K11 Analysis of Amphetamine and Methamphetamine in Whole Blood by Solid Phase Extraction and Gas Chromatography - Mass Spectrometry

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Attendees will learn an acceptable method for the solid-phase extraction of amphetamine and methamphetamine from blood matrix as an alternative to liquid/liquid extraction methods.

This presentation will impact the forensic community and/or humanity by demonstrating a solid-phase extraction procedure, which yields acceptable results, is time efficient (taking an average of 2.5 hours from start to completion), and requires a small amount of sample and limited amounts of organic solvents.

The purpose of this study was to demonstrate that the Cerex Polycrom Clin II solid-phase extraction column (SPEware, San Pedro, CA) provides acceptable extraction of amphetamine (AMP) and methamphetamine (MAMP) from whole blood. The Cerex Polycrom Clin II solid phase extraction (SPE) column is packed with a patented divinylbenzene polymer that is highly cross-linked and functionalized to perform in dual mode utilizing hydrophobic and cation exchange mechanisms.

The procedure requires pre-treatment of the blood samples (1 mL) with 2 mL of a phosphate buffer (pH 6), vortex mixing for 30 seconds, followed by sonication for 10 minutes, and then centrifugation for 6 minutes. Samples were then added to the SPE columns and washed with deionized water, pH 6-phosphate buffer, methanol, and ethyl acetate. During the wash procedures, the samples were vacuumed at 2-5 psi. The columns were dried at full vacuum for 3 minutes and then eluted with ethyl acetate containing 2% concentrated ammonium hydroxide. A solution of 1% HCl in methanol was added to the extracts, vortex mixed, and evaporated to dryness. The dried residues were derivatized with acetic anhydride and transferred to auto-sampler vials and analyzed by GC/MS (HP6890 GC, HP5973 MS) utilizing selected ion monitoring. Deuterated internal standards were used for the quantitation of AMP and MAMP. Ions monitored for the acetyl derivatives were (underlined ions are used for quantitation): AMP: 118, 44, 177; d_{11}-AMP: 128, 48, 188; MAMP: 100, 58, 191: d_{14}-MAMP: 107, 65, 205.

The linearity study exhibited an upper limit of linearity at 4000 ng/mL, and lower limit of detection and quantitation at 10 ng/mL for both analytes. Correlation coefficients were 0.9994 and 0.9993 for AMP and METH, respectively. Daily linear regression calibration curves for each analyte yielded correlation coefficients of 0.9995 or greater along the dynamic range. Carryover was not observed at 10,000 ng/mL. Extraction efficiencies at 100 ng/mL averaged 92% and 91% for AMP and MAMP, respectively. Precision was evaluated on three separate days at 50 ng/mL and 200 ng/mL with 5 replicates at each concentration. The within-run precision yielded average responses from 47 – 51 ng/mL (%CV 1.1 – 5.8) and 202 – 207 ng/mL (%CV 3.9 – 8.6) for AMP, and 47 – 55 ng/mL (%CV – 4.6) and 199 – 205 ng/mL (%CV 3.8 – 8.7) for MAMP. The between run precision for AMP produced CV results of 5.5% and 6.0% at the 50 ng/mL and 200 ng/mL levels respectively. The between-run precision for MAMP produced CV results of 7.3% and 6.1% at the 50 ng/mL and 200 ng/mL levels, respectively.

A small comparative study was conducted using this SPE procedure on preserved whole blood samples that had been previously analyzed by a liquid-liquid extraction method. Good agreement was observed between these two procedures. Correlation studies yielded correlation coefficients of 0.9732 for AMP and 0.9966 for MAMP. The results of the comparative study were analyzed statistically using a two-tailed Student's t-test. The calculated t values were 1.714 for AMP and 0.5068 for MAMP and the critical t values were 2.086 for AMP and 2.048 for MAMP at the 95% confidence level. The t-test indicates there is no significant statistical difference between the results from the two methods.

An interference study was conducted using a blank control and spiked blood samples at 50 ng/mL of AMP and MAMP. The following drugs were added to these controls at a concentration of 10,000 ng/mL: 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxy-N-ethyl-amphetamine, phenylpropanolamine, ephedrine, pseudoephedrine, propylhexadrine, fenfluramine, mecathinone, and p-methoxymethamphetamine. Phentermine, propylhexadrine, and fenfluramine were found to cause interferences with chromatography of the target analytes. If these drugs are anticipated, an alternative derivatization process, such as a fluorinated derivative, can be used to resolve these interferences.

This solid-phase extraction procedure yields acceptable results, is time efficient (taking an average of 2.5 hours from start to completion), and requires a small amount of sample and limited amounts of organic solvents.

Solid Phase Extraction, GC/MS, Methamphetamine

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