K72 Assessment of Postmortem Liver Samples Using a Validated Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction for Fentanyl and Metabolites With Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis

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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 postmortem liver specimens using LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by relaying a procedure that utilizes the unique properties of a QuEChERS extraction with modifications to allow for the extraction of fentanyl and metabolites from challenging postmortem matrices.

The opioid crisis in the United States has a serious impact on 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 28,466 deaths in 2017 (a 915% increase). In a forensic postmortem toxicological workflow, various specimens are often submitted for testing, including urine, blood, liver, kidney, and other tissues. For medicolegal death investigations, the interpretation of toxicological findings is a key component to establish the cause of death of an individual.

Common practices for tissue extractions involve Solid Phase Extraction (SPE) or Liquid-Liquid Extraction (LLE), but the process for these techniques can be time consuming and often involve tissue homogenization using blenders that could allow cross-contamination. In recent years, a QuEChERS extraction protocol was introduced in the market to deal with samples with a high content of fatty materials. By its nature, the liver contains many lipids that can contribute to matrix effects and be problematic for instrumentation. QuEChERS is ideal for tissue extractions due to its two-part process that allows for a direct extraction with organic solvent in the first stage, followed by a dispersive-solid phase extraction step. QuEChERS embraces the advantages of LLE, direct extraction from tissue with an organic solvent and cost effectiveness, combined with the advantages of SPE, reduction of matrix effects and impurities, without sacrificing selectivity or robustness.

In this study, the use of a modified QuEChERS extraction was demonstrated for testing a 0.2g liver sample. Homogenization was achieved using a Retsch® MM 200 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 quantification method was performed on an Agilent® 6470 Triple Quad Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) 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).

The validation of a QuEChERS extraction protocol is presented in this study as an alternative analytical method for efficient extraction and detection of fentanyl and its primary metabolites, norfentanyl and 4-ANPP. The LC/MS/MS analysis was validated following the American Academy of Forensic Sciences Standards Board (ASB) standard 036 guidelines. The evaluated parameters include selectivity, matrix effects, linearity, bias, precision, processed sample stability, and proof of applicability using authentic fentanyl case samples.

The validation of the quantitative method for fentanyl, norfentanyl, and 4-ANPP was performed with a Limit Of Detection (LOD) of 0.1µg/kg and an Analytical Measurement Range (AMR) of 0.5 to 100µg/kg. Overall, the QuEChERS technique met all the acceptable criteria set by Matuszewski et al. with recoveries higher than 90.2%, matrix effects greater than 80.4%, and process efficiencies greater than 76.7% with %CV values below 12.8% for all target analytes.[1] The authentic case samples ranged in fentanyl concentrations from 56.6 to 462.3µg/kg with a mean of 149.2µg/kg (*n*=10). The range of norfentanyl concentrations was 1.9 to 50.0µg/kg with a mean of 14.1µg/kg (*n*=10). The range of 4-ANPP concentrations was 3.2 to 23.7µg/kg with a mean of 7.5µg/kg (*n*=7).

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

Reference(s):

1. Matuszewski, B.K, Constanzer, M.L, and Chavez-eng, C.M. (2003). Strategies for theAssessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS, *Anal. Chem.* 75, 3019-3030.

QuEChERS, Fentanyl, Liver

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