K25 Analysis of Acetyl Fentanyl in Postmortem Blood and Urine Specimens by Gas Chromatography/Mass Spectrometry (GC/MS)

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After attending this presentation, attendees will better understand the quantitation of acetyl fentanyl in biological specimens utilizing Solid Phase Extraction (SPE) and GC/MS. In addition, attendees will better understand the postmortem distribution of acetyl fentanyl.

This presentation will impact the forensic science community by providing the first reported analytical method for the quantitative analysis of acetyl fentanyl in postmortem blood and urine specimens.

In 2013, the Centers for Disease Control and Prevention issued an alert regarding a new illicit drug, acetyl fentanyl. Acetyl fentanyl was implicated in the death of 15 decedents in Rhode Island from March 2013 through December 2013.[1] Acetyl fentanyl is an analog of fentanyl, and its pharmacological effects include altered mood, euphoria, respiratory depression, and central nervous system depression. Acetyl fentanyl is a μ-opioid receptor agonist with potency reportedly 15 times greater than morphine, but one-third the potency of fentanyl, based on animal studies.[2]

Due to the novelty of the analyte, it was important to develop a sensitive and specific method for the extraction of acetyl fentanyl from postmortem specimens. This study followed method validation procedures published by the Scientific Working Group for Forensic Toxicology (SWGTOX) to develop and validate a method to quantify acetyl fentanyl in postmortem blood and urine.

SPE (SPEware CEREX Trace -B) was utilized to isolate acetyl fentanyl from the biological matrices. A 1.0mL sample of blood and urine was aliquoted and fortified with isotope-labeled internal standard solution containing $^{13}C_6$-acetylfentanyl. The samples were buffered with pH 6.0, 0.1M phosphate buffer, vortexed, and centrifuged. The samples were transferred to preconditioned SPE columns. The columns were sequentially washed with deionized water, 100mM acetic acid, methanol, and dried for five minutes under N_2. The analytes were eluted with a 78:20:2 (*v:v:v*) methylene chloride:isopropanol:ammonium hydroxide elution solvent. The eluents were dried at 37°C under N_2, reconstituted with ethyl acetate, and submitted for GC/MS analysis.

The GC/MS parameters included splitless injection with an initial temperature of 140°C, hold for 0.5 minutes, ramp at 30°C/minute to the final temperature of 320°C with a final hold time of seven minutes. The total run time was 13.5 minutes. The helium flow rate was 1mL/minute. GC/MS analysis was conducted in Selected Ion Monitoring (SIM) mode utilizing the following ions: acetyl fentanyl, *m/z* 231,188, 146 and $^{13}C_6$-acetyl fentanyl, *m/z* 237, 194, 152. The assay was linear from 1.0ng/mL to 50ng/mL. The lower limit of quantitation was defined as the lowest non-zero calibrator, 1.0ng/mL, and the experimental limit of detection was 0.5ng/mL and 0.75ng/mL in blood and urine, respectively.

Specimens were obtained from the Rhode Island Office of State Medical Examiners and University of Florida Health Pathology Laboratories — Forensic Toxicology Laboratory for the analysis of acetyl fentanyl. The deaths occurred from March to December 2013. Acetyl fentanyl was detected in 14 decedents: nine males and five females, aged 23-57 years old. The results of the acetyl fentanyl analyses are shown in Table 1. According to the results indicated, acetyl fentanyl demonstrates postmortem redistribution with heart-to-femoral blood concentration ratios ranging from 0.97-2.84, with a mean of 1.59 in a group of eight fatalities.

Table 1. Acetyl fentanyl concentrations from casework.

	Femoral Blood (ng/mL)	Heart Blood (ng/mL)	Antemortem Blood (ng/mL)	Postmortem Urine (ng/mL)	Ratio of Heart-to- Femoral Blood Concentration
Mean	338.6	386.7	136.6	3705.3	1.59
Range	89-945	17-915	57-178	41-9825	0.97-2.84

SPE followed by GC/MS analysis was shown to be an acceptable method for the quantitation of acetyl fentanyl in postmortem blood and urine specimens, and the assay provided a sensitive and specific method while limiting interferences typically found in postmortem blood and urine.

Acetyl Fentanyl, Method Validation, GC/MS