K1 Hair Analysis of Amphetamine Using SPE and LC/MS/MS

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After attending this presentation, attendees will learn about the extraction of amphetamine (and related compounds) in hair using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to provide data on these compounds in hair samples.

This presentation will impact the forensic science community by offering analysts in forensic facilities a method that permits small samples of hair to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both LC/MS/MS and solid phase extraction.

Method: Extraction (SPE) was performed on mixed mode column (C8/SCX) conditioned with methanol, deionized water, and pH 6 buffer (3mL, 3mL and 1mL, respectively) prior to sample loading. Samples of decontaminated hair (10mg) were digested in 1M NaOH (containing deuterated analogues) for one hour at 70°C. The samples were cooled and glacial acetic acid (100µL) was added. Each solution was adjusted to pH 6 with 0.1M phosphate buffer (4mL) and applied to a conditioned SPE column. After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1M), and methanol (3mL of each, respectively). Each SPE column was dried and eluted with 3mL of a solvent consisting of methylene chloride/isopropanol/ ammonium hydroxide (78:20:2). After elution, 200µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC/MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine, methamphetamine, MDA, and MDMA (and deuterated analogues), respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1 mm biphenyl analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 30% acetonitrile in four minutes and then back to 5% for re-injection. The total run time for each analysis was less than five minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of amphetamine related compounds.

Results: The limits of detection/quantification for this method were determined to be 0.05ng/ mg and 0.1ng/mg, respectively for amphetamine (and other analogues). The method was found to be linear from 0.1ng/mg to 10 ng/mg ($r2>0.999$). Data is presented to show that recoveries of amphetamine were found to be greater than 95% for all the amphetamine analogues. Interday and Intraday analysis of the amphetamine analogues were found to < 8% and < 12%, respectively. Matrix effects were determined to be < 6% for the amphetamine analogues. Concentrations of amphetamine in real samples of hair were found to range from 1.2ng/mg to 1.3ng/mg. Other amphetamine analogues (methamphetamine, MDA, and MDMA) were not found to be present in the hair samples.

Conclusion: The use of this new procedure for the analysis of amphetamine and related compounds will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC/MS/MS to provide data from small amounts of hair sample.

Hair, LC/MS/MS, SPE

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