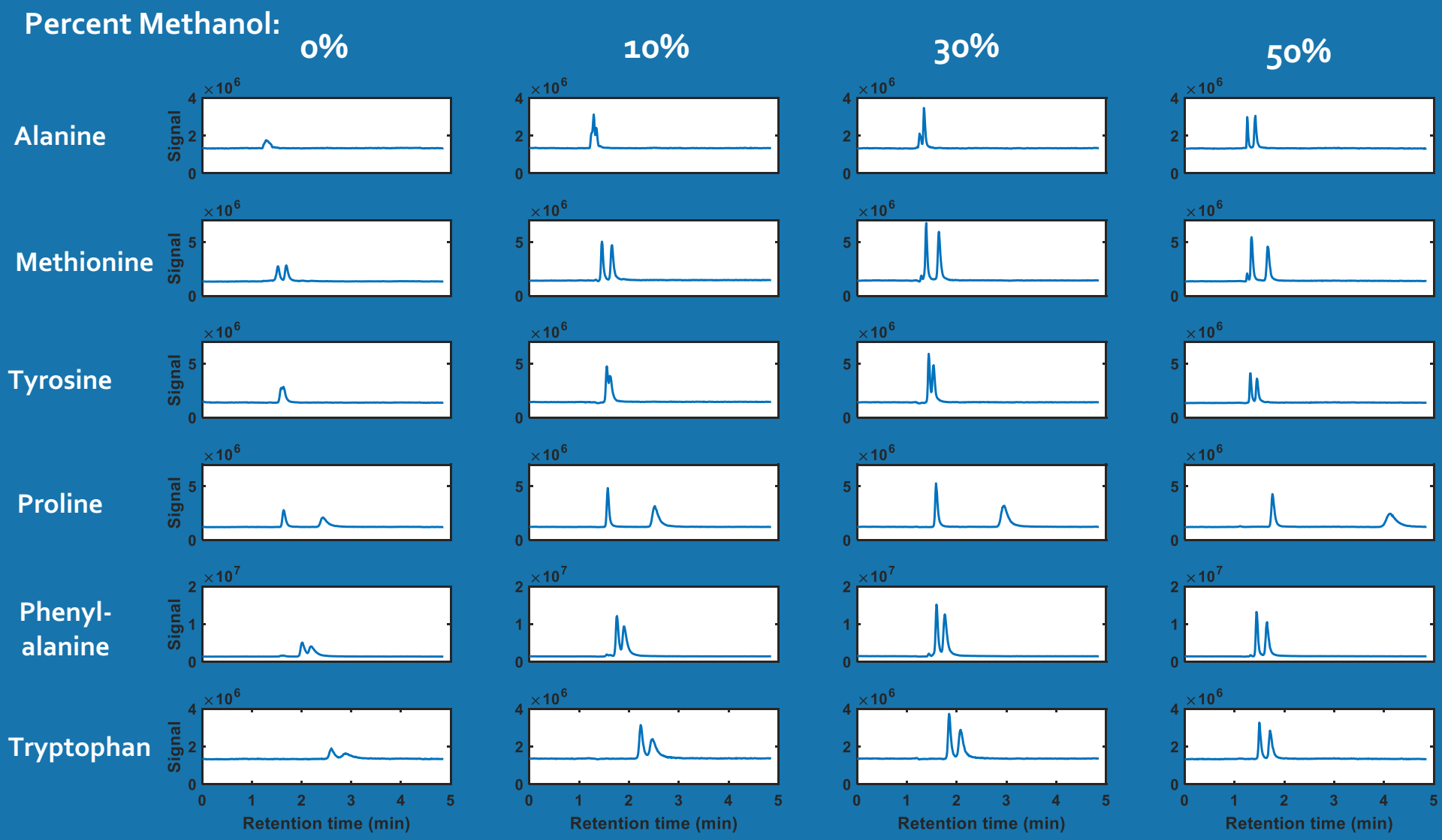


How to extract overlapping peaks

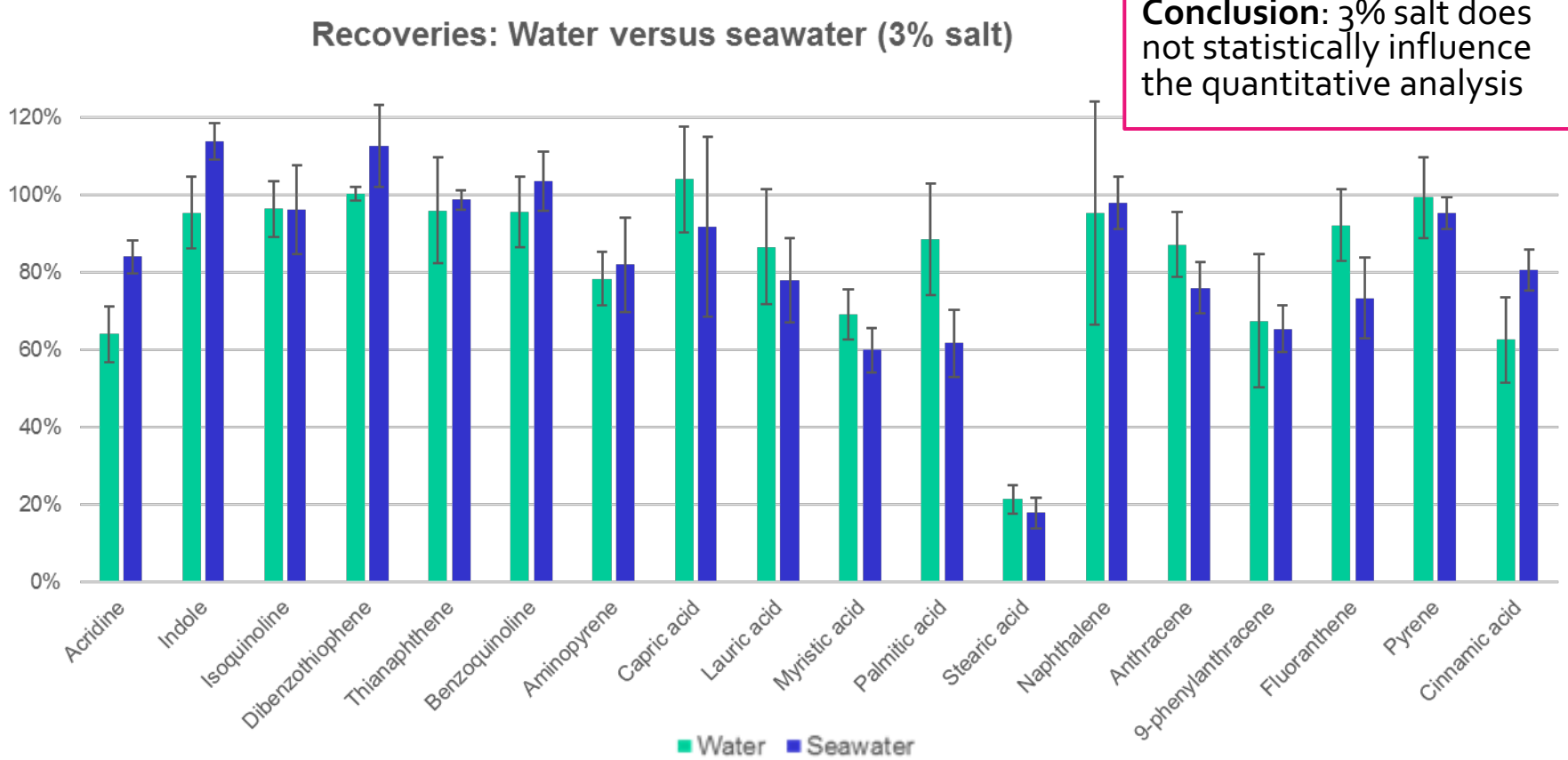


For a truly complex (>1000 analytes) sample, there's no single technique available today that will give baseline separation of that many species. Because of this, we choose to use a hyphenated technique – where peaks can be effectively deconvoluted unless they are exactly on top of one another (see figure). Unambiguous characterization of the composition and structure of an analyte is obtained through comparing the peak's elution time with its characteristic mass spectrum fingerprint.

