

Special Issue

Unique Structural/Stereo-Isomer and Isobar Analysis of Novel Fentanyl Analogues in Postmortem and DUID Whole Blood by UHPLC–MS–MS

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Abstract

The presented analytical method enabled the Toxicology Department at the Cuyahoga County Medical Examiner's Office to identify 26 and quantitatively report 24 compounds in 500 μ L of whole blood, including fentanyl analogues (fentalogues) such as methoxyacetyl fentanyl (MeOAF) and cyclopropyl fentanyl (CPF). This second-generation method (FG2) was developed with the objective to improve the existing analysis (FG1) by decreasing sample size, lowering limits of detection (LOD) and lower limit of quantitation, minimizing ion suppression and resolving chromatographic interferences. Interferences may occur in the analysis of fentanyl, MeOAF, CPF, 3-methylfentanyl (3MF), butyryl fentanyl and isobutyryl fentanyl due to isobars and structural or geometric isomerism with another analogue or metabolite. The isomeric and isobaric fentalogues were grouped into three sets. The LOD established for Set 1 [MeOAF, *para*-methoxyacetyl fentanyl, *para*-fluoro acryl fentanyl (isobar), fentanyl carbamate], 2-furanyl fentanyl, Set 2 [CPF, (*E*)-crotonyl fentanyl] and carfentanil was 0.0125 ng/mL. The LOD established for *N*-methyl norfentanyl, norfentanyl, norcarfentanil, despropionyl fentanyl (4-ANPP), acetyl fentanyl, β -hydroxy fentanyl, benzyl fentanyl, acryl fentanyl, alfentanil, fentanyl, *para*-fluoro fentanyl, Set 3 [(\pm)-*trans*-3MF, (\pm)-*cis*-3MF, isobutyryl and butyryl fentanyl], *para*-fluoroisobutyryl fentanyl, sufentanil, phenyl fentanyl and cyclopentenyl fentanyl was 0.0625 ng/mL. Seven-point linear calibration curves were established between 0.025 and 4.0 ng/mL for the 8 analytes with the lower LOD and 0.125 and 20 ng/mL for the 18 analytes with the higher LOD. 4-ANPP and cyclopentenyl fentanyl met qualitative reporting criteria only. The results for five postmortem and two driving under the influence of drugs authentic case samples are presented. To the authors' knowledge, FG2 is the first published method that achieved baseline resolution of the nine structural/stereo isomers and one isobar by ultra-high performance liquid chromatography–MS–MS and provided quantitative validation data for nine compounds. FG2 may be used as the new baseline for future isomers that need to be chromatographically separated.