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Paper

A novel facile method by using polyetheretherketone as a solid phase extraction material for fast quantification of urinary monohydroxylated metabolites of polycyclic aromatic hydrocarbons

Yinping Li,[a] Xue Li*[a,b] and Zhen Zhou[a,b]

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A novel facile method by using polyetheretherketone (PEEK) as a solid phase extraction (SPE) material to extract urinary monohydroxylated metabolites of polycyclic aromatic hydrocarbons (OH-PAHs) has been successfully demonstrated. Nine urinary OH-PAHs containing 2 to 5 benzene rings in their molecular structures have been quantified with a piece of 117-cm PEEK tubing (500 μm ID) for offline SPE followed by HPLC-FD. Limits of quantification for 4- and 5-ring OH-PAHs (e.g., 1-hydroxypyrene, 3-hydroxybenzo[a]pyrene) were 0.6 μg L^{-1} while for 2- and 3-ring congeners (e.g., hydroxylated phenanthrenes) were 5.0 μg L^{-1}. This result is comparable to those of conventional methods whereas much less time is required for sample pretreatment (2.5 min), indicating the potency of PEEK as a promising SPE material for the fast pretreatment of urinary OH-PAHs. The analytical strategy by using PEEK-based SPE will facilitate the high throughput analysis of urinary OH-PAHs demanded by PAHs risk assessment. Besides, since OH-PAHs with more rings (e.g., 3-hydroxybenzo[a]pyrene) can be efficiently adsorbed to the PEEK material, this method will be especially helpful for the risk assessment of these congeners, which are regarded with high carcinogenic potency.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of widespread environmental contaminants, and have aroused great concerns due to their mutagenic and carcinogenic effects.[1,2] PAHs can be produced from incomplete combustion of organic materials, and emitted from both natural and anthropogenic sources, e.g., volcanic eruptions, forest fires, coke plants, steel foundries, smoking, broiled foods and even medical treatment like coal tar,[3,4] resulting in either occupational or non-occupational exposure to PAHs.

Even though biomarkers for both external and internal exposures have been used for health risk assessment of PAHs, urinary monohydroxylated metabolites of PAHs (OH-PAHs) have been most widely considered. Compared with other biomarkers, OH-PAHs are a group of stable metabolites, and excel in response to multiple routes of exposure at an early stage.[5–8]

Up to now, a number of analytical techniques have been developed for the analysis of urinary OH-PAHs, including high performance liquid chromatography coupled with fluorescence detection (HPLC-FD),[9–11] gas chromatography mass spectrometry (GC-MS),[12–14] liquid chromatography MS (LC-MS),[15,16] LC tandem MS (LC-MS/MS).[17,18] Electrochemical,[19,20] spectroelectrochemical,[21,22] chemiluminescence[23] and immunochemical methods[24] have also been reported for the detection of urinary OH-PAHs. Regarding the methods mentioned above, sensitivity has been improved by several orders of magnitude, and also more OH-PAHs have been taken into account. However, time-consuming and laborious sample pretreatment could not be avoided yet, and intrinsically hampers the high throughput analysis of urinary OH-PAHs demanded by PAHs risk assessment.

Sample pretreatment is an indispensable step for the detection of urinary OH-PAHs. This is because: (i) OH-PAHs are excreted in urine at trace levels (e.g., 0.1–500 μg L^{-1}),[25,26] and need to be concentrated before instrumental analysis; (ii) high levels of organic and inorganic matrix compounds (around 37,057 mg L^{-1} in total)[27] could be removed during sample pretreatment, which will benefit method sensitivity and repeatability/reproducibility.

The application of an adsorption material that has similar chemical structure to OH-PAHs may improve the extraction efficiency, leading to less time consumption during sample pretreatment. Morishima found that polyetheretherketone (PEEK) tubing can effectively adsorb dibutyl phthalate, which contains a benzene ring in its molecular structure.[28] PEEK is an organic polymer thermoplastic, and has a chemical structure characterized by a benzene-ring backbone interconnected with functional groups of ethers and ketones (Fig. 1).[29] With this regard, PEEK could also be a proper solid phase adsorption material to extract urinary OH-PAHs, and this assumption has been proved quite recently, i.e., fast quantification (< 2 min) of urinary 1-hydroxypyrene (1-OHPyr) has been successfully achieved by using PEEK tubing coupled to MS/MS.[30,31]

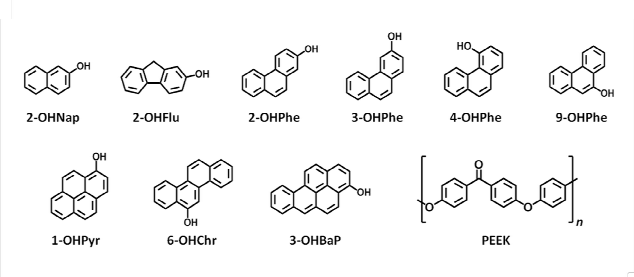

Fig. 1 Molecular structures of nine OH-PAHs and PEEK.

