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Solid phase extraction as a cleanup step before microextraction of diclofenac and mefenamic acid using nanostructured solvent

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ABSTRACT

A new pretreatment method, solid-phase extraction combined with supramolecular solvents, was proposed for the first time for extraction of diclofenac (DIC) and mefenamic acid (MEF) from urine and water samples. Supramolecular solvent (SUPRAS) is a nano-structured liquid, generated from the amphiphiles through a sequential self-assembly process occurring on two scales, molecular and nano. SUPRAS tested were generated from solutions of reverse micelles of decanoic acid (DeA) in tetrahydrofuran (THF) by addition of water, which acted as the coacervating agent.

In the present study, due to matrix effect, drugs were first extracted from the samples by SPE. The extracted analytes were then eluted from the sorbent with THF, and the eluate was subjected to SUPRAS formation (SUPRASF) process. Finally, the analytes in the SUPRAS were separated and determined by HPLC-UV. Several parameters affecting the SPE-SUPRASF process were investigated and optimized. The new method provides enrichment factors in the range of 431–489 for MEF and DIC, respectively. Calibration plots are linear in the range of 2–200 $\mu g\,L^{-1}$ for MEF and 1–200 $\mu g\,L^{-1}$ for DIC, with correlation of determination (r^2) ranging from 0.996 to 0.999. The method was successfully applied for extraction and determination of analytes in urine and water samples and relative recoveries of the studied compounds were obtained in the range of 90.4–103.8%.

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1. Introduction

Pharmaceuticals have become recognized as relevant environmental contaminants in the course of the last decade [1]. From all the pharmaceuticals reported in the literature, the classes of non-steroidal acidic anti-inflammatory drugs (NSAIDs) are the most frequently mentioned as environmental pollutants [2]. NSAIDs, including substances such as diclofenac [2-[(2,6-dichlorophenyl)-amino-phenyl]acetic acid] and mefenamic acid [2-[(2,3-dimethyl-phenyl)amino]benzoic acid], which are widely used for treatment of pain and fever and therefore constitute the active ingredient in many common painkillers [3]. These NSAIDs are acidic compounds with pK_a values between 3.5 and 4.5. The acid group is essential in inhibition of the cyclooxygenases COX-1 and COX-2, the basic enzymes in biosynthesis of prostaglandins (responsible for swelling and pain) [4].

Determination of NSAIDs can be performed by various techniques, such as high-performance liquid chromatography (HPLC) [5–8], gas chromatography–mass spectrometry (GC–MS) [9–10], spectrophotometric method [11], micellar electrokinetic capillary chromatography (MEKC), and capillary electrochromatography (CEC) [12,13]. HPLC is the most common method that is used for separation and determination of these compounds. The analysis of drugs in a complex matrix such as urine without sample preparation is very difficult. In general, sample preparation and concentration of the target analytes are often needed before analysis. Up to now, several procedures have been developed for preconcentration of NSAIDs from sample matrices including liquid–liquid extraction (LLE) [14] and solid-phase extraction (SPE) [15–17]. SPE offers unquestionable advantages compared with the traditional LLE technique, such as greater extraction efficiencies and lower consumption of organic solvents. However, it hardly reduces the large times spent for sample preparation and the large volumes of sample required for analysis [18]. Solid-phase microextraction [19,20], stir bar sorptive extraction (SBSE) [21], and liquid-phase microextraction [22–24] have been also applied for extraction of NSAIDs.

Supramolecular solvents (SUPRASs) constitute environment friendly alternative to molecular organic ones for analytical extractions [25]. They are nano-structured liquids generated from amphiphiles through a sequential self-assembly process occurring on two scales, molecular and nano. They made up of three-dimensional aggregates with regions of different polarity that offer a number of interactions for analyte solubilisation (i.e., ionic, hydrogen bond, π-cation, dipole–dipole, hydrophobic, etc.) that making them suitable for extraction of organic compounds in a

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wide polarity range. On the other hand, the large concentration of surfactant and, therefore, of binding sites they contain (typically 0.1–1 mg μL^{-1}), allows achieving high enrichment factors using low solvent volumes [26]. SUPRASs made up of reverse micelles of decanoic acid (DeA) dispersed in tetrahydrofuran (THF)-water have been introduced by Pérez-Bendito et al. [27]. Recently, Rubio et al. reviewed both theoretical and practical aspects related to the use of SUPRASs in analytical extractions reported over the last decade [28].

