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Quantification of 1-hydroxypyrene in undiluted human urine samples using magnetic solid-phase extraction coupled with internal extractive electrospray ionization mass spectrometry

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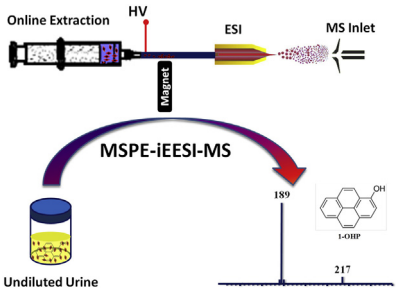
HIGHLIGHTS

- Coupling of magnetic solid-phase extraction with internal extractive electrospray ionization mass spectrometry is shown.
- 1-Hydroxypyrene in raw human urine samples was effectively enriched by polypyrrole-coated Fe_3O_4 magnetite nanocomposites.
- High throughput quantitative detection of urinary 1-OHP for health risk assessment of PAHs exposure was achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental contaminants raising worldwide concerns due to their carcinogenic effects. In this study, 1-hydroxypyrene (1-OHP, the most widely used biomarker of internal dose of PAHs exposure) in undiluted human urine samples (10 mL) was selectively enriched by polypyrrole-coated Fe_3O_4 magnetite nanocomposites (termed as Fe_3O_4@Ppy, 1 mg) and then directly eluted by the electrospraying solvent (acetone/benzene/acetic acid (v/v/v, 90/10/1); 100 uL) biased with −3.5 kV to produce the deprotonated 1-OHP anions for mass spectrometric analysis. The method established here significantly improved the current performance for detection of urinary 1-OHP, providing the speed for a single sample analysis within 4 min, the limits of detection (LOD) of 0.0001 μg L^{-1}, the linear response range of 0.001–5.000 μg L^{-1} ($R^2 = 0.9994$), recovery rates of 90.6–96.1%, and relative standard deviation (RSD, n = 6) values between 2.9% and 8.0%. Human samples including raw human urine collected from 10 healthy volunteers (5 smokers and 5 nonsmokers) and 7 lung cancer patients have been successfully analyzed, showing that magnetic solid-phase extraction (MSPE) coupled with internal extractive electrospray ionization mass spectrometry (iEESI-MS) is an

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Magnetic solid-phase extraction (MSPE)
Health risk assessment

alternative strategy for high throughput quantitative detection of urinary 1-OHP for health risk assessment of PAHs exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the most widespread organic pollutants, which are of worldwide concern due to their carcinogenic, mutagenic, and teratogenic toxicity [1,2]. PAHs in the environment are found primarily in soil, sediment, oily substances, and particulate matter suspended in air. In addition to their production from fossil fuels, they are also formed by a variety of incomplete combustion of carbon-containing fuels such as wood, fat, and tobacco [3–5]. Moreover, studies have shown that high levels of PAHs are also found in cooked foods, for example, in meat cooked at high temperature such as barbecuing or grilling [6,7]. Great concerns have been raised on the toxicity of PAHs due to their carcinogenic, mutagenic, and teratogenic effects [1,2]. Thus, evaluation of the internal dose of PAHs in human body is of vital importance for health risk assessment of PAHs exposure. Among many biomarkers for the evaluation of PAHs exposure, urinary 1-Hydroxypyrene (1-OHP, the main metabolite of pyrene) is the most widely used biomarker for evaluating of the internal dose of PAHs exposure from multiple routes [8,9].

To date, conventional analytical methods including fluorescence spectroscopy [10], liquid chromatography-mass spectrometry (LC-MS) [11], gas chromatography-mass spectrometry (GC–MS) [12], immunochemical assays [13], and electrochemical sensor technologies [14] have been applied in the urinary 1-OHP analysis. However, laborious multistep sample pretreatments (e.g., centrifugation, diluting, and chemical extraction etc.) were routinely required in previously reported strategies, which limited the high-throughput analysis of urinary 1-OHP. Clearly, facile and efficient strategy for the analysis of 1-OHP is urgently demanded in high-throughput evaluation of internal dose of PAHs exposure.

Ambient ionization mass spectrometry (MS) has emerged as a versatile solution for high-throughput analysis of practical raw samples with merits of high speed, good selectivity, high sensitivity, and minimum sample pretreatment [15–17]. Ambient ionization methods including desorption electrospray ionization (DESI) [18], direct analysis in real time (DART) [19], laser ablation electrospray ionization (LAESI) [20], low temperature plasma (LTP) [21], dielectric barrier discharge ionization (DBDI) [22], extractive electrospray ionization (EESI) [23], and paper spray [24], desorption atmospheric pressure chemical ionization (DAPCI) [25], etc., are attracting more and more attention in the community of analytical study, with dramatically improved performance for qualitative analysis. However, quantification analysis of trace analytes in complex raw samples requires more efforts, especially in cases where trace levels of analytes (e.g., urinary 1-OHP) must be quantitatively measured with high throughput. Therefore, fast and easy-to-use sample pretreatment methods are preferably introduced in coupling with ambient ionization mass spectrometry. For example, suitable sample preparation methods, such as solid-phase microextraction (SPME) [26], paper chromatography (PC) [27], thin layer chromatography (TLC) [28], and thin-film microextraction (TFME) [29], etc., have been introduced prior to ambient ionization without affecting analytical efficiency.

