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RESEARCH ARTICLE

Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction and Capillary Electrophoretic Determination of Tramadol in Human Plasma

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Abstract: ***Background*:** Tramadol, (±)-trans-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol, is a synthetic centrally acting analgesic used in the treatment of moderate to chronic pain. Tramadol, like other narcotic drugs, is used for the treatment of pain and may also be abused. Its overdose can cause adverse effects such as dizziness, vomiting, and nausea. The aim of this paper is to develop a sample preparation method for the determination of tramadol in human plasma samples, followed by CE analysis.

***Methods*:** Ultrasound assisted-dispersive liquid-liquid microextraction using binary mixed extractant solvent (chloroform and ethyl acetate) was used for the extraction of one hundred microliters of tramadol spiked human plasma samples and in real human plasma samples obtained from the patients with abuse of tramadol. After evaporation of the extractant solvent, the residue was reconstituted in 100 µL deionized water and subsequently analyzed by CE-UV.

***Results*:** The developed method has remarkable characteristics, including simplicity, good repeatability and appreciable accuracy. Under the best extraction conditions, a low limit of detection at 7.0 µg per liter level with good linearity in the range of 0.02-10 µg mL^{-1} was obtained.

***Conclusion*:** UA-DLLME, using a binary mixed extraction solvent, was established for the determination of tramadol in human plasma samples *via* the CE method with UV-detection. In addition, the analysis of tramadol in some plasma samples of patients with abuse of tramadol indicated that the method has acceptable performance for the determination of tramadol in plasma samples, which indicates that the method is suitable for clinical applications.

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1. INTRODUCTION

Tramadol, (±)-trans-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol, is a synthetic centrally acting analgesic used in the treatment of moderate to chronic pain. Analgesic effect of tramadol is due to its binding to opioid receptors in the central nervous system and inhibition of the reuptake of norepinephrine and serotonin [1]. Tramadol is quickly and almost completely absorbed after oral administration, but its absolute bioavailability is only 65-70% because of first-pass metabolism [2]. The non-metabolized drug, which is approximately 10-30% of the parent drug, is excreted in the urine. The therapeutic plasma concentration of tramadol is in the range of 0.1-1 µg mL^{-1} and its comatose lethal concentration is > 2 µg mL^{-1} [3]. Tramadol like other narcotic drugs is used for the treatment of pain and also may be abused. Its overdose can cause adverse effects such as dizziness, vomiting, and nausea [4]. Therefore, a sensitive and efficient analytical method is necessary for the determination of tramadol in biological samples in clinical contexts and diagnostic research.

The analytical methods used for the determination of tramadol in biological samples involve spectrophotometry [5], electrochemical techniques [6, 7] and several chromatographic techniques such as high-performance liquid chroma-

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tographic (HPLC) coupled to ultraviolet [8], fluorescence [9, 10], and tandem mass spectrometry (MS/MS) [11-13] detection, gas chromatography (GC) in combination with flame ionization detection [14] or mass spectrometry [15, 16], and capillary electrophoresis (CE) technique [17-19]. Although spectrophotometric and electrochemical techniques are rapid and simple, their sensitivity and selectivity are questionable. HPLC and GC techniques exhibit good sensitivity and specificity, but GC often needs pre-column derivatizations of the analytes to improve their GC characteristics and in the case of HPLC, the use of large amounts of organic solvents is inevitable [20]. CE offers many advantages, including good resolution, low consumption of samples and reagents, small sample volume, and automation [21], which have provided a remarkable impetus to the use of this technique for analysis of tramadol. However, sample matrix affects CE results and analysis of biological samples are not feasible without any pretreatment step. Thus, sample preparation is an essential step to clean up and eliminate interfering compounds found in the matrices.