SPE is the most popular clean-up technique due to factors such as convenience, cost, and simplicity and also it is the most accepted sample pretreatment method today [29]. The principal goals of SPE are trace enrichment (concentration), matrix simplification (sample clean-up), and medium exchange [30]. Although SPE methodologies often render high extraction yields, but it suffers from two main drawbacks: (i) in commercial sorbents (e.g. C18, etc), selectivity is relatively low and many interfering species might be co-eluted [31]. Thus other sorbents or extra-step microextraction methods are required for improvement of clean-up and selectivity [17]. (ii) The consumption of organic solvents is relatively low in SPE compared with LLE, but to obtain high extraction efficiency, evaporation of the eluent after extraction is required [32]. Therefore, extra step is needed in sample preparation for drying which therefore requires more time. For this purpose combination of SPE and SUPRASF extraction is an educated choice to overcoming of these drawbacks, as a new pretreatment method for extraction of DIC and MEF from complex matrices. The effects of different variables on SPE-SUPRASF efficiency were studied and optimized. After optimization, the method followed by HPLC-UV was applied for extraction and determination of DIC and MEF in urine and water samples.

2. Experimental

2.1. Chemicals and reagents

Standards of diclofenac (DIC, $pK_a=4.1$) and mefenamic acid (MEF, $pK_a=4.2$) were kindly donated from the Department of Medical Sciences of Tehran University (Tehran, Iran). THF was supplied by Merck (Darmstadt, Germany). Decanoic acid was obtained from Fluka (Buchs, Switzerland). Other reagents were of analytical grade and obtained from Merck. The ultra-pure water was prepared by a model Aqua Max-Ultra Youngling ultra-pure water purification system (Dongan-gu, South Korea). HPLC grade methanol and acetonitrile were purchased from Caledon (Ontario, Canada). Microliter syringes (25-500 μL) were purchased from Hamilton (Bonaduz, Switzerland). A Sepand Teb Azema centrifuge (Tehran, Iran) was used for phase separation. SPE cartridges with a reverse C18 stationary phase (sorbent mass 500 mg, volume 6 mL, 10 mm height × 15 mm i.d.,) were obtained from Supelco (Bellefonte, PA, USA).

Stock standard solutions of each analyte were prepared separately by dissolving proper amounts of each drug in methanol at 1000 μg mL^{-1} and stored at 4 °C. Mixtures of standard working solutions for extraction at different concentrations were prepared by dilution with ultra-pure water for optimization of parameters. The working solutions were freshly prepared by diluting the mixed standard solutions in ultra-pure water for the concentrations required.

2.2. Apparatus

Chromatographic analysis was performed with a HPLC instrument including a Varian 9012 HPLC pump (Walnut Creek, CA, USA), a six-port Cheminert HPLC valve from Valco (Houston, TX, USA) with a 20-μL sample loop and equipped with a Varian 9050 UV–Vis detector. Chromatographic data were recorded and analyzed using ChromanaCH software, version 3.6.4 (Tehran, Iran). An ODS-3 column (50 mm × 4.6 mm, with 5-μm particle size) from MZ-Analysentechnik (Mainz, Germany) was applied to separate DIC and MEF under isocratic elution conditions. A mixture of 50 mmol L^{-1} ammonium acetate buffer (pH 5.2) and acetonitrile (50:50) for 10 min and 100% acetonitrile for 5 min at a flow rate of 1 mL min^{-1} were used as mobile phase and the analytes were detected at 285 nm.

The SPE extraction was carried out in a Visiprep™ manifold from Supelco (Bellefonte, PA, USA) coupled to a vacuum pump from Vacuubrand (Wertheim, Germany).

