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Quantification of selected monohydroxy metabolites of polycyclic aromatic hydrocarbons in human urine

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An analytical method was developed to quantitatively determine selected monohydroxy metabolites of polycyclic aromatic hydrocarbons (PAHs) in human urine. The procedure included enzymatic hydrolysis to cleave the conjugated metabolites, solid-phase microextraction enrichment, and gas chromatography-mass spectrometry analysis. The method proved to be sensitive enough to detect the selected PAH metabolites in human urine.

polycyclic aromatic hydrocarbons, metabolites, human urine, solid-phase microextraction, GC/MS

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well known environmental pollutants, and many of them are carcinogenic or co-carcinogenic compounds. PAHs are ubiquitous in the environment, and they are formed during the incomplete combustion of organic material. Humans are mainly exposed to PAHs through occupational exposure, passive and active smoking, ingesting food and water containing PAHs, and inhaling polluted air [1]. Depending on the source of exposure, the uptake of PAHs occurs through inhalation, dermal absorption, or ingestion [2,3].

A useful and direct method for assessing the exposure to and uptake of PAHs by humans is to measure PAH metabolites in urine [4–6]. Methods have been developed to directly measure the concentrations of specific PAH metabolites in urine. Concentrations of PAH metabolites in urine give a more accurate estimate of the amounts of the parent PAHs that have been taken up by an individual than concentrations of parent PAHs in ambient air give. This is because the PAH metabolite concentrations in urine allow the total internal PAH doses from exposure to PAHs through the inhalation, ingestion, and other routes to be estimated [7]. It is often difficult to determine PAH metabolites because they are present at trace concentrations and are difficult to separate. A number of analytical methods, including high-performance liquid chromatography (HPLC) [8–15], liquid chromatography-mass spectrometry [16], liquid chromatography-tandem mass spectrometry [17–20], and gas chromatography-mass spectrometry (GC/MS) [21], have been used to determine PAH metabolites in urine samples to allow the metabolites to be used as biomarkers for PAH exposure and allow the potential effects of PAHs on human health to be investigated. Most methods for analyzing PAH metabolites in urine are similar to the method that was published by Jongeneelen *et al*. in 1987 [22]. Studies of PAH metabolites that were performed using HPLC and GC/MS have been summarized by Jongeneelen.

Although HPLC and liquid chromatography-mass spectrometry methods have been widely used to analyze PAH metabolites, there is some interest in using GC/MS to determine PAH metabolites. Analytes are generally separated more effectively using GC than using LC, but the sample

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preparation techniques that have been used in published GC methods are time-consuming and require considerable amounts of solvents and other materials [23,24]. Moreover, the separation of PAH metabolites using GC always requires the metabolites to be derivatized before analysis [25–27]. The aim of the work was to develop a simple method for determining selected monohydroxy metabolites of PAHs in human urine without the need to derivatize the metabolites.

2 Experimental

2.1 Chemicals and solvents

Seven target PAH metabolites, 1-hydroxypyrene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 9-phenanthrol, 1-naphthol, and 2-naphthol, were obtained from Sigma-Aldrich (USA). Acetonitrile (chromatographic grade) was obtained from Tedia (USA). A stock standard solution of each PAH metabolite (at a concentration of 1000 mg/L) was prepared in acetonitrile.

β-Glucuronidase/arylsulfatase (type HP-2, from *Helix pomatia*) was purchased from Sigma-Aldrich (USA). Sodium acetate (analytical grade) was obtained from Beijing Chemical Factory (China). Pure (HPLC grade) water was obtained by purifying deionized water using a Milli-Q system (EMD Millipore, USA) fitted with a 0.22 μm glass fiber filter.

2.2 Urine sample collection

Male and female chemistry research students (22–32 years old) from Beijing Lab provided urine samples. The students were informed of the objectives of the study. The students were divided into two groups, one containing the students (performing complex sample pretreatment and chromatographic analysis on PAHs and so on) that were likely to have been affected by sources of pollution and the other containing the students (performing cell analysis) that did not live near to sources of pollution. The urine specimens were collected just before and just after the traditional Chinese Spring Festival holiday. The samples were collected at 10 a.m., after the volunteers had eaten breakfast, and they were kept at −20 °C until they were analyzed.