Thus, this study aims at coupling the PEEK tubing to HPLC-FD for the analysis of nine urinary OH-PAHs that contains 2 to 5 benzene rings in their chemical structures (Fig. 1). Besides, adsorption efficiency of these nine OH-PAHs to the PEEK material has also been investigated, which could provide helpful information for further method improvement. HPLC-FD has been widely used for the detection of OH-PAHs, and therefore PEEK-based SPE followed by HPLC-FD is promising to reder a high throughput analytical platform demanded by the risk assessment of PAHs exposure.

Materials and Methods

A. Chemicals and materials

2-Hydroxynaphthalene (2-OHNap), 2-hydroxyphenanthrene (2-OHPhe), 9-OHPhe and 1-OHPyr were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). 3-OHPhe, 4-OHPhe, 2-hydroxyfluorene (2-OHFlu) and 3-hydroxybenzo[a]pyrene (3-OHBaP) were bought from Toronto Research Chemicals (Ontario, Canada). 6-Hydroxychrysene (6-OHCHr) was obtained from AccuStandard (New Haven, CT). β-Glucuronidase and carbazole (KZ) were the commercial products from Sigma-Aldrich (St Louis, MO, USA). HPLC grade of methanol (MeOH) 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). All chemicals were directly used without further treatment.

Standard solutions, hydrolyzed urine samples and spiked hydrolyzed urine samples were prepared according to the procedures reported previously.[26,31,32]

B. Sample pretreatment by applying PEEK-based SPE

Different pieces of PEEK tubing (1/16 inch OD, Agilent, USA) with various inner diameters (130, 180, 250 and 500 μm ID) and lengths (17, 44, 100, 117.0 and 133 cm) were used. Sample pretreatment was carried out as follows: (i) the PEEK tubing was firstly washed with 500 μL of MeOH and 500 μL of ultrapure water subsequently; (ii) 2 mL of spiked hydrolyzed urine sample was loaded into the tubing; (iii) the tubing was washed with 500 μL of ultrapure water; and (iv) finally, 500 μL of MeOH was applied to elute OH-PAHs adsorbed to the inner wall surface of the tubing. All the aforementioned solvents were directly injected into the PEEK tubing through a stainless steel union (bore size 250 μm) by using a 250-μL Hamilton syringe. 10–20 sec was taken for each injection and less than 2.5 min was required for the whole pretreatment procedure.

Afterwards, the eluting solution was collected and concentrated to a final volume of 200 μL under a gentle stream of nitrogen (purity ≥ 99.999%). 5 μL of 1.38 mg L^{-1} KZ methanol solution was added into the concentrated solution while KZ was used as an internal standard (IS).[33] Unspiked hydrolyzed urine sample was used as a control sample, and 3–6 technical replicates were performed for each sample. It is noteworthy that in this study all PEEK tubings were recycled and no deactivation of the PEEK tubings was observed.

C. HPLC-FD Analysis

According to the optimized HPLC and FD conditions reported in our previous work,[32] nine OH-PAHs and KZ (IS) were detected by using Waters 2695 HPLC coupled with Waters 2475 FD. The injection volume was 20 μL and the oven temperature was 40 °C. OH-PAHs and KZ were separated on a ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) while the following MeOH-Water gradient elution was applied: 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 flow rate of the mobile phase was set to 0.8 mL min^{-1}. The Excitation (Ex)/Emission (Em) wavelengths for OH-PAHs and KZ 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 (Table S1†).

he system containing PEEK-based SPE coupled to HPLC-FD is illustrated in Fig. S1†.

Results and Discussion

A. Detection of urinary OH-PAHs by using PEEK-based SPE coupled to HPLC-FD

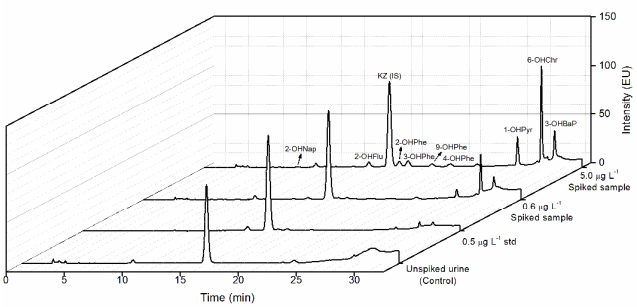

Fig. 2 HPLC-FD chromatograms of urine samples and the standard solution.

