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Ultrasound-Assisted Surfactant-Enhanced Emulsification Microextraction-HPLC Determination of Low-Levels of Thiopental in Serum and Urine Samples

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An ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) coupled with high performance liquid chromatography-diode array detection has been developed for the extraction and determination of thiopental in serum and urine samples. A simple microextraction method based on emulsification of organic extraction solvent in aqueous sample using an ionic surfactant and without any organic solvent was evaluated for preconcentration and extraction of trace amounts of thiopental. The surfactant was used as carrier and disperser agent simultaneously. The analyte was converted into their ion-pair complexes with tetra butyl ammonium bromide and then extracted into an organic solvent (chloroform) dispersed in aqueous solution. Some parameters affect the extraction efficiency, such as the type and volume of the extraction solvent, the type and concentration of the surfactant, sample pH, the ultrasound emulsification time and salt addition. These parameters were investigated and optimized. Under optimum conditions, the limit of detection (LOD) and enrichment factor for this technique were 0.084 ng mL^{-1} and 174 respectively.

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Introduction

Thiopental [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid] is an ultra-short-acting barbiturate used to induce anesthesia in man and animals [1]. It is used for intensive-care patients with head injuries to reduce intracranial hypertension [2] and is useful to prevent and treat brain ischemia [3]. As a result, monitoring of the serum concentrations is important in this patient population. Several different analytical methods have been used to assay thiopental levels in plasma or serum, including voltammetry [4], high-performance liquid chromatography (HPLC) [5-9], gas chromatography (GC) [10–12], gas chromatography-mass spectrometry (GC–MS) [13], high performance thin-layer chromatography (HPTLC) [14] and high-performance capillary electrophoresis (HPCE) [15]. However, in many cases, owing to matrix interference and insufficient instrumental detection limit for trace thiopental in real biological samples, direct chromatographic separation and determination of thiopental is difficult [9,14]. In order to obtain accurate, reliable and sensitive results, a separation / preconcentration method is required prior to chromatographic detection. In recent years, a number of solvent microextraction approaches have been developed that include single-drop microextraction (SDME) [16–18] and liquid-phase microextraction (LPME) [19–21]. These methods, which have been developed as alternatives to solid-phase microextraction (SPME), to avoid some of the problems associated with SPME, as it is limited to aqueous samples (because of low-stability of fibers in organic solvents), it cannot be used for highly concentrated analytes, the coated fibers used are more or less expensive, and it has limited lifetimes for some applications. Additionally, the automated SPME systems (primarily coupled to gas chromatography) are expensive and normally out of the reach of most laboratories [16-21].

In 2006, Assadi and coworkers developed a novel LPME technique called dispersive liquid–liquid microextraction (DLLME) [22]. The advantages of this novel method include very short extraction time, ease of operation, low cost, and high enrichment factors. Since its introduction, DLLME has been successfully applied to the determination of trace organic pollutants and metal ions in different media [23–26]. In addition, in ultrasound-assisted emulsification microextraction (USAEME) method, reported by Montes et al, [23], a few microliter volume of water-immiscible extraction solvent is dispersed into sample aqueous solution by ultrasound-assisted emulsification, without any dispersive solvent. To diminish emulsification time and enhance extraction efficiency, the use of a surfactant as emulsifier in USAEME has been reported [24]. It should be noted that the surfactants have been widely used in extraction processes and separation sciences [27–34]. In order to enhance efficiency of DLLME techniques, surfactant is added into the aqueous donor phase solution [28, 29]. Also, to increase the extraction efficiency of the hydrophilic drugs, carrier-mediated DLLME has been reported [30]. In this technique, ionic surfactants (as carrier) are added to the sample solution.

In the present study, the applicability of a cationic surfactant as a dispersion agent for organic solvents in UAEME combined with HPLC-DAD was considered for the preconcentration and determination of traces of thiopental in human serum samples. There are several factors affecting the extraction process including type and volume of extraction solvent, the type and concentration of surfactant, pH, ionic strength, time of ultrasonication, and the duration of centrifugation. The optimization was carried out using working solutions containing 100 ng mL^{-1} of thiopental. The chromatographic peak area, which is related to the number of moles of extracted analytes into the organic solvent, was used to evaluate the extraction efficiency under different experimental conditions.

Materials and Methods

Reagents

Sodium thiopental was provided by Naprod Ltd. (Maumbai, India). HPLC-grade methanol, acetonitrile, chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2) chlorobenzene (C_6H_5Cl) and sodium chloride (analytical grade) were purchased from Merck

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and sigma-Aldrich companies. Sodium dodecylsulfate (SDS), Triton X-100, cetyltrimethyl ammonium bromide (CTAB), Aliquat 366, tetraheptylammonium bromide (THAB), N-dodecyl trimethylammonium bromide (DDTMAB) and tetrabutylammonium bromide (TBAB) were purchased from Merck. Other chemicals were purchased from Merck. Double distilled water was used for the preparation of aqueous solutions.

Instrumentation

Chromatographic analysis was carried out on an Agilent 1200 HPLC system equipped with a diode array detector (California, USA). An auto sampler injector was used for the sample injection. The separation of the analytes was carried out on an Agilent Eclipse plus C18 column (5 µm, 4.6 mm × 150 mm). A CH_3CN-phosphate buffer (10 mM, pH; 6.2) (42:58, %v/v) mixture at a flow rate of 1.0 mL/min was used as the mobile phase in isocratic elution mode. The injection volume was 30 µL and the detection wavelength was 280 nm. Analyte was weighed with a Mettler AE200 electronic balance (Switzerland). The samples were ultrasonically irradiated in a water bath at 150 W and 40 kHz using an ultrasonic instrument (Fungilab). All the glassware used throughout this work was washed with deionized water and acetone and then dried at room temperature.