2.3 Sample pretreatment

A schematic of the sample pretreatment procedure is shown in Figure 1. A 3 mL aliquot of a urine sample was added to 5 μL of β-glucuronidase/arylsulfatase and 5 mL of 0.1 mol/L

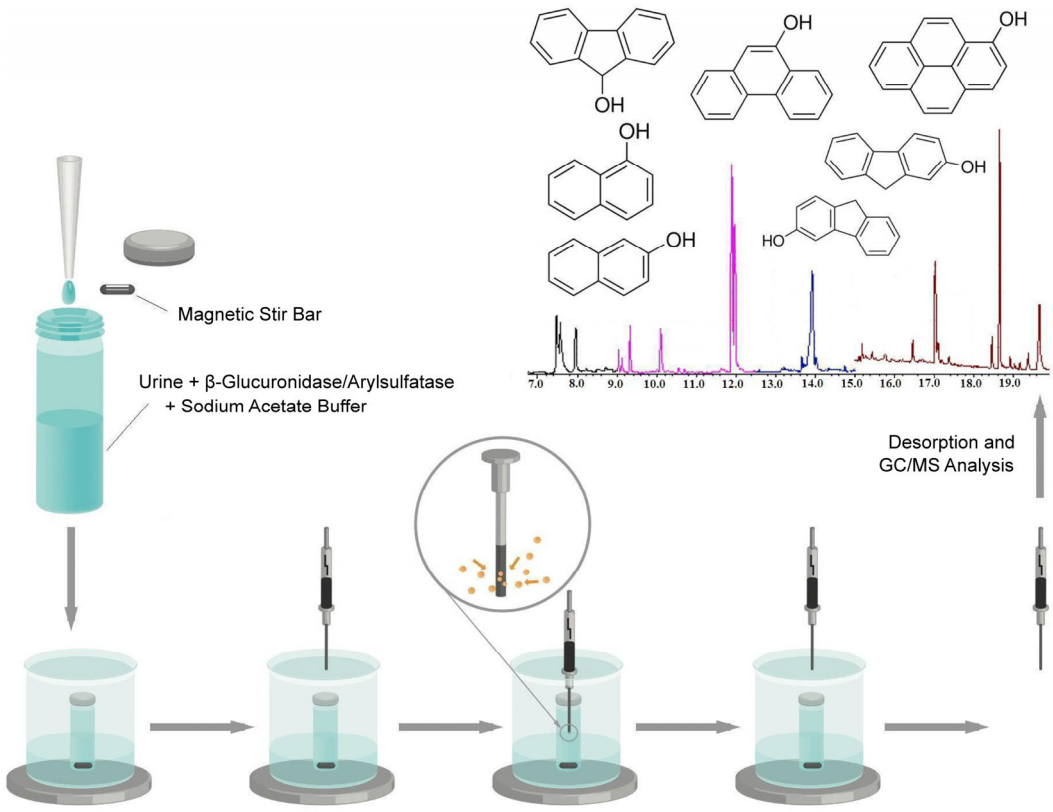

Figure 1 Schematic of the sample pretreatment procedure. Urine was added to β-glucuronidase/arylsulfatase and sodium acetate buffer. The buffered sample was then enzymatically hydrolyzed at 37 °C for about 2 h. Solid phase microextraction (SPME) was then performed by immersing an SPME fiber (coated with a 85 μm thick polyacrylate layer) into the liquid sample (kept at 35 °C) for 45 min while the sample was magnetically stirred. The SPME fiber was then analyzed by transferring it to a hot gas chromatograph injection port and allowing the chemicals it had sorbed to desorb for 3 min.

sodium acetate buffer (pH 4.97). A magnetic stirring bar that had been carefully cleaned was placed in the sample vial, and the vial was sealed with a crimped cap with a PTFE/silicon septum. The buffered sample was then enzymatically hydrolyzed at 37 °C for about 2 h and stored at −20 °C until it was analyzed.

