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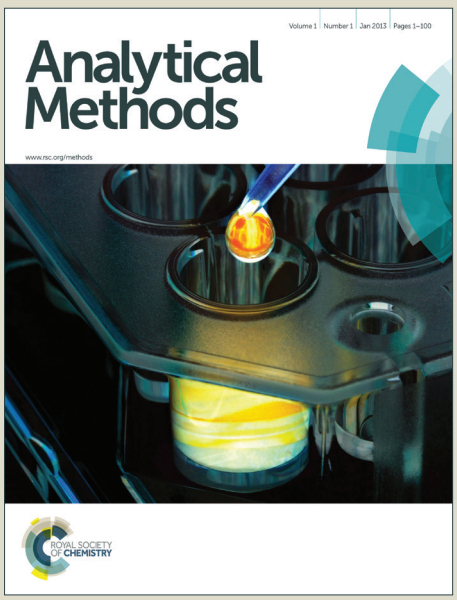

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Hollow fiber supported liquid phase microextraction combined with maltodextrin-modified capillary electrophoresis for determination of citalopram enantiomers in urine samples

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Abstract

A simple and highly sensitive method that involves three-phase hollow fiber supported liquid-phase microextraction (HF-LPME) coupled with maltodextrin (MD)-modified capillary electrophoresis (CE) was successfully developed for the separation and determination of trace concentration of citalopram (CIT) enantiomers in urine samples. Results of enantioseparation with CE showed that the R-enantiomer of CIT migrated faster than the S-enantiomer. The analyte was extracted into 1-octanol that was immobilized in the wall pores of a porous hollow fiber from 4.0 mL of aqueous sample, pH 12.5 (donor phase), and was back extracted into the acceptor phase (pH 2.2) that located in the lumen of the hollow fiber. A Box-Behnken design (BBD) and the response surface methodology (RSM) were used for the optimization of different parameters on the extraction efficiency. The limits of quantification and detection for both R-CIT and S-CIT were 10 and 30 ng mL^{-1}, respectively. The method was reproducible so that intra and inter day RSDs% (n = 5) were less than 6.9%. Finally, this method was successfully applied to determine the concentration of CIT enantiomers in real urine samples.

1 Introduction

Citalopram (CIT), a potent and highly selective serotonin reuptake inhibitor, is an interesting alternative in the treatment of depressive patients as it appears to have few and mild side effects compared to the classical antidepressants.[1-3] CIT was first introduced into therapy as a racemic drug because it has one chiral centre and therefore exists in the (R) and (S) forms (Figure 1). However, in vitro studies in rat brains have shown that the pharmacological effect of CIT primarily lies on the S–CIT enantiomer, where as the R–CIT enantiomers is considered to be pharmacologically inactive and could counteract the activity of the S-enantiomer.[4] Additionally, compared to the racemate, preclinical studies have postulated that the S-CIT is more efficacious [5] and even 150-times more potent than the R-CIT.[6] Thus, it seems that the concentration of R-CIT would be more than S-CIT in urine or plasma samples obtained from a patient under treatment with racemic CIT tablets.

The development of a rapid and specific method allowing the screening and the determination of this new antidepressant drug in biological fluids could be of great interest either in therapeutic drug monitoring use or in toxicological screening in the case of suicide involving this compound.[7] Determination of CIT has already been carried out using chromatographic methods such as high-performance liquid chromatography (HPLC-UV) [8-11], capillary electrophoresis (CE) [12-14], capillary electrochromatography (CEC) [15], and gas chromatography (GC-MS) [16, 17]. One of the important steps in an analytical method is the extraction of the compounds of interest from the sample matrix. The study of CIT bioavailability requires a highly sensitive analytical method because of the low plasma levels (15-620 nM for the racemate) resulting from therapeutic doses.[2] Thus, sample preconcentration and cleanup must be carried out prior to the determination step.

Liquid–liquid extraction (LLE), solid-phase extraction (SPE) and solid-phase microextraction (SPME) are the most common techniques for isolation and enrichment of CIT prior to chromatographic analysis.[18-25] These methods have many disadvantages, as they are tedious, labor-intensive and time-consuming. Formation of emulsion is a major drawback during LLE process. This method also requires the use of large amount of highly purified solvents, which are often hazardous and leads to the production of toxic laboratory waste. Although SPE is less time-consuming than LLE, it still requires an appreciable amount of toxic solvent for analyte desorption. SPME, which was introduced by Pawliszyn and his group in 1990 [26] is a solvent-free, simple and fast method and sampling can be carried out directly under field conditions. The main drawbacks of SPME are its expensive fibers and when reused, there is a potential for sample carry over between runs, which could invalidate the results. SPME's lifetime is limited because the solid-phase materials degrade with usage.[27, 28]

