The validation and use of an accredited routine method for the simultaneous analysis of cocaine and two breakdown products in oral fluid by LC-MS-MS

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AIMS: The analysis of drugs of abuse in oral fluid poses a challenge due to limited sample volume available for analysis and the requirement to detect drugs at lower concentrations. The advent of tandem mass spectrometric detection techniques has enabled analysts to meet both these challenges. The aim of the work described here was to develop a sensitive and robust method suitable for the routine analysis of cocaine, benzoylecgonine and cocaethylene in oral fluid in an accredited laboratory.

METHODS: The analytes of interest were extracted by solid phase extraction (SPE) using a mixed mode SPE cartridge. Analysis was carried out using a Varian LC-MS-MS. Each analyte was determined by multi reaction monitoring (MRM) of two transitions per ion (cocaine m/z 304 to 182 and 105; benzoylecgonine m/z 290 to 168 and 105; cocaethylene m/z 318 to 196 and 150). Deuterated internal standards were used for the quantitation of each analyte. Calibration standards at 0, 15, 30, 60 120 and 180 ng/ml were used, and each sample, standard and control was spiked with deuterated internal standard at 120 ng/ml. The validation of the method involved determination of linearity; detection and quantitation limits; robustness; assay drift and the precision of the method. The method was used for the analysis of 437 samples from criminal justice sources.

RESULTS: The method is linear for each analyte over the range of LOD – 180 ng/ml. The detection limit for each analyte is 1 ng/ml, with the quantitation limit as low as 2 ng/ml. The retention time of the peaks of interest varied by as little as 0.16% and the transition ion ratio variation was a low as 2.2%. The assay drift was less than 3%. The intra-assay precision was approximately 3% and the inter-assay precision 4%. Following analysis of 437 samples using this method, 60.9% were negative; 27.2% were positive for benzoylecgonine alone; 11.4% were positive for benzoylecgonine and cocaine; 0.5% was positive for all three analytes.

CONCLUSION: This method is suitably sensitive and robust for the routine analysis of cocaine and metabolites in oral fluid in an accredited laboratory. There is scope for future development of this method to include the pyrolysis products of cocaine present following the use of crack cocaine, and to decrease the sample volume used for analysis.

KEYWORDS: *Cocaine, Oral fluid, LC-MS-MS*

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