Journal of Chromatography B, 879 (2011) 2978–2983

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Extraction and determination of opium alkaloids in urine samples using dispersive liquid–liquid microextraction followed by high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 13 June 2011
Accepted 26 August 2011
Available online 31 August 2011

Keywords:
Dispersive liquid–liquid microextraction
Opium alkaloids
High-performance liquid chromatography
Urine analysis

ABSTRACT

A simple, rapid and sensitive method based on dispersive liquid–liquid microextraction (DLLME) combined with high-performance liquid chromatography–ultraviolet detection (HPLC-UV) was used to determine opium alkaloids in urine samples. Some effective parameters on extraction were studied and optimized. Under the optimum conditions, enrichment factors and recoveries for different opiates are in the range of 63.0–104.5 and 31.5–52.2%, respectively. The calibration graphs are linear in the range of 0.50–500 $\mu g\,L^{-1}$ and limit of detections (LODs) are in the range of 0.2–10 $\mu g\,L^{-1}$. The relative standard deviations (RSDs) for 200 $\mu g\,L^{-1}$ of morphine, codeine and thebaine, 5.0 $\mu g\,L^{-1}$ of papaverine and 10.0 $\mu g\,L^{-1}$ of noscapine in diluted urine sample are in the range of 2.8–6.1% ($n = 7$). The relative recoveries of urine samples spiked with alkaloids are 84.3–106.0%. The obtained results show that DLLME combined with HPLC-UV is a fast and simple method for the determination of opium alkaloids in urine samples.

1. Introduction

Opium is partially dried latex obtained from opium poppy cultivated mainly in Asia, South America and part of Europe [1]. Opiate and their derivatives are very potent analgesics commonly used as therapeutic agents. Some of these compounds are also frequently abused as illicit drugs [2]. Opiates can be classified into the three following series. The first one is constituted of the poppy alkaloids, including morphine, codeine, thebaine, noscapine and papaverine; the second category mainly included semi-synthetic or synthetic derivatives of morphine such as pholcodine, ethylmorphine (codethyline) and dextromethorphan which are used in therapy as antitussives and analgesics; the third class is composed of narcotic compounds including diacetylmorphine (heroine), buprenorphine and methadone [3], usually employed as substitutes in treatment of addiction.

Many techniques are already available for the quantification of opiates and their derivatives. Most of these use gas chromatography–mass spectrometry (GC–MS) [4–7], high-performance liquid chromatography (HPLC) [8–10], capillary electrophoresis (CE) [11–14] and electrochemical [15,16] analysis. GC–MS is often used because of its sensitivity, but the necessity of sample derivatization and the cost of the technique itself are restricting its applicability. On the other hand, HPLC appears as a technique that could separate a wide range of analytes without any chemical pretreatment. As such, it has become the preferred technique in most applications, using a variety of detection methods such as ultraviolet [17,18], fluorescence [19,20], diode array detection [2,8], chemiluminescence [9] and most recently, mass spectrometry [21–24].

Quantitative analysis of trace levels of opium alkaloids is still a significant challenge demanding a rapid and effective sample preparation procedure prior to analysis. Analytical procedures such as liquid–liquid extraction (LLE) [25], solid-phase extraction (SPE) [26–28] and ionic liquid-based aqueous two-phase system [1] have been developed for the determination of opium alkaloids. However, LLE usually requires some poisonous volatile organic solvents. SPE is a method with good purification and concentration effects, but it requires a solvent desorption step with traditional volatile organic solvents and the pretreatment processes are relatively time-consuming. Sometimes sample recovery is not satisfactory. Therefore, the development of simple and environmental friendly pretreatment methods is of great interest.

Dispersive liquid–liquid microextraction (DLLME) developed by Assadi and co-workers [29] is based on the formation of tiny droplets of the extractant in the sample solution using water–immiscible organic solvent (extractant) dissolved in a water–miscible organic dispersive solvent. Extraction of the analytes from aqueous sample into the dispersed organic droplets takes place. Rapidity, high enrichment factor, high extraction recovery,

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doi:10.1016/j.jchromb.2011.08.033

simplicity of operation and low cost are some of the advantages of this method. The performance of DLLME was illustrated by extraction of different organic and inorganic compounds from water samples [30–44]. Among these, DLLME is widely applied to the preparation of environmental water samples and rarely applied to the analysis of drugs in complex biological fluids [45,46].