In this study, polypyrrole-coated Fe_3O_4 magnetite (termed as Fe_3O_4@Ppy) was employed as the sorbent for magnetic solid-phase extraction (MSPE) of urinary 1-OHP in undiluted human urine samples, and then the Fe_3O_4@Ppy material was treated as a bulk sample, which was directly analyzed by internal extractive electrospray ionization mass spectrometry (iEESI-MS) [30–32]. Urinary 1-OHP levels in undiluted human urine samples donated by 10 healthy volunteers and 7 lung cancer patients were successfully measured in this study, suggesting that the procedure proposed here could be a promising strategy for rapid detection of urinary 1-OHP required for health risk assessment of PAHs exposure.

2. Experimental section

2.1. Chemicals and materials

1-Hydroxypyrene was purchased from AuccStandard Inc. (New Haven, CT 06513, U.S.A.) with the highest grade available. Both methanol and acetic acid were HLPC grade and bought from ROE Scientific Inc. (Newark, U.S.A). Acetone, benzene, pyrrole, sodium dodecyl sulfate (SDS), ethylene glycol (EG), ethanol, ethylene diamine (ED), ferric trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferric chloride ($FeCl_3$), and sodium acetate (NaAc) were purchased from Sinopharm Chemical Reagent (Shanghai, China). Deionized water used for the experiments was provided by ECUT chemistry facility.

2.2. Preparation and characterization of polypyrrole-coated Fe_3O_4 magnetite nanocomposites

Polypyrrole-coated Fe_3O_4 magnetitc nanocomposites were prepared according to our previous publication [33,34]. Firstly, Fe_3O_4 magnetic nanoparticles were obtained. In the preparation of Fe_3O_4 magnetic nanoparticles, NaAc (15.0 g) and ED (50 mL) were added in 100 mL $FeCl_3 \cdot 6H_2O$ ethylene glycol solution (0.05 g/mL). After vigorous vortexing for 30 min, the homogeneous mixture was sealed in a Teflon-lined stainless-steel autoclave (200 mL) under 200 °C heating condition for 8 h, and then cooled to room temperature (25 °C). The collected product was cleaned several times using water/ethanol for the matrixes clean up, and then vacuum-dried at 60 °C for 6 h. Thus, Fe_3O_4 magnetic nanoparticles were obtained. Secondly, the produced Fe_3O_4 magnetic nanoparticles were then coated with pyrrole according to the method described by Luo et al. with some modifications [35]. Mixture of Fe_3O_4 (1.0 g), FeCl3 (9.1 g), and deionized water (100 mL) were loaded to a 250 mL flask under continuously shaken (150 shakes/min) in a water bath at 25 °C for 3 h, and then pyrrole (0.5 mL) and SDS solution (20 mL, 5.85 wt%) were added in the mixture. The mixture was keeping shaking for an additional 10 h. The final product was magnetically collected, and cleaned using water/ethanol repeatedly for the matrixes clean up before vacuum-dried (at 60 °C for 6 h).

The property of the Fe_3O_4@Ppy nanocomposite has been reported in our recently literature [33,34,36]. The Fe_3O_4@Ppy nanocomposites were characterized by using a JEM-100CXII transmission electron microscope (TEM, Jeol, Japan) and a Thermo Nicolet 670FT-IR spectrometer (Boston, U.S.A.). TEM images showed that the morphologies of Fe_3O_4 nanoparticles were well-dispersed and had a uniform size distribution with a spherical diameter of about 65 nm. IR spectra of Fe_3O_4@Ppy nanocomposites showed the bands at 1552 and 1040 cm^{-1} could be assigned to the C–N ring stretching vibrations of the pyrrole ring, and the peaks at

1175, 894, and 785 cm^{-1} were related to the C–H in-plane and out-of plane vibrations. These results are consistent with literature [33,35], which further demonstrating the existence of Ppy on the Fe_3O_4 particles.

2.3. Urine samples

Human urine samples were donated by 10 healthy volunteers from ECUT campus (including 5 smokers and 5 nonsmokers), and 7 lung cancer patients (used have smoking habit, but quitted at least 3 months ago). Urine samples of the lung cancer patients were provided by Second Affiliated Hospital of Nanchang University with all patients' informed consent. The clinical investigations of lung cancer patients' urine samples were approved by the Medical Ethnics Committee in the Hospital Institutional Review Board of the Second Affiliated Hospital to Nanchang University, Nanchang, P. R. China, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The collected urine samples were contained in 100 mL glass sample vials, and stored at −80 °C in refrigerator before use.

