

ORIGINAL ARTICLE

Determination of Carbamazepine in Biological Samples Using Ultrasound-Assisted Emulsification Micro-extraction and Gas Chromatography

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ABSTRACT: In this study, simple and efficient ultrasound-assisted emulsification microextraction (USAEME) combined with gas chromatography-flame ionization detector (GC-FID) was developed for the preconcentration and determination of carbamazepine in biological samples. In this method, the fine droplets of 1-octanol were formed and dispersed in the sample with the help of ultrasonic waves, which accelerated the formation of the fine cloudy solution without using disperser solvents. Several factors influencing the extraction efficiency such as the nature and volume of organic solvent, extraction temperature, ionic strength and centrifugation time were investigated and optimized. The new method (USAEME) provided detection limits of $0.6 \mu\text{g L}^{-1}$ and $1.2 \mu\text{g L}^{-1}$ in urine and plasma samples, respectively. The calibration graphs were linear in the range of $2.5\text{-}500 \mu\text{g L}^{-1}$ and $5.0\text{-}500 \mu\text{g L}^{-1}$ in urine and plasma, respectively. This proposed method was successfully applied to the analysis of carbamazepine in biological samples.

INTRODUCTION

Carbamazepine is an iminostilbene derivative used for more than three decades as the drug of first choice for

the treatment of trigeminal neuralgia and also for both generalized and partial seizures, due to rapid control of

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excessive cerebral electrical discharges and the low incidence of acute and chronic toxicity [1].

Carbamazepine is almost entirely metabolized by the CYP450 enzyme system in the liver. It induces cytochrome P450 isoenzymes as well as UDPglucuronyl transferase and may inhibit CYP2C19. Carbamazepine undergoes autoinduction (via CYP3A4). Certain drugs (cimetidine, diltiazem, verapamil, and erythromycin) can increase carbamazepine serum levels. On the other hand, drugs that accelerate hepatic metabolism (phenytoin, phenobarbital, primidone, oxcarbazepine) will decrease serum concentrations of carbamazepine. Taking carbamazepine simultaneously with lamotrigine may increase the likelihood of neurotoxic side effects [2].

Because of its low therapeutic index and increasing number of carbamazepine intoxications there is a need for routine measuring of carbamazepine concentration in blood and tissue samples [3].

The most applying methods for determination of carbamazepine in biological materials use high performance liquid chromatography (HPLC-UV, HPLC-DAD) and immunoassay (FPIA). Gas chromatography with mass spectrometry and liquid chromatography with mass spectrometry methods have been described in the literature [4-6].

Liquid-liquid extraction (LLE) [7, 8], solid-phase extraction (SPE) [9-11] and stir bar-sorptive extraction (SBSE) [12, 13] have been used as sample preparation methods for the determination CBZ in biological fluids. Either a more recent technique, introduced by Rezaee et al., which does not involve the use of a fiber or a syringe, has been termed as Dispersive liquid-liquid microextraction (DLLME) [14]. As the name suggests, it is based on a ternary component solvent system similar to homogeneous liquid-liquid extraction and cloud point extraction. In DLLME, a cloudy solution is formed when an appropriate mixture of extraction solvent and disperser solvent is quickly injected into the

sample. Thus, a high turbulence is produced. This turbulent regimen gives rise to the formation of small droplets, which are dispersed throughout the aqueous sample. Emulsified droplets have a large interfacial area. Only water-immiscible extraction solvents with higher density than water are used to ease their collection as they settle below the aqueous phase after centrifuging. Organic solvents (such as carbon tetrachloride, chloroform or chlorobenzene) are generally used as the extractants in DLLME and are toxic [15-21].

