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Hollow Polymer Nanospheres and Fe_3O_4@TFPA-Bd-COF as a Mixture Adsorbent in Microextraction by Packed Sorbent for Extraction of BTEX Biomarkers in Urine

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A new and fast sample preparation method was developed to extract Hippuric acid (HA), Trans,trans-muconic acid (tt-MA), Mandelic acid (MA), and m-Methylhippuric acid (m-MHA) in urine samples using Hollow polymer nanospheres (HPSs), Covalent organic frameworks (COFs), and their mixture (HPS:COF) as an adsorbent, combined with high-performance liquid chromatography-ultraviolet spectrophotometry (HPLC-UV). X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to study the features of the adsorbents. The effect of important factors such as temperature and pH of the sample, amount of sorbent, conditioning and washing solvents, type and volume of desorption solvent, sample volume, and the number of extraction cycles were investigated to achieve the optimal conditions with microextraction by packed sorbent (MEPS) procedure. The applicability of the proposed method was then validated in the laboratory. Finally, the target analytes in the urinary samples of gas station workers were examined. The data analysis showed that using an HPS:COF mixture improves the extraction efficiency in optimal conditions compared to the case where the COF adsorbent is used alone (extraction efficiency, 81-87.5%). Also, a good linear dynamic range (0.1-1000 μg ml^{-1} for m-MHA), a low detection limit (0.02 μg ml^{-1} for tt-MA), and acceptable intra-day and inter-day precision (1.4-3.6 % and 4.5-8.9 %, respectively) were obtained under similar conditions. Overall, HPS: COF-MEPS exhibited excellent extraction efficiency (85.4-93.3%) and has the potential to replace previous methods in the biomonitoring of BTEX biomarkers in urine.

Keywords: BTEX biomarkers, HPSs: COF adsorbent, HPLC-UV, MEPS method, Urine samples

INTRODUCTION

Benzene, toluene, ethylbenzene, and xylene (BTEX) are among the most well-known volatile organic compounds [1]. These organic compounds have several adverse health impacts, such as probable neurological and cancer induction effects, fatigue, weakness, confusion, nausea, and loss of appetite [2]. Benzene is reported as a probable human carcinogen (leukemia and aplastic anemia), and ethylbenzene has been mentioned as a suspected carcinogen [3,4]. These compounds usually enter the body via respiratory tracts and, inside the body, are metabolized to their specific biomarkers such as trans,trans-muconic acid, mandelic acid, hippuric acid, and methylhippuric acid (Table 1) [5].

The accurate extraction and determination of BTEX compounds with different chemical structures and a wide

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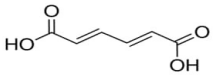
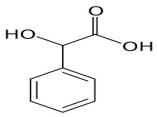
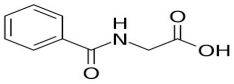
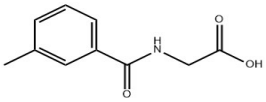
Table 1. The Properties, Chemical Structure, and Concentration of the Target Analytes

Analytes	Chemical structure	logP	pKa	Studied concentration	Ref.
Trans, trans-muconic acid (tt-MA)		0.49	3.87	0.02-100	[23]
Mandelic acid (MA)		0.9	3.75	0.1-320	[24]
Hippuric acid (HA)		0.53	3.59	0.1-500	[25]
m-Methylhippuric acid		1.04	3.74	0.1-1000	[26]

range of polarities is challenging. The conventional procedures for BTEX biomarkers from urine samples, such as the liquid-liquid extraction (LLE), only extract a nonpolar or polar analyte because the polarity of the solvent used for extraction mainly determines the extraction. Another conventional method, solid phase extraction (SPE), uses nonpolar adsorbents (mainly C_{18}) to extract the nonpolar target analytes [6,7]. Hence, the extraction of chemical compounds with different polarity ranges, such as BTEX biomarkers, is unsuitable *via* the SPE and LLE methods and with the common adsorbents. The present work proposed an alternative procedure to extract BTEX biomarkers with various polarities based on the packed sorbent microextraction and using a mixture adsorbent (HPSs:COF) from urine samples.

Microextraction by packed sorbent is a modern sample preparation method that carries the sampling, extraction, and injection of target analytes into gas or liquid chromatography via an automated sampling and injection device [8]. This method uses the main principles of SPE, although it miniaturizes the analysis process by utilizing small amounts of samples, solvents, and sorbents [9]. Microextraction by packed sorbent allows reusing the adsorbent (more than 100 times) without loss in its extraction efficiency. In addition, it can lower solvent consumption, sample volume, and extraction time, making it a green procedure. This method has been widely and successfully used to extract and determine a wide range of target analytes from different biological and environmental matrixes [10]. The long-term and frequent use of common MEPS sorbents are not noticeable, and the sorbent should be changed after a few extractions. Thus, it is highly fascinating to establish novel adsorbents which can simply and rapidly remove analytes from aqueous systems [11-15].

Covalent organic frameworks (COFs) and hollow polymer nanospheres (HPSs) have recently attracted much attention in the field of sample preparation [16,17]. COFs are characterized by their great surface area, low density, good selectivity, tunable pore size, and structural stability [18,19]. These frameworks have been used as adsorbents in SPE [20], solid-phase microextraction (SPME) [21], and magnetic solid-phase extraction (M-SPE) [22]. In contrast, HPSs are nanospheres containing a hollow cavity with a functional shell layer and tunable size. HPSs have been used for pollutant purification, drug delivery, active material encapsulation, support catalysts, and gas storage [17].

In the present work, we study the use of a new type of magnetized imine-linked COF (Fe_3O_4@TFPA-Bd), HPS, and their mixture (HPS:COF) in MEPS to extract and determine the BTEX biomarkers (Hippuric acid (HA),

Trans,trans-muconic acid (tt-MA), Mandelic acid (MA), and m-Methylhippuric acid (m-MHA)) with various polarity (log P from 0.49 to 1.04) from urine samples. The most important parameters influencing the analysis of tt-MA, MA, m-MHA, and HA were optimized, and the proposed method was validated through a laboratory study. Finally, the BTEX biomarkers in urinary samples of gas station workers were determined at Hamadan city (Iran).

EXPERIMENTAL

Chemicals

Chemicals used in this study were obtained from the following sources: MA, tt-MA, HA, m-MHA, and 2,4-dihydroxybenzoic acid (DA) were supplied from Merck (Schuchardt, Germany); formaldehyde (37%), Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Sodium citratedehydratese ($Na_3Cit \cdot 2H_2O$), Benzidine (Bd), and Tris (4formyl phenyl) amine (TFPA) were obtained from Sigma-Aldrich (Louis, USA). HPLC grade methanol, ethanol, propanol, acetonitrile, acetone, acetic acid glacial, oleic acid, sodium acetate, hydrochloric acid, ammonia solution (25%), tetrahydrofuran (THF), ethylene glycol (EG) and dimethyl sulfoxide (DMSO), were provided from Merck (Darmstadt, Germany).

Instruments

Agilent model 1260 HPLC instrument was employed to perform chromatographic analyses. The device was armed with a UV-Vis detector (1200 infinity model) set at 254 nm and 259 nm for extracting the HA, m-MHA, and MA, tt-MA, respectively. The separation was performed using a C_{18} column (3.5 µm, 4.6 mm × 100 mm, reversed-phase), and at room temperature. A mixture of water/acetonitrile/ acetic acid (84:16:0.025, v/v/v %) and a mixture of water/methanol/acetic acid (69:30:1, v/v/v %) was used for extracting m-MHA, and HA/tt-MA, and MA in urine, respectively. The mobile phases were daily prepared and an isocratic eluting at a flow rate of 0.7 ml min^{-1} was used to analyze the samples (injection volume of 20 µl). A needle exchangeable syringe (500 µl, Hamilton, USA) was used as the MEPS syringe. Deionized water was obtained from a purification device (TKA, Germany).