Hollow fiber supported liquid phase microextraction (HF-LPME) proposed by Pedersen-Bjergaard and Rasmussen[29] could solve the aforementioned problem effectively. In this mode, porous hollow fibers made of polypropylene were used to protect the extraction solvent. The extraction solvent is not in direct contact with the sample solution, so the samples may be stirred or vibrated vigorously without any loss of the extraction solvent. Micro pores of the hollow fiber would prevent large molecules and other impurities from entering into the extraction solvent in the lumen[30]. Moreover, the disposable hollow fiber could avoid cross-contamination. Thus, HF-LPME is a more robust and reliable alternative to single drop microextraction (SDME), which may provide high preconcentration, short extraction time and excellent sample clean-up capability.[31-33]

In most of the previous studies revealed to LPME, one variable at a time method (OVAT) has been used for optimizing different parameters affecting response. In a one-dimensional method, one factor is optimized while the other factors are held constant; and factors are optimized one by one. While such a method is easy to design and implement, it does not examine interactions between factors, and thus it is incapable of finding the true optimum conditions. Statistical experimental design, such as response surface methodology (RSM), was proposed to overcome these limitations. RSM integrates experimental design and data analysis, offering an efficient approach for yield optimization. Thus, RSM coupled with Box–Behnken design (RSM-BBD) was selected for the current study due to its: (1) ability to process multiple variables; (2) capability of evaluating the interactions between factors; and (3) avoidance of extreme conditions. Although, there are some reports that experimental design was applied to optimize the experimental parameters of HF-LPME.[34-36]

In this work, for the first time, HF-LPME as a new microextraction technique and maltodextrin (MD) as chiral selector were used for preconcentration, separation and determination of CIT enantiomers by CE. During the method development, no literature data on enantioseparation of CIT with MD mediated CE was available. The main goal was to make the best use of the advantages and strengths of both techniques in order to develop a powerful chiral method for quantification of CIT enantiomers present in low concentrations in biological matrices. Different variables affecting HF-LPME were optimized with the BBD and RSM. Finally, the optimized procedure was employed to determine CIT enantiomers in real urine samples.

2 Experimental

2.1 Reagents and materials

Racemic CIT was obtained from Dr. Abidi laboratories (Tehran, Iran) and was used without further purification. MDs with dextrose equivalents (DE) of 4–7, 13–17 and 16.5–19.5 were purchased from Fluka (Buchs, Switzerland). Analytical grade H_3PO_4, $NaH_2PO_4.2H_2O$, Na_2HPO_4, NaOH, HCl, 1-octanol, 1-octane, cyclohexane, hexadecane and toluene were purchased from Merck (Darmstadt, Germany). The Q3/2 Accurel polypropylene hollow fiber membrane (600 mm i.d., 200 mm wall thickness and 0.2 mm pore size) was purchased from Membrana Company (Wuppertal, Germany). HPLC grade water was obtained through a Milli-Q® system (Millipore, Milford, MA, USA) and was used for preparation of all solutions. To prevent capillary blockage, all buffers were filtered through 0.45 µm filter membranes (Millipore, Bedford, MA, USA).

2.2 Standard and real sample solutions

Stock solution of racemic CIT (1000 mg L^{-1}) was prepared in HPLC-grade water. The stock solution was protected from light using aluminum foil and stored for a month at 4 ^{0}C with no evidence of decomposition. Then, the required working standard solutions were freshly prepared by appropriate dilution of the stock solution to the required concentrations. The urine samples were obtained from the Clinic of Taleghani Hospital (Tehran, Iran) and were diluted at 1:3 ratio using HPLC-grade water. Urine 1 sample was collected from a person who had not used CIT at all, and urine 2, 3 and 4 samples were collected from a patient who was under treatment with CIT. All real sample experiments were performed in compliance with the guideline of Clinic of Taleghani Hospital.

2.3 CE equipment

CE experiments were carried out with a ^{3D}CE capillary electrophoresis equipment (Agilent Technologies, Waldbronn, Germany) equipped with a UV–vis diode array detector (DAD). Detection was performed at 214 nm. Column temperature was controlled by an air thermostating system. Prior to use, the capillary was conditioned for 20 min with 0.5 M HCl, 5 min with water, 30 min with 0.5 M NaOH and another 5 min with water. Additionally, the capillary was washed for 2 min with 0.5 M NaOH, 1 min with water and 2 min with the running buffer with positive pressure applied at the injection end before each run. Acquisition of electropherograms was computer-controlled by Chemstation software (Rev. A.09.01, Agilent Technologies). The analytes were injected at the anodic end by applying pressure (60 mbar × 5 s).

