FAST SCREENING OF URINARY METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS BY USING POLYETHERETHERKETONE FOR SOLID PHASE EXTRACTION

Li X[1]*, Li YP[1]

[1]Institute of Environmental Pollution and Health, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, PR China

Introduction

Urinary hydroxylated metabolites of polycyclic aromatic hydrocarbons (OH-PAHs) are a group of important metabolites of carcinogenic PAHs, and have been widely used as biomarkers for assessing health risk of human exposure to PAHs[1]. Up to now, numerous methods have been developed for the detection of urinary OH-PAHs; however, novel analytical strageties for high throughput detection of urinary OH-PAHs are still required considering that a large amount of samples (e.g., thousands of) need to be analyzed for PAHs risk assessment. A recent study found that urinary OH-PAHs can be readily adsorbed to the material of polyetheretherketone (PEEK)[2]. PEEK is an organic hydrophobic polymer thermoplastic, characterized with a chemical structure consisting of a benzene-ring backbone interconnected by functional groups of ethers and ketones[3]. With this regard, a fast screening method by using PEEK tubing for solid phase extraction (SPE) coupled to high performance liquid chromatography-fluorescence detector (HPLC-FD) was investigated to detect nine urinary OH-PAHs containing 2–5 rings in this study.

#

Materials and methods

1-Hydroxypyrene (1-OHPyr), 2-hydroxynaphthalene (2-OHNap) and 3-/4-/9-hydroxyphenanthrene (3-/4-/9-OHPhe) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 6-Hydroxychrysene (6-OHCHr) was obtained from AccuStandard (New Haven, CT). 2-Hydroxyfluorene (2-OHFlu) and 3-hydroxybenzo[a] pyrene (3-OHBaP) were bought from Toronto Research Chemicals (Ontario, Canada). β-Glucuronidase and carbazole (KZ) were commercial products from Sigma-Aldrich (St Louis, MO, USA). Methanol (MeOH, HPLC grade) was from Merck KGaA (Darmstadt, Germany). Ultrapure water (resistivity 18.2 MΩ · cm) was supplied by a Milli-Q Gradient A10 ultrapure water purification system (Millipore Inc, USA). Quantifications of OH-PAHs were carried out with Waters 2695 liquid chromatograph (HPLC) equipped with Waters 2475 fluorescence detector (FD). An Agilent ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 μm) was used as HPLC column.

A piece of 117-cm PEEK tubing (1/16 in. o.d. × 0.50 mm i.d., Agilent, USA) was used for SPE. Sample pretreatment was carried out as follows (Figure 1): (i) the PEEK tubing was first washed subsequently with 500 μL of MeOH and 500 μL of ultrapure water; (ii) 2 mL of spiked hydrolyzed urine sample was loaded into the PEEK tubing; (iii) the tubing was further washed with 500 μL of ultrapure water; (iv) finally, 500 μL of MeOH was applied to elute OH-PAHs adsorbed to the inner wall surface of the tubing. The eluting solution was concentrated to a final volume of 200 μL under a gentle stream of nitrogen. 5 μL of 1.38 mg L^{-1} KZ was added into the solution as internal standard (IS). All the aforementioned solutions were manually and directly injected into the PEEK tubing through a stainless steel (SS) union (bore size 0.25 mm) with a 250-μL Hamilton syringe. Moreover, the same piece of PEEK tubing has been used for all the experiments performed in this study, and no deactivation of inner wall surfaces of PEEK tubing has been observed over the entire study period. The preparation of spiked enzymatic hydrolyzed urine samples was carried out according to the procedure reported previously[4–5].

For HPLC-FD analysis: the flow rate was set to 0.8 mL/min, the injection volume was 20 μL and the oven temperature was 40 °C. Nine OH-PAHs and KZ were separated on ZORBAX SB-C18 column by using MeOH-Water gradient elution: 0–20 min 60% MeOH, 20–26 min a linear gradient from 60% to 95% MeOH, 26–33 min 95% MeOH, 33–34 min a linear gradient from 95% to 60% MeOH and 34–44 min 60% MeOH. The

Excitation (Ex)/Emission (Em) wavelengths for individual OH-PAHs and KZ (IS) were 227/355 nm (2-OHNap), 275/330 nm (2-OHFlu), 250/360 nm (2/3-OHPhe and KZ), 265/351 nm (4-OHPhe), 252/356 nm (9-OHPhe), 343/380 nm (1-OHPyr), 262/371 nm (6-OHChr) and 368/428 nm (3-OHBaP), respectively.

