



Presentation

Determination of Hydroxylated PAHs in Marine Sediments and Fish Bile by LC-MS/MS

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- **Aim**
  - To measure 9 hydroxylated polycyclic aromatic hydrocarbons in sediments and fish bile.
  - These data would be used to identify sources of contamination to the marine environment.
- **Challenges**
  - Chromatography – Congeners of hydroxylated PAHs are difficult to resolve on a chromatographic column.
  - Lack of Analytical Standards – The list of available analytical standards does not match the list of environmental contaminants found in affected environments. This is particularly true for conjugated hydroxylated PAHs.
  - Cleanup – Bile extracts are matrix intensive and require aggressive cleanup.
- **Methods**
  - Solvent extraction with sonication
  - Solid phase clean up
  - LC-ESI-MS/MS

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PAHs, OH-PAHs, and Conjugated OH-PAHs

- **Sources**
  - PAHs are commonly found in oils and oil products, and are generated through the combustion of almost any organic material, including coal and wood.
  - Patterns in the abundance of the various molecules can be linked to the products used in combustion, thus helping to identify potential sources.
- **Sinks**
  - PAHs are relatively water insoluble and readily partition to sediments.
  - From both sediment and water, PAHs are ingested by fish directly or from through bioaccumulation.
  - PAHs are rapidly metabolized to form hydroxylated PAHs (OH-PAHs). This is phase I metabolism and occurs in the liver.
  - Hydroxylated PAHs are then conjugated to make the xenobiotic more water soluble and more easily excreted. This is phase II metabolism. Studies have shown that glucuronide conjugates are the major phase II metabolites for OH-PAHs

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Hydroxylated PAHs and Conjugates Investigated

Analyte	CAS #	Abbreviation
1-Hydroxynaphthalene	90-15-3	1-OH-Nap
2-Hydroxynaphthalene	135-19-3	2-OH-Nap
1-Hydroxypyrene	5315-79-7	1-OH-Pyr
2-Hydroxyfluorene	2443-58-5	2-OH-Flu
1-Hydroxyphenanthrene	2433-56-9	1-OH-Phe
2-Hydroxyphenanthrene	605-55-0	2-OH-Phe
3-Hydroxyphenanthrene	922510-20-1	3-OH-Phe
4-Hydroxyphenanthrene	7651-86-7	4-OH-Phe
9-Hydroxyphenanthrene	484-17-3	9-OH-Phe
1-Hydroxynaphthalene glucuronide	17238-47-0	1-OH-Nap-Glu
1-Hydroxypyrene glucuronide	154717-05-2	1-OH-Pyr-Glu
1-Hydroxypyrene-d9 (Internal Standard)	132603-37-3	1-OH-Pyr-d9
1-Hydroxynaphthalene-d7 (surrogate)	124251-84-9	1-OH-Nap-d7

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Determination of OH-PAHs in Sediments and Bile

- Sediment Sample extraction
  - A 1 g sample is weighed into an amber glass jar and spiked with surrogates
  - Methanol is added
  - The samples are sonicated then centrifuged.
- Sediment Sample extraction
  - A 0.1 g sample is weighed into a centrifuge tube and spiked with surrogates
  - Acidified acetonitrile is added
  - The samples are vortexed then centrifuged.
- Sample clean-up
  - An aliquot is transferred to a culture tube and diluted 9:1 with DI water
  - This solution is extracted using either Waters Oasis HLB or Phenomenex Strata-X
  - The cartridge is eluted with 1 mL methanol and instrument IS is added.
  - 1 mL of this extract is filtered using 0.45µM PTFE filter

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Determination of OH-PAHs in Sediments and Bile

- Chromatography
  - Chromatography is preformed using a Shimadzu Prominence UPLC
  - 10 µL is injected onto a column from Phenomenex. The column was held at 65°C
  - 5mM ammonium acetate, methanol and acetonitrile are used as mobile phases
  - Run times were 10 min
- Mass Spectrometry
  - Mass Spectrometry was performed with an AB Sciex API 5000
  - The source was operated in ESI negative mode
  - The source temperature was kept at 650°C.

