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Dispersive liquid–liquid microextraction prior to field-amplified sample injection for the sensitive analysis of 3,4-methylenedioxymethamphetamine, phencyclidine and lysergic acid diethylamide by capillary electrophoresis in human urine

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ABSTRACT

A novel capillary zone electrophoresis (CZE) with ultraviolet detection method has been developed and validated for the analysis of 3,4-methylenedioxymethamphetamine (MDMA), lysergic acid diethylamide (LSD) and phencyclidine (PCP) in human urine. The separation of these three analytes has been achieved in less than 8 min in a 72-cm effective length capillary with 50-μm internal diameter. 100 mM NaH_2PO_4/Na_2HPO_4, pH 6.0 has been employed as running buffer, and the separation has been carried out at temperature and voltage of 20 °C, and 25 kV, respectively. The three drugs have been detected at 205 nm. Field amplified sample injection (FASI) has been employed for on-line sample preconcentration. FASI basically consists in a mismatch between the electric conductivity of the sample and that of the running buffer and it is achieved by electrokinetically injecting the sample diluted in a solvent of lower conductivity than that of the carrier electrolyte. Ultrapure water resulted to be the better sample solvent to reach the greatest enhancement factor. Injection voltage and time have been optimized to 5 kV and 20 s, respectively. The irreproducibility associated to electrokinetic injection has been correcting by using tetracaine as internal standard. Dispersive liquid–liquid microextraction (DLLME) has been employed as sample treatment using experimental design and response surface methodology for the optimization of critical variables. Linear responses were found for MDMA, PCP and LSD in presence of urine matrix between 10.0 and 100 ng/mL approximately, and LODs of 1.00, 4.50, and 4.40 ng/mL were calculated for MDMA, PCP and LSD, respectively. The method has been successfully applied to the analysis of the three drugs of interest in human urine with satisfactory recovery percentages.

1. Introduction

Amphetamine and its derivatives are powerful stimulants of the central nervous system and they have a notorious reputation in the illicit drug market. Chronic use of amphetamines often leads to hallucinations and psychosis, as well as dysphoria and depression upon withdrawal [1]. It has been recognized an increased consumption amongst youth of methylenedioxy- and methoxy-substituted amphetamines, of which the pharmacology in humans is yet under investigation [2]. Renal excretion is the major elimination route of amphetamines, and because of that their plasma half-life is highly dependent on the acidity of the urine [3].

Lysergic acid diethylamide (LSD), is considered one of the most powerful hallucinogenic drugs. Small doses of LSD result in a number of psychotropic effects. After oral administration, LSD is extensively metabolized in the liver and less than 1% of the drug is eliminated unchanged in urine [4]. The determination of LSD in biological fluids is considered a challenging analytical problem for forensic laboratories due to the extremely low doses and its extensive metabolism. After ingestion of typical street doses (40–120 μg), the concentration of LSD in urine falls to <1.00 μg/L within few hours [4].

Phencyclidine (PCP) was initially developed in the 1950s by Parke Davis & Company as a dissociative anesthetic. In the 1950s it was tested as intravenous anesthetic but due to its severe side effects, it was withdrawn from the clinical use and employed in veterinary surgery until 1978. In the 1960s PCP became popular as a recreational drug. Symptomatic blood concentrations range from about 4.00 to 100 ng/mL. PCP is metabolized by the liver to

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various hydroxylated metabolites, but a portion of PCP is also excreted unchanged in urine. The cutoff concentration for both screening and confirmation is 25.0 ng/mL of unchanged drug [5]. PCP is a lipid soluble drug and its window of detection in urine is huge in comparison with other drugs. The mean detection window of PCP in urine after last use is 14 days [6].

It is usual in the analysis of drugs of abuse to perform first a screening of the samples, usually by immunological methods and then subjecting the positive samples to quantitative and confirmatory analysis. Liquid or gas chromatography coupled to mass spectrometry (LC–MS or GC–MS) or tandem mass spectrometry ($LC–MS^2$ or $GC–MS^2$) are by far the most widely employed techniques for this purpose [7].

Capillary electrophoresis (CE) presents potential advantages in forensic science [8–11], mainly for the determination of drugs of abuse in biological fluids and tissues [12], including rapidity of the method, minimal need of sample, negligible consumption of organic solvents, relatively low cost, high mass sensitivity and suitability for coupling with MS. Capillary zone electrophoresis (CZE) has demonstrated its capacity for the analysis of MDMA, PCP and LSD in human fluids coupled to UV [13,14], electrochemical [15] or MS-detection [16,17]. Non-aqueous capillary electrophoresis (NACE) and micellar electrokinetic chromatography (MEKC) are also reported as suitable modes for the analysis of MDMA [18] and LSD [19], respectively. Fluorescence detection is known to be highly selective and sensible and it has been successfully employed coupled to CE for the analysis of LSD [20].

