



Coupling of micro-solid-phase extraction and internal extractive electrospray ionization mass spectrometry for ultra-sensitive detection of 1-hydroxypyrene and papaverine in human urine samples

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Received: 10 January 2019 / Revised: 15 February 2019 / Accepted: 20 March 2019
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Abstract

Quantification of ultra-trace analytes in complex biological samples using micro-solid-phase extraction followed by direct detection with internal extractive electrospray ionization mass spectrometry (μ SPE–iEESI–MS) was demonstrated. 1-Hydroxypyrene (1-OHP) and papaverine at attomole levels in human raw urine samples were analyzed under negative and positive ion detection mode, respectively. The μ SPE was simply prepared by packing a disposable syringe filter with octadecyl carbon chain (C18)-bonded micro silica particles, which were then treated as the “bulk sample” after the analytes were efficiently enriched by the C18 particles. Under the optimized experimental conditions, the analytes were readily eluted by isopropanol/water (80/20, V/V) at a high voltage of ± 4.0 kV, producing analyte ions under ambient conditions. The limit of detection (LOD) was 0.02 pg/L (9.2 amol) for 1-hydroxypyrene and 0.02 pg/L (5.9 amol) for papaverine. The acceptable linearity ($R^2 > 0.99$), signal stability (RSD $\leq 10.7\%$), spike recoveries (91–95%), and comparable results for real urine samples were also achieved, opening up possibilities for quantitative analysis of trace compounds (at attomole levels) in complex bio-samples.

Keywords μ SPE · Urine · Enrichment · Ultra-sensitive · iEESI–MS/MS · Detection limit

Introduction

Quantification of ultra-trace analytes (sub-ng– μ g/L) such as the metabolites of pharmaceuticals, carcinogenic chemicals, and many other noxious compounds in complex biological samples is of increasing interest in life science, food safety, drug discovery, and other related fields [1–5]. For instance, 1-hydroxypyrene is an important urinary biomarker for

evaluation of exposure to polycyclic aromatic hydrocarbons (PAHs); papaverine is clinically used as a bronchodilator to relax smooth muscles, smooth musculature of the larger blood vessels [6]. However, to date, the behaviors and function mechanism of these compounds in human body are still not fully understood. Therefore, a robust analytical method is urgently needed to examine and evaluate urinary 1-hydroxypyrene and papaverine at ultra-trace levels.

Solid-phase extraction (SPE) [7, 8] and solid-phase microextraction (SPME) [9–11] are a kind of efficient sample enrichment technique, which is based on the interactions between analytes and adsorbents, including π – π , electrostatic, and hydrophobic interactions [12]. However, the amount of analytes extracted by SPME is limited, depending on the equilibrium between analytes and adsorbent (either a small fiber or tube coated with sorptive material). In contrast, SPE allows enrichment of a larger volume of samples by using various packing materials, e.g., porous particles with large surface areas [12]. Previous studies have shown that the amount of analytes extracted via SPE would be significantly higher (two

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00216-019-01794-2>) contains supplementary material, which is available to authorized users.

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orders of magnitude) than that extracted via SPME [12, 13], ensuring a much lower detection limit. Recently, SPE was integrated with paper spray ionization for pre-concentration of drugs at nanogram-per-liter to microgram-per-liter levels; however, only a small volume (10–100 μL) of biosample [14] was demonstrated, preventing further improvement of sensitivity. Therefore, highly efficient detection of analytes at extremely low (attomole) levels remains a challenge for mass spectrometry analysis. Since large-volume urine samples are noninvasively available [15], urine is often preferably employed to evaluate drug abuse and chemical hazard exposure.

Pre-concentration with SPE, followed by (LC/GC-)MS detection [16–19], was a common strategy for sensitive analysis of various species. In recent years, various ambient mass spectrometries (AMS) [20–23] were developed and widely applied due to their high sensitivity, high specificity, highly efficient ionization, and minimized (or without) sample pretreatment, e.g., desorption electrospray ionization (DESI) [1, 2], direct analysis in real time (DART) [24], extractive electrospray ionization (EESI) [25], microwave plasma torch (MPT) [26, 27], and desorption atmospheric pressure chemical ionization (DAPCI) [28]. Among these techniques, EESI was one of the most efficient ionization methods which used two separate sprayers, one to nebulize the sample solution and the other to produce charged microdroplets of solvent. Liquid–liquid extraction occurred when the formed microdroplets of samples and charged solvent collided. Consequently, the desired analytes were extracted and ionized more efficiently. This technique allowed direct and sensitive detection of analytes without being influenced by complex matrices [20, 25, 29–34]. As an improved EESI, internal extractive electrospray ionization mass spectrometry (iEESI) facilitates direct MS analysis of bulk samples (e.g., tissue, leaf, fruit) by infusing extraction solvent (e.g., methanol, water) into the bulk sample through an inserted capillary. A stable electrospray plume is then generated and transferred to the MS inlet. Considering that the matrix effect and ionization suppression are difficult to be thoroughly eliminated in AMS, it is of sustainable interest to improve the sensitivity and reliability of mass spectrometry, even with affordable compromise on the analytical speed.

Herein, a disposable micro-solid-phase extraction (μSPE) device for processing a large volume of urine samples was prepared by a wet packing method. The μSPE was simply prepared by packing a cheap syringe filter with octadecyl carbon chain-bonded micro silica particles (C18), which pre-concentrated the trace analytes such as 1-hydroxypyrene and/or papaverine from a raw urine sample when it flowed through the μSPE device. After enrichment, the μSPE device loaded with analytes was treated as the “bulk sample,” which was then directly analyzed by internal extractive electrospray ionization mass spectrometry (iEESI–MS) for direct MS/MS characterization and quantification. The linearity, detection

limit, and spike recovery were investigated. The method was successfully applied to analyzing real urine samples collected from different people with consents.

Experimental

Chemicals and reagents

1-Hydroxypyrene (99%) was purchased from AuccStandard Inc. (New Haven, CT 06513, USA). Papaverine ($\geq 98\%$) was a gift from Jiangxi provincial public security criminal science and technology research institute. The stock solutions of 1-hydroxypyrene (5 $\mu\text{g/L}$) and papaverine (1 $\mu\text{g/L}$) were prepared using methanol. Reagents of acetonitrile, methanol, acetic acid, and acetone, of analytical grade, were purchased from Merck (Darmstadt, Germany). Ultrapure water (18.2 $\text{M}\Omega/\text{cm}$) was produced by a Milli-Q device (Millipore, Milford, MA). The SPE packings (C18, 40–75 μm in particle size, specific surface area of 300 m^2/g) and disposable syringe (5 mL and 30 mL, respectively) were purchased from Changchun Boda Weiye Instrument Co., Ltd. (Changchun, China). A disposable syringe filter (with an aperture inner diameter of 0.44 μm , filter diameter of 13 mm) was purchased from Tianjin Navigator Lab Instrument Co., Ltd. (Tianjin, China).

