A95 LC-MS/MS Analysis of Psilocybin and Psilocin in Mushrooms: SPE Approach

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After attending this presentation, attendees will learn about the extraction of psilocybin and its metabolite (psilocin) from seized mushrooms using readily available solid phase extraction (SPE) cartridges and tandem mass spectrometry. Use of this SPE method will permit analysts to provide data on both compounds in samples.

This presentation will impact the forensic science community by offering analysts in forensic facilities a method that permits samples of mushroom to be analyzed in a clean format with minimal matrix effects and excellent analytical characteristics in terms of both SPE and LC- MS/MS.

This novel extraction (SPE) procedure was performed on a commercially available mixed mode column (C8/SCX) that had previously been conditioned with methanol, deionized water, and pH 6 phosphate buffer (0.1 M (3 mL, 3 mL and 1 mL, respectively)) prior to sample loading. The methanolic mushroom samples (1 mL) were adjusted to pH 6 with 0.1 M phosphate buffer (5 mL) and an internal standard was added (ethyl morphine) to the resulting solution. After loading the sample onto the SPE under gravitational flow, the sorbent was washed with deionized water and methanol (3 mL of each, respectively). Each SPE column was eluted with 3 mL of a solvent consisting of ethyl acetate containing 2% (v/v) ammonium hydroxide followed by 3 mL of methanol containing 4% (v/v) ammonium hydroxide. The individual eluates were collected in separate glass tubes under gravitational flow, evaporated to dryness (under nitrogen), and dissolved in a mobile phase (250 µL). These individual solutions were combined for analysis by LC- MS/MS in positive multiple reaction monitoring (MRM) mode. Data is presented for MRM's of psilocybin, psilocin, and ethyl morphine, respectively.

Liquid chromatography was performed in gradient mode employing a 50 mm x 2.1 mm C18 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% acetonitrile for re-injection. The total run time for each analysis was less than five minutes. In this presentation, representative chromatograms are shown to illustrate the efficiency of the

chromatography and analysis along with calibration curves and representative chromatograms of real mushroom samples.

Results: The limits of detection/quantification for this method were determined to be 50 ng/ g and 100 ng/ g, respectively for both psilocybin and psilocin. The method was found to be linear from 100 ng/ g to 2000 ng/ g (r2>0.999). Data is presented to show that recoveries of psilocybin and psilocin were found to be greater than 85% for both compounds. Interday and Intraday analysis of psilocybin and psilocin were found to be

< 5% and < 8%, respectively. Matrix effects in this procedure were determined to be < 6%. Details of real samples (10) showing concentration of both compounds are given at the presentation.

Conclusion: The use of this new procedure for the analysis of psilocybin and psilocin using both SPE and LC-MS/MS will be of great use to analysts in the field of forensic drug analysis as the concentrations of both drugs can now be reported rather than just the psilocin value alone, as is currently provided by laboratories using gas chromatography coupled to mass spectrometry.

Hallucinogens, SPE, LC-MS/MS

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