Type of Sorbent

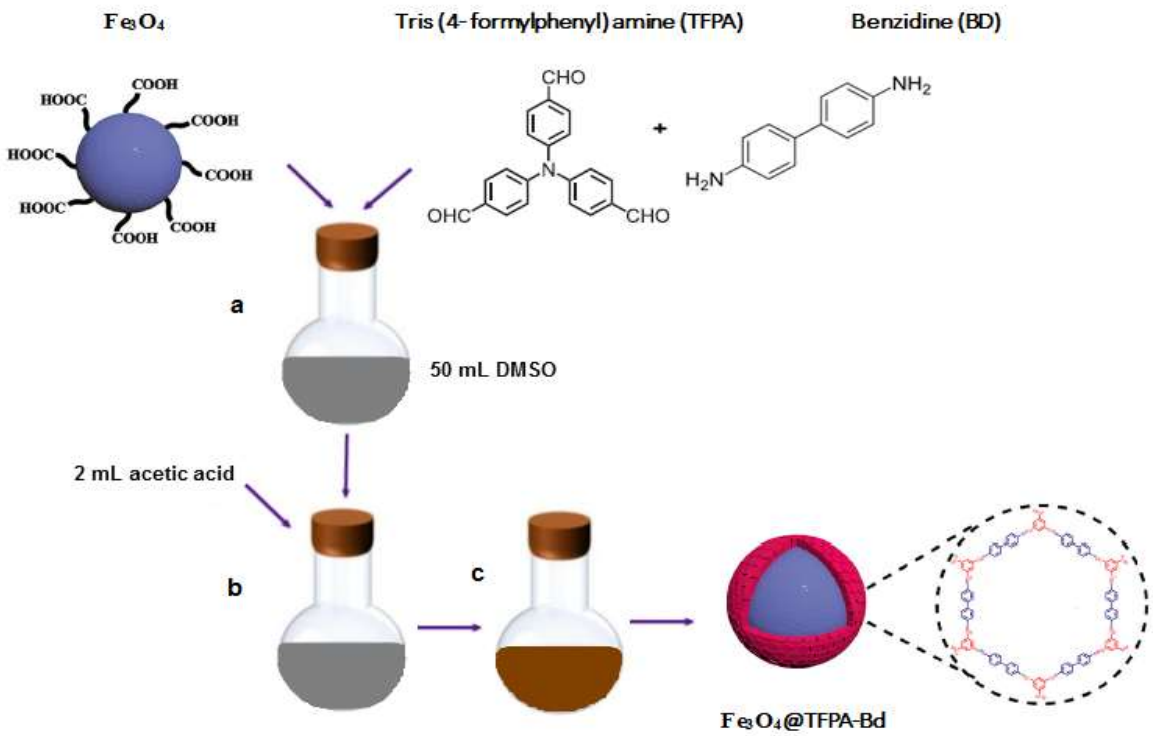
The success of the MEPS procedure in extracting the target analytes mostly depends on the selection of a proper sorbent. The attraction of a compound by a sorbent occurs mainly via weak chemical interactions, including π-π, van der Waals interactions, electrostatic, and hydrogen bonding [22, 27-29]. In this study, the adsorbents (HPSs, COF, and HPSs: COF) were investigated by packing the solid sorbent into the MEPS syringe. The adsorbents with various polarities were selected to encompass a large range of polarities. The synthesis methods of the HPS, as well as Fe_3O_4 nanoparticles, were mentioned in previous studies published by the authors [30,31]. Briefly, the core-shell structured Fe_3O_4@TFPA-Bd nanobeads were synthesized through the precipitation polymerization of Bd and TFPA for coating Fe_3O_4 NPs with COF shells. First, 75 mg of Fe_3O_4, 24.3 mg of TFPA, and 41.5 mg of Bd were added to 50 ml DMSO under ultrasound. Then, 2 ml acetic acid was added to the above solution, and the yellow mixture was transformed into a Teflon autoclave and heated for 72 h (120 °C). The products were collected via a magnet, washed with methanol and THF, and dried (at 25 °C). The synthetic process to prepare Fe_3O_4@TFPA-Bd nanobeads is represented in Scheme 1.

The preparation process of HPS is also simple and fast: First, 15.0 mmol of formaldehyde and 5.0 mmol of DA were resolved in 200 ml of deionized water. A 10 ml aqueous solution including 105 µl of oleic acid and 360 µl of ammonia dilution (25%) was added to the above solution dropwise (35 °C). Next, it was transferred into a high-pressure autoclave and maintained at 150 °C for 4 h. The products were obtained after centrifugation, rinsing with ultrapure water and ethanol (6 times), and drying (60 °C) [17].

The mixed adsorbent was prepared by adding HPSs first into the MEPS syringe, followed by adding COF to the mixture. About 30 mg of solid sorbent was packed in the MEPS syringe between two PTFE frits and then used for extracting tt-MA, HA, MA, and m-MHA from urine samples.

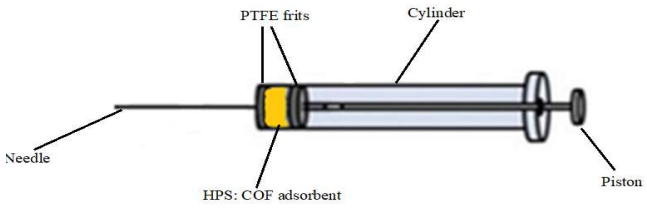
Preparation of MEPS Syringe

The packed sorbent microextraction syringe was set up under the approach introduced by Mohamed Abdel-Rehim [32]. First, two polytetrafluoroethylenes (PTFE) filter pieces with dimensions equal to the inner diameter of the syringe cylinder were supplied. A piece of the prepared filter was



Scheme 1. Diagram of the synthetic process to create COF nanobeads

placed into the syringe cylinder and packed by the syringe piston. Next, 30 mg of the HPSs: COF sorbent (50:50 w:w) was carefully transferred into the cylinder. In the end, another piece of the PTFE filter was moved into the cylinder and pressed by the piston to the extent that the sorbent was placed properly inside the syringe cylinder (Scheme 2).



Scheme 2. Diagram of MEPS syringe preparation

Adsorption Mechanism

A new extraction method was developed, and a suitable adsorbent was introduced by investigating the features of the analytes, sample, and sorbent. We first activated the packed sorbent with a suitable solution to open the alkyl chains from the tangled state. The solid bed was then exposed to a solution similar to the sample. As a result, a full-contact was established between the analytes and the adsorbent surface in later exposures. After passing the sample from the packed bed, the analytes were adsorbed on its surface by strong hydrophobic interactions, van der Waals, and π-π stacking. Next, disturbances with the analytes are washed by passing a suitable solution from the sorbent. Eventually, passing through a suitable solution, the adsorbed analytes on the solid phase were eluted and injected into an HPLC-UV device.

Standard Solutions

MA, HA, tt-MA, and m-MHA stock solutions were prepared at 1000 µl/ml by dissolving the required values of the compounds in urine. Calibration solutions were provided from the stock solutions by successively diluting the stock solutions with a proper volume of urine in an appropriate linear range. We also prepared quality control samples (QCs) according to Table 2.

