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Combination of counter current salting-out homogenous liquid–liquid extraction and dispersive liquid–liquid microextraction as a novel microextraction of drugs in urine samples

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ABSTRACT

The counter current salting-out homogenous liquid-liquid extraction (CCSHLLE) joined with the dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME–SFO) has been developed as a high preconcentration technique for the determination of different drugs in urine samples. Amphetamines were employed as model compounds to assess the extraction procedure and were determined by high performance liquid chromatography–ultraviolet detection (HPLC–UV). In this method, initially, NaCl as a separation reagent is filled into a small column and a mixture of urine and acetonitrile is passed through the column. By passing the mixture, NaCl is dissolved and the fine droplets of acetonitrile are formed due to salting-out effect. The produced droplets go up through the remained mixture and collect as a separated layer. Then, the collected acetonitrile is removed with a syringe and mixed with 30.0 μL 1-undecanol (extraction solvent). In the second step, the 5.00 mL K_2CO_3 solution (2% w/v) is rapidly injected into the above mixture placed in a test tube for further DLLME–SFO. Under the optimum conditions, calibration curves are linear in the range of 1–3000 μg L^{-1} and limit of detections (LODs) are in the range of 0.5–2 μg L^{-1}. The extraction recoveries and enrichment factors ranged from 78 to 84% and 157 to 168, respectively. Repeatability (intra-day) and reproducibility (inter-day) of method based on seven replicate measurements of 100 μg L^{-1} of amphetamines were in the range of 3.5–4.5% and 4–5%, respectively. The method was successfully applied for the determination of amphetamines in the actual urine samples. The relative recoveries of urine samples spiked with amphetamine and methamphetamine are 90–108%.

1. Introduction

Sample preparation is an important analytical step especially for the determination of drugs in complex matrices, commonly encountered in biological analysis [1]. The solution to this problem emerged early with the use of separation and extraction techniques, which offered not only the ability to isolate the target drugs from the sample solution, thus reducing, controlling or even eliminating the interferences originally present, but also the opportunity for these drugs to be pre-concentrated and determined at very low levels [2]. The separation of drugs or medicines from biological matrices is one of the most important objects in investigations on the toxicological and pharmaceutical properties of drugs [3]. Among the biological samples, urine is the primarily preferred specimen for drug testing because specimen collection is simple and non-invasive and drugs and their metabolites tend to be present in relatively high concentrations [4]. However, urine matrices are very complex, and therefore, a suitable sample preparation method aimed at separating the matrix and enriching the target drugs is necessary to obtain the reliable analytical results.

Several procedures have been developed for the separation and preconcentration of different drugs of abuse from biological sample matrices, such as liquid–liquid extraction (LLE) [5], solid-phase extraction (SPE) [6–8], solid-phase microextraction (SPME) [9–11], liquid-phase microextraction (LPME) [12–14], supercritical fluid extraction (SFE) [15], stir-bar sorptive extraction (SBSE) [16] and dispersive liquid-liquid microextraction (DLLME) [17–20]. LLE and SPE are time-consuming and expensive, while LLE method requires high volume of potentially toxic organic solvents, which is hazardous to health. SPME is also expensive, its fiber is fragile and

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has limited lifetime and sample carry-over can be a problem. The disadvantages of LPME are as follows: fast stirring would tend to break up the organic drop; air bubble formation; extraction is time-consuming and equilibrium could not be attained after a long time in most cases. SFE and SBSE can also be relatively expensive and time-consuming [21]. In DLLME, the choice of the extraction solvent is its main drawback and solvents with the densities higher than water are required and further, they are not often compatible with reverse phase HPLC. In addition, the high density extraction solvents, being mostly halogenated, are generally hazardous to laboratory personnel and the environment [22,23].

Recently, a new microextraction method was developed, which is DLLME integrated with the solidification of a floating organic drop (DLLME–SFO) [24]. In DLLME–SFO, the extraction solvent after DLLME, was collected in the top of the test tube and was then cooled by inserting it into an ice bath for 5 min. The solidified extraction solvent was transferred into a suitable vial and immediately melted at room temperature; then it was finally injected into a suitable instrument. The performance of DLLME–SFO was illustrated by extraction of different organic and inorganic compounds [25–30]. In the previous research, we applied DLLME–SFO for extraction and preconcentration of amphetamines in urine samples [23]. Despite many benefits of the DLLME–SFO, the pretreatment and dilution of urine samples is its main drawback. Because of decrease in matrix effect, urine samples should be pretreated and diluted before DLLME–SFO.