Real sample preparation

Blank urine and serum samples were provided by healthy volunteers in our lab. According to the method by Shamsipur and Fattahi [29], for the sedimentation of undesirable compounds in the bottom of the conical test tube, these samples were kept frozen at −20 °C before extraction process. The frozen urine and serum samples were thawed at room temperature. The serum sample (2 ml) was diluted with 4ml methanol for precipitation of plasma proteins. After centrifuging of the serum sample for 5 min., the precipitated proteins were separated. Then, the clear supernatant layer was filtered through a 0.45µm milli-pore filter to obtain protein-free human serum sample.

Extraction procedure

The experimental procedure for the ultrasound-assisted surfactant-enhanced emulsification microextraction is shown in Fig. 1. Aliquots of 2 mL human serum sample were used for analysis, spiked with thiopental. In all of experiments, the pH was adjusted with 0.1 M HCl and NaOH solutions. Then, it was diluted with water (to final volume of 4 mL) and the DLLME procedure was followed. The supernatant was placed in a 15 mL screw-cap polypropylene (PP) test tube with conical bottom. To the sample solution was injected 100 µL of $CHCl_3$, as extraction solvent, containing 5% (w/v) of TBAB as carrier and emulsifier. After manual shaking (3-5 s), the resulting mixture was immersed into an ultrasonic bath at 25±3°C for 2 min sonication. The cloudy solution contained very fine droplets of CHCl3 dispersed in the aqueous sample, and the analytes were extracted into the fine droplets. The emulsion was disrupted by centrifugation at 4500 rpm for 4 min and the organic phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was completely transferred to another test tube with conical bottom and then evaporated to dryness under a mild nitrogen stream. Finally, the extract was re-dissolved in 21 µL of the mobile phase. Acrodisc 13-mm syringe filters with0.2-µm nylon membrane (Pall Corp., MI, USA) were used for filtration of sample extracts prior to the injection in the HPLC system.

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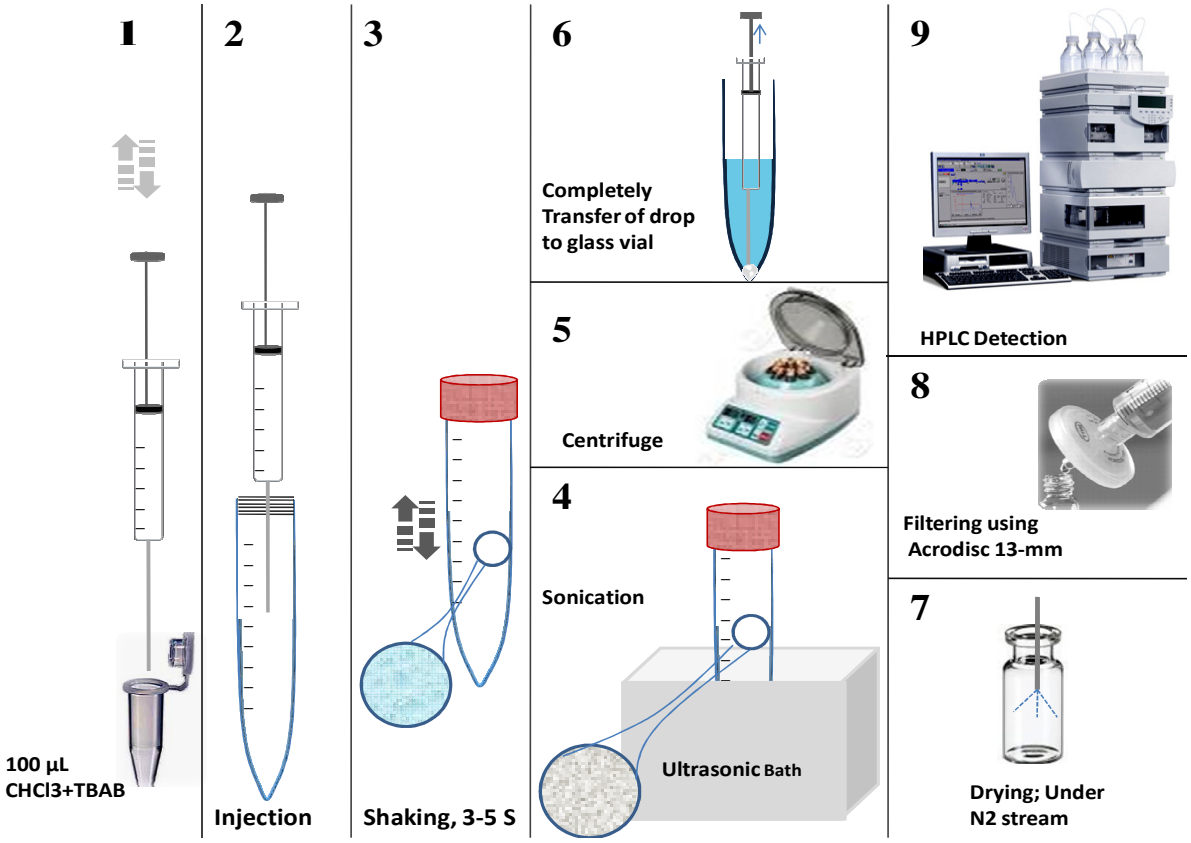

Fig. 1. Schematic of the proposed method.