Real Samples

End-of-shift urine samples (urine samples collected at the end of the work- week) were obtained from 5 healthy male workers exposed to BTEX compounds in gas stations, in

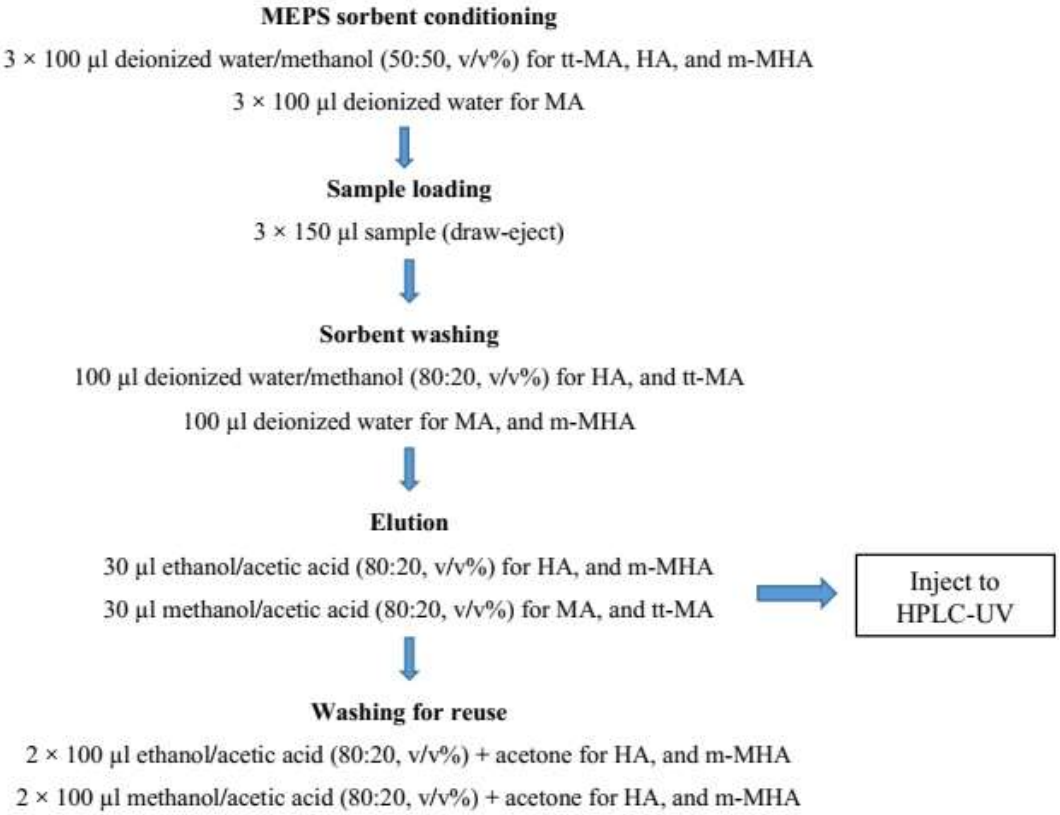
Table 2. The Quality Control Samples (QCs)

	The concentration of QCs (µg ml^{-1})		
Analytes	Low	Medium	High
tt-MA	0.02	50	100
HA	0.1	250	500
MA	0.5	160	320
m-MHA	0.1	500	1000

Hamadan city in July 2021. At the time of extraction, the collected samples were transferred to room temperature and an aliquot of them was homogenized by centrifugation, then diluted with water.

Extraction Procedure

In this study, mixed sorbent was prepared *via* adding HPSs first into the MEPS syringe, followed by the addition of COF. 30 mg of solid sorbent was packed in the MEPS syringe between two PTFE frits and then used for extracting tt-MA, HA, MA and m-MHA from urine samples. The mixed sorbent was activated by 100 µl of water/methanol (50:50, v/v%) solution (three times) and then conditioned with deionized water in the same condition. Next, a loading sample was carried out for 150 µl of a spiked urine sample by drawing up and down through the syringe and then discarding it (three times). The sample loading was performed at an approximate speed of 10 µl s^{-1} to ensure proper contact between the adsorbent and the target analytes [32]. In the washing step, packed sorbent was washed with 100 µl of deionized water/methanol mixture (80:20, v/v%) to eliminate the biological interferences. Finally, the adsorbed analytes on the solid sorbent were eluted with 30 µl of the ethanol-acetic acid mixture (80:20, v/v%), and then injected into the HPLC-UV system. To eliminate or reduce the carry-over effect of reusing the packed sorbents, eluting solution followed by an acetone solution was used after each extraction (Scheme 3).



Scheme 3. Diagram of the extraction procedure

Optimization of MEPS Procedure

The optimization of the most effective parameters for selected adsorbents was carried out in triplicates, and the average amounts were considered. The extraction efficiency percentage was calculated to achieve the optimum amounts. EE (%) was obtained employing the replication approach [33] according to Eq. (1).

$$EE\ (\%) = \frac{P_f}{P_s} \quad (1)$$

Where P_f is the peak area of the analyte in the first extraction and P_S refers to the summation of analyte peak areas in the replications.

RESULTS AND DISCUSSION

Structure Characterization of COF and HPS

In this research, the Fe_3O_4 NPs were created *via* a solvothermal reaction that allows coating these nanoparticles with the COF shell. Next, the COF framework was formed at room temperature *via* a Schiff-base condensation reaction of Tb and Bd in a DMSO solution.

The HPSs were created using a weak interaction-induced acid-base assembly. Moreover, the functional shells were prepared *via* the polymerization of formaldehyde and DA in the presence of ammonia.

FTIR, XRD, SEM, and TEM were performed to assess the morphological and dimensional structures of the adsorbents. The successful construction of the HPSs was confirmed in a previous article published by the authors [31]. The crystal structure of the COF nanobeads was investigated by an XRD pattern (Fig. 1a). The assessment was performed in the 2θ range from 1 to 40°. As shown in Fig. 3a, two characteristic peaks of nanobeads at 30 and 35° were observed in the sample, verifying good crystallinity of the COF [22]. Also, the morphological structure of the adsorbent was characterized by a TEM image (Fig. 1b). Results show a distinct core-shell structure of Fe_3O_4@TFPA-Bd NBs with a dark core of Fe_3O_4 NPs and a gray COF layer.

