K9 Development and Validation of a Confirmatory Method for Six Novel Psychoactive Substances (NPS) in Whole Blood Using Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry (UPLC/MS/MS)

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After attending this presentation, attendees will be able to implement a method for the confirmation of six prevalent NPS in whole blood including methylone, dimethylone, ethylone, butylone, 4-Fluoroamphetamine (4-FA), and alpha-Pyrrolidinopentiophenone (alpha-PVP).

This presentation will impact the forensic science community by providing evidence of a robust and reliable analytical method capable of quantifying the target compounds and meeting the requirements for method validation established by the Scientific Working Group for Forensic Toxicology (SWGTOX). The method focuses on novel compounds with which forensic laboratories have relatively limited experience in routine casework.

The use of NPS within the United States has recently been a focus of media attention due to drug-related deaths, emergency room visits, and large-scale hospitalizations or medical aid calls. Due to the increase in use, and a lack of resources for the analysis of some of the most currently abused compounds, there is a significant need to develop toxicological procedures for the measurement of these analytes in forensic specimens. The assays can then be used to study the prevalence of NPS use in various populations and the investigation of specific forensic cases. In addition, given the proliferation of NPS drugs in the United States marketplace with very similar structures and identical molecular formula, there is an urgent need for methods that can distinguish between closely related compounds, isomers, and isobars.

The increasing popularity of NPS and recreational research chemicals is easily documented by monitoring online forums and discussion groups of Electronic Dance Music (EDM) festival attendees and other groups associated with EDM culture. This method was developed and validated for the confirmation of the most prominent drugs identified from screening samples collected at an EDM event. The purpose of this project was to develop a method using UPLC/MS/MS for the confirmation of six NPS including methylone, dimethylone, ethylone, butylone, 4-FA, and alpha-PVP, which had screened positive in biological specimens (blood, urine, and oral fluid) collected from attendees at an EDM festival.

Samples (0.5mL) were prepared for analysis using a basic liquid-liquid extraction with 0.1M borate buffer (pH=10.4) and n-butyl chloride:ethyl acetate (70:30). The organic phase was evaporated to dryness and the samples were reconstituted in 90:10 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B). Chromatographic separation was achieved on a UPLC/MS/MS system (Waters® Acquity UPLC coupled with a Waters® Quattro Micro API mass spectrometer) using positive electrospray ionization and multiple reaction monitoring mode with an Acquity UPLC® BEH C18 (50mm x 2.1mm, 1.7µm) at 50ºC. Mobile phase was introduced into the system in a gradient programmed with 15% B, isocratic for one minute, that was linearly increased to 35%B over five minutes, increased again to 90% B over one minute before the gradient was returned to the initial conditions and held for 2.9 minutes. The flow was set to 0.2mL/min with a total run time of eight minutes.

The method was developed to be a highly sensitive assay, with optimal run time. The method was validated following guidelines set forth by the SWGTOX. The method was linear between 5ng/mL and 500ng/mL and had a defined limit of quantitation and detection of 5ng/mL for all analytes. Recovery for all analytes was greater than 85%. The method was free from carryover at five and ten times the highest calibrator, from interferences from matrix effects, and from interferences from commonly encountered and related analytes at various levels (n=20). The within-run precision ranged from 3.2% to 5.6% for the low control at 15ng/mL and 2.0% to 4.2% for the high control at 350ng/mL. For the low and high controls respectively, the between run precision ranged from 4.4% to 7.7%, and 2.6% to 6.5%, and the accuracy ranged from -3.2% to 3.7% and -0.8% to -5.8%. The method was matrix matched to urine and was found to be acceptable, producing a between-run precision of 1.8% to 12.5%, and 2.2% to 8.3% for the low and high controls, respectively.

The method was applied for the confirmation and quantitation of the target drugs in blood samples from 17 human subjects whose samples had screened positive for these substances.

NPS, UPLC/MS/MS, Cathinones

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