K60 Distinguishing Cyclopropylfentanyl From the Analogs Crotonylfentanyl and Methacrylfentanyl in Two Dimensions: The Utilization of Liquid Chromatography and Mass Spectrometry

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Learning Overview: After attending this presentation, attendees will: (1) be aware of two fentanyl analogs that could potentially be mistaken for cyclopropylfentanyl, and (2) have the tools to distinguish them.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by: (1) providing modes of separation for cyclopropylfentanyl and closely related analogs, and (2) identifying potential pitfalls involving isobaric analogs in New Psychoactive Substances (NPS) identification.

Background: In 2017, Sweden experienced a series of 72 deaths involving the fentanyl analog cyclopropylfentanyl. Identification was complicated by the two isobaric analogs crotonyl- and methacrylfentanyl, which produced similar high-resolution Mass Spectrometry (MS) -spectra and similar retention times in the screening assay.

Goal: The goal of this study was to establish methodology that could distinguish the three analogs by Liquid Chromatography (LC) and/or MS to verify the presence of cyclopropylfentanyl in autopsy cases.

Methods: A screen for chromatographic selectivity was conducted. Five different Ultra High-Performance Liquid Chromatography (UHPLC) columns (50x2.1, <2µm) including Waters® HSS T3, CSH Phenyl Hexyl, Protein BEH C4 and BEH C8 as well as Kinetex® PFP from Phenomenex, were screened using two different organic modifiers as B-phase (either acetonitrile or methanol with 0.05% formic acid) and two different A-phases (0.05% formic acid in either water or 10mM ammonium formate) using a generic gradient from 5%–65% organic modifier over 8min at 0.7ml/min. A 2min gradient was optimized based on the results of the screen.

As fragmentation of the analogs was very similar (major fragments in Figure 1a), ion ratios of major fragments were investigated to distinguish the analogs. Reference ion ratios were established as the average of ten replicates analyzed by Multiple Reaction Monitoring (MRM) on an AB SCIEX® API 4500 LC/MS/MS. The separation was performed using a Waters® BEH Phenyl column (50x2.1mm, 1.7µm) with 0.05% formic acid in 10mM ammonium formate and methanol as mobile phases.

The stability of ion ratios over time was investigated by analyzing data from 68 cyclopropylfentanyl autopsy cases analyzed over seven months. The standard deviation and the 99% confidence interval (±2.6 SD) were calculated. As no case data was available for crotonyl- and methacrylfentanyl, the same Relative Standard Deviation (RSD) was assumed.

Results: The best separation was obtained on a Waters® Protein BEH C4 column using 0.05 formic acid in 10mM ammonium formate and 0.05% formic acid in methanol as mobile phases. Separation with an optimized 2min gradient from 15-30% methanol is shown in Figure 1b.

Average ion ratios (m/z 188/105) for cyclopropyl-, crotonyl-, and methacrylfentanyl were 1.46, 1.94 and 2.21, respectively. The RSD for the cyclopropyl ion ratio was 5.1%. Probability profiles were plotted in Figure 1c including the 99% confidence interval (±2.6 SD). Cyclopropylfentanyl could be distinguished from the other analogs while an overlap existed between crotonyl- and methacrylfentanyl.

Analogs with closely related isobaric analogs is a growing concern given the increasing number of NPS, especially as laboratories may not be aware of potential analogs at the time of method validation. By utilizing selective chromatographies and carefully selecting acceptance criteria for ion ratios, the confidence in NPS identification can be increased.

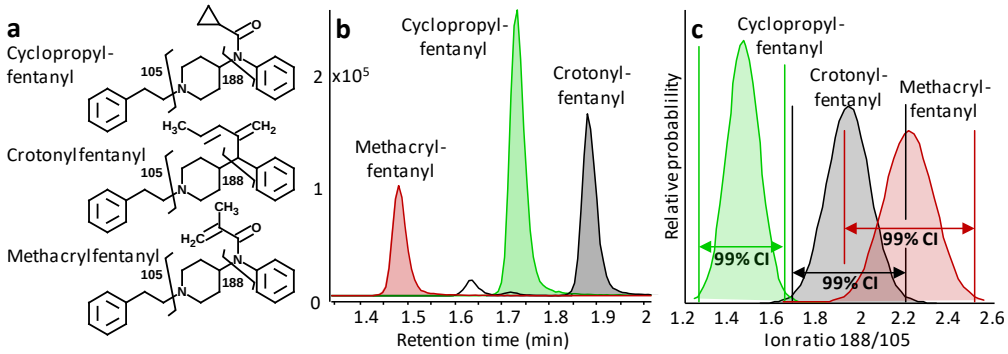

Figure 1 – Structures of the fentanyl analogs (a), optimized chromatography (b), and Confidence Intervals (CI) of m/z 188/105 ion ratios (c).

Conclusion: This study illustrates that even though cyclopropyl-, crotonyl-, and methacrylfentanyl have the same nominal mass and are structurally similar, it was possible to distinguish them by their retention time on a BEH C4 column.

It was also possible to distinguish cyclopropylfentanyl from crotonyl- and methacrylfentanyl by the difference in their respective m/z 188/105 ion ratios. However, crotonyl- and methacrylfentanyl could not be distinguished from each other using only ion ratios. These approaches could potentially be used to distinguish other NPS drugs from isobaric analogs and provide more reliable identifications in toxicological casework.

Cyclopropylfentanyl, Crotonylfentanyl, Methacrylfentanyl