μSPE –iEESI–MS

The concept of μSPE –iEESI–MS was schematically illustrated in Fig. 1, mainly including three parts: fabrication of μSPE , sample loading, and iEESI–MS analysis, respectively. Figure 1(a) demonstrates the setup of the μSPE device. In brief, a disposable syringe filter was assembled to a 5-mL disposable syringe, allowing the whole assembly to coaxially align inside a centrifuge tube (50 mL). One milliliter of packing slurry, namely the μSPE packing material (C18) suspended in methanol (1 mg/mL), was loaded into the reservoir of the small syringe. Then, the tube was tightly covered and centrifuged at a speed of 6000 rpm for 1 min (Beckman Coulter Inc., CA, USA), making the SPE packings (C18) to be evenly packed in the syringe filter. The filter, after it was washed with water (1 mL), was installed to a 30-mL syringe for urinary sample loading. For example, the undiluted human urine was added into the syringe reservoir (20 mL each time, five times in total, Fig. 1(b)), and then centrifuged at 6000 rpm for 10 min after each sample loading, allowing the analytes to be thoroughly captured by the C18 material inside the filter (Fig. 1(c)). After sample loading, the filter was then treated as the “bulk sample” (Fig. 1(d)) and fluxed with deionized water (1 mL) at 6000 rpm for 1 min. After matrix elimination, the analyte-loaded filter was directly eluted with isopropanol/water (80/20, V/V) solution to produce the analyte ions (Fig.

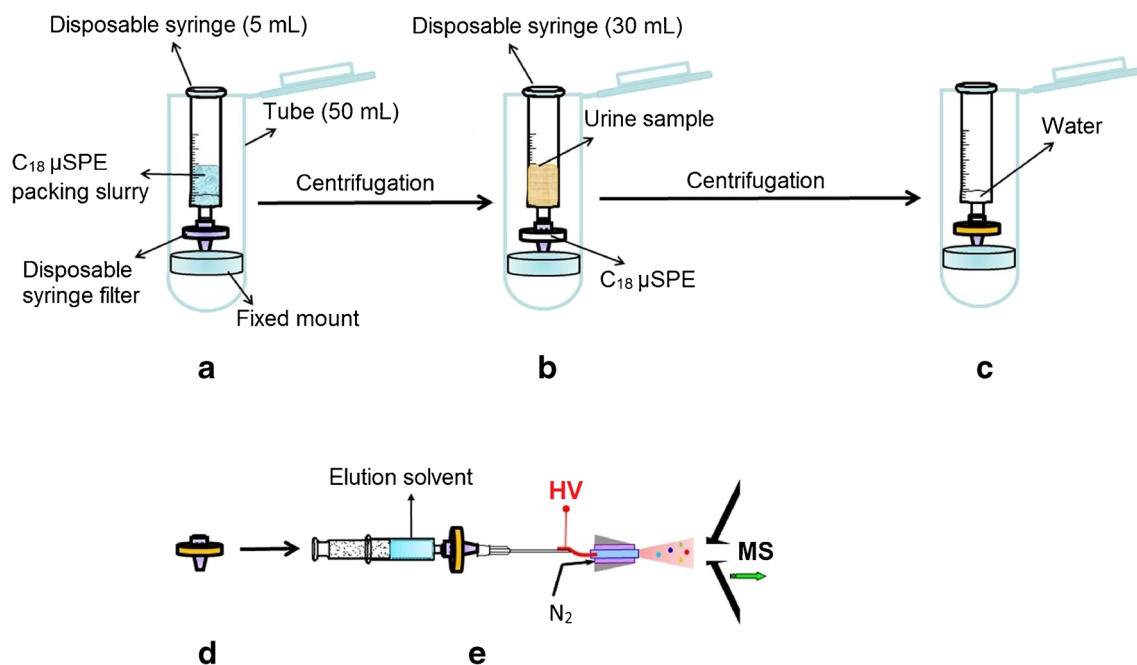


Fig. 1 The concept and experimental setup of μ SPE-iEESI-MS

1(e)) for mass analysis. Note that for the steps shown in Fig. 1(a–c), the sample preparation was conducted in parallel with multiple tubes (e.g., 6) in this work. Thus, the overall time for μ SPE preparation was substantially reduced. The procedure facilitates the removal of a complex matrix of raw urine at the maximal degree with minimal sample loss.

An Orbitrap Fusion™ Tribrid™ mass spectrometer (Thermo Scientific, San Jose, CA, USA), which was coupled with a homemade μ SPE-iEESI source, was used as a detector. The positive or negative ion detection mode was used for MS analysis. The ionization voltage was set at ± 4.0 kV accordingly, and the capillary temperature was set at 320 °C. Mass spectra were recorded in the mass range of m/z 50–500, with a maximum ion injection time of 100 ms. Collision-induced dissociation (CID) experiments were performed by applying 30–40% collision energy (40% for 1-hydroxypyrene and 30% for papaverine) for 30 ms to the precursor ions, which were isolated with a mass-to-charge ratio width of 1.2 Da. For better CID efficiency, the activation Q value was thus adjusted to 0.35 and collision energy was set to 40% during the CID process. The MS/MS spectra were collected with a recording time of 30 s.

HPLC conditions

The concentrations of 1-hydroxypyrene and papaverine were also measured by HPLC-MS/MS. Chromatographic separation (HPLC, Agilent 1260) was performed using an Eclipse Plus C18 column (3.5 μ m, 4.6 \times 100 mm). A mixture of acetonitrile and water (75:25, v/v) with a flow rate of 0.8 mL min^{-1} was used as eluent. Sample pretreatment for

HPLC-MS/MS analysis was detailed in the [Electronic Supplementary Material \(ESM\)](#).