Results and discussion

In the conventional DLLME method, a volume of about 0.1-1 mL of the disperser solvents (e.g., methanol, acetonitrile, or acetone), which are miscible with the organic solvents, are used to disperse a non-polar extraction solvent in an aqueous sample. The addition of this relatively large volume of the organic solvent, which is miscible with water, leads to the reduced extraction efficiency of the method due to an increase in the solubility of the analytes in the sample solution. As previously described, in ultrasound-assisted surfactant-enhanced emulsification microextraction [30], the surfactant is added into the aqueous phase in order to enhance of dispersion of extraction solvent in the aqueous sample. The solvent is emulsified in the presence of ultrasonic radiation (1-5 min sonication). In comparison with USAEME [23], the addition of surfactant, accelerates the formation of fine droplets of the extraction solvent in aqueous sample, hence decreasing the extraction time. Therefore, as it will be seen later, quick equilibrium (less than 30s) can be achieved due to the fast transition of analytes from aqueous phase to extraction solvent.

Optimization of UASEME

The extraction efficiency of UASEME procedure depends on some important experimental parameters, which should be investigated in detail. The effects of type and volume of extraction solvent, type and concentration of surfactant, effect of the salt addition, ultrasonic time, extraction temperature and sample pH were studied. In order to obtain the optimized extraction condition, extraction recovery (ER) was used to evaluate the optimum condition. %ER was defined as the percentage of the total analyte (n_0) extracted into the sedimented phase (n_{sed}). Accordingly, calculation of the extraction recovery, as analytical response, was carried out using the following equation:

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$$\% ER = n_{sed}/n_0 = c_{sed} \times v_{sed}/c_0 \times v_{sam} \quad (1)$$

Where C_{sed} and C_0 are the concentrations of analyte in sedimented phase and initial concentration of analyte in aqueous sample, respectively. C_{sed} is determined from a calibration curve which was obtained using direct injection of standard solutions. V_{sed} and V_{sam} are the volumes of sedimented phase and aqueous sample, respectively.

The preconcentration factor (PF) was defined as the ratio between the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte (C_0) in the aqueous sample, as follows:

$$PF = c_{sed}/c_0 \quad (2)$$

Combination of eqs. (1) and (2) gives:

$$ER\% = PF \times v_{sed}/v_{sam} \times 100 \quad (3)$$

Selection of the surfactant and pH

The pH value of solution determines the dominant form of the analytes. For analytes with both acidic and basic functional groups, cationic and anionic forms are dominant in acidic and basic ranges of pH, respectively. The pK_a value of thiopental is 7.4 [31], where pK_a is assigned to the thiol group. Since thiopental have both acidic and basic functional groups, they are ionizable and form ion pair complex with any of the surfactants. Therefore, the extraction efficiency will be affected by ion pair formation.

In the present study, the pH was selected in a range that the surfactants form the ion pair complex as an emulsified medium. Therefore, to achieve the best conditions, the effect of pH and surfactant type was investigated synchronously. For this purpose, seven types of surfactant, namely the anionic (SDS) non-ionic (Triton X-100) and cationic (CTAB, Aliquat 336, TBAB, THAB and DDTMAB) in different pHs were investigated. To study the effect of surfactant type, 100 µL extraction solvent (chloroform) containing 5% (w/v) of each surfactant was added into the 4 mL of spiked serum sample containing 100 ng mL^{-1} of the thiopental. Based on the obtained results illustrated in Fig. 2, the cationic surfactants give the best extraction recoveries. Thus, CTAB, Aliquat 336, TBAB, and DDTMAB at basic pHs (7-9) upon addition to emulsifying chloroform in sample solution, forms ion pair complexes with analyte ions and efficiently extract them into the organic solvent. As a result, among the seven surfactants investigated, TBAB and SDS gave the highest and lowest peak area for thiopental. At pH > 9 the extraction was decreased, most possibly due to competition of OH^- with the analytes to form ion pair with cationic surfactant. Therefore, TBAB as an anionic carrier and pH = 8.1 were selected as the optimal options.

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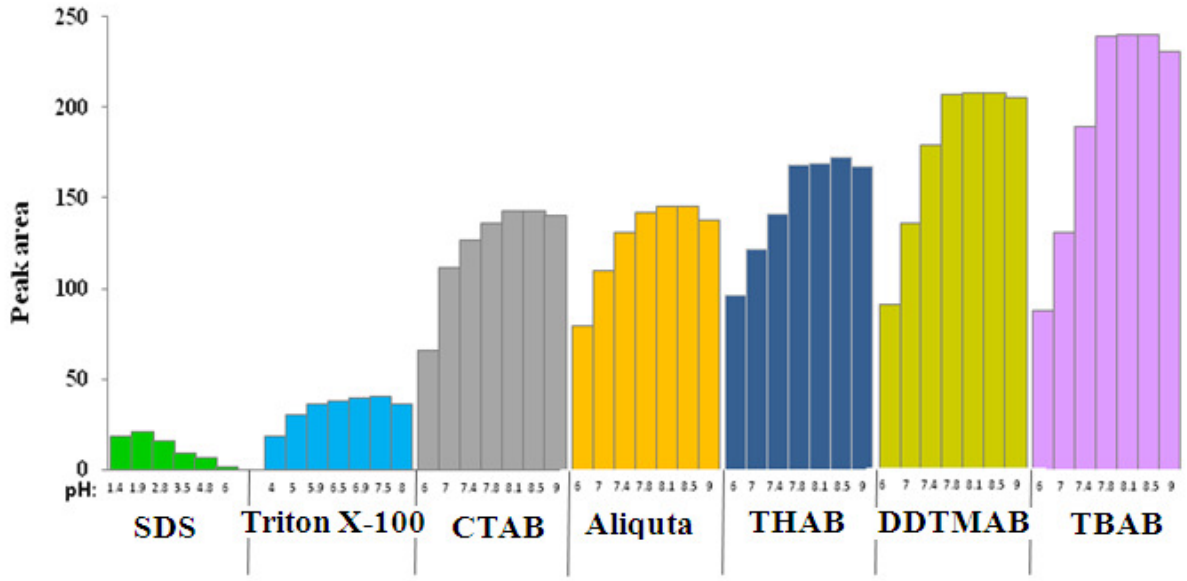

