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Surfactant enhanced liquid-phase microextraction of basic drugs of abuse in hair combined with high performance liquid chromatography

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Abstract

The aim of this study was to evaluate the performance of a technique for simultaneous testing of hydrophilic abuse drugs in hair. The analysis of, codeine and methadone in morphine hair included incubation in methanol (5 h, 50 °C), Surfactant enhanced liquid-phase microextraction (SE-LPME) and HPLC analysis. This study has demonstrated that SE-LPME constitute a real alternative to the other liquid-phase microextraction methods, for pre-concentration and extraction of hydrophilic drugs in biological samples and has shown the advantages of these optimized methodologies over the traditional microextraction techniques. For these drugs recoveries in the range of 57.5–93.7 were obtained from hair. The drugs were enriched by a factor of 61–128 during SE-LPME. Linearity (r^2, 0.9982–0.9997) was obtained in the range of 50–500 μg/l for morphine and 10–500 μg/l for codeine and methadone.
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Keywords: Basic drug of abuse; Surfactant enhanced liquid-phase microextraction; High performance liquid chromatography; Hair analysis

1. Introduction

Hair analysis to detect drug abuse is a new perspective in forensic toxicology [1,2]. In 1979, for the first time Baumgartner et al. [3] reported the detection of opiates in hair and this was followed by several reports on the detection of other drugs in hair by different methods like, solid phase extraction, solid phase microextraction and GC–MS, or tandem MS [4–7]. As biological matrix such as plasma and urine, hair gives particular advantages such as; the stability of a specimen, non-invasive sampling, broad time detection window and it can be stored and transported without specific tanks [8]. But, drug determination in the human hair and/or biological fluids is often complicated by low analyte concentration and the complex sample matrix. Because of this, sample preparation is crucial in drug analysis and includes both analyte pre-concentration and sample clean-up. Recently, Pedersen–Bjergaard and Rasmussen introduced an alternative concept for three-phase microextraction, as a powerful sample preparation technique for drug analysis, based on the use of disposable low-cost porous hollow fibers made of polyproylene [9–11]. In this sample preparation technique, analytes are extracted through an aqueous solution (donor phase) into an organic liquid immobilized within the pores of the hollow fiber before they are trapped with the aqueous acceptor phase, that is contained within the lumen of the porous hollow fiber and thus microextracts are not in direct contact with the sample solution.

The extraction involves pH adjustment of the sample solution to a pH where the analytes are uncharged. The analytes are extracted through the organic phase immobilized in the pores of the hollow fiber and into the aqueous acceptor phase, that has a pH where the analytes are charged preventing them from back diffusion into the organic solvent [12,13]. Hydrophobic analytes are easily extracted into organic sol-

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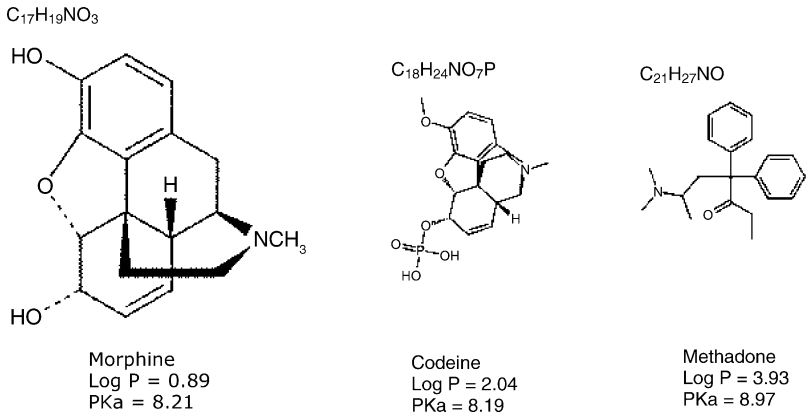

Fig. 1. Structure of the tested drugs and their pK_a and log $P_{o/w}$ values [8,38].