Another extraction procedure, namely homogeneous liquid–liquid extraction (HLLE), utilizes a phase separation phenomenon in a homogeneous solution and a very small collected phase is resulted. One version of HLLE is salting-out homogenous liquid–liquid extraction (SHLLE) which has been used for extraction and preconcentration of the selected analytes from aqueous samples [31,32]. It is worthy to note that the enrichment factor using SHLLE is often low, which still cannot be satisfied for the requirement of the ultra-trace residue analysis. In principle, SHLLE combined with DLLME can provide a solution to this problem. Farajzadeh and co-workers introduced a new version of SHLLE, namely counter current salting–out homogenous liquid–liquid extraction (CCSHLLE) and its combination with DLLME for the extraction and preconcentration of some pesticides from fruit juices and aqueous samples [33,34]. Not only does the combination result in a high enrichment factor, but it can be also used in complex matrices.

The aim of this work is the combination of CCSHLLE and DLLME–SFO, as a sample-preparation method for high performance liquid chromatography (HPLC). Amphetamine (AP) and methamphetamine (MA) were chosen as model analytes to investigate the feasibility of the improved CCSHLLE–DLLME–SFO technique. To the best of our knowledge, for the first time, the CCSHLLE–DLLME–SFO is developed and applied to the analysis of amphetamines in human urine without pretreatment and dilution of the samples.

2. Experimental

2.1. Reagents and standards

Standards of amphetamines were obtained from Cerilliant (Round Rock, TX, USA) as 1 mg mL^{-1} methanol solutions. The amphetamines stock standard solution was prepared in methanol at the concentration levels of 1.00 mg L^{-1} for AP and MA. Afterwards, they were stored in a freezer at −20 °C. Working standard solutions were prepared daily by diluting the stock solution with methanol. The ultra-pure water (six times distilled) was purchased from Shahid Ghazi Company (Tabriz, Iran). Methanol (for spectroscopy), acetone (Suprasolv for gas chromatography), acetonitrile (Hyper grade for liquid chromatography), acetic acid, sodium dihydrogenphosphate, sodium dodecyl sulfate, sodium chloride, 1-undecanol, *n*-hexadecane, 2-dodecanol and 1-decanol were obtained from Merck (Darmstadt, Germany).

Drug free urine sample (blank) collected from healthy volunteer in our lab was used for the study. Actual human urine samples taken from four young people who were suspicious to consumption of amphetamines were stored at −20 °C and analyzed within 48 h after of collection without any previous treatment or filtration.

2.2. Instrumentation

Quantitative analysis of the amphetamines was performed on a Knauer HPLC system (Berlin, Germany) equipped with a Smartline-1000 binary pumps and Smartline-UV-2500 detector variable wavelength programmable, an on-line solvent vacuum degasser and manual sample injector fitted with a 20 μL injection loop (model 7725i, Rheodyne, Cotati, CA, USA). Chromatographic separation was achieved on an ODS-3 column (25 cm × 4.0 mm, with 5 μm particle size) from Waters (Milford, MA, USA). The mobile phase consisted of 80% buffer containing 10.0 mmol L^{-1} sodium phosphate monobasic and 0.50 mmol L^{-1} sodium dodecyl sulfate and 20% acetonitrile. The pH of the aqueous buffer in the mobile phase was adjusted to pH 5.5. 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 210 nm. The Hettich Zentrifugen (EBA20, Tuttlingen, Germany) was used for centrifugations. Chromatographic data were recorded and analyzed using Chromgate software version 3.1.

2.3. Extraction procedure

In the first step, a 10-mL glass syringe barrel was cleaned with pure water and then a frit was placed in the bottom of the barrel and installed a stopcock. Afterward 4 g NaCl was poured into the barrel and slightly compressed with the syringe plunger. A 5.0 mL of urine sample (spiked or not with amphetamines) was mixed with one milliliter acetonitrile and passed through the barrel at a flow rate of 0.6 mL min^{-1}. By passing the above homogenous solution through the barrel, fine droplets of acetonitrile were formed at the interface of solid (NaCl) and solution due to dissolution of salt into solution (salting-out effect). The produced droplets moved through the remained solution to top of the barrel and floated on the surface of solution as a separated layer due to lower density of acetonitrile with respect to water. During this step, the analytes were extracted into the fine droplets of acetonitrile. After passing all aqueous solution, the stopcock was closed. The volume of the acetonitrile (separated phase) on the top of remained NaCl solid was about 0.50 ± 0.03 mL. Subsequently, the organic phase obtained from the first step was transferred into a 10-mL glass test tube and 34.0 μL 1-undecanol (extraction solvent) was added to the test tube. Then, K_2CO_3 solution (2% w/v, 5.00 mL) were rapidly injected into a test tube, using a 5.00-mL syringe (gastight, Hamilton, Reno, NV, USA). A cloudy solution, resulting from the dispersion of the fine 1-undecanol droplets in the aqueous solution, was formed in the test tube and the mixtures were centrifuged for 4 min at 4200 g. Accordingly, the organic solvent droplet was floated on the surface of the aqueous solution due to its low density. The sample vial was there after put into an ice bath for 5 min; at this time, the floated solvent was solidified because of the low melting point (14 °C). The solidified solvent was transferred into a conical glass sample cup where it was melted immediately. Finally, 25 μL of the extractant was collected with a syringe and injected onto the HPLC–UV.