Various procedures such as liquid-liquid extraction (LLE) [22], solid-phase extraction (SPE) [1, 23], solid phase microextraction (SPME) [24, 25] and dispersive liquid-liquid microextraction (DLLME) [26-28] have been developed for the extraction of tramadol from biological matrices. LLE and SPE are time-consuming and laborious and require large volumes of organic solvents. SPME consumes no extraction solvent, but it is associated with other disadvantages such as limited lifetime and fragility of the fiber, cost and sample carry-over [29]. Although among liquid phase microextraction techniques, DLLME uses larger volumes of solvents, it has emerged as a powerful sample preparation with simple, rapid and low-cost characteristics. However, the original configuration of DLLME, termed as classical DLLME, suffers from some drawbacks and limitations that are in continuous revision, namely: (i) organic solvents denser than water are employed as extractants which limit finding a suitable extraction solvent, (ii) emulsification requires a dispersant solvent which competes with the extractant for the analyte, thus reducing enrichment factor, and (iii) centrifugation is necessary to separate phases after microextraction.

In order to overcome the limitation of finding an efficient extraction solvent, a binary mixed solvent has been used as the extractant instead of a single-component solvent [30]. In this method, two kinds of extraction solvents (a mixture of low and high density solvents) can be used simultaneously, whereas in the conventional DLLME, the choice of extraction solvent is limited to the solvents which are heavier than water such as chlorinated solvents [27, 31]. Furthermore, the use of ultrasonic radiation is another improvement in DLLME. Ultrasonic radiation is known to be a powerful aid accelerating the formation of a fine cloudy dispersive mixture, which significantly increases the extraction efficiency and reduces the equilibrium time [32].

The aim of this paper is to develop a sample preparation method for the determination of tramadol in human plasma samples, followed by CE analysis. Thus, special attention was paid to the careful evaluation and selection of DLLME parameters. The proposed ultrasound assisted-dispersive liquid-liquid microextraction (UA-DLLME) with a binary mixed extractant was applied for the determination of tramadol in plasma samples of patients with abuse of tramadol. To the best of our knowledge, there is no report for tramadol extraction from plasma samples prior to CE analysis and this paper is the first report of the application of DLLME for the preparation of plasma samples in tramadol determination using CE-UV detection.

2. EXPERIMENTAL

2.1. Chemicals & Solutions

Tramadol was obtained from Temad Co. (Tehran, Iran). Acetonitrile (ACN), acetone, methanol, chloroform ($CHCl_3$), sodium tetraborate decahydrate ($Na_2B_4O_7.10\ H_2O$) and boric acid (H_3BO_3) were purchased from Scharlau (Barcelona, Spain). Carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2), ethanol, ortho-phosphoric acid, sodium chloride, sodium hydroxide and tris (hydroxymethyl) aminomethane (Tris) were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade and used as received. Deionized water (Shahid Ghazi Pharmaceutical Co., Tabriz, Iran) was used throughout the experiment.

The stock solution of 100 μg mL^{-1} tramadol was prepared in deionized water and stored in a refrigerator at 4°C. Working solutions for tramadol were prepared by serial dilutions of stock solution with plasma and subjected to UA-DLLME procedure.

2.2. Instruments

CE experiments were carried out using an Agilent 7100 CE system (Waldbronn, Germany) equipped with a DAD detector. Data analysis and instrumental control were performed using Agilent Chemstation software (Waldbronn, Germany). An uncoated fused-silica capillary with an effective length of 41.5 cm and 50 μm I.D (50 cm total length; Agilent Technology) was used for separation. A vortex from Labtron Company (Tehran, Iran), Hettich centrifuge (Tuttlingen, Germany) and Alex machine ultrasonic bath model AXUY-01D (Istanbul, Turkey) operated at 32 kHz were used throughout the extraction procedure. pH measurements were performed with a Metrohm pH-meter (model 774). All weighing was done by an electronic analytical balance model AB204-S (Mettler Toledo Company, Switzerland).