The urine samples were thawed at room temperature (24 °C). For the purpose of preparing a blank urine sample to establish quantitative method of MSPE-iEESI-MS, urine samples from non-occupationally exposed volunteer were selected, and any potential 1-OHP in the urine sample (10 mL) was removed by adding plenty of Fe_3O_4@Ppy nano-material (1 g) inside for a MSPE process. Then, the suspect Fe_3O_4@Ppy nano-materials were removed with the assistance of an external magnet. Thus, the residual urine after removing 1-OHP was used as a blank urine sample. A series of 1-OHP standard solutions containing 0–5 μg L^{-1} of 1-OHP was prepared by serial dilution of 200 μg L^{-1} of 1-OHP in methanol solution. No centrifugation, filtration, or dilution was performed on any samples.

2.4. Magnetic solid phase extraction coupled with iEESI-MS analysis

The experimental protocol of MSPE-iEESI-MS was schematically shown in Fig. 1. The MSPE was carried out in a 15-mL glass vial filled with 10 mL undiluted urine sample and 1 mg Fe_3O_4@Ppy nanocomposite. The sample mixture was heavily vortexed for 1 min for Fe_3O_4@Ppy to capture 1-OHP from the urine. After the vortexing, the suspension mixture was loaded in a syringe (volume of 5 mL), and the nanocomposites were magnetically gathered to the inner wall of the syringe with an external magnet. The urine waste was discharged into a glass beaker. After twice repeats of the collection, all the magnetic nanocomposites with 1-OHP were completely gathered on the inner wall of the syringe. To avoid the urine matrix interference during the electrospray ionization of 1-OHP, the magnetic nanocomposites inside the syringe were washed using 1 mL deionized water for twice. After loading with 100 μL extraction solution (acetone/benzene/acetic acid (v/v/v, 90/10/1); 100 μL), the syringe was gently shaken for 10 s, allowing that the 1-OHP adsorbed on Fe_3O_4@Ppy was completely eluted to form a 1-OHP solution suitable for ESI purpose. While the 1-OHP solution was pumped through a capillary for ESI, a magnet was positioned outside the capillary before the Fe_3O_4@Ppy reached the ESI nozzle (Fig. 1).

It was noted that a full retention of the magnetic nanoparticles could be realized with the assistance of a strong NdFeB magnet (N50, 1.41–1.45 T) positioned outside of the capillary. To demonstrate the full retention of the magnetic nanoparticles, an indirect proof experimental approach was designed. Different from the experimental setup of described MSPE-iEESI process, the nitrogen sheath gas was turned off and the elution solution was replaced by water (100 μL). Blank urine sample was spiked with 0.01 μg L^{-1} 1-OHP. In the first step, a 1-mL glass vial was used to collect the water elutant. Then, an NdFeB magnet was used try to magnetically collect the any potential escaped magnetic nanoparticles in the collected water eluant. The supernatant was loaded in a 200 μL syringe for electrospray mass spectrometry (ESI-MS) analysis for the detection of any potential 1-OHP in the water eluant. The result showed that non 1-OHP was detected in the water elutant, which indicated that water is not suitable of the 1-OHP elution, i.e., any potential 1-OHP molecules were still adsorbed on the surface of these potential escaped magnetic nanoparticles. In the second step, all the supernatant water was removed away from these potential escaped magnetic nanoparticles with the assistance of an external NdFeB magnet (These potential escaped magnetic nanoparticles were left on the bottom of the glass vial.). Then, elution solution of acetone/benzene/acetic acid (v/v/v, 90/10/1; 100 μL) was added in the glass vial for the elution of any 1-OHP on the surface of these potential escaped magnetic nanoparticles. After shaken of 1 min, the acetone/benzene/acetic acid (v/v/v, 90/10/1; 100 μL) eluant was separated and loaded in a syringe for ESI-MS analysis. The result showed that characteristic signal of *m/z* 189 was absent from the MS^2 spectra window of parent ion *m/z* 217. The experimental results demonstrated that there was no magnetic nanoparticles in the collected water eluant as mentioned above. Thus, a full retention of the magnetic nanoparticles was realized in the MSPE-iEESI process. Therefore, all the Fe_3O_4@Ppy were held by the external magnet and no nano-particle reached the ion entrance of the mass spectrometer instrument.

