K2 Extraction and Analysis of AM2201 Metabolites in Urine: A Drugs and Driving Case

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After attending this presentation, attendees will learn about the extraction and analysis of the hydroxy metabolites of AM2201 (a newer fluorinated synthetic cannabinoid) from urine using readily available Solid Phase Extraction (SPE) cartridges and tandem mass spectrometry.

This presentation will impact the forensic science community by offering forensic toxicology analysts and chemists involved with drugs and driving cases more information about the analysis of this synthetic cannabinoid (AM2201 hydroxylated metabolites) employing LC/MS/MS and solid phase extraction.

Method: In this method, urine (1mL samples (calibrators, controls, and test samples)) containing internal standard (THC-d_3/AM2201-OH-d_5) was enzymatically hydrolysed with β-glucuronidase before being adjusted to pH6 with 0.1M phosphate buffer. The samples were then applied to pre-conditioned SPE mixed mode columns (C_8-Weak Anion Exchange). The SPE columns were conditioned with methanol, dionized (DI) water, and 0.1M pH6 phosphate buffer (3mL, 3mL, 1mL, respectively). After loading the samples onto the columns, the SPE sorbents were washed with DI water and pH6 phosphate buffer containing 20% acetonitrile (v/v) (3mL of each) and then SPE columns were dried under full vacuum for 5 min. The analytes were eluted with 3mL of a solvent mixture consisting of ethyl acetate containing 10% methanol. The eluates were evaporated to dryness using nitrogen gas at 40°C and dissolved in 100µL of a mixture of: 95% aqueous formic acid (0.1%) and 5% acetonitrile (containing 0.1% formic acid). The samples were analyzed by tandem mass spectrometry using positive Multiple Reaction Monitoring mode (MRM) and gradient liquid chromatography. Liquid chromatography was performed on a 50x2.0mm C_{18} analytical column with a guard column of the same chemistry. The mobile phase employed consisted of **A** aqueous formic acid (0.1%) and **B** acetonitrile (containing 0.1% formic acid). The gradient was started at 5% **B** and increased 90% **B** in 4 min, after which it was decreased to 5% **B** and kept until 5 min. The flowrate of the mobile phase was 0.5mL per min. Each analytical run was completed in 5 min.

In this presentation, representative chromatograms and calibration curves are shown to illustrate the efficiency of the chromatography and analysis of AM2201 and its metabolites.

Results: The limits of detection/quantification for the SPE method were determined to be 0.5ng/mL and 1.0ng/mL, respectively for the analytes The method was found to be linear from 1.0ng/ mL to 100ng/ mL (r^2>0.999). Data is presented to show that the recoveries of the AM2201 metabolite were found to be greater than 90%. Interday and intraday analysis were found to <7% and <10%, respectively. Matrix effects were determined to be <5%. No parent drug was found in the test sample. Results of the metabolite concentrations are shown in the presentation.

Conclusion: The use of the information given in this new procedure for the analysis of the metabolites AM2201 will be of great use to analysts in the field of forensic toxicology as it demonstrates the use of SPE/LC/MS/MS to provide valuable data regarding the metabolites of one of the newer synthetic cannabinoids

AM2201, SPE, LC/MS/MS

 ** Presenting Author*