A fiber coated with an 85 μm thick polyacrylate layer (Shanghai ANPEL Scientific Instrument Co., China) was used for the SPME procedure. The SPME fiber was desorbed for 3 min at 270 °C before each use in an offline GC injector inlet. The SPME procedure was performed by immersing the fiber into the liquid sample (kept at 35 °C) for 45 min while the sample was magnetically stirred. The sample was either urine or urine spiked with a standard solution. The fiber was then analyzed by transferring it to the hot GC injection port and allowing the chemicals it had sorbed to desorb for 3 min.

2.4 Optimized GC/MS conditions

The PAH metabolites were determined using a GC/MS QP2010 instrument (Shimadzu, Japan). Separation was achieved using an RTX-5MS fused silica capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness; Restek, USA). The carrier gas was helium. The initial GC oven temperature was 100 °C, which was held for 2 min; this then increased to 160 °C (at a rate of 15 °C/min) followed by an increase to 295 °C (rate 10 °C/min). This final temperature of 295 °C was held for 0.5 min. The split/splitless GC injector was kept at 270 °C. The mass spectrometer was operated in electron impact ionization mode, and the ionization energy was 70 eV. The ion source temperature was 250 °C, and the GC-MS interface was kept at 280 °C. The analyses were performed in selected ion monitoring mode. The typical retention times that were found and the *m*/*z* ratios of the quantification and confirmation ions for the PAH metabolites are shown in Table 1.

3 Results and discussion

3.1 Quality control and assurance

The PAH metabolites in each sample were identified and quantified by comparing the retention times and heights of the peaks in the sample chromatogram and the PAH metabolite peaks in the calibration standard chromatograms. The calibration standards were seven target metabolites spiked into urine samples at different concentrations. The calibration curves had correlation coefficients of 0.9919–0.9998.

The recoveries of PAH metabolites spiked into urine were determined. Three spiked samples were prepared and unspiked samples were also analyzed. The mean concentration in the unspiked samples was subtracted from the mean concentration in the spiked recovery samples, and the recoveries of the target PAH metabolites were 85%–118%.

The limits of detection were defined as the PAH metabolite standard concentration that gave a signal-to-noise ratio ⩾3 in selected ion monitoring mode. The limits of detection for the seven PAH metabolites were between 0.03 and 0.16 ng/mL.

3.2 Analysis of PAH metabolites in urine

Total ion chromatograms of a spiked urine sample (with a 1 ng/mL PAH metabolite standard added) and the unspiked urine sample are shown in Figure 2. The pretreatment procedure and the GC/MS conditions needed to be optimized as carefully as possible to allow the PAH metabolites to be well separated and have good peak shapes. The PAH metabolite concentrations found in ten different urine specimens provided by research students are shown in Table 2.

The data shown in Table 2 are summarized as histograms in Figures 3 and 4. The total PAH metabolite concentrations in the urine samples from three volunteers before and after the Spring Festival are shown in Figure 3. Different concentrations were found in the samples from the three volunteers, but the concentrations were higher in the sample taken before the Spring Festival than in the sample taken after the Spring Festival for all three of the volunteers. This was possible because Beijing is more seriously polluted than are other cities, and the three students left Beijing to other cities (which air quality index values are lower than Beijing e.g. Taizhou in Jiangsu Province, Qingdao in Shandong Province) for the Spring Festival. The total PAH metabolite concentrations found in the samples provided by the volunteers are shown in Figure 4 with the volunteers divided into two groups, those that were most likely to be affected by

Table 1 Typical retention times that were found and the *m*/*z* ratios of the quantification and confirmation ions for the polycyclic aromatic hydrocarbon metabolites