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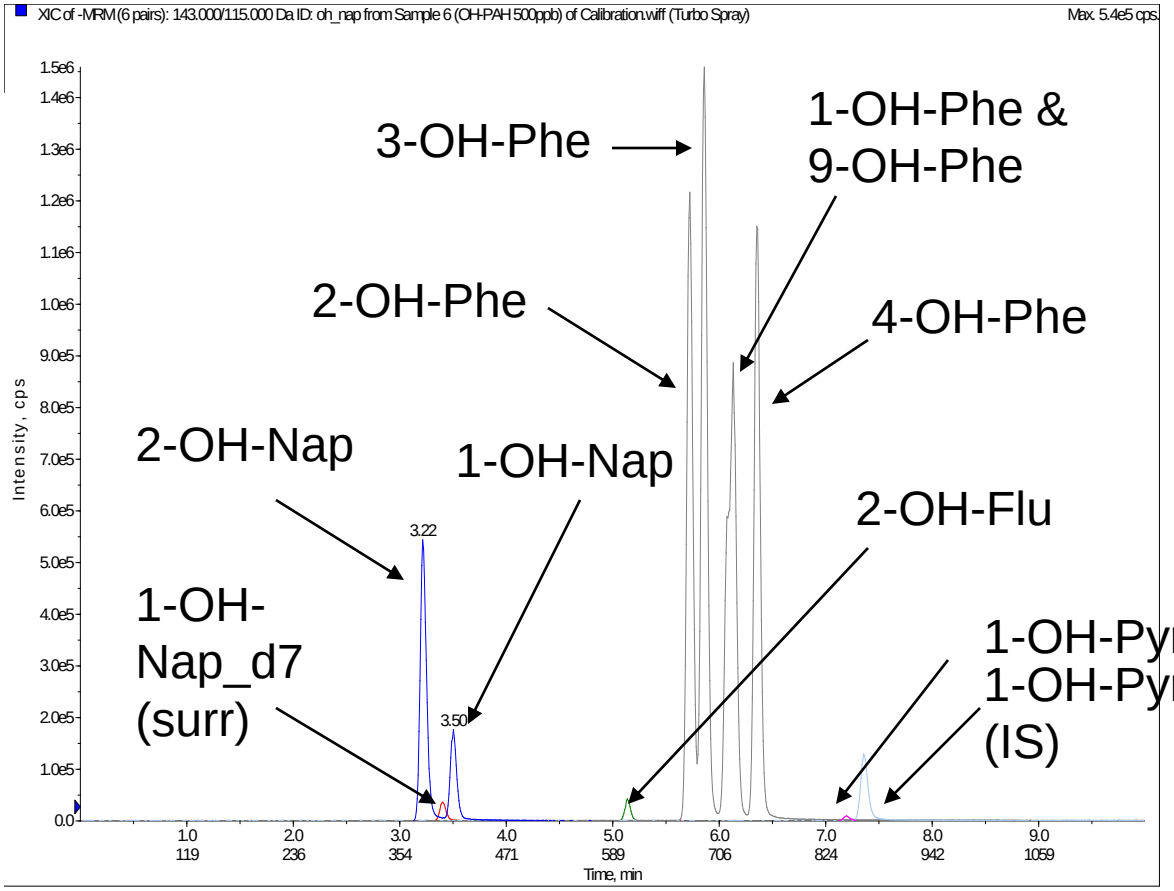
