Optimal Quantitative Analysis for Urinary Biomarkers of Exposure of Polycyclic Aromatic Hydrocarbons that Vary in Ring Number: 1-Hydroxypyrene (1-OHP), 3-Hydroxyphenanthrene (3-OHPh), and 2-Hydroxy- Benz[c]phenanthrene (2-OHBcPh)

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Goal of the Study

Critically evaluate and unify all phases of the analytical process for analysis of widely different PAH metabolites with emphasis on both the number of samples and quantitative results.

Hydroxy Polyaromatic Hydrocarbons

- Considered to be bio-indicators of exposure to total PAHs
- Assay of urinary 3-OHBaP has been suggested to assess PAH carcinogenic risk, but there are 11 other structural isomers and concentration is low
- 1-OHP proposed as a bio-indicator of exposure to PAH
- Assumes concentration ratio of 1-OHP and other OHPAHs neither varies with time or source
- Other urinary biomarkers should be studied to truly assess PAH exposure

Analytical Background (Assay of OHPAHs)

- More than 40 reports during the past 10 years

  seldom quantitative recovery/high precision

- Pre-derivatization HRGC vs. HPLC

  100% yield required/aqueous matrix

- Sample clean up via solid phase extraction

  no urinary clean up step is 100% effective

- Mass spectrometric vs. fluorescence detection

  MS/MS is more selective/fluorescence is more sensitive

- Standardization: external vs. internal

  no universal standard/desirable standards not always available

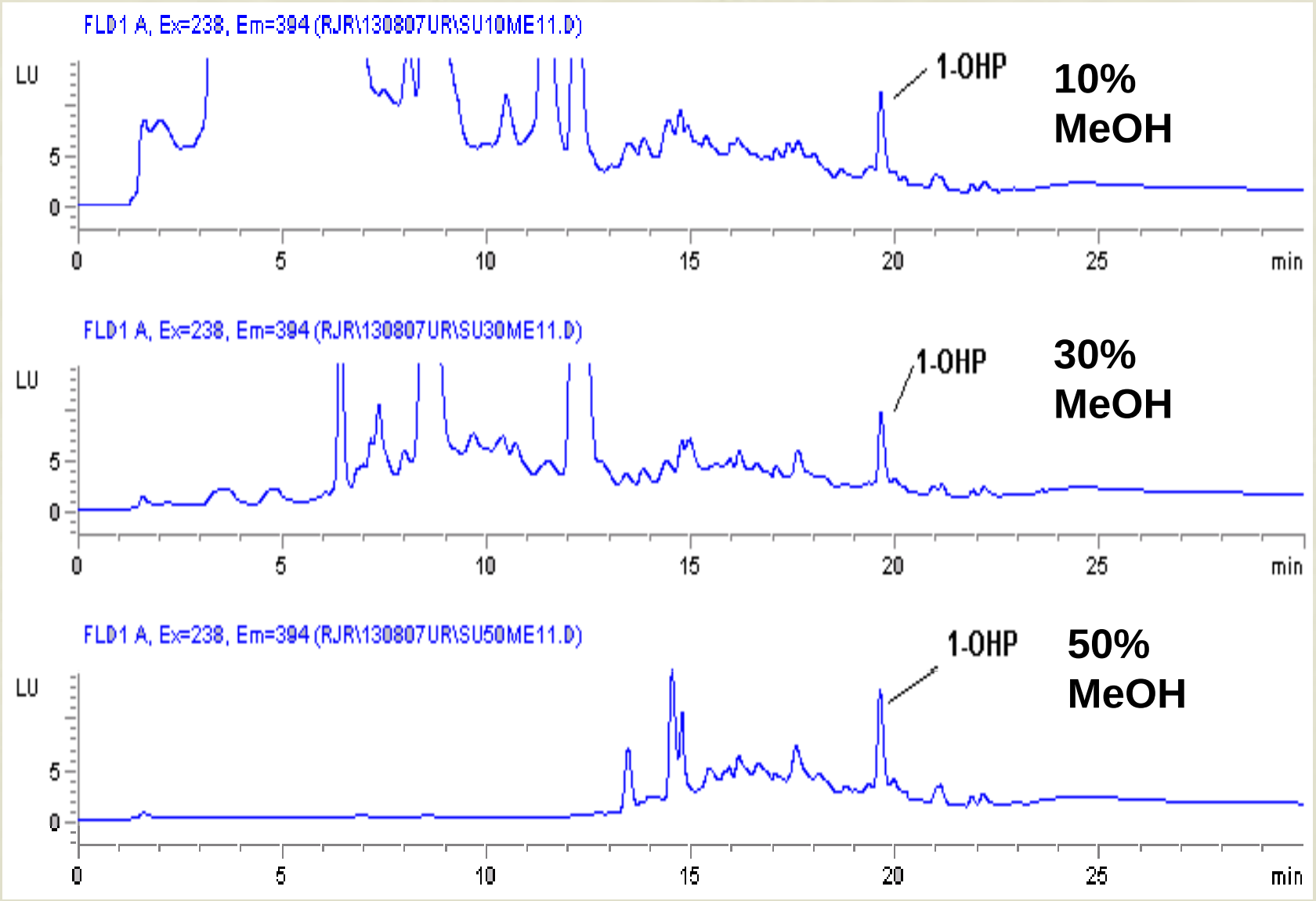
Benchmarks for our Analysis

- Effective enzymatic hydrolysis
- Efficient sample clean up
- High resolution chromatography
- Selective detection
- Sensitive response
- Fast turn-around time

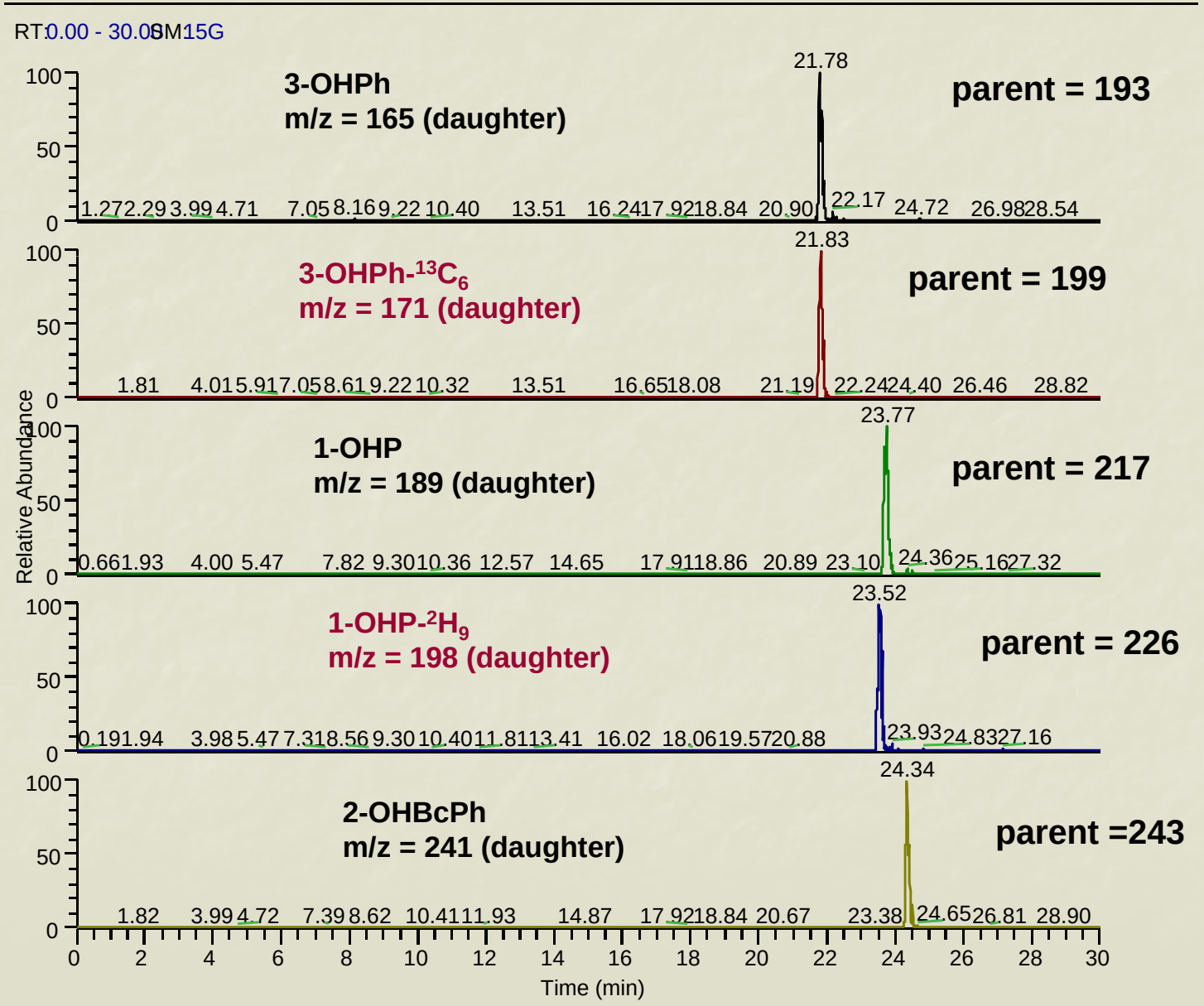
Observations Leading to a Viable Analytical Method