Ultrasound-assisted emulsification microextraction (USAEME) is based on the application of ultrasonic radiations for accelerating the emulsification phenomenon. On application of ultrasonic radiation, the solution becomes turbid due to the dispersion of extraction droplets into the aqueous phase. The emulsification process favors the mass transfer of analytes from aqueous phase into the organic phase, which leads to the enhanced extraction efficiency of analytes in minimum amount of time. Thereby combining the benefits of microextraction and ultrasonic radiations the USAEME is derived as a fast and efficiency microextraction technique for extractions of trace analytes from the liquid medium [22]. In fact, this preconcentration technique has been developed by Regueiro et al. [23], who successfully applied it to determine synthetic musk fragrances, phthalate esters and lindane in aqueous samples. Saleh et al. applied low-density organic solvent in USAEME for the determination of PAHs in water samples [24]. In 2012, USAEME method combined with the GC-FID was used for the determination of amphetamines compounds in urine samples and under the optimized conditions; good recovery, linearity, and reproducibility were obtained [25]. Rezaee et al. reported the application of ultrasound-assisted dispersive liquid-liquid microextraction method for the trace analysis of methyl tert-butyl ether in the water samples. The performance of the proposed method in MTBE extraction from the

different water samples with various matrixes was excellent and no matrix effect was observed [26]. Recently, homogeneous liquid-liquid microextraction via flotation assistance was used for the determination of abamectin from water samples. In this method, toluene at microliter volume level and acetone were used as extraction and homogeneous solvents, respectively. In this research, a special extraction cell was designed to facilitate collection of the low-density extraction solvent. No centrifugation was required in this procedure. The water sample solution was added into the extraction cell, which contained an appropriate mixture of extraction and homogeneous solvents. Using air flotation, extraction solvent was collected at the conical part of the designed cell [27].

At the best of our knowledge, none of the published papers reports the use of USAEME for the extraction and determination of carbamazepine in biological samples. The aim of this work is the application of the USAEME technique combined with the GC-FID for the extraction and determination of carbamazepine in biological samples. A series of parameters influencing the extraction recovery was investigated systematically.

MATERIALS AND METHODS

Chemicals and reagents

CBZ standard was provided by Aras two pharmaceutical companies (Tehran, Iran). Proper amount of CBZ was dissolved in methanol to obtain a stock solution of analyte with a concentration of 250 mg L⁻¹. Working standard solutions were freshly prepared by diluting the standard solution of the analyte with the deionized water to the required concentration. All the stock solutions were stored at 4 °C and were stable at least for 4 weeks. Toluene, 1-octanol, 1-undecanol, 1-dodecanol and NaCl were obtained from Merck (Darmstadt, Germany). The water used was purified on a Nanopure ultra-pure water purification system (Nano pure, USA). The tap water was obtained from our laboratory (Tehran, Iran). The

urine sample was obtained from a healthy individual and was collected in disposable polyethylene containers and kept at 4 °C before analysis. A frozen human plasma sample was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran), thawed and allowed to reach room temperature.

Apparatus

A 40 kHz and 0.138 kW ultrasonic water bath with temperature control (Tecno-GazSpA, Italy) was applied to emulsify the organic solvent. One hundred and 25 µL Hamilton syringes (Bonaduz, Switzerland) were used to inject the organic solvent into the samples. Twenty milliliters home-designed centrifuge glass vials were used for extraction and collection procedure (Figure 1). A 10.0 µL Hamilton gas-tight syringe was applied for the collection of floated organic solvent and injection into the GC. A gas chromatograph (Agilent GC-7890) equipped with a split/splitless injector system and flame ionization detector, was used for separation and determination of target analyte. Ultra-pure helium gas (99.999%, Air products, UK) was passed through a molecular sieve and oxygen trap (Crs, USA) and was used as carrier gas with a flow rate of 2 mL min⁻¹. The injection port was held at 250 °C and operated in the splitless mode for 1 min then split valve was opened and split ratio of 1:5 was applied. Separation was carried out on a DB5, 25 m × 0.32 mm i.d. and 0.25 µm film thickness from SGE (Victoria, Australia) capillary column. The oven temperature was kept at 100 °C for 1 min and then increased to 280 °C at the rate of 20 °C/min and was held for 3 min. The FID oven temperature was maintained at 270 °C. Hydrogen was generated by hydrogen generator (OPGU-2200S, Shimadzu) for FID at a flow rate of 40 mLmin⁻¹. The flow of air (99.999%, Air products) for FID was 400 mL min⁻¹.

