K59 The Quantitative Analysis of Fentanyl and Fentanyl Analogs in Hair

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Learning Overview: After attending this presentation, attendees will know how to detect fentanyl use through hair analysis. Attendees will also know how to identify the presence of fentanyl analogs and their metabolites in hair.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting a method for the detection and quantitation of fentanyl, fentanyl analogs, and their metabolites in hair. Data on the prevalence of fentanyl analogs that are also appearing in fentanyl users' hair will also be presented.

Hypothesis: Fentanyl, fentanyl analogues, and their metabolites are detectable in the hair of fentanyl users. The fentanyl can be extracted and analyzed via microwave extraction, solid phase preparation, and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis.

An LC/MS/MS method for the analysis of fentanyl and fentanyl analogues in hair was developed. The assay was validated for fentanyl, norfentanyl, acetyl fentanyl, acetyl norfentanyl, furanyl fentanyl, furanyl norfentanyl, carfentanil, norcarfentanil, butyryl fentanyl, butyryl norfentanyl, valeryl fentanyl, and para-fluoro-butyryl fentanyl. The method was validated for precision, accuracy, linearity, carryover, interference, matrix suppression, recovery, and dilution integrity.

The fentanyl was extracted from the hair via microwave extraction in 9:1 methanol:trifluoroacetic acid. The solid phase clean-up was performed with DPX-CX tips on a Hamilton® STARlet liquid handler. The samples were sequentially washed with 0.1M acetate buffer pH 4.0, methanol/0.1 M HCl (4:6), and methanol, and eluted with 80:20:3 methylene chloride:isopropanol:ammonium hydroxide. The eluent was dried and reconstituted in mobile phase. The analysis was performed on a QTRAP® 6500+ LC/MS/MS with a Shimadzu® LC30-AD binary pump system and PAL-HTCxt with a DLW wash system. A Phenomenex® Kinetex® C18 150 x 2.1 mm, 1.7 µm particle size, 100 Å pore size Ultra Performance Liquid Chromatography (UPLC) column using a gradient elution with 0.1% formic acid in water and 0.1% formic acid in methanol was employed for chromatographic separation.

The presence of fentanyl was confirmed in 110 samples that had previously been screened by enzyme immunoassay for fentanyl. The sample pool utilized for the screening consisted predominantly of samples that had previously identified as positive for heroin metabolite (6-monoacetylmorphine, morphine). Many of the samples were also positive for other street drugs, mostly cocaine and methamphetamine.

The median concentration for the fentanyl was 132.5pg/mg. The median concentration for norfentanyl was 10.4pg/mg. The average ratio of fentanyl to norfentanyl was 20:1.

Many of the 110 positive samples contained other fentanyl derivatives. Furanyl fentanyl was detected in 55% of the samples; and butyryl fentanyl, carfentanil, and acetyl fentanyl were detected in a smaller fraction of the samples. No valeryl fentanyl or para-fluoro butyryl fentanyl was detected in any of these samples. The normetabolites of the fentanyl derivatives were also detected in samples with high concentrations (typically >100pg/mg) of the parent drug.

No samples were identified that had fentanyl analogs to the exclusion of fentanyl. This is somewhat due to poor cross-reactivity of some of the fentanyl analogs in the immune-assay, although butyryl fentanyl, valeryl fentanyl, furanyl fentanyl, and acetyl fentanyl have 100% or better cross-reactivity.

This method was used to successfully extract and analyze fentanyl, fentanyl analogs, and their metabolites in hair. Samples were identified containing fentanyl, furanyl fentanyl, carfentanil, butyryl fentanyl, and acetyl fentanyl.

Fentanyl, Analogs, Hair