2.4 Extraction procedure

The hollow fiber was cut manually and carefully into 8.0 cm lengths. All of the hollow fiber segments were sonicated in acetone for 20 min to remove any possible contaminants and then dried at ambient temperature prior to use. Each fiber was used only once to avoid any possible memory effects. The hollow fiber was then dipped in 1-octanol for 15 s to impregnate the pores in the fiber walls with the organic solvents, and excess of solvent was removed with a medical wipe. After impregnation, the lumen of the hollow fiber was filled with 20 μL of the acceptor phase (pH 2.2) using a microsyringe insofar as the excess of acceptor phase was removed from its end and then the end of the hollow fiber was sealed using a pair of hot flat-tip pliers. A 4.0 ml aqueous sample solution (pH 12.5) containing 100 ng ml^{-1} of each enantiomer was poured into sample vial. The sample vial was placed on a magnetic stirrer with a stirring rate of 1000 rpm and the prepared hollow fiber with the microsyringe was immersed into the aqueous sample

solution. After extraction for a prescribed period of time (28 min), hollow fiber was taken out from the sample solution. The sealed end of the hollow fiber was cut with scissors, and the acceptor phase was collected with a microsyringe, and then transferred to a microinsert vial which was placed into the sample tray of a CE system for analyses.

2.5 Data analysis and statistical methods

In order to obtain optimal condition and investigate the interaction of variables, a BBD was employed. The experimental design matrix and data analysis were performed by the Statgraphics Plus Package, version 5.1.

2.6 Calculation of enrichment factor, extraction recovery and relative recovery

The enrichment factor (EF) was defined as the ratio of the final analyte concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte in the donor phase ($C_{i,d}$):

$$EF = \frac{C_{f,a}}{C_{i,d}} \quad (1)$$

Recovery (R) was calculated according to the following equation for each analyte:

$$R\% = \frac{n_{f,a}}{n_{i,d}} \times 100 = \left(\frac{V_a}{V_d}\right)\left(\frac{C_{f,a}}{C_{i,d}}\right) \times 100 \quad (2)$$

where $n_{i,d}$ and $n_{f,a}$ are the number of moles of analyte originally present in the donor phase and the number of moles of analyte finally collected in the acceptor phase, respectively. V_a is the volume of acceptor phase and V_d is the volume of donor phase.

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

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

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

concentration of known amount of standard which was spiked into the real sample, respectively.

3 Results and discussion

3.1 Separation of CIT enantiomers

MDs are complex malto-oligo and polysaccharide mixtures, which were obtained from starch by partial acid and/or enzymatic hydrolysis.[37,38] When used in CE, MDs were found to enable highly efficient chiral separations of a broad range of acidic and basic compounds.[37-39] Thus, in this work MD as a simple and inexpensive chiral selector was applied for enantioseparation of CIT enantiomers. Different interactions of chiral analytes with the helical structure of the MD emerge as the basis of the enantioselectivity. The change in conformation from a flexible coil to a helix in the presence of chiral analytes and buffer salts may play an important role in selective interactions. The helical structure of the MDs mimics the cavity responsible for chiral recognition by cyclodextrins.[40, 41] To obtain the optimum enantioseparation of the CIT, effective experimental parameters, such as DE of MD, pH of the back ground electrolyte (BGE), chiral selector concentration, capillary column temperature and applied voltage were optimized. The R-enantiomer of CIT was used for identifying migration order of enantiomers in MD mediated CE. Results showed that the R-enantiomer of CIT migrated faster than the S-enantiomer. In all optimization for enantioseparation, the concentration of each enantiomer was 10 mg L^{-1}.

MDs with different DE values (4-7, 13-17 and 16.5-19.5) were evaluated for their chiral selector activity. MD with DE value 4-7 separated the enantiomers with better resolution and longer migration times. Since MD is a neutral polysaccharide, hydrophobic and hydrogen bonds are the most important interactions responsible for separating the enantiomers and MDs with lower DE value and longer oligomeric chain have more sites to interact with the analyte molecules which can explain the observed results.[42] Thus, in the next experiments MD with DE 4–7 was used as chiral selector.

The BGE pH is one of the most important parameters in CE analysis because of its effect on the electroosmotic flow (EOF) and ionization state of the analyte. Different pH values in the range of 2.0-7.0 were investigated in order to obtain the optimum amount based on the enantio-resolution and rational migration time (Table 1). It was observed that Rs decreased with the increase of buffer solution pH in the range of 2.0 to 7.0. This may easily be explained by an increase in the EOF. Considering the migration time and peak shapes, a buffer solution of pH 5.0 was used in the following experiments.