2.3. Sample preparation

(a) Urine sample: To obtain calibration curve and figures of merit, human urine sample was collected from healthy 30 years old adult male volunteer. It should be noted that all ethical and human rights guidelines in the sampling procedure were obeyed. The sample was filtered through a 0.45-μm pore size cellulose acetate filter from Millipore (Madrid, Spain). The filtrate was collected in a glass container, which was carefully cleaned with hydrochloric acid and washed with deionized water and stored at 4 °C to prevent bacterial growth and proteolysis. Then, 15 mL of the urine sample was spiked with mixed standard solution to obtain desired concentration and diluted to 30 mL with deionized water. In the following, proper amount of HCl solution (0.1 mol L^{-1}) was added to achieve pH value of 2.5. These samples were subsequently submitted to SPE-SUPRASF procedure. Urine samples were obtained from healthy volunteers (29 and 42 years old), one of them consumed a single oral dose of DIC (100 mg), while others consumed MEF (250 mg). These samples were collected 2 and 5 h after administration of tablets. The urine volumes were also recorded.

(b) Water samples: Different water samples, including tap water from our lab (Tehran, Iran), and waste water sample from a pharmaceutical factory (Tehran, Iran) were collected and the SPE-SUPRASF method was applied to extract the drugs. Each water sample was filtered to remove any suspended material. For preconcentration, pH values of the samples were adjusted at 2.5 using HCl solution (0.1 mol L^{-1}) before extracting them by the described procedure. Before the analysis, the water samples were stored in a dark place at 4 °C in an amber glass bottle that was previously rinsed with ultra-pure water and methanol.

2.4. SPE-SUPRASF procedure

SPE of DIC and MEF from water samples was carried out using 10 mm height × 15 mm i.d., 500 mg of C18 sorbent with 6 mL syringe barrels from Supelco. As a pretreatment step, the SPE column bed was conditioned with 3.0 mL acetonitrile and 3.0 mL water. 30 mL of the water sample, containing 20 μg L^{-1} of DIC and MEF were acidified by HCl (0.1 mol L^{-1}) to pH value of 2.5 to change the drug into their undissociated forms. The sample was loaded into the SPE column at a flow rate of about 7 mL min^{-1} with the aid of a vacuum pump. Then, the column was rinsed by 3.0 mL water to remove the matrix interferences. The columns were then dried under vacuum in the manifold system from Supelco for 5 min. The extracted drugs in the SPE column were eluted by 1.5 mL THF and the eluent solution was collected and 30 mg of DeA was added. Afterwards, an aliquot of 10 mL ultra-pure water with pH value adjusted at 2.0 by dropwise addition of HCl (0.1 mol L^{-1}) was poured into a 12 mL homemade

centrifuge tube, which is designed for collection of low density organic solvents [33]. The solution that was obtained from SPE step was quickly injected into the aqueous solution using a 5 mL gastight syringe from Hamilton. The SUPRAS, made up of reverse micelles of DeA dispersed in THF: water spontaneously formed and separated from the THF: water solution as an immiscible liquid. The mixture was centrifuged at 5000 rpm for 5 min to accelerate complete separation of the two immiscible liquids. The coacervate, located at the top of the glass tube, was withdrawn using a microsyringe and injected into the HPLC instrument for subsequent analysis. The total time for SPE-SUPRASF procedure was about 25 min.

2.5. Calculations of preconcentration factor and relative recovery

The preconcentration factor (PF) was defined as the ratio of the final analyte concentration in the acceptor phase ($C_{SUPRASF}$) to the initial concentration of analyte (C_0) in the sample solution:

$$PF = \frac{C_{SUPRASF}}{C_0} \quad (1)$$

Relative recovery (RR%) was acquired from the following equation:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (2)$$

where C_{found}, C_{real}, and C_{added} are concentration of analyte after addition of a known amount of standard into the real sample, the concentration of analyte in real sample, and the concentration of a known amount of standard, which was spiked into the real sample, respectively.

3. Results and discussion

For SPE-SUPRASF extraction of DIC and MEF from aqueous solutions, several parameters that control the optimal performance of extraction were investigated and optimized using one variable at a time method. Peak area of each drug in HPLC chromatogram was selected as signal in the optimization process.

