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Research article

Simultaneous determination of cocaine/crack and its metabolites in oral fluid, urine and plasma by liquid chromatography-mass spectrometry and its application in drug users

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ABSTRACT

Introduction: A single LC-MS equipment was used to validate three methods for simultaneously analyzing cocaine (COC), benzoylecgonine (BZE), cocaethylene (CE), anhydroecgonine methyl ester (AEME) and anhydroecgonine (AEC) in oral fluid (OF), urine and plasma.
Methods: The methods were carried out using a Kinetex HILIC column for polar compounds at 30 °C. Mobile phase with isocratic condition of acetonitrile: 13 mM ammonium acetate pH 6.0: methanol (55:35:10 v/v/v) at 0.8 mL/min flow rate was used.
Results: After buffer dilution (OF) and protein precipitation (urine and plasma), calibration curve ranges were 4.25–544 ng/mL for oral fluid and 5–320 ng/mL for urine and plasma with correlation coefficients (r) between 0.9947 and 0.9992. The lowest concentration of the calibration curves were the lower limit of quantification. No major matrix effect could be noted, demonstrating the efficiency of the cleaning procedure.
Discussion: The methods were fully validated and proved to be suitable for analysis of 124 cocaine and/or crack cocaine users. Among the subjects, 56.5% reported daily use of cocaine in the previous three months. Results show a high prevalence of the analytes, with BZE as the most prevalent (94 cases), followed by COC (93 cases), AEC (70 cases), CE (33 cases) and AEME (13 cases). In addition, the concentration of BZE in urine was higher compared to OF and plasma found in the real samples, showing the facility of accumulation in chronic users in matrices with a large detection window.

1. Introduction

Drugs of abuse have extended to be a worldwide problem. According to the World Drug Report 2015 (UNODC, 2015), it is estimated that 5.2% (range: 3.4–7.0%) of the world's population used an illicit drug in 2013 - almost a quart of a billion people. In South America, cocaine is still one of the drugs of most concern, mainly because its consumption continues to increase as opposed to most either regions worldwide - where the consumption remains stable or decreases. The most important producers are Colombia, Peru and Bolivia. Brazil has the largest cocaine market in South America due to its large cocaine consumption and its geographical position, which makes it a convenient gateway to traffic cocaine to Europe.

The use of validated analytical methods to detect drugs in biological fluids is an important tool in the actual scenario. Several matrices, such as blood, urine, oral fluid, sweat, hair, nail and vitreous humor can be used for this purpose. Although there are several matrices to be used, the most common are blood components (whole blood, plasma, serum) and urine. Blood is considered the “gold-standard” for analysis due to a good correlation between blood drug concentrations and pharmacological effects (Langel et al., 2013), while urine is mainly used to monitor illicit drug use in drug treatment, criminal justice, and workplace drug-testing, since it provides a long detection window for drug abuse (Dams, Murphy, Lambert, & Huestis, 2003). The main disadvantages of blood

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and urine are the difficulty and risks to collect and the easy adulteration, respectively. Oral fluid has been highlighted recently because of its many advantages, such as: it is easy and non-invasive to collect by professionals and non-professionals, and has a good correlation with blood regarding several classes of drugs. Disadvantages include small volume of samples and low drug concentrations, which demands high method sensitivity (Chu et al., 2012; Concheiro, Gray, Shakleya, & Huestis, 2010; Martí-Álamo, Mancheño-Franch, Marzal-Gamarra, & Carlos-Fabuel, 2012; Montesano et al., 2015). Together, the three matrices are strongly recommended for screening and confirmatory analyses in forensic toxicology.

Currently, hyphenated chromatographic techniques are the most used to analyze cocaine in oral fluid, urine and plasma (Cardona, Chaturvedi, Soper, & Canfield, 2006; Institóris, Angyal, Árok, Kereszty, & Varga, 2012; Johansen & Bathia, 2007; Liu et al., 2014; Montesano et al., 2015). Liquid chromatography coupled to mass detector (LC–MS) has some notable differences from gas chromatography coupled to mass detector (GC–MS) such as its capacity to analyze polar, non-volatile and thermally labile compounds (all characteristics of cocaine and derivatives). The same analysis by GC–MS would require a lengthy derivatization procedure and sample preparation (Couchman & Morgan, 2011; Perez et al., 2016; Stout, Bynum, Mitchell, Baylor, & Ropero-Miller, 2009). There are many methods in literature that analyze anhydroecgonine methyl ester (AEME) and anhydroecgonine (AEC), the main pyrolysis products of cocaine (COC) in oral fluid, urine and plasma by gas chromatography (Carvalho, Chasin, & Carvalho, 2008; Cognard, Bouchonnet, & Staub, 2006; Jager & Andrews, 2001; Jager & Andrews, 2002; Paul, Lalani, Bosy, Jacobs, & Huestis, 2005; Wang, Darwin, & Cone, 1994). It has been demonstrated that COC thermally degrades during gas chromatography particularly at high gas chromatograph injector port temperatures (> 210 °C) (Bell & Nida, 2015; Cardona et al., 2006; González, Carnicero, de la Torre, Ortuño, & Segura, 1995; Kraemer & Paul, 2007; Toennes, Fandino, Hesse, & Kauert, 2003) which does not occur when using liquid chromatography. Few studies have analyzed pyrolysis products in liquid chromatography tandem mass spectrometry (Concheiro et al., 2010; Dams et al., 2003; Jeppesen, Busch-Nielsen, Larsen, & Breindahl, 2015; Langman, Bjergum, Williamson, & Crow, 2009) and the majority of them performed extraction using SPE, however, there are no methods published in a single-stage LC-MS with no sample extraction to this purpose. Therefore, the aim of the present study was to validate and apply three methods to analyze cocaine/crack cocaine and its metabolites in biological samples by LC-MS prioritizing fastness, robustness, sensitivity and low-cost.