Urine samples

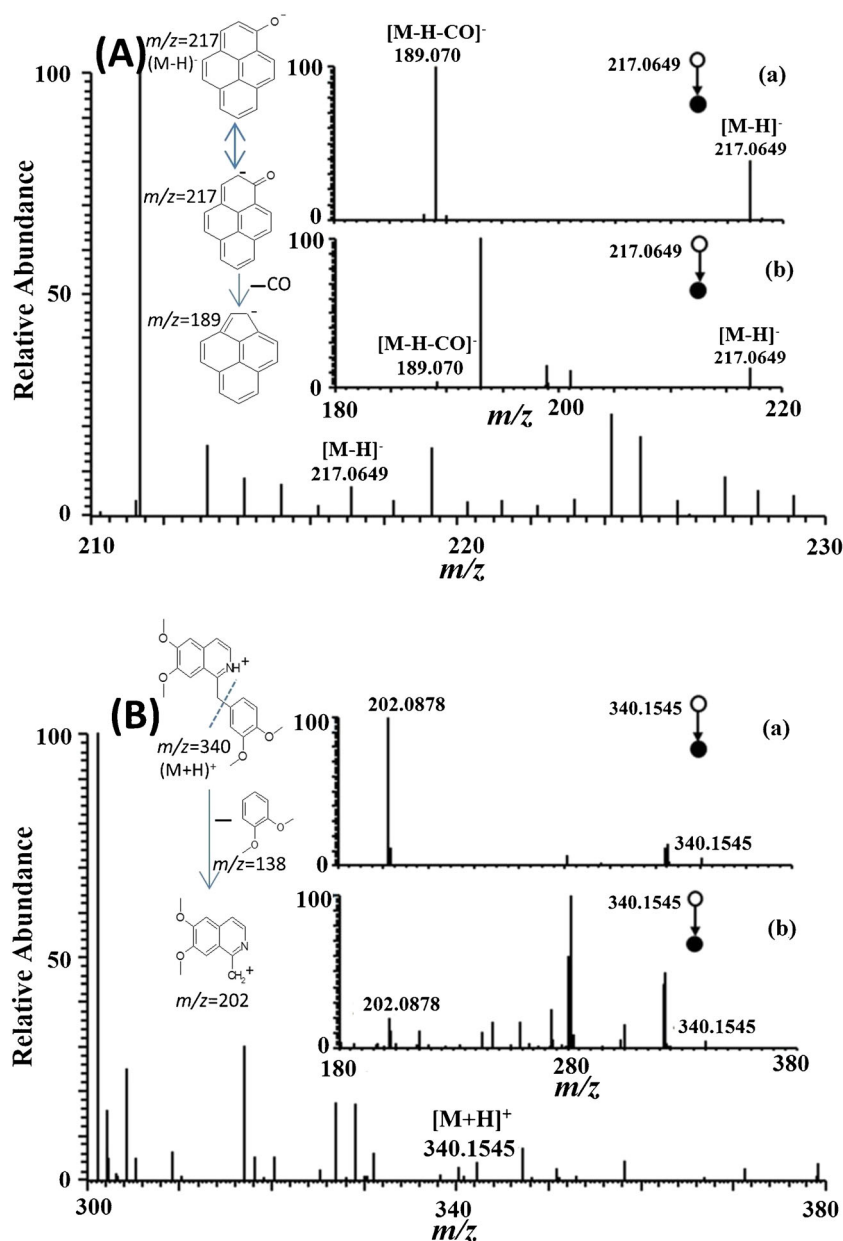
Standard solutions of 1-hydroxypyrene and papaverine were prepared by appropriate dilution of the stock solutions using methanol. The urine samples in which none of the targeted compounds was detected were used as the blank urine samples. Spiked samples were prepared by adding different concentrations of analytes into blank urine samples. Real urine samples from five healthy volunteers were collected with their consents before and after metabolism for about 12 h since they had sufficient barbecue foods. Another six undiluted raw urine samples were donated from a local Bureau of Drug Abuse Control (BDAC), where the samples were collected from six adults exposed to papaverine with different dosages. All of the urine samples were stored in -80 °C before use. One hundred milliliters of each urine sample was firstly pre-concentrated through μ SPE, and followed by direct analysis using iEESI-MS.

Results and discussion

Characteristic spectra of analytes in urine samples

1-Hydroxypyrene has a strong tendency to be deprotonated. Thus, its quantitative detection was demonstrated by μ SPE-iEESI-MS under the negative ion detection mode. As the reference experiment, the mass spectra of authentic 1-

Fig. 2 (A) The mass spectral signals of 1-hydroxypyrene. The deprotonated 1-hydroxypyrene (m/z 217.0649) yielding the characteristic fragment of m/z 189.070. (a) 1-Hydroxypyrene in standard solution (0.1 $\mu\text{g/L}$); (b) 1-hydroxypyrene-spiked urine sample (0.01 ng/L). (B) The mass spectral signals of papaverine. The protonated papaverine yielding the fragment ions of m/z 202.0878. (a) papaverine in standard solution (0.1 $\mu\text{g/L}$); (b) papaverine-spiked urine sample (0.05 ng/L)



hydroxypyrene spiked into the blank urine sample were recorded using a working solution of 1-hydroxypyrene of 0.01 ng/L. As a result (Fig. 2A), the predominant peak at m/z 217.0649 observed in the full-scan mass spectrum was attributed to the deprotonated molecular ions $[M-H]^-$ m/z 217.0649 (theoretical value of m/z 217.0647, Δm 0.92 ppm). The deprotonated 1-hydroxypyrene formed a stable four-ring structure, which was scarcely decomposed into the fragments during the CID experiments. The main fragment ions of protonated 1-hydroxypyrene were detected at m/z 189.070 ($[M-H-CO]^-$) (Fig. 2A, inset), which were formed by the neutral loss of CO (28 Da) from the precursor ions [35].

Since papaverine is apt to form positive ions, it was detected by $\mu\text{SPE-iEESI-MS}$ under the positive ion detection

mode. The protonated molecular ion of papaverine (Fig. 2B) was clearly detected at $[M+H]^+$ m/z 340.1545 (theoretical value m/z 340.1543, Δm 0.58 ppm) when the urine sample containing 0.05 ng/L papaverine was analyzed under the optimized conditions. In the MS/MS spectrum (Fig. 2B, inset), the characteristic fragment ions of m/z 202.0878 were detected with high abundance, which were derived from the protonated papaverine by the loss of the benzyl moiety [35].

