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Research Article

Development of Sodium Sulfate Induced Water Based Dispersive Liquid–Liquid Microextraction for the Extraction of Four Tricyclic Antidepressants in Urine Samples Prior to Their Determination by Gas Chromatography–Mass Spectrometry

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Abstract

Background: Because of the narrow therapeutic range of tricyclic antidepressant drugs, their determination in biological samples is of great importance. In this work, a fast and environment friendly sample pretreatment method based on a dispersive liquid–liquid microextraction was developed for the extraction and preconcentration of four tricyclic antidepressants including nortriptyline, amitriptyline, desipramine, and clomipramine in urine prior to their determinations by gas chromatography–mass spectrometry.

Methods: In the suggested method, an appropriate mixture of Na_2SO_4 solution (as phase separation agent and disperser) containing isopropanol (extraction solvent) is rapidly injected into an alkaline aqueous sample solution containing Na_2SO_4 and the analytes. As a result, a cloudy mixture is formed and the tiny droplets of the extractant containing the extracted analytes are collected on the surface of the aqueous phase after centrifuging. Finally, an aliquot of the collected organic phase is removed and injected into the separation system for the quantitative analysis.

Results: Under the optimum conditions, the enrichment factors and extraction recoveries were in the ranges of 380–440 and 76–88%, respectively. The limits of detection and quantification were obtained in the ranges of 11–24, and 41–75 ng/L, respectively. The relative standard deviations of the proposed method were ≤ 6.1% for intra– (n=6) and inter–day (n=4) precisions at a concentration of 100 ng/L of each analyte.

Conclusion: The introduced method was satisfactorily utilized for the simultaneous determination of the selected tricyclic antidepressant drugs in the patient's urine samples.

Introduction

Antidepressant drugs are widely utilized in psychiatric clinics to treat the depression and improve the quality of patient's life.[1] Tricyclic antidepressants (TCAs) are belong to the first generation of psychotropic drugs which are still widely utilized throughout the world in order to treat depression.[2] These drugs inhibit the reuptake of serotonin and norepinephrine in the central nervous system.[3] Therapeutic drug monitoring is highly recommended to achieve the best therapeutic concentration with at least overdose and adverse problems. The narrow therapeutic ranges of TCAs has multiplied the importance of their determination in biological samples (plasma, serum or urine) of patients. Therefore, the development of an efficient, environmental friendly, and rapid analytical approach for the quantification of TCAs in biological samples is of great importance. The concentration of these drugs in biological fluids are mostly quantified using chromatographic techniques such as liquid[4,5] and gas[6, 7] chromatography. However, because of the complex matrices of biological samples and trace levels of the

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analytes in them, an efficient and sensitive pretreatment approach is required before their analysis by above–mentioned analytical instruments. Conventionally, sample preparation is carried out by liquid–liquid extraction or solid–phase extraction. However, both of them have various disadvantages such as consumption of extensive hazardous organic solvents and being tedious and time–consuming[8,9] In order to overcome these problems, several pretreatment methods based on solid phase microextraction (SPME)[10] and liquid phase microextraction (LPME)[11,12] have been developed. SPME is a novel microextraction method that has been used for TCAs.[13] This technique is simple, fast, solventless, and efficient but suffers from some drawbacks like fragility and limited life time of fibers and sample carry over. Single drop microextraction (SDME) is the first LPME method in which a single drop of organic solvent is exposed to the headspace or directly into the sample matrix and the analytes can be concentrated into this droplet. Up to now, several reports on the application of this method in determination of TCAs in biological samples[14,15] and wastewater[16] were published. SDME is limited by the relatively long extraction time as well as droplet instability resulting from the attachment of the microdroplet from the needle when changing extraction conditions. Hollow–fiber liquid–phase microextraction (HF–LPME) is the other sample preparation method in which the analytes are transferred from sample solution into the organic layer filled in the pores of a suspended or directly contacted hollow fiber.[17,18] In 2006, a new mode of LPME named dispersive liquid–liquid microextraction (DLLME) has been developed.[19] In DLLME, a mixture of extraction and disperser solvents is hastily injected into a sample solution. Consequently, the small droplets of the extractant is formed and the analytes are extracted into them.[20-23] DLLME shows several advantages like rapidity, low cost, simplicity of operation, and relatively high recovery and enrichment factors (EFs). Therefore, this method has been used for the determination of organic compounds,[19] metal ions,[24] and drugs[25-28] in different samples. Also, DLLME was used for determination of TCAs in biological samples in the previously published papers.[27,29-41] Even though, these method removes the main deficiencies of the customary extraction approaches but it also requires toxic organic solvents like chlorinated or aromatic solvents howbeit much less compared to the previous methods.