2. Materials and methods

2.1. Chemicals and reagents

Standards of cocaine hydrochloride (COC), benzoylecgonine (BZE), anhydroecgonine methyl ester (AEME) and anhydroecgonine (AEC) were donated by the Instituto Nacional de Criminalística (Brasília, DF, Brazil) through an official partnership. Cocaethylene (CE) and cocaethylene D-3 (CE-D3) standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol and ammonium acetate were obtained from Merck (Frankfurt, Germany), all with analytical grade. Ultrapure water was obtained using a Milli-Q Plus system of Millipore (Bedford, MA, USA). “Multi Drugs” devices to collect oral fluid were obtained from Alere Inc. (Massachusetts, USA). Oral fluid, urine and plasma drug-free samples were donated by volunteers.

2.2. Equipment

Agilent 1260 infinity LC system equipped with G1311B quaternary pump, G1329B autosampler, G1314F UV/VIS detector and G1316A thermostatizer coupled to Agilent 6120B series mass detector was used (Agilent Technologies, Palo Alto, CA, USA). Chemstation software (v. B.04.03) was used for data analysis. Eppendorf centrifuge, model 5430R (Hamburg, Germany) was used to prepare the samples.

2.3. Preparation of reference solutions

Stock solutions of COC, BZE, CE and AEME were prepared at 1 mg/mL in acetonitrile and AEC was prepared at 1 mg/mL in methanol. Work solutions were prepared by dilution in a mixture of ultrapure water and acetonitrile (80:20). All solutions were kept at − 20 ± 2 °C. Calibration curves were prepared daily by adding known concentrations of work solutions to 100 μL (oral fluid) and 200 μL (urine and plasma) drug-free matrices. Quality controls were prepared at 4.25 ng/mL (lower limit of quantification - LLOQ), 12.75 ng/mL (low quality control - LQC), 85.00 ng/mL (middle quality control - MQC), 442.00 ng/mL (high quality control - HQC) and 1088.00 ng/mL diluted to 272.00 ng/mL (dilution quality control - DQC) in drug-free oral fluid. Urine and plasma quality controls were prepared at concentrations of 5.00 ng/mL (lower limit of quantification - LLOQ), 15.00 ng/mL (low quality control - LQC), 50.00 ng/mL (middle quality control - MQC), 260.00 ng/mL (high quality control - HQC) and 640.00 ng/mL diluted to 160.00 ng/mL (dilution quality control – DQC).

2.4. Sample preparation

2.4.1. Oral fluid

An aliquot of one hundred microliters of oral fluid (blank, calibrators, quality controls and real samples) were added to polypropylene conical tubes (1.5 mL) with 10 μL of IS, 70 μL of buffer and tubes were vortex for 20 s. Samples were centrifuged at 14.000 rpm at 4 °C for 20 min. An aliquot of 100 μL of supernatant was transferred directly into a vial.

2.4.2. Urine

An aliquot of two hundred microliters of urine (blank, calibrators, quality controls and real samples) were added to polypropylene conical tubes (1.5 mL) with 10 μL of IS, 100 μL of acetonitrile and tubes were vortex for 20 s. Samples were centrifuged at 14.000 rpm at 4 °C for 30 min and the supernatant (100 μL) filtered (PTFE 13 mm 0.22 μm) directly into a vial.

2.4.3. Plasma

An aliquot of two hundred microliters of plasma (blank, calibrators, quality controls and real samples) were added to polypropylene conical tubes (1.5 mL) with 10 μL of IS, 400 μL of acetonitrile and tubes were vortex for 20 s. Samples were centrifuged at 14.000 rpm at 4 °C for 30 min and the supernatant (100 μL) filtered (PTFE 13 mm 0.22 μm) directly into a vial.

2.5. Ethics

This study was formally approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (14-0266). A verbal and signed consent was obtained from all volunteers after detailed explanation of the research process, and accepted for participation assuring that all the data were kept confidential and anonymous.

2.6. Liquid chromatography–mass spectrometry (LC–MS)

All parameters and specifications of the equipment and mobile phase were adopted from a previously validated method developed by our research group for another biological matrix (D'Avila et al., 2015). The column used in the analyses was a Phenomenex Kinetex HILIC (150 mm × 4.6 mm, particle size of 2.6 μm) (Torrance, CA, USA) maintained at 30 °C. The flow used was 0.8 mL/min, mobile phase consisting of acetonitrile: ammonium acetate 13 mM pH 6.0: methanol