3.1. Optimization of supramolecular solvent microextraction

Decanoic acid ($pK_a = 4.8 \pm 0.2$) is sparingly soluble in water (e.g. $\sim 0.2\ g\ L^{-1}$), while it dissolves well in THF and self-assembles as reverse micelles having 4–8 nm diameter according to a sequential-type self-association model. Addition of water (pH 1–4) to these solutions causes partial desolvation of the reverse micelles, which facilitates micelle–micelle interaction and leads to formation of larger aggregates. As a result, these aggregates become insoluble in the water: THF solution separate as an immiscible liquid. So, water, a non-solvent for the DeA, is the inductor agent of the coacervation. At a microscopic level, the coacervate consists of spherical droplets, made up of a variable number of reverse micelles, dispersed in the water: THF continuous phase. The water content is only about ~1–2%, and it is expected to be either in the micellar core or mixed with THF in the continuous phase [34]. The excellent dissolution properties of reverse micelles and the low volume of the coacervates obtained make them very attractive to be used in analytical extractions. In order to set up efficient extraction schemes, it is important to understand the intermolecular forces driving the extraction process. Hydrocarbon chains of DeA molecules forming aggregates in these SUPRASs extend into and are surrounded by the THF, while their carboxylic groups are solvated by water in the interior of the aggregates. DeA reverse micelles provide a two-fold mechanism for substrate solubilisation, namely hydrophobic interactions in the surfactant tails at the micellar surface and hydrogen bonds in the polar head groups at the micellar core [35]. Consequently, the expected driving forces for the extraction were van der Waal's interactions between the hydrocarbon chains of the DeA and the NSAIDs aromatic framework, and hydrogen bonds, on account of the acceptor and donor groups of the analyte.

The pH value of the sample is a significant factor, which may affect the extraction recovery of the analytes and determines their state. Acidification of the sample is usually required to have the neutral forms of these compounds and thus increasing recoveries. Also, the coacervation phenomenon occurs from protonated alkyl carboxylic acids; so extractions must be carried out at pH values below 4 [27]. At higher pH values, solubilisation of deprotonated DeA molecules in the water-THF phase in equilibrium with the SUPRAS occurred and that resulted in reduction of formed SUPRAS volume. Therefore, the effect of pH on microextraction of analytes was studied in the range of 1–4. A 30 mg of DeA was dissolved in 1.5 mL THF and injected into the homemade glass tube containing 10.0 mL sample solution using a gastight syringe. Immediately, the SUPRAS was formed and the mixture was centrifuged at 5000 rpm for 5 min to accelerate the phase separation. Twenty microliters of the collected phase was taken using a 50-μL microsyringe and directly injected into the HPLC instrument. As can be seen in Fig. 1, the best extraction efficiency of the analytes was obtained at pH 2.0.

Addition of salt is widely used in microextraction techniques to improve the partitioning of analytes into the organic through salting out effect. To investigate the influence of salt addition on performance of SUPRASF extraction, various experiments were performed by adding different amounts of NaCl (0–15% w/v) into sample solution with pH value adjusted at 2.0. The results showed that extraction recovery dramatically decreased in the presence of salt (Fig. 2). The presence of salt could change the physical properties of the Nernst diffusion film and viscosity of aqueous solution, thus, reducing the rate of diffusion of the MEF and DIC into the micellar phase. Therefore, the presented method is not suitable for extraction of target analytes from saline samples. As a result, a pre-cleaning step is needed in sample preparation before SUPRASF.

SPE is a wildly used sample preparation technique, commonly used for clean-up and preconcentration in biological and environmental samples analysis. Therefore, SPE combined with SUPRASF can provide a solution to this problem.

3.2. Optimization of SPE conditions

3.2.1. Effect of flow rate of the sample solution

Flow rate of the sample solution through the solid phase is an effective parameter to control the analysis time. It must be low enough to perform an effective separation and high enough to

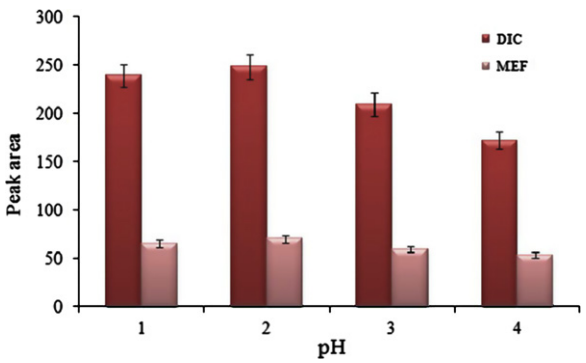

Fig. 1. Influence of the sample pH in SUPRASF extraction on the peak area of the drugs. Extraction conditions: volume of the aqueous solution for SUPRAS, 10 mL; DeA 30 mg; volume of THF, 1.0 mL.