The aim of the present work was to suggest a new and green version of DLLME without using common disperser and extraction solvents. The proposed method was utilized for the extraction and preconcentration of four TCAs from urine samples before their quantification by gas chromatography–mass spectrometry (GC–MS) in order to achieve high EFs and low limits of detection (LODs). In this method a low toxic organic solvent named isopropanol is used instead of halogenated, aromatic or other toxic solvents consumed in the traditional DLLME. On the other hand, water is used as a disperser solvent in this study. Indeed, in this work a green DLLME method is performed in the presence of Na_2SO_4 in which water is used as a disperser in DLLME. As far as we know, this is the first time that water is used as a disperser which makes the proposed method environment friendly. In addition, isopropanol is used as an extraction solvent which is much safer than halogenated solvents utilized in the traditional DLLME. Because of the low density of extractant compared to water, a home–made device was designed and utilized as an extraction vessel to simplify the collection of the extractant after performing the suggested approach.

Materials and Methods

Reagents and solutions

Desipramine was supplied from Pars Darou Co. (Tehran, Iran). Amitriptyline and nortriptyline were purchased from Daroupakhsh Co. (Tehran, Iran). Clomipramine and imipramine which used as internal standard (IS) were supplied from Amin Pharmaceutical Co. (Isfahan, Iran). Sodium sulfate (Na_2SO_4), ammonia (25%), hydrochloric acid (37%), sodium hydroxide, methanol, isopropanol, acetonitrile (ACN), and *n*–propanol were from Merck (Darmstadt, Germany). A stock solution of analytes was prepared in methanol (10 mg/L of each drug). This solution was diluted with deionized water to prepare working standard solutions. To increase the repeatability of the suggested approach and provide an acceptable precision, imipramine (as an IS) at a concentration of 5 mg/L was added into the extraction solvent throughout this study.

Apparatus

The quantification of the analytes was carried out on an Agilent 6890N gas chromatograph (Agilent Technologies, CA, USA) coupled to a 5973 mass–selective detector. Injection was performed in a pulsed spilt mode (split ratio of 1:10) with a sampling time of 1 min. Helium (99.9999%, Gulf Cryo, United Arab Emirates) was utilized as the carrier gas (at a constant flow rate of 1.0 mL/min). An HP–5MS fused–silica capillary column (5% diphenyl–95% dimethylsiloxane, 30 m × 0.25 mm i.d., and a film thickness of 0.5 µm) (Agilent Technologies, Illinois, USA) was utilized for separation the drugs. The column oven temperature programming was as follows: primary temperature 60 °C (held for 3 min), then ramped at a rate of 15 °C/min to 300 °C (held for 4 min). The ionization was made by electronic impact at 70 eV. The other operational conditions of MS were: ionic source temperature: 250 °C; mass range: m/z 30–400; transfer line temperature: 260 °C; detector voltage: –1700 V; and acquisition rate: 20 Hz. To analysis the analytes, the following ions were opted: m/z 44, 114, and 208 for desipramine; m/z 58, 202, and 215 for amitriptyline; m/z 58, 85, and 269 for clomipramine; m/z 44, 214, and 232 for nortriptyline; and m/z 58, 193, and 234 for IS. Metrohm pH meter model 654 (Herisau, Switzerland) was used in pH measurements. A Hettich centrifuge (model D–7200, Kirchlengern, Germany) was utilized in order to speed up phase separation procedure.

Samples

Blank urine samples were collected from volunteers who had not consumed any drug for about two months. The other samples were obtained from patients who treated

with a tablet containing 25 mg of each drug, twice a day. All of the samples were collected within 24 h from the first oral administration. The pH of urine samples were adjusted at 10.0 using an ammoniacal buffer (0.5 M) and after that introduced to the suggested approach. All sample donors have been informed on details of the drugs and signed a consent form which was confirmed by the Ethical Committee of Tabriz University of Medical Sciences which was confirmed by the Ethical Committee of Tabriz University of Medical Sciences and registered with the approval code of IR.TBZMED.REC.1397.492.