All the experiments were carried out using a linear trap quadruple (LTQ) mass spectrometer (Thermo Scientific, San Jose, CA, U.S.A.) coupled with a homemade MSPE-iEESI source. Mass spectra were collected in the mass range of *m/z* 50–400 with a negative ion detection mode. The electrospray solution was pumped through the Fe_3O_4@Ppy particles at a flow rate of 15 μL min^{-1} using a syringe pump (Harvard Apparatus, Holliston, MA, U.S.A.). The ionization voltage was set at −3.5 kV, and the heated LTQ capillary was maintained at 300 °C. The pressure of nitrogen sheath

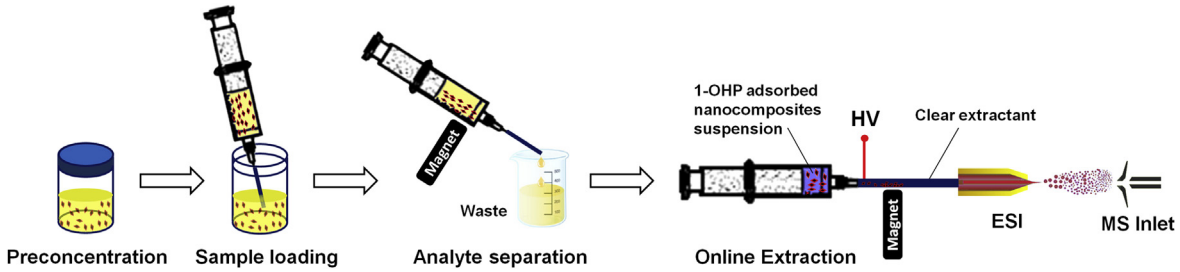

Fig. 1. Schematic illustration of the concept and protocol of MSPE-iEESI-MS.

gas was 1.2 MPa. Collision induced dissociation (CID) experiments were carried out for MS/MS analysis. During the CID experiments, precursor ions were isolated with a window width of 1.5 Da, and the activation Q (AQ) and normalized collision energy (NCE) were set to 0.40 and 40% respectively according previous study of 1-OHP [37]. Other parameters were set to default LTQ instrument values.

3. Result and discussion

3.1. Qualitative detection of urinary 1-OHP by MSPE-iEESI-MS

The MSPE-iEESI-MS full scan spectrum (*m/z* 50–400) of human urine sample has been shown in the supporting information as Fig. S1. A relative clean full mass scan spectrum was obtained, which should be attributed to matrix clean up in the MSPE-iEESI process. To exclude potential false positives, qualitative detection of authentic 1-OHP (5 μg L^{-1}) spiked into a blank urine sample was performed using MSPE-iEESI-MS/MS experiments. As the results, urinary 1-OH was detected as the deprotonated molecules of *m/z* 217 in the full scan mass spectrum (Fig. 2), which yielded characteristic fragments of *m/z* 189 (inset of Fig. 2) by the loss of CO under the collision-induced dissociation conditions, resulting in a very stable ionic 4-ring structure in the gas phase. This observation was consistent with literature [37,38].

Because the polypyrrole coatings possessed a highly π-conjugated structure and good hydrophobicity, Fe_3O_4@Ppy nanocomposites showed excellent performance for the 1-OHP extraction from various types of liquid samples [33,34,36]. For better performance, the ratio of Fe_3O_4@Ppy nanocomposites to the urine sample containing 1-OHP were optimized using undiluted human urine as the samples. In this respect, different amounts of Fe_3O_4@Ppy sorbent ranging from 0.1 to 50.0 mg were experimentally investigated using MSPE-iEESI-MS/MS. The highest signal intensity of the fragment ions (*m/z* 189) was obtained when the solid-to-liquid ratio was 1/10 mg/mL (Fig. 3a). Thus, 1.0 mg Fe_3O_4@Ppy nanocomposites was selected as optimal amount for subsequent experiments. A maximum signal obtained here should attribute to a fixed amount of urine sample solution (10 mL) and eluent (100 μL) was used in this study. In the 1-OHP adsorption case, the more analytes adsorbed on per unit mass material, the higher the signal intensity would be obtained. However, when the amount of Fe_3O_4@Ppy sorbent was low (such as 0.1/10 (mg/mL) in this study), the quantity of Fe_3O_4@Ppy sorbent were inadequate to catch all the 1-OHP molecules in the urine sample. Therefore, the signal intensity of the fragment ions (*m/z* 189) was increased with the increasing amount of Fe_3O_4@Ppy sorbent used for the 1-OHP adsorption. While, in the 1-OHP elution case, when the amount of Fe_3O_4@Ppy sorbent was extremely large (such as 50/10 (mg/mL) in this study), the fixed 100 μL eluent would be not enough to obtain an efficient elution. Also, the aggregation effect of magnetic Fe_3O_4@Ppy sorbent would be more serious when the density of Fe_3O_4@Ppy sorbent was too high (such as 50 mg Fe_3O_4@Ppy sorbent in 100 μL eluent for the case of 50/10 (mg/mL)), which hinders the elution of 1-OHP. Thus, a lower 1-OHP signal level was obtained. The volume of the extraction solution (i.e., the solution for electrospray ionization) for elution of 1-OHP from the Fe_3O_4@Ppy nanocomposites, the composite of the solution, and the flow rate of the solution were also optimized to achieve the highly efficient elution and good ionization efficiency. Considering the parameters such as chemical property of 1-OHP, polypyrrole coated Fe_3O_4@Ppy nanocomposites, and ionization efficiency, solvents of a relatively high hydrophobicity were preferred for the 1-OHP extraction. Tentative elution solutions including methanol, acetone, acetone/benzene, and acetone/benzene/acetic acid were tested. Finally, optimized solution of acetone/benzene/acetic acid (v/v/v, 90/10/1; 100 μL) at a flow rate of 15 μL min^{-1} was employed for MSPE-iEESI analysis, since the solution showed satisfactory performance to improve the target signal of *m/z* 189 (Fig. 3b).