The chiral selector concentration is a main experimental parameter and its optimization is usually critical for a successful enantiomer separation. The effects of MD concentration on the enantioseparation of CIT were investigated using 50 mM phosphate buffer solution (pH 5.0) over a concentration range of 1.0 to 20.0% w/v. The migration time of all the enantiomers increased with increasing concentration of MD. Increases in the drug-chiral selector complex adduct and in viscosity of the BGE were the main causes of the retarded migration for enantiomers. The resolution increased as MD concentration rose, but reached maximum at 15.0% MD addition. In order to obtain a rational resolution and migration time, 15% w/v was chosen as the optimum MD concentration.

The effect of applied voltage on the enantioseparation of CIT was investigated using 50 mM phosphate solution (pH 5.0, 15% (w/v) MD with DE 4–7) in the range of 12 to 22 kV. Considering the good resolution and fast migration time, a voltage of 18 kV was used in the following experiments.

We also investigate the effect of the temperature (ranged from 15 to 30°C) on the enantioresolution and sensitivity was systematically investigated. Based on the results, 20°C was chosen to carry out the experiments.

The optimum conditions for the chiral separation of CIT enantiomers are as follows: buffer solution, 50 mM phosphate (pH 5.0, 15.0% (w/v) MD with DE 4-7); applied voltage 18 kV and temperature of 20°C. Figure 1S (Supplementary information) shows the typical electropherogram in optimized CE conditions.

3.2 Preliminary experiments for development of LPME process

Initially, the influence of the composition of the SLM and ionic strength on extraction efficiency was separately evaluated by OVAT methodology. Then, the influence of the other factors (extraction time, pH of the acceptor and donor phases), were evaluated by BBD which is a kind of experimental design methodology. The reason of selection of these factors are described in Supplementary information. In all optimization experiments, the concentration of each enantiomer was 100 ng mL^{-1}.

3.2.1 Optimization of organic liquid membrane composition

An organic solvent suitable for the HF-LPME should have low solubility in water, good compatibility with polypropylene hollow membrane and high partition coefficient between the analytes and the solvent.[30] Based on the above considerations, five organic solvents including cyclohexane, toluene, hexadecan, 1-octanol and 1-octane were evaluated for extraction efficiencies. Other factors were maintained constant during the optimization (stirring rate of 1000 rpm, 15 min as extraction time, acceptor phase pH 3.0 and donor phase pH 11.0). Table 2 shows that among different solvents examined, 1-octanol has the best extraction efficiency. Moreover, 1-octanol demonstrated low solvent loss, and was immobilized in the fiber pores within seconds. Therefore, 1-octanol was chosen as the extracting solvent in this investigation.

3.2.2 Salt effect

It was reported that the addition of salt reduces the solubility of the analytes in the donor phase and enhance their partitioning into the organic phase (salting out effect). In the present work, various amounts of NaCl (0–15%, w/v) were added to the sample solution. The obtained results showed that the best extraction efficiency was achieved when no NaCl was added. This is probably due to the increase of the viscosity of the bulk solution (donor phase) which decreases the diffusion rate and tends to restrict the movement of the analyte from the bulk solution to the organic phase. Therefore, further experiments were performed without the addition of salt.

3.3. Optimization by BBD

The experiments were performed according to BBD which is a kind of response surface methodology RSM. It is well known that RSM is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response.[43, 44]

Thus, in this step, a BBD was applied for the optimization of three factors (extraction time, pH of the acceptor and donor phases) and investigate the interaction among these variables.

The total number of experiments (N) was calculated as follows:

$$N = 2k\,(k-1) + Cp \qquad (5)$$

where k is the number of variables and C_p is the number of centre points.[45] Thus, experimental design consists of 16 experiments with four center points (in order to allow the estimation of pure error) and allows calculations of the response function at intermediate levels and enables estimation of the system performance at any experimental point within the studied range. The examined levels of these factors are given in Table 3.

The Analysis of variance (ANOVA) results produced the Pareto chart of the main effects and their interactions which were shown in Fig. 2. Since the obtained results showed that there was

no discrimination between extractions of two enantiomers, only one chart was chosen as a representative example of the enantiomers. According to this figure, three factors and the interaction between some of them showed statistically significant effects at the $p < 0.05$ level. Also, this figure shows pH of the acceptor phase has the large influence on the peak area and a negative effect upon the extraction. The pH of the donor phase and the extraction time showed a positive and significant effect on the extraction efficiency. In fact, as other microextraction techniques, HF-LPME is a type of equilibrium extraction. The analyte is partitioned between the source and receiving phases until the equilibrium is established. Hence, the positive effect of extraction time is corresponding to the equilibrium, which is established at longer time. Since CIT is a weak basic compound (pK_a = 9.58), the source phase should be sufficiently basic to keep the analyte in its neutral form and reduce its solubility in the source phase. On the other hand, pH of the acceptor phase should be selected to ensure full ionization of the analytes to ensure that the ionized analyte is prevented from being back extracted into the organic phase. According to the overall results of the optimization study, the following experimental conditions were chosen: pH of the acceptor phase, 2.2; pH of the donor phase, 12.5; and extraction time, 28 min.

