B28 The Effect of Modifications to the Core Fentanyl Structure on the Observed Product Ion Spectra

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Learning Overview: After attending this presentation, attendees will understand how the abundance of diagnostic product ions can be used to predict the region of modification of novel Fentanyl-Related Compounds (FRCs). Attendees will also learn about how multistage Mass Spectrometry (MS^n) can be used to determine differences in structures of isobaric product ions.

Impact on the Forensic Science Community: This presentation will benefit the forensic science community by providing a deeper understanding of spectral interpretation of fentanyl analogs in tandem mass spectrometry.

The central hypothesis is that by identifying trends or correlations between certain types of modification and the abundance of diagnostic product ions, analysts could use these trends to help determine the structure of novel FRCs from their mass spectra.

During a previous study, Davidson et al. found evidence for a novel R-group transfer in the Collision-Induced Dissociation (CID) spectra of protonated fentanyl analogs from an Electrospray Ionization (ESI) source.[1] Davidson et al. found that a product ion at *m/z* 244 likely formed through the transfer of the propionaldehyde group of fentanyl from the aniline moiety to the piperidine nitrogen. The proposed mechanism involves a nucleophilic attack of the carbonyl carbon by the lone pair on the piperidine nitrogen atom. This transfer is followed by cleavage of the aniline ring and the formation of a double bond on the piperidine ring. Using MS^3 data, Davidson et al. also showed that the product ion at *m/z* 244 readily loses cyclobutene (54 Da) to form a subsequent fragment at *m/z* 190.

In a subsequent study, Davidson et al. observed the fragmentation behavior of fentanyl, fentanyl d5, and 14 other FRCs and found that only 11 of these compounds gave a product ion at *m/z* 244 or its equivalent mass.[2] The current work focuses on a quantitative analysis of the previously reported spectra with the addition of selected fentanyl analogs that were expected to influence the R-group transfer in systematic ways.

Of the currently studied fentanyl analogs, the amide R-group transfer pathway contributes less than 1% of the abundance of product ion spectra, so this pathway is minor relative to other dominant pathways. However, quantitative comparisons of the R-group transfer pathways show that larger electron donating groups on the R2 (amide) region tend to enhance the abundance of the product ion at *m/z* 244 relative to the core structure of fentanyl. Also, modifications at the R3 and R4 positions (the piperidine ring and alkyl chain, respectively) tend to encourage competitive pathways and hinder the abundance the R-group transfer. R1 modifications appear to have little effect overall on the abundance of the product ion at *m/z* 244 or its equivalent. Modifications on the R5 position (phenyl ring) has a negligible effect on the abundance of the R-group transfer because this modification region is distant from the rearrangement region. The use of MS^n allowed for the detection of these trends in the product ion spectra and an improved understanding of how diagnostic product ion abundances can be used to determine areas of modification on novel FRCs.

Reference(s):

1. J. Tyler Davidson, Zachary J. Sasiene, Glen P. Jackson. The characterization of isobaric product ions of fentanyl using multi-stage mass spectrometry, high-resolution mass spectrometry and isotopic labeling. *Drug Test Anal* 2020, 12 (4), 496-503.
2. J. Tyler Davidson, Zachary J. Sasiene, Glen P. Jackson. The Influence of Chemical Modifications on the Fragmentation Behavior of Fentanyl and Fentanyl-Related Compounds in Electrospray Ionization Tandem Mass Spectrometry *Drug Test. Anal.* 2020, 12 (7), 957-967.

Fentanyl-Related Compounds, Predictive Identification Method, Tandem Mass Spectrometry

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