K16 The Development and Validation of a Method for Cocaine/Crack Cocaine Biomarkers in Human Oral Fluid, Urine, and Plasma by Liquid Chromatography/Mass Spectrometry (LC/MS) and Its Application in Drug Users

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After attending this presentation, attendees will be able to implement a method for cocaine/crack cocaine biomarkers in three biological matrices and assess analytical results from the analysis of plasma, urine, and oral fluid within a population of drug users.

This presentation will impact the forensic science community by providing simpler and faster extraction techniques made possible with LC/MS, which will provide evidence of analytical methods capable of quantifying the target compounds and also provide data on the temporal trends of cocaine/crack cocaine use within this population.

Drugs of abuse, including cocaine, are responsible for many social and economic problems worldwide. Within a global context, Brazil has a negative role in the cocaine market and is known as a trafficking country, besides having a high rate of cocaine and crack/cocaine users. Liquid chromatography coupled to mass detector (LC/MS) has some notable differences from gas chromatography coupled to mass spectrometry (GC/MS) such as its capacity to analyze polar, non-volatile, and thermally labile compounds.[1,2] There are methods in the literature for the analyses of the pyrolysis products of cocaine anhydroecgonine methyl ester (AEME) and anhydroecgonine (AEC) in oral fluid, urine, and plasma by GC/MS and LC/MS/MS but there are no methods that use a single-stage LC/MS.[3,4] The goal of this research was to develop, validate, and apply three bioanalytical methods in the analysis of cocaine (COC), benzoylecgonine (BZE), cocaethylene (CE), AEME, and AEC in oral fluid, urine, and plasma by LC/MS prioritizing speed of analysis, robustness, and low-cost.

Validation experiments were performed on an Agilent LC 1260 coupled to an Agilent 6120B mass spectrometer operating in ESI+ mode using a Kinetex HILIC column Phenomenex (150mm x 4.6mm, 2.6mm) at 30°C. Isocratic elution of ACN:MeOH:CH_3COONH_4, 13mm, pH 6.0 (55:10:35) as mobile phase, flow as set to 0.8mL/min with a total run of 13 minutes and injection volume was 10µL. Samples were prepared by buffer dilution (oral fluid) and protein precipitation using acetonitrile (urine and plasma), followed by centrifugation, filtration, and injection. The methods were validated following the guidelines set forth by the RDC 27/2012 (ANVISA) and supplemented by SWGTOX and FDA guidelines.

Cocaine and/or crack cocaine users (*n*=124) were recruited at service centers specializing in drug addiction in the city of Porto Alegre, Brazil. The study received institutional review approval for human subject studies. Data collection was obtained through interviews conducted within the first 24 hours after admission. Subjects were asked to donate samples of blood, urine, and oral fluid. All biological samples were collected at the same time range (mean interval of 1 hour ± 20min). Samples were stored at -80 ± 2°C until analysis.

Calibration curves were linear in the range of 4.5ng/mL to 544ng/mL (oral fluid) and 5ng/mL to 320ng/mL (urine and plasma) and the experimental detection limit ranged from 1ng/mL to 3.4ng/mL for all analytes. The

between run variability ranged from 0.9% to 8.5% for the low control, and 0.6% to 13.5% for the high control. For the low and high controls respectively, accuracy ranged from 0.7 to 13.6% and 0.1% to 12.8%. The method was free from carryover, interferences from matrix effects, and interferences from commonly encountered related analytes (*n*=10).

The sample comprised of 118 males and 6 females (mean age 34 ± 9, 47.6% Caucasians, 56.5% unmarried). Subjects were mostly low-educated (62.9% with less than eight years of schooling) and unemployed (58%). Regarding substance abuse, the majority of the subjects (56.5%) reported daily use of cocaine in the previous three months. COC was detected in 93 subjects, BZE in 94, CE in 33, AEME in 13 and AEC in 70 subjects. In 37.6% of the cases where COC was detected, its use was confirmed in all three matrices. BZE was detected in three matrices in almost half of the total samples (45.7%). The prevalence decreases in crack metabolites (AEME and AEC) and CE, which could be detected more frequently only in urine.

The methods were successfully validated and proved to be suitable to detect and quantify cocaine in oral fluid, urine and plasma, in all types of utilization: salt form (COC and BZE biomarkers), alkaline form (AEME and AEC biomarkers), and concomitant use with ethanol (CE).

Reference(s):

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Cocaine, LC/MS, Drug Users