Also, RSM was applied to analyse the effect of independent variables on the response (Fig. 3). This figure also depicts 2D contour plot on the basis of the model equations, which displays the interaction between independent variables and assists in determining the optimum operating condition for the desirable responses. The peak area for CIT increased along with increasing the HCl concentration of the acceptor phase and time of extraction, but they were not affected with increasing pH of the donor phase (Fig. 3).

3.4 Method validation

Under optimum conditions, limit of detection (LOD), regression equation, correlation of determination (r^2), dynamic linear range (DLR), preconcentration factor (PF), and extraction recovery (R%) were obtained. The LOQ (S/N = 10) was estimated to be 30 ng mL^{-1} for both enantiomers, while the limit of detection (S/N = 3) was 10 ng mL^{-1} for both enantiomers (Table 4). Repeatability or intra day precision was investigated by injecting five replicate of a standard solution (100 ng mL^{-1}) and inter day precision were assessed by injecting the same sample over five consecutive days. Intra and inter day precision extractions varied between 3.6 and 6.9% for both enantiomers (Table 4). In order to calculate the enrichment factor of each enantiomer, five replicate extractions were performed at optimal conditions from aqueous solution containing 100 ng mL^{-1} of each enantiomer. The enrichment factor was calculated as the ratio of the final concentration of the analyte in the acceptor phase and its concentration in the original solution. The very good enrichment factors were 142 for both enantiomers.

3.5 Extraction of CIT enantiomers from urine samples

Due to the importance of analysis of drugs in biological samples, the proposed method was applied to the determination of CIT enantiomers in urine samples. The urine samples were diluted at 1:3 ratios using HPLC grade water, and their pH values were adjusted at 12.5. Then, 4.0 mL of each solution was transferred into the sample vial and LPME process was applied. The experimental results revealed that no drugs were found in urine samples. Validation results were shown in Table 5. Figure 4 represents the electropherograms of the nonspiked (blank) urine 1 sample and urine 1 sample spiked with 100 ng mL^{-1} of each enantiomer. These electropherograms reveal a good cleanup of the enantiomers as no interfering peak is observed. The method was compared with the other previous works (Table 6). This technique demonstrated several advantages compared to the other extraction methods. As compared to the SPE and stir

bar sorptive extraction (SBSE), the consumption of organic solvents in this technique is minimal. Also, HF-LPME eliminate possible carry-over problems because of the hollow fiber not expensive and can be discarded after each extraction. This serves to maintain high reproducibility and repeatability. In addition, the small pore size prevents large molecules in the matrix and insoluble particles in the donor phase to enter the acceptor phase. Thus, HF-LPME was more suitable for the analysis of more complex matrix samples like human plasma. Although, LC–MS–MS can create high sensitivity but this instrument is very expensive and not accessible as a routine instrument. Therefore, CE–UV coupling with HF-LPME can provide good and sensitive results for determination of these enantiomers in urine samples.

4. Concluding remarks

In the present work, HF-LPME was combined with MD modified CE for the enantioseparation of CIT in urine samples. The method described here represents a very simple and efficient analytical method for the preconcentration and enantioseparation of CIT enantiomers. The cost and time of the sample preparation step as well as consumption of toxic organic solvents were minimized without affecting the sensitivity of the method. The developed method had high enrichment factors and low detection limits for CIT enantiomers. The extraction unit (hollow fiber) was a disposable device utilized only for a single extraction. This was a major advantage, because memory effect, carry over effects and cross-contamination were totally eliminated. Finally, it is concluded that this method has acceptable precision and is a highly sensitive technique for the separation, preconcentration and determination of trace amounts of CIT enantiomers in real urine samples. Therefore, proposed method could be suitable for routine use and pharmacokinetic studies.

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Figure Legends:

Figure 1. Chemical structure of CIT. The asterisk denotes the chiral centre.

Figure 2. Pareto chart of the main effects in the BBD.

Figure 3. (A) RSM and (B) contour plot obtained by plotting of pH of the acceptor phase versus the pH of the donor phase, (C) RSM and (D) contour plot obtained by plotting of pH of the acceptor phase versus the extraction time.

Figure 4. Electropherograms obtained after LPME from nonspiked urine 1 sample and urine 1 sample spiked with 100 ng mL^{-1} of each enantiomer. LPME conditions: 1-octanol as SLM, 28 min as extraction time, pH of the acceptor phase 2.2 and pH of the donor phase 12.5. CE conditions: applied voltage: 18 kV; temperature: 20 ^{0}C; injection: 60 mbar 5 s; separation solution: 50 mM phosphate buffer pH 5.0 containing 15.0% (w/v) MD with DE 4-7 as chiral selector.