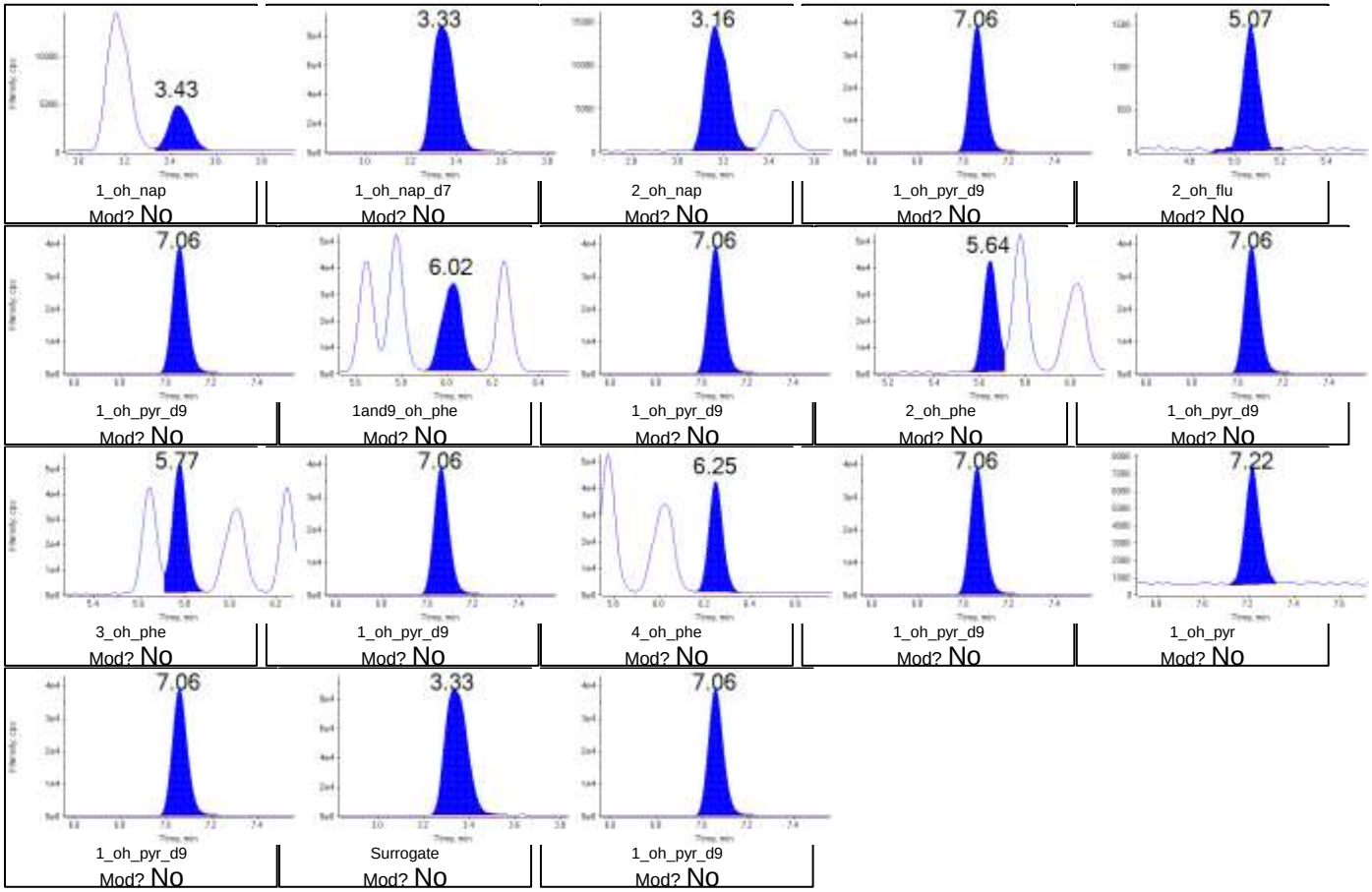
Total Ion Chromatograms



