K71 Identification and Quantification of Classic, Prescription, and Synthetic Opioids in Hair by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will better understand how hair can be used as a matrix to identify and quantify different types of opioids, including fentanyl and derivatives, using Solid Phase Extraction (SPE) and LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees with a validated method for the confirmation of a comprehensive panel of opioids, including classic, prescription, and synthetic opioids, in hair. The current widespread misuse of both prescription and non-prescription opioids in the United States has reached an epidemic status. According to the United States Department of Health and Human Services, every day more than 130 people in the United States die after overdosing on opioids, and 2.1 million had an opioid use disorder in 2016. Drug testing tools to monitor opioid use and abuse habits in both the living and postmortem are necessary to understand the opioid use pattern, identify risk groups, and cause of death. Hair is becoming an alternative matrix of increasing interest in forensic toxicology to investigate drug use and abuse patterns due to its long window of detection. Hair growth rate is about 1cm/month; therefore, segmental hair analysis provides information of drug exposure from at least one to several months before samples collection.

Hypothesis: Highly sensitive and specific identification and quantification of a comprehensive panel of opioids in hair samples can be achieved utilizing SPE as a sample preparation and LC/MS/MS as an instrumental technique.

Method: This procedure allows for the confirmation of 27 opioids (8 classic and prescription opioids and 19 synthetic opioids) in 25mg of hair. After hair decontamination, the sample was pulverized using a beadmill. To 25mg of this powder, 15 internal standards (morphine-d3, codeine-d3, 6-acetylmorphine-d3, hydrocodone-d6, hydromorphone-d3, oxycodone-d3, fentanyl-d5, norfentanyl-d5, EDDP-d3, methadone-d3, oxymorphone-d3, tramadol-13C-d3, sufentanyl-d5, isobutyryl fentanyl-d5, and valeryl fentanyl-d5) and 2mL of digestion solution (0.01% formic acid + 2.5mM ammonium formate in water and methanol, 95:5, v/v) was added. The hair sample was digested overnight at 37°C in a heating block. The digestion was cleaned of interferences using a Phenomenex® Strata™-XC Strong Cation SPE cartridge. The elutants were dried under nitrogen and reconstituted using the initial mobile phase concentration (A:B, 95:5, v/v) containing 0.01% formic acid + 2.5mM ammonium formate (A) and acetonitrile (B) prior to LC/MS/MS analysis.

The chromatographic separation was performed in gradient mode employing a 100 x 2.1mm Kintex® C-18 analytical column with a 1.7um particle size and mobile phases A and B. The gradient was programmed to run from 5% to 20% acetonitrile for 3min, 20% to 28% for 11min, 28% to 95% for 10min, then back to 5% for 1min, along with a 5min equilibration for a total run time of 30min. These chromatographic conditions were capable of resolving closely eluting isomers, such as 3-methylfentanyl, butyryl fentanyl, and isobutyryl fentanyl. The Shimadzu® mass spectrometer triple quadrupole LC/MS-8050 was employed in electron spray ionization positive mode, and two multiple reaction monitoring transitions were acquired per analyte.

The Limit Of Quantification (LOQ) was established to be 1pg/mg (3-methyl-fentanyl, 4-anpp, 4-methoxy-butyryl-fentanyl, acetyl fentanyl, acryl-fentanyl, alfentanil, butyryl fentanyl, carfentanil, EDDP, fentanyl, furanyl-fentanyl, isobutyryl fentanyl, MT-45, para-fluorobutyryl fentanyl, sufentanil, U-47700, and valeryl fentanyl), 10pg/mg (6-acetylmorphine, hydrocodone, methadone, morphine, norfentanyl, oxycodone, oxymorphone, and tramadol), or 100pg/mg (codeine and hydromorphone). The method was fully validated following the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The parameters evaluated were linearity, limit of detection and LOQ, imprecision, accuracy, carryover, matrix effects, extraction efficiency, process efficiency, interferences, and autosampler stability.

Conclusion: A sensitive and specific method was developed for the identification and quantification of 27 classic, prescription, and synthetic opioids in hair by LC/MS/MS.

Opioids, Hair, LC/MS/MS