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Investigation of a QuEChERS-Based Method for Determination of Polycyclic Aromatic Hydrocarbons in Rat Plasma by GC–MS

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Abstract

Owing to the toxic effects of polycyclic aromatic hydrocarbons (PAHs) on humans and the environment, their sensitive biomonitoring is essential and significant. In this work, a sensitive, simple and rapid bioanalytical method was established for the simultaneous determination of 13 PAHs in rat plasma depending on QuEChERS (Quick, easy, cheap, effective and rugged method) as a preliminary step and gas chromatography–mass spectrometry (GC–MS) for identification. QuEChERS procedure was optimized where acetonitrile was employed for plasma samples extraction, which was further cleaned using primary secondary amine as the sorbent material. Optimization of GC–MS conditions was performed to produce optimum selectivity of the proposed method. The method was fully validated for rat plasma samples where recoveries, matrix effects, limit of quantitation (LOQ), linearity and precision were evaluated. Linearity range was 5–100 ng/mL for most of the 13 analytes. Average recoveries of the 13 PAHs ranged between 85.57% and 109.64% in fortified rat plasma with standard deviations <8.91 except for anthracene that showed 19.24. The limits of detection and LOQs for the 13 compounds ranged from 0.045 to 0.372 ppb and from 0.137 to 1.128 ppb, respectively. The established method was successfully implemented to perform a minor toxicokinetic study in intraperitoneally dosed rats (0.25 and 2 mg/kg in vegetable oil). The 13 PAHs were tracked in rat plasma samples for 6 h after administration, and most of the target compounds were recognized in plasma samples only at the higher dose.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are omnipresent environmental contaminants renowned for their potential toxicity and carcinogenicity (1). They are stable compounds, composed of two or more fused aromatic rings (2). Since PAHs mostly originate from both natural and anthropogenic sources, for example, fossil fuel combustion, oil spills and industrial emissions, they have a widespread distribution that raises significant environmental and public health concerns (3, 4).

Many studies addressed the hazards of PAHs on human health, and various toxic effects have been reported, including endocrine disruption, immunosuppression, teratogenicity and carcinogenicity (5). The severity of response depends on several factors such as the duration of exposure, the route of exposure and the concentration of PAHs during exposure. Concurrent health issues and age also play a role in aggravating PAH toxicity (5). Given the right conditions, PAHs can accumulate in sediments, water bodies, air and food (6) and can enter the body through direct ingestion, dermal contact or inhalation (7). This implied the importance of large-scale biomonitoring to assess the population exposure and hazard caused by such compounds (8).

Several analytical methods have been introduced to check PAHs in numerous sources such as human urine, saliva (9) and water samples (10) using liquid chromatography–mass spectrometry; soil

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samples (11) and cocoa butter (12) by HPLC (High Performance Liquid Chromatography)-fluorimetric detection; soil samples (13), tap water, river water, well water and rainwater (14) by HPLC-UV, human plasma and saliva (15) by GC–FID (16); and infant formula (17), milk powder, yoghurt (18), cosmetics (19), water samples (20) and black, green, red and white tea (21) using gas chromatography–mass spectrometry (GC–MS).

In this work, we are studying the investigation of QuEChERS (Quick, easy, cheap, effective and rugged method) combined with GC–MS for the prompt separation and determination of PAHs from rat plasma. QuEChERS was initially developed as an all-inclusive sample preparation method consisting of dispersion, extraction and clean-up. It has been successfully used to extract different classes of compounds including pesticides and pharmaceuticals from a wide range of matrices (22, 23). In addition, it has proven to be fast and inexpensive in the extraction of PAHs from various matrices like water, air and food products (24–26).

There are multiple merits for QuEChERS over most traditional techniques. To begin with, acetonitrile-based samples prepared by QuEChERS can be introduced into GC as well as liquid chromatography. Since acetonitrile is soluble in water, it can penetrate water-based matrices for the withdrawal of the objective compounds. The solvent is adequately separated from the aqueous matrix when sodium chloride or magnesium sulfate is added. Moreover, the QuEChERS method is very simple, and its procedure is effortless. In addition to that, it is an energy-efficient process, which is due to the lower extraction solvent, lab space and fewer dishwashing needs than those needed for other approaches, and thus increasing the potential of decreasing the time and expenses of routine laboratory analysis (27).

In this study, we describe a method that combines QuEChERS with GC–MS to address simultaneously 13 PAHs in rat plasma. Being quick and cheap without excessive sample loss, the described method may be utilized in human biomonitoring studies of PAHs. The method was applied successfully to perform a minor toxicokinetic study for the degradation of the 13 PAHs in real rats. According to the available data, this is the first method to be introduced for the measurement of the 13 PAHs, simultaneously, in rat plasma, utilizing QuEChERS in sample preparation with subsequent toxicokinetic study in small animals as an attempt for quantitation of these compounds in trace levels of small biological samples.

Experimental

Materials

Pure standards

Fluorene, phenanthrene, pyrene, anthracene, fluoranthene, indeno [1,2,3-cd] pyrene, benzo [b] flouranthene, benzo [k] flouranthene, benzo [a] anthracene, benzo [a] pyrene, chrysene, dibenzo [a,h] anthracene and benzo [g,h,i] perylene were purchased from Sigma-Aldrich (Germany). All analytes were guaranteed to be >99% pure. Chemical structures of the studied compounds are presented in Figure 1.