Chiral separation (LC-MS)



3. Separation of Organics: End-to-End Demonstration

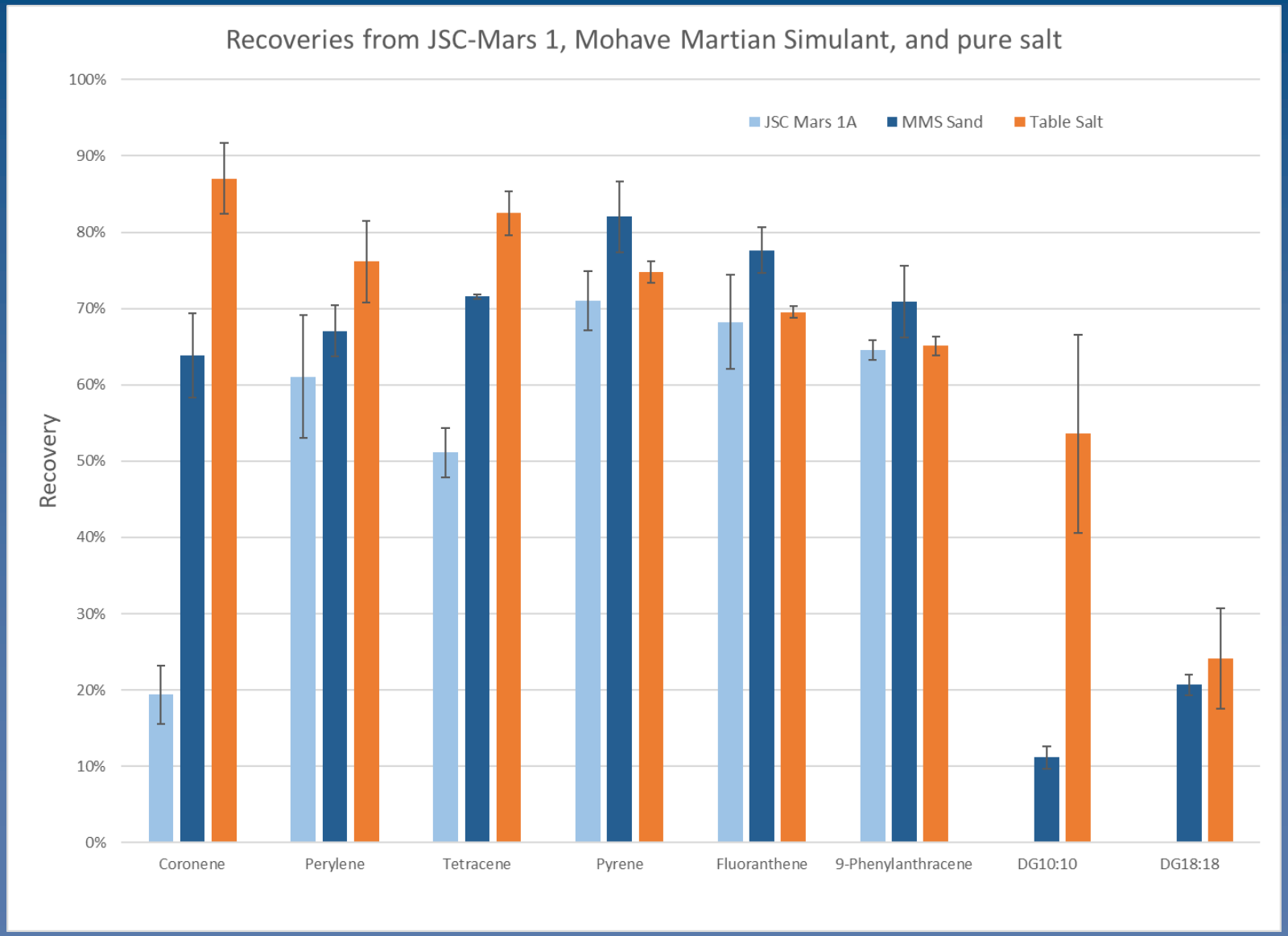


Conclusion: 3% salt does not statistically influence the quantitative analysis

Water and seawater samples spiked with 45-80 ppb for each PAH/heterocycle and 220-450 ppb of each FFA (n=3).