2.3. Electrophoretic Procedure

The new capillary was preconditioned by sequentially flushing with 1.0 mol L^{-1} NaOH (30 min), deionized water (30 min) and background electrolyte (BGE) (30 min). Between runs, the capillary was flushed successively with 0.1 mol L^{-1} NaOH for 1 min, then deionized water for 1 min, and conditioned with BGE for 2 min. A 50 mmol L^{-1} borate buffer adjusted to pH 10.6 was used as BGE. Sample solutions and BGE were stored in a refrigerator at 4°C and filtered through a 0.20-μm pore size PTFE filter (Chromafil, Germany). Samples were electrokinetically injected at 15 kV for 30 s. Electrophoretic separation was performed under 15 kV and

the temperature was 20°C. Detection was carried out at 215 nm and peak area was used for quantitative calculations.

2.4. Plasma Samples

Drug-free human plasma was supplied by the Iranian Blood Transfusion Research Center (Tabriz, Iran) and frozen at -20°C until analysis. Real plasma samples were obtained from patients with abuse of tramadol who referred to Sina Hospital (Tabriz, IRAN), and stored at -20°C. Written informed consents approved by the Ethics Committee of Tabriz University of Medical Sciences were obtained from all sample donors.

2.5. UA-DLLME Procedure

One hundred microliters of the tramadol spiked plasma sample was placed in 2.5 mL polypropylene microtube and then 100 µL of 0.1 mol L^{-1} NaOH solution and 300 µL of acetonitrile (as plasma protein precipitating agent and disperser solvent) were added. Next, the mixture was vortexed for 30 s and centrifuged at 4,000 rpm for 10 min. The clear supernatant was transferred into the glass conical-bottom test tube and mixed with 5 mL of an aqueous solution containing 2% (w/v) of NaCl. Afterward, a mixed solution of 200 µL ethyl acetate and 150 µL chloroform as extraction solvents was rapidly injected into the above solution using a syringe. The test tube was immersed in an ultrasonic water bath for 10 min. After centrifugation at 5,000 rpm for 10 min, the sedimented organic phase in the bottom of the conical tube was transferred to a microtube and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 100 µL deionized water and subsequently analyzed by CE-UV. Real plasma samples were also subjected to this extraction procedure.

3. RESULTS AND DISCUSSION

3.1. Optimization of CE-UV System

In order to achieve the highest sensitivity for the determination of tramadol, some variables affecting the analytical signal, including composition, concentration, and pH of BGE, separation temperature and voltage for a 2 µg mL^{-1} tramadol solution were studied using a one-factor-at-a-time approach. Three buffer systems (Tris, phosphate and borate) were tested as the running buffers. It was found that the signal of tramadol in borate buffer was more sensitive and repeatable than those obtained in other buffers. CE separation is also influenced by the pH of the running buffer. Hence, the effect of the pH was investigated in the range between 5 and 11. The results indicated that the highest analytical signal and the best peak shape were achieved at pH 10.6. It is worth noting that in the pH ranges, more than 11 and less than 5, tramadol peak broadens and overlaps with other peaks, so its determination is not possible. Moreover, buffer concentration was tested in the range of 10-100 mmol L^{-1}. As shown in Fig. (**1A**), the signal intensity of tramadol increases with increasing buffer concentration from 10 to 50 mmol L^{-1} and then decreases with a further increase of buffer concentration. It should be noted that by increasing buffer ionic strength, baseline noise was observed and peak broadening occurred because of the Joule heating.

CE analyses are deeply affected by temperature due to the dependence of buffer viscosity, resistance, and dielectric constant on temperature [17]. The effect of cassette temperature was investigated in the range of 5-30°C. By the increase of temperature, the electrophoretic mobility increases and migration time decreases. It is found that the best signal and migration time were achieved at 20°C. The effect of the applied voltage was also evaluated in the ranges of 10-25 kV. On the basis of the results, it can be concluded that 15 kV provides the highest signal intensity and it was chosen as an optimized value (Fig. **1B**). At higher voltages, the Joule heating *caused by* the current flow through the buffer leads to decreased signals.