Chemical reagents

All chemicals used during this study were of analytical grade, and the solvents were of HPLC grade. Dichloromethane and acetonitrile were purchased as HPLC grade from Chem-Lab (Belgium). Sodium chloride was obtained from El-Nasr Pharmaceutical Chemicals Co (Abu-Zaabal, Cairo). Primary secondary amine (PSA) (Sepra™ (50 μm, 70A) Bulk Packing was purchased from Phenomenex (USA).

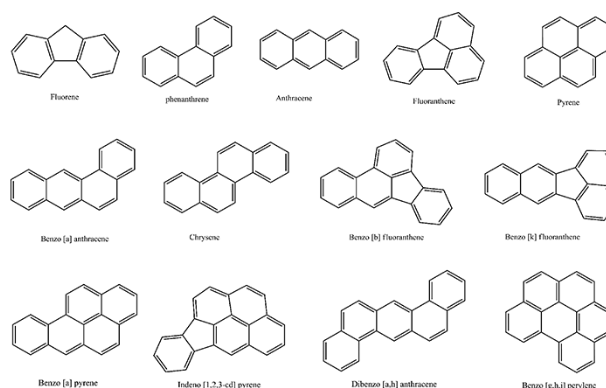

Figure 1. Chemical structure of the studied compounds.

Anhydrous magnesium sulfate was obtained from Fisher Chemical (UK). Adult male Sprague-Dawley rats (weighing 150–200 g) were purchased from a private animal house (Cairo, Egypt). Vacutainer EDTA (Ethylenediaminetetraacetic acid) tubes (Vacuette K3E) were purchased from Greiner-Bio-One GmbH (Germany).

Methods

Apparatus

A 5975C inert XL EI/CI MSD gas chromatography system (Agilent Technologies, Santa Clara, CA, USA) supplied with Triple-Axis Detector and electron ionization source (70 eV) was utilized for analysis. Mass Hunter software was employed for data processing and system control. Chromatographic separation was accomplished using a DB-5MS-fused silica column (30 m × 0.25 mm I.D., 0.25-μm film) (Agilent J & W) and a constant helium flow at 1 mL/min (90 kPa). GC inlet was operated at 280°C in pulsed splitless mode, with injection volume of 2-μL sample. The temperature program commenced at 90°C, accompanied by a 30°C/min gradual increase to 170°C, 10°C/min gradual increase to 290°C and then held for 5–10 min, resulting in a run time of 25.67 min. The transfer line was operated at 280°C. The MS source was maintained at 230°C, and quadrupole was maintained at 150°C. The MS system was routinely set in selective ion monitoring mode, with a solvent delay of 3 min using separate ions to identify and confirm the analytes.

- Super High Speed Refrigerated Centrifuge Z36 HK (Hermle Labortechnik, Germany).
- Advanced Vortex mixer ZX3 (F20230176, Alfa Medical Westbury, China).

GC–MS separation and identification

Identity of PAHs in the samples was confirmed by the retention time and abundance of target and qualifier ions in the authentic PAHs standards as well as qualifier-to-target ion ratios. The selected target and qualifier ions and their ratios used to quantify and identify the 13 PAHs are shown in Supplementary Table A1.

Solutions and standards

The working solution containing the PAH mixture was prepared by diluting a standard stock solution (100 μg/mL) in dichloromethane to produce a final concentration of (1 μg/mL). Serial dilution was prepared from the standard working solution to produce final concentrations of 5, 7, 20, 30, 40, 50, 80, 90 and 100 ng/mL. Quality control (QC) solutions were set at 10, 60 and 70 ng/mL. All dilutions

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were carried out using dichloromethane. Fresh working solutions were prepared for each analysis or validation day. All solutions were maintained at −20°C when unused.

Spiked and real samples

Exact volumes (50–1,000 μL) of the standard working solution (1 μg/mL) were spiked into 500 μL of blank plasma. Standard concentrations were 5, 7, 20, 30, 40, 50, 80, 90 and 100 ng/mL in blank plasma after sample preparation. QC samples were 10, 60 and 70 ng/mL.

Plasma samples were withdrawn from rats following administration of PAH mixture (0.25, 2 mg/kg, IP (intraperitoneally)). Blood samples were obtained from the venous sinus where the rat is restrained, the neck scruffed gently and the eye made to protrude. A capillary tube is placed dorsally, laterally or medially. Blood is permitted to flow by capillarity into the capillary tube. Biological samples were transferred to EDTA-containing vacutainers and maintained at −80°C until use. QC samples and calibration standard samples were daily freshly prepared.

Sample preparation

Pretreatment of samples was carried out by QuEChERS procedure where 500 μL of plasma samples (spiked or blank) was vortexed in a 15-mL Teflon tube with 7 mL acetonitrile. Half gram of sodium chloride and 2 g anhydrous magnesium sulfate were added and vortexed for another 5 min. The tube was centrifuged at a high speed (6,000 rpm) for 20 min. The upper layer containing acetonitrile was then moved to a clean-up tube comprising 0.6 g anhydrous magnesium sulfate and 0.07 g PSA, which is vortexed for 1 min and centrifuged at 6,000 rpm for another 20 min. The purified extract was dried at 40°C on a hot water bath and finally reconstituted in 10 mL dichloromethane for GC–MS analysis.

Method validation

Method validation was conducted in agreement with the ICH (International Conference on Harmonisation) guidelines (28). Selectivity ($n = 6$) was assessed by correlating chromatograms of blank plasma, with chromatograms of plasma fortified at lower limit of quantitation (LLOQ, 5 ng/mL for most of the analytes). Linearity was evaluated using standard samples within the range of 5–100 ng/mL for plasma.