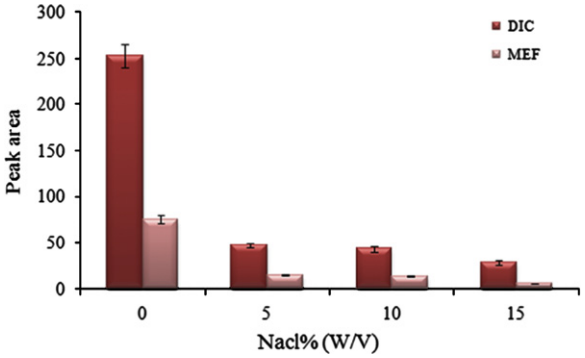

Fig. 2. Effect of salt addition on SUPRASF extraction and the peak area of the drugs. Extraction conditions: as in Fig. 1; except that pH value of the aqueous solution is 2.

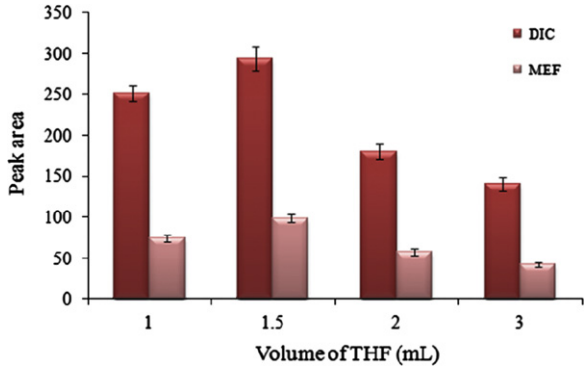

Fig. 3. Effect of the elution solvent volume on the peak area of the drugs. Extraction conditions: water sample volume for SPE, 30 mL; sample solution flow rate, 7 mL min^{-1}; pH of the aqueous solution for SPE, 2.5; volume of the aqueous solution for SUPRASF extraction, 10 mL; pH of the aqueous solution for SUPRASF extraction, 2.0; DeA amount, 30 mg; concentration of the drugs 20 μg L^{-1}.

shorten the extraction time reasonably. The effect of flow rate on the extraction efficiency was studied in the range of 1–10 mL min^{-1}, as mentioned in Section 2.4. The results showed that the quantitative recovery was obtained at flow rates from 1 to 7 mL min^{-1} and then decreased. According to the results, 7 mL min^{-1} was chosen as the optimal flow rate of the sample solution in the subsequent experiments.

3.2.2. Influence of sample pH in SPE

The pH value of the sample is an important factor, which may affect the extraction recovery of the analytes owing to its effect on the forms of the drugs existing in the aqueous solution. Acidification of the sample is usually required to change the drugs into their neutral form in the solution. The effect of pH on the extraction efficiency of the drugs was investigated in the pH range of 2–4. The results obtained indicated that the maximal extraction efficiencies were achieved at pH value of 2.5.

3.2.3. Breakthrough volume

To study the breakthrough volume, different sample volumes (30, 60, 100, 150, and 200 mL) containing 20 μg of each drug were passed through the SPE column and after elution of the drugs using 1.5 mL THF, the sample was subjected to SUPRASF procedure. It was found that recoveries of the analytes were almost constant up to sample volume of 200 mL. However, in order to reduce the analysis time, a sample volume of 30 mL was selected for the subsequent experiments.

3.2.4. Effect of salt addition on SPE of the drugs

To investigate the effect of salt concentration on extraction recovery of the drugs, the experiments were performed using sample solutions containing 0–15% (w/v) NaCl. The results demonstrated that salt addition had no significant effect on the extraction recoveries. Therefore, the proposed method can be employed for preconcentration of the drugs from saline solutions.

3.2.5. Influence of elution solvent volume

When combining SPE with SUPRASF, the elution solvent of SPE must be miscible with water, dissolve alkyl carboxylic acids, and permit aggregate formation of the amphiphiles. Therefore, THF, which displays these properties, was selected as the SPE elution solvent. To evaluate the THF volume required, the elution was investigated in the volume range of 1.0–3.0 mL. The best peak areas for the drugs were obtained using 1.5 mL THF (Fig. 3).