In the present paper, DLLME was applied to the extraction and preconcentration of five major opium alkaloids in urine samples prior to their determination by HPLC-UV. The results indicated that DLLME is an efficient extraction technique to analyze opium alkaloids in urine samples.

2. Experimental

2.1. Reagents and standards

Pure samples of morphine, codeine, papaverine, noscapine and thebaine were obtained from Active Pharmaceutical Ingredients Manufacturer of Narcotic and Non-narcotic Products (TEMAD, Tehran, Iran). HPLC-grade solvents acetone, methanol, acetonitrile and chloroform were obtained from Rankem (New Delhi, India). Acetic acid, chlorobenzene, sodium carbonate, sodium dihydrogen phosphate, sodium dodecyl sulfate and sodium chloride were obtained from Merck (Darmstadt, Germany). The ultra-pure water (six times distillated) was purchased from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran).

Stock standard solutions of opium alkaloids were prepared in methanol (10.0 mL) with concentration levels of 1000 mg L^{-1} for morphine, codeine and thebaine, 100 mg L^{-1} for noscapine and 50 mg L^{-1} for papaverine, and were stored in a freezer at −20 °C. Working solutions were obtained by appropriate dilution of the stock standard solutions.

Blank urine sample (drug-free) was collected from a healthy volunteer and actual urine sample was obtained from the Clinic of Emam Reza Hospital (Kermanshah, Iran), and stored at −20 °C prior to use.

2.2. Apparatus

Chromatographic separations were carried out on a Cecil 1100 series HPLC equipped with a CE-1100 HPLC pump (Cambridge, UK), an on-line solvent vacuum degasser, a Cecil CE-1100 variable-wavelength UV detector (Cambridge, UK) and a model 7725, Rheodyne manual sample injector fitted with a 20 μL injection loop (Cotati, CA, USA). Separations were carried out on a H5-ODS C18 column (25 cm × 4.6 mm, with 5 μm particle size) from Anachem (Luton, UK). The mobile phase consisted of 55% buffer containing 10.0 mM sodium phosphate monobasic and 0.70 mM sodium dodecyl sulfate and 45% acetonitrile. The pH of the aqueous buffer in the mobile phase was adjusted to pH 6.56 using sodium hydroxide. A mobile phase flow-rate of 1.0 mL min^{-1} was used in isocratic elution mode and the detection was performed at the wavelength of 285 nm.

The Hettich Zentrifugen (EBA20, Tuttlingen, Germany) was used for centrifugations. Prior to use, all 10-mL screw cap conical bottomed glass test tubes (extraction vessels) were maintained at 500 °C in furnace (Carbolite, model CWF 1200, UK) to remove any organic compound.

2.3. Extraction procedure

For the DLLME, an aliquot of 5.00 mL of a diluted urine sample containing 200 μg L^{-1} of morphine, codeine and thebaine, 5.0 μg L^{-1} of papaverine, and 10.0 μg L^{-1} of noscapine was placed in a 10-mL screw cap conical bottomed glass test tube and then 0.50 mL Na_2CO_3 (10%, w/v) was added. Then the injection of 1000 μL acetone (disperser solvent) containing 88.0 μL chloroform (extraction solvent) to water samples was performed rapidly by a gastight 2.50 mL syringe (Hamilton, Nevada, USA), which resulted in dispersed fine droplets of chloroform to form a cloudy solution. In this step, the analytes were extracted into the fine droplet of chloroform, in a few seconds. After centrifugation for 3 min at 5000 rpm, fine droplets of extraction solvent were sedimented at the bottom of the conical test tube. After centrifuging, the sedimented phase (about 30 ± 3 μL) was completely transferred into another test tube and after evaporation of the solvent in a water bath, the residue was dissolved in 30 μL of mobile phase and injected into the HPLC.