Extraction procedure

Ten mL of sample was placed in a home-designed centrifuge glass vial (Fig. 1, a). Then, 20.0 µL 1-octanol

was injected into solution and sample was sonicated for thirty second at 25°C in ultrasonic bath (Figure 1, b). As a result, oil-in-water emulsions of 1-octanol in water were formed. After centrifuging at 3500 rpm for 6 min, the organic solvent droplet was floated on the surface of the aqueous solution due to low density below water. After separation of the two phases, a few microliters of

doubly distilled water were added into the vial through the glass tube fixed on the side of the vial (Figure 1, c). The floated organic solvent was raised into the capillary tube attached to the top of the vial and collected by a gas-tight syringe (Fig. 1, d). Two microliters of collected organic solvent was injected into GC-FID instrument.

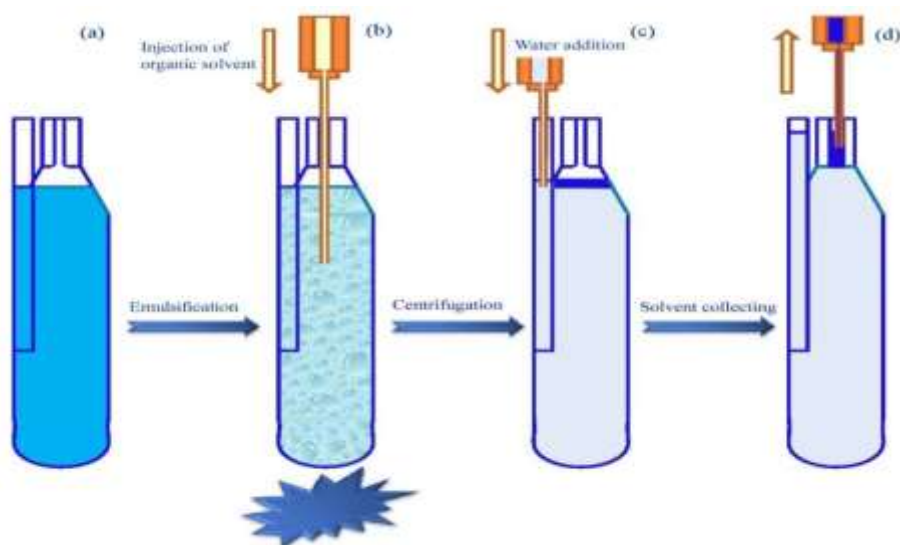


Figure 1. Schematic representation of the proposed method (a) sample solution in the home-designed emulsification glass vial without salt addition, (b) simultaneous injection and dispersion of 20.0 µL 1-octanol into sample, (c) addition of a few µL of doubly distilled water into the vial and (d) collection of 1-octanol transferred into the capillary tube at the top of the vial (about 6 µL)

RESULTS AND DISCUSSION

In this research, USAEME combined with GC-FID was developed for the determination of CBZ in biological samples. In order to obtain a high recovery and enrichment factor, the effect of different parameters such as type of extraction solvent and volume of its, centrifugation time, temperature and salt addition were examined and optimum conditions were selected.

Selection of extraction solvent

The selection of a suitable extraction solvent is critical for the USAEME process. In the USAEME, the

extraction solvent should have following characteristics: 1) lower density than that of water, 2) low solubility in water, 3) the ability to extract interest analyte. Based on these requirements, four organic solvent candidates, including toluene, 1-undecanol, 1-dodecanol and 1-octanol were investigated. The results (Table 1) revealed that the extraction recovery obtained for the analyte using 1-octanol were higher than those with the other solvents were. Therefore, 1-octanol was selected as the extraction solvent for the study.

Table 1. Extraction efficiency (%) of different extraction solvents evaluated for the extraction of the target analyte^a

Compound	Extraction efficiency			
	1-Octanol	1-Undecanol	Toluene	1-Dodecanol
CBZ	63	47	22	31

^aExtraction conditions: extraction solvent volumes, 20.0 μL 1-octanol, 10.0 1-undecanol, 14.0 toluene, 12.0 1-dodecanol; concentration of analyte, 100 $\mu\text{g L}^{-1}$.