Table 1

The effects buffer pH on the migration time (min) and resolution of CIT enantiomers.

pH	t_R	t_S	Rs
2	22.12	24.01	8.45
3	14.87	15.90	7.31
4	8.30	8.91	5.05
5	5.92	6.45	4.48
6	4.50	4.78	2.1
7	3.12	3.20	1.2

[a] t_R and t_S show migration times of R-CIT and S-CIT, respectively.

Table 2

Extraction recovery of CIT enantiomers with different organic solvents in the SLM.

Parameter	Enantiomer	Organic solvent			
		1-octanol	hexadecan	toluene	1-octane
Recovery % (EF)[a]	R-CIT	54 (108)	14 (28)	12 (24)	42 (84)
	S-CIT	53 (106)	14 (28)	11 (22)	42 (84)

[a] The number of replicates (n = 3).

Table 3

The experimental variables and levels of the BBD.

Variable	Key	Level		
		Lower	Central	Upper
pH of acceptor phase	A	2	4	6
Extraction time (min)	B	5	17.5	35
pH of donor phase	C	11	12	13

Table 4

Figures of merit of LPME in drug-free water and urine samples.

Sample	Analyte	R^2	LOQ[a]	LOD[a]	Linearity[a]	EF	Recovery[b]	RSD%[c]	
								Intra day	Inter day
Water	R-CIT	0.9988	30	10	30-300	142	71	3.6	4.2
	S-CIT	0.9988	30	10	30-300	142	71	3.6	4.1
Urine	R-CIT	0.9978	60	20	60-300	118	59	5.9	6.7
	S-CIT	0.9975	60	20	60-300	116	58	5.8	6.9

[a] Concentration is based on ng mL^{-1}.

[b] Recovery was obtained for 100 ng mL^{-1} of each enantiomer (n = 5).

[c] Intra day and inter day RSDs% were obtained by five replicate measurements for 100 ng mL^{-1} of each enantiomer.

Table 5
Determination of CIT enantiomers in real urine samples.

Sample		R-CIT (ng mL^{-1})	S-CIT (ng mL^{-1})
Urine 1	Initial concentration (ng mL^{-1})	n.d[b]	n.d[b]
	RR%[a]	97	97
	RSD% (n=3)	4.3	4.2
Urine 2	Initial concentration (ng mL^{-1})	n.d[b]	n.d[b]
	RR%[a]	92	91
	RSD% (n=3)	5.7	5.8
Urine 3	Initial concentration (ng mL^{-1})	n.d[b]	n.d[b]
	RR%[a]	89	89
	RSD% (n=3)	5.9	6.1
Urine 4	Initial concentration (ng mL^{-1})	n.d[b]	n.d[b]
	RR%[a]	95	94
	RSD% (n=3)	4.5	4.4

[a] 100 ng mL^{-1} of each CIT enantiomer was added to calculate relative recovery percent (RR%).
[b] n.d, not detected.

Table 6

Comparison of analytical performance data of proposed method with other methods applied for the analysis of CIT.

Method	Analyte	Sample preparation	Sample type	Organic solvent volume (µL)	LOD[a]	Linear range[a]	RSD%	Ref.
HPLC	CIT	SPME	Urine	-	10	50-200	10	[24]
HPLC	CIT	SME/BE	Plasma	100	0.3	1-130	10.1	[11]
HPLC	R-CIT S-CIT	SPE	Plasma	5000	10	100-500	10	[10]
LC/MS/MS	R-CIT S-CIT	SBSE	Urine	3000	2	-	12	[8]
CE-UV	R-CIT S-CIT	HF-LPME	Urine	10	20 20	60-300 60-300	5.9 5.8	Proposed method

[a] All concentrations are based on ng mL^{-1}.

