The Analysis of Hydroxy Polycyclic Aromatic Hydrocarbons in Human Urine by Isotope Dilution Online Solid Phase Extraction Followed by High Performance Liquid Chromatography Tandem Mass Spectrometry

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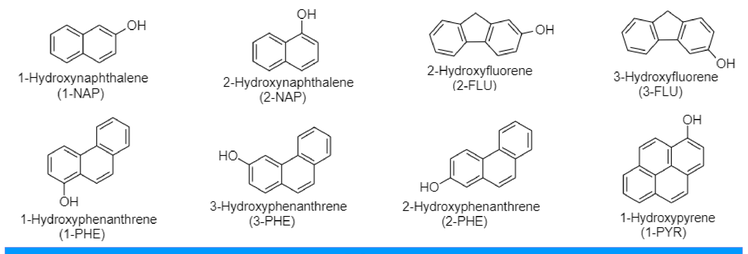
Abstract

Polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion organic materials. These PAHs are widespread, and many are suspected carcinogens with no established threshold levels for carcinogenicity[1]. Biomonitoring of PAHs is relevant for environmental public health because of the widespread exposure of PAHs to humans. Some PAHs are metabolized in the human body and excreted in urine. The reference CDC method[2] is adapted for the quantitation of eight hydroxy polycyclic aromatic hydrocarbons (HO-PAHs) in human urine. The method employs isotope dilution, online solid phase extraction (SPE), followed by high performance liquid chromatography tandem mass spectrometry HPLC-MS/MS). The concentration of eight PAHs (1-hydroxynaphthalene, 2- hydroxynaphthalene, 2-hydroxyfluorene, 3- hydroxyfluorene, 1-hydroxyphenanthrene, 2- & 3-hydroxyphenanthrene, 1-hydroxypyrene) can be determined for 500 subjects as part of the program. The chromatography of the samples showed baseline separation of six analytes by the current gradient program. Excellent linearity achieved for all analytes (R^2>0.997) in linear range: 1-NAP: 0.977-100, 2-NAP: 0.0488-100, 1,2,3-PHE 2&3-FLU: 0.0122-25.0 and 1-PYR: 0.0244-25.0 ng/ml.

Introduction

The measurement of HO-PAHs in human urine is the method of choice in determining exposure to PAHs from multiple routes. [2] The metabolism of PAH molecules is complex and are metabolized by multiple enzymes through three known major pathways. HO-PAH is excreted in urine by one pathway. An on-line SPE LC-MS/MS for the quantitation of HO-PAH in human urine was implemented in the Environmental and Chemical Laboratory Services (ECLS) laboratory according to the CDC method. [3] The method is complex and involves over 20 preparative steps, which includes enzymatic hydrolysis of the precursor target analytes for 18 hours at 37 °C. Several major challenges were overcome before successfully implementing the method. A major challenge is the lack available of neat standards. The refence method uses standards dissolved in methanol; however, only standards dissolved in toluene is available. Toluene is immiscible in aqueous standards and the analytes partitions differently in aqueous and organic phases. A mixed solvent mixture by combining ethanol in standards and mixing by shaking, enabled adequate analyte partitioning into the aqueous phase and produced acceptable standards that resulted in excellent calibration curves for all analytes (R^2>0.997). This mixed solvent and mixing approach was also applied to the urine specimens and produced acceptable recoveries at low and high spiked pooled urine specimens. Consistent with the refenced method, all target analytes were adequately separated, however using different guard and analytical columns. Analyte sensitivity and specificity is acceptable, and the limit of detection is consistent or lower than the reference method.

Target analytes



Aims

- Adopt online SPE-LC-MS/MS methods to measure eight PAHs in human urine, based on CDC's existing method (# 6705.02.)
- Optimize LC-MS/MS conditions to increase sensitivity and selectivity
- Optimize sample preparation procedures to improve recovery
- Validate the method that will be used for population in NJHANES study

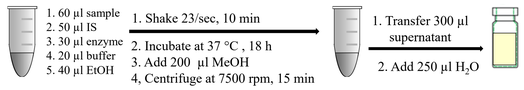
References

1. Moorthy, B.; Chu, C.; Carlin, D. Poly Aromatic Hydrocarbons: From Metabolism to Lung Cancer. *Toxsci*, 145(1), **2015,** 5-15.

2. Hetch, S.; Carmella, S.; Yoder, A.; Chen, M.; Li, Z.; Le, C.; Dayton, R.; Jensen, J.; Hatsukami, D. Comparason of Polymorphism in Genes Involved in Polycyclic Aromatic Hydrocarbon Metabolism with Urinary Phenanthrene Metabolite Ratio in Smokers. *Cancer Epideniol Biomarkers*. **2006**, 15, 10.