2.4. Sample preparation

Blank urine sample (drug-free) was provided by healthy volunteer in our lab, which not exposed to any drug for at least 6 months. Actual urine sample was collected from a person who was addicted to opium, kindly provided by the Clinic of Emam Reza Hospital (Kernanshah, Iran). Urine samples were kept frozen at −20 °C before analysis. The frozen urine samples were thawed at room temperature and centrifuged for 10 min at 5000 rpm. White lipidic solid was sedimented in the bottom of the conical test tube, probably due to the co-sedimentation of the matrixes (such as carbamide and uric acid) in urine. The supernatants were transferred into clean glass tube and filtrated through a 0.45 μm filter. A 2.0 mL volume of this solution was diluted to 5.0 mL (for decreasing matrix effects) and 0.5 mL of Na_2CO_3 (10%, w/v) was added. The resulting solution was then subjected to the DLLME process.

2.5. Optimization of DLLME procedure

Those parameters affecting the DLLME procedure, including the nature and volume of the extraction and the disperser solvents, amount of Na_2CO_3, salt addition and extraction time, were optimized. It should be noted that the optimization procedure was conducted using spiked samples. The enrichment factor (EF) was defined as the ratio of the analyte concentration in the sedimented phase to the analyte concentration in the aqueous sample. The analyte concentration in the sedimented phase was calculated from the direct calibration graph (0.2–20 mg L^{-1} of opium alkaloids in methanol). Extraction recovery (%R) and relative recovery (%RR) were calculated according to equations described before [29,30].

3. Results and discussion

3.1. Selection of extraction solvent

Some characteristics such as low solubility in water, extraction capability of interested compounds, good chromatographic behavior and higher density than water, provided extra limitations on the selection of extraction solvent in the conventional DLLME method. Thus, chloroform (density = 1.48 g mL^{-1}, boiling point = 61.2 °C, solubility in water at 20 °C = 8 g L^{-1}) and chlorobenzene (density = 1.1 g mL^{-1}, boiling point = 131.6 °C, solubility in water at 20 °C = 0.4 g L^{-1}) were examined as extraction solvent. In order to select the best extraction solvent, a series of sample solutions were studied by using 1000 μL acetone containing 52 μL and 88 μL chlorobenzene and chloroform, respectively. The volume of the sedimented phase for both extraction solvents were 30.0 μL. According to the results given in Table 1, chloroform showed higher extraction efficiency than chlorobenzene. It is probably because of higher solubility of opium alkaloids in chloroform in comparison with chlorobenzene. Also, evaporation of chloroform is easier than the chlorobenzene. Therefore, chloroform was selected as the extraction solvent. It is interesting to note that, since the extraction

Table 1
Efficiency of different extraction solvents evaluated for extraction of opium alkaloids by DLLME.[a]

Compound	Recovery (%)	
	Chlorobenzene mean (RSD%) ($n=3$)	Chloroform mean (RSD%) ($n=7$)
Morphine	28.7 (4.1)	31.5 (6.1)
Papaverine	35.3 (5.1)	48.9 (2.8)
Codeine	38.3 (7.2)	42.7 (5.7)
Noscapine	48.8 (3.9)	52.2 (3.6)
Thebaine	46.0 (3.9)	49.2 (4.6)

[a] Extraction conditions: sample volume, 5.00 mL; concentration of Na_2CO_3, 1% (w/v); volume of acetone as disperser solvent, 1000 μL; volumes of extraction solvent, 88.0 μL chloroform and 52.0 μL chlorobenzene; volume of sedimented phase, 30.0 ± 3 μL; room temperature; concentration of morphine, codeine and thebaine, 200 μg L^{-1}; noscapine, 10.0 μg L^{-1}; papaverine, 5.0 μg L^{-1}.

of alkaloids was carried out at very low concentrations, the distribution coefficients and corresponding recoveries were found to be more or less concentration independent.