Effect of centrifugation time

Centrifugation is essential to separate extraction solvent from aqueous solution in USAEME, because centrifugation time may affect the volume of floated phase. The effect of the centrifugation time on the extraction efficiency was examined from 2 to 20 min at 3500 rpm. The experimental results showed that the best performance was obtained at 3500 rpm for 10 min. At higher centrifugation times (>15 min), the volume of collected solvent was decreased.

Effect of volume of extraction solvent

The effect of the volume of the extracting solvent on the proposed method of CBZ was investigated in the range of 20.0-44.0 μL . According to figure 2, by increasing the volume of 1-octanol, preconcentration factor decrease, because the volume of collected solvent increases. Hence, high preconcentration factor are obtained using the 20.0 μL volume of extraction solvent. In the following studies, 20.0 μL was selected as the optimal volume of extraction solvent.

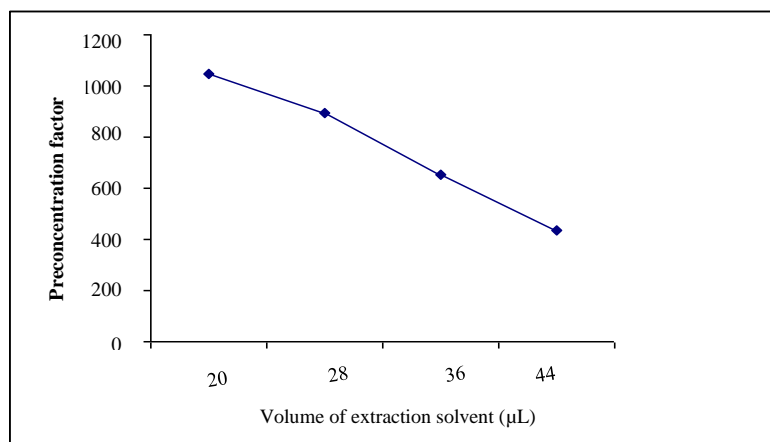


Figure 2. Effect of extracting solvent volume on the preconcentration factor. Conditions: sample solution: 10 mL of 100 $\mu\text{g L}^{-1}$ of the analyte; solution temperature: 25 ± 3 °C; dispersion time: 30 second; centrifugation time: 10 min

Salt addition

The influence of ionic strength was evaluated at 0-8% (w/v) NaCl levels while other parameters were kept constant. The experimental result showed that salt addition had no significant effect on the extraction efficiency of the analyte. Therefore, all the following experiments were carried out without adding salt.

Effect of emulsification-extraction temperature

Temperature affects organic solvent solubility in water as well as the emulsification phenomenon. Thus, this affects the mass-transfer process and the extraction efficiency. To determine the influence of the extraction temperature, extraction producers were done in different temperatures such as 20, 25, 35, 40 and 50 °C. The results are shown in Figure 3. The highest extraction efficiency was obtained at the range of 20°C - 25°C, but

in higher temperature (35-50 °C), extraction recoveries decreased. This event is possible because of the decrease

in distribution coefficient (K_D) in higher temperature. Hence, 25°C was used for further experiments.

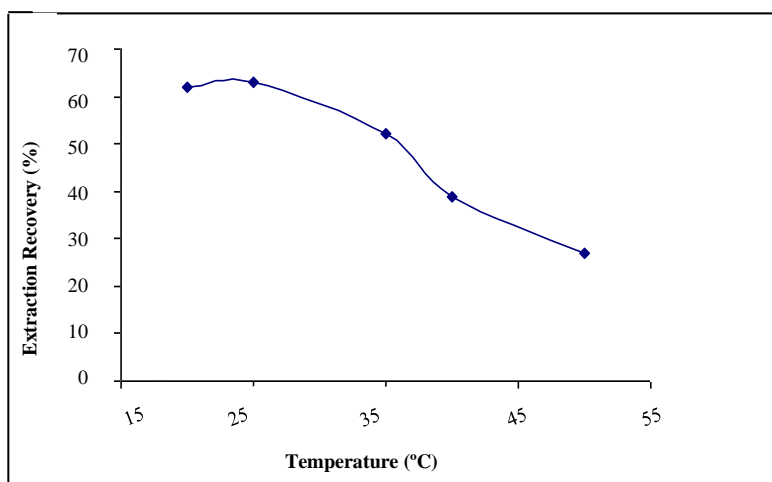


Figure 3. Effect of extraction temperature on the extraction efficiency. Conditions: sample solution: 10 mL of 100 $\mu\text{g L}^{-1}$ of the analyte; volume of organic phase: 20.0 μL ; dispersion time: 30 second; centrifugation time: 10 min