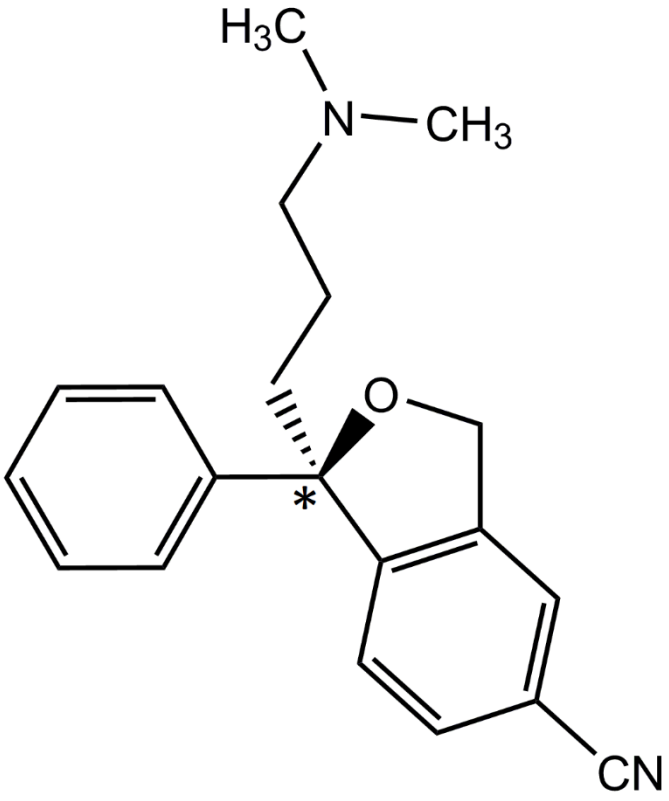

Figure 1

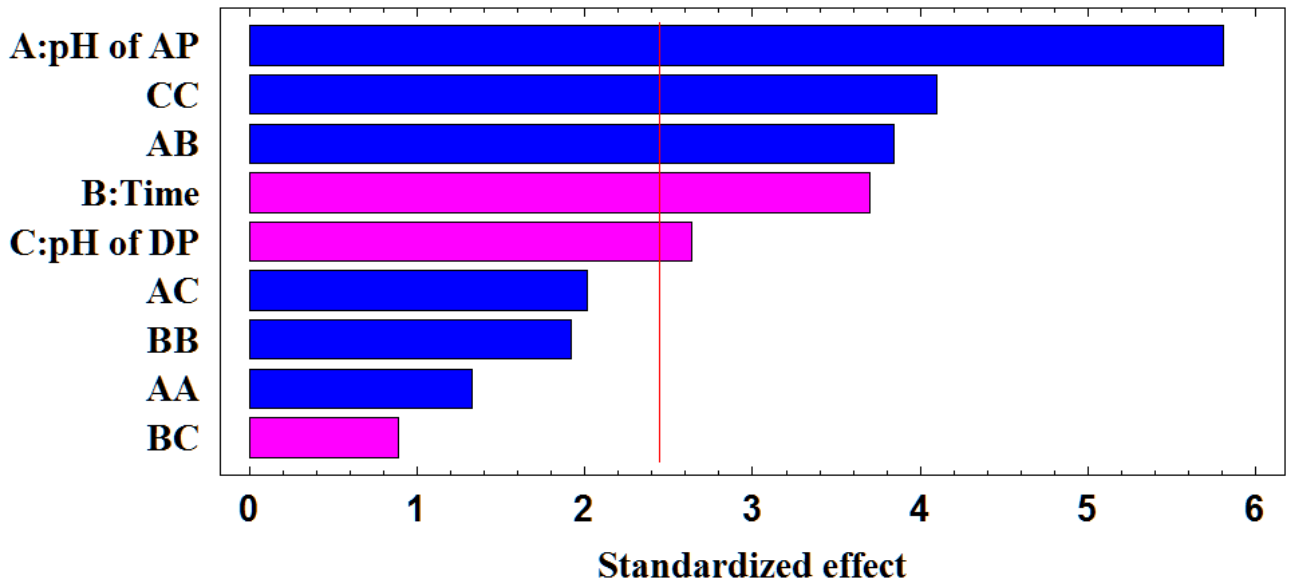

Figure 2