3.2. Selection of disperser solvent

As explained before [32], the disperser solvent should be miscible in water and dissolve the extraction solvent. Therefore, acetone, acetonitrile and methanol were examined as disperser solvent and the effect of these solvents on the performance of DLLME was investigated. For this purpose, various experiments were performed by using 1000 μL of each disperser solvent containing 88.0 μL of chloroform as extraction solvent. Considering the sedimented phase volume, it was found that with combination of chloroform–acetonitrile, the sedimented phase volume was very higher than 30.0 μL and the cloudy state was not formed well; whereas, in the case of chloroform–methanol and chloroform–acetone, the sedimented volume was about 30.0 μL. Therefore, acetone and methanol could be suitable canditates as disperser solvents for further studies. The results showed that the recoveries are almost equal for acetone (31.5–52.2%) and methanol (32.0–51.5%). Finally, among these two solvents, acetone was chosen because of less toxicity and low cost.

3.3. Effect of volume of extraction solvent

In order to study the effect of volume of extraction solvent on the performance of the presented DLLME procedure, different volumes of chloroform (68–118 μL at 10-μL intervals) and a constant volume of dispersive solvent (acetone, 1000 μL) were tested. In this study, all experiments were performed in triplicates ($n=3$). It was found that with the increase in volume of chloroform from 68.0 to 118.0 μL, the volume of the sedimented phase increased from 10.0 to 60.0 μL. According to Fig. 1 and considering the experimental errors on the data points, the extraction efficiency of analytes found to increases by increasing volume of chloroform up to 88.0 μL; while, further increase in volume of chloroform caused a small decrease in the extraction efficiency. The volume of extraction solvent should be selected so that high enrichment factor and recovery are obtained. Thus, in the following studies, 88.0 μL of chloroform was used as an optimal volume of the extractant solvent.

3.4. Effect of volume of disperser solvent

Variation of the volume of acetone causes changes in the volume of sedimented phase. To obtain a constant volume of the sedimented phase (i.e., 30 ± 3 μL), the volume of acetone and chloroform were changed, simultaneously. For obtaining optimized volume of acetone, various experiments were used by using different volumes of acetone (250, 500, 1000, 1500 and 2000 μL) containing 71.5, 77.0, 88.0, 100.0 and 113.0 μL chloroform, respectively. All experiments were performed in triplicates ($n=3$). Fig. 2 shows the resulting plots of recovery of opium alkaloids versus the volume of acetone. By considering the experimental errors on the data points of Fig. 2 plots, it is seen that at low volume of disperser solvent, acetone cannot disperse the extraction solvent properly and the cloudy solution is not formed completely. Meanwhile, a high volume of acetone will also result in diminished extraction efficiency, most probably due to decreased solubility of analytes in water. Thus, according to the results, 1000 μL of acetone was chosen as the optimum volume of disperser solvent.

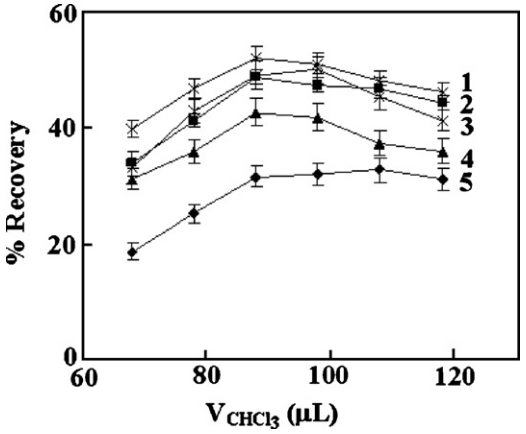

Fig. 1. Effect of the volume of chloroform as extraction solvent on the recovery of opium alkaloids: (1) noscapine, (2) papaverine, (3) thebaine, (4) codeine, (5) morphine. Extraction conditions: sample volume, 5.00 mL; disperser solvent (acetone), 1000 μL; Na_2CO_3, 1% w/v; volume of sedimented phase, 30 ± 3 μL; concentration of morphine, codeine and thebaine, 200 μg L^{-1}; noscapine 10.0 μg L^{-1}, papaverine 5.0 μg L^{-1}.