Under gradient elution conditions used in the experiment, nine OH-PAHs together with KZ were successfully separated on the ZORBAX SB-C18 column (Fig. S2†). A series of OH-PAHs mix standard solutions with concentrations ranging from 0.02−100.00 μg L^{-1} were analyzed. Calibration curves with the linear range of 0.20−100.00 μg L^{-1} (R^2 = 0.9988−1.0000) were obtained for individual OH-PAHs. As instrumental detection limit (IDL) and quantification limit (IQL) are defined for signal-to-noise ratios (S/N) of 3 and 10, respectively, IDLs of nine OH-PAHs were estimated to be 0.02−0.10 μg $L^{-1}$and IQLs were 0.20−0.50 μg L^{-1}; the intra-day relative standard deviations (RSDs) (n = 6) of IQLs were 3.4−10.2% (Table 1).

Table 1 Linear range and R^2 of calibration curves, IDLs and IQLs, and RSDs for nine OH-PAHs.

OH-PAHs	Linear range (μg L^{-1})	R^2	IDL (μg L^{-1})	IQL (μg L^{-1})	RSD (%) (n = 6)
2-OHNap	0.50–98.00	1.0000	0.05	0.50	4.4
2-OHFlu	0.50–100.00	1.0000	0.05	0.50	4.1
2-OHPhe	0.50–100.00	1.0000	0.05	0.50	3.4
3-OHPhe	0.50–102.00	1.0000	0.05	0.50	5.0
4-OHPhe	0.50–104.00	0.9988	0.10	0.50	10.2
9-OHPhe	0.50–100.00	0.9998	0.10	0.50	5.2
1-OHPyr	0.20–103.00	1.0000	0.03	0.20	3.9
6-OHChr	0.20–102.00	1.0000	0.03	0.20	4.7
3-OHBaP	0.20–90.00	0.9994	0.02	0.20	9.1

Hydrolyzed urine samples with spike concentrations of 0.6, 1.0, 2.0, 5.0, 10.0 and 50.0 μg L^{-1} were treated by using a 117-cm piece of PEEK tubing (1/16 inch OD × 500 μm ID) followed by HPLC-FD. Limit of quantification (LOQ) of 0.6 μg L^{-1} was obtained for 1-OHPyr, 6-OHChr and 3-OHBaP. This result is comparable to those reported by using conventional methods; on the other hand, much less time and sample volume are used in this study (Table S2†). LOQs for 2-, 3-, 4- and 9-OHPhe was 2.0 μg L^{-1}, and for 2-OHFlu and 2-OHNap was 5.0 μg L^{-1} (Fig. 2). Furthermore, RSDs (n = 4) indicated that for 1-OHPyr, 6-OHChr and 3-OHBaP, repeatability became better at spike concentrations ≥ 2.0 μg L^{-1}, and for 2-OHFlu, 2-OHNap and OH-Phes, repeatability was improved when spike concentrations ≥ 5.0 μg L^{-1} (Table S3†).

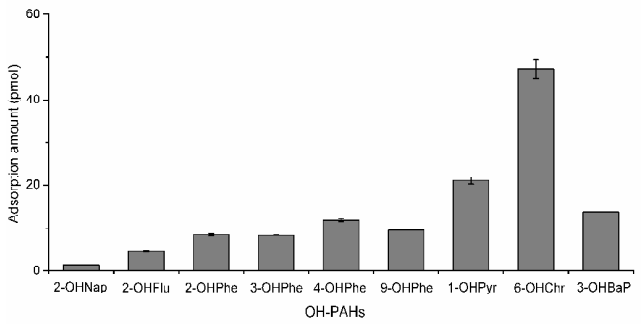

Fig. 3 Adsorption of nine urinary OH-PAHs to the PEEK tubing.