vents from the donor aqueous phase, but hydrophilic and polar analytes have low solubility in the water immiscible organic solvents. Therefore, these analytes are difficult to extract by three-phase LPME. For enhancing the analyte solubility in the organic solvents, we used non-ionic surfactants. It is well known that surfactant, or surface-active agents, are amphiphilic molecules, the head of which is polar, or hydrophilic, and the tail hydrophobic. The tail is generally a hydrocarbon chain with different member of carbon atoms and may be linear or branched, and also contain aromatic rings. The surfactant molecules can be associated in aqueous solution to form molecular aggregates called micelle, the minimum concentration of surfactant required for this phenomenon to occur is called critical micellar concentration (CMC). One of the most important properties of these compounds is their good capacity to solubilize solutes of different character and nature [14–16]. These solutes may interact electrostatically, hydrophobically or by a combination of both effects. This capacity of the surfactants to solubilize different compounds has been used to develop the extraction and the pre-concentration of organic compounds and for bio-analysis of different basic drugs as model compounds.

The model drugs, morphine, codeine and methadone were selected to present a broad range of hydrophilicity, see log $P_{o/w}$ values, Fig. 1.

2. Experimental

2.1. Chemicals and reagents

The drugs, methadone hydrochloride, codeine phosphate, were obtained from Sigma (St., Louis, Mo, USA). Morphine sulfate was obtained from H. Lundbeck (Copenhagen, Denmark) and all drugs were gifts from the Ministry of health and cure (Center of Khorasan, Iran) and administration of Justification (Khorasan, Iran). Methanol was purchased from Fluka (Buchs SG, Switzerland). The other compounds were from Merck (Darmstadt, Germany). Triton X-100 was obtained from Merck, Tween 20 and Nonoxynol-9 were from Sigma. These compounds were all of analytical grade. The Deionized water and solutions were filtered by a Milli-Q filtering system (Millipore).

2.2. Hair samples

A bulk of blank hair, necessary for method development and validation, was obtained from a men hairdresser's shop. The absence of opiate was verified.

Hair samples were collected from 20 men ranging from 16 to 45 years old. They were captured by the police and for most of them, screening tests were positive for drug of abuse. Some of the addicted persons were under therapeutic treatment.

A standard of hair of about 5 mm in diameter was cut from close to the scalp at the vertex posterior area, folded in aluminium foil, and the proximal and distal ends marked. Samples 2–4 cm long was selected for analysis.

2.3. Hair analysis

The hair, was washed with different solvents as follow: 20 ml dichloromethane, 15 ml acetone, 15 ml methanol, 10 ml methanol, at room temperature for 5 min and then it was dried. The last washing solvent was tested with GC for checking residual content of opiates.

2.4. Digestion of hair matrix

The washed and dried hairs was finally cut into approximately 1 mm pieces and digested by the following procedure; 2 ml methanol as an extracting solvent was added to 50 mg of hair, in a 10 ml screw-cap tube. The pH was adjusted to 7.4 by phosphate buffer solution. The samples were incubated at 50 °C for 5 h [17]. In case of a remaining solid matrix, extracts were filtered. The remaining was rinsed with 0.5 ml ethanol and both fractions were evaporated to dryness at 40 °C under a steam of nitrogen.

2.5. Stock and working solutions

Stock solutions containing 1 mg/ml of morphine sulfate, codeine phosphate and methadone hydrochloride were prepared, in methanol and stored at 4 °C. Standard calibration curves were obtained by adding calculated amounts of the standards into methanolic solution of 50 mg finely cut blank hair. These spiked samples were digested and the calibration curves were obtained. Limit of detection (LOD) and limit of quantification (LOQ) of the analytes were determined by decreasing concentrations of spiked samples until signal to noise ratio (S/N) of 3 and 10 were obtained, respectively.

