K67 The Real Heroin in South Florida: The Detection of a Fentanyl Analog in Postmortem Specimens Using Liquid Chromatography (LC) -Ion Trap Tandem Mass Spectrometry (MS/MS)

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After attending this presentation, attendees will better understand a new fentanyl analog, ß-hydroxythiofentanyl, that has been linked to eight fatal cases in Miami-Dade County. The detection of this fentanyl analog has previously gone undetected and is presumably being distributed as heroin.

This presentation will impact the forensic science community by providing vital information regarding a new, deadly compound that is complicated to detect using a basic streamlined Gas Chromatography/Mass Spectrometry (GC/MS) blood drug screen.

Fentanyl and its analogs are commonly known as potent synthetic opioids that exhibit powerful and rapid analgesic onset. Fentanyl itself was first introduced into the medical community as an analgesic with a potency approximately 75-125 times that of morphine. The chemical structure of fentanyl allows manufacturers to create analogs that also possess powerful analgesic properties. Although fentanyl and its analogs have legitimate medical use, they are also abused recreationally as interchangeable, substitute, or cutting agents for heroin. Numerous derivatives of fentanyl are sold on the street as synthetic heroin or, more popularly, China White. Due to the potency of fentanyl and its analogs, the abuse of these compounds increases the potential for accidental overdose, especially if consumers are unaware of what they are ingesting. In 2015, the Miami-Dade Medical Examiner Department (MDME) observed an increase in deaths due to fentanyl toxicity (up 300% compared to the previous year) and observed several cases in which the fentanyl analog, β-hydroxythiofentanyl, was implicated in the cause of death. The majority of the cases contain history which indicates heroin use, and none of the deaths reported are related to the abuse of prescription fentanyl.

Approximately 30 medical examiner cases previously screened by GC/MS for the presence of fentanyl and opiates were submitted to a more comprehensive and sensitive screening method. Case blood was extracted via solid-phase extraction using mixed-mode United Chemical Technologies CleanScreen® columns and a positive pressure manifold. Analysis was performed using a Thermo Scientific™ Dionex™ UltiMate® 3000 Ultra High-Performance Liquid Chromatography (UHPLC) coupled to a Bruker amaZon Speed Ion Trap Mass Spectrometer (MS) equipped with Bruker ToxTyper® software. UHPLC separation was achieved over a nine-minute data collection time, using gradient elution on a C18 column (100mm x 2.1mm, 2.2µm) at 40oC using 2mM ammonium formate, 0.1% formic acid, water, and acetonitrile (99:1) mobile phase. Positive mode electrospray ionization MS analysis was performed using UltraScan mode between 70m/z and 800m/z. A custom ToxTyper® acquisition method targeting a panel of analgesics, including, but not limited to, morphine, oxycodone, hydrocodone, heroin, 6-monoacetylmorphine, methadone, buprenorphine, fentanyl, norfentanyl, β-hydroxythiofentanyl, acetylfentanyl, sufentanil, and butyrylfentanyl was utilized. The acquisition method uses an embedded precursor list, which triggers MS/MS and/or MS^3 on the targeted compounds in question, if present in the specimen. β-hydroxythiofentanyl was found to elute at 3.8 minutes with a targeted parent mass of 359m/z. An MS/MS breakdown was performed on the 359 ion, producing a main product ion of 341m/z, indicative of a water loss. The 341 ion was finally introduced to an MS^3 analysis, where a distinct spectral profile was achieved.

Out of the 30 cases analyzed, 10 contained the fentanyl analog, β-hydroxythiofentanyl. Detection of β-hydroxythiofentanyl was confirmed using an in-house library entry created from a certified reference standard, as well as retention time, parent ion, and daughter ion spectra. Two of the cases positive for β-hydroxythiofentanyl had a history of intravenous drug use and were negative for any other compounds, including fentanyl and other opiates. The remaining 8 cases also had a history of intravenous drug use, and the β-hydroxythiofentanyl was detected in addition to heroin and/or fentanyl in the postmortem specimens.

These cases represent some of the first reported β-hydroxythiofentanyl-involved deaths in Florida. The detection of the analog in routine screening procedures using GC/MS presented a problem and required the use of LC/MS analysis to make a definitive identification.

In all cases, it appeared as if norfentanyl was detected in the blood by GC/MS; however, norfentanyl was only confirmed by LC/MS in those cases in which fentanyl was also present. Norfentanyl, in the absence of fentanyl in routine GC/MS analysis, may be an indicator of the presence of β-hydroxythiofentanyl and require further confirmation.

Postmortem, Fentanyl, ß-hydroxythiofentanyl

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