Calibration curves were plotted as peak areas versus concentrations. Repeatability (within a day precision) ($n = 5$), reproducibility (between-day precision) ($n = 15$) (relative standard deviation (% RSD)) and accuracy (% recovery) were determined by analyzing QC samples at 10, 60 and 70 ng/mL.

Recovery

Absolute recoveries were assessed as the amount of sample extracted relative to that of a standard prepared in dichloromethane. Samples were prepared ($n = 3$) at low QC (10 ng/mL), mid QC (30 ng/mL) and high QC (90 ng/mL) levels, and were compared to the equivalent standards in dichloromethane. Recoveries were computed for each of the three extractions.

Animal treatment and real samples

Male adult Sprague-Dawley rats (7 weeks, 150–200 g, $n = 5$) were used for toxicokinetic study, where rats were kept in polystyrene cages with wood chip bedding in the animal facility of Faculty of Pharmacy, Ain Shams University, under controlled temperature, humidity and illumination. Rats were provided with plentiful supply of clean drinking water, yet they were deprived of food for 24 h before the experiment and were adapted to the animal facility habitat for few days before beginning the study.

Intraperitoneal doses of 0.25 and 2 mg/kg were provided in vegetable oil. Samples were gathered at specific timepoints (0.5, 1, 2, 4 and 6 h) and stored in a deep freezer until their manipulation. Blood samples were then moved to anticoagulant-coated tubes and instantaneously centrifuged at 4,000 rpm for 10 min at 4°C for plasma separation. All samples were instantaneously treated using the previous method and analyzed.

Ethical statement

Experimental procedures and animal handling were conducted in compliance with guidance from the National Research Council for the care and use of laboratory animals (29) and were accepted by the Research Ethics Committee at Faculty of Pharmacy, Ain Shams University.

Results and Discussion

Sample preparation development

Optimization of extraction and clean-up procedures

The initial attempts using solid-phase extraction (SPE) (with dichloromethane:acetone in the ratio 1:1 as extraction solvent) and liquid–liquid extraction (LLE) procedures (with acetone, cyclohexane and methylene chloride) failed because multiple interferents were observed in the extracting solution, which obviously worsened the limit of detection (LOD). In addition to that, only 10 out of the 13 PAHs could be identified. Moreover, uncleaned extract of plasma with co-extractives might spoil the chromatographic column. Thus, it was essential to use a universal method as QuEChERS to obtain satisfactory results.

QuEChERS technique gives the advantage of reducing the time required for preparing samples in comparison to other conventional methods. In addition, when conventional methods were used for such a complex matrix, it was essential to incorporate a clean-up procedure with columns packed with aluminum oxide, silica gel or florisil, which was tedious and labor-intensive.

The extraction solvent was necessary to precipitate plasma proteins. The following solvents were tested: acetonitrile, dichloromethane and hexane. In the latter two cases, the extract included a great proportion of co-extractives. On the contrary, on utilizing acetonitrile, there was minimal co-extraction of interferents. Furthermore, addition of salts after acetonitrile extraction enhanced separation between aqueous and organic phases (30). The highest efficiency for PAH extraction was achieved by acetonitrile, so it was chosen for further studies. However, the crucial step was the determination of its volume. Different volumes were tested (3, 5, 7 and 9 mL), and it was observed that as the volume increases, recoveries increase but to a certain limit. Upon trial of 9 mL acetonitrile, lower recoveries were obtained for the last few eluted compounds. Thus, 7 mL was the optimum volume as shown in Figure 2.

In the QuEChERS method, dispersive SPE is utilized for extract purification. The objective of the sorbent is to adsorb interferents and maintain PAHs in the extract. The PSA sorbent was the optimum choice. PSA binds co-extractives such as sugars and fatty acids and allows more efficient separation of the studied PAHs from lipids. In brief, PSA permits isolation of PAHs from fats; thus, interferents can be adsorbed by the sorbents while analytes are kept in the extract. In