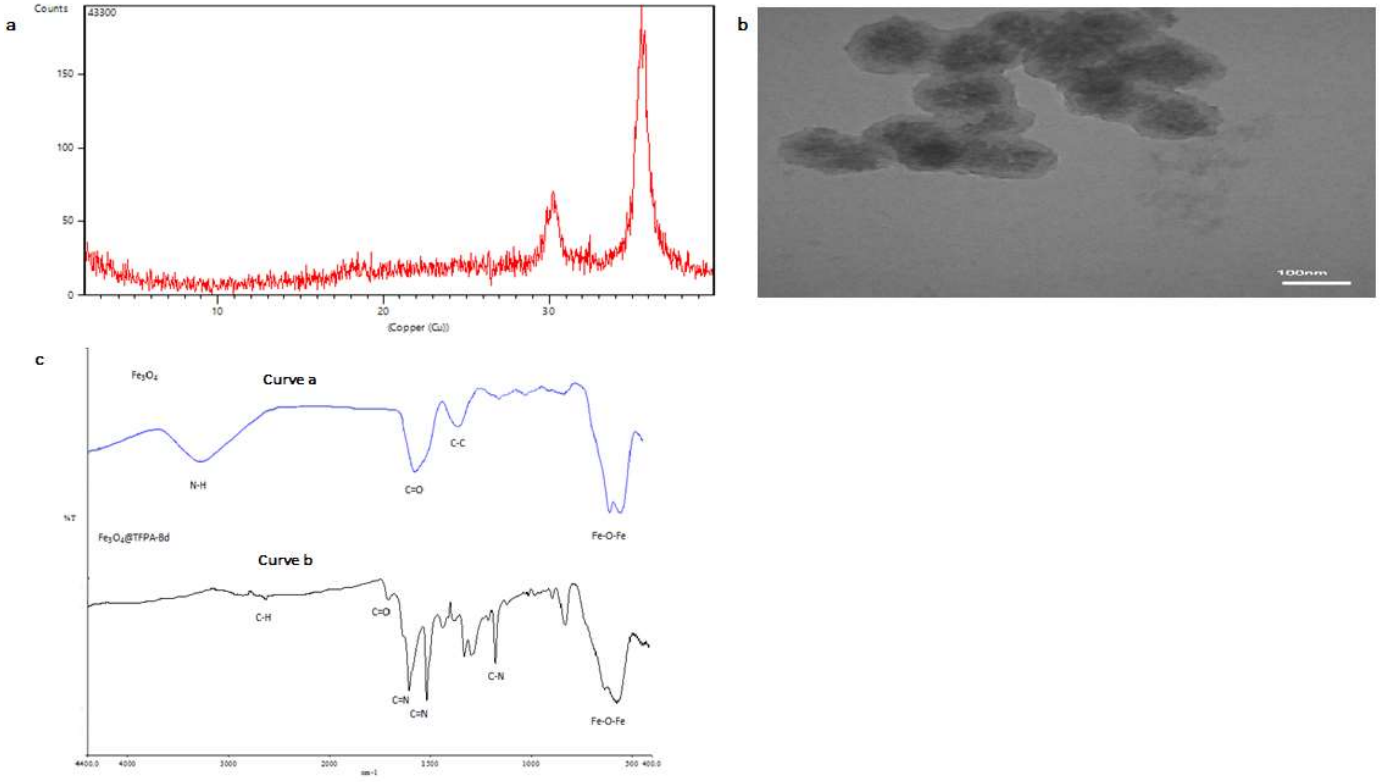


Fig. 1. XRD pattern (a), TEM image (b), and (c) FTIR spectra of COF nanobeads.

FT-IR spectra were employed to confirm the successful synthesis of the COF shell (Fig. 1c). The appearance of a strong peak at 565 cm^{-1} indicates the presence of the Fe-O-Fe. Also, absorption bands around 1412, 1618, and 3384 cm^{-1} confirm the presence of the carboxyl groups on the surface of Fe_3O_4 NPs (Curve a). Compared with Curve a, the spectrum of COF shows the new characteristic peak of C=N vibrations at 1598 cm^{-1} and C=N at 1621 cm^{-1}, which correspond to the Imine-link and Schiff-link C with N. The stretching vibration of C=N at 1621 cm^{-1} confirms the formation of the COF shell on the surface of Fe_3O_4 NPs (Curve b).

The pore volume and specific surface area of the COF nanobeads were found to be 0.65 $cm^3\ g^{-1}$ and 202.18 $m^2\ g^{-1}$, respectively, which are higher than those of the Fe_3O_4 nanoparticles, *i.e.*, 0.16 $cm^3\ g^{-1}$ and 40.5 $m^2\ g^{-1}$, respectively [22]. In this regard, HPSs with hollow core sizes ranging from 30 to 80 nm and diameters ranging from 100 to 200 nm can be synthesized [17].

Extraction Parameters Optimization

Several factors including conditioning solution, amount of sorbent, washing solution, sample volume, extraction cycles, type and volume of eluting solution, pH, and temperature of the sample were examined to evaluate parameters affecting the extraction efficiency of each analyte. The extraction parameters optimization was performed by analysis in triplicate via the QCs samples.

Conditioning solution. Before the extraction function, the MEPS cylinder should be washed with an organic solution to activate the sorbent for better compound diffusion, as well as to condition the sorbent and clean it from pollutants. Therefore, multiple usually used preconditioning solutions, including methanol, deionized water, acetonitrile, propanol, and deionized water/methanol mixture (50:50 v/v%) were investigated. The deionized water/methanol mixture (50:50 v/v%) was the best preconditioning solution for the HPSs: COF sorbent in the extraction of tt-MA, HA, and m-MHA from urine samples. However, deionized water showed better performance for MA extraction.

Amount of sorbent. The effect of the sorbent amount on the recovery of the target analytes was evaluated in the range of 10 to 50 mg. The highest recovery was achieved when the amount of mixture adsorbent increased up to 30 mg, exceeding which the recovery declines. The obtained result is attributed to the enhancement of active surface region and results in rather existing sites for encapsulated target analytes. The lower extraction efficiency above the optimal point is due to weak cleaning of the adsorbed target analytes *via* a specified volume of the elution solution.

Washing solution. Several solutions include deionized water, deionized water- propanol (80:20 v/v%), deionized water- acetic acid (90:10 v/v%), deionized water-acetonitrile (80:20 v/v%), and deionized water-methanol (80:20 v/v%) were tested to eliminate matrix interferences from the HPSs: COF sorbent. The investigation was performed via analysis in triplicate by the spiked urine samples. The highest recovery and clean chromatograms were achieved when a deionized water-methanol mixture (80:20 v/v%) was used for extracting tt-MA and HA (Fig. 3a).

Type and volume of eluting solution. After extraction, the target analytes were eluted with the aid of adding a proper organic solution. The used solution should be able to interrupt the interaction between the analytes and the sorbent. Organic solutions, including methanol, ethanol, or acetonitrile, effectively desorb organic compounds from solid sorbents [24,34]. Additionally, previous studies have shown that the acidity of eluting solvent plays a significant role in the extraction of organic compounds from sorbents bed [35].

For this purpose, multiple solvents such as ethanol-acetic acid (80:20, v/v%), methanol-acetic acid (80:20, v/v%), acetonitrile-acetic acid (80:20, v/v%), deionized water-acetic acid (50:50, v/v%), and deionized water-hydrochloric acid (50:50, v/v%) in the different volume (*i.e.*, 10, 20, 30, 40 and 50 µl) were examined. The highest peak area for HA and m-MHA was found with 30 µL of ethanol-acetic acid (80:20, v/v%) (Fig. 3b).

Sample volume and extraction cycle. The effects of five sample volumes (30, 60, 90, 120 and 150 µl) and five extraction cycles (1, 2, 3, 4 and 5 cycles) on the extraction of the analytes were studied in triplicate for QCs samples. The sample loading was performed at an approximate speed of 10 µl s^{-1}. As can be seen in Figs. 2c-d, the best results were achieved when 150 µl of the sample with 3 numbers extraction cycles were used.