The concentration of analytes in the hair blank samples for validation were 20, 50, 100, 300, 500, and 1000 ng/ml. All solutions stored at 4 °C and protected from light.

2.6. HPLC system

The HPLC system used in this work was a Waters (Millipore. Co, Milford, MA, USA) and consisted of a Waters (488) Tune able absorbance detector and a Waters 746 integrator.

The monolithic silica columns were evaluated in reversed-phase HPLC. These showed lower plate heights and much lower pressure drops [18,19] than the conventional columns packed with the 5 μm C_{18} silica particles. Therefore, we used of a Chromolith performance RP-18e column (4.6 mm diameter 100 mm length, 2 μm macro-pore size and 13 nm meso-pore size) from Merck (Darmstadt, Germany). A RP-18 guard column was fitted upstream of the analytical column.

The mobile phase consisting of 10 mM KH_2PO_4 at pH 3-acetonitrile (93:7) which was filtered by Milli-Q filtering system, was delivered by a Waters LC-600 HPLC pump.

The flow rate of the mobile phase was 3 ml/min and the UV detection wavelength was set at 211 nm.

2.7. LPME equipment

The experimental setup is illustrated in Fig. 2. 2.0 cm length of polypropylene hollow fiber (1200 μm I.D., a wall thickness of 150 μm, a pore size of 0.2 μm and a porosity of 70%) flame-sealed at the one end and was plunged into the organic solvent for 5 min to immobilize the pores, and then the excess of the solvent was removed. 3.0 ml sample solution (first phase) was held in a 5.0 ml sample vial, and the polypropylene hollow fiber, impregnated with the organic solvent (second phase) was adjusted and immersed in the sample solution, perfectly. Then, 10 μl the acceptor phase was added into the internal hole of the hollow fiber by a microsyringe.

The hollow fiber was shaped with a star liked profile using heat press. This shape was selected for increasing the contact area of the hollow fiber with donor and acceptor solutions and keeping volume of internal hole of the fiber at minimum.

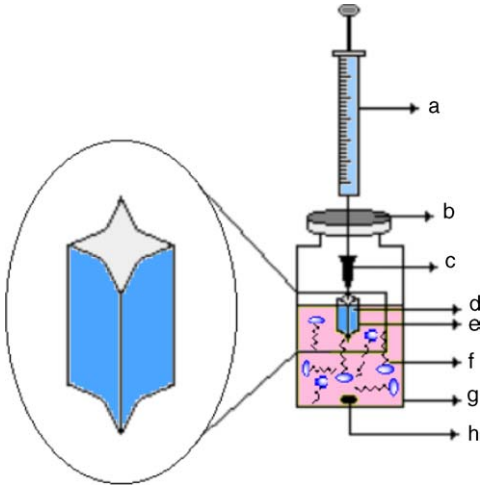

Fig. 2. SE-LPME extraction device: (a) HPLC syringe; (b) vial cover; (c) conical guide; (d) acceptor phase (pH 2.0); (e) 2 Cm, hollow fiber with star liked profile; (f) donor phase (pH 10.0); (g) glass vial and (h) stirring bar.

The length of the hollow fiber was reduced to 2.0 cm and the reduced length was compatible with small sample volumes, which are highly relevant in some analytes in the biomedical and environmental applications. In addition, enrichment of the analyte increases with increasing the volume ratio of sample solution to acceptor solution [20].

A conical guide was placed on the top of the fiber to ensure that the microsyringe needle was effectively guided into the fiber. A 25 μl micro-syringe, with a cone tip (0.49 mm O.D.) (Hamilton, Reno, NV, USA) was used for delivery and removal of the acceptor phase. Before each extraction, the syringe was rinsed with acetone and then with de-ionized water for 10 times to avoid the analyte carry-over and air bubble formation. Prior to use the fiber was dept into acetone for 3 h to remove the contaminations. An aluminium foil was used to cover the vial during extraction to prevent the evaporation of the organic phase. The solution was agitated with a stirring rate of 1000 rpm during the extraction process. Because of the fiber is very inexpensive, we used from any fiber in one period of extraction, thus was avoided the sample carry-over.

