EXTRACTION AND ANALYSIS OF AM2201 METABOLITES IN URINE: A DRUGS AND DRIVING CASE

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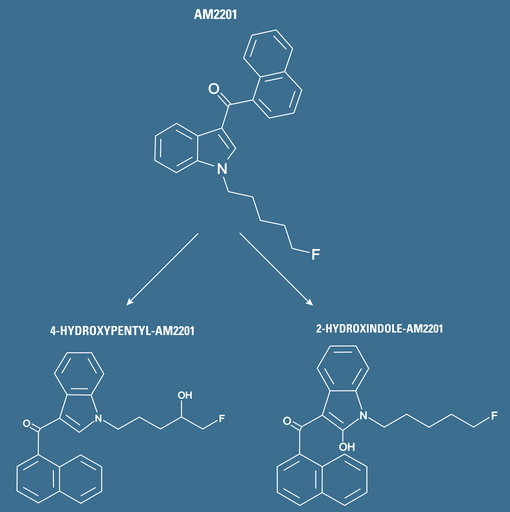
INTRODUCTION

Synthetic Cannabinoids are now widely established in the area of forensic toxicology. Their analysis is a challenge to laboratories undertaking testing in biological fluids. In this study, analysis of a newer drug (AM2201) and its metabolites in the urine of a motor vehicle operator in the Commonwealth of Massachusetts is described. The challenge facing analysts is not only identifying the correct synthetic cannabinoid metabolite in the urine sample but extracting, and quantifying the concentration of the compound.
After viewing this poster 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.

MATERIALS AND CHEMICALS

AM2201, 4-Hydroxypentyl-AM2201, 4-Hydroxypentyl-AM2201-d_5, 2-Hydroxyindole-AM2201 were obtained from Cayman Chemicals (Ann Arbor, MI). Acetonitrile, ethyl acetate, methanol, and phosphate buffer (pH 6) were obtained from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained from Acros Chemicals (via Fisher Scientific). All chemicals were of ACS quality. Solid phase extraction columns (CSTHC206 (6mL, 200 mg)) were obtained from UCT Inc., (Bristol, PA).

STRUCTURES:



ANALYTICAL METHOD

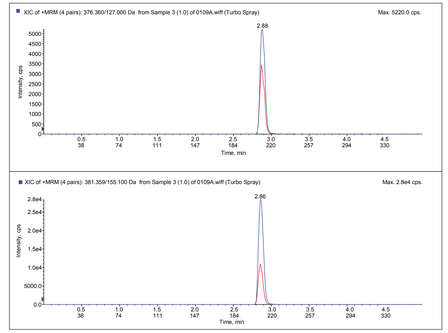
In this method, urine (1 mL samples (calibrators, controls, and test samples) containing internal standard (AM2201-OH-d_5) was enzymatically hydrolysed with β-glucuronidase before being adjusted to pH 6 with 0.1 M phosphate buffer. The samples were then applied to Clean Screen® THC columns. The SPE columns were conditioned with methanol, dionized (DI) water, and 0.1 M pH 6 phosphate buffer (3 mL, 3 mL, 1 mL, respectively). After loading the samples onto the columns, the SPE sorbents were washed with DI water and pH 6 phosphate buffer containing 20% acetonitrile (v/v) (3 mL of each). SPE columns were then dried under full vacuum for 5 minutes. The analytes were eluted with 3 mL of ethyl acetate containing 10% methanol. The eluates were evaporated to dryness using nitrogen gas at 40°C and dissolved in 100 µL of methanol.