Effect of sample pH. The pH of the sample considerably affects the extraction procedure and its change causes the existing form of analytes in the solution to change [27]. In

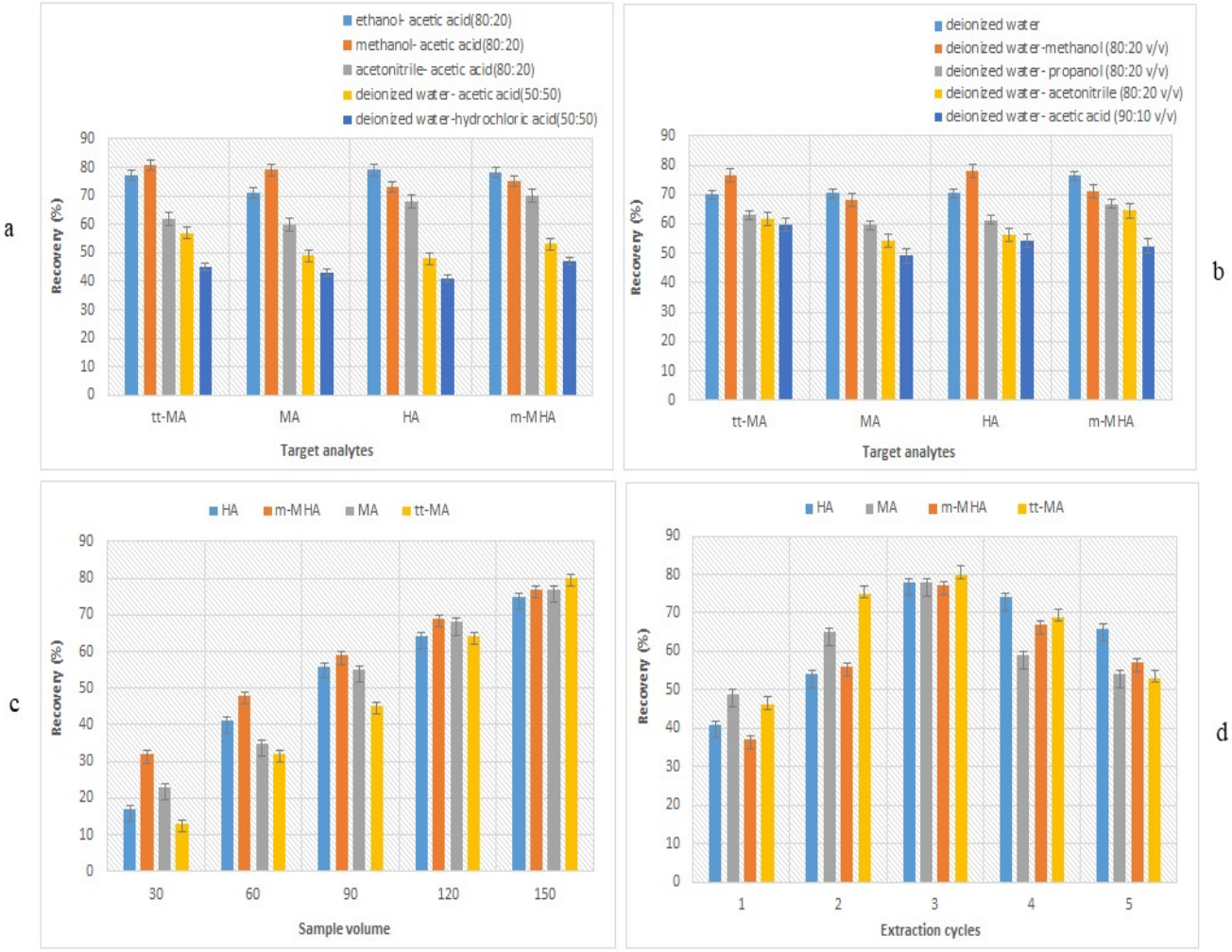


Fig. 2. Effect of a) washing solutions b) elution solutions c) sample volumes, and d) extraction cycles on the extraction of tt-MA, MA, HA, and m-MHA in urine samples. Experimental conditions: temperature = 30 °C; pH = 6; the amount of sorbent, 30 mg.

this study, the sample pH effect on the extraction was studied between 2 and 10 for all target analytes. The sodium hydroxide (NaOH) and hydrochloric acid solutions (HCl) were used to adjust the pH in investigation values. The extraction efficiency of the procedure was dependent on pH since a decrease in recovery of tt-MA, MA, and HA was observed at pH > 2 (Fig. 3a). Increasing the pH further, the EE (%) did not improve and it decreased considerably at pH > 6. The low pH of the sample leads to the adsorption of the target analytes onto sorbent due to the predominant negative charges on HPSs: COF. When the pH increases, the charge of organic matter with linking groups becomes more negative. As a result, at higher pH values, the repulsive forces between BTEXs and nanobeads increase, reducing the amount of organic matter adsorbed on the HPS:COF adsorbent.

The Zeta potential of the adsorbent was measured within the pH range of 2-10. Three measurements were done for each sample, and the average was considered the zeta potential of the mixture adsorbent. The isoelectric point (*i.e.*, the pH at which the negative surface charge equals the positive surface charge) was investigated using these measurements. It was found that the surface charge of HPS:COF becomes more negative with pH, probably due to

the deposition of more hydroxide ions on the adsorbent surface.

Effect of sample temperature. Temperature changes may affect the extraction rate *via* changing diffusion coefficients [36]. Optimal temperature changes the kinetics and thermodynamics of the extraction and accelerates the transfer of compounds from the sample to the solid sorbent. The influence of temperature was evaluated in the range of 10-50 °C (at 10 °C intervals). Hippuric acid, Trans, trans-muconic acid, and m-Methylhippuric acid showed a significant increase in recovery percentage at 20 °C. Moreover, the optimum temperature for extraction of mandelic acid was obtained at 30 °C.

The solubility changes and the rate of migration of analytes in aqueous and organic phases by temperature may change the diffusion coefficients of analytes. Hence, different extraction efficiencies were achieved with various temperatures.

Optimized values for HPSs and COF. The optimized values for HPSs and COF adsorbents are mentioned in previous studies [30,31] by the authors. Under optimized circumstances, the recovery percentage achieved using HPS were: tt-MA (104.9%), MA (105.5%), HA (102.6%), and m-MHA (81.8%). The extraction efficiency was calculated for each analyte via the peak area achieved from the chromatograms of the HPLC system (Eq. (1)). About COF, the extraction efficiency was: tt-MA (81.6%), MA (81.0%), HA (83.8%), and m-MHA (87.5%).