Procedure

To 5 mL of ammoniacal buffer (C=0.1 M, pH=10.0) spiked with 20 µg/L of the drugs or pretreated urine sample (see Sec. 2.3) placed into a 10–mL glass test tube, 1.5 g Na_2SO_4 was added and manually shaken to dissolve. Afterward, 1.0 mL deionized water containing Na_2SO_4 (30%, *w/v*) was mixed with 120 µL isopropanol containing 5 mg/L IS and rapidly injected into the sample solution. Consequently, the fine droplets of isopropanol containing the extracted analytes were formed. After centrifuging for 5 min at 5000 rpm, 10 ± 0.5 µL of the organic solvent was collected on the surface of the aqueous phase. 1 µL of this solvent was taken and injected into the GC–MS. The suggested microextraction procedure is schematically presented in Figure 1.

Results and Discussion

In this step, different experimental parameters affecting the proposed method performance and efficiency such as extraction solvent type and volume, disperser volume, salt concentration in disperser and aqueous phase, pH of aqueous phase, and centrifuging rate and time were carefully investigated and optimized.

Optimization of extraction solvent type and volume

Opting an appropriate extractant has a great role in all microextraction methods including DLLME. The performance and selectivity of the proposed method for the studied analytes are strongly affected by this parameter. The selected extraction solvents in the proposed method must meet several criteria including high extraction capability of the selected analytes, the ability to form a separate organic phase in the presence of a salt, low solubility in water, being environment friendly, and density lower than water. Considering these characteristics, ACN, isopropanol, and *n*–propanol were investigated as the extraction solvent in this study. For investigating this parameter, 130 µL of each solvent containing 5 mg/L of IS, is mixed separately with 0.75 mL deionized water containing Na_2SO_4 (25%, *w/v*). This solution was rapidly injected into an alkaline aqueous solution (pH=10.0) spiked with the analytes (20 µg/L, each drug) containing Na_2SO_4 (25%, *w/v*). By this action, the extraction solvent was dispersed and the tiny droplets of extractant containing the extracted analytes were formed and collected on the surface of the aqueous phase after centrifuging. According to the results (Figure 2), by comparing the ratio of the analyte peak area to the IS peak area for various extraction solvents, isopropanol is the best extractant among the evaluated solvents. Thus, isopropanol was opted for the further experiments.

The volume of extractant is another vital parameter that can affect the extraction recoveries (ERs) and EFs of the analytes and subsequently LODs of the proposed approach. To investigate this parameter, different volumes of isopropanol (130, 140, 150, and 160 µL) were investigated.

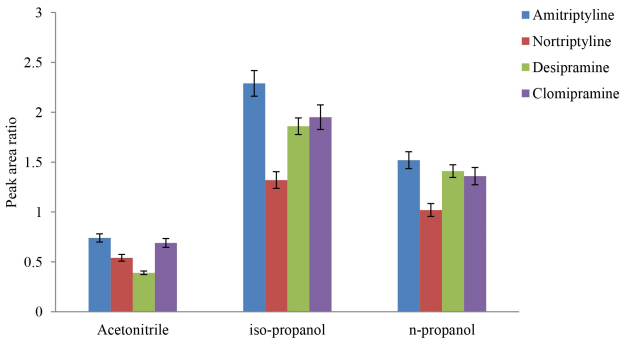

Figure 2. Selection of extraction solvent. Extraction conditions: aqueous sample volume, 5 mL ammoniacal buffer (C=0.1 M, pH=10.0) spiked with the selected analytes at 20 µg/L of each analyte; extraction solvent volume, 130 µL; disperser solvent (volume), deionized water (0.75 mL); Na_2SO_4 concentration in disperser and aqueous solution (25%, *w/v*); centrifugation rate, 5000 rpm; and centrifugation time, 5 min. The error bars show the minimum and maximum of three repeated determinations.

