K1 Analysis of Amphetamine on Swabs and Oral Fluid Sampling Device: A SPE Approach

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After attending this presentation, attendees will learn about the extraction of amphetamine from an oral swab and an oral fluid sampling device using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to compare results from both types of sampling methods.

This presentation will impact the forensic science community by offering analysts in forensic toxicology data from methods that permit samples of oral fluid material to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both SPE and LC-MS/MS.

Method: Extraction (SPE) was performed on mixed mode column (C_8/SCX) conditioned with methanol, deionized water, and pH 6 buffer (3 mL, 3 mL, and 1 mL, respectively) prior to sample loading. Oral samples (swabs/ fluid sampling device) were taken 1 hour after administration of prescribed amphetamine. The swabs were extracted with methanol and adjusted to pH 6 with 0.1 M phosphate buffer (5 mL). The samples from the sampling device were extracted into 3 mL of a proprietary formulated buffer (pH 7) containing a non-azide preservative. A 1 mL aliquot was buffered with 5 mL of 0.1 M phosphate buffer. To both sets of sample an internal standard was added (amphetamine-d_5). After loading the sample, the sorbent was washed with deionized water, acetic acid, and methanol (3 mL of each, respectively). Each SPE column was eluted with 3 mL of a solvent consisting of dichloromethane-isopropanol-ammonium hydroxide (78:20:2). An aliquot of this solvent was treated (details presented) with the mobile phase and analyzed by LC-MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of amphetamine and the internal standard, respectively.

Liquid chromatography was performed in gradient mode employing a 50 mm x 2.0 mm C_{18} analytical column and a mobile phase consisting of acetontitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 minutes and then back to 5% for re-injection. The total run time for each analysis was less than 5 minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis.

Results: The limits of detection/quantification for this method were determined to be 5 ng/ mL and 10 ng/ mL, respectively for amphetamine. The method was found to be linear from 10 ng/ mL to 500 ng/ mL (r^2>0.999). Data is presented to show that recovery of amphetamine was found to be > 94 %. Interday and Intraday analysis of amphetamine were found to be < 4% and < 6%, respectively. Matrix effects were determined to be < 4%. Analysis of the subject swab concentrations ranged from 16 to 129 ng/ mL (mean: 46 ng/ mL (sample size =10)), while the oral sampling device ranged from 52 to 846 ng/ mL (mean: 132 ng/ mL (sample size =10)).

Conclusion: The use of this procedure for the analysis of amphetamine adds to the body of knowledge regarding the analysis of amphetamine. The data should be of great use to analysts in the field of forensic toxicology employing oral fluid analysis, as it demonstrates how far the horizons of oral fluid sampling can be expanded as it permits a direct comparison between oral swabs and oral sampling devices in relation to the analysis of amphetamine after oral administration. The novelty of this study is the originality of the compare and contrast approach (as demonstrated by the presented data) to the analysis of amphetamine using readily available swabs and commercially available oral fluid devices. This limited study indicates the range of concentrations of the drug that can be achieved using either system. **Amphetamine, SPE, LC-MS/MS**

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