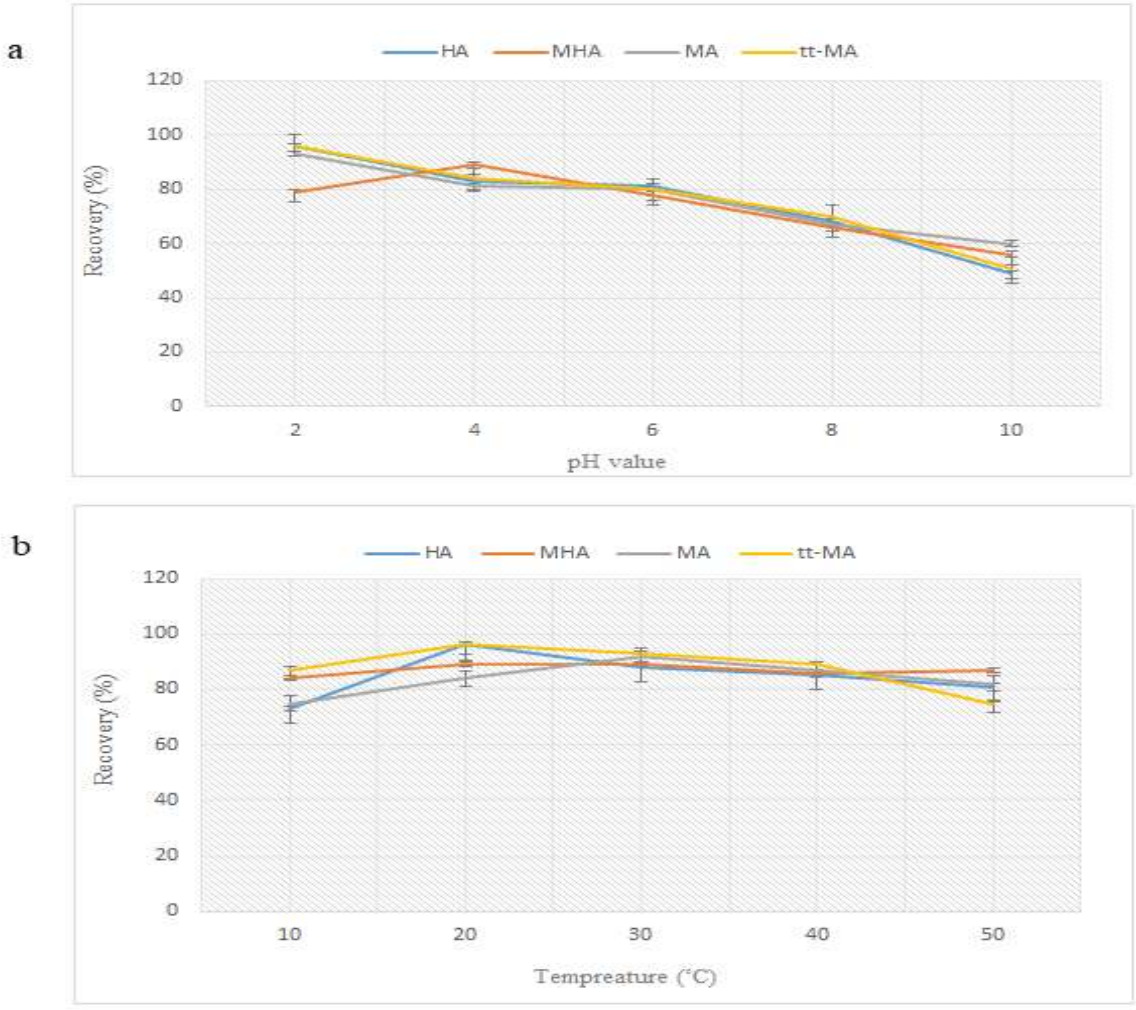


Fig. 3. Effect of a) pH, and b) temperature on the extraction of tt-MA, MA, HA, and m-MHA in urine samples.

Effect of mixed-sorbent

A mixture of HPS and COF was used as sorbents for increasing the recovery percentage of not well-extracted analytes by a single sorbent (*e.g.* m-MHA when HPS was utilized and tt-MA, MA, and HA when COF was employed to extract). Investigation of the effect of mixed-sorbent was performed according to the optimized circumstances adopted for single sorbents. Method validation was carried out by spiked urine samples (low, medium, and high concentrations QC samples. Linear dynamic range (LDR) was obtained using five concentrations of standard solutions (0.05-100 µg ml^{-1} for tt-MA, 0.5-320 µg ml^{-1} for MA, 0.1-500 µg ml^{-1} for HA, and 0.1-1000 µg ml^{-1} for m-MHA). The calibration curves were plotted by drawing the peak area against the concentration of each analyte. The calibration curves were linear with a proper determination coefficient ($R^2 > 0.98$) in the investigated concentration range. The limit of quantification (LOQ) and limit of detection (LOD) were studied based on 10:1 and 3:1 signal-to-noise ratios, respectively (Table 3). The precision (intra-day and inter-day), explained as RSD% was calculated in three concentration levels (low, medium, and high) of QC samples for each analyte. Acceptable intra-day and inter-day precision (RSD < 8.9%) were achieved (Table 3). The extraction efficiency of tt-MA, MA, HA, and m-MHA from urine samples was evaluated using spiking three concentrations of standard solutions within the urine sample. Good extraction efficiencies (ranging from 85.4 to 93.3%) were obtained for all target analytes.

Comparison of Efficiency between Single and Mixed Sorbents

A comparison of the extraction efficiency by the sorbents investigated is given in Fig. 5. As can be seen, HPS and the mixture sorbent provided better extraction efficiency compared to COF. Results showed that tt-MA, MA, and HA were not extracted with high efficiency when using COF as a sorbent. In contrast, these compounds were extracted well and with high efficiency when using mixed sorbent (HPS:COF) and HPS. In the case of HPS sorbent, high extraction efficiency for less polar compounds showed strong π-π stacking, hydrogen bonding, and Van der Waals interactions of these compounds with the nanochannels of HPS. The highest extraction efficiencies were achieved for tt-MA, MA, and HA when HPS was employed. In Fig. 4, the extraction efficiencies obtained for different adsorbents are compared. The lowest EE (%) was achieved for a compound with higher polarity (m-MHA) using HPS sorbent. However, COF and HPS: COF sorbents offer a higher recovery for m-MHA. The adsorption capacity of the COF sorbent is mainly attributed to the hydrophobic interaction (*e.g.*, van der Waals and π-π stacking interactions) between the COF coating and target analytes.

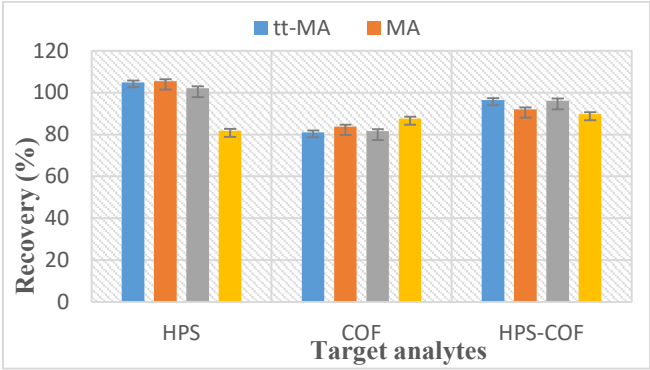


Fig. 4. The extraction efficiency (%) for different adsorbents.

Table 3. Method Validation for Spiked Urine Samples Using Mixed Sorbent

Parameters	tt-MA	MA	HA	m-MHA
Liner dynamic range (µg ml^{-1})	0.05-100	0.5-320	0.1-500	0.1-1000
Limit of detection (LOD) (µg ml^{-1})	0.02	0.5	0.1	0.1
Limit of quantification (LOQ) (µg ml^{-1})	0.05	1	0.3	0.2
Intra-day precision (RSD%)	1.4	3.2	3.6	2.2
Inter-day precision (RSD%)	5.5	8.9	4.5	8.6
Extraction efficiency (%)	91.7	87.4	93.3	85.4
Determination coefficient (r^2)	0.9901	0.9873	0.9890	0.9941

Table 4. Comparison of the HPS: COF-MEPS Proposed Procedure with Previously Reported HPLC-UV Methods for Determination of the Target Analytes

Preparation method	Sample	Analytes	LDR (μg ml^{-1})	LOD (μg ml^{-1})	EE (%)	Ref .
SAX-SPE	Urine	MA	50-1600	4	97-100	[37]
MIP-MEPS	Urine	MA	0.2-20	0.06	> 88.8	[38]
MIP-SPE	Urine	tt-MA	0.3-10	0.1	87-112	[39]
MOF-MEPS	Urine	tt-MA	0.01-50	0.001	86-98.5	[23]
LLE	Urine	HA	0.0-25	0.01	> 92.7	[40]
D-μSPE	Urine	HA	5-200	0.02	> 97	[41]
Derivatization	Urine	MHA	20-320	20	81.7-85.7	[42]
HF-LPME	Urine	MHA	0.01-50	0.002	89-98	[43]
HPS:COF-MEPS	Urine	tt-MA	0.05-100	0.02	96.4	Present
HPS:COF-MEPS	Urine	MA	0.5-320	0.5	92.0	Present
HPS:COF-MEPS	Urine	HA	0.1-500	0.1	96.1	Present
HPS:COF-MEPS	Urine	m-MHA	0.1-1000	0.1	89.7	Present

