K39 Comprehensive Analysis of 34 Fentanyl Analogs Including Carfentanil From Liver Tissue Using Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis

Kylea M. Mathison, West Virginia University, Morgantown, WV 26506; Joseph A. Cox, MS*, Morgantown, WV 26508; Colby E. Ott, MS, Shippensburg, PA 17257; Joseph A. DelTondo, DO, Allegheny General Hospital, Pathology, Pittsburgh, PA 15212; James C. Kraner, PhD, Office of the Chief Medical Examiner, Charleston, WV 25302; Kristen M. Bailey, MS, Office of the Chief Medical Examiner, Charleston, WV 25302; Myron A. Gebhardt, MS, Office of the Chief Medical Examiner, Charleston, WV 25302; Luis E. Arroyo, PhD, West Virginia University, Morgantown, WV 26506-6121*

Learning Overview: After attending this presentation, attendees will better understand a QuEChERS extraction protocol utilized for the quantitative analysis of 34 different fentanyl analogs from liver tissue and detection using an LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a comprehensive procedure for the analysis of emerging fentanyl analogs extracted from postmortem liver tissue through the use of a safe method that reduces potential contamination while maintaining sensitivity.

Opioid overdoses have increased in recent years, and according to the National Institute on Drug Abuse currently more than 130 people die from an opioid overdose each day within the United States. The analysis of these analogs from biological specimens pose an analytical challenge to practitioners due to the acute lethal intoxication concentrations, continuous variations of analogs, and wide variations in chemical structure. Additionally, Postmortem Redistribution (PMR) of concentrations of drugs such as fentanyl within a deceased individual can occur with time and has the potential to change the concentration in the peripheral blood, while studies have shown that PMR is not as frequently occurring in the liver. Therefore, based on the PMR tendencies of fentanyl, liver or other organ tissues are needed to provide the medical examiner the proper data to make an informed decision on the manner or cause of death when fentanyl is present and may provide a more adequate indication of antemortem concentrations compared to blood.

Liver specimens are often homogenized with bladed devices that can require costly disposable parts or require cleaning to avoid contamination. Here, this study is proposing to use a disposable single tube with steel beads for homogenization. Presented in this study is a QuEChERS method to extract fentanyl and fentanyl analogs from human liver samples.

This method used prepackaged extraction powder containing magnesium sulfate and sodium acetate, 0.1g of liver tissue, three stainless steel balls, and 2mL dispersive-Solid Phase Extraction (SPE) tubes, containing 25mg of Primary Secondary Amine (PSA), 25mg end-capped octadecylsilane ($C18_{EC}$), and 150mg magnesium sulfate. Homogenization was achieved using a high-speed mixer mill with homemade attachments for 1.7mL centrifuge tubes and 4.5mm stainless steel balls for pulverization, reducing the risk of cross contamination by producing a homogenized sample in a single disposable tube. The quantitation method was performed on an Agilent® 6470 LC/MS/MS system coupled with a 1290 Infinity® II LC system. Chromatographic separation was achieved on a Zorbax® Eclipse Plus™ C18 RRHD 3.0x100mm, 1.8µm column with 0.1% formic acid and 5mM ammonium formate in water (mobile phase A), and 0.1% formic acid in methanol (mobile phase B).

An evaluation of a QuEChERS extraction procedure is presented in this study as an alternative analytical method for efficient extraction and detection of fentanyl and fentanyl analogs. The evaluated parameters include selectivity, matrix effects, linearity, bias, precision, and proof of applicability using authentic fentanyl case samples. Average recoveries for the 34 fentanyl analogs were 101.1% and 104.4% for low (1µg/kg) and high (50µg/kg), respectively. Average matrix effects for the 34 fentanyl analogs were 99.3% and 98.4% for low and high, respectively.

The comprehensive extraction and LC/MS/MS method developed for analysis of liver tissue for fentanyl and fentanyl analogs is precise, sensitive, and reproducible for complex forensic matrices such as liver tissue.

Fentanyl Analogs, QuEChERS, Postmortem Liver