Fig. 2. The Effect of sample pH and surfactant on peak area of the analyte. Thiopental concentration, 100 ng mL^{-1}; sample volume, 4 mL; ultrasonic time,120 s; 100 µL of chloroform as extraction solvent; surfactant concentration, (4%); centrifuging time, 5 min.

Effect of extraction solvent

The selection of a suitable extraction solvent is critical for the DLLME process. Based on the characteristics required, including higher density than water, low melting point, low water solubility and high extraction capability of target compounds [24], the extraction solvents tested were as follows: chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), dichloromethane (CH_2Cl_2), and chlorobenzene (C_6H_5Cl). As shown in Fig. 3, the highest extraction efficiency was obtained when using chloroform as an extraction solvent, which was then selected as extraction solvent.

Influence of extraction solvent volume

In order to study the effect of volume of extraction containing 5% (w/v) TBAB on the performance of the presented UASEME procedure, the volume of $CHCl_3$ was varied in the range from 20 to 80 µL. Fig. 4 displays the effect of volume of chloroform on the extraction efficiency of thiopental. When the volume of the extraction solvent was increased, the extraction recoveries were increased until 60 µL. At higher volumes than 60 µL, the recoveries were remained more or less constant or slightly decreased. From the obtained results, 60 µL of $CHCl_3$ was chosen for further studies.

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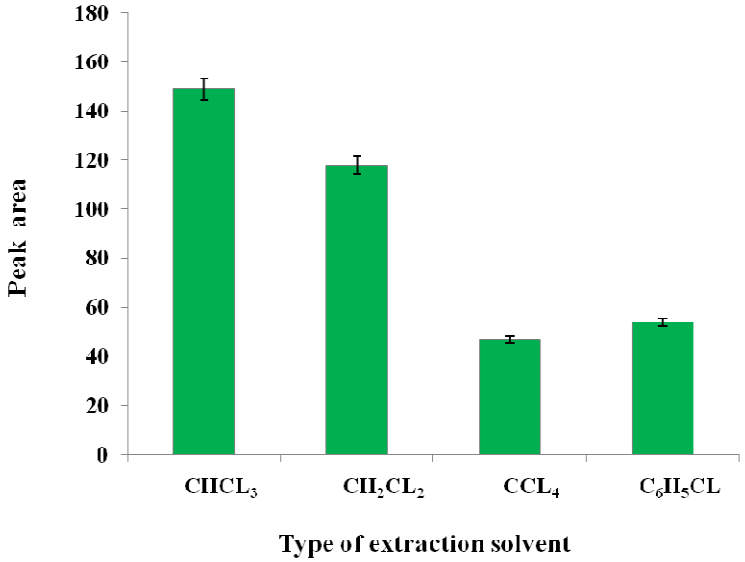

Fig. 3. The Effect of extraction solvent on the microextraction efficiency. Extraction conditions: thiopental concentration, 80 ng mL^{-1}; sample volume, 4 mL; extraction solvent, 100 µL; TBAB(5%); pH (8.1) ; ultrasonic time, 120 s; centrifuging time, 5 min.

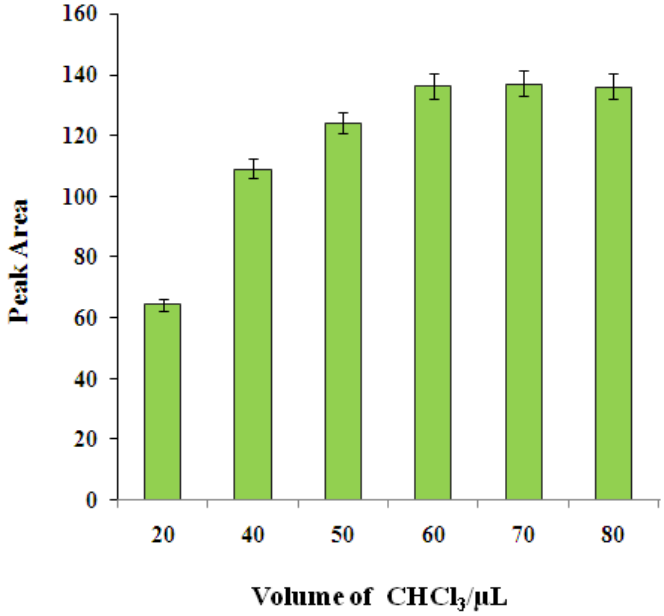

Fig. 4. The Effect of extraction solvent volume on the microextraction efficiency: Extraction conditions; thiopental concentration, 70 ng mL^{-1}; sample volume, 4 mL; extraction solvent ($CHCl_3$); TBAB (5%); pH (8.1) ; ultrasonic time, 120 s; centrifuging time, 5 min.