2.8. LPME procedure

Three millilitres of donor phase, with pH 10.0 (as described in Sections 2.4 and 2.5) was added into a 5.0 ml vial. The hollow fiber was dipped into *n*-octanol for 5.0 min and then the excess of the solvent was carefully removed. Subsequently, 10.0 μl of HCl solution (acceptor phase, pH 2) were injected into the lumen of the hollow fiber with a microsyringe. This fiber was placed into the sample solution present in the vial.

The samples were stirred at 1000 rpm for 40 min. After the extraction, the total volume of acceptor phase was injected into HPLC with monolithic column for further analysis.

2.9. Calculation of extraction recoveries and analyses enrichments

The extraction recovery (R), was calculated by the following equation:

$$R = \left(\frac{n_{\mathrm{a,final}}}{n_{\mathrm{s,initial}}}\right) 100\% = \left(\frac{V_{\mathrm{a}} C_{\mathrm{a,final}}}{V_{\mathrm{s}} C_{\mathrm{s,initial}}}\right) 100\% \quad (1)$$

where $n_{\mathrm{s,\,initial}}$ and $n_{\mathrm{a,\,final}}$ are the number of moles of analytes originally present in the sample and finally collected in the acceptor solution, respectively. V_{a}, is the volume of acceptor phase and V_{s}, the volume of sample, $C_{\mathrm{a,\,final}}$, the final concentration of analyte in the acceptor phase, and $C_{\mathrm{s,\,initial}}$, is the initial concentration of analyte within the sample.

The analyte enrichment factor (EF) was calculated by the following equation:

$$\mathrm{EF} = \frac{C_{\mathrm{a,final}}}{C_{\mathrm{s,initial}}} \quad (2)$$

These calculations are previously reported [4,21].

3. Results and discussion

3.1. Theoretical notations

The main aim of three-phase LPME is to increase enrichment and clean-up of analytes from environmental samples and biological fluids, prior to HPLC or CE. In this technique, the aqueous acceptor phase is injected directly into the HPLC or CE without further changes. These considerations affect the selection of phases as the most important factor in this technique.

In the LPME device, sample solution and acceptor phase are separated by membrane. The contact area between donor and organic phases is limited because of the large volume of sample respect to the organic phase immobilized in the pores of the hollow fiber. This situation limited the extraction and furthermore, some ionic analytes are highly water-soluble and have an insignificant tendency to migrate towards a lipophilic membrane. Therefore, the approach of adding the surfactants directly to the sample solution was investigated.

Surfactant enhanced transport through a liquid membrane depends on a number of parameters such as the nature and concentration of the surfactant, partition coefficient of the analyte under these conditions, properties of the organic solvent, sample agitation rate, etc.

The extraction in this mode is separated into three stages. The first involves extraction of the analyte from the sample solution to the organic phase immobilized in the pores of hollow fiber. The sample solution is added to buffer solution containing non-ionic surfactant. The buffer adjusted the pH of the sample solution to a pH where the analytes are uncharged and neutral, to advance formation of a hydrophobic species. In this experiment the sample solution has a pH of 10.

The second stage is extraction of the analyte into the membrane phase and diffusion of the analyte-surfactant through the membrane.

The third stage involves back extraction of analyte to the acceptor phase. In the meantime, at the interface between the donor and the organic phase, non-ionic surfactant molecules (near, but under the critical micellar concentration limit) gathered and enhanced the analyte transfer into the organic phase. The fraction of surfactant molecules, according to their size and tendency for organic solvent, also are transferred into the organic phase and in this phase, form reverse micelle. This phenomenon occurs because the volume of the organic solvent is smaller than the sample solution (donor phase) and surfactant enriched in this phase and raise up to the CMC. Thus the analyte dissolve strongly by the micelles and is prevented from the back extraction into the donor phase.