3.5. Effect of buffer concentration

To investigate the effect of quantity of Na_2CO_3 (for adjusting pH and ionic strength) on the performance of DLLME, various experiments were added in the presence of different amounts of Na_2CO_3 (0–2%) to the sample solution. Other experimental conditions were kept constant and all experiments were performed in triplicates ($n=3$). Fig. 3 shows that the recovery of opium alkaloids increases,

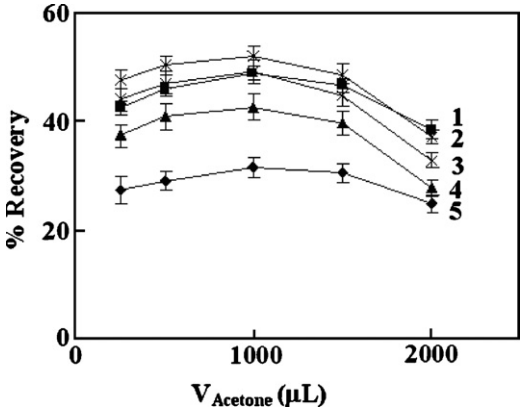

Fig. 2. Effect of the volume of acetone as disperser solvent on the recovery of opium alkaloids: (1) papaverine, (2) noscapine, (3) thebaine, (4) codeine, (5) morphine. Extraction conditions: sample volume, 5.00 mL; varying volumes of acetone: 250, 500, 1000, 1500 and 2000 μL containing 71.5, 77.0, 88.0, 100.0 and 113.0 μL chloroform, respectively; Na_2CO_3, 1% (w/v); volume of sedimented phase, 30 ± 3 μL; concentration of morphine, codeine and thebaine, 200 μg L^{-1}; noscapine 10.0 μg L^{-1}, papaverine 5.0 μg L^{-1}.

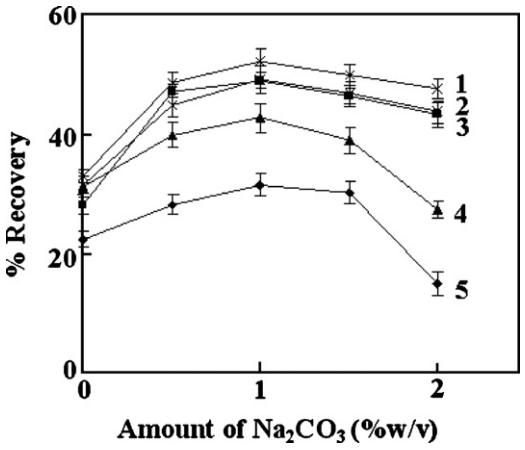

Fig. 3. Effect of the amount of Na_2CO_3 on the recovery of opium alkaloids: (1) noscapine, (2) thebaine, (3) papaverine, (4) codeine, (5) morphine. Extraction conditions: sample volume, 5.00 mL; disperser solvent (acetone), 1000 μL; extraction solvent (chloroform), 88.0 μL; volume of sedimented phase, 30 ± 3 μL; concentration of morphine, codeine and thebaine, 200 μg L^{-1}; noscapine 10.0 μg L^{-1}, papaverine 5.0 μg L^{-1}.

within the experimental errors on the data points, by increasing percentage of Na_2CO_3 from 0% to 1%. However, further increase in percentage of Na_2CO_3 leads to a decrease in the extraction efficiency. According to the results, a 1% Na_2CO_3 was chosen as the optimum amount of the salt. It is worth mentioning that in the presence of 1% Na_2CO_3, the pH of sample solution was 10.7.

3.6. Effect of salt addition and extraction time

Salt addition is frequently used to adjust the ionic strength, improve the extraction efficiency and reduce the detection limit. The effect of ionic strength on the opium alkaloids extraction efficiency by DLLME was examined in the absence and presence of NaCl over the concentration range of 0–5% (w/v). The results obtained from these experiments revealed that the salt addition did not influence the enrichment factor significantly for any of the analytes. As a consequence, all the extraction experiments were carried out without salt addition. The practicability of the method was also confirmed in saline samples up to 5% (w/v) NaCl.

