A Combined Method for the Analysis of Barbiturates and 11-nor-9-carboxy Δ⁹ THC in Urine by LC/MS/MS
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Introduction

Many laboratories are discovering the efficiency and ease of running larger advanced toxicology panels by liquid chromatography/tandem mass spectrometry (LC/MS/MS) as opposed to the traditional screen then confirm model. Forgoing complicated sample preparation involving solid phase extraction (SPE) for a dilute and shoot methodology is another industry trend. The vast majority of the compounds in our 54-analyte advanced toxicology panel are run using positive mode; however, barbiturates and 11-nor-9-carboxy Δ⁹ THC perform better in negative mode. With the rise in benzodiazepine use, abuse of barbiturates has declined, although not eliminated. Clinically, barbiturates are still commonly prescribed to treat seizure disorders and migraines and there is still a need to test for them. In addition, it is important to be able to test for both the synthetic version of cannabis that can be prescribed, and the natural version that in some jurisdictions can be legally prescribed, while remaining illicit in others. Traditionally, analyzing both barbiturates and 11-nor-9-carboxy Δ⁹ THC would require separate sample preparation and two separate instrument runs. We developed an assay to combine five common barbiturates and a THC metabolite into one effective panel with minimal sample preparation.

Materials and Methods

Patient urine samples arrived at our Troy, MI facility via second day air. An aliquot was then centrifuged at 220 x g for 5 minutes. Next, the urine was hydrolyzed with 2.5% β-Glucuronidase Type HP-2 enzyme from Helix pomatia. The samples were then diluted with a 50/50 water/methanol mixture spiked with the internal standards 11-nor-9-carboxy Δ⁹ THC-d₄ and pentobarbital-d₄ from Cerilliant.

Sample preparation was simple and efficient. Separation of the analytes was adequate (Graph 2). Butobarbital and pentobarbital did not separate chromatographically, but can easily be separated by their different mass-to-charge ratios and subsequent loss.

Results

Barbiturates tested were: butabarbital, butalbital, pentobarbital, phenobarbital, and secobarbital. The THC metabolite was 11-nor-9-carboxy Δ⁹ THC. Samples were run utilizing electrospray ionization and negative multiple reaction monitoring (MRM) mode on a Micromass Ultima coupled to an Alliance 2795 HPLC Autosampler. Separations were performed using a Pinnacle® DB C18 column 5µm 150mm x 2.1mm and carried out at 45°C. The mobile phases consisted of water and acetonitrile with an ammonium hydroxide modifier. The run time was 10 minutes.

The linearity for all compounds was at least 0.995 (R²) for at least 150 laboratories. A series of 30 injections gave a %RSD of under 16% for each compound. Graph 3 shows the repeatability for 11-nor-9-carboxy Δ⁹ THC which is 3.58% RSD. The lower limits of quantitation (LLOQ) varies for the barbiturates between 10-100 ng/mL, while the LLOQ for 11-nor-9-carboxy Δ⁹ THC was 3 ng/mL. The single to noise ratio for the barbiturates and 11-nor-9-carboxy Δ⁹ THC were both over 15 at the lower end of the calibration curve (Graph 4). No ion suppression studies were performed at this time. The results were comparable to our previous methods that involved running each compound class independently.

Summary and Conclusion

In conclusion, we were able to successfully combine two commonly run negative mode assays into one efficient panel with minimal sample preparation, and low limits of quantitation.

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