K3 A Validated PCI GC/MS Method for the Quantification of Amphetamine, Opiates, Cocaine and Metabolites in Human Postmortem Brain

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After attending this presentation, attendees will learn about a sensitive and specific method for the simultaneous detection and quantification of amphetamine, opiates, cocaine, and cocaine metabolites. The presentation will allow an attendee to evaluate the performance characteristics and implement the assay in their laboratory.

This presentation will impact the forensic community and/or humanity by demonstrating an assay which provides reproducible recovery and quantification of amphetamine, morphine, codeine, 6-acetylmorphine, cocaine, benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, and anhydroecgonine methyl ester in human brain tissue. The assay has application in forensic and postmortem toxicology laboratories.

Determination of drug concentrations in human brain has applications in forensic and postmortem toxicology and in biological studies of cellular responses to drug exposure. Direct measurement of drug and metabolite concentrations in discrete brain regions also is used to study mechanisms of drug action, regional distribution, and preferential accumulation of drugs. Most quantification methods have focused on a single class of drugs, such as cocaine, amphetamines, or opiates. The objective of this study was to develop and validate a reliable extraction and quantification method for multiple classes of drugs in brain tissue.

The method employs ultrasonic homogenization of brain tissue in pH 4.0 sodium acetate buffer and solid phase extraction (SPE) utilizing copolymeric octyl/benzyl sulfonic acid extraction columns. Extracts were concentrated and derivatized with *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) and *N,O-bis*(trimethyl) trifluoroacetamide (BSTFA). GC/MS analyses were performed with an Agilent 6890 gas chromatograph interfaced with a 5973 mass-selective detector. Analyte separation was achieved on an HP-1MS capillary column (30 m x 0.32 mm i.d., 0.25 µm film thickness) with helium carrier gas. Initial column temperature of 70^{O}C was held for 1.00 min, increased to 175^{O}C at 30^{O}/min, ramped to 250^{O}C at 23^{O}/min and increased to a final temperature of 310^{O}C at 18^{O}/min that was held for 5.00 min. The MS was operated in PCI mode with methane reactant gas. Target and qualifier ions acquired for each analyte and deuterated internal standard were: amphetamine 158, 250; amphetamine-*d10* 162, 245; ecgonine methyl ester 314, 256; ecgonine methyl ester-*d3* 317, 259; anhydroecgonine methyl ester 182, 210; ecgonine ethyl ester 328, 196; cocaine 304, 182; cocaine-*d3* 307, 185; cocaethylene 318, 196; cocaethylene-*d3* 321, 199; codeine 282, 356; codeine-*d3* 285, 359; benzoylecgonine 404, 282; benzoylecgonine-*d3* 407, 285; morphine 456, 382; morphine-*d3* 459, 385; 6-acetylmorphine 382, 470; and 6-acetylmorphine-*d3* 385, 473, respectively.

Developing a validated method for simultaneous quantification of multiple drug analytes in human brain required optimization of numerous factors. First, a technique for successful tissue disruption coupled with an efficient extraction methodology was required. This was addressed by brief ultrasonic homogenization of 0.10 g of tissue in pH 4.0 sodium acetate buffer followed by centrifugation. SPE was rapid and reproducible with suitable recoveries, and required small volumes of organic solvents. Second, the need to quantify multiple analytes at low concentrations required reliable chromatographic separation of analytes and a suitably specific and sensitive detection method. A third objective was to utilize instrumentation readily available in most research and forensic toxicology laboratories, which was met by a bench-top GC/MS operated in PCI mode. Each analyte was adequately resolved from other analytes or from tested interferents with the chromatographic parameters described. Positive chemical ionization GC/MS in SIM mode provided sensitive and specific quantification.

Linearity, carryover, limits of detection and quantification, selectivity, extraction efficiency, precision and accuracy were investigated to evaluate method integrity. The limits of detection and limits of quantification for all analytes were 50 pg/mg of brain. Calibration curves were linear to 1000 pg/mg for anhydroecgonine methyl ester and 6-acetylmorphine, and to 2000 pg/mg for all other analytes. Precision and accuracy were evaluated over the linear range with four QC materials at target concentrations of 120, 240, 480, and 1600 pg/mg. Accurate quantification and precision is achieved over the linear dynamic range of the assay with accuracy ranging from 89.5% to 113.7%, and inter-assay precision, as percent relative standard deviation, ranging from 3.0 to 16.6%.

The method provided adequate and reproducible recovery of amphetamine, morphine, codeine, 6-acetylmorphine, cocaine, benzoylecgonine, ecgonine methyl ester, ecgonine ethyl ester, cocaethylene, and

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anhydroecgonine methyl ester from human brain tissue. The assay was developed to identify and quantify drugs in human postmortem brain tissue and to identify drug users and validate controls for microarray analysis of the transcriptional neurobiology of drug abuse.

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