The extraction time (i.e., the interval time between the time of injection of a mixture of disperser solvent and extraction solvent, before the time starting to centrifuge) is an important factor that may affect the analytes' extraction efficiency from aqueous phase into the organic phase. Thus, the variation in enrichment factor of opium alkaloids as a function of extraction time was studied in the range of 0.1–10 min. The resulting data show that the extraction time has no significant effect on the extraction efficiency for all the target compounds. It was found that, after the formation of the cloudy solution, the contact area between the extraction solvent and the aqueous phase was considerably large, delineating why the extraction equilibrium could be established very fast.

3.7. Quantitative analysis

The characteristics of the calibration curves, summarized in Table 2, were obtained under the optimized conditions. The resulting calibration graphs were linear over the concentration ranges of 20–500 μg L^{-1} for morphine, 30–500 μg L^{-1} for codeine and thebaine, 1.5–50 μg L^{-1} for noscapine and 0.5–25 μg L^{-1} for papaverine. The values of the correlation coefficients (r^2) ranged from 0.9987 to 0.9998. The repeatability was studied by extracting the spiked samples of 200 μg L^{-1}concentration of morphine, codeine and thebaine, 10.0 μg L^{-1} of noscapine and 5.0 μg L^{-1} of

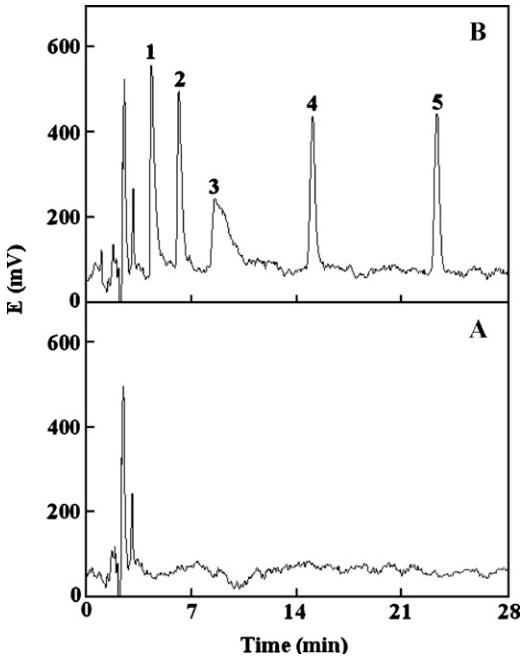

Fig. 4. (A) Chromatogram of blank urine sample. (B) Chromatogram of spiked urine sample at concentration levels of 300 μg L^{-1} for morphine (1), codeine (3) and thebaine (5), 15.0 μg L^{-1} for noscapine (4) and 7.5 μg L^{-1} for papaverin (2). Extraction conditions: similar to those in Fig. 1, except for a chloroform volume of 88.0 μL.

papaverine. The relative standard deviations (RSDs) were calculated to be in the range of 2.8–6.1% for 7 repeated experiments. The limits of detection (LODs), based on a signal-to-noise ratio (S/N) of 3, varied from 0.2 to 10.0 μg L^{-1}. Moreover, the enrichment factors and the recovery of opium alkaloids were between 62.0 and 104.5, and between 31.5 and 52.2%, respectively.