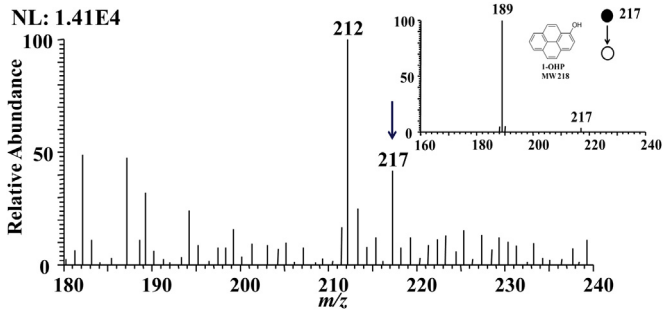

Fig. 2. Mass spectral signals of 1-OH spiked into human urine (5 μg L^{-1}). The inset was the MS/MS spectrum of the isolated ions of *m/z* 217.

3.2. Quantitative analysis of urinary 1-OHP

A series of blank urine samples (10 mL) was spiked with 1-OHP standard solutions to make working solutions containing 0.0005–5.0000 μg L^{-1} of 1-OHP for MSPE-iEESI-MS/MS analysis. The signal intensity of *m/z* 189 was linearly responded with 1-OHP concentrations over the range of 0.001–5.000 μg L^{-1} ($R^2 = 0.9994$) (Fig. 4). The limit of detection (LOD) of 1-OHP in undiluted urine sample (10 mL) defined by a signal-to-noise ratio (S/N) of 3 were 0.0001 μg L^{-1}. The relative standard deviations (RSDs) of six replicates for the 1-OHP concentrations ranging from 0.001 to 5.000 μg L^{-1} were below 8.0% (Table 1). Surprisingly, sub-ppt level of 1-OHP in undiluted human urine samples could be successfully detected using MSPE-iEESI-MS/MS, which was greatly owing to the high performance of Fe_3O_4@Ppy nanocomposites in the preconcentration of 1-OHP from urine, the large volume of urine sample (10 mL), as well as the specially unified sample loading process of MSPE-iEESI. Remarkable sensitivity of urinary 1-OHP detection was provided by the proposed method, showing the potential application in the evaluation of trace level internal dose of PAHs exposure of human beings. Furthermore, the recoveries of 1-OHP in 4 practical samples were experimentally evaluated. As the result, the 1-OHP concentrations detected in the untreated raw human urine samples were 0.0195–0.0326 μg L^{-1}, and the recoveries of 1-OHP from the 4 raw urine samples were determined to be 90.6–96.1% (Table 2). Note that a single sample analysis was completed within 4 min, and the analysis throughput can be further improved if all the urine samples of a large set could be processed on MSPE simultaneously. To validate the analytical ability of MSPE-iEESI-MS, conventional method of off-line MSPE-ESI-MS was performed to detect the parallel 1-OHP spiked urine samples. As shown in Table S1, the results demonstrate that the presented MSPE-ESI-MS method is of good measurement accuracy comparing with conventional method.

3.3. Fast determination of urinary 1-OHP in practical urine samples

Human urine samples from 10 healthy volunteers (including 5 smokers and 5 nonsmokers) were selected in this study to estimate the health risks of internal dose of PAHs exposure via cigarette smoking. As shown in Table 3, the 1-OHP concentrations detected in urine samples collected from non-smokers were 0.0195–0.0326 μg L^{-1}. These values were significantly lower than those, 0.0453–0.1278 μg L^{-1}, found from the regular smokers. The