Materials and Methods

Sample preparation

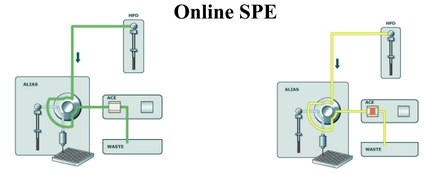


Gradient program

	time	Flow	0.1 mM NH_4F in H2O 0.2 (A, %)	0.1 mM NH_4F in MeOH (B, %)
1	00:00:01	0.50	99	1
2	00:03:30	0.50	99	1
3	00:03:54	0.50	60	40
4	00:04:20	0.50	55	45
5	00:05:00	0.60	50	50
6	00:08:00	0.60	45	55
7	00:12:00	0.80	40	60
8	00:18:00	0.80	35	65
9	00:19:30	0.80	30	70
10	00:20:00	1.00	15	85
11	00:21:00	1.00	15	85
12	00:22:00	1.00	10	90
13	00:24:00	1.00	5	95
14	00:24:30	1.00	5	95
15	00:24:36	1.00	99	1
16	00:27:00	1.00	99	1

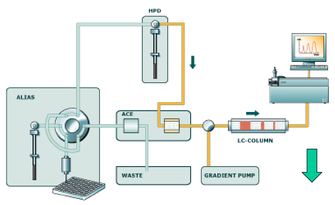
On-line SPE cartridge: Oasis Symbiosis/Prospekt-2, WAX 10 x 1 mm
Analytical guard column: Agilent Zobax Eclips PAH 4.6 x 12.5 mm 5- micron
Analytical column: Agilent Zobax Eclips PAH 4.6 x 100 mm 3.5- micron

Online SPE



1. 2 x 2.0 ml MeOH
2. 2 x 2.0 ml ACN
3. 2.0 ml 0.1 % formic acid in H_2O

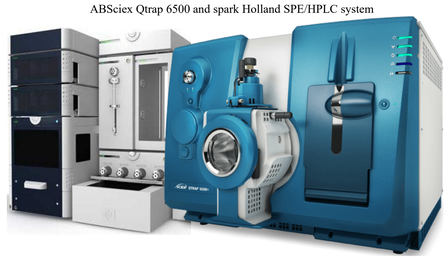
4. 450 µl sample introduced in SPE cartridge with 1.5 ml 0.1 % formic acid in in H_2O



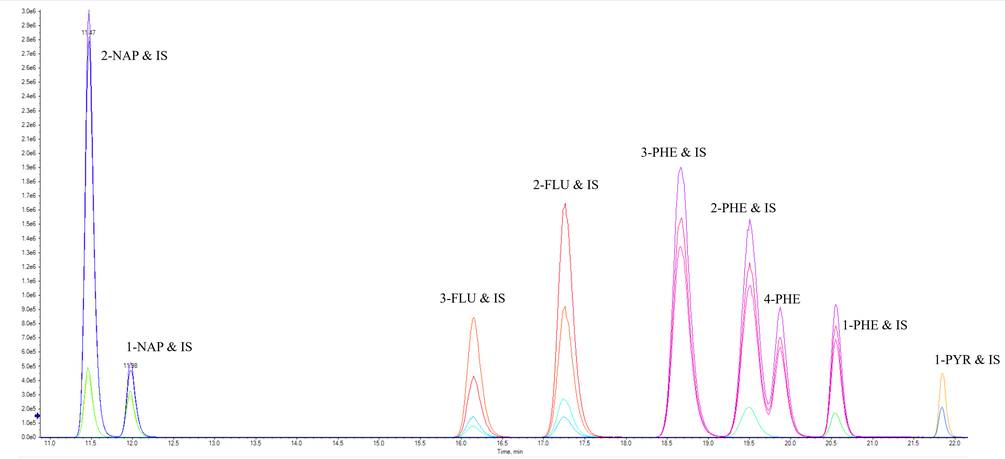
5. The analytes are eluted from the SPE cartridge onto the guard column with 350 µl MeOH. The analytes are separated in the analytical column by the LC gradient program.