Comparison of the Method with Previously Reported HPLC-UV Procedures

In Table 4, the analytical performance of the HPS:COF-MEPS developed procedure was compared with the previously reported HPLC-UV methods. The developed procedure is simple, fast, and unlike traditional SPE and LLE methods, it uses a small volume of solvents and required a short sample preparation time. The evaluated analytes showed good extraction efficiency with the HPS: COF-MEPS procedure (89.7-96.4%). The method demonstrated a high ability to extract analytes with various polarities, which is a very good strong point compared to other developed methods. The existing methods for the analysis of the target analytes required different extraction and separation conditions, and as a result, they make the investigation more time-consuming and expensive. The main advantage of the proposed procedure is its ability to extract all target analytes from urine samples, an advantage not seen in other previously developed procedures using MEPS. For instance, in the MIP-MEPS procedure, each adsorbent is specific to one type of analyte, and with each adsorbent, only one compound can be extracted. Besides, in the MOF-MEPS procedure, the final elution of biomarkers at low concentrations of metal framework adsorbents is difficult, and small amounts remain in the adsorbent. Additionally, the synthesis process in the present study is very simple and fast, and the adsorbent can be easily eluted and prepared for subsequent extraction.

Method Application to Real Samples Analysis

The developed procedure was applied to five human urine samples, obtained from gas stations in Hamadan (west of Iran). Table 5 shows the obtained experimental results. Urine samples of non-smoker workers exposed to BTEX compounds were analyzed to extract tt-MA, MA, HA, and m-MHA. All the urine concentrations obtained from volunteers' samples were in the calibration range of the developed

Table 5. The Average Concentration of tt-MA, MA, HA, and m-MHA in Five Different Urine Samples from Gas Stations

	Concentration (μg ml^{-1}) (RSD%)			
Sample	tt-MA	MA	HA	m-MHA
1	0.85 (3.3)	24.5 (4.9)	4.4 (5.5)	2.9 (5.0)
2	1.2 (4.2)	73.1 (4.7)	7.1 (3.9)	5.9 (3.4)
3	3.0 (2.1)	58.9 (6.6)	7.5 (7.3)	3.4 (5.5)
4	2.3 (4.3)	43.7 (3.8)	3.8 (4.8)	4.1 (4.9)
5	1.1 (2.5)	22.4 (4.0)	6.3 (3.2)	1.6 (6.2)

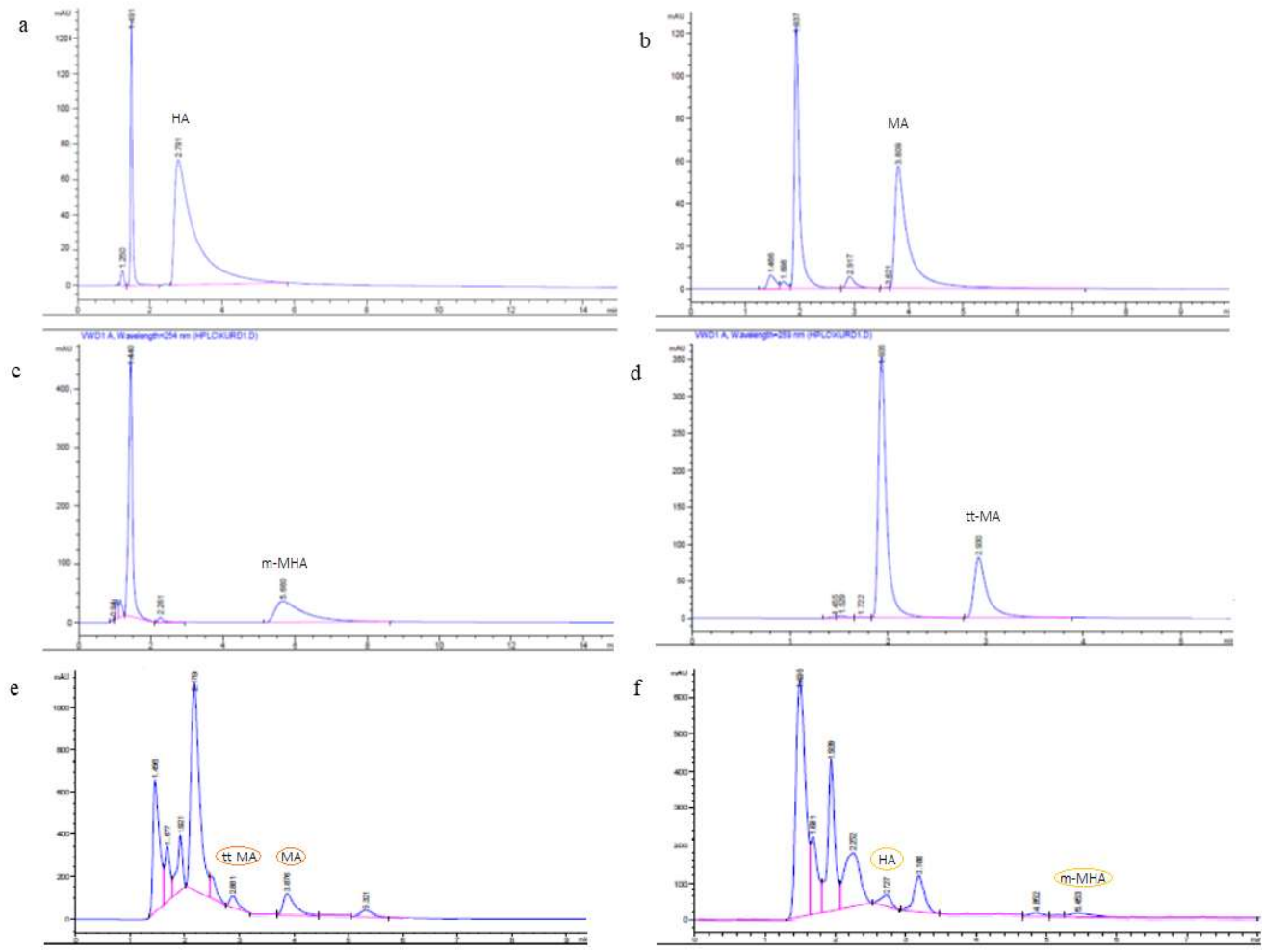


Fig. 5. HPLC chromatograms of a) blank sample spiked with HA at QCs concentration, b) blank sample spiked with MA at QCs concentration, c) blank sample spiked with m-MHA at QCs concentration, d) blank sample spiked with m-tt-MA at QCs concentration, e, and f) urine sample of a BTEX-exposed worker after the HPS: COF-MEPS procedure.

HPS:COF-MEPS method. These results demonstrate that the developed procedure can be reliably employed for the extraction of target analytes in the urine matrix.

The chromatograms of HA, MA, m-MHA, and tt-MA from a blank sample spiked at QCs concentration and urine sample of a BTEX-exposed non-smoker worker are shown in Figs. 5a-f. The results showed that the proposed method has an acceptable extraction performance and all analytes can be extracted without any interference.