Effect of surfactant concentration in extraction solvent

Surfactant concentration is another important parameter for effective extraction. The influence of the TBAB concentration was investigated by changing its concentration to 0.5, 1.0, 2.0, 3.0, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.5%, respectively. The surfactant molecules can be associated in an aqueous solution to form molecular aggregates called micelle. The effect of TBAB concentration on extraction efficiency is shown in Fig. 5A. As can

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be seen, extraction efficiency for thiopental was increased with increasing surfactant concentration up to 6%, beyond which it remained constant. So, the 6% w/v was selected as the optimum concentration of TBAB.

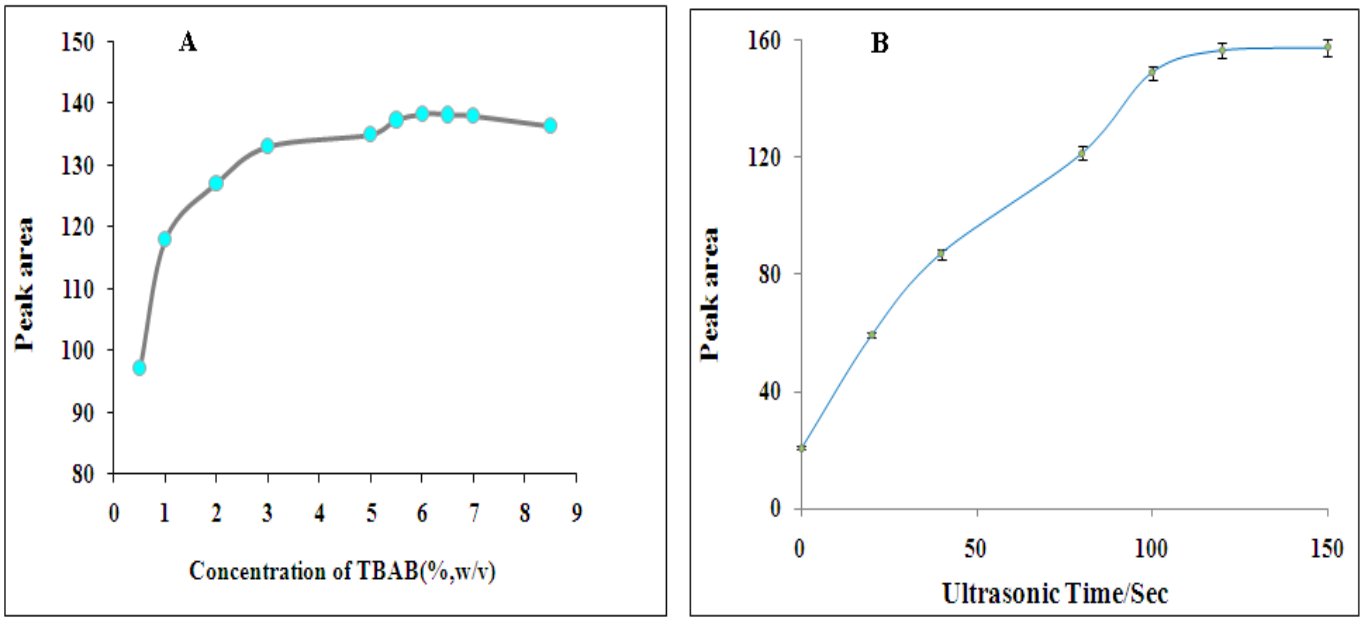

Fig. 5. The effect of cationic surfactant concentration (TBAB) on the extraction efficiency (A) and effect of ultrasonic time (B): Extraction conditions: thiopental concentration, 80 ng mL^{-1};sample volume, 4 mL; chloroform volume (60 µL); pH (8.1); centrifuging time, 5 min.

Effect of ultrasound extraction time and extraction temperature

Ultrasound extraction time is one of the main factors in SAUSEME as in most extraction procedures. It affects both emulsification and mass transfer processes, and thus influences the extraction recovery of the analytes. The ultrasound extraction time was defined as the time interval between the addition of the extraction solvent to the sample (the start of the sonication) and the end of the sonication. The effect of ultrasound extraction time was studied over the time intervals of 0 and 150 seconds. The results shown in Fig. 5B indicated that the extraction recoveries are increased strongly from 0-100 s and slightly from 100-120 s. By increasing the extraction time before 120 second, and after that, remained almost constant. Therefore, 120 second of sonication time was chosen for further experiments.

Temperature could also affect both the mass transfer and emulsification process, thus influencing the extraction efficiency. The effect of extraction temperature was studied over different temperatures ranging from 25 to 45 ºC. In the whole, at a temperature range of 25 to 45 ºC, the emulsification was easily achieved and remained during the whole extraction time. This may be due to the fact that the contact surface between the organic solvent and the aqueous phase is very large and mass transfer is not a limiting factor for the extraction. For the convenience of the experiment, the extractions were carried out at room temperature (25±2 ºC).

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Effect of salt addition

To evaluate the possibility of salting out effect, the extraction recovery was studied over the NaCl concentration range from 0 to 10% (w/v), while the other parameters were kept constant. The obtained result showed that the salt addition has no significant effect on the extraction recovery of analyte. Hence, NaCl was not added in all subsequent experiments.