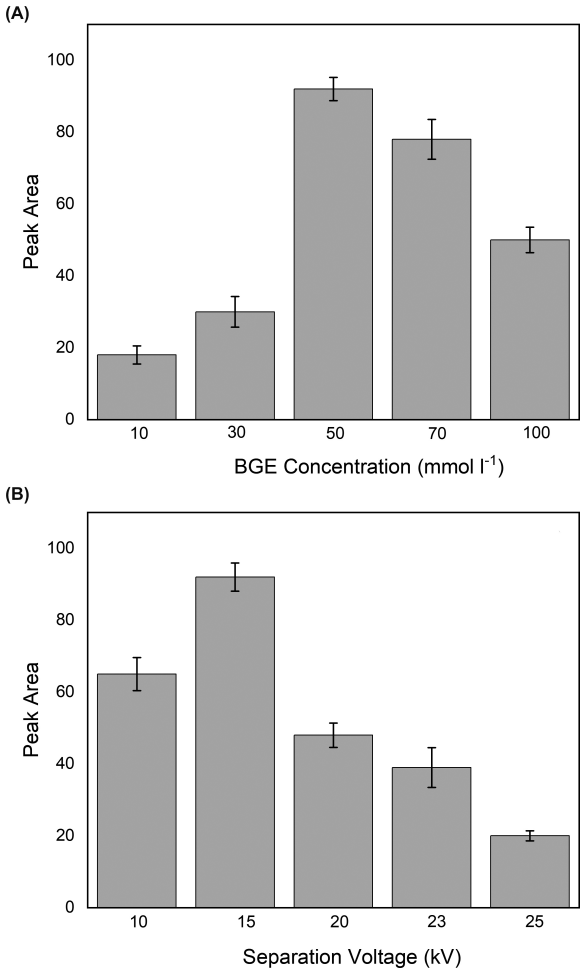

Fig. (1). The effect of (**A**) BGE concentration and (**B**) separation voltage on CE response of 2 µg mL^{-1} tramadol. Experimental conditions: capillary, 50 cm (41.5 cm effective length); UV detection at 215 nm; temperature, 20°C; separation voltage, 15 kV for A; BGE, borate buffer (pH 2.2); BGE concentration, 50 mmol L^{-1} for B, electrokinetic injection at 15 kV for 30 s. The error bars indicate the SD (n = 3).

3.2. Optimization of DLLME Parameters

To obtain the maximum extraction efficiency, the effect of different parameters such as type and volume of high den-

sity extraction solvent, the volume of ethyl acetate as low density extraction solvent, the type and volume of disperser solvent and ionic strength was investigated by varying a parameter while the others were kept constant. A 2 μg mL^{-1} tramadol spiked plasma sample was used in the optimization procedure and all experiments were repeated three times.

3.2.1. Effect of Type and Volume of High Density Extraction Solvent

As the extractant solvent of the DLLME, the selected organic solvent should be of high density, low water solubility, and should have the high extraction capability of the target analyte. Furthermore, because of the incompatibility of most solvents with capillary columns (such as halogenated solvents), the extraction solvent should have high volatility for easy removal after extraction [33]. Several solvents with a higher density than water, such as chloroform, carbon tetrachloride and dichloromethane, were investigated in the extraction procedure. According to the results, the best performance was achieved by using chloroform as an extraction solvent; hence chloroform was used in the subsequent experiments.

The extraction solvent volume has an important effect on the extraction efficiency and is usually studied in order to reduce the final organic phase volume and consequently obtaining a high concentration of the analyte. To test the effect of chloroform volume on performance of the presented extraction procedure, different volumes of chloroform varying from 70 to 200 μL were subjected to the DLLME procedure. As shown in Fig. (**2**), the extraction recovery increased with increasing chloroform volume up to 150 μL. Hence, 150 μL was used in the following experiments.