For the urine samples with spike concentrations ranging from 0.6 to 50.0 μg L^{-1}, the adsorption amount of OH-PAHs increased with the increase of the number of benzene rings in OH-PAHs molecular structures (except for 3-OHBaP). This result suggested that OH-PAHs with more rings, e.g., 6-OHChr that contains 4 rings, were more readily adsorbed to the PEEK tubing than the congeners with a smaller number of rings, e.g., a 2-ring congener of 2-OHNap (Fig. 3). The possible explanation is that OH-PAHs with more rings in their structures have lower polarity than the congeners with fewer rings, and thus are more easily adsorbed to the hydrophobic surface of the PEEK tubing. This property of PEEK-based SPE would be especially attractive for the risk assessment of PAHs with more rings, for these congeners pose higher health risk due to their greater carcinogenic potency.[1,2]

To summarize, the present analytical strategy by using PEEK-based SPE coupled to HPLC-FD can be applied to quantify 1-OHPyr, 6-OHChr and 3-OHBaP at their urinary concentrations ≥ 0.6 μg L^{-1}; for 2-OHNap, 2-OHFlu and four OH-Phe isomers, the urinary concentrations need to be ≥ 5.0 μg L^{-1} in order to be quantified. The sample pretreatment procedure is efficient and simple. Only 2.5 min is required for the whole sample pretreatment procedure, including cleaning tubing, loading sample, extracting OH-PAHs, removing matrix compounds and eluting adsorbed OH-PAHs. Moreover, less amount of solvent is used, e.g., 1 mL of MeOH and 1 mL of ultrapure water were consumed for cleaning tubing, removing matrix compounds and eluting OH-PAHs. This would result in less time used for SPE as well as evaporation/contraction processes.

B. Adsorption of OH-PAHs to the PEEK material

It is important to enhance the adsorption of OH-PAHs and meanwhile decrease the loss of adsorbed OH-PAHs while removing matrix compounds. High adsorption and low loss will contribute to lower LOQs, higher recovery and better repeatability/reproducibility. Since the adsorption of OH-PAHs is achieved when samples contact the inner wall surface of the PEEK tubing, and the inner wall surface area (S) is related to the inner diameter (D) and length (L) of the PEEK tubing (Eq. (1)), effects of D and L on the adsorption of OH-PAHs were investigated.

$$S = \pi DL \quad (1)$$

For comparing adsorption efficiency of 2- to 5-ring OH-PAHs, the parameter named z-score (Z) was introduced. Considering the data set with the mean X_{ave} and standard deviation (SD), Z of a data point x was calculated according to Eq. (2).

$$Z = (x - X_{ave})/SD \quad (2)$$

As presented in Eq. (2), Z measures the distance of x from X_{ave} in terms of SD. This is also called standardization of the data. The mean and standard deviation values of the standardized data set become 0 and SD of 1, respectively; meanwhile the shape properties of the original data set is retained (MATLAB R2012b). By using Z, data sets with different units can be put on the same scale before further analysis. Thus, this merit of Z analysis will ease the comparison of adsorption efficiencies, for the adsorption results varied greatly among OH-PAHs congeners with different numbers of rings (Fig. 3).

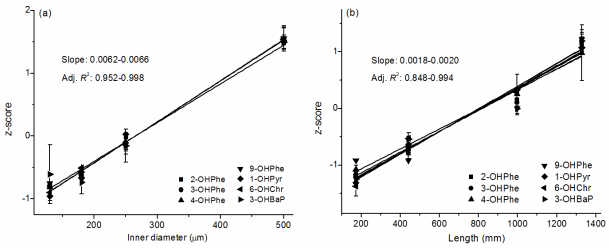

Fig. 4 Effects of (a) ID and (b) length on the adsorption of urinary OH-PAHs to the PEEK tubing.

150-cm long PEEK tubing with IDs of 130, 180, 250 and 500 μm, or 500-μm (ID) PEEK tubing with lengths of 17, 44, 100, 117 and 133 cm were used to treat 1 mL of 10 μg L^{-1} spiked hydrolyzed urine sample. *Z* was calculated based on the adsorption amounts of OH-PAHs, and then plotted against IDs or lengths. The adjusted R^2 of linear regressions resulting from the plots of ID effects (Fig. 4a) and length effects (Fig. 4b) were in the range of 0.952−0.998 and 0.848−0.994, respectively, indicating *Z* linearly increased with the increasing ID or length. Moreover, the slope values of linear regressions for ID effects (0.0062−0.0066) are more than three times higher than those for length effects (0.0018−0.0020), which implied the effects of ID are more significant than the effects of length. Values of slope and adjusted R^2 were provided in Table S4†.

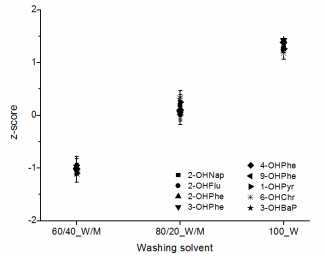

Fig. 5 Effects of solvents used to remove matrix compounds on the adsorption of OH-PAHs.