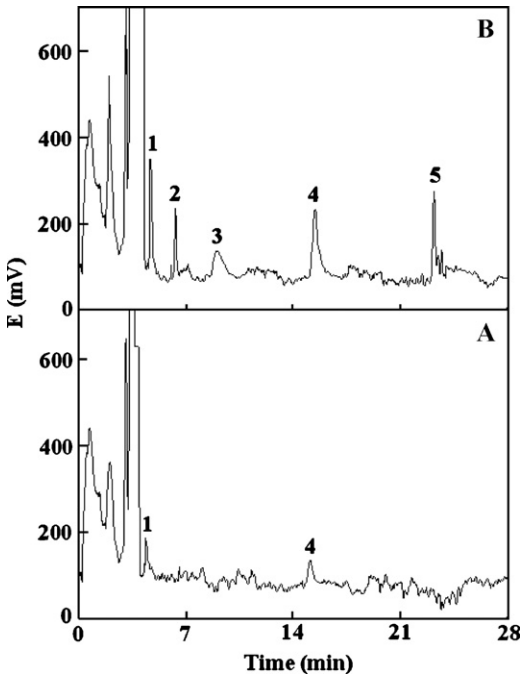

Fig. 5. (A) Chromatograms of actual urine sample. (B) Chromatograms of spiked actual urine sample at concentration levesl of 100 μg L^{-1} for morphine (1), codeine (3) and thebaine (5), 5.0 μg L^{-1} for noscapine (4) and 2.0 μg L^{-1} for papaverin (2): extraction conditions are similar to those of Fig. 1, except for a chloroform volume of 88.0 μL.

Table 2
Quantitative results of DLLME and HPLC-UV of opium alkaloids from spiked blank urine samples.[a]

Compound	RSD%[b] ($n=7$)	EF[c]	Recovery (%)	LR[d] ($\mu g\,L^{-1}$)	R^2[e]	LOD[f] ($\mu g\,L^{-1}$)
Morphine	6.1	63.0	31.5	20–500	0.9990	7.0
Papaverine	2.8	97.8	48.9	0.5–25	0.9994	0.2
Codeine	5.7	85.0	42.7	30–500	0.9987	10.0
Noscapine	3.6	104.5	52.2	1.5–50	0.9998	0.5
Thebaine	4.6	98.4	49.2	30–500	0.9988	10.0

[a] Extraction conditions: sample volume, 5.00 mL; concentration of Na_2CO_3, 1% (w/v); volume of acetone as disperser solvent, 1000 μL; volume of chloroform as extraction solvent, 88.0 μL; volume of sedimented phase, 30.0 ± 3 μL; room temperature.
[b] RSD% at a concentration of 200 $\mu g\,L^{-1}$ for morphine, codeine and thebaine, 10.0 $\mu g\,L^{-1}$ for noscapine and 5.0 $\mu g\,L^{-1}$ for papaverine.
[c] Enrichment factor.
[d] Linear range.
[e] Correlation coefficient.
[f] Limit of detection for a S/N = 3.

Table 3
Relative recoveries and standard deviations of opium alkaloids from spiked blank urine samples and actual urine samples.[a]

Compounds	Blank urine sample				Actual urine sample		
	Added ($\mu g\,L^{-1}$)	Found (SD,[b] $n=3$) ($\mu g\,L^{-1}$)	Relative Recovery (%)	Concentration of alkaloids (SD,[b] $n=3$) ($\mu g\,L^{-1}$)	Added ($\mu g\,L^{-1}$)	Found (SD,[b] $n=3$) ($\mu g\,L^{-1}$)	Relative recovery (%)
Morphine	200	179.2 (25.2)	89.6	13 (1.5)	100	97.7 (8.5)	86.4
	300	288.3 (22.5)	96.1	13 (1.5)	200	201.5 (18)	94.6
Papaverine	5	4.6 (0.68)	92.0	n.d.[c]	2	2.1 (0.3)	105.0
	7.5	6.4 (0.8)	85.3	n.d.	5	5.1 (0.5)	102.0
Codeine	200	168.7 (26.5)	84.3	n.d.	100	89.1 (9.2)	89.1
	300	265.7 (28.0)	88.5	n.d.	200	188.6 (21.5)	94.3
Noscapine	10	10.6 (1.8)	106.0	1.1 (0.2)	5	6.5 (0.8)	106.5
	15	15.2 (2.5)	101.3	1.1 (0.2)	10	12.0 (1.5)	108.1
Thebaine	200	188.3 (18.17)	94.1	n.d.	100	84.6 (7.1)	84.6
	300	273.6 (28.2)	91.2	n.d.	200	202.6 (15.4)	101.3

[a] Extraction conditions: sample volume, 5.00 mL; concentration of Na_2CO_3, 1% (w/v); volume of acetone as disperser solvent, 1000 μL; volume of chloroform as extraction solvent, 88.0 μL; volume of sedimented phase, 30 ± 3 μL; room temperature.
[b] Standard deviation.
[c] n.d., not detected.