Effects of extraction solvent compositions and flow rates

For better performance of $\mu\text{SPE-iEESI-MS}$, parameters including compositions of extraction solvent, sorbent amount,

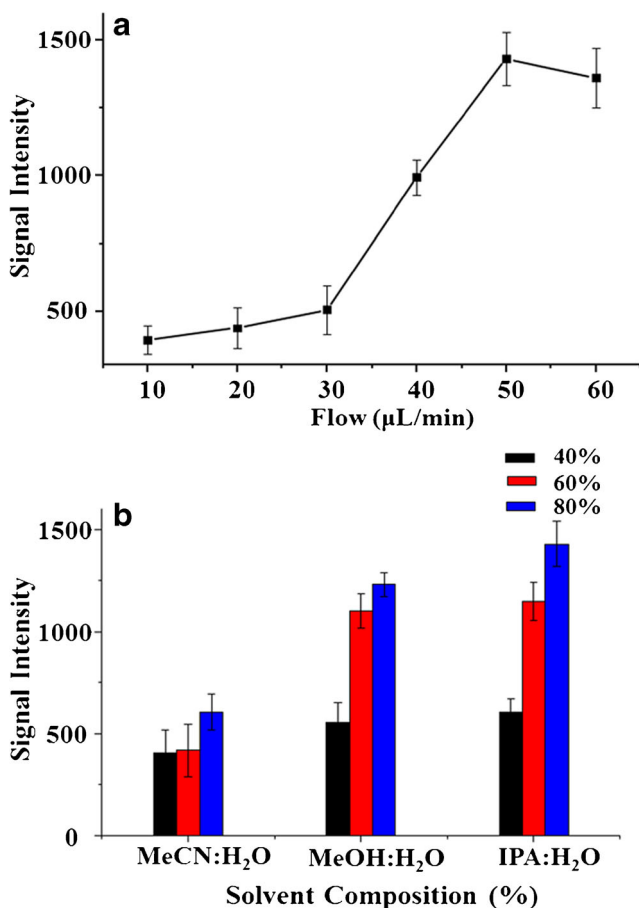


Fig. 3 Optimization of experimental conditions. (a) Effects of the elution solvent flow rate; (b) effects of the elution solvent compositions for μ SPE-iEESI-MS. The error bars represent standard deviations of three replicates ($n = 3$)

and the flow rates of extraction solvent were experimentally optimized using the characteristic fragments (m/z 189.070) of MS/MS experiments. The signal intensity increased notably when the eluent flow rates increased from 10 to 50 μ L/min (Fig. 3a), probably because more analytes were eluted when a higher solvent flow rate was used. However, the signal intensity decreased when the flow rate was higher than 50 μ L/min, probably due to the fact that a large amount of solvent would also dilute the analyte. Compromisingly, the solvent flow rate was optimized to be 50 μ L/min, with an infusion time of 3 min per sample. That is, the solvent consumption was estimated to be 150 μ L.

The influence of the composition of elution solvents was also investigated. A series of eluents including methanol/water (20/80, 40/60, 60/40, 80/20, V/V), acetonitrile/water (20/80, 40/60, 60/40, 80/20, V/V), isopropanol/water (20/80, 40/60, 60/40, 80/20, V/V) were evaluated by μ SPE-iEESI-MS/MS. Generally, increasing the organic content resulted in better signal intensity (Fig. 3b); thus, the ratio of 80/20 (V/V) was selected as the optimal condition. On the other hand, compared with methanol and acetonitrile, isopropanol brought

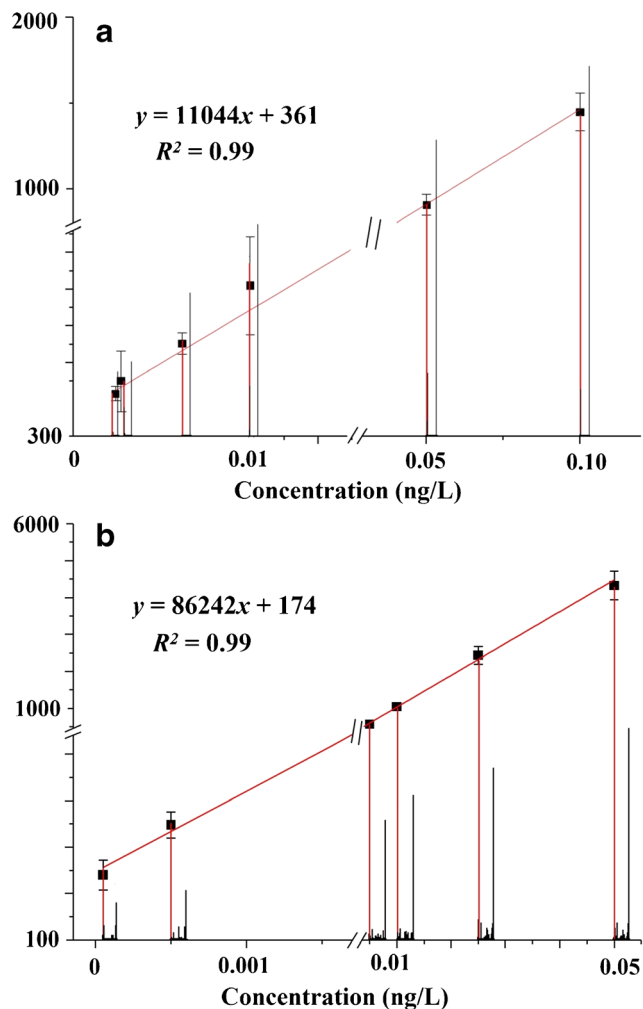


Fig. 4 The calibration curves of μ SPE-iEESI-MS/MS for 1-hydroxypyrene (m/z 189.070) and papaverine (m/z 202.0878). The error bars represent standard deviations of three replicates ($n = 3$). (a) 1-Hydroxypyrene in urine samples. The characteristic fragments (m/z 189.070) are indicated in red, while the precursor ions of 1-hydroxypyrene (m/z 217.0649) are in black. (b) Papaverine in urine samples. The characteristic fragments (m/z 202.0878) are in red, while the precursor ions of papaverine (m/z 340.1565) are in black

lower background noises and better stability of electrospray [36], probably because isopropanol performed better on the physicochemical features such as water-miscible, volatility, low surface tension, and chemically inactive than the other two compounds.