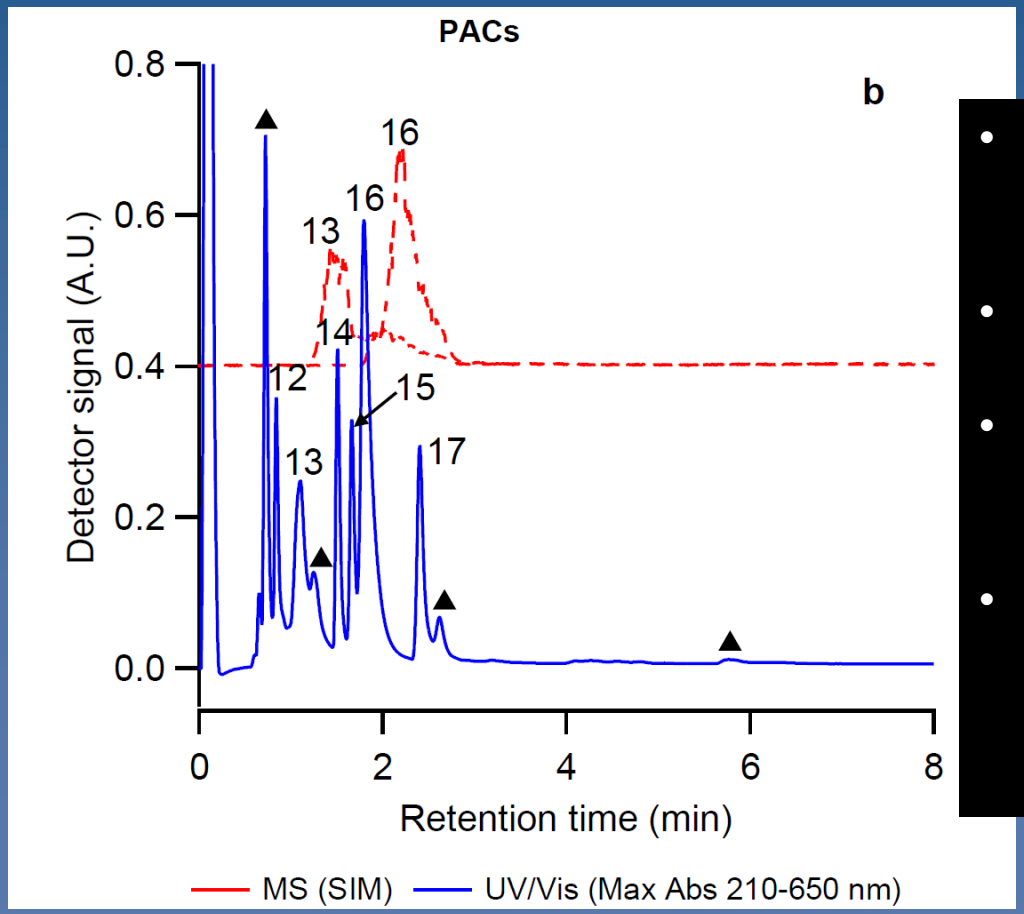
No salt effects seen with extractions of nonpolar molecules.
Currently working on trapping options for polar molecules to minimize salt transfer.

Effect of sample matrix on recovery



SFE-SFC-MS

Not included in COLDTech



- The MS peaks should be as narrow as the DAD peaks!
- Concentrations in DAD trace = **~1 ppb**
- Concentrations in MS trace = **~20 ppt**

- **We think we have a solution for getting the best of both worlds**