	Component	Retention time (min)	Quantification ion (*m*/*z*)	Confirmation ions (*m*/*z*)
1	1-Naphthol	7.463	144	115, 116
2	2-Naphthol	7.565	144	115, 116
3	9-Hydroxyfluorene	10.098	182	181, 152
4	3-Hydroxyfluorene	11.892	182	181, 152
5	2-Hydroxyfluorene	11.966	182	181, 152
6	9-Phenanthrol	13.927	194	165, 166
7	1-Hydroxypyrene	17.025	218	189, 94

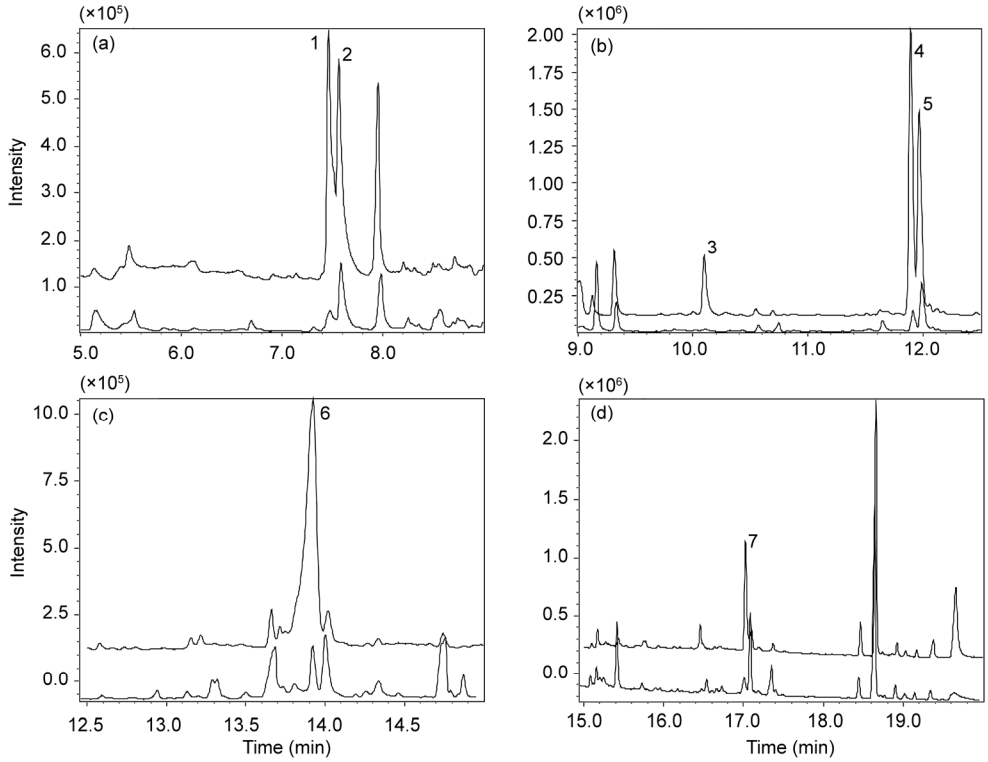

Figure 2 Total ion chromatograms of a spiked urine sample (with a 1 ng/mL polycyclic aromatic hydrocarbon (PAH) metabolite standard added) (upper chromatogram) and the unspiked urine sample (lower chromatogram). The four sections of the chromatogram ((a)–(d)) are provided so that all of the PAH metabolites are shown. Peak 1: 1-naphthol; Peak 2: 2-naphthol; Peak 3: 9-hydroxyfluorene; Peak 4: 3-hydroxyfluorene; Peak 5: 2-hydroxyfluorene; Peak 6: 9-phenanthrol; Peak 7: 1-hydroxypyrene.