Effect of pH

The pH of sample solution was changed in the range of 4-10, by using NaOH, KH_2PO_4 and HCl. The results show that, by increasing the pH from 4 to 7.5, the peak area of CBZ increases and from 7.5 to 10, the peak area of CBZ slightly decreases. It may be explained that, in the pH=7.5, analyte is largely neutral and it is obvious that neutral form of organic compound has a greater tendency to be extracted into the organic solvent compared to the ionized form.

Method performance

Analytical performance

The figures of merit of the proposed method are shown in Table 2. The calibration curve of CBZ with a linear range of 1.0-500 $\mu\text{g L}^{-1}$ and a suitable coefficient of determination ($r^2=0.9997$), were obtained under the optimized condition in aqueous sample. The relative standard deviations (RSD, n=4) for CBZ extraction and its determination was 6.2% in aqueous sample. The limit of detection (LOD), based on signal-to-noise (S/N) of 3 was 0.3 $\mu\text{g L}^{-1}$ in aqueous sample. The calibration graphs were linear in the range of 2.5-500 $\mu\text{g L}^{-1}$ and 5.0-500 $\mu\text{g L}^{-1}$ with detection limits of 0.6 $\mu\text{g L}^{-1}$ and 1.2 $\mu\text{g L}^{-1}$ in urine and plasma samples, respectively.

Table 2. Quantitative results of USAEME and GC-FID method for CBZ compound

Sample	Linear range ($\mu\text{g L}^{-1}$)	LOD ^a ($\mu\text{g L}^{-1}$)	RSD (%) ^b	R ^{2c}
Water	1.0-500	0.3	6.2	0.9997
Urine	2.5-500	0.6	8.7	0.9983
Plasma	5.0-500	1.2	10.3	0.9976

^a LOD, limit of detection for S/N=3.

^bRSD, relative standard deviation (n=4).

^ccoefficient of determination

Table 3 compares proposed method with other extraction methods for the determination of CBZ. The comparison of extraction time of the proposed method with stir bar sorptive extraction (SBSE) [29] and solid-phase microextraction (SPME) [30] for the extraction of CBZ indicates that this novel method has a very short

equilibrium time comparing to the mentioned methods and the extraction time needed for the proposed method is a few seconds. Quantitative results of proposed method are better than solid-phase extraction (SPE) [28], SPME and SBSE in plasma sample.

Table 3. Comparison of the proposed method with other extraction methods for the determination of CBZ

Methods	R.S.D.%	Dynamic linear range ($\mu\text{g L}^{-1}$)	Limit of detection ($\mu\text{g L}^{-1}$)	Extraction time (min)	Ref.
SPE-HPLC-UV	6.8 (plasma)	2.0-40 ($\mu\text{g mL}^{-1}$) (plasma)	25 (plasma)	-	[28]
SBSE-HPLC-UV	<8.8 (plasma)	0.08-40 ($\mu\text{g mL}^{-1}$) (plasma)	-	50	[29]
SPME-HPLC-UV	<7 (plasma)	0.2-20 ($\mu\text{g mL}^{-1}$) (plasma)	-	20	[30]
USAEME-GC-FID	6.2 (water)	1.0-500 (water)	0.3 (water)	A few seconds	This work
	8.7 (urine)	2.5-500 (urine)	0.6 (urine)		
	10.3 (plasma)	5.0-500 (plasma)	1.2 (plasma)		

Extraction of the carbamazepine from the aqueous sample

To demonstrate the performance of the present method, it was utilized to determine the analyte concentration in

tap water. The obtained results are given in Table 4. The relative recovery for the spiked sample ($2.0 \mu\text{g L}^{-1}$) is acceptable.

