K39 Long-Term Stability Assessment of Fentanyl Analogs in Blood Using Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/QTOF/MS)

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Learning Overview: After attending this presentation, attendees will better understand the stability of fentanyl analogs in blood specimens over a nine-month time frame.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees with a novel fentanyl analog quantification method and knowledge of fentanyl analog stability.

Fentanyl analogs played a major role in proliferating the opioid epidemic in the United States. While fentanyl analog use has recently decreased due to regulation, overdose deaths caused by these drugs are not uncommon. With high rates of overdose deaths, forensic toxicology laboratories often experience backlogs, resulting in extended sample storage time. To address this issue, a quantitative method was developed and validated for fentanyl analogs in blood using LC/qTOF/MS). The method was then applied to a long-term stability study (nine months) to assess fentanyl analogs in blood at various temperatures.

Analytes of interest were methoxyacetylfentanyl, acetylfentanyl, acrylfentanyl, 4-ANPP, fentanyl, furanylfentanyl, p-fluorofentanyl, cyclopropylfentanyl, 3-methylfentanyl, carfentanil, butyrylfentanyl, 4-fluoro-isobutyrylfentanyl, and valerylfentanyl. Blood samples (250µL) were subjected to a previously published solid-phase extraction procedure. Quantification was performed using an Agilent® Technologies 1290 Infinity liquid chromatograph coupled to an Agilent® Technologies 6530 Accurate Mass TOF/MS operated in targeted acquisition mode.

For long-term stability, potassium oxalate and sodium fluoride-preserved blood (100mL) was fortified at a low (10ng/mL) and a high (80ng/mL) concentration, and aliquots were subjected to the following conditions: frozen (-20°C), refrigerated (4°C), room temperature (~25°C), and elevated temperature (35°C). All samples were stored in glass vacutainer tubes, except frozen, which were stored in plastic falcon tubes. Samples were analyzed fresh (t_0) and after 24h, 48h, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and then monthly (for 9 months). Stability samples were extracted and analyzed using the present validated method. Analyte concentrations were compared to t_0, and analytes were considered stable if compounds quantified within ±20%.

The method was validated according to American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036. Calibration ranges were 1–100ng/mL for all analytes. Limits Of Detection (LOD) were 0.5ng/mL for all analytes. Precision and bias values were determined to be acceptable for all analytes (within ±7.2%CV and ±15.2%, respectively). Matrix effects exhibited minor ion enhancement for all analytes, except carfentanil and 4-ANPP in the low quality control (ion enhancement >25%). No carryover, endogenous, or exogenous interferences were encountered. Short-term stability studies were performed to establish time points for the long-term portion of the study. All analytes remained stable under the following conditions: 48h in the autosampler, 24h room temperature, and 72h refrigerated. Acrylfentanyl was unstable at room temperature (>±20% bias).

Over the first three months, the fentanyl analogs of interest (except acrylfentanyl) remained stable under room temperature and refrigerated conditions at both concentrations (84.0%–112.5%). While most fentanyl analogs remained stable under frozen conditions, downward trends were observed after two weeks (4 freeze/thaw cycles). At elevated temperatures, most analytes were stable for one week at both concentrations (74.2%–112.6%). Acrylfentanyl was determined to be unstable after 24h under elevated (<30%) and room temperature (<50%) conditions, after 48–72h under refrigerated (59.5%–82.0%) conditions, and four weeks under frozen (73.0%–78.0%) conditions.

This research presents a validated method for the quantification of fentanyl analogs in blood using LC/qTOF/MS and has determined analyte stability in blood at four temperatures over the first three months of an in-progress nine-month study. While fentanyl analogs are observed stable for months under various temperature conditions, optimal storage is recommended under refrigeration. In the event of improper storage or handling, subjecting samples to elevated temperatures may remain stable for about one week. However, acrylfentanyl was determined to be extremely unstable and immediate analysis would be required for a suspected case.

Fentanyl Analogs, Postmortem Blood, Stability

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