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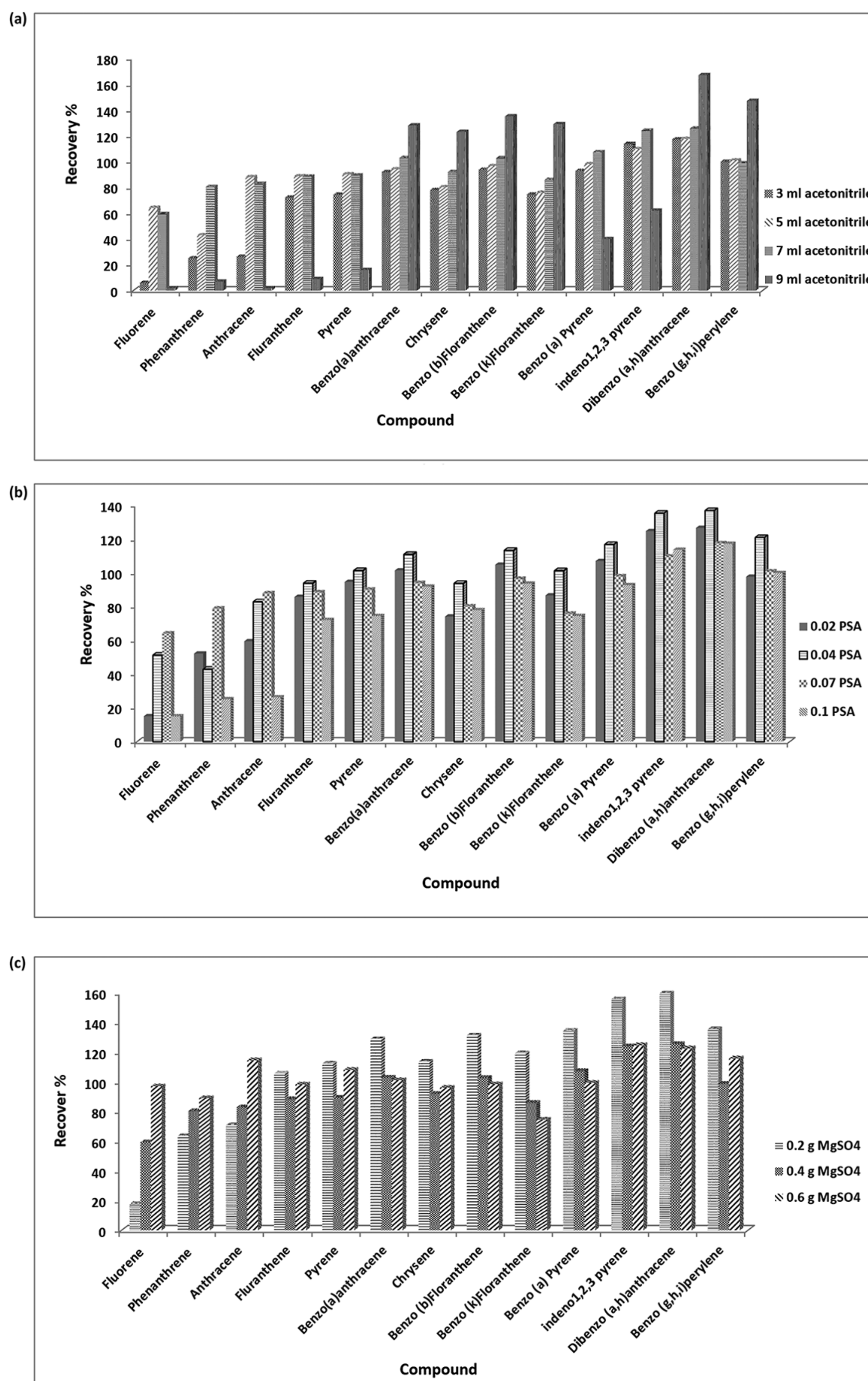

Figure 2. Effect of clean-up step on the extraction efficiency of the compounds from spiked rat plasma using different amounts of acetonitrile, PSA and $MgSO_4$. (a) Effect of acetonitrile volume. (b) Effect of PSA amount. (c) Effect of $MgSO_4$ amount.

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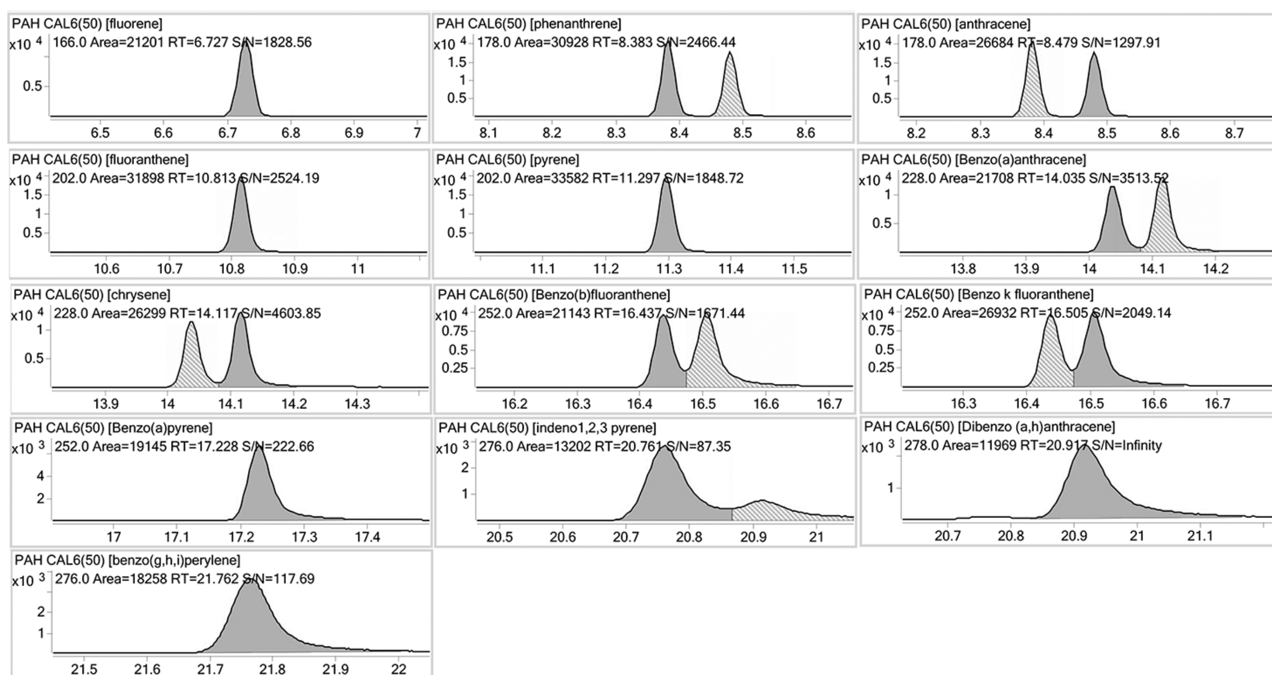

Figure 3. Extracted ion chromatograms of 13 PAHs (50 ng/mL) obtained during GC–MS calibration.

our method, the required quantity of PSA was increased to 0.07 g, which produced the highest recoveries as shown in Figure 2. Similar to the volume of acetonitrile, different amounts of PSA (0.02, 0.04, 0.07 and 0.1 g) were tested, where the recoveries of the studied analytes increased by increasing the amount of PSA up to 0.07 g.

