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

Mojtaba Shamsipur*, Nazir Fattahi

Department Chemistry, Razi University, Kermanshah, Iran

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,

* Corresponding author. Tel.: +98 21 66908033; fax: +98 21 66809030.
E-mail address: mshamsipur@yahoo.com (M. Shamsipur).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
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