Extracted SRM Chromatograms

Quantitative Peak Review **OH-PAH 5ppb**



? Before **?** After

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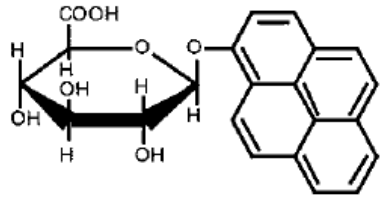
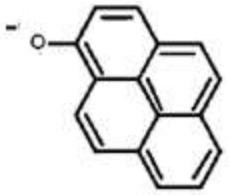
Predictive determination of Hydroxylated PAH conjugates

- Currently we only have conjugate standards for 1-hydroxynaphthalene glucuronide and 1-hydroxypyrene glucuronide
- The parent ion of the glucuronide conjugate is the parent ion OH-PAH + m/z 176
- The daughter ion of the conjugate is the parent ion of the OH-PAH
- This is true for both 1-OH-Pyr-Glu and 1-OH-Nap-Glu
- Therefore we predict this will be true for all OH-PAH-Glu. That is that the collision cell deconjugates the OH-PAH
- Conjugation shifts the retention of the OH-PAHs in a predictable manner



1-hydroxypyrene glucuronide

CID



1-hydroxypyrene

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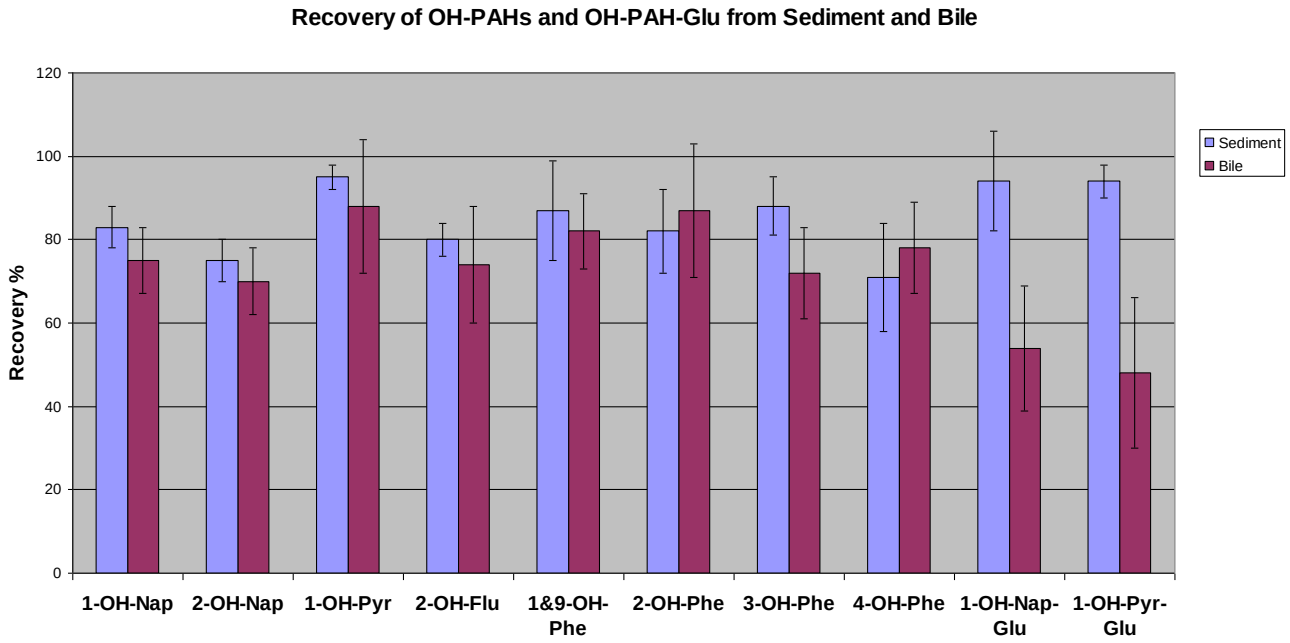
Results

- All OH-PAHs and OH-PAH-Glucuronides were recovered from sediment and bile at >40%.



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Future Work

- Significant ionization suppression of the instrument IS was observed. A more aggressive cleanup should be investigated.
- Find more environmentally relevant analytical standards e.g. Hydroxychrysene and Hydroxybenzo(a)pyrene
- Find more conjugated OH-PAH analytical standards.
- Obtain BCR-720 (sediment-exposed flounder bile) and/or BCR-721 (oil-exposed plaice bile)
- Alternatively find some fish bile from a PAH contaminated site
- Better resolution of hydroxylated phenanthrenes may be possible under altenative chromatographic conditions.
- Ion ratio confirmation could be possible by identifying second daughter ions for each target analyte.

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Conclusions

- LCMSMS can measure hydroxylated PAHs and conjugated hydroxylated PAHs in sediments and fish bile.
- While not ideal, we may be able to qualitatively measure hydroxylated PAH metabolites without matching analytical standards.

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Thank you

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