CONCLUSIONS

An alternative procedure for the extraction of analytes with various polarity by combined sorbent of HPS: COF in the MEPS model was illustrated *via* tt-MA, MA, HA, and m-MHA as target analytes. The effective parameters were optimized to achieve the best experimental results in terms of validation variables. The developed procedure relies on the desirable features of the MEPS technique, including easy sample preparation and short analysis duration. However, the use of mixed HPS:COF adsorbent with the features of reusability, easy clean-up, and the ability to extract all target analytes with various polarities is the reason for its superiority over other methods developed with MEPS. Additionally, the method allows accurate determination of analyzed analytes, so it can be used as an alternative to

conventional methods.

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REFERENCES

[1] S.K. Chauhan, N. Saini, V.B. Yadav, Int. J. Adv. Res. Sci. Eng. Technol. 1 (2014) 667.

[2] W.H. Organization, WHO Guidelines for Indoor air Quality: Selected Pollutants, World Health Organization. Regional Office for Europe, 2010.

[3] I.A.f. R.o. Cancer, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans 60 (1994).

[4] P.C. Lu, S. Shahbaz, L.M. Winn, J. Appl. Toxicol. 40 (2020) 1018.

[5] N.B. Hopf, J. Kirkeleit, M. Bråtveit, P. Succop, G. Talaska, B.E. Moen, Int. Arch. Occup. Environ. Health 85 (2012) 261.

[6] N. AlMasoud, S.M. Wabaidur, Z.A. Alothman, A.A. Ghfar, T.S. Alomar, Desalination and Water Treatment 195 (2020) 222.

[7] M.A. Habila, Z.A. ALOthman, E. Yilmaz, M. Soylak, Int. J. Environ. Anal. Chem. 98 (2018) 171.

[8] X. Shan, L. Zhang, H. Ye, J. Shao, Y. Shi, S. Tan, L. Zhang, K. Su, Bioanalysis 13 (2021) 1569.

[9] A. Abdel-Rehim, M. Abdel-Rehim, Biomed. Chromatogr. 27 (2013) 1188.

[10] W. Du, G. Zhao, Q. Fu, M. Sun, H. Zhou, C. Chang, Food Chem. 145 (2014) 789.

[11] Z. Bahiraee, S. Fathi, M. Safari, Environ. Prog. Sustain. Energy (2022) e13971.

[12] M. Samimi, M. Safari, Environ. Prog. Sustain. Energy 41 (2022) e13859.

[13] N. Orachorn, O. Bunkoed, Talanta 228 (2021) 122237.

[14] M. Safari, Y. Yamini, Talanta 221 (2021) 121648.

[15] H. Ehzari, M. Safari, M. Samimi, Talanta 234 (2021) 122698.

[16] M. Wang, M. Gao, K. Zhang, L. Wang, W. Wang, Q. Fu, Z. Xia, D. Gao, Microchim. Acta 186 (2019) 1.

[17] G.-H. Wang, Q. Sun, R. Zhang, W.-C. Li, X.-Q. Zhang, A.-H. Lu, Chem. Mater. 23 (2011) 4537.

[18] N. Li, J. Du, D. Wu, J. Liu, N. Li, Z. Sun, G. Li, Y. Wu, Trends Analyt. Chem. 108 (2018) 154.

[19] L.M. Salonen, S.R. Pinela, S.P. Fernandes, J. Louçano, E. Carbó-Argibay, M.P. Sarriá, C. Rodríguez-Abreu, J. Peixoto, B. Espiña, J. Chromatogr. A 1525 (2017) 17.

[20] J. Liu, E. Zong, H. Fu, S. Zheng, Z. Xu, D. Zhu, J. Colloid Interface Sci. 372 (2012) 99.

[21] Y. Peng, W.K. Wong, Z. Hu, Y. Cheng, D. Yuan, S.A. Khan, D. Zhao, Chemistry of Materials 28 (2016) 5095.

[22] L. Chen, M. Zhang, F. Fu, J. Li, Z. Lin, J. Chromatogr. A 1567 (2018) 136.

[23] R. Rahimpoor, A. Bahrami, D. Nematollahi, F.G. Shahna, M. Farhadian, J. Iran. Chem. Soc. 17 (2020) 2345.

[24] R. Rahimpoor, A. Bahrami, D. Nematollahi, F.G. Shahna, M. Farhadian, J. Chromatogr. B Biomed. Appl. 1114 (2019) 45.

[25] M.M. Moein, A. El-Beqqali, M. Javanbakht, M. Karimi, B. Akbari-Adergani, M. Abdel-Rehim, J. Chromatogr. A 1372 (2014) 55.

[26] Z. Pirmohammadi, A. Bahrami, D. Nematollahi, S. Alizadeh, F. Ghorbani Shahna, R. Rahimpoor, Biomed. Chromatogr. 34 (2020) e4725.

[27] M.N. Jajuli, G. Herzog, M. Hébrant, N.E. Poh, A.A. Rahim, B. Saad, M.H. Hussin, RSC Adv. 11 (2021) 16297.

[28] M. Kah, G. Sigmund, F. Xiao, T. Hofmann, Water Res. 124 (2017) 673.

[29] M.B. Ahmed, J.L. Zhou, H.H. Ngo, M.A.H. Johir, L. Sun, M. Asadullah, D. Belhaj, J. Hazard. Mater. 360 (2018) 270.

[30] N. Kurd, A. Bahrami, A. Afkhami, F.G. Shahna, M.J. Assari, M. Farhadian, J. Chromatogr. B Biomed. Appl. 1197 (2022) 123197.

[31] N. Kurd, A. Bahrami, A. Afkhami, M.J. Assari, M. Farhadian, J. Iran. Chem. Soc. (2022) 1.

[32] M. Abdel-Rehim, Anal. Chim. Acta 701 (2011) 119.

[33] A.M. Ramezani, Y. Yamini, M. Nazraz, J. Food Composit. Anal. 98 (2021) 103806.

[34] N. Alizadeh, H. Zarabadipour, A. Mohammadi, Anal. Chim. Acta 605 (2007) 159.

[35] P. Rocío-Bautista, C. Martínez-Benito, V. Pino, J.

Pasán, J.H. Ayala, C. Ruiz-Pérez, A.M. Afonso, Talanta 139 (2015) 13.

[36] M.A. Farajzadeh, M.R.A. Mogaddam, H. Ghorbanpour, J. Chromatogr. A 1347 (2014) 8.

[37] S. Shahtaheri, M. Abdollahi, F. Golbabaei, A. Rahimi-Froushani, F. Ghamari, J. Environ. Health 1 (2004) 70.

[38] E. Soleimani, A. Bahrami, A. Afkhami, F.G. Shahna, Arch. Toxicol. 92 (2018) 213.

[39] S. Jamaleddin Shahtaheri, F. Ghamari, F. Golbabaei, A. Rahimi-Froushani, M. Abdollahi, Int. J. Occup. Saf. Ergon. 11 (2005) 377.

[40] Y. Ohashi, T. Mamiya, K. Mitani, B. Wang, T. Takigawa, S. Kira, H. Kataoka, Anal. Chim. Acta 566 (2006) 167.

[41] M. Behbahani, S. Bagheri, F. Omidi, M.M. Amini, Microchim. Acta 185 (2018) 1.

[42] P. Kongtip, J. Vararussami, V. Pruktharathikul, J. Chromatogr. B Biomed. Sci. Appl. 751 (2001) 199.

[43] F. Ghamari, A. Bahrami, Y. Yamini, F.G. Shahna, A. Moghimbeigi, Toxicol. Environ. Chem. 99 (2017) 760.