K10 Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction of Novel Psychoactive Substances (NPS) From Biological Matrices

Ashley N. Kimble, BS, 9581 Fontainebleau Boulevard, Miami, FL 33172; and Anthony P. DeCaprio, PhD, Florida International University, International Forensic Research Institute, 11200 SW 8th Street, Miami, FL 33199*

The goal of this presentation is to describe the findings of using QuEChERS as a quicker and less expensive alternative for the extraction of NPS from biological matrices. This presentation will cover a broad range of drug classes and the trends observed. An optimized extraction method that encompasses as many NPS as possible will also be presented.

This presentation will impact the forensic science community by presenting an alternative to currently used extraction techniques for NPS that includes added benefits, such as decreased costs and time spent on sample preparation.

This presentation is intended to demonstrate the potential of QuEChERS ("catchers") as an extraction technique for various NPS in biological fluids (urine and whole blood). Although extraction techniques for common drugs of abuse are well studied, developing extraction methods specifically targeting NPS is needed due to the high prevalence of NPS in forensic casework. A validated screening/confirmatory triggered Multiple Reaction Monitoring (tMRM) method for 826 NPS by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) recently developed in the lab is being used to analyze all extracts for this study. The method allows for the screening of a wide variety of NPS drug classes and metabolites, with a focus on synthetic stimulants and cannabinoids due to their current importance in forensic toxicology casework.

Most biological fluids require an extraction step before analysis to avoid unwanted matrix effects and to protect instrumentation. Some commonly used extraction/purification techniques are Solid Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and dilute/crash and shoot. These techniques can be expensive, time consuming, and may not eliminate all matrix effects. Extraction methods ideally should be inexpensive and relatively fast to allow for high throughput in forensic toxicology labs.

QuEChERS has the potential to be a desirable alternative to these techniques because it can decrease overall costs, matrix effects, and time. QuEChERS was originally developed to simplify and speed up sample prep to extract pesticides from fruits and vegetables. QuEChERS uses a dispersive SPE (d-SPE) technique that increases the sample's contact with the sorbents. This increased contact allows for a more effective extraction than classic SPE. It is specifically designed for highly aqueous matrices and, therefore, can be a very useful technique for forensic toxicology work. While QuEChERS has been previously used as a technique for extracting common drugs of abuse from urine and blood, to date it has not been applied to extraction of NPS on this scale.

In this study, a modified Bond Elute kit was utilized. Blank human urine spiked with 29 different NPS at three different concentrations (5ng/mL, 20ng/mL, and 80ng/mL) was used for this work. The mixture included NPS from different drug classes, including synthetic cannabinoids, synthetic cathinones, tryptamines, and phenethylamines. The range of concentrations was selected to test the utility of the technique for authentic samples, which may contain very low concentrations of these compounds. The extraction process consisted of two major steps: a drying step with salts, followed by d-SPE. First, 3mL of the spiked urine was combined with 3mL acetonitrile, followed by the addition of salts (magnesium sulfate and sodium acetate) to dry the sample, which was then centrifuged at 4,400rpm for 5min. Then, 1mL of the acetonitrile layer was used for d-SPE. The acetonitrile was added to a centrifuge tube containing primary secondary amines, magnesium sulfate, and CI8 and centrifuged at 4,400rpm for 5min. A 100µL aliquot of the resulting supernatant was diluted with 100µL of internal standard mix and 300µL of High-Performance Liquid Chromatography (HPLC) water for analysis by LC/QqQ/MS. The total time for extraction was 15min, which is an improvement over most other extraction methods. An Agilent® 1290 Infinity® HPLC system and Agilent® 6460 QqQ/MS with Jet Stream Technology ESI was used with an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column for LC/MS/MS using the tMRM method.

Results demonstrate that QuEChERS is a promising technique for the extraction of tryptamines, phenethylamines, and synthetic cannabinoids, all of which exhibited recoveries >85% at all three concentration levels. This work is being continued with whole blood and additional NPS to further assess the technique's potential. QuEChERS is capable of extracting NPS from multiple drug classes in a single urine specimen and represents an appealing alternative to other extraction methods due to fewer transfer steps, quick extraction time, and decreased cost.

QuEChERS, Novel Psychoactive Substances, LC/MS

*Presenting Author