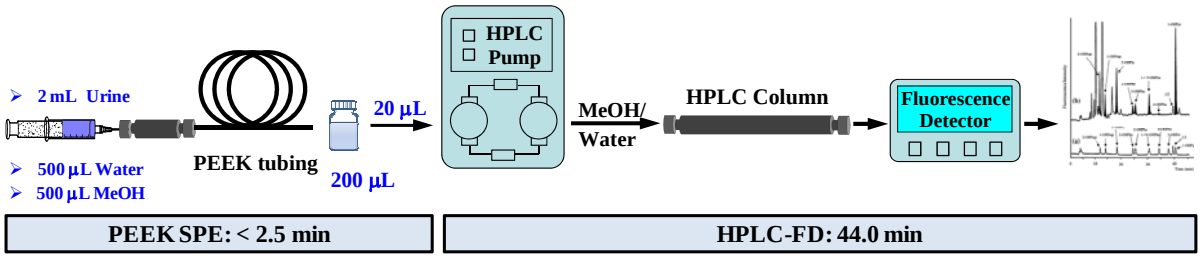

Figure1 Schematic diagram of the system that uses PEEK tubing for SPE coupled to HPLC-FD.

Results and discussion#

Based on the present gradient elution conditions, all nine OH-PAHs were successfully separated on the ZORBAX SB-C18 column; additionally, satisfied separation of OH-Phe isomers of 2- and 3-OHPhe were observed (Figure 2) compared with previous studies[6].

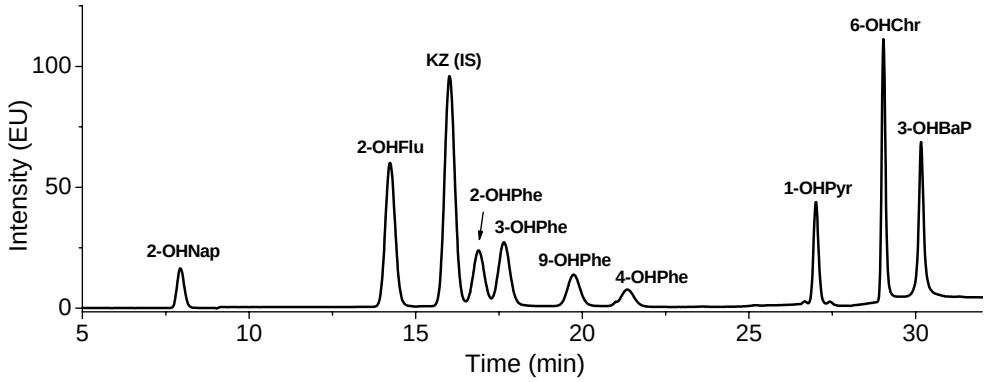

Figure2 HPLC Chromatogram of a mixed standard solution containing 10 µg L^{-1} of individual OH-PAHs and 34 µg L^{-1} of KZ (IS).

By analyzing a series of OH-PAHs mix standard solutions with concentrations ranging from 0.02−100 µg L^{-1}, calibration curves with linear ranges of 0.2−100 µg L^{-1} or 0.5−100 µg L^{-1} (determination coefficient R^2: 0.9988−1.0000) were obtained for individual OH-PAHs. Instrumental detetion limits (IDL) defined for a signal-to-noise ratio (S/N) of 3 and instrumental detetion limits (IQL) defined for an S/N of 10 of each OH-PAH were estimated to be 0.02−0.1 µg L^{-1} and 0.2−0.5 µg L^{-1}, respectively. The intra-day relative standard deviations (RSD) (n = 3) of IDL or IQL were in the range of 1.7−8.1%.

Table1 Linear range and R^2 of calibration curve, and IDL and IQL for nine OH-PAHs.

OH-PAHs	Linear range (µg L^{-1})	R^2	IDL (µg L^{-1})	IQL (µg L^{-1})	RSD (%) (n = 3)
2-OHNap	0.50–98.00	1.0000	0.05	0.50	3.3
2-OHFlu	0.50–100.00	1.0000	0.05	0.50	2.9
2-OHPhe	0.50–100.00	1.0000	0.05	0.50	2.7
3-OHPhe	0.50–102.00	1.0000	0.05	0.50	3.7
4-OHPhe	0.50–104.00	0.9988	0.10	0.50	3.8
9-OHPhe	0.50–100.00	0.9998	0.10	0.50	1.7
1-OHPyr	0.20–103.00	1.0000	0.03	0.20	8.1
6-OHChr	0.20–102.00	1.0000	0.03	0.20	7.6
3-OHBaP	0.20–90.00	0.9994	0.02	0.20	6.0

When 0.6 μg L^{-1} of a spiked urine sample was loaded to the PEEK tubing, 4−5 rings of OH-PAHs, including 1-OHPyr, 6-OHChr and 3-OHBaP have been successfully quantified while 3 rings of OH-PAHs, such as 2-OHFlu and 2-/3-/4-/9-OHPhes were detected (Figure 3a); as applying the spiked urine sample with a higher spike concentration of 2.0 μg L^{-1}, quantification of 2−3 rings of OH-PAHs has also been achieved (Figure 3b). RSDs (n = 4) of spiked urine samples with spike concentrations of 0.6−50 μg L^{-1} varied from 3.4−51.0%, and lower RSD values (better reproducibility) were obtained when spiked urine samples with higher spike concentration were analyzed (Table 2), e.g., RSDs of 5.0 and 2.0 μg L^{-1} spiked samples were 2.9−14.1% and 3.3−44.2%, respectively.