Adsorption capacity of C18- μ SPE

In order to evaluate the adsorption capacity of C18 packed μ SPE, a small volume (e.g., 1–20 mL, each time) of 1-hydroxypyrene standard solution (4 μ g/L) was sequentially loaded to the μ SPE device following the sample loading procedure. After each sample loading, the effluent was collected for HPLC-MS/MS detection as described in the

literature [37]. If the 1-hydroxypyrene was not detectable ($S/N = 3$) in the waste solution, the next loading of 1-hydroxypyrene standard solution (4 $\mu\text{g/L}$) would be repeated. Consequently, the adsorption capacity of C18- μSPE packings (1.0 mg in total) was found to be 0.4 μg for 1-hydroxypyrene, with the recovery of $91.1 \pm 7.6\%$.

Accordingly, the adsorption capacity of C18- μSPE for papaverine was evaluated to be more than 0.4 μg (the maximum value was not achieved), with the recovery of $93.1 \pm 3.7\%$. The results indicated that the μSPE used in this work could accommodate the targeted analytes in 100 mL of urine samples with sufficient capacity.

Influence of matrix effect by μSPE

Since the complex matrices of urine, including salts and other compounds, may cause significant background disturbance and suppress the electrospray ionization efficiency of target analytes [36], it is necessary to evaluate the influence of matrices. For the analysis of undiluted raw urine samples spiked with 0.01 ng/L 1-hydroxypyrene or papaverine, dominant signals were confidently detected by $\mu\text{SPE-iEESI-MS/MS}$, and no detectable suppression on the analytes was observed. These findings suggested that the analytes were selectively trapped by μSPE , and therefore, the matrices in raw urine samples imposed no obvious influence on urinary analysis of analytes by $\mu\text{SPE-iEESI-MS/MS}$.

Linearity of $\mu\text{SPE-iEESI-MS/MS}$

Under the optimized experimental conditions, the linearity of each calibration curve was examined for different concentration ranges with at least six standard points (100 mL for each data point). 1-Hydroxypyrene was analyzed in the concentration range of 0.0001–0.1 ng/L by $\mu\text{SPE-iEESI-MS/MS}$ under the negative ion detection mode. Noteworthy, the

calibration curves for quantification were constructed by peak intensity of characteristic ions of analytes at the same time duration for control procedures. Extracted ion current (EIC) obtained in the way as reported [38] for 1-hydroxypyrene at different concentrations (0.0001–0.1 ng/L) showed good stability (ESM Fig. S1). The signal intensity of the characteristic fragment $[\text{M-H-CO}]^-$ m/z 189.070 responded linearly to 1-hydroxypyrene concentration levels over the range of 0.0001–0.1 ng/L, with a correlation coefficient of 0.99, as shown Fig. 4a.

Similarly, papaverine was studied in the concentration range of 0.00005–0.05 ng/L under positive ion detection mode. The result showed that the characteristic signal of papaverine at m/z 202.0878 was also linearly correlated with the papaverine concentrations over the range of 0.00005–0.05 ng/L ($R^2 = 0.99$, Fig. 4b).

Limit of detection of $\mu\text{SPE-iEESI-MS/MS}$

The LOD of this method was calculated following the process described in literature [39] using a sample with an analyte level at 0.0001 ng/L ($n = 20$, $S/N = 3$). As a result, the LOD for 1-hydroxypyrene and papaverine was about 0.02 pg/L (9.2 amol, $n = 20$, $S/N = 3$) and 0.02 pg/L (5.9 amol, $n = 20$, $S/N = 3$), respectively (see ESM for detailed calculations). The LOD values obtained by $\mu\text{SPE-iEESI-MS}$ were far lower than those obtained with HPLC-MS for urine analysis (ESM Table S1) [40–42]. To date, the LOD of conventional HPLC-MS/MS was about 0.0001–0.5 $\mu\text{g/L}$ [35] [40, 41, 43–47] for 1-hydroxypyrene and 0.02–1.6 $\mu\text{g/L}$ [48–51] for papaverine (ESM Table S1), respectively. Probably, the highly efficient μSPE packing material (C18, with a surface area up to 300 m^2/g), large volume of urine sample (100 mL) concentrated, and small volume of elution (~ 150 μL) in this study were the main reasons, which ensured the highest degree of enrichment of various analytes without being diluted.

Table 1 Analytical results for $\mu\text{SPE-iEESI-MS/MS}$ analysis of spiked urine samples

Analyte	Spiked conc. (ng/L)	Signal intensity (cps)			Mean value	Standard deviation	RSD (%)
1-Hydroxypyrene	1.0E-4	359	348	368	358	11	3.1
	5.0E-4	406	390	329	375	41	10.3
	0.005	415	443	421	426	15	3.3
	0.01	469	508	538	505	97	6.9
	0.05	963	915	843	907	60	6.6
	0.1	1368	1405	1576	1450	110	7.7
Papaverine	5.0E-5	186	170	154	170	16	9.4
	1.0E-4	218	214	240	224	14	6.3
	0.005	514	558	632	568	60	10.7
	0.01	1083	976	1176	1078	100	9.3
	0.025	2366	2689	2234	2433	239	9.8
	0.05	4054	4771	4167	4330	385	8.9

Table 2 The analytical results of five urine samples collected before and after the barbecue

Volunteers	Diet	μ SPE-iEESI-MS/MS						HPLC-MS/MS		
		Signal intensity		Mean value	Concentration (ng/L)	RSD (%)	Values found ^a (ng/L)	RSD (%)	RE ^b (%)	
1-MYG	B	396	344	356	365	0.0004	6.1	–	–	–
	A	423	461	467	450	0.0081	4.3	–	–	–
2-LN	B	399	401	365	388	0.0024	4.3	–	–	–
	A	496	521	445	487	0.0114	6.5	0.0100	6.1	17.5
3-YLN	B	382	465	386	411	0.0045	9.3	–	–	–
	A	617	574	593	595	0.0212	3.0	0.0182	8.3	14.1
4-WXX	B	400	431	440	424	0.0057	4.0	–	–	–
	A	794	812	632	746	0.0349	10.9	0.0311	4.4	10.8
5-LAY	B	452	500	493	482	0.0110	4.4	0.0100	5.7	15.5
	A	926	875	728	843	0.0436	10	0.0396	3.5	9.2

A samples collected after barbecue, B samples collected before barbecue

^aThe data are the mean value of three replicate measurements for each sample

^bRE% = (values found by μ SPE-iEESI-MS/MS – values found by HPLC-MS) \div values found by μ SPE-iEESI-MS/MS \times 100%

Additionally, the direct analysis of analyte-loaded μ SPE using iEESI and the high sensitivity of the MS/MS detector may also play a role in ensuring an ultra-low detection limit. It is worth noting that this work aimed at developing an effective method for ultra-trace analysis, though a relatively large amount of sample (100 mL) and long sample preparation time (10 min \times 5 aliquots) were needed. However, one can also obtain a satisfactory LOD as needed by condensing fewer aliquots (e.g., 25 mL) [35].