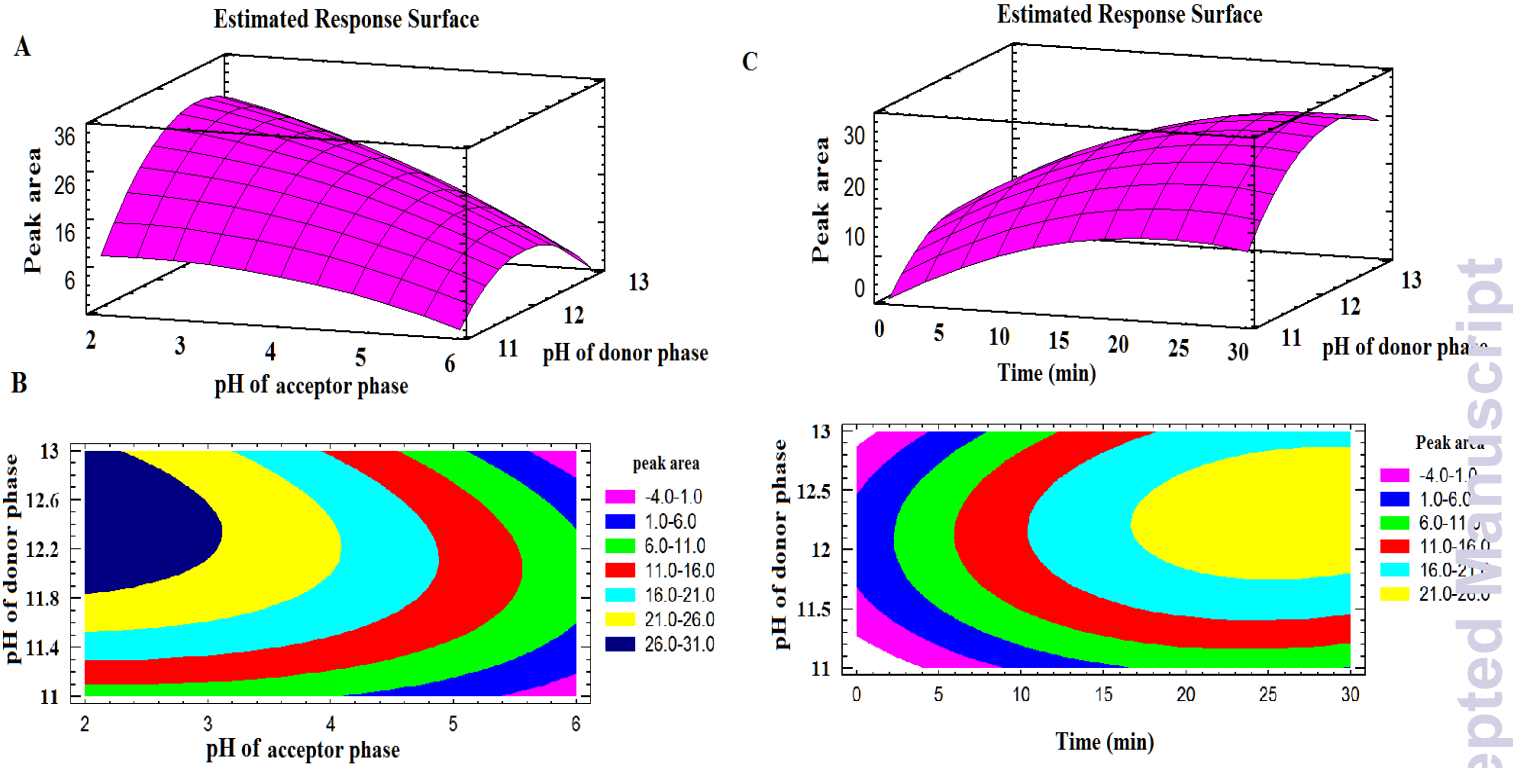

Figure 3

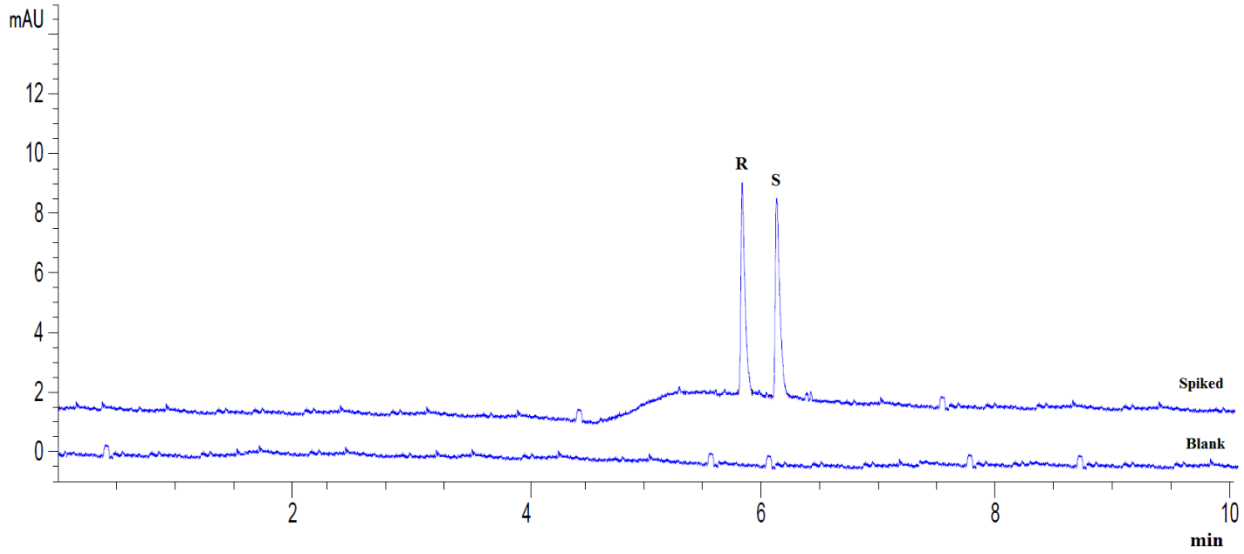

Figure 4