As mentioned before, the addition of salts in QuEChERS method was necessary for the separation of the water-organic phase. However, the use of higher amounts of salts after acetonitrile addition decreased the volume of the supernatant and thus was not applicable. So, 2 g of magnesium sulfate was used in the extraction step, while 0.6 g was used in the clean-up step after optimization using different amounts (0.2, 0.4 and 0.6 g) as shown in Figure 2.

A literature review was performed to compare the developed method with others as shown in Supplementary Table A2. The most commonly used methods are conventional techniques used for extraction of human blood, plasma and serum such as LLE, SPE, solid-phase microextraction and thermal extraction. It can be deduced from the collected data that no previously reported methods were used for extraction of PAHs from rat plasma, and the LOD of PAHs in rat plasma with the QuEChERS procedure (0.045–0.372 ng/mL) was lower than that with LLE and SPE. Comparing our method to the reported method using QuEChERS for determination of PAHs in green turtle blood (31), our method has the advantage of studying the effect of various parameters on extraction efficiency such as different concentrations of salts and adsorbents, as well as different concentrations of PSA (with the advantage of economic use of PSA alone without the need for C18 and the elimination of freezing out step) and optimizing the concentration of each used chemical for achieving satisfactory detection of the 13 PAHs in rat plasma.

GC–MS method development

Method development in this work commenced by using a different column (HP-5 MS capillary column coated with 5% phenylmethylsiloxane), which produced longer run time and did not identify all peaks; however, the best choice was DB-5 MS-fused silica capillary column where the last compound was identified at only 20.9 min. Extracted ion chromatograms showing retention times of the studied compounds and the mass spectra of pure samples showing target and qualifier ions used for identification are presented in Figures 3, 4 and A4.

Different temperature programs were attempted, and the optimum was as per a previously reported method (32).

Method validation

Selectivity

The selectivity ($n = 6$) was evaluated by analyzing representative chromatograms obtained from both blank and spiked matrices with LLOQ standards (5 ng/mL) to assess the potential interferences from endogenous components. No intrusion was observed, from internal components in the matrix, at the retention times of 13 PAHs in blank samples as shown in Supplementary Figure A1.

Linearity and sensitivity

Calibration curves were constructed from peak areas against the corresponding spiked concentrations as indicated in Supplementary Figure A2 and Table I, which revealed valid linear responses ($r > 0.995$) from 5 to 100 ng/mL for most of the target analytes and a few from 7 to 90 ng/mL. The LODs and limits of quantitation (LOQs) for the 13 compounds ranged from 0.045 to 0.372 ppb and from 0.137 to 1.128 ppb, respectively, as presented in Table I. The sensitivity of the method was expressed by the LLOQ, which was the lowest concentration within 20% precision and accuracy. The analyte signal-to noise (S/N) ratio was also used to evaluate the sensitivity.

Precision and accuracy

Repeatability (within a day precision) ($n = 5$), reproducibility (between-day precision) ($n = 15$) and accuracy ($n = 5$) were computed using QC samples at 10, 60 and 70 ng/mL in rat plasma. Precision was evaluated by % RSD, while accuracy was evaluated

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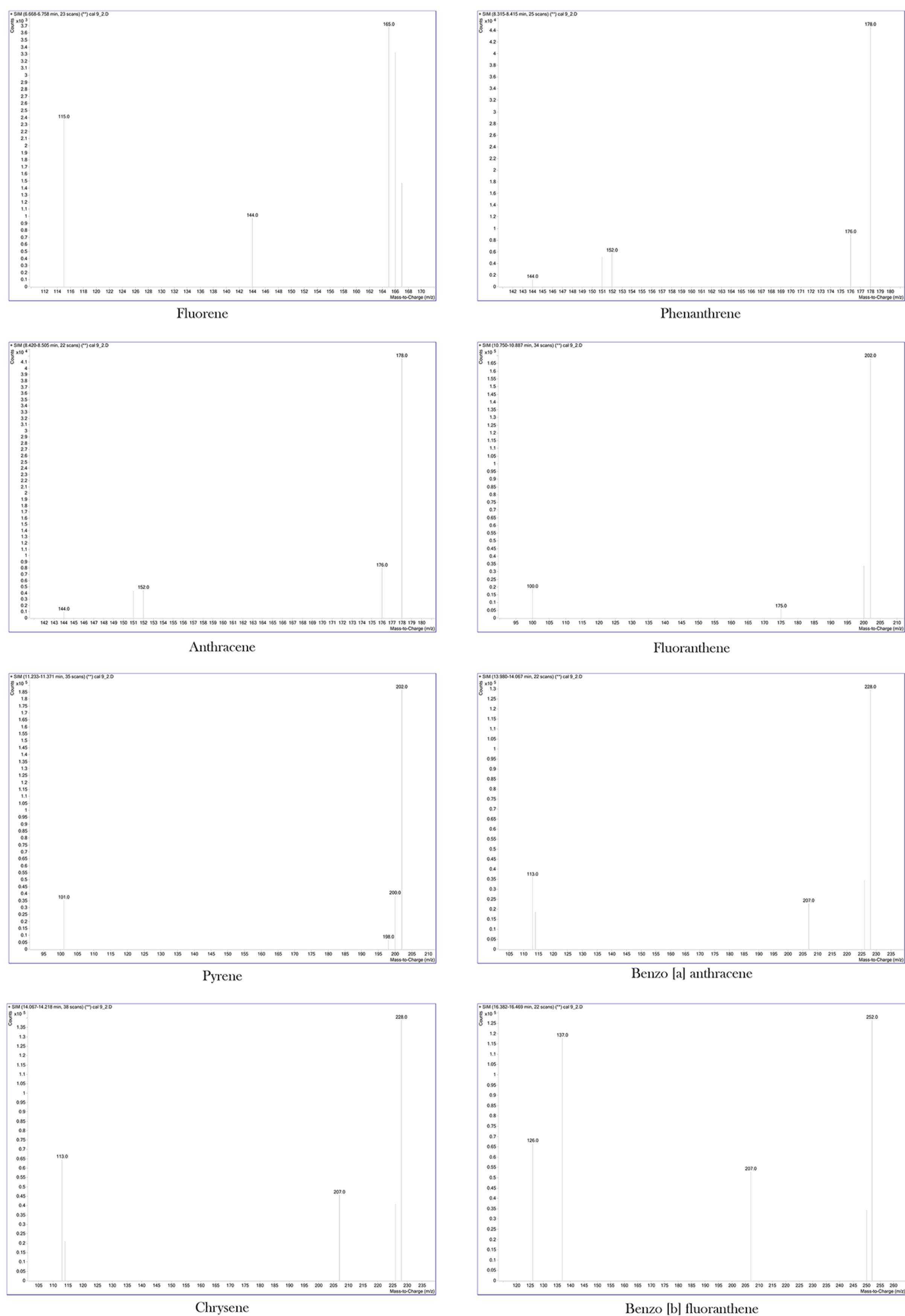

