K33 Analysis of Alpha PVP, MePPP, Naphyrone, and Seven Related Pyrrolidinophenones in Blood by LC/TOF and LC/MS/MS

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After attending this presentation, attendees will be able to describe the characteristics and effects of pyrrolidinophenones, optimum methods for their analysis in blood using a screen and confirmation combination of Liquid-Chromatography/Time-of-Flight (LC/TOF) and Liquid Chromatography/Electrospray Ionization/Tandem Mass Spectrometry (HPLC/ESI/MS/MS), and to take into account limitations on its analysis in interpreting results.

This presentation will impact the forensic science community by describing a comprehensive assay for the detection of this emerging class of New Psychoactive Substances (NPS).

Pyrrolidinophenones are an emerging group of designer drugs that are appearing in NPS. Compounds in this class are characterized by a propiophenone backbone attached to a pyrrolidine group, with various substituents on the aromatic ring and modifications at the alkyl chain producing the related compounds. One of the most popular compounds of this group that is being recreationally abused is alpha-pyrrolidinopentiophenone (alpha-PVP), which is a designer stimulant that can cause many adverse effects, including acute psychosis with delusions as well as other medical issues including cardiovascular problems, seizures, and hyperthermia. Other compounds of the pyrrolidinophenone class have been seen on the market. Alpha-PVP and MePPP have both been reported in synthetic drug material and alpha-PVP, MDPPP, and MPHP have all been reported in case histories of individuals using “bath salts” or “legal high” products. Due to the emerging nature of this group of compounds, it is necessary to develop testing for the detection of pyrrolidinophenones in forensic toxicology.

This method was developed for the detection of ten different compounds in the pyrrolidinophenone class: alpha PVP, pyrovalerone, naphyrone, alpha-PBP, alpha-PPP, MePPP, MDPPP, MOPPP, MPBP, and MPHP. Since many of the pyrrolidinophenones have similar molecular structures, weights, and formulas, it was important to develop a sensitive and reliable method for the determination of these compounds in biological matrices for forensic toxicology.

Pyrrolidinophenones were extracted from whole blood by a single step liquid-liquid extraction. Samples are extracted with a 0.1M borax buffer (pH 10.4) and purified by liquid-liquid partition with a lamotrigine extraction solvent (30% ethyl acetate in n-butyl chloride). Spiked samples were analyzed by LC/TOF mass spectrometry for screening purposes. For the LC/TOF, the mobile phases consisted of 0.05% formic acid in 5mM ammonium formate and 0.05% formic acid and the analysis was performed on a ZORBAX® Eclipse Plus C18 Rapid Resolution HT 3.0 x 100mm column. Target compounds were detected and reported from accurate scan data using MassHunter Qualitative and Personal Compound Database and Library (PCDL) software. Retention time data was obtained from blank matrices spiked at a cutoff concentration with reference material for each drug. A confirmatory technique for biological matrices was also developed using HPLC/ESI/MS/MS. Infusions of each analyte were performed to determine daughter ions; transitions had to be carefully selected due to some compounds having the same molecular mass and almost identical retention times. HPLC conditions for the LC/MS/MS method included ammonium formate vs methanol at 0.40mL/min on an ACQUITY® UPLC™ BEH C18 2.1 x 100mm column. A serial dilution was evaluated for a calibration curve; the final calibration range is 5-500ng/mL. Characterization experiments regarding linearity, matrix-matching, and recovery were successfully completed. This panel separates and identifies ten compounds that have similar structures, molecular weights, and retention times.

This research was the first documented attempt to develop a comprehensive panel for pyrrolidinophenones, which are increasing in popularity for abuse in the new designer drug movement. This panel was developed for identifying these compounds in biological matrices for forensic toxicological analysis. These findings suggest that the combination of LC/TOF and LC/MS/MS provides a valuable approach to the sensitive and specific analytical identification and measurement of pyrrolidinophenones in forensic casework.

Pyrrolidinophenone, Novel Psychoactive Substances, Toxicology

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