Table 4. Determination of carbamazepine (CBZ) in different spiked samples

Sample	Concentration of CBZ ($\mu\text{g L}^{-1}$)	Added CBZ ($\mu\text{g L}^{-1}$)	Found CBZ ($\mu\text{g L}^{-1}$) \pm SD ^b (n = 3)	Relative recovery (%)
Tap water	n.d. ^a	2.0	1.85 \pm 0.1	92.5
Urine	n.d.	5.0	4.4 \pm 0.4	88.0
Plasma	n.d.	10.0	8.7 \pm 1.0	87.0

^aNot detected.

^bStandard deviation

Extraction of the carbamazepine from human urine and plasma sample

Due to the importance of the analysis of CBZ in biological samples, the proposed method was applied to determine the concentration of CBZ in plasma and urine samples, the obtained results are summarized in Table 4. In order to reduce the matrix effect, the urine sample

was diluted to 1:1, using deionized water. Samples of plasma were dissolved in suitable amount of acetonitrile

for reducing the matrix effect and then were centrifuged. After filtering, they were diluted ten times for USAEME procedure. The chromatograms of the urine sample spiked at $5.0 \mu\text{g L}^{-1}$ concentration level and plasma sample spiked at $10.0 \mu\text{g L}^{-1}$ concentration level of the CBZ are shown in figures 4 and 5, respectively. In addition, the obtained results for the spiked urine and plasma samples (Table 4) show that these matrices little effect on the performance of USAEME procedure.

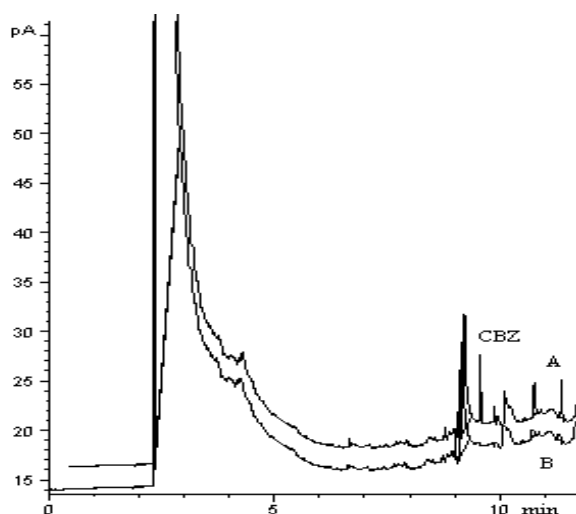


Figure 4.GC-FID chromatograms of (B) before spiking with analyte in urine, (A) $5.0 \mu\text{g L}^{-1}$ spiked of analyte in urine after extraction via proposed method at optimum conditions

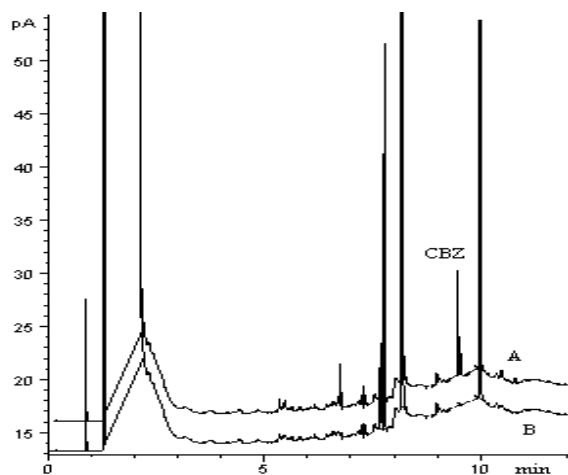


Figure 5.GC-FID chromatograms of (B) before spiking with analyte in plasma, (A) $10.0 \mu\text{g L}^{-1}$ spiked of analyte in plasma after extraction via proposed method at optimum conditions

CONCLUSIONS

Ultrasound-assisted emulsification micro extraction (USAEME) combined with GC-FID detection allows tackling the determination of CBZ in biological fluids and water samples in a simple way. The method is simple, rapid and inexpensive. The performance of this procedure in the CBZ extraction from biological fluids was excellent. The developed method was sensitive, reproducible and linear over a wide range for determination of CBZ from biological and water samples.