Figure 4. Mass spectra of 13 PAHs (50 ng/mL) obtained during GC–MS calibration.

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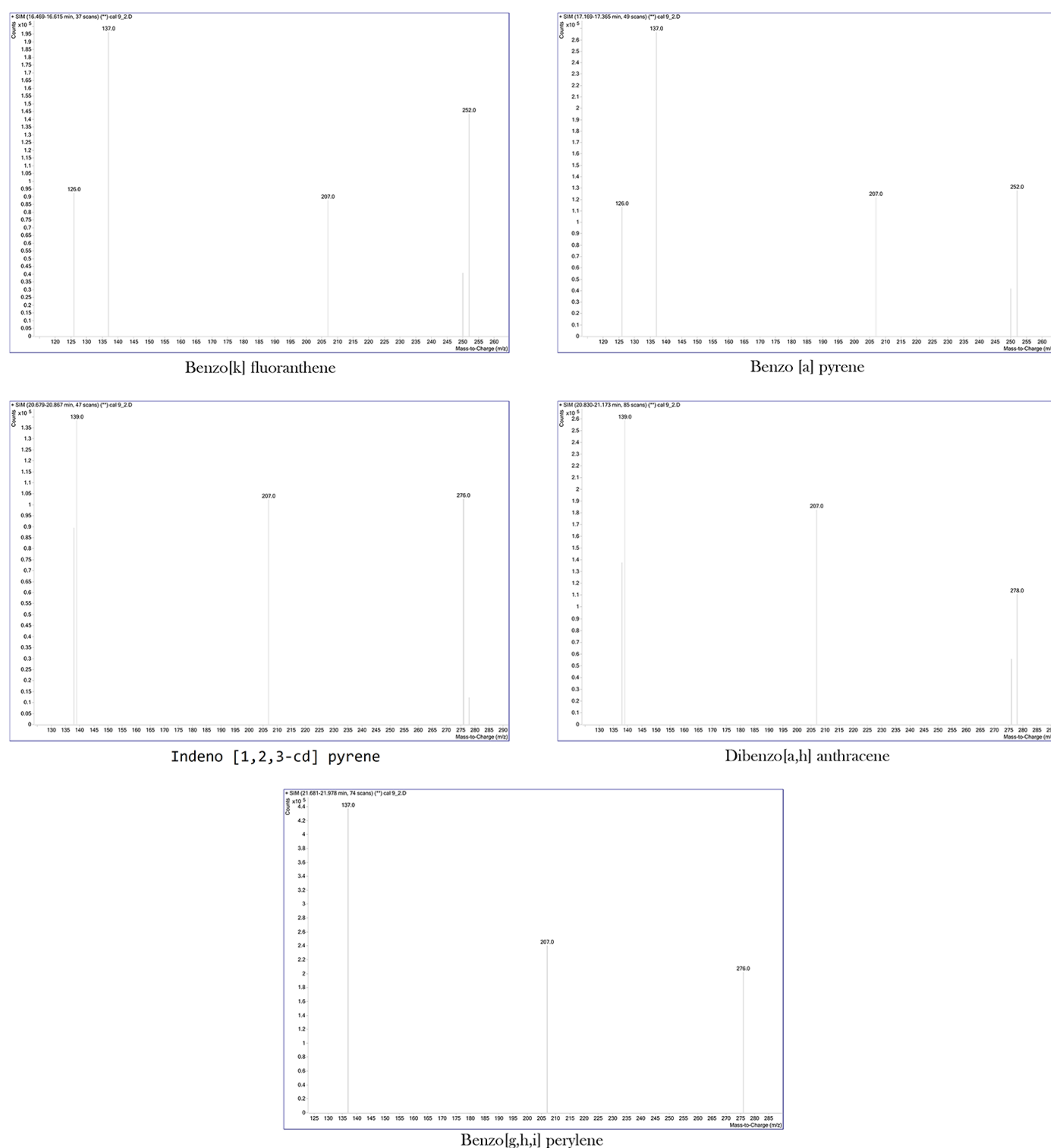

Figure 4. (Continued)

between measured concentrations and true concentrations. Supplementary Table A3 shows recovery percent (Rec %), where response is accurate (80–120%) for each compound in plasma samples whereas the % RSD values are <2.71% for the QC samples for all analytes as shown in Supplementary Tables A4 and A5. The values are <15% for the QC samples and <20% for the LLOQ for all analytes in plasma matrix according to the ICH guidelines for bioanalytical method validation (28).

