K26 Screening for K2: Monitoring JWH-018, 073, 081, and 250 and Some Prominent Metabolites by HPLC-MS/MS

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The goal of this presentation is to show a strategy for detecting synthetic cannabinoids in a urine matrix with quadrupole-linear ion trap technology. Attendees will learn the identity of the some of the prominent metabolites and their characteristic ions.

This presentation will impact the forensic science community by showing how synthetic cannabinoids and their metabolites can be detected with a hybrid quadrupole-linear ion trap mass spectrometer even in the absence of standards. Additionally, the work will give forensic scientists confidence in the analysis of such compounds with mass spectral library search techniques.

Objectives: To expand K2/spice screening method to include JWH-81 and JWH-250 metabolites using a quadrupole-linear ion trap, to identify the metabolites and generate a spectral library. Human liver microsomes and hepatocytes were incubated with the parent drugs (JWH-018, 073, 081, 250) to generate phase I and phase II metabolites. After predicting the precursor and product ions using a metabolite identification software package the incubated samples were analyzed using the multiple reaction monitoring to trigger acquisition of product ion spectra. Spectral comparison of the metabolite spectra to those of the parent drugs allowed for the identification of the metabolites. The objective of the work was to produce a highly sensitive screening method for JWH-81 and JWH-250 metabolites and parent drugs to augment our current method that screens for JWH-73 and JWH-18 metabolites and parent drugs.

Materials and Methods: A state of the art quadrupole linear ion trap and High Performance Liquid Chromatography (HPLC) were used to acquire the metabolite mass spectra. The initial multiple reaction-monitoring list was generated from a metabolite identification software package. Samples were analyzed on a biphenyl hplc column using an acetonitrile gradient with 0.1% formic acid to aid ionization. Standards were prepared in synthetic urine matrix and samples were prepared by diluting them with acetonitrile prior to centrifugation at 21,000 rcf. When the multiple reactions monitoring transition generated a signal above a set threshold, the linear ion trap obtained the product ion spectrum on the metabolite precursor. Once the metabolite identification was confirmed through spectral analysis, the product ion spectra were added to a spectral library.

Result: Multiple metabolites of each parent drug were found including alkyl chain hydroxylations, demethylations, indole ring hydroxylations, carboxylations and hydrogenations. The glucuronide conjugates of these phase I metabolites were identified in the hepatocyte incubation of JWH-250. The mass spectral analysis allowed for the identification of the prominent metabolites as hydroxylations on the aromatic rings or the aliphatic chain portions of the molecules. The addition of 17 daltons to the recorded m/z values for the metabolite parent ion and the fragment ions versus those of the drug as well as the absence of other changes in the m/z values led to this conclusion. Representative product ion spectra were added to a compound database against which spectra from physiological samples could be searched using mass spectral software. Using dilute and shoot sample preparation allowed for rapid and sensitive sample analysis with good purity scores on spectral matches though sensitivity could be increased with enzyme hydrolysis of the glucuronide metabolites. When the microsome incubation solutions were spiked into urine samples, metabolites of JWH-018, JWH-73, JWH-081, and JWH-250 could be uniquely identified through a library search algorithm.

Conclusion: Many common metabolites of JWH-250 and JWH-081 were identified using the quadrupole linear ion trap. A spectral library of the most common metabolites was generated, and this library was used in a screening method to detect metabolites in urine samples. The method provides for the high sensitivity screening of physiological samples for JWH-018, JWH-073, JWH-081, and JWH-250 metabolites as well as the parent drugs.

K2, Spice, Mass Spectrometry

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