At the interface between the organic phase and the acceptor phase, the micelle releases the analyte into the acceptor phase. The acceptor solution has an acidic pH and the analyte molecules are ionized within the acceptor solution, they are prevented from re-entering the organic solvent in the pores of the hollow fiber. Since the volume of donor phase is very small, the analyte is pre-concentrated within the acceptor solution, the non-ionic surfactant has not inclination for going to the strong acidic acceptor phase.

In order to test this model several experiments were carried out. The initial experiments were based on mixing 3.0 ml of working solution containing the 1 μg/ml of analytes with pH 10.0. The volume of acceptor solution was 10.0 μl. Throughout the experiments the sample solution were stirred for 40.0 min.

3.2. Optimization of surfactant enhanced liquid-phase microextraction (SE-LPME)

In SE-LPME, extraction needs to be carried out under conditions in which the pre-concentration factor will be the maximum or the extraction yield will be 100%. This goal depends on the various factors and the extraction process can be also altered by different factors such as: organic solvent, extraction time, stirring rate, pH, concentration and nature of the surfactants, etc. The effect of these factors on the percentage of extraction of the analyses studied therefore needs to be established.

3.2.1. Organic solvent

Organic solvent to be immobilized as liquid membrane is an important agent in SE-LPME, and several factors have to be taken into consideration.

The analyte in the sample solution (donor phase) should have high partition coefficients into the organic solvent in the pores of the membrane. Solvents of low viscosity are preferred as low viscosity provides large diffusion through the membrane. In addition, the water solubility should be as

Table 1
Effect of organic solvent on the extraction recovery

Organic phase	Morphine	Recovery (%)	
		Codeine	Methadone
Benzyl alcohol	11	34	39
1-Octanol	39	48	67
Amyle alcohol	35	39	55
Iso-propanol	10	12	11
Dodecyl acetate	34	41	48

Sample solution: 3 ml of test solution containing analyses without surfactant, pH 10.0. Acceptor phase: 10.0 μl HCl, pH 2.0.

low as possible and the solvent should have a high boiling point avoid evaporation during experiment.

In this work, organic solvents such as benzyl alcohol, *n*-octanol, amyle alcohol, iso-propanol, dodecyl acetate, have been examined and *n*-octanol showed higher analyte enrichment than the others. The enrichment factors are presented in Table 1, and further studies were focused on *n*-octanol as organic liquid membrane.

3.2.2. Extraction time

The recovery percentage depends on the time that the analytes are in contact with the organic phase into the pores of hollow fiber and the acceptor solution. It has been reported that longer equilibration times do not have any significant effect on the extraction parameters [22,23] and in this work we observed that an equilibration time of 40.0 min is sufficient to obtain a good extraction. The results were showed in Fig. 3

3.2.3. pH and ionic strength

The extraction involves pH adjustment of the sample solution to a pH where the analyses are uncharged, because the analyses must be extracted through the organic solvent and uncharged molecules have a better tendency for going to organic membrane. The acceptor solution has a pH where the analytes are charged preventing them from back diffusion into the organic solvent. Also, in the donor phase, ionic form of a neutral molecule normally dose not interact with the non-ionic surfactant as strongly as its natural form, and a smaller amount of the analytes are therefore extracted [24,25]. Thus pH should be adjusted to ensure that the neutral molecular forms of the analytes are present prior performing the microextraction step. For practical applications, pH

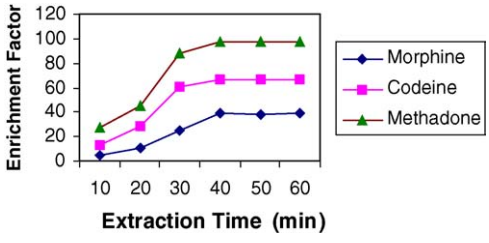

Fig. 3. The effect of extraction times, on the enrichment factors of drugs under SE-LPME procedure.