Extraction recovery and enrichment factor for UASEME

The enrichment factor (EF) and the extraction recovery (ER) for this UASEME were calculated according to the same equations, as mentioned in Ref. [26]. As a result, under the optimum conditions, the enrichment factor and extraction recovery were 174 and 87%, respectively. The reason for high enrichment factor and good extraction recovery in UASEME, could be due to the fact that there is no need to use dispersive solvent, which could reduce the partition coefficients of the analytes between the extraction solvent and aqueous samples.

Validation of the method

Under the optimum conditions, some parameters of the proposed UASEME–HPLC-DAD method such as linearity, limits of detection (LODs), enrichment factor (EF) and reproducibility was investigated. As shown in Table 1, calibration curves were drawn in the concentration range of 0.15–550 ng mL^{-1}. The limit of detection (LOD) was calculated using the calibration curve methodology with a signal-to-noise ratio of 3 (the ratio between the peak intensity and the noise intensity was used), while limits of quantification (LOQ) values was calculated using a signal-to-noise ratio of 10. The resulting values are reported in Table 1. Good linear range was obtained for thiopental, with correlation coefficients (r) 0.9931.The LOD and LOQ were evaluated as 0.086 ng $mL^{-1}$and 0.15 ng mL^{-1}, respectively. The precision of the method was investigated by determining intra-day (repeatability) and inter-day (reproducibility) RSDs of the analysis. The inter-day precision was performed over three days (Table 1). To assess the performance of this method, linear range and LOD were compared with those of othermethods used for the analysis of thiopental (Table 2). As is seen, in comparison to the other reported methods for the determination of thiopental, the proposed method shows a relatively low LOD.

Table 1. Regression line, quantitative characteristics, intra-day precision and inter-day precision for the developed method (n=5).

Drug	Linear range	LOD	LOQ	EF	r^2 value	Intra day		Inter day	
	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})			Added	RSD (%)	Added	RSD (%)
Thiopental	0.15-550	0.084	0.15	174	0.9931	2.5	3.76	2.5	6.17
						12.0	2.95	12.0	5.72
						95.0	2.30	95.0	4.55

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Table 2. Comparison of the analytical performance of the present work with the recently reported values.

Method	Real sample	Linear range (ng mL^{-1})	LOD (ng mL^{-1})	Ref.
cathodic stripping voltammetry	serum and urine sample	2.6-26	0.052	[4]
reversed-phase high-performance liquid chromatography	serum sample	200-1×10^5	200	[6]
high-performance liquid chromatography using iodine–azide reaction as a postcolumn detection system	urine sample	10.6-265	5.3	[7]
reversed-phase high-performance liquid chromatography	serum sample	0- 2×10^4	10	[8]
reversed-phase high-performance liquid chromatography	serum sample	1×10^3–1×10^5	200	[9]
Gas Chromatographic Determination of Thiopental in Plasma Using an Alkali Flame Ionization Detector	serum sample	0 -1×10^4	-	[10]
High-Performance Thin-Layer Chromatography	serum sample	1×10^3–1×10^5	500	[14]
This work	serum and urine sample	0.15-550	0.086	-

Real sample analysis

The proposed method was firstly applied to the determination of concentration of thiopental in blank human serum and urine samples. The results showed that the analyzed samples were free from thiopental. These samples were then spiked with the standards of thiopental to assess the matrix effects while, all experiments were performed in triplicates (n = 3). Fig. 6 shows the obtained chromatograms of the blank human serum and urine samples and that spiked with 12.0 ng L^{-1} of thiopental. Accuracy was calculated as the relative recoveries (% recovery) for the analysis of known amounts of thiopental added to biological samples (urine and serum, samples) using the proposed method (Table 3). Based on the obtained results, by using UASEME the matrix of different samples has low effects on the recoveries.

Effect of biological matrix

The complex matrix in serum and urine samples would cause a negative effect on the recovery of analytes under ordinary conditions. Thus, a main way was to dilute the biological samples at a risk of further decreasing the analytical sensitivity of analytes [32,33]. In order to validate the applicability of the proposed sample preparation technique for real biological sample matrices, the optimal conditions previously described were evaluated by comparing the analytical signals of the target drugs in blank biological samples and aqueous standards both spiked with the same concentrations of the analyte. The experimental results indicated that nodifference of analytical signals of target drug was observed between aqueous standards and biological/diluted biological samples. Thus, the results obtained imply that UASEME has high ability of resisting the interference of biological matrices.

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Table 3. Determination of thiopental in biological samples (n = 3).

Sample	Sample No.	Added(ng mL^{-1})	Found± SD (ng mL^{-1})	Recovery %
Serum	1	0.40	0.38 ± 0.02	94.5
		12.0	11.58 ± 0.34	96.5
	2	0.40	0.39 ± 0.01	97.5
		12.0	11.82 ± 0.21	98.5
	3	0.40	0.38 ± 0.01	96.0
		12.0	12.18 ± 0.24	101.5
	4	0.40	0.37 ± 0.02	91.5
		12.0	11.71 ± 0.30	97.6
Urine	1	0.40	0.42 ± 0.01	104.2
		12.0	12.27 ± 0.20	102.2
	2	0.40	0.42 ± 0.01	104.0
		12.0	11.82 ± 0.23	98.5
	3	0.40	0.39 ± 0.012	97.5
		12.0	11.69 ± 0.38	97.4