- Enzymatic hydrolysis of the OHPAH glucuronide was not detrimental to analyte recovery
- MS/MS response steadily declined with number of urine injections
- Environmental and pharmaceutical SPE C_{18} phases gave similar results
- Many background components fluoresce; thus more efficient sample clean-up will be required for quantification using fluorescence detection
- Internal standardization with isotopically labeled OHPAHs leading to relative recovery is preferred
- Fluorescence detection is not feasible due to both insufficient selectivity and chromatographic resolution.

HPLC-FD of 1-OHP spiked (5.0 ng/5 mL) into urine sample, incubated, and cleaned using various percentages of MeOH



HPLC-MS/MS (negative ion mode) for Three Analytes and Two Internal Standards in Aqueous Matrix



Percent Recovery of OHPAH's Spiked in Urine Using External Standards

OHPAH	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	% Rec.	%RSD
3-OHPh	53	68	73	87	93	85	76	19
1-OHP	32	47	38	47	39	42	41	14
2-OHBcPh	27	28	30	38	40	38	34	18

1- 1 mL of 0.2M sodium acetate at pH=5 was added

2- 10 µL of enzyme added

3- Incubated for 16 hours

4- Passed through SPE cartridge conditioned with 3 mL of MeOH, 3 mL of 1% formic acid, next urine passed through cartridge – Washed with 10 mL H_2O, and then with 3 mL of 50/50 MeOH/H_2O – Eluted with 1.5 mL of MeOH

Percent Recovery of OHPAH's Spiked in Urine Using 1-OHP-$^2H_9$ as an Internal Standard

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Avg (%RSD)
3-OHPh	163	105	138	191	148	149 (21)
1-OHP	105	95	97	101	98	99 (4)
2-OHBcPh	78	69	67	72	72	72 (5)

1- Samples were spiked at 2.5 ng/mL in 5 mL urine

2- 1 mL of 0.2M sodium acetate at pH=5 was added

3- 10 µL of enzyme added

4- Incubated for 16 hours

5- Passed through SPE cartridge conditioned with 3 mL of MeOH, 3 mL of 1% formic acid, next urine passed through cartridge – Washed with 10 mL H_2O, and then with 3 mL of 50/50 MeOH/H_2O – Eluted with 1.5 mL of MeOH

Percent Recovery of OHPAHs Spiked in Urine Using 3-OHPh-$^{13}C_6$ and 1-OHP-$^{2}H_9$ as Internal Standards

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	% Rec. (% RSD)
3-OHPh	103	107	131	140	106	106	116 (13)
1-OHP	109	127	118	112	104	104	113 (8)
2-OHBcPh	90	74	92	88	105	93	90 (11)

Application of Analytical Method for Analysis of Urine (in progress)

- Smokers of leading Lights cigarette vs. Non-smokers
- Six subjects per group
- Three males, nine females
- Ages 28-62
- Twenty-four hour urine collection
- 1-OHP, 3-OHPh, 2-OHBcPh, 1-naphthol, and 2-naphthol were measured
- Duplicate analysis performed twice separated by four weeks (i.e. two sample sets)
- Sample analysis conducted on a blinded, randomized basis

Conclusions

- An optimal method for the quantitative analysis of multiple urinary biomarkers of exposure to polycyclic aromatic hydrocarbons has been developed and validated
- The method involves enzymatic hydrolysis of OHPAH conjugates, SPE clean-up and negative ion HPLC-MS/MS
- Accurate quantitation of 1-Hydroxypyrene, 3-Hydroxyphenanthrene, 2-Hydroxy- Benz[c]phenanthrene, 1-Naphthol and 2-Naphthol requires multiple isotopically labeled internal standards
- Based on preliminary results, differences between smokers and non-smokers observed for 1-Hydroxypyrene, 3-Hydroxyphenanthrene, and 1-Naphthol
- Based on preliminary results, differences were not observed for 2-Naphthol and 2-Hydroxy-Benz[c]phenanthrene

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