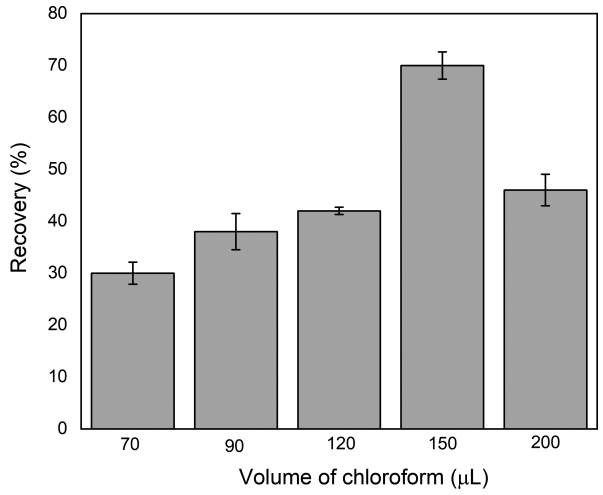

Fig. (2). Effect of the volume of chloroform on the extraction efficiency. Conditions: concentration of tramadol: 2 μg mL^{-1}, Ethyl acetate: 100 μL, disperser solvent: 300 μL acetonitrile. The error bars indicate the SD (n = 3).

3.2.2. Effect of Ethyl Acetate and its Volume

Since chloroform alone did not provide satisfactory results in extracting tramadol (recovery < 40% using 150 μL chloroform), in order to improve extraction efficiency, ethyl acetate was used as a lighter solvent to form a binary mixed extractant with chloroform. Binary solvents should form a miscible mixture with high density to be collected at the bottom of the conical bottom test tubes after centrifuging. To evaluate the effect of the ethyl acetate volume, different volumes of ethyl acetate in the range of 30-250 μL were mixed with 150 μL of chloroform and used in the extraction procedure. The results indicated that the recovery of the analyte increased markedly with the increasing volume of the ethyl acetate. However, when the volume of the ethyl acetate reached 200 μL and higher, the recoveries remained constant. Therefore, 200 μL of ethyl acetate was chosen.

3.2.3. Effect of Type and Volume of the Disperser Solvent

The nature and volume of the solvent that can be used as a disperser are important to achieve good extraction efficiency. Dispersive solvents should be miscible solvents with both aqueous samples and extraction solvents and could disperse the extraction solvents to fine droplets in the aqueous phase to help the target analyte transfer from the sample matrix into the organic phase. The solvents commonly used to precipitate plasma proteins respond to these demands. Thus, they can be applied as a disperser solvent and precipitating agent simultaneously. In this trend, the consumption of organic solvents can be reduced as much as possible. Methanol, ethanol, acetonitrile and acetone were studied as a dispersive solvent. For this purpose, 300 μL of this solvent was added to 200 μL of the alkaline spiked plasma samples. After the protein precipitating step, the obtained clear supernatant solution was used as a disperser in the next steps. Among the investigated solvent, acetonitrile gave good extraction efficiency. Therefore, acetonitrile was applied in the extraction procedure. The effect of the volume of acetonitrile was also investigated (Fig. **3**). It is found that the precipitation of plasma proteins does not take place completely when the volume of acetonitrile is less than 300 μL; however, higher volumes of acetonitrile decrease the extraction efficiency. Hence, 300 μL of acetonitrile was used in this work.

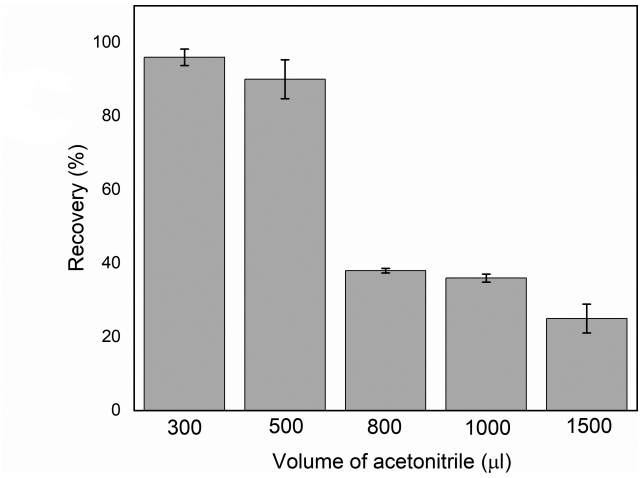

Fig. (3). Effect of the volume of the disperser solvent on the extraction efficiency. Extraction conditions: concentration of tramadol: 2 μg mL^{-1}, binary extraction solvents: 150 μL chloroform and 200 μL ethyl acetate, salt amount: 2% (w/v) NaCl. The error bars indicate the SD (n = 3).