Repeatability and recovery of μ SPE-iEESI-MS/MS

As shown in Table 1, the relative standard deviations (RSD, $n = 3$) for 1-hydroxypyrene detection ranged from 3.1 to 10.3% in the concentration range of 0.0001–0.1 ng/L, indicating a good repeatability for 1-hydroxypyrene. Furthermore, the recovery for 1-hydroxypyrene was estimated based on the quantity of 1-hydroxypyrene detected as a percentage of

the amount added to the sample. With two different concentrations of 0.001 and 0.25 ng/L spiked to real urine samples, recoveries of 91 and 95% were obtained, showing good performance of μ SPE-iEESI-MS for urinary analysis.

As for papaverine, a similar analytical procedure was conducted. The relative standard deviations (RSD, $n = 3$) for papaverine were between 6.3 and 10.7% (Table 1). With two different concentrations of 0.0001 and 0.0025 ng/L spiked to real urine samples, recoveries of 95 and 106% were obtained, respectively. The results also showed good analytical performance of μ SPE-iEESI-MS for papaverine analysis.

μ SPE-iEESI-MS/MS analysis of real urine samples

The approach was also applied to the analysis of real urine samples, and the results were compared with HPLC-MS/MS analysis. Table 2 summarizes the analytical results of five urine samples collected from five volunteers before and after

Table 3 The analytical results of six urine samples for papaverine detection

Code of drug users	μ SPE-iEESI-MS/MS						HPLC-MS/MS		
	Signal intensity		Mean value	Concentration (ng/L)	RSD (%)	Values found ^a (ng/L)	RSD (%)	RE ^b (%)	
1	811	923	987	907	0.0085	9.8	0.0068	4.2	20
2	1080	1010	1130	1073	0.0104	5.6	0.0098	2.0	5.7
3	443	425	465	444	0.0031	4.5	0.0017	8.2	45
4	444	424	387	418	0.0028	6.9	0.0012	7.9	57
5	196	207	203	202	0.0003	2.8	–	–	–
6	879	900	965	915	0.0086	4.9	0.0071	5.0	17.4

^aThe data were the mean value of 3 replicate measurements for each sample

^bRE% = (values found by μ SPE-iEESI-MS/MS – values found by HPLC-MS) \div values found by μ SPE-iEESI-MS/MS \times 100%

barbecue foods. The results showed that before the barbecue, only trace levels of 1-OHP were detectable (0.0004–0.011 ng/L) by μ SPE–iEESI–MS/MS, but with a notable increase after the barbecue (0.008–0.044 ng/L). The results were generally in agreement with that of the HPLC–MS/MS analysis, though in some samples the analytes could not be detected by HPLC–MS/MS (see [ESM](#) for more information). Table 3 shows the analytical results of another six urine samples donated by the local BDAC where urinary analyses were often conducted to monitor drug abuse. It can be seen that different concentration levels of papaverine (0.0003–0.01 ng/L) were detected using μ SPE–iEESI–MS/MS, with the RSD less than 10% ($n \geq 3$). It is worth noting that the values detected by HPLC–MS/MS were slightly lower than those obtained by μ SPE–iEESI–MS/MS, probably because the concentrations of papaverine were generally much lower (less than 0.01 ng/L) in the analyzed samples and thus mass loss may easily occur during sample manipulation. As a result, the μ SPE–iEESI–MS/MS was proven to be a more reliable method than HPLC–MS/MS in this case, showing potential applications in urine analysis at ultra-trace levels.

Conclusions

In this study, a facile μ SPE device was prepared using a disposable syringe filter, which was subsequently treated as a “bulk sample” for direct iEESI–MS analysis. The developed method can be reliably used for rapid quantitative detection of trace analytes in undiluted human raw urine, with the LOD down to attomol levels in either negative or positive ion detection mode. The main advantage of this approach is that it allowed close study of the behavior of 1-hydroxypyrene and papaverine in the human body at ultra-trace levels. The μ SPE device enabled efficient enrichment of trace analytes. On the other hand, direct analysis with iEESI–MS avoided offline elution and mass loss of analytes. Therefore, the μ SPE–iEESI–MS method established in this work was a promising analytical tool for sensitive quantification of various ultra-trace analytes in complex matrix samples.

Funding This work was supported by the National Natural Science Foundation of China (No. 21605016, 21705017), International Science & Technology Cooperation Program of China (No. 2015DFA40290), and Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT) (No. IRT_17R20).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in this work involving human participants were in accordance with the ethical standards of the research committee of East China University of Technology and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants from the local Bureau of Drug Abuse Control (BDAC) included in the study.