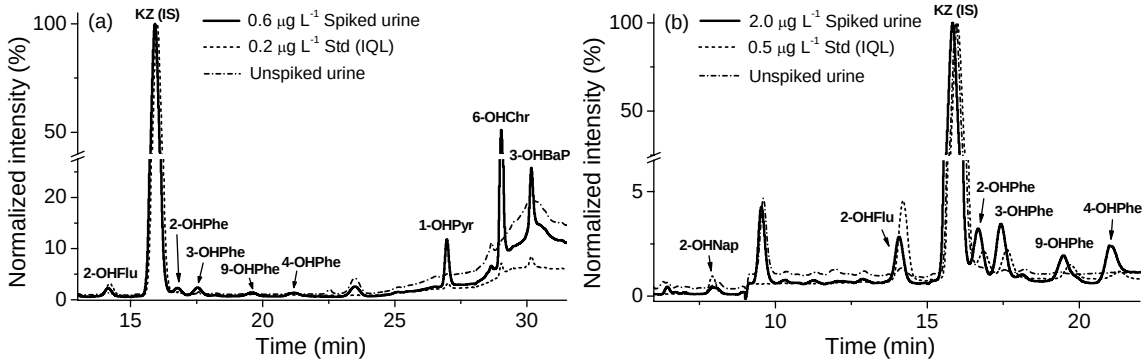

Figure 3 HPLC chromatograms of spiked urine, standard solution (IQL) and unspiked urine, showing that urinary 4–5 ring OH-PAHs (a) and 2–3 ring OH-PAHs (b) can be successfully quantified with present method.#

Table 2 Relative standard deviations (RSDs) of four measurements of spiked urine samples containing 0.6–50 μg L^{-1} of OH-PAHs.

OH-PAH	0.6 μg L^{-1}	1.0 μg L^{-1}	2.0 μg L^{-1}	5.0 μg L^{-1}	10.0 μg L^{-1}	50.0 μg L^{-1}
2-OHNap	n.a.	n.a.	n.a.	14.1	17.2	4.7
2-OHFlu	n.a.	n.a.	n.a.	8.3	11.7	5.7
2-OHPhe	31.1	24.5	7.6	2.9	4.9	5.5
3-OHPhe	42.6	43.1	20.3	3.7	4.8	5.3
4-OHPhe	51.0	45.7	15.4	12.5	14.4	15.3
9-OHPhe	n.a.	n.a.	44.2	5.6	9.6	11.0
1-OHPyr	21.2	14.6	4.0	5.6	5.8	3.4
6-OHChr	33.1	27.8	3.3	6.6	11.8	5.2
3-OHBaP	7.9	9.3	9.0	13.8	12.3	16.3

Therefore, the above results indicate that: (i) the present analytical strategy by using PEEK tubing for SPE pretreatment is applicable to fast screening/quantifying urinary OH-PAHs, in regard with only 2.5 min was required for sample pretreatment; (ii) this method would be promising for occupationally or even non-occupationally exposed people, for the lowest concentrations of urinary OH-PAHs can be quantified in this work were 0.6 and 2.0 μg L^{-1} for 4−5 rings and 2−3 rings of OH-PAHs, respectively, despite of no optimization of pretreatment procedure; (iii) the method is more sensitive for high molecular weight (HMW) OH-PAHs than low molecular weight OH-PAHs (HMW). This would be especially attractive for PAHs risk exposure, for HMW PAHs lead to higher risk due to greater carcinogenic potency; (iv) finally, method sensitivity can be further imporved as PEEK tubing is used as online SPE column, for all adsorbed OH-PAHs rather than an aliquot can be delivered to the detector (in the present method the injection volume is 20 μL while the volume of the final concentrated solution is 200 μL).

Furthermore, it is probably necessary to carry out more studies on the application of PEEK tubing as online SPE column to facilitate high-throughput analysis of urinary OH-PAHs. The advantages of using PEEK tubing as online SPE column include low back pressure, easy operation/maintenance, availability of well-developed commercial products, and relatively low cost. Besides, it will be also interesting to investigate the extraction of organic compounds with different polarities and functional groups by using PEEK material.

Acknowledgements
National Natural Science Foundation of China (No. 21107066), Special Funds for the Development of National Major Scientific Instruments and Equipment (2011YQ170067), Young Teachers Program of Universities in Shanghai (2012) and Innovation Foundation of Shanghai University.

References

1. Strickland P, Kang DH. (1999); *Toxicol Lett.* 108:191−199
2. Li X, Zenobi R. (2013); *Anal Chem*. 85: 3526−3531
3. Awaja F, Bax DV, Zhang S, James N, McKenzie DR. (2013); *Plasma Processes Polym*. 9: 355−362
4. Li X, Fang XW, Yu ZQ, Sheng GY, Wu MH, Fu JM, Chen HW. (2013); *Anal Methods*. 5: 2816−2821
5. Wang Y, Zhang WB. Dong YL, Fan RF, Sheng GY, Fu JM. (2005); *Anal Biom Chem*. 383(5): 804−809
6. Kuusimaki L, Peltonen Y, Mutanen P, Peltonen K, Savela K. (2004); *Int Arch Occ Env Hea*. 77: 23−30