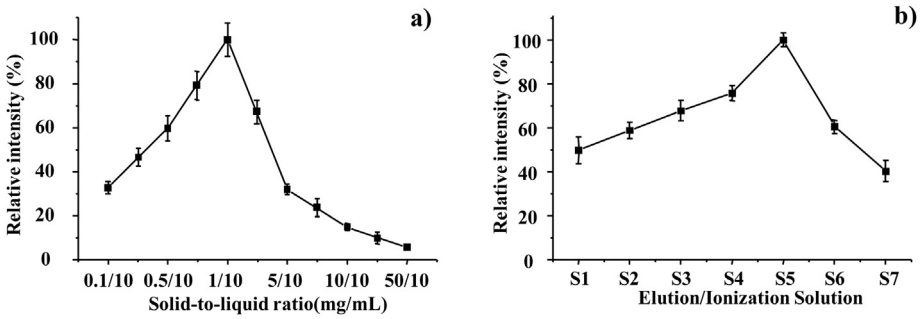

Fig. 3. Optimization of the experimental conditions. a) Signal levels with different solid-to-liquid ratio between Fe_3O_4@Ppy nanocomposites and urine solution. The total amounts of Fe_3O_4@Ppy ranging from 0.1 to 50.0 mg were applied to the blank urine samples (10 mL) spiked with 5 μg L^{-1} 1-OHP. b) Signal levels obtained from blank urine samples spiked 0.1 μg L^{-1} 1-OHP against the chemical composition of the 1-OHP elution/ionization solution (S1, methanol; S2, Acetone; S3, acetone/benzene (v/v: 9/1); S4, acetone/benzene/acetic acid (v/v/v, 90/10/0.5); S5, acetone/benzene/acetic acid (v/v/v, 90/10/1); S6, acetone/benzene/acetic acid (v/v/v, 90/10/2); S7, acetone/benzene/acetic acid (v/v/v, 90/10/5).).

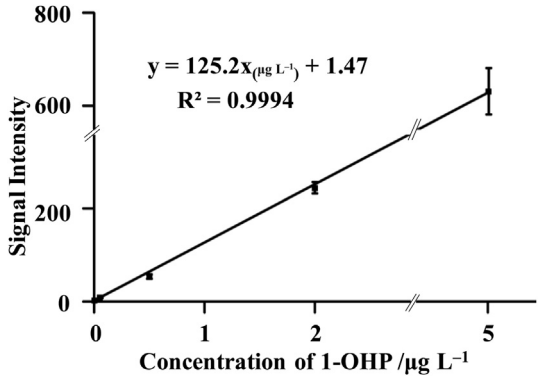

Fig. 4. The intensity levels of the characteristic fragment (*m/z* 189) of 1-OHP against the concentrations (μg L^{-1}) of 1-OHP in urine sample solution. The error bars indicate the standard deviations (RSDs) of six replicates.

experimental findings indicated that tobacco smoke is an unnegligible source of PAHs exposure. Note that the Intra-group differential data might be related with the individual metabolic differences and potential environmental exposures of PAHs (e.g., industrial pollution, vehicle exhaust, and foods habits like charbroiled meats ingestion); a large set of samples would be necessary to understand the difference better.

Cigarette smoking contributes heavily to lung cancer cases [39–41]. In this study, urine samples donated by 7 lung cancer patients (used to have smoking habit for decades, but quitted smoking for more than 3 months before the samples were collected.) were tested to evaluate the internal dose of 1-OHP. Interestingly, the 1-OHP concentrations detected in the lung cancer patients' raw urine samples were 0.0103–0.0219 μg L^{-1} (Table 3), which were similar to the levels found in healthy nonsmokers. These data indicated that the high internal dose of PAHs caused by cigarette smoking was almost metabolized within 3 months.

Table 1
Analytical results for MSPE-iEESI-MS/MS analysis of blank urine samples and spiked urine samples.

Samples (μg L^{-1})	Signal intensity of *m/z* 189 of six replicates						Average (n = 6)	SD	RSD (%)
Blank urine[a]	1.21	1.68	1.26	1.41	1.53	1.73	1.47	0.22	14.6%
0.0005[b]	2.38	2.49	2.87	2.70	2.69	2.86	2.67	0.20	7.4%
0.0010[b]	2.71	2.73	2.74	2.72	2.84	2.91	2.78	0.08	2.9%
0.0500[b]	10.20	9.62	9.26	9.11	9.43	8.31	9.32	0.62	6.7%
0.5000[b]	56.90	53.90	58.40	58.70	48.90	49.80	54.43	4.30	7.9%
2.0000[b]	247.00	251.00	248.00	223.00	242.00	259.00	245.00	12.15	5.0%
5.0000[b]	603.00	601.00	596.00	710.00	780.00	597.00	631.17	50.41	8.0%

[a] Blank urine was obtained by adding plenty of (1 g) Fe_3O_4@Ppy nano-material inside for a MSPE process.
[b] Blank urine was spiked with a series concentration (μg L^{-1}) of 1-OHP.

Table 2
Recoveries obtained for MSPE-iEESI-MS/MS analysis of urinary 1-OHP.

Practical urine samples	Three replicates	$C_{unspiked}$ [a] (μg L^{-1})	Mean (n = 3)	SD	C_{spiked} [b] (μg L^{-1})	Mean (n = 3)	SD	Recovery (%)
Urine 1	1	0.0268	0.0244	0.0021	0.1272	0.1195	0.0076	95.1%
	2	0.0231			0.1192			
	3	0.0234			0.1121			
Urine 2	1	0.0254	0.0275	0.0018	0.1113	0.1192	0.0076	91.7%
	2	0.0287			0.1200			
	3	0.0285			0.1264			
Urine 3	1	0.0345	0.0326	0.0032	0.1336	0.1232	0.0092	90.6%
	2	0.0289			0.1161			
	3	0.0344			0.1200			
Urine 4	1	0.0186	0.0195	0.0012	0.1288	0.1155	0.0122	96.1%
	2	0.0208			0.1129			
	3	0.0189			0.1049			

[a] Practical urine samples were donated from non-smokers.
[b] The concentration of 1-OHP standard added to individual urine sample was 0.1 μg L^{-1}.