References

1. Cooks RG, Ouyang Z, Takats Z, Wiseman JM. Ambient mass spectrometry. *Science*. 2006;80(311):1566–70. <https://doi.org/10.1126/science.1119426>.
2. Takats Z, Wiseman JM, Gologan B, Cooks RG. Mass spectrometry sampling under ambient conditions with desorption electrospray ionization. *Science* (80-). 2004;306:471–3. <https://doi.org/10.1126/science.1104404>.
3. Cui Y, Wei Q, Park H, Lieber CM. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science* (80-). 2001;293:1289–92. <https://doi.org/10.1126/science.1062711>.
4. Deng J, Yang Y, Fang L, Lin L, Zhou H, Luan T. Coupling solid-phase microextraction with ambient mass spectrometry using surface coated wooden-tip probe for rapid analysis of ultra trace perfluorinated compounds in complex samples. *Anal Chem*. 2014;86:11159–66. <https://doi.org/10.1021/ac5034177>.
5. Reyes-Garcés N, Gionfriddo E, Gómez-Ríos GA, Alam MN, Boyac E, Bojko B, et al. Advances in solid phase microextraction and perspective on future directions. *Anal Chem*. 2018;90:302–60. <https://doi.org/10.1021/acs.analchem.7b04502>.
6. Peng Z, Song W, Han F, Chen H, Zhu M, Chen Y. Chromatographic tandem mass spectrometric detection of papaverine and its major metabolites in rat urine. *Int J Mass Spectrom*. 2007;266:114–21. <https://doi.org/10.1016/j.ijms.2007.07.013>.
7. Dénes J, Katona M, Hosszú A, Czuczny N, Takats Z. Analysis of biological fluids by direct combination of solid phase extraction and desorption electrospray ionization mass spectrometry. *Anal Chem*. 2009;81:1669–75. <https://doi.org/10.1021/ac8024812>.
8. Wang H, So PK, Yao ZP. Direct analysis of herbal powders by pipette-tip electrospray ionization mass spectrometry. *Anal Chim Acta*. 2014;809:109–16. <https://doi.org/10.1016/j.aca.2013.11.060>.
9. Deng J, Yang Y, Xu M, Wang X, Lin L, Yao ZP, et al. Surface-coated probe nanoelectrospray ionization mass spectrometry for analysis of target compounds in individual small organisms. *Anal Chem*. 2015;87:9923–30. <https://doi.org/10.1021/acs.analchem.5b03110>.
10. Ahmad S, Tucker M, Spooner N, Murnane D, Gerhard U. Direct ionization of solid-phase microextraction fibers for quantitative drug bioanalysis: from peripheral circulation to mass spectrometry detection. *Anal Chem*. 2015;87:754–9. <https://doi.org/10.1021/ac503706n>.
11. Ouyang G, Vuckovic D, Pawliszyn J. Nondestructive sampling of living systems using in vivo solid-phase microextraction. *Chem Rev*. 2011;111:2784–814. <https://doi.org/10.1021/cr100203t>.
12. Basheer C, Alnedhary AA, Rao BSM, Valliyaveetil S, Lee HK. Development and application of porous membrane-protected carbon nanotube micro-solid-phase extraction combined with gas chromatography/mass spectrometry. *Anal Chem*. 2006;78:2853–8. <https://doi.org/10.1021/ac060240i>.
13. López-Blanco MC, Cancho-Grande B, Simal-Gándara J. Comparison of solid-phase extraction and solid-phase microextraction for carbofuran in water analyzed by high-

- performance liquid chromatography–photodiode-array detection. *J Chromatogr A*. 2002;963:117–23.
14. Zhang C, Manicke NE. Development of a paper spray mass spectrometry cartridge with integrated solid phase extraction for bioanalysis. *Anal Chem*. 2015;87:6212–9. <https://doi.org/10.1021/acs.analchem.5b00884>.
 15. Gómez-Ríos GA, Reyes-Garcés N, Bojko B, Pawliszyn J. Biocompatible solid-phase microextraction nanoelectrospray ionization: an unexploited tool in bioanalysis. *Anal Chem*. 2016;88:1259–65. <https://doi.org/10.1021/acs.analchem.5b03668>.
 16. Hu B, So PK, Yao ZP. Electrospray ionization with aluminum foil: a versatile mass spectrometric technique. *Anal Chim Acta*. 2014;817:1–8. <https://doi.org/10.1016/j.aca.2014.02.005>.
 17. Hu B, Xin GZ, So PK, Yao ZP. Thin layer chromatography coupled with electrospray ionization mass spectrometry for direct analysis of raw samples. *J Chromatogr A*. 2015;1415:155–60. <https://doi.org/10.1016/j.chroma.2015.08.055>.
 18. Spietelun A, Kloskowski A, Chrzanowski W, Namieśnik J. Understanding solid-phase microextraction: key factors influencing the extraction process and trends in improving the technique. *Chem Rev*. 2013;113:1667–85. <https://doi.org/10.1021/cr300148j>.
 19. Yao ZP. Characterization of proteins by ambient mass spectrometry. *Mass Spectrom Rev*. 2012;31:437–47. <https://doi.org/10.1002/mas.20346>.
 20. Chen H, Talaty NN, Takács Z, Cooks RG. Desorption electrospray ionization mass spectrometry for high-throughput analysis of pharmaceutical samples in the ambient environment. *Anal Chem*. 2005;77:6915–27. <https://doi.org/10.1021/ac050989d>.
 21. Wiseman JM, Ifa DR, Song Q, Cooks RG. Tissue imaging at atmospheric pressure using desorption electrospray ionization (DESI) mass spectrometry. *Angew Chem Int Ed*. 2006;45:7188–92. <https://doi.org/10.1002/anie.200602449>.
 22. Venter A, Neffliu M, Graham Cooks R. Ambient desorption ionization mass spectrometry. *TrAC Trends Anal Chem*. 2008;27:284–90. <https://doi.org/10.1016/j.trac.2008.01.010>.
 23. Chen H, Gamez G, Zenobi R. What can we learn from ambient ionization techniques? *J Am Soc Mass Spectrom*. 2009;20:1947–63. <https://doi.org/10.1016/j.jasms.2009.07.025>.
 24. Cody RB, Laramée JA, Durst HD. Versatile new ion source for the analysis of materials in open air under ambient conditions. *Anal Chem*. 2005;77:2297–302. <https://doi.org/10.1021/ac050162j>.
 25. Chen H, Wortmann A, Zhang W, Zenobi R. Rapid in vivo fingerprinting of nonvolatile compounds in breath by extractive electrospray ionization quadrupole time-of-flight mass spectrometry. *Angew Chem Int Ed*. 2007;46:580–3. <https://doi.org/10.1002/anie.200602942>.
 26. Hu L, Liang J, Chingim K, Hang Y, Wu X, Chen H. Early release of 1-pyrroline by *Pseudomonas aeruginosa* cultures discovered using ambient corona discharge ionization mass spectrometry. *RSC Adv*. 2016;6:8449–55. <https://doi.org/10.1039/c5ra24594j>.
 27. Chen H, Zheng J, Zhang X, Luo M, Wang Z, Qiao X. Surface desorption atmospheric pressure chemical ionization mass spectrometry for direct ambient sample analysis without toxic chemical contamination. *J Mass Spectrom*. 2007;42:1045–56. <https://doi.org/10.1002/jms.1235>.
 28. Zhang X, Jia B, Huang K, Hu B, Chen R, Chen H. Tracing origins of complex pharmaceutical preparations using surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal Chem*. 2010;82:8060–70. <https://doi.org/10.1021/ac100407k>.
 29. Chen H, Sun Y, Wortmann A, Gu H, Zenobi R. Differentiation of maturity and quality of fruit using noninvasive extractive electrospray ionization quadrupole time-of-flight mass spectrometry. *Anal Chem*. 2007;79:1447–55.
 30. Chen H, Venter A, Cooks RG (2006) Extractive electrospray ionization for direct analysis of undiluted urine, milk and other complex mixtures without sample preparation. *Chem Commun* 2042–2044. <https://doi.org/10.1039/b602614a>.
 31. Chen H, Yang S, Li M, Hu B, Li J, Wang J. Sensitive detection of native proteins using extractive electrospray ionization mass spectrometry. *Angew Chem Int Ed*. 2010;49:3053–6. <https://doi.org/10.1002/anie.200906886>.
 32. Chen H, Yang S, Wortmann A, Zenobi R. Neutral desorption sampling of living objects for rapid analysis by extractive electrospray ionization mass spectrometry. *Angew Chem Int Ed*. 2007;46:7591–4. <https://doi.org/10.1002/anie.200702200>.
 33. Chen H, Zenobi R. Neutral desorption sampling of biological surfaces for rapid chemical characterization by extractive electrospray ionization mass spectrometry. *Nat Protoc*. 2008;3:1467–75. <https://doi.org/10.1038/nprot.2008.109>.
 34. Luo M, Hu B, Zhang X, Peng D, Chen H, Zhang L, et al. Spectrometry for sensitive detection of uranyl species in natural water samples. *Anal Chem*. 2010;82:282–9.
 35. Zhang H, Lu H, Huang H, Liu J, Fang X, Yuan BF, et al. Quantification of 1-hydroxypyrene in undiluted human urine samples using magnetic solid-phase extraction coupled with internal extractive electrospray ionization mass spectrometry. *Anal Chim Acta*. 2016;926:72–8. <https://doi.org/10.1016/j.aca.2016.04.033>.
 36. Nišavić M, Hozic A, Hameršak Z, Radić M, Butorac A, Duvnjak M, et al. High-efficiency microflow and nanoflow negative electrospray ionization of peptides induced by gas-phase proton transfer reactions. *Anal Chem*. 2017;89:4847–54. <https://doi.org/10.1021/acs.analchem.6b04466>.
 37. Qi F, Qian L, Liu J, Li X, Lu L, Xu Q. A high-throughput nanofibers mat-based micro-solid phase extraction for the determination of cationic dyes in wastewater. *J Chromatogr A*. 2016;1460:24–32. <https://doi.org/10.1016/j.chroma.2016.07.020>.
 38. So PK, Ng TT, Wang H, Hu B, Yao ZP. Rapid detection and quantitation of ketamine and norketamine in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry. *Analyst*. 2013;138:2239–43. <https://doi.org/10.1039/c3an36641c>.
 39. Yang S, Ding J, Zheng J, Hu B, Li J, Chen H, et al. Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Anal Chem*. 2009;81:2426–36. <https://doi.org/10.1021/ac900063u>.
 40. Ramsauer B, Sterz K, Hagedorn H-W, Engl J, Scherer G, McEwan M, et al. A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of phenolic polycyclic aromatic hydrocarbons (OH-PAH) in urine of non-smokers and smokers. *Anal Bioanal Chem*. 2011;399:877–89. <https://doi.org/10.1007/s00216-010-4355-7>.
 41. Lankova D, Urbancova K, Sram RJ, Hajslova J, Pulkrabova J. A novel strategy for the determination of polycyclic aromatic hydrocarbon monohydroxylated metabolites in urine using ultra-high-performance liquid chromatography with tandem mass spectrometry. *Anal Bioanal Chem*. 2016;408:2515–25. <https://doi.org/10.1007/s00216-016-9350-1>.
 42. Tang C, Tan J, Fan R, Zhao B, Tang C, Ou W, et al. Quasi-targeted analysis of hydroxylation-related metabolites of polycyclic aromatic hydrocarbons in human urine by liquid chromatography–mass spectrometry. *J Chromatogr A*. 2016;1461:59–69. <https://doi.org/10.1016/j.chroma.2016.07.051>.
 43. Zhang H, Xu H. Electrospun nanofibers-based online micro-solid phase extraction for the determination of monohydroxy polycyclic aromatic hydrocarbons in human urine. *J Chromatogr A*. 2017;1521:27–35. <https://doi.org/10.1016/j.chroma.2017.09.035>.
 44. Kang HG, Jeong SH. 1-OH-pyrene and 3-OH-phenanthrene in urine show good relationship with their parent polycyclic aromatic