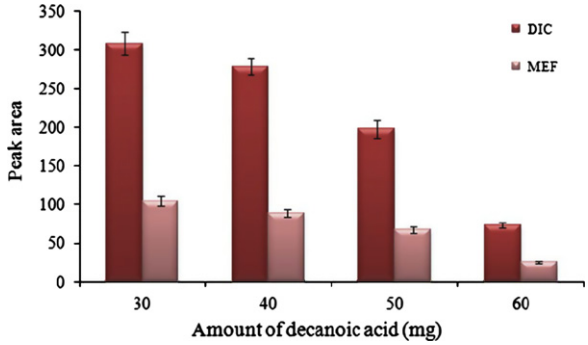

Fig. 4. Effect of DeA amount on the peak area of the analytes. Extraction conditions: as in Fig. 3; eluent solvent (THF) volume, 1.5 mL.

3.2.6. Effect of DeA amount

The volume of SUPRASF that is used for extraction can greatly influence the extraction of the drugs. Therefore, different amounts of DeA (30–60 mg) in a fixed amount of THF (1.5 mL) were dissolved. The results are shown in Fig. 4. It can be seen that the peak areas of the drugs were decreased by increasing DeA amount due to increasing the volume of the standing phase. Therefore, 30 mg of DeA was used in the further experiments.

3.3. Quantitative analysis

To evaluate practical applicability of the proposed SPE–SUPRAS technique, linearity, relative standard deviations (RSDs), limits of detection, and preconcentration factors (PFs) were investigated by extraction of DIC and MEF from water and urine samples under the optimal conditions, whose results are summarized in Table 1. Based on the signal to noise ratio of 3 (S/N=3), limits of detection (LODs) of DIC and MEF were determined as 0.4, 1.0 and 3.0, 7.0 μg L^{-1} in the water and urine samples for DIC and MEF, respectively.

3.4. Analysis of water and urine samples

In order to evaluate applicability of the developed extraction method for analysis of DIC and MEF in real samples with complex matrices, water and urine samples were selected and the drugs were extracted using the proposed method under the optimal conditions. Sample preparation for real samples was performed according to Section 2.3.

Two types of water samples (tap water and waste water) were analyzed by HPLC-UV after SPE-SUPRASF procedures. The results

Table 1
SPE-SUPRASF extraction performance and validation data.

Sample	Analyte	R^2	LOD ($\mu g\,L^{-1}$)	LR ($\mu g\,L^{-1}$)	RSD[a] (%)	PF
Water (deionized)	DIC	0.999	0.4	1.0–200	4.0	489
	MEF	0.996	1.0	2.0–200	4.6	431
Urine	DIC	0.996	3.0 (1.5)[b]	7.0–300	6.2	65 (130)[b]
	MEF	0.993	7.0 (3.5)	10.0–300	5.4	61 (122)

[a] RSD% was calculated at 10, 20 $\mu g\,L^{-1}$ ($n=3$) in water and urine samples, respectively.
[b] Without dilution.

Table 2
Determination of DIC and MEF in water samples.

Sample	Drug	$C_{initial}$	C_{added}	C_{found}	RSD (%)	RR (%)	Error%
Tap water	DIC	n.d[a]	10.0	10.2	7.2	102.0	+2.0
			25.0	24.1	5.6	96.4	−3.6
			50.0	46.9	5.2	93.8	−6.2
	MEF	n.d	10.0	9.7	6.7	97.0	−3.0
			25.0	23.8	6.1	95.2	−4.8
			50.0	48.0	5.4	96.0	−4.0
Waste water	DIC	27.3	10.0	36.7	4.9	94.0	−6.0

[a] Not detected.