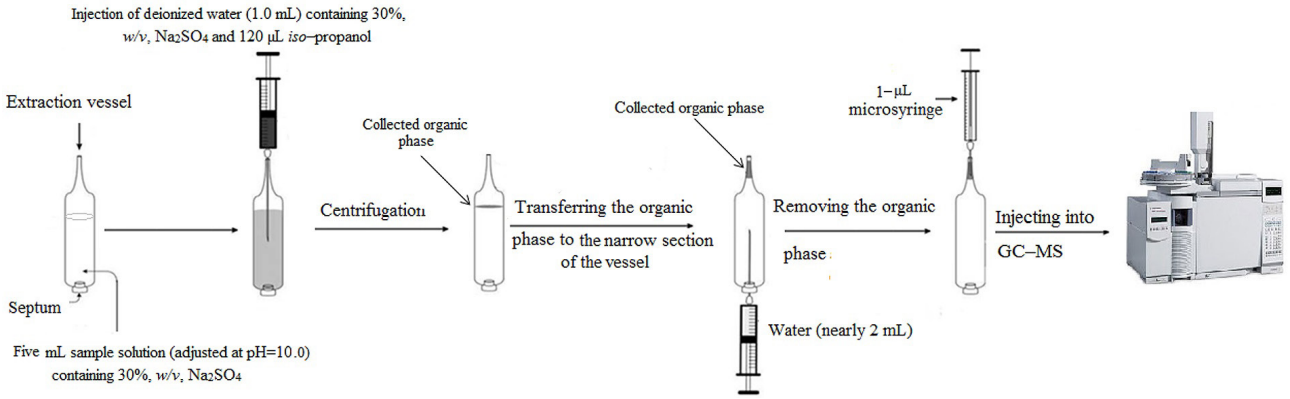

Figure 1. Procedure and extraction vessel used in the presented method.

Considering the outcomes by increasing the volume of isopropanol from 130 to 150 µL, the volume of the collected organic phase was increased (from 10 to 29 µL) and thereby, analytical signals of the proposed method decreased which can be attributed to the dilution effect. Therefore, 130 µL was chosen as the optimum volume of the extractant.

Optimizaton of disperser solvent volume

To reach high preconcentration of analytes in the suggested method, the optimization of disperser volume is necessary. The main role of the disperser solvent is dispersing the extraction solvent into the aqueous phase in order to afford a very large contact area and accelerates the extraction of the analytes into the extraction solvent. In this work, deionized water was used as a green, environment friendly, and low–cost disperser solvent for dispersing isopropanol into the sample solution as tiny droplets in the presence of Na_2SO_4.

To optimize the volume of disperser, various volumes of deionized water (0.25, 0.50, 0.75, 1.00, and 1.25 mL) were evaluated while the other experimental conditions except isopropanol volume (101, 112, 130, 144, and 157 µL for 0.25, 0.50, 0.75, 1.00, and 1.25 mL of deionized water, respectively) were kept constant. The obtained results (Figure 3) indicate that the extraction efficiency of the proposed method for the target analytes enhances as the volume of the deionized water increases from 0.25 to 1.00 mL and then decreases. So, 1.00 mL of deionized water was opted for the accomplishment of the next experiments.

Salt addition

Salt addition is one of the important factors that should be investigated in the microextraction procedures. This phenomenon can decrease the solubility of the extraction solvent into the aqueous sample, which leads to an increase in the volume of the collected phase. Also, salting out effect can be observed for the analytes by reducing the solubility as a result of ionic strength enhancement. The salt addition was used in two parts of the presented work. In this work, Na_2SO_4 was selected due to its higher solubility into the aqueous sample (0.44 g/mL at 20 °C) compared to the other salts like sodium chloride (0.36 g/mL) and potassium chloride (0.25 g/mL).[42] This phenomenon leads to more ionic strength enhancement in the presence of Na_2SO_4.

In the first part, Na_2SO_4 was added into deionized water (disperser) to prevent the reduction of ionic strength of aqueous sample after injection of the mixture of deionized water and isopropanol into it. To optimize this parameter, various concentrations of Na_2SO_4 (15–40%, *w/v*) were added into deionized water, while the other parameters, except isopropanol volume (160, 152, 144, 134, 126, and 120 µL of isopropanol for 15, 20, 25, 30, 35, and 40 % *w/v*, of Na_2SO_4, respectively) were kept constant during the microextraction procedure. The obtained outcomes (Figure 4) indicate that the extraction efficiency of analytes enhances with increasing Na_2SO_4 concentration till 30% (*w/v*) and after that decreases. Thus, 30% (*w/v*) Na_2SO_4 was opted for the accomplishment of the next experiments.

In the second part, Na_2SO_4 was added into the aqueous solution in order to evaluate the effect of ionic strength on the extraction efficiency of the selected analytes. In this step, different concentrations of Na_2SO_4 (15–40%, *w/v*) were added into the aqueous sample solution. To reach a constant volume of the collected phase (10 µL), the following studies were carried out using various volumes of isopropanol (165, 149, 134, 120, 112, and 105 µL of isopropanol for 15, 20, 25, 30, 35, and 40 %, *w/v*, of Na_2SO_4, respectively). As

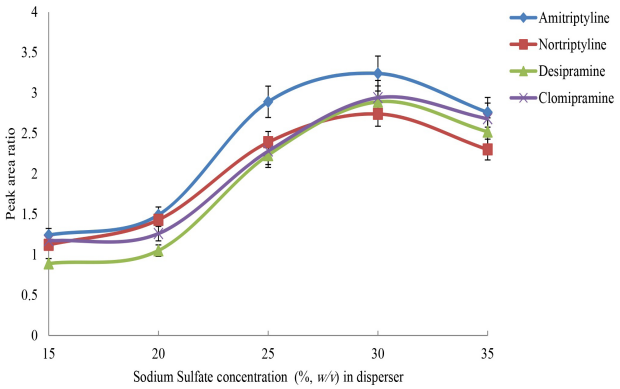

Figure 4. Optimization of Na_2SO_4 concentration in disperser. Extraction conditions: are the same as used in Fig. 3, except 1.0 mL deionized water was used as the disperser solvent.

