K19 A Comparison of Multiple Extraction/Purification Methods for Novel Psychoactive Substances (NPS) From Biological Matrices

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Learning Overview: The goal of this presentation is to demonstrate the results of comparing multiple extraction/purification methods for NPS in biological fluids (urine and whole blood).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by optimizing an extraction technique that is capable of handling high throughput and NPS of many different classes, which could greatly benefit forensic toxicology laboratories.

This research was designed to develop/optimize an extraction method capable of isolating a wide variety of NPS from biological fluids. This was accomplished by comparing multiple extraction methods, including online Solid Phase Extraction (SPE), classical SPE, crash/dilute and shoot, and (Quick, Easy, Cheap, Effective, Rugged, and Safe) QuEChERS. The focus of this work was to statistically compare the extraction/purification methods based on drug recovery, drug retention, reproducibility, minimization of matrix effects, time, and overall cost. Although extraction techniques for common drugs of abuse are well studied, developing extraction methods specifically targeting NPS is needed due to the increasing prevalence of NPS in forensic casework.

Dilute-and-shoot was accomplished by diluting urine samples with High-Performance Liquid Chromatography (HPLC) water using a 1:5 dilution followed by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) analysis. For crash-and-shoot, 600µL of cold acetonitrile (-20°C) was added to 200µL of whole blood, vortexed, and centrifuged for 5min at 7,000rpm. After being centrifuged, the supernatant was removed and added to an LC vial, evaporated to dryness under a gentle flow of nitrogen, then reconstituted in 200µL of methanol for analysis. Classical SPE was performed following a method previously developed in the laboratory using a positive-pressure SPE apparatus and Agilent® Bond Elut Plexa PCX cartridges. Online SPE utilized an Agilent® 1290 Flex Cube LC unit with a Bond Elut (BE) online polymeric sorbent material (PLRP-S) cartridge.

QuEChERS has been shown to be a successful technique for extracting common drugs of abuse from biological fluids, but also has potential for extracting NPS. QuEChERS is an appealing alternative due to fewer transfer steps, quick extraction time, and decreased cost when compared to other approaches. This work used an in-house developed mini one-pot QuEChERS kit. Samples were added to pre-weighed components ($MgSO_4$, NaCl, PSA, C18), shaken by hand, vortexed, and centrifuged at 7,000 rpm for 5min. After centrifugation, the acetonitrile layer was removed and added to an LC vial, dried, and reconstituted in 200µL of methanol. An Agilent® 1290 Infinity® HPLC system and Agilent® 6460 QqQ/MS with Jet Stream Technology Electrospray Ionization (ESI) operated in positive mode was used for analysis, along with an Agilent® Zorbax® Rapid Resolution HD Eclipse Plus™ C18 column for chromatographic separation. All samples were analyzed using a triggered Multiple Reaction Monitoring (tMRM) method that is validated for the detection of multiple NPS. All methods were tested using a mix of 36 NPS, which included compounds and metabolites from different drug classes, at three different concentrations (5ng/mL, 20ng/mL, and 80ng/mL). The four different extraction procedures for NPS in blood and urine were evaluated and compared using two-way Analysis Of Variance (ANOVA) to assess significant differences. If the ANOVA showed a significant difference, a Tukey Honest Significant Difference (HSD) test was completed to determine which specific methods were significantly different.

Findings demonstrated that NPS recoveries from urine with QuEChERS and dilute-and-shoot were not statistically different, while recoveries from blood were significantly higher with QuEChERS than with crash-and-shoot. For example, most synthetic cannabinoids demonstrated recoveries from urine above 75% with QuEChERS. QuEChERS, when used for both blood and urine, showed a decrease in matrix effects for all classes of NPS when compared to crash/dilute-and-shoot. While online SPE is an efficient option, it provided low recoveries for many classes of NPS, especially synthetic cannabinoids. Total time required from extraction start to analysis varied from 5min (dilute-and-shoot) to 3h (classical SPE), while costs varied from relatively inexpensive (dilute-and-shoot and QuEChERS) to expensive (online SPE and classical SPE, due to instrumentation and consumable costs, respectively). Although dilute-and-shoot was the quickest and most cost effective, it is a crude method that can leave matrix components, which can damage instrumentation and lead to unwanted ion suppression and enhancement. In conclusion, results showed that QuEChERS provided the best combination of extraction capability, elimination of matrix effects, time, and cost for application to NPS analysis.

Extraction Methods, LC/QqQ/MS, Novel Psychoactive Substances