Finally, the loss of adsorbed OH-PAHs during the removal of matrix compounds was studied by using solvents with different compositions, including 60/40 (V/V) water: MeOH, 80/20 (V/V) water: MeOH and 100% water. 2 mL of 20 μg L^{-1} spiked urine sample was loaded to a 117-cm piece of PEEK tubing (500 μm ID). *Z* increased obviously with the decrease of the volume of MeOH used in the solvent (Fig. 5), and the loss of adsorbed OH-PAHs is the least while 100% water was used to remove matrix compounds.

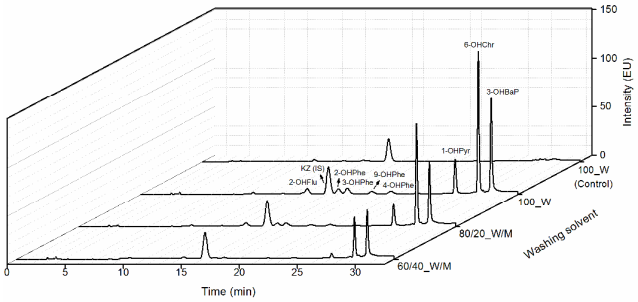

Fig. 6 HPLC-FD chromatograms of urine samples when different solvents are used to remove matrix compounds.

In addition, the baseline of the chromatogram while applying 100% water was almost the same as those by applying 60/40 or 80/20 water: MeOH (Fig. 6). As a result, when HPLC-FD is used for detection, 100% water can be used as the washing solvent, for the loss of adsorbed OH-PAHs is minimized while satisfactory removal of matrix compounds would also be obtained.

Therefore, by using a 117-cm piece of PEEK tubing (1/16 inch OD × 500 μm ID) for sample pretreatment, the method developed in this study is practical for detecting urinary OH-PAHs for occupationally exposed populations (Table S5†). As for non-occupationally exposed people, LOQs of the method need to be further improved by: (i) using PEEK tubing with smaller ID or longer length; (ii) increasing sample volume; and (iii) using other PEEK materials that could provide larger contact area. For example, a commercial product of PEEK particle with an average diameter of 500 μm (Fig. S3†) was tentatively used for sample pretreatment instead of PEEK tubing, and recoveries ranging from 22.2–88.2% were obtained for nine OH-PAHs (Table S6†). Furthermore, investigation on the interaction between OH-PAHs and PEEK material should be considered, because it can lead to better understanding of the adsorption process and benefit PEEK adsorption performance.

Conclusions

In this study, the application of PEEK as a novel SPE material to extract urinary OH-PAHs has been validated, and a facile method for fast quantification of nine urinary OH-PAHs containing 2 to 5 benzene rings in their molecular structures have been successfully developed. LOQs of the method for 4- and 5-ring OH-PAHs (1-OHPyr, 6-OHChr and 3-OHBaP) are 0.6 μg L^{-1}, which are comparable to those of conventional methods; and meanwhile much less time is consumed for sample pretreatment (2.5 min). These results suggest that the analytical strategy by using PEEK-based SPE for sample pretreatment is promising for the fast screening/quantification of urinary OH-PAHs, which is highly demanded by PAHs risk assessment. Besides, OH-PAHs with more rings (e.g. 1-OHPyr, 6-OHChr and 3-OHBaP) can be efficiently adsorbed to the PEEK material. Since congeners with more rings pose higher health risk due to their greater carcinogenic potency,[1,2] this method would be especially attractive for the risk assessment of high-risk PAHs. Finally, PEEK-based SPE may also facilitate the investigation on PAHs kinetics[34,35] and metabolism[36] related to PAHs exposure.

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Notes and references

[a] *Institute of Environmental Pollution and Health, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China.*

[b] *Institute of Atmospheric Environmental Safety and Pollution Control, Jinan University, Guangzhou 510632, China. Fax: +86-20-85225991; Tel: +86-20-85221076; E-mail: zlpamylee@gmail.com.*

† Electronic Supplementary Information (ESI) available: Figures of (i) schematic diagram of PEEK-HPLC-FD analytical system, (ii) HPLC chromatogram of the standard solution containing OH-PAHs and KZ (IS), and (iii) the commercial product of PEEK particle. Tables of (i) Ex/Em values for the detecting OH-PAHs, (ii) LOQ, and time and sample volume used for sample pretreatment when PEEK and C18 are used as

SPE material, respectively, (iii) SDs based on the measurements of urine samples, (iv) slope and adjusted R^2 (Adj. R^2) concerning ID and length effects, (v) occurrence of urinary OH-PAHs in occupationally exposed workers previously reported, and (vi) recoveries obtained for OH-PAHs when PEEK particle and tubing were used, respectively. See DOI: 10.1039/b000000x/

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