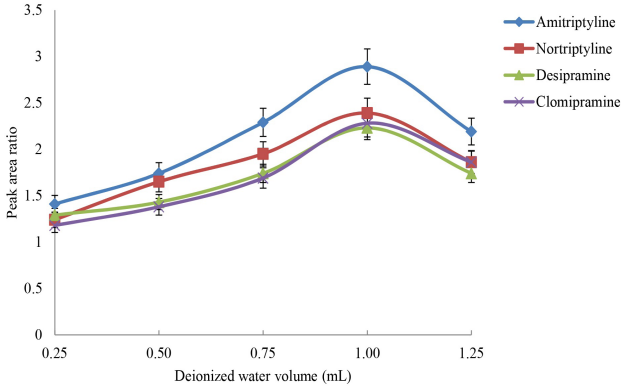

Figure 3. Optimization of disperser volume. Extraction conditions: are the same as used in Fig. 2, except *iso*–propanol was used as the extraction solvent. The error bars show the minimum and maximum of three repeated determinations.

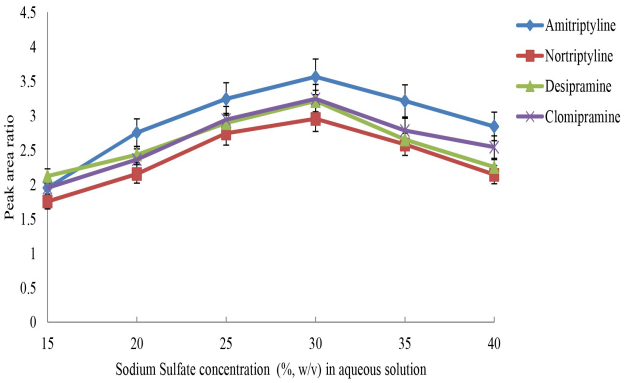

Figure 5. Optimization of ionic strength of aqueous phase. Extraction conditions: are the same as used in Fig. 4, except 30%, *w/v,* Na_2SO_4 was dissolved into the disperser.

shown in Figure 5, the extraction efficiency of the analytes increases with the concentration of Na_2SO_4 up to 30% and then decreases. So, 30%, *w/v,* Na_2SO_4 was selected as the optimum. It should be mentioned that in both parts, in the concentrations less than 15%, *w/v* of Na_2SO_4, no organic layer was collected on the aqueous phase.

Study of pH

The pH of aqueous solution can possesses significant influence on the ERs of the analytes which are susceptible to hydrolysis due protonation including the studied drugs. The influence of pH was investigated using different experiments designed by changing pH of aqueous solution from 6 to 12 with the help of 0.1 M HCl or NaOH solutions. The obtained data demonstrated that (Figure 6), the extraction efficiency of drugs improved with the increasing pH till 10.0 and then remained approximately constant. Considering the pK_a values of analytes,[43,44] at pHs lower than pK_a, the analytes were converted to the related conjugated acids and they will have less tendency to be extracted into the extractant. Therefore, the pH of samples was adjusted at 10.0 for subsequent experiments. In the following experiments, an ammoniacal buffer (C=0.5 M, pH=10.0) was utilized for the pH adjustment.

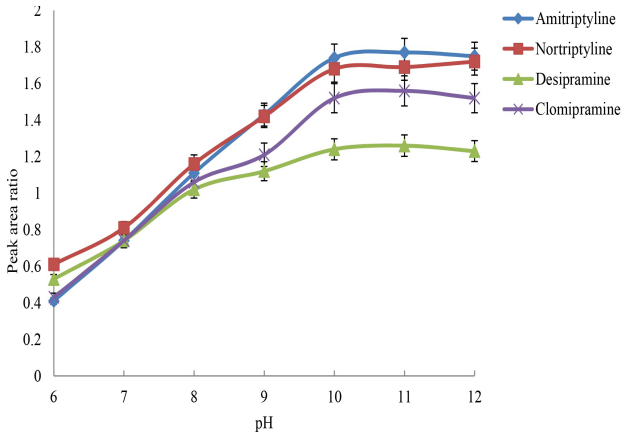

Figure 6. Study of pH effect. Extraction conditions: are the same as used in Fig. 5, except 30%, *w/v*, Na_2SO_4 was dissolved into the aqueous phase and its pH was changed in the range of 6–12.