Table 1. Accuracy and precision of the proposed method for the determination of tramadol in plasma samples (n=3).

Nominal Concentration (μg mL^{-1})	Found Concentration (μg mL^{-1})	Recovery (%)	RSD (%)
0.5	0.44 ± 0.005	88 ± 1.0	2.8
2	2.1 ± 0.25	105 ± 12.6	5.7
7	7.2 ± 0.52	103 ± 7.4	1.0

3.2.4. Effect of Salt Addition

Ionic strength is an effective parameter in the DLLME procedure, which was evaluated by adding sodium chloride. To study the effect of ionic strength on extraction performance, different amounts of NaCl in a range of 0-10% (w/v) were added to the sample solution. The results indicated that without the addition of NaCl, after centrifugation, the volume of the sedimented organic phase at the bottom of the conical tube was very little so that it was not collected reproducibly. The volume of sediment phase increases along with the increasing salt concentration, which was because of the decreased solubility of the extraction and disperser solvents in the aqueous phase. However, a high concentration of NaCl leads to decrease extraction efficiency due to the reduction of the diffusion rate of the analyte into the extracting solvent. In the case of 2% NaCl, a good result in terms of extraction efficiency and the volume of the sediment phase was observed. Thus, 2% of NaCl was used in subsequent experiments.

3.2.5. Effect of Stirring Mode

The formation of the cloudy solution in DLLME can be accelerated by different stirring modes, including shaking, vortex and ultrasound assisted. Although shaking is commonly used for this purpose, dispersion of a small volume of extractant into the aqueous phase takes place hardly in this case. Previous literature demonstrated that shaking and vortex do not lead to the formation of stable and reproducible cloudy solution [32, 34]. In this work, the effect of shaking, vortex and ultrasonic radiation as stirring mode was also investigated. It was found that cloudy solution formation occurs uniformly and reproducibly using an ultrasonic bath. Accordingly, the ultrasonic process was applied in the DLLME procedure.

3.3. Analytical Figures of Merit

To conduct quantitative analysis and to evaluate the method on the aspects of the linear range and the detection limit, the calibration curve was constructed using tramadol spiked plasma samples. The average of three calibration curves constructed on three days was used for linearity investigations. Two calibration graphs for the quantification of tramadol were obtained in the range of 0.02-10 μg mL^{-1}; a calibration graph with the equation; A= 232.71C + 0.7131 for tramadol concentration in the range of 0.02 to 0.2 μg mL^{-1} and a calibration graph with the equation; A = 16.47C + 46.55 for tramadol concentration in the range of 0.2 to 10 μg mL^{-1} where A and C correspond to the peak area and concentration of tramadol in μg mL^{-1}, respectively. A steeper line with a larger slope indicates a more sensitive measurement. The sensitivity indicates the response of the instrument to changes in analyte concentration or a measure of a method's ability to distinguish between small differences in concentration at different samples. The less steep calibration graph indicates decreasing sensitivity at higher concentrations, which may be due to declining extraction efficiency. The limit of detection (signal/noise=3) and limit of quantification (signal/noise=10) of the proposed method according to the lower linear range (0.02-0.2 μg mL^{-1}) of the method were found to be 0.007 μg mL^{-1} and 0.02 μg mL^{-1}, respectively. The preconcentration factor of the represented method is 1.0 since the initial amount of the plasma sample and the final amount of reconstituted solvent are 100 μL.