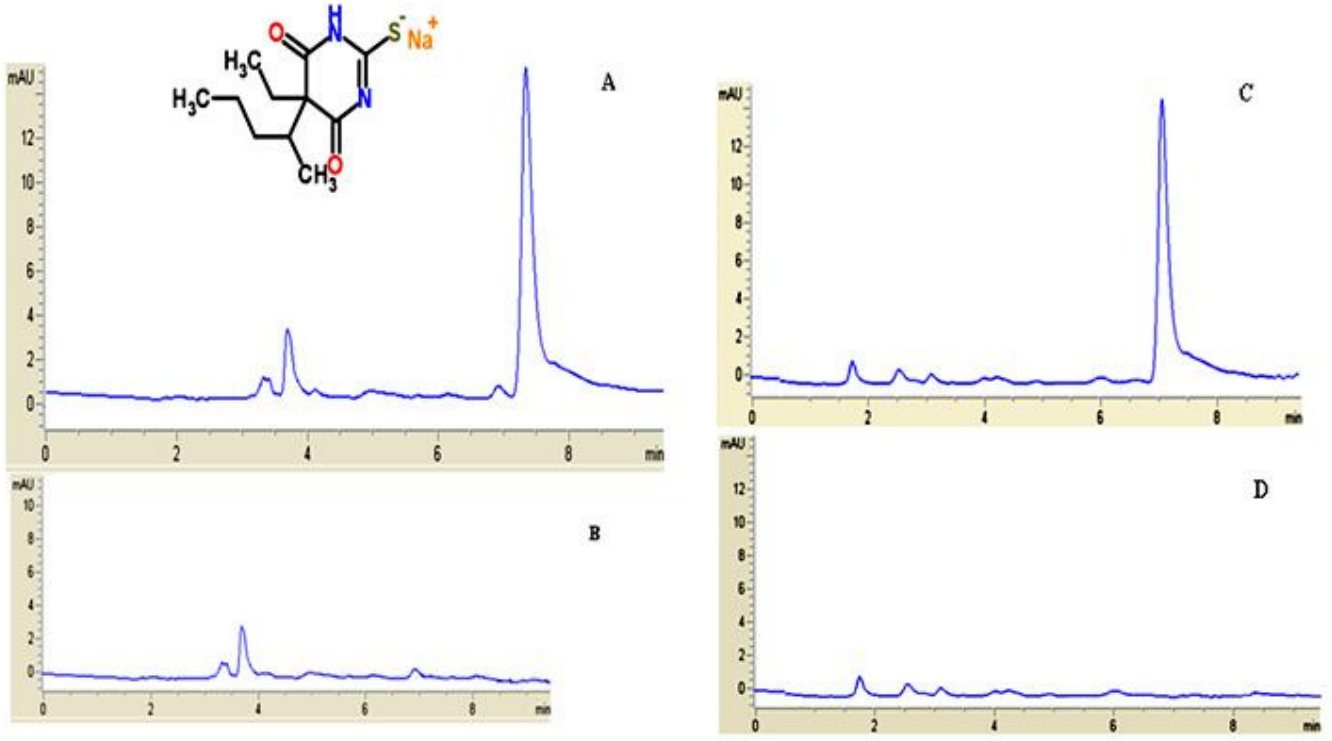

Fig. 6. HPLC-UV chromatograms of the spiked serum and urine, samples (A and C, respectively) and non-spiked serum and urine, samples (B and D, respectively) and (B), spiked samples by 12 ng mL^{-1} of the target analyte, after UASEME.

Conclusion

In the present study, a cationic surfactant was used as a disperser agent in a DLLME procedure named ultrasound-assisted surfactant-enhanced emulsification microextraction (UASEME) as a sample preparation step before determination of thiopental in biological samples by HPLC-UV. The experimental results revealed that this method provides high recovery and preconcentration factor within a short time and good linearity over

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the investigated concentration range. Compared to other microextraction methods, this method uses smaller volumes of low-toxicity extraction solvent and also avoids use of disperser solvent and other specially designed equipment to collect the extractants. This study illustrates the application of UASEME, which facilitates concentration of thiopental present at low concentrations in biological matrices.

Conflict of interest

Authors do not have any conflict of interest with the commercial identities mentioned in this article.

References

[1] Russo H, Bressolle F. Pharmacodynamics and pharmacokinetics of thiopental. Clinical pharmacokinetics. 1998;35:95-134.

[2] Marshall LF, Shapiro HM, Rauscher A, Kaufman NM. Pentobarbital therapy for intracranial hypertension in metabolic coma Reye's syndrome. Critical care medicine. 1978;6:1-5.

[3] Hirotani T, Kameda T, Kumamoto T, Shirota S, Yamano M. Protective effect of thiopental against cerebral ischemia during circulatory arrest. The Thoracic and cardiovascular surgeon. 1999;47:223-238.

[4] Ali AMM, Farghaly OA, Ghandour MA. Determination of thiopentone sodium in aqueous and biological media by cathodic stripping voltammetry, Analytical Chimica Acta. 2000;412: 99-110.

[5] Marty P, Arnoux P, Cano JP. Micromethod for Determination of Thiopental in Biological Fluids by High Performance Liquid Chromatography. Application to Therapeutic Follow-Up and Pharmacokinetic Study. Analytical Letter. 1988; 21:2241-2249.

[6] Coppa G, Testa R, Gambini AM, Testa I, Tocchini M, Bonfigli AR. Fast, simple and cost-effective determination of thiopental in human plasma by a new HPLC technique. Clinica chimica acta, 2001;305:41-45.

