K17 Liquid Chromatography/Quadrupole Time-Of-Flight (LC-QTOF) Screening for Fentanyl Analogs in Whole Blood and Oral Fluid

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Learning Overview: After attending this presentation, attendees will better understand the role of high-resolution Mass Spectrometry (MS) in the identification of novel fentanyl analogs in biological samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a unique screening methodology for the detection of fentanyl analogs in blood and oral fluid.

Fentanyl analogs are responsible for an increasing number of opioid related deaths in the United States. Routine forensic analyses are often unable to detect these analogs due to the low concentrations and similar molecular structures. To address this problem, a comprehensive screening method was developed and validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for 14 fentanyl analogs in whole blood and oral fluid using LC-qTOF/MS.

Blood (0.25mL) or oral fluid (1mL containing 1:3 oral fluid: Quantisal® buffer) were fortified with deuterated internal standards, diluted with phosphate buffer, and extracted with a polymeric solid-phase extraction column. After washing, analytes were eluted with 5% ammonium hydroxide in ethyl acetate, dried, and reconstituted in mobile phase. An Agilent® Technologies 1290 Infinity® LC coupled to an Agilent® Technologies 6530 Accurate Mass TOF/MS was used for analysis in two separate acquisition modes: TOF mode and All Ions Fragmentation (AIF) mode. Personal Compound and Database Libraries (PCDLs) were produced in-house containing all analytes of interest (*n*=14), as well as other drugs of abuse (*n*=53). Both matrices were validated according to SWGTOX Standard Practices for Method Validation in Forensic Toxicology guidelines. For proof of applicability, authentic postmortem blood (*n*=30) and antemortem oral fluid samples (*n*=18) were extracted and analyzed as described above.

Chromatographically, all analytes eluted before seven minutes. Baseline resolution was achieved for most analytes. Butyrylfentanyl and isobutyryl fentanyl were unable to be distinguished by the LC system, which is a common issue encountered in toxicological analyses. To address this problem, certified reference standards were analyzed using an Agilent® Technologies 6890N Gas Chromatograph (GC) coupled to an Agilent® Technologies 5975B MS equipped with an Agilent Technologies DB-5 (30m x 0.25mm x 0.25µm) column. The GC was able to fully separate these isomeric compounds 12.5min method.

The Limits Of Detection (LOD) for all analytes in blood were 0.1-0.25ng/mL and 0.1-1.0ng/mL in TOF and AIF modes, respectively. In oral fluid, the LOD were 0.25ng/mL and 2.5ng/mL in TOF and AIF modes, respectively. No carryover or interferences (exogenous or endogenous) were observed. Matrix effects in blood were considered acceptable for most analytes with minor ion enhancement of 1%–14.4%, while the nor- analytes (metabolites) exhibited ion suppression >25%. Matrix effects in oral fluid were considered acceptable for all analytes with ion suppression and enhancement ranging from -11.7%–13.3%. Stability was assessed after 24 hours in the autosampler (blood at 22°C and oral fluid at 4°C) and refrigerator (blood at 4°C). All analytes were determined to be stable under each condition, except alfentanil in OF (>25% loss in autosampler). Authentic postmortem blood samples (*n*=30) were positive for: furanyl fentanyl (*n*=16), 4-ANPP (*n*=15), cis-methyl fentanyl (*n*=4), fentanyl (*n*=3), and valeryl fentanyl (*n*=1). Additional drugs of abuse detected included methamphetamine (*n*=2), cocaine (*n*=2), ketamine (*n*=2), 6-MAM (*n*=7), alprazolam (*n*=5), morphine (*n*=8), codeine (*n*=7), hydrocodone (*n*=2), etizolam (*n*=3), amitriptyline (*n*=1), buprenorphine (*n*=1), zolpidem (*n*=1), meperidine (*n*=1), and U-47700 and its metabolites (n-desmethyl-U47700 and n,n-didesmethyl-U47700) (*n*=15). Oral fluid samples (*n*=18) collected from arrestees under an Institutional Review Board (IRB) -approved protocol did not contain any fentanyl analogs. However, additional drugs of abuse detected included methamphetamine (*n*=15), amphetamine (*n*=7), cocaine (*n*=4), 6-MAM (*n*=4), morphine (*n*=2), codeine (*n*=1), alprazolam (*n*=1), and mephedrone (*n*=1).

This research presents two validated screening methods for fentanyl analogs in whole blood and oral fluid using LC-qTOF analysis with low limits of detection. In addition, this research presents an alternative GC/MS application for the separation of fentanyl analogs, specifically, butyryl and isobutyryl fentanyl.

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Fentanyl Analogs, Postmortem Blood, Oral Fluid