Investigation of centrifuging rate and time

Centrifugation is noteworthy procedure in order to achieve a rapid and complete separation of the extractant droplets from sample solution. Rate and time of centrifugation were investigated in the ranges of 2000–7000 rpm and 1–6 min, respectively. The outcomes revealed that the extraction capability of analytes enhanced with increasing centrifugation rate and time till 5000 rpm and 5 min, respectively, and after that remain unchanged. Therefore, 5000 rpm and 5 min were selected for the accomplishment of the next studies.

Method validation

In this step, international guidelines and protocols [45,46] were utilized to validate the suggested approach considering parameters including LOD, limit of quantification (LOQ), intra– and inter–day precisions, linearity, selectivity, EFs and ERs.

Linearity and calibration curves

The linearity of the developed approach was investigated by preparing matrix–matched calibration curves based on peak area ratio (analyte to IS) *versus* the analyte concentration. The LOD and LOQ values were evaluated on the basis of the signal–to–noise ratios (S/N) of 3 and 10, respectively. The lower limit of quantification (LLOQ) was reported as the lowest concentration on the calibration curve that could be determined with a relative standard deviation (RSD) ≤ 20% and an accuracy of 80–120%. The outcomes are listed in Table 1. Broad linearities were achieved with coefficients of determination ≥0.9993.

Selectivity

Selectivity studies evaluate the effects of interferences which can potentially be available in urine to indicate the ability of the approach to measure the analytes in the presence of these components. In order to investigate this parameter, six urine samples from various volunteers who expressed that they had not taken drugs were analyzed. The responses of the analytes were compared with those of the analytes at the LLOQ. No significant interference was observed at the retention times and peaks areas of the analytes.

Table 1. Quantitative features of the developed method for the selected TCAs.

Analyte	LOD [a]	LOQ [b]	LLOQ [c]	LR [d]	r^2 [e]	RSD% [f]		EF ± SD [g]	ER ± SD [h]
						Intra–day	Inter–day		
Amitriptyline	13	47	22	22–2000000	0.9996	4.2	5.5	440 ± 25	88 ± 5
Nortriptyline	24	75	37	37–2000000	0.9994	3.3	6.1	380 ± 15	76 ± 3
Desipramine	11	41	19	19–2000000	0.9993	2.3	5.4	380 ± 15	76 ± 3
Clomipramine	15	49	26	26–2000000	0.9993	4.1	5.3	405 ± 20	81 ± 4

a) Limit of detection (S/N=3) (ng/L).
b) Limit of quantification (S/N=10) (ng/L).
c) Lower limit of quantification (S/N=5) (ng/L).
d) Linear range (ng/L).
e) Coefficient of determination.
f) Relative standard deviation for intra– (n=6) and for inter–day (n=4) precisions at a concentration of 100 ng/L of each analyte.
g) Enrichment factor ± standard deviation (n=3).
h) Extraction recovery ± standard deviation (n=3).

Precision and accuracy

In fact, the precision is expressed as the measurement of the random errors. The precision of the approach defined as RSD was investigated by doing the approach on six (for intra–day) and four (for inter–day) quality control (QC) samples at a concentration of 100 ng/L of each drug and ranged from 2.3–4.2 and 5.3–6.1%, respectively.

Calculation of EF and ER

EF and ER have been utilized for investigating the performance of the suggested approach. EF is expressed as

$$EF = C_{coll} / C_0 \qquad \text{Eq. (1)}$$

$$ER = \frac{n_{coll}}{n_0} \times 100 = \frac{c_{coll} \times v_{coll}}{c_0 \times v_{aq}} \times 100 = EF \times \frac{v_{coll}}{v_{aq}} \times 100 \qquad \text{Eq. (2)}$$

the ratio of the analyte concentration in the collected phase (C_{coll}) found from calibration curve equation to its primary concentration in the aqueous solution (C_0):
ER is expressed as the percentage of the total analyte amount (n_0) which is extracted into the collected phase (n_{coll}): Where V_{coll} and V_{aq} are volumes of the collected organic phase and aqueous solution, respectively. As it can be seen from Table 1, high EFs and ERs in the ranges of 380–440 and 76–88%, respectively, are obtainable.