should differ from the pK_a values of the analyses by at least 2 units [32]. The influence of pH on recovery percentages is not very important for those compounds that do not present in ionic form. However, in the case of the hydrophilic drug when working with pH modified on pH 10.0, the percentage extraction is better for most solutes. Thus the pH 10.0, was selected for the sample solution, higher basic solution may cause degradation of the surfactant. The pH of the solution in acceptor phase should be kept at around 2.0. The pH in the acceptor phase is low to ensure that the non-ionic surfactant is not trapped within this phase.

The addition of inert salt can facilitate the phase separation process for some non-ionic surfactants with salting out effect [26,27]. Due to using NaCl–NaOH solution for pH adjustment of the donor phase (on pH 10.0), it is not necessary to use additional NaCl for salting effect, because NaCl has two roles in this case, one as part of buffering solution for pH control and the second role is as a salting out effect. It was examined that, the addition of excess amount of NaCl (2.5%) to the donor phase, was not significant effect on the recoveries of the drug analysis.

Furthermore, the results obtained indicated that the addition of salt cause an increase in the extraction of more polar solutes while the recovery of the less polar compounds are not affected [25,28].

3.2.4. Stirring rate

Stirring rate as we described before [29,30]. Facilitate the mass transfer of analyses through the phases, thus the sample solution were agitated at 1000 rpm, using a magnetic stirrer. The stirring rate of above 1000 rpm, was not suitable due to making air bubbles on the surface of the hollow fiber.

3.2.5. Nature and concentration of surfactant

A correct choice of surfactant is fundamental for obtaining a satisfactory pre-concentration and extraction process. When selecting the surfactant, consideration should be given to its interaction with the analytes and matrix, as well as the solubility of the analytes. So far, nonionic and zwitterionic surfactants have been applied for liquid-phase separation while the use of charged surfactants species is still in question [31]. The non-ionic surfactants could be a good choice for extracting of the drugs in our analysis (Table 2). In addition, the surfactant phase is compatible with the water–organic mobile phase usually employed in HPLC and in most cases, is UV transparent too.

Surfactant concentration is an important parameter for effective extraction. The extraction efficiency of relative non-polar organic compounds can reach to about 100% even when very low surfactant concentrations are used [31]. We have carried out a comparative study of three different nonionic surfactants, Triton X-100, Tween-20 and Nonoxynol-9, in the extraction and pre-concentration of abuse drugs, in the hair analysis. The results obtained indicated that Triton X-100 have been shown better result than the others and the enrichment factors can be increased as a function of the surfactant

Table 2
Characteristics of non-ionic surfactants

Surfactant	Commercial name	Linkage	CMC[a]
Polyoxyethylene 9-5 octylphenyl ether	Triton X-100	(*p*-Diisobutylphenoxy-polyethoxy)ether	0.24
Poly(oxy-1,2-ethanediyl),a-(4-nonylphenyl)-w-hydroxy	Nonoxynol-9	(Nonylphenoxy-polyethoxy ethanol)ether	0.085
Polyoxyethylene sorbitan monolaurate	Tween-20	(Polysorbate 20)ether-ester	0.065

[a] Critical micellar concentration (CMC) values given in mM [41,42].

concentration, but in the case of more polar compounds it will be more. We observed that, when the surfactant concentration, in the donor solution, was reached over its CMC, the extraction efficiency decreased sharply. It is due to great interaction of drug molecule with micelles. When an analyte is added into the micelle solution (in which, surfactant concentration is over the CMC) a fraction of it incorporated into the micelle and this complex cannot passes, completely through the hollow fiber pores.