Recovery

Absolute recoveries ($n = 3$) for the 13 PAHs were determined to be within 85.57%–109.64% in fortified rat plasma with standard deviations (SDs) <8.91 except for anthracene with SD of 19.24 as shown in Table II. The absolute recoveries varied for different analytes; however, they were congruous, precise and reproducible at the three concentrations for the same compound.

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Table I. Results of Validation Parameters for the Determination of the 13 PAHs by the Proposed Method

Name of PAH	Range (ng/mL)	Mean± standard deviation (SD)	RSD	Regression equation	Correlation coefficient (r)	Intraday precision (% RSD)	Interday precision (% RSD)	Limit of detection (LOD[a], ppb)	Limit of quantitation (LOQ[b], ppb)
Flourene	7–100	99.95 ± 2.09	2.09	$y = 368.49x + 2{,}043.80$	0.9993	1.47	2.56	0.37	1.11
Phenanthrene	7–90	100.02 ± 1.95	1.95	$y = 569.51x - 1{,}659.90$	0.9992	0.79	2.70	0.14	0.44
Anthracene	7–100	100.07± 0.95	0.95	$y = 356.05x - 214.86$	0.9997	0.78	0.97	0.11	0.34
Flouranthene	5–100	99.60± 1.45	1.46	$y = 596.26x + 1{,}661.50$	0.9992	2.63	2.32	0.05	0.14
Pyrene	5–100	99.85± 2.07	2.07	$y = 638.93x + 1{,}907.70$	0.9992	2.57	1.28	0.21	0.64
Benzo [a] anthracene	5–100	100.05± 1.89	1.89	$y = 528.74x + 593.40$	0.9994	1.73	1.37	0.26	0.77
Chrysene	5–100	100.07± 2.05	2.05	$y = 565.1x + 223.34$	0.9983	1.13	1.02	0.10	0.31
Benzo [b] flouranthene	5–100	99.68± 1.88	1.89	$y = 474.84x + 1{,}583$	0.9995	1.08	1.69	0.09	0.26
Benzo [k] flouranthene	5–90	99.91± 1.16	1.16	$y = 507.68x + 820.79$	0.9998	0.57	0.35	0.08	0.25
Benzo [a] pyrene	5–90	100.10± 1.54	1.54	$y = 461.26x + 913.47$	0.9999	1.16	1.79	0.13	0.39
Indeno [1,2,3-cd] pyrene	5–90	99.78 ±2.72	2.72	$y = 385.63x - 400.11$	0.999	0.94	1.01	0.35	1.06
Dibenzo [a,h] anthracene	5–90	99.99± 2.02	2.02	$y = 361.86x - 380.78$	0.9992	0.77	2.23	0.37	1.13
Benzo [g,h,i] perylene	5–90	99.87 ± 1.30	1.30	$y = 458.26x - 65.93$	0.999	1.02	0.16	0.36	1.09

LOD and LOQ are determined based on signal-to-noise ratio.
[a]LOD = 3 × S/N.
[b]LOQ = 10 × S/N.

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Table II. The Absolute Recoveries for the Determination of the 13 Compounds at Different Spiking Levels in Rat Plasma Using the Proposed Method ($n = 3$)

Name of compound	Spiked level (ng/mL)	Found (ng/mL)	Recovery %[a]
Flourene	10	9.35	93.49
	30	28.57	95.33
	90	79.76	88.63
	Rec ± SD		92.45 ± 3.42
Phenanthrene	10	8.31	83.09
	30	27.03	90.11
	90	75.15	83.57
	Rec ± SD		85.57 ± 3.94
Anthracene	10	10.45	104.50
	30	34.14	113.80
	90	69.13	76.81
	Rec ± SD		98.37 ± 19.24
Flouranthene	10	9.76	97.58
	30	25.62	85.39
	90	73.31	81.45
	Rec ± SD		88.14 ± 8.41
Pyrene	10	9.85	98.50
	30	25.53	85.11
	90	73.47	81.63
	Rec ± SD		88.42 ± 8.90
Benzo [a] anthracene	10	11.64	116.45
	30	32.76	109.21
	90	89.75	99.72
	Rec ± SD		108.46 ± 8.30
Chrysene	10	9.68	96.78
	30	28.4	94.67
	90	79.15	87.94
	Rec ± SD		93.13 ± 4.62
Benzo [b] flouranthene	10	10.49	104.88
	30	33.99	113.28
	90	91.30	101.44
	Rec ± SD		106.53 ± 6.09
Benzo [k] flouranthene	10	9.53	95.33
	30	24.83	82.77
	90	71.29	79.22
	Rec ± SD		85.77 ± 8.46
Benzo(a) pyrene	10	10.73	107.28
	30	31.41	104.70
	90	83.79	93.10
	Rec ± SD		101.69 ± 7.55
Indeno [1,2,3-cd] pyrene	10	10.02	100.23
	30	31.22	104.08
	90	99.21	110.24
	Rec ± SD		104.85 ± 5.05
Dibenzo [a,h] anthracene	10	11.69	116.96
	30	33.14	110.46
	90	91.36	101.51
	Rec ± SD		109.64 ± 7.75
Benzo [g,h,i] perylene	10	11.20	112.00
	30	32.96	109.87
	90	86.32	95.91
	Rec ± SD		105.93 ± 8.74

[a] Average of three determinations.

