K37 The Development and Validation of a Method for the Analysis of Novel Emerging Opioids

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After attending this presentation, attendees will be able to describe a Scientific Working Group for Forensic Toxicology (SWGTOX) - compliant approach to method validation for the analysis of designer opioid compounds in a variety of forensic samples using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) technology.

This presentation will impact the forensic science community by demonstrating the capability of an analytical method that can be used to simultaneously analyze 17 novel emerging opioid compounds, many of which have only recently appeared on the market.

In recent years, an increasing number of novel opioids have appeared on the illicit drug market and have been linked to the growing opioid crisis in the United States. In 2015, butyryl fentanyl was ranked in 12th place of the most frequently identified narcotic analgesics category in the National Forensic Laboratory Information System (NFLIS). By mid-year 2016, butyryl fentanyl was replaced by furanyl fentanyl, U-47700, and 3-methylfentanyl, which were ranked 12th, 13th , and 14th, respectively.

A method is described for the analysis of 19 of the most current novel opioid drugs in whole blood and serum, and 17 analytes in urine using LC/MS/MS. Blood and serum were analyzed quantitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, U-47700, U-50488, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. Urine samples were analyzed qualitatively for butyryl fentanyl/isobutyryl fentanyl, MT-45, AH-7921, furanyl fentanyl, para-fluorofentanyl, ortho-fluorofentanyl, para-fluorobutyryl fentanyl/FIBF, 4-methoxybutyryl fentanyl, 4-ANPP, alpha-methyl fentanyl, 4-methylphenethyl acetyl fentanyl, acryl fentanyl, valeryl fentanyl, carfentanil, and beta-hydroxythiofentanyl. The isomer pairs butyryl fentanyl/isobutyryl fentanyl and para-fluorobutyryl fentanyl/FIBF are not chromatographically separated in this method and are reported as a pair.

The method was validated according to a SWGTOX-compliant procedure, which for the quantitative portion evaluated precision and accuracy, limit of detection, lower limit of quantitation, linearity, stability in matrix and on-instrument, robustness, an evaluation of interfering compounds, matrix matching, dilution integrity, carry-over, matrix effect, and extraction efficiency. The validation for the qualitative portion of the method evaluated precision around the decision concentration (cut-off) stability in matrix and on-instrument, sensitivity and specificity, robustness, evaluation of interfering compounds, matrix effect, and extraction efficiency.

Sample preparation consisted of protein precipitation followed by solid phase extraction using Agilent® Plexa™ PCX 3mL/60mg extraction columns. The analytical method consisted of separation using a ZORBAX® RX-SIL (3mm x 100mm, 1.8 micron) column coupled with an Optimize EXP filter (0.2 micron) and a gradient elution utilizing ammonium formate, pH 4.0 (Mobile Phase A), acetonitrile (CH3CN), LC/MS grade (Mobile Phase B and weak wash), and formic acid in deionized water, 0.1% (strong wash). The analysis was performed on a Waters® ACQUITY® TQD MS/MS with a Waters® ACQUITY® Ultra Performance LC system.

This method produced data that met the acceptance criteria established for the validation. The quantitative portion of the analysis produced controls within 25% of target value, while the qualitative portion produced 94.1% sensitivity and 100% specificity during the validation. During the validation, it was determined that all analytes were stable in blood at room temperature for at least two weeks, and at refrigerated and frozen conditions for 30 days, with the exception of acryl fentanyl, which was only stable for one day at room temperature and one week refrigerated. In serum, it was determined that all analytes were stable at room temperature for at least two weeks, and 30 days refrigerated and frozen, except acryl fentanyl, which was stable for two days at room temperature, and MT-45, which was stable for one week at room temperature. In urine, it was determined that all analytes were stable for a minimum of one week at room temperature, and 30 days refrigerated and frozen.

Designer Opioids, Forensic Toxicology, LC/MS

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