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REFERENCES

1. Tsatsakis A.M., Psillakis T.K., Tzatzarakis M., Kourtopoulos H., Paritsis N., 1997. Carbamazepine levels in the hair of patients under longterm treatment: a preliminary study. *Clin Chim Acta.* 263, 187–195.
2. Carbamazepine. Interactions with other medications. Professional. epilepsy.com/medications
3. Dordevic S., Kilibarda V., Stojanovic T., 2009. Determination of carbamazepine in serum and saliva samples by high performance liquid chromatography with ultraviolet detection. *Vojnosanit Pregl.* 66, 347-352.
4. Minkova G., Getova D., 2001. Determination of carbamazepine and its metabolite carbamazepine-10,11-epoxide in serum with gas chromatography mass spectrometry. *Methods Find Exp Clin Pharmacol.* 23, 481–485.
5. Zhu Y., Chiang H., Wulster-Radcliffe M., Hill R., Wong P., Kissinger C.B., Kissinger P.T., 2005. Liquid chromatography/tandem mass spectrometry for the determination of carbamazepine and its main metabolite in rat plasma utilizing an automated blood sampling system. *J Pharm Biomed Anal.* 38, 119-125.
6. Van Rooven G.F., Badenhorst D., Swart K.J., Hundt H.K., Scanes T., Hundt A.F., 2002. Determination of carbamazepine and carbamazepine 10, 11-epoxide in human plasma by tandem liquid chromatography–mass spectrometry with electrospray ionisation. *J Chromatogr B.* 769, 1-7.
7. Romanlyshyn L.A., Wichmann J.K., Kucharczyk N., Shumaker R.C., Ward D., Sofia R.D., 1994. Simultaneous determination of felbamate, primidone, phenobarbital, carbamazepine, two carbamazepine metabolites, phenytoin and one phenytoin metabolite in human plasma by high performance liquid chromatography. *Ther Drug Monit.* 16, 90-99.
8. Kouno Y., Ishikura C., Homma M., Oka K., 1993. Simple and accurate high-performance liquid chromatographic method for the measurement of three antiepileptics in therapeutic drug monitoring. *J Chromatogr A.* 622, 47-52.
9. Patil K.M., Bodhankar S.L., 2005. Simultaneous determination of lamotrigine, phenobarbitone, carbamazepine and phenytoin in human serum by high-performance liquid chromatography. *J Pharm Biomed Anal.* 39, 181-186.
10. Liu H., Delgado M., Forman L.J., Eggers C.M., Montoya J.L., 1993. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. *J Chromatogr B.* 616, 105-115.
11. Loughrin H., 2006. Comparison of solid-Phase microextraction and stir bar sorptive extraction for the quantification of malodors in wastewater. *Agric J Food Chem.* 54, 3237-3241.

