Abstract

The United States and numerous other countries worldwide are currently experiencing a public health crisis due to the abuse of illicitly manufactured fentanyl (IMF) and its analogues. This manuscript describes the development of a liquid chromatography-tandem mass spectrometry-based method for the multiplex detection of $N=24$ IMF analogues and metabolites in whole blood at concentrations as low as $0.1−0.5$ ng mL$^{-1}$. These available IMFs were fentanyl, norfentanyl, furanyl norfentanyl, remifentanil acid, butyryl norfentanyl, remifentanil, acetyl fentanyl, alfentanil, AH-7921, U-47700, acetyl fentanyl 4-methylphenethyl, acrylfentanyl, para-methoxyfentanyl, despropionyl fentanyl (4-ANPP), furanyl fentanyl, despropionyl para-fluorofentanyl, carfentanil, (±)-cis-3-methyl fentanyl, butyryl fentanyl, isobutyryl fentanyl, sufentanil, valeryl fentanyl, para-fluorobutyryl fentanyl, and para-fluoroisobutyryl fentanyl. Most IMF analogues ($N=22$) could be easily distinguished from one another; the isomeric forms butyryl/isobutyryl fentanyl and para-fluorobutyryl/para-fluoroisobutyryl fentanyl could not be differentiated. $N=13$ of these IMF analogues were quantified for illustrative purposes, and their forensic quality control standards were also validated for limit of detection ($0.017−0.056$ ng mL$^{-1}$), limit of quantitation ($0.100−0.500$ ng mL$^{-1}$), selectivity/sensitivity, ionization suppression/enhancement ($87−118\%$), process efficiency ($60−95\%$), recovery ($64−97\%$), bias ($<20\%$), and precision ($>80\%$). This flexible, time- and cost-efficient method was successfully implemented at the Montgomery County Coroner’s Office/Miami Valley Regional Crime Laboratory in Dayton, Ohio, where it aided in the analysis of $N=725$ postmortem blood samples collected from February 2015 to November 2016.