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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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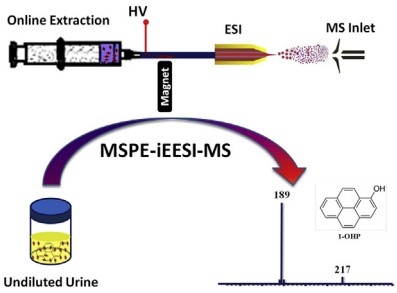
HIGHLIGHTS

- 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