[7] Zakrzewski R., Ciesielski W. Determination of thiopental in urine sample with high-performance liquid chromatography using iodine–azide reaction as a post column detection system, Journal of Chromatography A. 2005;305:327-332.

[8] Celardo A. Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography, Journal of Chromatography A. 1990;527:220-225.

[9] Russo H, Allaz JL, Bressolle F. High-performance liquid chromatographic assay for thiopental in human plasma: Application to pharmacokinetic studies. Journal of Chromatography B: Biomedical Sciences and Applications. 1997;694:239-245.

[10] Sennello LT, Kohn FE. Gas chromatographic determination of thiopental in plasma using an alkali flame ionization detector. Analytical chemistry. 1974;46:752-5.

[11] Külpmann W. Gas-chromatographic method for the determination of thiopental in serum. Fresenius' Zeitschrift für analytische Chemie. 1982;311:409.

[12] Jung D, Mayersohn M, Perrier D. Gas-chromatographic assay for thiopental in plasma, with use of a nitrogen-specific detector. Clinical chemistry. 1981;27:113-115.

[13] Kuepper U, Birgel A, Musshoff F, Madea B. Detection of benzodiazepines and barbiturates using a validated HS-SPME/GC-MS method. 2007;299-305.

[14] Sanganalmath PU, Nagaraju PM, Mohan BM. HPTLC method for the assay of thiopental in post-mortem blood in a fatal case of suicide. Journal of pharmaceutical and biomedical analysis. 2013;80:89-93.

[15] Meier P, Thormann W, Determination of thiopental in human serum and plasma by high-performance capillary electrophoresis—micellarelectrokinetic chromatography, Journal of Chromatography A, 1991;559:505-513.

[16] Psillakis E, Kalogerakis N. Developments in single-drop microextraction. TrAC Trends in Analytical Chemistry. 2002;21:54-64.

[17] Xu L, Basheer C, Lee HK. Developments in single-drop microextraction. Journal of Chromatography A. 2007;1152:184-192.

[18] Liu H, Dasgupta PK. Analytical chemistry in a drop. Solvent extraction in a microdrop. Analytical Chemistry. 1996;68:1817-1821.

[19] Pedersen-Bjergaard S, Rasmussen KE. Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid–liquid extraction. Journal of Chromatography A. 2008;1184:132-142.

[20] Hyötyläinen T, Riekkola ML, Sorbent-and liquid-phase microextraction techniques and membrane-assisted extraction in combination

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with gas chromatographic analysis: a review, Analytical Chimica Acta. 2008;614:27-35.

[21] Barri T, Jönsson J-Å. Advances and developments in membrane extraction for gas chromatography: Techniques and applications. Journal of Chromatography A. 2008;1186:16-38.

[22] Rezaee M, Assadi Y, Milani Hosseini MR, Aghaee E, Ahmadi F, Berijani S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. Journal of Chromatography A. 2006;1116:1-9.

[23] Montes R, Rodríguez I, Ramil M, Rubí E, Cela R. Solid-phase extraction followed by dispersive liquid–liquid microextraction for the sensitive determination of selected fungicides in wine. Journal of Chromatography A 2009;1216:5459-5466

[24] Wu Q, Zhou X, Li Y, Zang X, Wang C, Wang Z. Application of dispersive liquid–liquid microextraction combined with high-performance liquid chromatography to the determination of carbamate pesticides in water samples. Analytical and bioanalytical chemistry. 2009;393:1755-61.

[25] Chen H, Chen H, Ying J, Huang J, Liao L. Dispersive liquid–liquid microextraction followed by high-performance liquid chromatography as an efficient and sensitive technique for simultaneous determination of chloramphenicol and thiamphenicol in honey. Analytica chimica acta. 2009;632:80-85.

[26] Tsai WC, Huang SD. Dispersive liquid–liquid microextraction with little solvent consumption combined with gas chromatography–mass spectrometry for the pretreatment of organochlorine pesticides in aqueous samples. Journal of Chromatography A. 2009;1216:5171-5175.

[27] Rubio S, Pérez-Bendito D. Supramolecular assemblies for extracting organic compounds. TrAC Trends in Analytical Chemistry. 2003;22: 470-485.

[28] Sarafra-Yazdi A, Es'haghi Z. Antibiotic-assisted three-phase liquid-phase microextraction of aromatic amines from aqueous solutions combined with high-performance liquid chromatography. Journal of Analytical Chemistry. 2006;61:787-793.

[29] Shamsipur M, Fattahi N. Extraction and determination of opium alkaloids in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography. Journal of Chromatography B. 2011; 879:2978-2983.

[30] Regueiro J, Llompart M, Garcia-Jares C, Garcia-Monteagudo JC, Cela R. Ultrasound-assisted emulsification–microextraction of emergent contaminants and pesticides in environmental waters. Journal of Chromatography A. 2008;1190:27-38.

[31] M. Wood, A. J. Wood, Drugs and anesthesia: pharmacology for anesthesiologists. M. D. Baltimore,: Williams & Wilkins; 1983.

[32] Samanidou VF, Nika MK, Papadoyannis IN.,Development of an HPLC method for the monitoring of tricyclic antidepressants in biofluids, Journal of Seperation Science. 2007;30:2391-2398.

[33] Cruz-Vera M, Lucena R, Cárdenas S, Valcárcel M. Combined use of carbon nanotubes and ionic liquid to improve the determination of antidepressants in urine samples by liquid chromatography. Analytical and bioanalytical chemistry. 2008;391:1139-45.

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