Real samples analysis

Usability of the introduced approach was evaluated by analyzing four urine samples collected from patients who had treated with a tablet containing 25 mg of each drug twice a day. It should be mentioned that each patient only had consumed one of the studied drugs. Figure 7 reveals typical GC–selected ions monitoring (SIM)–MS chromatograms of patients urine samples after carrying out the introduced approach along with a blank urine sample and direct injection of a standard solution of analytes (25 mg/L) and IS (5 mg/L) prepared in methanol. After three determinations of each sample using standard addition method, the found concentrations of clomipramine, desipramine, nortriptyline, and amitriptyline in the mentioned urine samples were 6.3 ± 0.4, 4.8 ± 0.2, 5.9 ± 0.1, and 7.3 ± 0.3 µg/L, respectively. To investigate matrix effect in blank urine sample, the added–found method was utilized and sample was spiked with drugs at three concentrations (100, 200, and 500 ng/L). Mean relative recoveries (the recoveries obtained for the analytes in the urine sample compared to those obtained in deionized water spiked at the related concentrations) were calculated and summarized in Table 2. Considering the outcomes, this method presents good relative recoveries ranging from 82–94%.

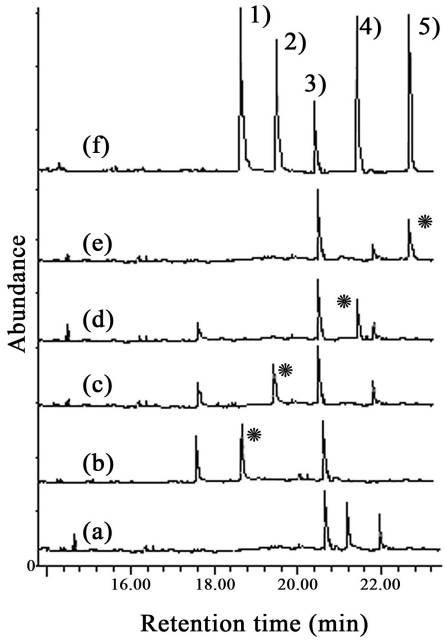

Figure 7. Typical GC–SIM–MS chromatograms of (a) blank urine sample, (b) urine sample of a patient treated with amitriptyline, (c) urine sample of a patient treated with nortriptyline, (d) urine sample of a patient treated with desipramine (e) urine sample of a patient treated with clomipramine, and (f) direct injection of standard solution of the selected analytes at a concentration of 10 mg/L of each analyte and IS at a concentration of 5 mg/L prepared in methanol. Peak identification, 1) amitriptyline, 2) nortriptyline, 3) imipramine (IS), 4) desipramine, 5) clomipramine.

Table 2. Study of matrix effect in the proposed method in the blank urine sample spiked at different concentrations.

Analyte	Added (ng/L	Found (ng/L)	Mean relative recovery ± standard deviation (n=3)
Amitriptyline	100	82 ± 3	82 ± 3
	200	188 ± 8	94 ± 4
	500	450 ± 15	90 ± 3
Nortriptyline	100	91 ± 3	91 ± 3
	200	186 ± 4	93 ± 2
	500	455 ± 20	91 ± 4
Desipramine	100	89 ± 2	89 ± 2
	200	186 ± 6	93 ± 3
	500	450 ± 15	90 ± 3
Clomipramine	100	89 ± 3	89 ± 3
	200	168 ± 10	84 ± 5
	500	450 ± 10	90 ± 2

Comparison of the developed approach with others

The figures of merit of the introduced approach in quantification of studied drugs were compared with those of the published approaches considering LOD, LR, RSD, and EF and the data are summarized in Table 3. As it can be seen, the RSDs, LODs, and EFs of the suggested approach are comparable or better than those reported for the other approaches. These outcomes indicate that the introduced approach is efficient, sensitive, and reliable technique for the extraction of the analytes from urine samples.

Conclusion

In this study, an efficient and green microextraction method in combination with GC–MS was introduced for extraction and determination of four TCAs in human urine samples. In the suggested approach isopropanol was utilized as an extractant instead of highly toxic solvents utilized in

Table 3. Comparison of the proposed method with other approaches in the extraction and determination of the selected drugs.

