## K36 Quantitation of Fentanyl and Metabolites From *Lucilia Sericata* Larvae and Liver Tissue Using a Modified Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction With Analysis by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

*Joseph A. Cox, MS*, Pasadena, TX 77504; Colby Ott, BS, Shippensburg, PA; Luis E. Arroyo, PhD, Department of Forensic and Investigative Sciences, Morgantown, WV 26506-6121*

**Learning Overview:** After attending this presentation, attendees will better understand a modified QuEChERS extraction combined with a sensitive method for determining concentrations for fentanyl and two known metabolites, norfentanyl, and despropionyl fentanyl (4-ANPP), in tissue toxicology specimens using LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by relaying a novel procedure that utilizes the unique extraction properties of a QuEChERS extraction with modifications to allow for the extraction of drugs from challenging matrices. Analytical separation and detection was conducted on a Triple Quad LC/MS/MS that provides the required sensitivity and selectivity of the target analytes.

The opioid crisis in the United States has a serious impact on our society. According to the National Institute on Drug Abuse (NIDA), more than 100 people die every day of opioid overdose. Even more concerning is the increase of overdose deaths involving synthetic opioids (e.g., fentanyl and fentanyl analogs), with increases from 3,105 deaths in 2013 to 19,413 deaths in 2016 (625% increase). Under special circumstances (e.g., when blood or urine are not available due to decomposition or exsanguination), liver samples are regularly the first choice for toxicological analysis, mainly due to the organ's ease of collection at autopsy and homogenization for drug extraction. On the other hand, fly larvae have been used as a toxicological analysis matrix when traditional matrices are not available due to skeletonization or to corroborate findings in extremely decomposed tissues. The succession that occurs on a corpse after death is a relatively confined and closely packed ecosystem that is typically restricted to the remains and close proximity. If xenobiotics are present in the body, they will be ingested by the organisms feeding on the corpse. Therefore, any concentrations identified in insect tissue can be indicative of drugs present in the tissue they were feeding on.

Common practices for tissue extraction involve a Solid Phase Extraction (SPE) or Liquid-Liquid Extraction (LLE), but the process for these techniques can be time consuming and often involve tissue homogenizers using blenders that could allow cross contamination. In recent years, the QuEChERS extraction protocol was introduced in the market to deal with samples with high a content of fatty materials, especially for food analysis. This sample preparation technique avoids the risk of cross contamination by producing a homogenized sample into a single disposable tube. QuEChERS has being reported as a rugged sample preparation method in blood analysis and more recently in liver tissues. This study proposed using a simple sample homogenization system consisting of a high-speed mixer mill with disposable stainless-steel balls contained in a homemade plastic holder. This setup helped to reduce the sample size to a few grams and improve the homogenization steps. A modified QuEChERS extraction was used with a 0.2g sample (liver and larvae) amount. Homogenization was achieved using a high-speed mixer mill (Retsch MM 200) with a homemade attachment for 1.5mL centrifuge tubes and 4.5mm stainless steel balls. The quantification method was performed on an Agilent® 6470 Triple Quad LC/MS/MS system. Chromatographic separation was achieved on a ZORBAX® Eclipse Plus™ C18 RRHD 3.0mm x 100mm, 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). *Lucilia sericata* eggs (250) were placed to feed on beef liver tissue homogenate (200g) with four concentrations of fentanyl (0µg/Kg, 2. µg/Kg, 50µg/Kg, 250µg/Kg) and allowed to develop to third instars. Thirty specimens from each treatment were heat killed in water, rinsed to remove any potential residual drug, then dried, measured (weight, length, and width) and stored at -80°C for toxicology analysis.

A validation of the LC/MS/MS method for fentanyl, norfentanyl, and 4-ANPP was performed with a Limit Of Detection (LOD) of 0.1ppb and an Analytical Measurement Range (AMR) of 0.5ppb to 250ppb. Accuracy for all three compounds at the lowest calibrator were above 82 (±0.88) % with a %RSD of less than 5% and for the highest calibrator above 94 (±0.47) % accuracy with a %RSD less than 0.5%. Linearity was acceptable for all three compounds as they were all better than 0.995. The average recovery for the liver extraction was greater than 75 (±1.4) % and the average recovery for the larvae extraction was greater than 71 (± 1.6) %.

The method was validated, and the calibration curves reconcile well with forensic toxicology criteria. The extraction and LC/MS/MS method developed for analysis of larvae and liver tissue for fentanyl, norfentanyl, and 4-ANPP is precise, sensitive, and reproducible at forensically relevant concentrations.

**QuEChERS, Fentanyl, *Lucilia sericata***