Table 3
Urinary 1-OHP concentrations in raw urine samples donated by healthy nonsmokers, healthy smokers, and lung cancer patients.

Tested volunteers		Urinary 1-OHP concentration (μg L^{-1})	Smoking dose
Nonsmokers	1	0.0244 ± 0.0021	N/A
	2	0.0275 ± 0.0018	N/A
	3	0.0326 ± 0.0032	N/A
	4	0.0195 ± 0.0012	N/A
	5	0.0248 ± 0.0022	N/A
Smokers	1	0.1278 ± 0.0017	About 20 cigarettes per day
	2	0.0669 ± 0.0035	About 20 cigarettes per day
	3	0.0873 ± 0.0049	About 4 cigarettes per day
	4	0.0453 ± 0.0076	About 2 cigarettes per day
	5	0.0651 ± 0.0049	About 20 cigarettes per day
Cancer patients	1	0.0188 ± 0.0021	Ever smoked, quit smoking about 3 months
	2	0.0219 ± 0.0013	Ever smoked, quit smoking about 10 years
	3	0.0191 ± 0.0015	Ever smoked, quit smoking about 6 months
	4	0.0210 ± 0.0002	Ever smoked, quit smoking about 6 months
	5	0.0129 ± 0.0027	Ever smoked, quit smoking about 6 years
	6	0.0172 ± 0.0013	Ever smoked, quit smoking about 1 years
	7	0.0103 ± 0.0011	Ever smoked, quit smoking about 5 months

4. Conclusion

Conclusively, the presented method is a promising strategy to combine a fast and easy-to-use sample pretreatment method and rapid mass spectrometric analysis for the high throughput interrogation of trace analytes in biological and environmental samples with complex matrix. In this study, a facile method for high throughput quantification of 1-hydroxypyrene in undiluted human urine samples was established by using magnetic solid-phase extraction coupled with internal extractive electrospray ionization tandem mass spectrometry. The Fe_3O_4@Ppy nanocomposite showed excellent performance to enrich trace urinary 1-OHP in undiluted human urine samples and good compatibility with internal extractive electrospray ionization mass spectrometric analysis. The results demonstrated that MSPE-iEESI-MS is a useful tool for rapid quantification of urinary 1-OHP for health risk assessment of PAHs exposure. Of specific interest for future studies are some other human metabolites from PAHs exposure that are also suitable for MSPE-iEESI-MS analysis, e.g., 3-hydroxybenzeo[a]pyrene [42,43] is of similar chemical structure as 1-OHP which has been proposed as an alternative biomarker for evaluating of the internal dose of PAHs exposure from multiple routes.

Notes

The authors declare no competing financial interest and no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.04.033.