Table 2 Polycyclic aromatic hydrocarbon metabolite concentrations (ng/mL) in the urine specimens that were analyzed

Target metabolite	Urine specimen [a]									
	No. 1		No. 2		No. 3		No. 4		No. 5	
	before	after	before	after	before	after	before	after	before	after
1-Naphthol	ND [b]	ND	ND	ND	0.11	0.06	ND	ND	0.1	ND
2-Naphthol	0.32	ND	ND	0.05	0.28	0.21	0.07	0.31	0.46	0.42
9-Hydroxyfluorene	ND	ND	ND	ND	0.43	0.95	0.26	ND	ND	0.34
3-Hydroxyfluorene	0.09	ND	ND	ND	0.09	0.08	0.04	0.06	0.07	ND
2-Hydroxyfluorene	0.36	0.11	ND	ND	0.47	0.46	0.09	0.26	0.21	0.22
9-Phenanthrol	0.29	0.22	ND	0.25	0.55	0.51	ND	0.31	0.4	0.31
1-Hydroxypyrene	0.2	ND	ND	ND	0.19	0.15	ND	0.14	0.23	0.57
	No. 6		No. 7		No. 8		No. 9		No. 10	
	before	after	before	after	before	after	before	after	before	after
1-Naphthol	0.1	ND	0.06	ND	ND	ND	ND	ND	ND	0.61
2-Naphthol	0.43	0.07	0.09	0.05	0.22	0.06	0.28	0.12	ND	4.23
9-Hydroxyfluorene	ND	ND	ND	ND	0.28	ND	1.39	ND	ND	0.12
3-Hydroxyfluorene	0.09	ND	ND	ND	ND	ND	0.12	0.05	0.05	0.44
2-Hydroxyfluorene	0.36	ND	0.18	0.14	0.38	0.08	0.71	0.31	0.15	0.81
9-Phenanthrol	0.65	0.2	0.28	0.25	0.18	ND	0.6	0.26	0.34	3.05
1-Hydroxypyrene	0.15	ND	ND	ND	ND	ND	0.15	ND	0.8	1.65

a) Before, sample collected before the Spring Festival; after, sample collected after the Spring Festival. b) ND, not detected.

sources of pollution and those that lived far from sources of pollution (each column represents the concentration found in one urine specimen). Three of the volunteers were included in the first group (likely to be affected by sources of pollution who performing complex sample pretreatment and chromatographic analysis on PAHs and so on) and four volunteers were in the second group (those living far from sources of pollution, and performing cell analysis). The

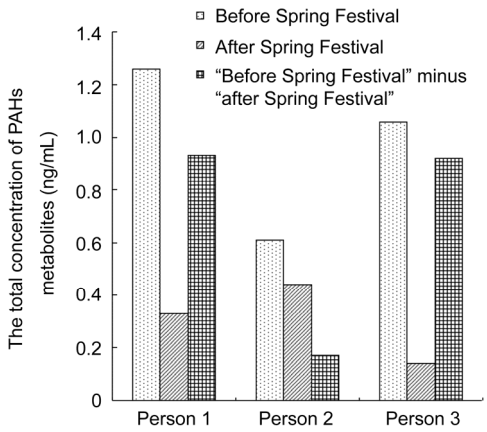

Figure 3 Total polycyclic aromatic hydrocarbon (PAH) metabolite concentrations in urine samples provided before and after the Spring Festival by three volunteers.

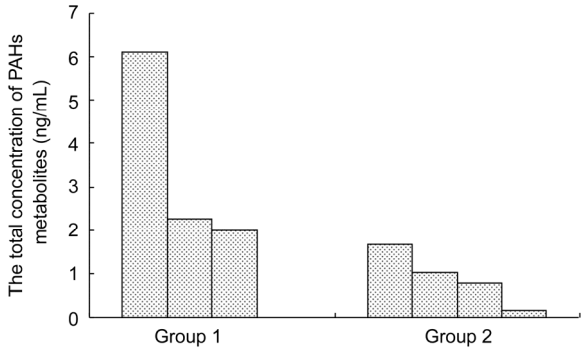

Figure 4 Total polycyclic aromatic hydrocarbon (PAH) metabolite concentrations in the two groups of volunteers. The volunteers in Group 1 were likely to be exposed to sources of pollution and the volunteers in Group 2 lived far from sources of pollution.