Furthermore, high concentration of surfactant result relatively high viscosity that cause reduction of drug extraction in to organic phase. Consequently, the data shows that Triton X-100 with optimized concentration of 0.2 mM is the best surfactant for these drugs analysis. Results are shown in the Table 3 and Fig. 4.

3.3. Analytical performance

Chargeable compounds can be successfully extracted into three-phase SE-LPME with expanded applicability range. As reported by previous researchers [6,7,32], LPME may have a good potential for the extraction of drugs from biological fluids. Quantitative determination of drugs in hair is strongly dependent on the method of digestion for hair sample and also on the blank hair matrix used for calibration. We have analysed the blank and sample hair from the examinants who were from the same aged category and sexuality.

In order to further investigation the potential, the linearity was checked in the range 50–500 ng/ml for morphine and 10–500 for the other analytes in hair. The limit of detections, were 5–20 μg/l ($n = 7$, S/N; 3) following analysis by HPLC. Limit of quantifications were 16–66 μg/l ($n = 5$), (S/N; 10), too. Linearity was observed with $r^2 > 0.99$ for analytes. Thus the concentration of drugs in the final SE-LPME extract was directly proportional with the concentration of drugs in the hair sample, and indicated that SE-LPME may be utilized for quantitative analysis of drugs in hair.

Calibration curve parameters for abuse drugs were reported in Table 4. As was shown in Table 5, precision of the method was tested at three concentration levels, and the RSD ranged between 1.27 and 8.20. This was comparable with data obtained for plasma, whole blood and urine [7,23,33].

The recovery after SE-LPME was determined in each case of samples and illustrated in Table 5. SE-LPME provided recoveries in the range 62–93% (concentration 100 μg/l), Tables 5 and 6. The analyte enrichments of 57–118 times.

There have been many publications describing the determination of abuse drugs by liquid chromatography [34,35], GC–MS [36], LC–MS [37] and by capillary electrophoresis [38], but the major advantages of SE-LPME-HPLC are simplicity, high selectivity and recovery for these compounds. For example, optimized recovery for morphine in plasma, in a proficient research, by carrier-mediated transport microextraction coupled with electrophoresis, is 57% (RSD < 10%, $n = 3$) [11]. Or for morphine in plasma, in the same microextraction technique coupled with LC–MS, recovery is reported 59% [37]. In the other work, the overall percent mean recovery for (*R*)-methadone in plasma by GC–MS is 72.0 [39].

Table 3
The effect of different non-ionic surfactants on the recovery (%) of abuse drugs

	Recovery (%)		
	Triton X-100	Tween-20	Nonoxynol-9
Morphine	62	45	63
Codeine	86	51	75
Methadone	93	74	89

Test solution: 100 μg/l of morphine, codeine and methadone and Concentration of surfactants in the solution is, 0.05 mM. RSD ($n = 3$) < 10%.

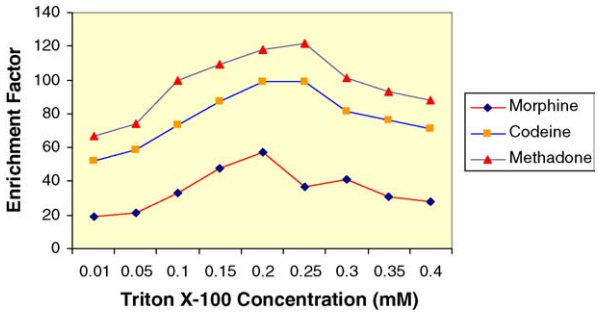

Fig. 4. The effect of surfactant concentration on the enrichment factors of drugs under SE-LPME procedure.