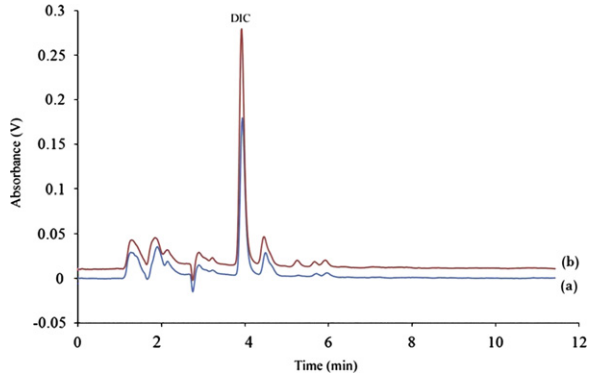

Fig. 5. Chromatograms of non-spiked (a) and 10 ng mL^{-1} diclofenac spiked (b) waste water sample.

showed that tap water sample was all free from the drug contaminations. However, DIC was detected to be 27.3 $\mu g\,L^{-1}$ in the waste water sample.

To investigate the relative recoveries, water samples spiked at concentrations of 10.0, 25.0 and 50.0 $\mu g\,L^{-1}$ were extracted under the optimized conditions (Table 2). The RR% and RSD% for the analytes were 93.8–102.0% and 4.9–7.2%, respectively. Fig. 5 shows chromatograms of the non-spiked and 10.0 $\mu g\,L^{-1}$ spiked waste water sample.

Urine samples belonged to healthy volunteers: one of them consumed a single oral dose DIC (100 mg) and the others consumed MEF (250 mg). The samples were taken from the volunteers 2 and 5 h after administration of the tablets. Moreover, urine volumes were recorded (as mention in Section 2.3). Table 3 provides the results of three-replicate urine analyses for two drug consumers and two healthy volunteers. It was found that concentration of DIC in urine sample 1 and concentration of MEF in urine sample 2 were 15.2 and 87.1 $\mu g\,L^{-1}$, respectively. To investigate accuracy of the method, urine samples were spiked with 25.0, 50.0, 80.0 and 100.0 $\mu g\,L^{-1}$ of the drugs. The RRs% obtained by the method were

Table 3
Determination of DIC and MEF in urine samples.

Sample	Drug	$C_{initial}$	C_{added}	C_{found}	RSD (%)	RR (%)	Error%
Urine 1	DIC	15.2	25.0	37.8	5.6	90.4	−9.6
	MEF	n.d[a]	25.0	23.4	7.4	93.6	−6.4
Urine 2	MEF	87.1	80.0	170.2	8.4	103.8	+3.8
Urine[b] 3	DIC	n.d	50.0	48.7	7.6	97.4	−2.6
	MEF	n.d	50.0	46.4	8.2	92.8	−7.2
Urine[b] 4	DIC	n.d	25.0	23.4	6.1	93.6	−6.4
			100.0	95.6	5.8	95.6	−4.4
	MEF	n.d	25.0	24.2	7.1	96.8	−3.2
			100.0	92.0	6.4	92.0	−8.0

[a] Not detected.
[b] Obtained from a healthy volunteer.

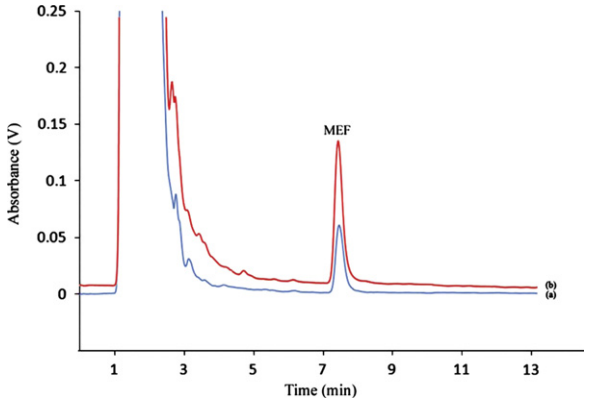

Fig. 6. Chromatograms of non-spiked (a) and 80 ng mL^{-1} spiked (b) urine samples obtained from the volunteer after administration of mefenamic acid (250 mg).