PAH metabolite concentrations were higher in the samples from all of the volunteers in the first group than in the samples from all of the volunteers in the second group, and the mean PAH metabolite concentrations in the samples from the two groups were very different. One volunteer in the second group was a special case. The PAH metabolite concentrations were lower in the urine samples provided by this volunteer than in the samples provided by any of the other volunteers. This was because this volunteer routinely wore a gas mask to decrease the amounts of pollutants inhaled. We concluded that it is likely to be beneficial to the health of an individual if he or she takes measures to protect against the inhalation of pollutants and spends as much time as possible far away from sources of pollution.

4 Conclusions

Measuring PAH metabolites in human urine is the preferred way of determining the recent exposure of an individual to PAHs, especially when multiple exposure routes have to be taken into account. The method presented here, which did not include a derivatization step, allowed hydroxylated PAH metabolites to be selectively enriched from a complex matrix before being analyzed. The limits of detection were sufficient for the PAH metabolites present in human urine to be quantified. The uncomplicated sample handling procedure and the robustness of the method allowed the method to be used for the reliable and rapid analysis of PAH metabolites in human urine. The method may allow other metabolites to be identified in human urine in the future.

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1 Liu Y, Hashi Y, Lin JM. Continuous-flow microextraction and gas chromatographic-mass spectrometric determination of polycyclic aromatic hydrocarbon compounds in water. *Anal Chim Acta*, 2007, 585: 294–299
2 Li K, Li HF, Maeda T, Lin JM. Solid-phase extraction with C30-bonded silica for analysis of polycyclic aromatic hydrocarbons in airborne particulate matters by gas chromatography-mass spectrometry. *J Chromatogr A*, 2007, 1154: 74–80
3 Li Z, Mulholland JA, Romanoff LC, Pittman EN, Trinidad DA, Lewin MD, Sjödin A. Assessment of non-occupational exposure to polycyclic aromatic hydrocarbons through personal air sampling and urinary biomonitoring. *J Environ Monit*, 2010, 12: 1110–1118
4 Strickland PT, Kang D, Bowman ED, Fitzwilliam A, Downing TE, Rothman N, Groopman JD, Weston A. Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas chromatography-mass spectrometry. *Carcinogenesis*, 1994, 15: 483–487
5 Zhu L, Xu H. Magnetic graphene oxide as adsorbent for the determination of polycyclic aromatic hydrocarbon metabolites in human urine. *J Sep Sci*, 2014, 37: 2591–2598
6 Jacob P, Wilson M, Benowitz N. Determination of phenolic metabolites of polycyclic aromatic hydrocarbons in human urine as their pentafluorobenzyl ether derivatives using liquid chromatography-tandem mass spectrometry. *Anal Chem*, 2007, 79: 587–598
7 Jacob J, Seidel A. Biomonitoring of polycyclic aromatic hydrocarbons in human urine. *J Chromatogr B*, 2002, 778: 31–47
8 Hollender J, Koch B, Dott W. Biomonitoring of environmental polycyclic aromatic hydrocarbon exposure by simultaneous measurement of urinary phenanthrene, pyrene and benzo[*a*]pyrene hydroxides. *J Chromatogr B*, 2000, 739: 225–229
9 Kim H, Cho SH, Kang JW, Kim YD, Nan HM, Lee CH, Lee H, Kawamoto T. Urinary 1-hydroxy pyrene and 2-naphthol concentrations in male Koreans. *Int Arch Occup Environ Health*, 2001, 74: 59–62
10 Kuusimaki L, Peltonen Y, Mutanen P, Peltonen K, Savela K. Urinary hydroxy-metabolites of naphthalene, phenanthrene and pyrene as markers of exposure to diesel exhaust. *Int Arch Occup Environ Health*, 2004, 77: 23–30
11 Schedl M, Wilharm G, Achatz S, Kettrup A, Niessner R, Knopp D. Monitoring polycyclic aromatic hydrocarbon metabolites in human urine: extraction and purification with a sol-gel glass immunosorbent. *Anal Chem*, 2001, 73: 5669–5676
12 Li H, Krieger RI, Li QX. Improved HPLC method for analysis of 1-hydroxypyrene in human urine specimens of cigarette smokers. *Sci Total Environ*, 2000, 257: 147–153
13 Toriba A, Chetiyanukornkul T, Kizu R, Hayakawa K. Quantification of 2-hydroxyfluorene in human urine by column-switching high per-