- hydrocarbons in muscle in dairy cattle. *Toxicol Res.* 2011;27:15–8. <https://doi.org/10.5487/TR.2011.27.1.015>.
45. 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;85:3526–31. <https://doi.org/10.1021/ac303402s>.
46. Jacob P, Wilson M, Benowitz NL. 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;79:587–98. <https://doi.org/10.1021/ac060920l>.
47. Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, et al. Measurement of urinary monohydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high-resolution mass spectrometry. *Anal Chem.* 2006;78:5744–51. <https://doi.org/10.1021/ac0606094>.
48. Musshoff F, Trafkowski J, Madea B. Validated assay for the determination of markers of illicit heroin in urine samples for the control of patients in a heroin prescription program. *J Chromatogr B Anal Technol Biomed Life Sci.* 2004;811:47–52. <https://doi.org/10.1016/j.jchromb.2004.03.072>.
49. Dams R, Benijts T, Lambert WE, De Leenheer AP. Simultaneous determination of in total 17 opium alkaloids and opioids in blood and urine by fast liquid chromatography-diode-array detection-fluorescence detection, after solid-phase extraction. *J Chromatogr B Anal Technol Biomed Life Sci.* 2002;773:53–61. [https://doi.org/10.1016/S1570-0232\(01\)00594-3](https://doi.org/10.1016/S1570-0232(01)00594-3).
50. Kikura-Hanajiri R, Kaniwa N, Ishibashi M, Makino Y, Kojima S. Liquid chromatographic-atmospheric pressure chemical ionization mass spectrometric analysis of opiates and metabolites in rat urine after inhalation of opium. *J Chromatogr B Anal Technol Biomed Life Sci.* 2003;789:139–50. [https://doi.org/10.1016/S1570-0232\(03\)00096-5](https://doi.org/10.1016/S1570-0232(03)00096-5).
51. Lombardo-Agüi M, Cruces-Blanco C, García-Campaña AM. Capillary zone electrophoresis with diode-array detection for analysis of local anaesthetics and opium alkaloids in urine samples. *J Chromatogr B Anal Technol Biomed Life Sci.* 2009;877:833–6. <https://doi.org/10.1016/j.jchromb.2009.01.041>.

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