Table 4
Calibration curve parameters for abuse drugs

$n = 3$	Slope	Intercept
Morphine		
Mean	0.002108	−0.000121
SD	0.000516	0.004811
Codeine		
Mean	0.001924	−0.000553
SD	0.000372	0.002341
Methadone		
Mean	0.005962	−0.000816
SD	0.000369	0.002517

Table 5
Performance of the SE-LPME method

Compound	Enrichment factor	RSD (%)	Linear range (μg/l)	Correlation coefficient (r^2)	LOD (μg/l) ($n = 7$)	LOQ (μg/l) ($n = 5$)	Relative recovery (%) 50 μg/l[a]	Relative recovery (%) 100 μg/l[a]
Morphine	57	8.20	50–500	0.9982	20	66	58	62
Codeine	99	2.05	10–500	0.9996	5	17	80	86
Methadone	118	1.27	10–500	0.9997	5	16	89	93

SE-LPME under optimum conditions. The concentration of analytes in the hair blank samples for validation were 20, 50, 100, 300, 500, and 1000 μg/l.
[a] Final concentration of each analyte after spiking in hair samples.

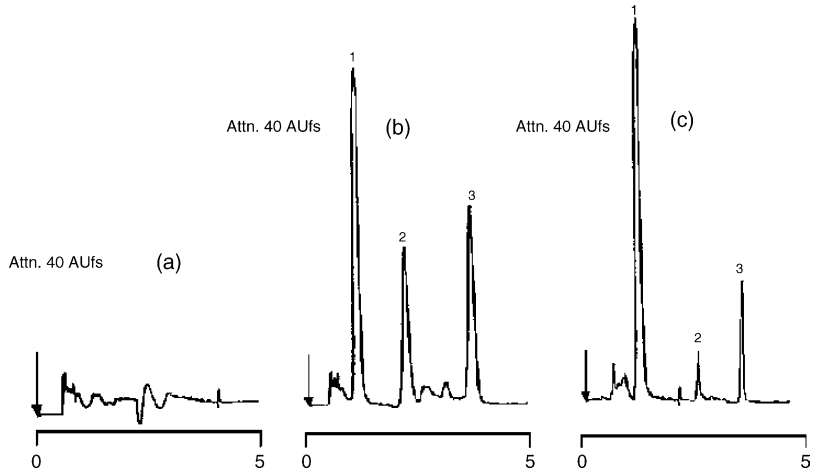

Fig. 5. HPLC-UV analysis of SE-LPME under optimum conditions: (1) morphine, (2) codeine and (3) methadone. (a) Drug free blank hair, (b) spiked blank hair with 50 mM of each analyte and (c) abuser sample hair.

Table 6
Concentrations (μg/l) of morphine, codeine and methadone in the hair of drug abusers

Compound	Concentration (μg/ml)	n	Precision (RSD%)
Morphine	64.43	20	7.16
Codeine	6.22	20	5.06
Methadone	33.21	20	2.27

Concentrations (μg/l) of morphine, codeine and methadone in the hair of drug abusers and the related chromatograms are shown in Table 6 and Fig. 5, respectively.

4. Conclusions

The aim of the present study was to develop and validate a rapid, sensitive, robust and reliable method for the quantitative determination of the drug abuse in human hair by HPLC and the results obtained with the method described above indicate that SE-LPME methodology is a good alternative extraction technique for hydrophilic drugs in hair and offers highly interesting advantages from an analytical point of view, such as possibility of extracting and pre-concentrating the analytes of different polarities.

Surfactants are less toxic and cheaper than the extractants used in LPME. The most commonly used surfactants are commercially available and, no analyte is lost in the process. The experimental operations involved in SE-LPME are very simple and the final surfactant-rich phase, if it could be diffused into the acceptor phase, is compatible with the mobile phase used in HPLC analysis. Moreover, this procedure offers several advantages over traditional extraction techniques such as; a reduction in extraction time (typically 20–45 min) this method is economical and easy to use.

In our method, we introduced a reliable qualitative and quantitative technique for abuse drug at low level of concentration in hair. In the mean time hair sample have some advantages over the other biological samples like urine and blood, such as long time of drug residence in the sample and low risk of side effect in transferring to examiner [40].

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