Method	Sample	LOD [a] (ng/L)	LR [b] (ng/L)	RSD [c] (%)	EF [d]	Ref.
DSPE–DES–AALLME–GC–MS [e]	Urine and plasma	32–60	108–5000000	3.0–5.0	–	29
LLLE–DLLME–GC–FID [f]	Plasma	1000–3000	10000–15000	3.9–6.5	79–98	30
DLLME–GC/MS [g]	Urine	1000–2000	–	2.3–9.9	–	31
AALLME–GC–FID [h]	Plasma	2000	20000–2000000	4.9–5.4	82–111	32
EME–DLLME–GC–FID [i]	Urine	3000	10000–500000	11.7	753	33
DLLME–GC–FID [j]	Plasma	5000–10000	2000–16000	5.6–6.4	740–1000	34
DLLME–HPLC–UV [k]	Urine	600	2000–100000	5.1–6.1	162–187	35
TDLLME–HPLC/UV [l]	Plasma	700–1000	2500–5000000	4.1–6.1	50–101	27
TA–DLLME–GC–FID [m]	Urine	2000–4000	8000–4000000	3.0–6.0	820–1070	36
USA–DLLME–GC–MS [n]	Blood	–	5000–15000	2.7–8.7	–	37
AALLME–SFO–GC/FID [o]	Wastewater	5000–7000	15000–2000000	<8.4	682–731	38
IL–DLLME–μ–SPE–HPLC–UV [p]	Environmental water	300–1000	1000–1000000	1.5–7.8	17–43	39
USA–DLLME–UPLC–PDA [q]	Plasma	4000–5000	–	2.4–7.5	5	40
USA–DLLME–HPLC–UV [r]	Plasma	6000–10000	20000–2000000	3.2–5.9	800–920	41
HF–LPME–GC–MS [s]	Whole blood	10000	20000–1200000	1.6–6.4	–	47
SPE–GC–MS [t]	Whole blood	300–1500	5000–1000000	3.8–4.2	–	48
In–tube SPME/HPLC–MS [u]	Urine Plasma	80–170 70–100	1000–500000 1000–500000	3.6–8.4 3.9–5.7	6.4–6.7	49
MSPE–HPLC–UV [v]	Plasma	40–80	500–1000000	2.1–2.5	220–250	50
SI–Water based–DLLME–GC–MS [w]	Urine	11–24	41–2000000	2.3–4.2	380–440	This method

a) Limit of detection.
b) Linear range.
c) Relative standard deviation.
d) Enrichment factor.
e) Dispersive solid phase extraction–deep eutectic solvent–air–assisted liquid–liquid microextraction–gas chromatography–mass spectrometry.
f) Liquid–liquid–liquid extraction–dispersive liquid–liquid microextraction–gas chromatography–flame ionization detection.
g) Dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry.
h) Air–assisted liquid–liquid microextraction–gas chromatography–flame ionization detection.
i) Electromembrane extraction–dispersive liquid–liquid microextraction–gas chromatography–flame ionization detection.
j) Dispersive liquid–liquid microextraction–gas chromatography–flame ionization detection.
k) Dispersive liquid–liquid microextraction–high performance liquid chromatography–ultraviolet detector.
l) Tandem dispersive liquid–liquid microextraction–high performance liquid chromatography–ultraviolet detector.
m) Temperature assisted–dispersive liquid–liquid microextraction–gas chromatography–flame ionization detection.
n) Ultrasonic assisted–dispersive–liquid–liquid microextraction–gas chromatography–mass spectrometry.
o) Air–agitated liquid–liquid microextraction–solidifiable organic solvent–gas chromatography–flame ionization detection.
p) Ionic liquid–dispersive liquid–liquid microextraction–micro–solid phase extraction–high performance liquid chromatography–ultraviolet detector.
q) Ultrasonic assisted–dispersive–liquid–liquid microextraction–ultra performance liquid chromatography–photodiode array detector.
r) Ultrasonic assisted–dispersive–liquid–liquid microextraction–high performance liquid chromatography–ultraviolet detector.
s) Hollow fiber–liquid phase microextraction–gas chromatography–mass spectrometry.
t) Solid phase extraction–gas chromatography–mass spectrometry.
u) In–tube–solid phase microextraction–high performance liquid chromatography–mass spectrometry.
v) Magnetic solid phase extraction–high performance liquid chromatography–ultraviolet detector.
w) Sodium sulfate induced water based–dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry.

conventional DLLME. In addition, deionized water and Na_2SO_4 were utilized as disperser and phase separation agent, respectively. The obtained outcomes indicated good precision, high sensitivity, ease of operation, and rapidity. Furthermore, consumption of a safe organic solvent like isopropanol at only μL level caused to decrease the risk for human health and environment.

Ethical Issues

All sample donors have been informed on details of the drugs and signed a consent form which was confirmed by the Ethical Committee of Tabriz University of Medical Sciences and registered with the approval code of IR.TBZMED.REC.1397.492.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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