Matrix effect

To evaluate the matrix effect, both solutions for standards and matrix (corresponding to blank plasma samples fortified after sample preparation) were injected and compared ($n = 3$). Matrix effect was then computed as shown in Table III, where accuracy was within ±15% of the nominal concentration. The types of matrix effects defined as enhancement or suppression could be determined where the matrix caused enhancement of all compounds except for

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Table III. Matrix Effect for the Determination of the 13 Compounds at Different Spiking Levels in Rat Plasma Using the Proposed Method ($n = 3$)

Name of compound	Spiked level (ng/mL)	Found (ng/mL)	Matrix effect (ME %)[a]
Flourene	7	5.89	84.12
	30	30.63	102.10
	90	89.08	98.98
Phenanthrene	7	5.73	81.80
	30	33.87	112.90
	90	86.44	96.04
Anthracene	7	7.92	113.20
	30	31.07	103.57
	90	92.09	102.32
Fluoranthene	7	7.41	105.84
	30	33.66	112.20
	90	93.22	103.58
Pyrene	7	7.59	108.40
	30	34.33	114.44
	90	92.13	102.37
Benzo [a] anthracene	7	8.07	115.24
	30	33.74	112.48
	90	102.16	113.52
Chrysene	7	7.89	112.71
	30	28.97	96.57
	90	91.22	101.36
Benzo [b] fluoranthene	7	8.01	114.42
	30	33.91	113.04
	90	98.33	109.25
Benzo [k] flouranthene	7	6.54	93.40
	30	29.15	97.16
	90	84.12	93.46
Benzo [a] pyrene	7	8.08	115.43
	30	34.18	113.92
	90	100.35	111.50
Indeno [1,2,3-cd] pyrene	7	8.10	115.71
	30	35.17	114.73
	90	103.98	115.53
Dibenzo [a,h] anthracene	7	8.08	115.43
	30	33.17	110.57
	90	103.62	115.13
Benzo [g,h,i] perylene	7	7.85	112.14
	30	34.42	114.72
	90	97.78	108.64

[a] Average of three determinations.

fluorene, phenanthrene and benzo [k] fluoranthene, where it caused suppression.

To counterbalance the matrix effect, matrix matched calibration curves were applied for quantitation.

Application to the analysis of real samples

To manifest the effectiveness of this procedure in an experimental situation, the proposed procedure was applied to the quantitation of compounds in rat plasma samples for a minor toxicokinetic study as presented in Table IV. Concentrations of the compounds extracted from rat plasma after intraperitoneal dosing of 0.25 and 2 mg/kg of rats ($n = 5$) over a 6-h time period is shown in Supplementary Figure A3. Most of the compounds were not detected at 0.25 mg/kg; however, the 13 compounds were recognized in plasma samples at 2 mg/kg, although some were not detected at 1 and 6 h. It is evident from Supplementary Figure A3 that all PAHs reached the peak concentration in the rat plasma at 1 h after administration followed by a gradual decrease. The peak concentration of anthracene was the highest followed by phenanthrene. Fluorene, fluoranthene and pyrene demonstrated similar peak concentrations. Anthracene peak concentration was twice as high as each of dibenzo [a,h] anthracene, chrysene, indeno [1,2,3-cd] pyrene and benzo [g,h,i] perylene that showed no significant difference from each other, while benzo [a] pyrene, benzo [b] fluoranthene and benzo [k] fluoranthene exhibited the least peak concentrations. As a result of the few data points analyzed, these values should be carefully managed and more studies with greater number of animals and extra toxicokinetic parameters are needed to provide an eventual interpretation.

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Table IV. Plasma Concentrations of 13 Polycyclic Aromatic Hydrocarbons in Plasma Samples Obtained from Male Rats after IP Administration of a Dose of 2 mg/kg ($n = 5$)

Analyte	Plasma concentrations (ng/mL) at different time intervals				
	0.5 h	1 h	2 h	4 h	6 h
Fluorene	3.08	19.52	3.96	2.43	0.57
Phenanthrene	8.96	26.63	18.19	13.01	7.57
Anthracene	6.68	35.01	11.57	11.09	7.01
Flouranthene	1.06	19.64	5.12	4.27	1.61
Pyrene	1.08	19.42	5.25	4.07	1.57
Benzo [a] anthracene	4.79	15.58	2.84	2.18	0.88
Chrysene	0.93	16.50	3.99	2.43	1.34
Benzo [b] fluoranthene	2.23	14.20	0.81	0.20	ND
Benzo [k] fluoranthene	0.46	15.03	1.72	0.80	ND
Benzo [a] pyrene	5.41	15.13	1.86	0.97	ND
Indeno [1,2,3-cd] pyrene	ND	16.28	3.80	3.25	0.43
Dibenzo [a,h] anthracene	0.58	16.37	3.85	3.21	1.59
Benzo [g,h,i] perylene	1.39	15.96	3.54	2.71	0.83

ND: not detected.

Conclusion

In the present study, a novel sensitive and reliable analytical method has been established and applied to the determination of 13 PAHs, simultaneously, in rat plasma.

The method exhibited perfect accuracy and precision and has been applied with acceptable success to a minor toxicokinetic study, following IP administration to rats. Thus, the developed method is applicable to biomonitoring in domestic exposures as well as in forensic and clinical studies.

Supplementary data

Supplementary data is available at *Journal of Analytical Toxicology* online.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Conflict of Interest

The authors declare that they have no conflict of interest.

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