K72 Rise in Fentanyl Derivatives Acetyl and Butyryl Fentanyl Detection in Blood and Serum Coinciding With Rise in Opiate and Novel Psychoactive Substances (NPS) Use

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After attending this presentation, attendees will be able to describe the emerging group of fentanyl analogs, the development of an analytical assay utilizing Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) for their detection in biological fluids, and be able to discuss concentrations of the drugs determined in blood from toxicology casework.

This presentation will impact the forensic science community by highlighting the new trend of fentanyl analogs that are being detected with increasing frequency in forensic casework.

With the rise in opiate use in the United States, it is not unexpected that there would be a corresponding rise in related drugs either contaminating or counterfeiting the heroin supply. Fentanyl is commonly seen as either a contaminant or a replacement product in street heroin; however, in recent times, certain additional fentanyl derivatives are garnering their own market share and appearing in forensic casework. Two of the fentanyl derivatives that have been detected in chemical and toxicological analyses are acetyl fentanyl and butyryl fentanyl. Reports of acetyl fentanyl began in 2013, with overdoses being reported in Rhode Island, Pennsylvania, and Louisiana. Butyryl fentanyl, a homologue of fentanyl, started to be identified in forensic casework in late 2014. Due to the quick proliferation of cases that were being linked to fentanyl derivatives, as well as other designer opioids, there was a need to develop an assay for the detection of these compounds in forensic toxicology specimens.

A quantitative procedure was developed for the identification and quantitation of four analytes (acetyl fentanyl, butyryl fentanyl, and two other designer opioids not related to fentanyl, MT-45 and AH-7921) using positive mode Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Extraction of these compounds was achieved using acetonitrile for protein precipitation, followed by adding phosphate buffer to the transferred supernatant, with a final step of solid phase extraction using cation exchange columns. Analytical separation was achieved using a silica-based, 2.1mm x 50mm, 1.8 micron column, using mobile phases of ammonium formate at pH4.0 and acetonitrile. Internal standards consisted of $^{13}C_6$-acetyl fentanyl and dueterated AH-7921.

The calibration curve was comprised of six calibrators, at 0.1ng/mL, 0.2ng/mL, 0.5ng/mL, 2ng/mL, 5ng/mL, and 10ng/mL. Calibration experiments (n=5) demonstrated acceptable performance, with correlation coefficients >0.999 for all four analytes. Between-run precision, total precision, and accuracy experiments for acetyl and butyryl fentanyl (n=15) demonstrated acceptable performance (< +/- 8.7%). The limit of detection was determined to be 0.003ng/mL in both blood and serum for acetyl and butyryl fentanyl. Both fentanyl derivatives are stable in blood in all tested conditions for up to 30 days; acetyl fentanyl and butyryl fentanyl are stable for up to 14 days in serum at room temperature, but 30 days at refrigerated and frozen conditions.

Of the designer opioids included in this panel, positives have been reported for both acetyl fentanyl and butyryl fentanyl. From July 2013 to June 2015, 183 cases of acetyl fentanyl use have been detected by the laboratory. Blood concentrations range from 0.11ng/mL to 3,800ng/mL, with average and median concentrations falling at 150ng/mL and 30ng/mL, respectively. Butyryl fentanyl has been detected in only two cases thus far, but testing for this drug was not implemented until May 2015. Butyryl fentanyl was found at 20ng/mL in one case and in a second case was detected at 3ng/mL, in addition to 0.65 ng/mL of acetyl fentanyl.

In 81 (44%) cases positive for acetyl fentanyl, fentanyl and/or norfentanyl were also detected. In 52 (64%) of these positive cases, both acetyl fentanyl and fentanyl were greater than 5ng/mL. In 15 (18%) cases, fentanyl was greater than 5ng/mL, accompanied by smaller amounts of acetyl fentanyl.

With the recent federal scheduling of acetyl fentanyl, it is unknown if that will deter future manufacturing, distribution, and use of this drug or if other analogs will replace its presence. Forensic toxicology laboratories as well as other members of the drug monitoring community must be cognizant of the expansion of fentanyl derivatives and the other designer opioids whose prevalence is increasing in forensic casework, and consider their analysis in cases where circumstances of death cannot be explained by traditional opioid drugs.

Acetyl Fentanyl, Butyryl Fentanyl, Opioid

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