B188 On the Fragmentation Behavior of Fentanyl and Its Analogs in Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS)

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Learning Overview: After attending this presentation, attendees will better understand the structures and mechanisms of formation of at least three distinct isomeric/isobaric fentanyl product ions observed at nominal mass *m/z* 188 in ESI-MS/MS. Attendees will learn about the concepts of multistage Mass Spectrometry (MS^n), accurate mass measurements with High Resolution Mass Spectrometry (HRMS), and isotopic labeling for structural elucidation of fentanyl and Fentanyl-Related Compounds (FRCs).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a better understanding of the fragmentation pathways of fentanyl and FRCs. Specifically, the identification of a novel isobaric fentanyl product ion at *m/z* 188 with ESI-MS/MS will enlighten this community about potential issues with the use of the *m/z* 188 fentanyl product ion for quantification with Multiple Reaction Monitoring (MRM).

Hypothesis: The central hypothesis is that the combination of MS^n, isotope labeling, and accurate mass measurements with HRMS will result in a deeper understanding of the fragmentation behavior of fentanyl and its analogs. Another hypothesis postulates that isomers having identical masses and different constitutional arrangements of atoms will provide measurable differences in their fragmentation patterns. A final hypothesis is that isotopic labeling (e.g., D for H and ^{13}C for ^{12}C) does not alter the fragmentation behavior of an ion in a meaningful way, other than by changing the *m/z* value(s) of the product ions that contain the isotope(s).

Methods/Results: Analyses involve the characterization of fragmentation pathways of fentanyl and FRCs using MS^n, accurate mass measurements with HRMS, and isotopic labeling. Specifically, the identification of isobaric fentanyl product ions of the base peak at *m/z* 188 with ESI-MS/MS was accomplished through the analysis of fentanyl, fentanyl-d5, and two 15N-fentanyl analogs. All compounds were analyzed using a Thermo Scientific™ LTQ™ Velos Pro™ with Heated Electospray Ionization (HESI) ionization source and an Agilent® Technologies 6538 UHD Accurate-Mass quadrupole Time-Of-Flight (qTOF) with a dual Electrospray Ionization (ESI) source.

Wichitnithad et al.[1] have shown that when the protonated precursor ion of fentanyl at *m/z* 337 is exposed to collisional activation, two distinct constitutional isomers of the product ion at *m/z* 188 are formed. The two constitutional isomers are formed via the intermediate at *m/z* 281. However, MS^n experiments in this work demonstrate another isobar at nominal mass *m/z* 188 that has a distinct elemental composition from the other structures based on accurate mass measurements. This third ion forms via the intermediate fragment at *m/z* 216 and is of interest because the abundance of *m/z* 188 is commonly used for quantification with Multiple Reaction Monitoring (MRM)[1], and one should be confident in the ions' identity and relative abundance.

MS^n data on the linear ion trap mass spectrometer reveals that there are three pathways of formation for the nominal mass of *m/z* 188, but that the isolation and MS^n fragmentation of *m/z* 188 from different precursors provides distinct fragment ions. For example, the pathway *m/z* 337→281→188→ provides a range of product ions consistent with the two distinct isomers at *m/z* 188 that were first described by Wichitnithad et al.[1]. However, the current work shows that the pathway *m/z* 337→216→188→ results in an exclusive fragment at *m/z* 132 and that fragmentation through the pathway *m/z* 337→244→188→ results in fragments at *m/z* 134 and *m/z* 120.

The proposed mechanism for the formation of the novel isobaric product ion involves the opening of the piperidine ring from the [M+H]+ fentanyl precursor followed by a 4-center-elimination of the saturated alkyl chain. The combination of MS^n, accurate mass measurements with HRMS, isotopic labeling, and differences in the MS3 product ion spectra allow for the identification of this novel fentanyl product ion and an enhanced understanding of the fragmentation behavior of fentanyl and FRCs.

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Reference(s):

1. Wisut Wichitnithad, Terence J. McManus, Patrick. S. Callery. Identification of isobaric product ions in electrospray ionization mass spectra of fentanyl using multistage mass spectrometry and deuterium labeling. *Rapid Commun. Mass Spectrom.*, 2010. 24(17): pp. 2547-53.

Seized Drugs, Mass Spectrometry, Isotopic Labeling

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