Accuracy and precision of the proposed method were also evaluated. For this purpose, plasma samples were spiked with three different concentrations of tramadol and subjected to the proposed method. The recoveries and relative standard deviation (RSD) values were obtained by performing three replicate measurements. As shown in Table **1**, the recoveries were in the range of 88-105%, indicating the suitability of the developed method for the analysis of tramadol in plasma samples (Table **2**). RSD values of all experiments were less than 5.7%. These results demonstrate that the developed method is both accurate and precise. Table **3** compares the main analytical characteristics (*i.e*., linear range, LOD and electrophoretic conditions) of the represented method for the determination of tramadol with some previously reported methods.

3.4. Real Sample Analysis

To evaluate the practical applicability of the developed method, it was used for the determination of tramadol in real human plasma samples of the patients with abuse of tramadol. It is worth noting that these patients also receive other drugs. Details of the patients and the level of tramadol in these samples are given in Table **2**. The correct calibration graph must be chosen based on the magnitude of the signal. The amount of tramadol in all the patient samples in Table **2** was determined based on the calibration graph with a larger linear range (0.2-10 μg ml^{-1}). Fig. (**4**) displays the electropherograms of tramadol-spiked plasma (a) and plasma sample of patient number 1 (c) under optimal UA-DLLME-CE. These results demonstrate that the developed method has acceptable performance for detecting tramadol in real plasma samples, which indicates that the method is suitable for clinical applications.

Table 2. Determination of tramadol in real plasma samples by the proposed method (n=3).

No.	Gender	Age (yr)	Other used Drugs (Administered)	Duration between Intake and Sampling Time (h)	Concentration (μg mL^{-1})
1	M	35	-	3.5	1.37 ± 0.12
2	M	38	Clonazepam, Nortriptyline, Alcohol	-	<LOQ
3	F	41	Oral contraceptive pill	3	<LOQ
4	F	60	Enalapril, Propranolol	5	<LOQ
5	M	27	Clonazepam	3	0.73 ± 0.08
6	M	43	Clonazepam, Citalopram	5.5	0.25 ± 0.03

Table 3. An overview of CE techniques for the determination of tramadol.

Extraction Method	Linear Range (mg/L)	LOD (mg/L)	Sample	Electrophoretic Conditions	Refs.
SPE- C18 cartridge	0.245-49.7	0.245	Urine	Capillary, fused-silica 57 cm (50 cm effective length) ×50 μm I.D.; separation buffer, 50 mM borate buffer, pH 10.35; detection, 214 nm; applied voltage, 20 kV; temperature, 20°C; detection: 195 nm injection, hydrodynamically with 0.5 psi for 10 s.	[35]
-	-	2.0	Plasma	Capillary, fused-silica 60 cm (47 cm effective length) ×50 μm I.D; separation buffer, 100 mM phosphate buffer, pH 8 containing 20% $w \cdot v^{-1}$ maltodextrin; detection, 214 nm; applied voltage, 16 kV; temperature, 25°C; injection, hydrodynamically at 65 mbar for 10 s.	[17]
LLE	0.5-20	0.1	Urine	Capillary, fused-silica polyacrylamide coated 40.5 cm (36.0 cm effective length) ×50 μm I.D.; separation buffer, 50 mM phosphate buffer, pH 2.5 containing 5 mM of carboxymethylated b-cyclodextrin; detection: 195 nm; applied voltage: 20 kV; Temperature: 25°C; injection, hydrodynamically with 5 psi for 1s.	[36]
-	5-100	1.5	Tablets	Capillary: fused-silica 60 cm (50 cm effective length) × 75 μm I.D; separation buffer, 50 mM borate buffer, pH 10.2 containing 10% $w \cdot v^{-1}$ maltodextrin; detection: 214 nm; applied voltage: 20 kV; temperature: 20°C; injection: hydrodynamically at 30 mbar for 5 s.	[37]
UA-DLLME	0.02-10	0.007	Plasma	Capillary: fused-silica 50 cm (41.5 cm effective length) × 50 μm I.D; separation buffer, 50 mM borate buffer, pH 10.6; detection: 215 nm; applied voltage: 15 kV; temperature: 20°C; injection: electrokinetically at 15 kV for 30 s	This work