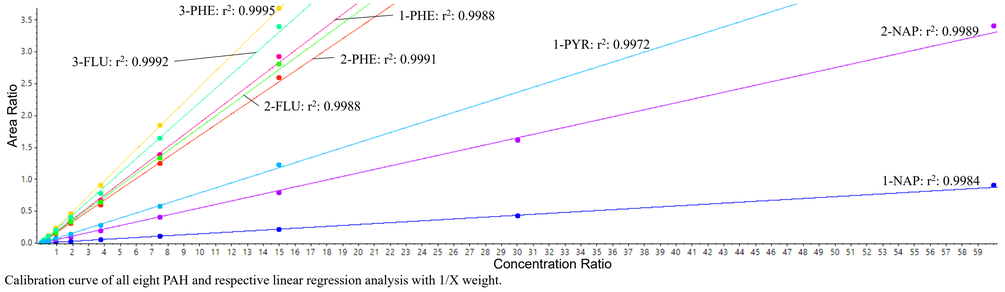
LC-MS/MS Instruments

ABSciex Qtrap 6500 and spark Holland SPE/HPLC system



Results

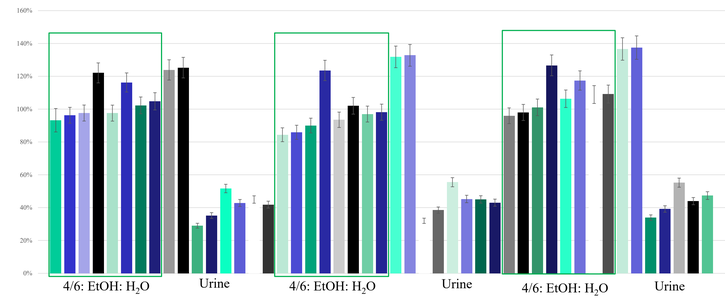




Calibration curve of all eight PAH and respective linear regression analysis with 1/X weight.

- ❖Baseline separation achieved for most PAH.
- ❖Excellent linearity achieved for all analytes (R^2>0.997) in linear range: 1-NAP: 0.977-100, 2-NAP: 0.0488-100, 1,2,3-PHE 2&3-FLU: 0.0122-25.0 and 1-PYR: 0.0244-25.0 ng/ml.

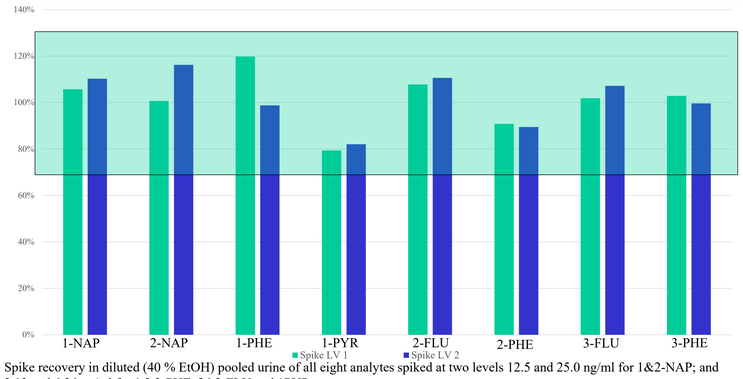
Results: Matrix Effects



Spike recovery in 4/6:EtOH/H_2O and in pooled urine. All eight analytes spiked at four levels 0.780, 6.25, 12.5, 25.0 ng/ml for 1&2-NAP and 0.200, 1.56, 3.13. 6.26 ng/ml for 1,2,3-PHE, 2&3-FLU and 1PYR.

- ❖There is a significant matrix effect observed between 4/6: EtOH/H_2O and in urine.
- ❖The PAHs recoveries in 4/6: EtOH/H_2O are good, and, in most cases, the recoveries are within 70-130%.
- ❖The PAHs recoveries in urine are inconsistent and are between 20-80%

Results: Matrix Matching



Spike recovery in diluted (40 % EtOH) pooled urine of all eight analytes spiked at two levels 12.5 and 25.0 ng/ml for 1&2-NAP; and 3.13 and 6.26 ng/ml for 1,2,3-PHE, 2&3-FLU and 1PYR.

- ❖Both spiking levels for all analytes spiking levels had recoveries within 70-130%.

Conclusions and Future Plans

- ❖An ultra sensitive online LC-MS/MS method was established at ECLS-NJDOH to measure PAHs metabolites in human urine.
- ❖Consistent ratio of 4/6 : EtOH/H_2O in the calibrators and samples is critical for good spike recoveries in urine.
- ❖The dynamic range was achieved to cover both specimens with excellent linearity (R^2>0.999)
- ❖Preliminary results showed acceptable accuracy (recovery between 70-130%)
- ❖The LOD and QC characterization will be determined to complete the validation.
- ❖This method in under validation and will be used to support NJHANES and other NJ Biomonitoring programs.

Acknowledgements

- This study was supported by the CDC State Biomonitoring Grant (#5 NU88EH001326-02-00) and New Jersey State Government.
- Thank each colleague in the Chemical Terrorism Lab and other NJ State Biomonitoring team members for their endless support.

Disclaimer: Contents and conclusions presented here are solely the responsibility of the authors and do not necessarily represent the views of CDC.

References

3. Centers for Disease Control and prevention. (2013-2014) Isotope Dilution Online Solid Phase Extraction High Performance Liquid Chromatography/Tandem Mass Spectrometry (online SPE-HPLC-MS/MS. Retrieved April 5, 2022, from Eight monohydroxy-polycyclic aromatic hydrocarbons (cdc.gov).