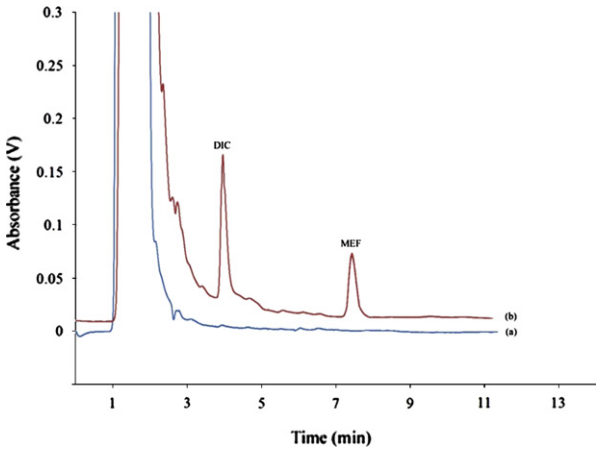

Fig. 7. Chromatograms of non-spiked (a) and 50 ng mL^{-1} spiked (b) urine sample.

in the range of 90.4–103.8%. Relative standard deviations for determination of the drugs in the examined real urine samples were also in the range of 5.6–8.4%. Fig. 6 demonstrates the chromatograms obtained from analyses of the urine sample collected from the volunteer who consumed one MEF tablet. Fig. 7 shows chromatograms of the non-spiked and 50.0 $\mu g\,L^{-1}$ spiked urine sample taken from the healthy volunteer.

4. Conclusions

Supramolecular solvents have outstanding properties for micro-extraction. They combine the capability of solubilising solutes in a

Table 4
Comparison of proposed method with other methods for extraction and determination of the drugs.

Analysis method	Matrices	Diclofenac			Mefenamic acid			Ref.
		LOD[a]	DLR	RSD%	LOD	DLR	RSD%	
SBSE–HPLC–DAD	Water	1.6	6.3–63.0	15	1.5	6.0–60.0	< 15	[2]
DLLME–LC–MS	Water	0.1	25–2000	6.0	–	–	–	[5]
SPME–LC–DAD	Water	1.5	4.0–50.0	5.9	–	–	–	[6]
HF–LPME–HPLC (DAD)	Urine	52.9	176.6–10,000	1.1	–	–	–	[7]
SBSE–HPLC (UV)	Urine	12.0	100–2000	5.5	–	–	–	[8]
SPE–GC–MS	Animal tissue	1.1 ng Kg^{-1}	3.3–10,000	5.1	0.4	1.3–10,000	4.9	[16]
HPLC	Plasma	–	–	–	15	25–2000	10.6	[36]
MCR–ALS–FS[b]	Urine	–	–	–	320	800–5000	–	[37]
SPE–HPLC-UV	Urine	7.0	20–1000	3	–	–	–	[38]
SPME–GC–MS	Water	–	–	–	1.3	–	10.0	[9]
SPE–HPLC-UV	Water	1.2 ng	5–80 ng mL^{-1}	5.1	–	–	–	[39]
MAE–SPE–GC–MS[c]	Soil	2 ng Kg^{-1}	6.5–20,000	5.2	0.9 ng Kg^{-1}	3.0–20,000	5	[40]
SPE–GC–MS	Water	0.001	–	6	0.001	–	10	[41]
SPE–HPLC-UV	Urine	50	100–10,000	6.4	50	100–10,000	6.4	[42]
SPE-SUPRAS	Water	0.4	1.0–200	4.0	1.0	2.0–200	4.6	**This work**
HPLC-UV	Urine	3.0	7.0–300	6.2	7.0	10.0–300	5.4	

[a] Concentrations were reported as $\mu g\, L^{-1}$.
[b] Fluorescence spectrometer.
[c] Microwave-assisted extraction.

wide polarity range with the ability to achieve high enrichment factors, mainly arising from the mixed-mode mechanisms and multiple binding sites they provide. In this research, SPE combined with supramolecular solvents made up of water-induced coacervation of DeA reverse micelle were proposed as valuable tools for extraction of DIC and MEF from water and urine samples. SPE as a clean-up method decreased the matrix effects. For comparison, some characteristics of previously reported methods such as LODs, LDRs, and RSD% for extraction and determination of DIC and MEF are summarized in Table 4. As can be seen, the proposed SPE-SUPRASF method has a good sensitivity and proper precision with a suitable dynamic linear range. Also, the LODs obtained for the drugs by the present method are comparable with those obtained by other methods. However this method is time consuming, but the proposed method provided high preconcentration factor without the need for solvent evaporation after the extraction, and the extract is directly injected into the HPLC loop. Concerning, satisfactory LODs, RSDs and good performance of the method in analysis of real samples showed that it could successfully be applied in complex matrices (such as highly saline solutions).

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