12. David F., Sandra P., 2007. Stir bar sorptive extraction for trace analysis. *J Chromatogr A*.1152, 54-63.
13. Hu Y., Zheng U., Zhu F., Li J., 2007. Sol-gel coated polydimethylsiloxane/beta-cyclodextrin as novel stationary phase for stir bar sorptive extraction and its application to analysis of estrogens and bisphenol A. *J Chromatogr A*.1148, 16-22.
14. Rezaee M., Assadi Y., MilaniHosseini M.R., Aghaee E., Ahmadi F., Berijani S., 2006. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J Chromatogr A*.1116, 1-9.
15. RahnamaKozani R., Assadi Y., Shemirani F., MilaniHosseini M.R., Jamali M.R., 2007. Part-per-trillion determination of chlorobenzenes in water using dispersive liquid-liquid microextraction combined gas chromatography-electron capture detection. *Talanta*.72, 387-393.
16. Farajzadeh M.A., Bahram M., Jonsson J.A., 2007. Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-diode array detection as an efficient and sensitive technique for determination of antioxidants. *Anal Chim Acta*.591, 69-79.
17. Bidari A., Zeini Jahromi E., Assadi Y., Milani Hosseini M.R., 2007. Monitoring of selenium in water samples using dispersive liquid-liquid micro extraction followed by iridium-modified tube graphite furnace atomic absorption spectrometry. *Microchem J*. 87, 6-12.
18. Shokoufi N., Shemirani F., Assadi Y., 2007. Fiber optic-linear array detection spectrophotometry in combination with dispersive liquid-liquid microextraction for simultaneous preconcentration and determination of palladium and cobalt. *Anal Chim Acta*. 597, 349-356.
19. Rezaee M., Yamini Y., Shariati S., Esrafil A., Shamsipur M., 2009. Dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-UV detection as a very simple, rapid and sensitive method for the determination of bisphenol A in water samples. *J Chromatogr A*.1216, 1511-1514.
20. Rezaee M., Yamini Y., Faraji M., 2010. Evolution of dispersive liquid-liquid microextraction. *J Chromatogr A*.1217, 2342-2357.
21. Yamini Y., Rezaee M., Khanchi A., Faraji M., Saleh A., 2010. Dispersive liquid-liquid microextraction based on the solidification of floating organic drop followed by inductively coupled plasma-optical emission spectrometry as a fast technique for the simultaneous determination of heavy metals. *J Chromatogr A*. 1217, 2358-2364.
22. Luque de Castro M.D., Priego-Capote F., 2007. Ultrasound-assisted preparation of liquid samples. *Talanta*.72, 321-334.
23. Regueiro J., Llompert M., Garcia-Jares C., Garcia-Monteagudo J.C., Cela R., 2008. Ultrasound-assisted emulsification-microextraction of emergent contaminants and pesticides in environmental waters. *J Chromatogr A*.1190, 27-38.
24. Saleh A., Yamini Y., Faraji M., Rezaee M., Ghambarian M., 2009. Ultrasound-assisted emulsification microextraction method based on applying low density organic solvents followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples. *J Chromatogr A*.1216, 6673-6679.
25. Rezaee M., Mashayekhi H.A., SadeghiGarmaroudi S., 2012. Simultaneous determination of amphetamine and related compounds in human urine using ultrasound-assisted emulsification microextraction and gas chromatography. *Anal Methods*.4, 3212-3218.
26. Rezaee M., Yamini Y., Mashayekhi H.A., Naeeni H.A., BashiriJubari M., 2011. Trace analysis of methyl tert-butyl ether in water samples using new ultrasound-assisted dispersive liquid-liquid micro extraction and gas chromatography-flame ionization detection. *J Chin Chem Soc*. 58, 332-339.

27. Rezaee M., Mashayekhi H.A., Mohammad Hosseini M., Haddadi H., 2014. Homogeneous liquid-liquid microextraction via flotation assistance followed by high-performance liquid chromatography as an efficient and sensitive technique for the determination of abamectin in aquatic samples. *J Liq Chromatogr R T.* 37, 2559-2570.
28. Bugamelli F., Sabbioni C., Mandrioli R., Kenndler E., Albani F., Raggi M.A., 2002. Simultaneous analysis of six anti-epileptic drugs and two selected metabolites in human plasma by liquid chromatography after solid-phase extraction. *Anal Chim Acta.*472, 1-10.
29. Queiroz R.H.C., Bertucci C., Malfara W.R., Dreossi S.A.C., Chaves A.R., Valerio D.A. R., Queiroz M.E.C., 2008. Quantification of carbamazepine, carbamazepine-10, 11-epoxide, phenytoin and phenobarbital in plasma samples by stir bar-sorptive extraction and liquid chromatography. *J Pharm Biomed Anal.* 48, 428-434.
30. Queiroz M.E.C., Silva S.M., Carvalho D., Lancas F.M., 2002. Solid-phase microextraction–liquid chromatography (SPME–LC) determination of lamotrigine simultaneously with carbamazepine and carbamazepine 10, 11-epoxide in human plasma. *J Sepsci.*25, 91-