INSTRUMENTAL CONDITIONS

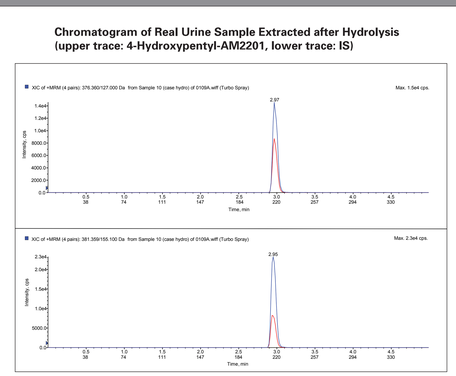
The samples were analyzed by tandem mass spectrometry using positive multiple reaction monitoring mode (MRM) and gradient liquid chromatography. Liquid chromatography was performed on a 50 x 2.0mm C_{18} analytical column with a guard column of the same chemistry. The mobile phase employed consisted of A aqueous formic acid (0.1%) and B acetonitrile (containing 0.1% formic acid). The gradient was started at 35% B and held for 0.5 minutes and increased 90% B in 4 minutes, after which it was decreased to 35% B and kept until 5 minutes when it was returned to 35% B. The flowrate of the mobile phase was 0.5 mL per minute. The instrument was readied to run after 5.1 minutes. Each analytical run was completed in 5 minutes.

Tandem mass spectrometry was performed using positive multiple reaction (MRM) mode. The following transitions were monitored (quantification ions underlined): AM2201: m/z: 360.2 to 155.1, 127.1, 4-Hydroxypentyl-AM2201 m/z: : 376.360 to 127.00, 154.00 , 4-Hydroxypentyl-d_5-AM2201 m/z: 381.359 to 126.00, 154.00, 2-Hydroxyindole-AM2201 m/z: 365.320 to 155.300, 127.200, respectively.

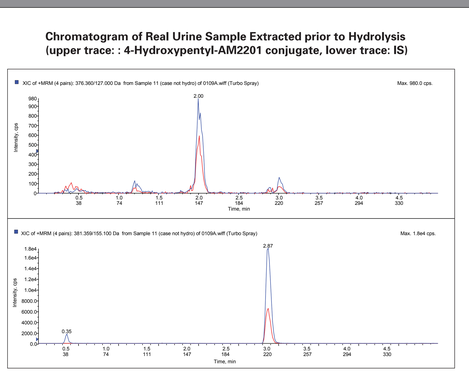
Chromatogram of 4-Hydroxypentyl-AM2201 extracted at LOQ (upper trace: 4-Hydroxypentyl-AM2201, lower trace: IS)



Chromatogram of Real Urine Sample Extracted after Hydrolysis (upper trace: 4-Hydroxypentyl-AM2201, lower trace: IS)



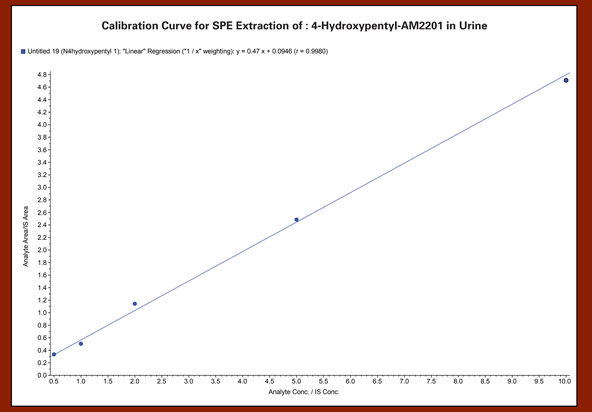
Chromatogram of Real Urine Sample Extracted prior to Hydrolysis (upper trace: : 4-Hydroxypentyl-AM2201 conjugate, lower trace: IS)



RESULTS

The limits of detection/quantification for the SPE method were determined to be 0.5 ng/ mg and 1.0 ng/ mL, respectively for the AM2201 and metabolites. The method was found to be linear from 1 ng/ mL to 100 ng/ mL (r^2>0.999). Recoveries of AM2201 and metabolites were found to be greater than 90%. Interday and intraday analysis of AM2201 and metabolites were found to < 7% and < 10%, respectively. No parent AM2201 was found in the urine of the driver, neither was any 2-Hydroxyindole-AM2201. A compound suspected to be AM2201 conjugate was observed in a non hydrolyzed sample (similar MRM transitions to 4-Hydroxypentyl-AM2201 but different retention time). The concentration of AM2201 metabolite (4-Hydroxypentyl-AM2201) was found to be 3.1 ng/ mL after hydrolysis.

Calibration Curve for SPE Extraction of : 4-Hydroxypentyl-AM2201 in Urine



CONCLUSION

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. This method also permits analysts to differentiate between AM2201, its metabolites and conjugates.

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