Sensitivity is the main "Achilles heel" of CE. Among the different strategies to overcome the lack of sensitivity inherent to CE, on-line preconcentration techniques allow to increase analyte mass loading, with the subsequent decreasing of detections limits [21,22]. This can be done through the manipulation of the composition of sample and background solutions together with different injection procedures. Among these pre-concentration methods, sample stacking is an example of efficient method and it has been widely explained in the literature [21,22]. Namely, field amplified sample injection (FASI) is based on in a mismatch between the electric conductivity of the sample and that of the running buffer and it is achieved by injecting the sample diluted in a solvent of lower conductivity than that of the carrier electrolyte. Upon application of the voltage (electrokinetic injection) an enhancement of the electric field strength occurs in the low-conductivity zone, which induces an increase of electrophoretic velocities. When electrokinetic injection is performed in normal polarity mode, cations experience the high field strength, move rapidly and concentrate in sharp bands in the tip of the capillary at the boundary between the sample and the separation buffer. Once in the separation buffer, the injected components of the sample migrate in different zones according to their charge/mass characteristics [23]. FASI has been successfully applied to the analysis of drugs of abuse in biological specimens such as human urine [24], hair [25–27] and blood [16] and also in other samples like banknotes [28].

The most important prerequisite for achieving the high sensitivity associated with FASI is that samples need to be free of electrolytes. Biological samples naturally present high salt and ion contents which is seriously detrimental for FASI [29]. On-line or off-line liquid–liquid extraction (LLE) [15,30] and solid phase extraction (SPE) [31,32] have been the most traditionally employed pre-treatment methods for sample clean-up and preconcentration of the analytes. However LLE or SPE present drawbacks like large consumption of toxic organic solvents, and being highly time consuming. Solvent microextraction (ME) methodologies are the current trend. They are considered green sample pretreatment techniques, since they involve important reduction of the organic solvent to aqueous phase ratio of LLE, with the subsequent production of less toxic organic waste. Dispersive liquid–liquid microextraction (DLLME) can be cited within these green techniques and it is a quite novel ME technique, initially developed by Rezaee et al., based on ternary solvents [33]. Basically it consists in the rapid injection of an appropriate mixture of extractive solvent plus a disperser agent, into an aqueous sample, resulting in the formation of a cloudy solution. Analytes of interest are extracted into the interior of extractive solvent droplets.

The potential of DLLME has not been very exploited as sample treatment technique prior to CE, and only some environmental analysis applications can be cited: these comprise the analysis of mercury(II) [34], sulfonamides [35] or fluoroquinolones in water samples [36]. Forensic applications of DLLME/CE are scarce yet and it only can be cited the determination illicit drugs by DLLME/CE-UV in banknotes, kraft paper, plastic bags and aluminum paper [37] or a toxicological screening of urine by DLLME/CZE-TOF [38].

As stated above, the three drugs object of this study (MDMA, PCP and LSD) are extensively metabolized and only a small proportion of the consumed amount is excreted unchanged in urine. In this paper we have not looked for their metabolites, but only for the "father drugs", because we consider that it is also necessary the establishment of analytical methods focused in the detection of the small amounts of unchanged drug, both for expert valuation and pharmacological and metabolic studies.

A novel DLLME/FASI-CZE-UV method has been developed for the analysis of MDMA, PCP and LSD in human urine samples. Experimental design (central composite design) and the response surface methodology (RSM) have been used in the development of the DLLME procedure. The proposed methodology has been validated in terms of linearity, limits of detection and quantification, repeatability and intermediate precision, and applied to the analysis of MDMA, PCP and LSD in real urine samples at the ng/mL level.

2. Materials and methods

2.1. Chemicals

Different buffer solutions at different concentrations and pH values were employed while optimizing the electrophoretic separation. The pH of all of them was adjusted by the addition of appropriate volumes of diluted solutions of sodium hydroxide or the corresponding acid.

Standard solutions containing 1000 mg/mL of (±)3,4-methylenedioxymethamphetamine in methanol, phencyclidine in methanol and lysergic acid diethylamide in acetonitrile were purchased from Cerilliant (Cerilliant Corp., TX, USA). Each standard solution was provided in an amber ampoule. As soon as they were received, the content was transferred to a 1.5-mL amber vial with hermetic close and stored at −20 °C. Tetracaine hydrochloride, employed as internal standard (I.S.) was purchased from Sigma (Sigma–Aldrich Co., Steinheim, Germany).

2.2. Instrumentation and software

Capillary zone electrophoresis experiments were performed with an HP^{3D} CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector. The software provided with the HP Chemstation version A.09.01 was used for instrument control and data acquisition.

All pH measurements were carried out with a pH meter (Crison model pH 2000, Barcelona, Spain) with a resolution of ±0.01 pH unit.

The software package The Unscrambler (v. 9.8, Camo AS, Oslo, Norway), running under Windows Vista, was used for the application of chemometrics.