3.8. Real sample analysis

The proposed method was firstly applied to determination of the concentration of opium alkaloids in blank urine samples, provide by one male volunteer in our lab. The results showed that the analyzed samples were free from opium alkaloids. These samples were then spiked with the standards of opium alkaloids at two different concentration levels to assess the matrix effects. Fig. 4 shows the obtained chromatograms of the blank urine sample and that spiked with 300 $\mu g\,L^{-1}$ of morphine, codeine and thebaine, 15.0 $\mu g\,L^{-1}$ of noscapine and 7.5 $\mu g\,L^{-1}$ of papaverine. The corresponding relative recoveries are summarized in Table 3. As seen, the relative recoveries for all opium alkaloids in spiked urine samples are between 84.3 and 106.0%.

An actual urine sample taken from an addicted person to opium was also subjected to the proposed procedure of DLLME, and analyzed in triplicate. The age of urine samples was approximately 24 h. It was found that the concentration of morphine and noscapine in the actual urine samples were 13.0 ± 1.5 $\mu g\,L^{-1}$ and 1.1 ± 0.2 $\mu g\,L^{-1}$, respectively, while no papaverine, codeine and thebaine was detected. In order to validate the method, two different concentrations of opium alkaloids were spiked into the actual urine sample and the recommended procedure was followed. The results of relative recovery and the concentrations obtained in spiked studies of the actual urine samples are also included in Table 3. Fig. 5 shows the resulting chromatograms of the actual urine sample and the corresponding spiked ones. These results demonstrated that the matrices of the analyzed real urine samples possess negligible effect on the proposed DLLME followed by HPLC-UV determination of the opium alkaloids.

3.9. Comparison of DLLME with other methods

In Table 4 are compared the figures of merits for the determination of opium alkaloids in urine samples by the proposed dispersive liquid–liquid microextraction and high-performance liquid chromatography-ultraviolet detection (DLLME-HPLC-UV) with those of the previously published methods including fast liquid chromatography-diode-array detection after solid phase extraction (SPE-FLC-DAD) [2], capillary zone electrophoresis with diode-array detection after solid phase extraction (SPE-CZE-DAD) [16] and solid phase extraction followed by high performance liq-

Table 4
Comparison of DLLME-HPLC-UV with other extraction methods for determination of opium alkaloids.

Method	LOD[a] ($\mu g\,L^{-1}$)	RSD[b] (%)	Extraction time (min)	Reference
SPE-FLC-DAD	1.6–7.6	4.9–7.7	>40	[2]
SPE-CZE-DAD	70–120	2.1–11.3	>20	[16]
SPE-HPLC-DAD	24–32	3.18–4.02	>20	[45]
DLLME-HPLC-UV	0.2–10	2.8–6.1	<4	This work

[a] Limit of detection.
[b] Relative standard deviation.

uid chromatography-diode-array detection (SPE-HPLC-DAD) [45]. As can be seen from Table 4, the relative standard deviations (RSDs) of the proposed method are about the same with those reported for the other methods. However, the limits of detection and extraction times of the proposed method are superior to those reported before. All these results indicate that the proposed DLLME-HPLC-UV is a sensitive, fast, reproducible and simple technique that can successfully be used for the preconcentration and determination of opium alkaloids in urine samples.

4. Conclusion

In this study, a novel DLLME-HPLC-UV method for the determination of opium alkaloids in urine samples has been evaluated. The optimum conditions of extraction performance have been obtained. The established method can be applied to the determination of concentration of opium alkaloids in real urine samples. The relative recoveries of those compounds studied in urine are from 82.2% to 108.6%. Adequate repeatability, reproducibility, linearity, and the absence of matrix effects demonstrated that the method is feasible for quantitative analysis of opium alkaloids in real urine samples, and could be used in routine analyses.

Acknowledgement

The support of this work by Iran National Science Foundation (INSF) is gratefully acknowledged.

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