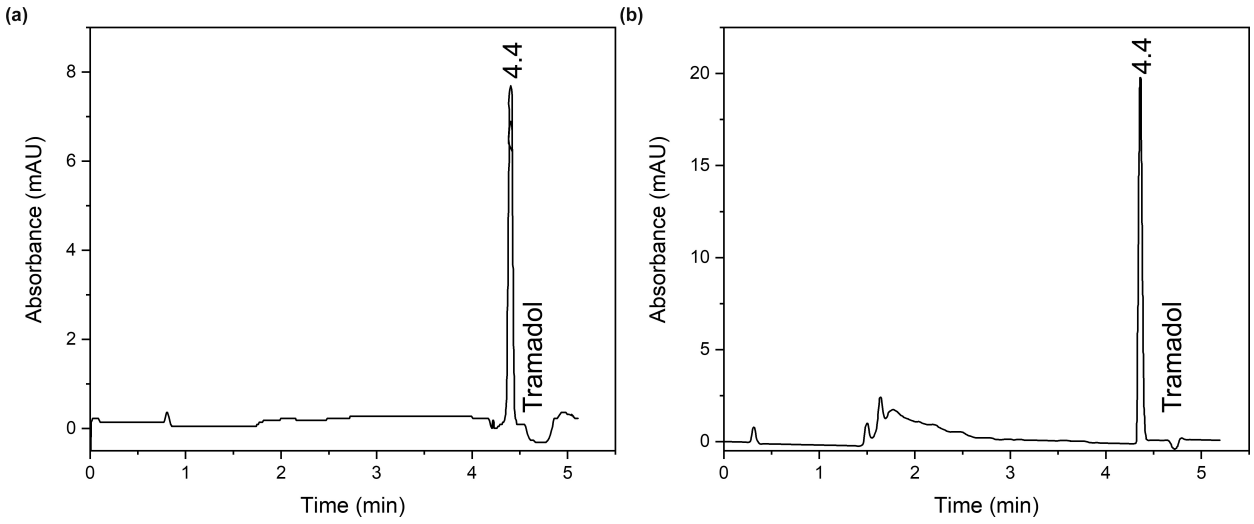

Fig. (4). Typical electropherograms of (**a**) tramadol-spiked plasma (with tramadol concentration of 2 μg mL^{-1}) and (**b**) plasma sample of patient number 1. Electrophoretic conditions: Capillary: fused-silica 50 cm (41.5 cm effective length) × 50 μm I.D; separation buffer, 50 mM borate buffer, pH 10.6; detection: 215 nm; applied voltage: 15 kV; temperature: 20℃; injection: electrokinetically at 15 kV for 30 s.

CONCLUSION

Herein, UA-DLLME, using a binary mixed extraction solvent, was established for the determination of tramadol in human plasma samples *via* the CE method with UV-detection. It is important to use an extraction technique prior to CE analysis to clean-up biological matrix. In the represented method, a small amount of plasma sample (100 µL) was applied at the extraction step and after solvent evaporation, it was reconstituted in 100 µl of deionized water (preconcentration factor =1). The developed method has remarkable characteristics, including simplicity, good repeatability and appreciable accuracy. Under the best extraction conditions, a low limit of detection at 7.0 µg per liter level with good linearity in the range of 0.02-10 µg mL^{-1} was obtained. In addition, the analysis of tramadol in some plasma samples of patients with abuse of tramadol indicated that the method has a great potential to be a reliable analytical technique for routine laboratory analysis.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the ethical committee of Tabriz University of Medical Sciences, Iran.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All research procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983.

CONSENT FOR PUBLICATION

Consent for research involving humans was signed by the participants.

AVAILABILITY OF DATA AND MATERIALS

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

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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