References

[1] T. Matikainen, G.I. Perez, A. Jurisicova, J.K. Pru, J.J. Schlezinger, H.Y. Ryu, J. Laine, T. Sakai, S.J. Korsmeyer, R.F. Casper, D.H. Sherr, J.L. Tilly, Nat. Genet. 28 (2001) 355–360.
[2] D.W. Nebert, T.P. Dalton, A.B. Okey, F.J. Gonzalez, J. Biol. Chem. 279 (2004) 23847–23850.
[3] S.S. Xu, W.X. Liu, S. Tao, Environ. Sci. Technol. 40 (2006) 702–708.
[4] W. Wilcke, Geoderma 141 (2007) 157–166.
[5] K. Srogi, Environ. Chem. Lett. 5 (2007) 169–195.
[6] D.K. Essumang, D.K. Dodoo, J.K. Adjei, Food Chem. Toxicol. 58 (2013) 86–94.
[7] B.H. Chen, Y.C. Chen, J. Agric. Food Chem. 49 (2001) 5238–5243.
[8] P. Strickland, D. Kang, P. Sithisarankul, Environ. Health Persp 104 (1996) 927–932.
[9] P. Strickland, D.H. Kang, Toxicol. Lett. 108 (1999) 191–199.
[10] H.-M. Yang, Y.-S. Wang, J.-H. Li, G.-R. Li, Y. Wang, X. Tan, J.-H. Xue, X.-L. Mao, R.-H. Kang, Anal. Chim. Acta 636 (2009) 51–57.
[11] K. Luo, Q. Gao, J. Hu, J. Chromatogr. A 1379 (2015) 51–55.
[12] N. Grova, G. Salquebre, B.M.R. Appenzeller, Anal. Bioanal. Chem. 405 (2013) 8897–8911.
[13] D. Knopp, M. Schedl, S. Achatz, A. Kettrup, R. Niessner, Anal. Chim. Acta 399 (1999) 115–126.
[14] X. Shen, Y. Cui, Y. Pang, H. Qian, J. Electroanal. Chem. 667 (2012) 1–6.
[15] G.A. Harris, A.S. Galhena, F.M. Fernandez, Anal. Chem. 83 (2011) 4508–4538.
[16] M.E. Monge, G.A. Harris, P. Dwivedi, F.M. Fernandez, Chem. Rev. 113 (2013) 2269–2308.
[17] H. Chen, G. Gamez, R. Zenobi, J. Am. Soc. Mass Spectrom. 20 (2009) 1947–1963.
[18] J. Laskin, B.S. Heath, P.J. Roach, L. Cazares, O.J. Semmes, Anal. Chem. 84 (2012) 141–148.
[19] J. Hajslova, T. Cajka, L. Vaclavik, Trac-trend. Anal. Chem. 30 (2011) 204–218.
[20] P. Nemes, A. Vertes, Anal. Chem. 79 (2007) 8098–8106.
[21] J.D. Harper, N.A. Charipar, C.C. Mulligan, X. Zhang, R.G. Cooks, Z. Ouyang, Anal. Chem. 80 (2008) 9097–9104.
[22] C. a. Guo, F. Tang, J. Chen, X. Wang, S. Zhang, X. Zhang, Anal. Bioanal. Chem. 407 (2015) 2345–2364.
[23] S. Pan, Y. Tian, M. Li, J. Zhao, L. Zhu, W. Zhang, H. Gu, H. Wang, J. Shi, X. Fang, P. Li, H. Chen, Sci. Rep. 5 (2015) 8725.
[24] H. Wang, J. Liu, R.G. Cooks, Z. Ouyang, Angew. Chem. Int. Ed. 49 (2010) 877–880.
[25] F.P.M. Jjunju, S. Maher, A. Li, S.U. Syed, B. Smith, R.M.A. Heeren, S. Taylor, R.G. Cooks, Anal. Chem. 87 (2015) 10047–10055.
[26] X. Wang, X. Li, Z. Li, Y. Zhang, Y. Bai, H. Liu, Anal. Chem. 86 (2014) 4739–4747.
[27] Y.-Q. Huang, J.-Q. You, Y. Cheng, W. Sun, L. Ding, Y.-Q. Feng, Anal. Methods 5 (2013) 4105–4111.
[28] S.S. Kanyal, T.T. Haebe, C.V. Cushman, M. Dhunna, T. Roychowdhury, P.B. Farnsworth, G.E. Morlock, M.R. Linford, J. Chromatogr. A 1404 (2015) 115–123.
[29] G.A. Gomez-Rios, J. Pawliszyn, Angew. Chem. Int. Ed. 53 (2014) 14503–14507.
[30] H. Zhang, L. Zhu, L. Luo, N. Wang, K. Chingin, X. Guo, H. Chen, J. Agric. Food Chem. 61 (2013) 10691–10698.
[31] H. Zhang, H. Gu, F. Yan, N. Wang, Y. Wei, J. Xu, H. Chen, Sci. Rep. 3 (2013) 2495.
[32] H. Zhang, K. Chingin, L. Zhu, H. Chen, Anal. Chem. 87 (2015) 2878–2883.
[33] Q. Gao, D. Luo, M. Bai, Z.-W. Chen, Y.-Q. Feng, J. Agric. Food Chem. 59 (2011) 8543–8549.
[34] D. Chen, H.-B. Zheng, Y.-Q. Huang, Y.-N. Hu, Q.-W. Yu, B.-F. Yuan, Y.-Q. Feng, Analyst. 140 (2015) 5662–5670.
[35] Y.L. Luo, L.H. Fan, F. Xu, Y.S. Chen, C.H. Zhang, Q.B. Wei, Mater. Chem. Phys. 120 (2010) 590–597.
[36] S.-N. Xu, Q. Zhao, H.-B. He, B.-F. Yuan, Y.-Q. Feng, Q.-W. Yu, Anal. Methods 6 (2014) 7046–7053.
[37] X. Li, X. Fang, Z. Yu, G. Sheng, M. Wu, J. Fu, H. Chen, Anal. Methods 5 (2013) 2816–2821.
[38] X. Li, R. Zenobi, Anal. Chem. 85 (2013) 3526–3531.

[39] F.R. Khuri, Cancer 121 (2015) 3049–3051.
[40] Z. Chen, R. Peto, M. Zhou, A. Iona, M. Smith, L. Yang, Y. Guo, Y. Chen, Z. Bian, G. Lancaster, P. Sherliker, S. Pang, H. Wang, H. Su, M. Wu, X. Wu, J. Chen, R. Collins, L. Li, China Kadoorie Biobank, C. K. B. C, Lancet 386 (2015) 1447–1456.
[41] J.M. Boyle, D.J. Tandberg, J.P. Chino, T.A. D'Amico, N.E. Ready, C.R. Kelsey, Cancer 121 (2015) 598–604.
[42] L. Yao, J. Yang, B. Liu, S. Zheng, W. Wang, X. Zhu, X. Qian, Anal. Methods 6 (2014) 6488–6493.
[43] D. Barbeau, A. Maitre, M. Marques, Analyst. 136 (2011) 1183–1191.