formance liquid chromatography with fluorescence detection. *Analyst*, 2003, 128: 605–610
14 Li YP, Li X, Zhou Z. A novel facile method using polyetheretherketone as a solid phase extraction material for fast quantification of urinary monohydroxylated metabolites of polycyclic aromatic hydrocarbons. *RSC Adv*, 2014, 4: 39192–39196
15 Barbeau D, Maître A, Marques M. Highly sensitive routine method for urinary 3-hydroxybenzo[*a*]pyrene quantitation using liquid chromatography-fluorescence detection and automated off-line solid phase extraction. *Analyst*, 2011, 136: 1183–1191
16 Ferrari S, Mandel F, Berset JD. Quantitative determination of 1-hydroxypyrene in bovine urine samples using high-performance liquid chromatography with fluorescence and mass spectrometric detection. *Chemosphere*, 2002, 47: 173–182
17 Li YH, Li AC, Shi HH, Zhou SL, Shou WZ, Jiang XY, Weng ND, Lauterbach JH. The use of chemical derivatization to enhance liquid chromatography/tandem mass spectrometric determination of 1-hydroxypyrene, a biomarker for polycyclic aromatic hydrocarbons in human urine. *Rapid Commun Mass Sp*, 2005, 19: 3331–3338
18 Li X, Zenobi R. Use of polyetheretherketone as a material for solid phase extraction of hydroxylated metabolites of polycyclic aromatic hydrocarbons in human urine. *Anal Chem*, 2013, 85: 3526–3531
19 Li X, Fang XW, Yu ZQ, Sheng GY, Wu MH, Fu JM, Chen HW. Direct analysis of urinary 1-hydroxypyrene using extractive electrospray ionization ion trap tandem mass spectrometry. *Anal Methods*, 2013, 5: 2816–2821
20 Yao L, Yang J, Liu BZ, Zheng SJ, Wang WM, Zhu XL, Qian XB. Development of a sensitive method for the quantification of urinary 3-hydroxybenzo[*a*]pyrene by solid phase extraction, dansyl chloride derivatization and liquid chromatography-tandem mass spectrometry detection. *Anal Methods*, 2014, 6: 6488–6493
21 Grimmer G, Jacob J, Dettbarn G, Naujack KW. Determination of urinary metabolites of polycyclic aromatic hydrocarbons (PAH) for the risk assessment of PAH-exposed workers. *Int Arch Occup Environ Health*, 1997, 69: 231–239
22 Jongeneelen FJ, Anzion RB, Henderson PT. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatogr*, 1987, 413: 227–232
23 Grimmer G, Jacob J, Dettbarn G, Naujack KW, Heinrich U. Urinary metabolite profile of PAH as a potential mirror of the genetic disposition for cancer. *Exp Toxic Pathol*, 1995, 47: 421–427
24 Yang WJ, Wang YW, Zhou QF, Tang HR. Analysis of human urine metabolites using SPE and NMR spectroscopy. *Sci China Chem*, 2008, 51: 218–225
25 Smith CJ, Walcott CJ, Huang WL, Maggio V, Grainger J, Patterson DG. Determination of selected monohydroxy metabolites of 2-, 3- and 4-ring polycyclic aromatic hydrocarbons in urine by solid-phase microextraction and isotope dilution gas chromatography-mass spectrometry. *J Chromatogr B*, 2002, 778: 157–164
26 Gmeiner G, Krassnig C, Schmid E, Tausch H. Fast screening method for the profile analysis of polycyclic aromatic hydrocarbon metabolites in urine using derivatisation-solid-phase microextraction. *J Chromatogr B*, 1998, 705: 132–138
27 Gmeiner G, Gartner P, Krassnig C, Tausch H. Identification of various urinary metabolites of fluorene using derivatization solid-phase microextraction. *J Chromatogr B*, 2002, 766: 209–218