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The characterization of isobaric product ions of fentanyl using multi-stage mass spectrometry, high-resolution mass spectrometry and isotopic labeling

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Abstract

This study uses a combination of multi-stage mass spectrometry (MSⁿ), accurate mass measurements – with high-resolution mass spectrometry (HRMS) – and isotopic labeling to characterize the fragmentation behavior of fentanyl and 4-ANPP. By understanding the fragmentation behavior of fentanyl and its analogs in more detail, toxicologists and seized drug analysts will be better poised to identify new and emerging fentalogs, which are increasingly common and deadly adulterants in the growing opioid crisis. Throughout the literature the product ion at m/z 188 is often the most abundant fragment in the mass spectrometric analysis of fentanyl and fentanyl analogs, and this fragment is used for both qualitative and quantitative determinations. Our work shows there are at least three different structures for the isobaric fentanyl product ions at m/z 188, and they each form and fragment via different pathways. The development of fragmentation mechanisms to explain the observed fragmentation pathways of fentanyl and its main precursor 4-ANPP helps contribute to the advancement of knowledge about fentanyl fragmentation and could provide important information for the identification of future fentanyl analogs.

KEYWORDS

drug identification, fentanyl, fragmentation mechanism, isotope labeling, mass spectrometry

1 | INTRODUCTION

Fentanyl and fentanyl analogs have emerged as some of the most deadly compounds from the growing opioid crisis in America.¹ Fentanyl is a synthetic opioid approved by the FDA as an analgesic and anesthetic, but licit and illicit fentanyl analogs end up on the drug market through theft, fraudulent prescriptions and illicit distribution by patients, doctors, and pharmacists.² Since 2013, fentanyl and fentanyl analogs have become increasingly common adulterants in heroin seizures, and their incredible efficacy for binding to opioid receptors in the body has caused accidental overdoses in almost every state.³ According to the 2017 National Laboratory Forensic Information System (NFLIS), more than 56,000 fentanyl drug reports were filed the year before by local and state forensic laboratories.⁴

The Janssen and Siegfried methods are the two main synthetic routes for clandestine synthesis of fentanyl.⁵ The Siegfried method is the easier of the two approaches, and it uses *N*-phenethyl-4-piperidone (NPP) as the starting material and produces 4-anilino-*N*-phenethylpiperidine (4-ANPP) as an intermediate to the fentanyl product. Investigators can benefit from knowing the synthetic route of a fentanyl seizure, and the synthetic pathway can be established by the identification of residual unreacted precursors in the seizure. The Siegfried method uses 4-ANPP and the Janssen method uses benzylfentanyl. According to the DEA, four of the five domestic fentanyl clandestine labs seized since 2000 used the Siegfried method or a modified version thereof.⁵

Whereas fentanyl and its synthetic precursors are Schedule II narcotics, the impact on public health has led fentanyl analogs such as α -methylfentanyl, 3-methylfentanyl, acetylfentanyl, butyryl fentanyl, and β -hydroxythiofentanyl to be listed as Schedule I narcotics.^{3,5,6} Figure 1 shows the generic chemical structure of fentanyl analogs. This core structure is conserved in almost all analogs. Paramethylphenethylacetylfentanyl is an example of a fentanyl analog generated through modification at location R1. Thiofentanyl, α -methylfentanyl, and β -hydroxyfentanyl are examples of fentanyl analogs generated through modification at location R2. Carfentanil and 3-methylfentanyl are examples of modifications at location R3. Sufentanil, alfentanil, and remifentanil all contain modifications at locations R2 and R3, where R3 refers to a substitution at any position on the piperidine ring. Alteration in the length of the aliphatic chain at location R4 differentiates butyrylfentanyl and acetylfenantyl analogs from fentanyl. Finally, substitutions such as a fluorine at location R5 generate fentanyl analogs such as para-fluorofentanyl.⁷

The differentiation of fentanyl analogs with electron ionization mass spectrometry (EI-MS) has proven to be difficult. Mallette et al.⁹ noted that 2-methylfentanyl and 3-methylfentanyl can only be distinguished based on the subtle differences in the relative abundance of ions at m/z 202, 203, 160, and 216. Similarly, cyclopropylfentanyl and crotonylfentanyl are only distinguishable by differences in the relative abundance of ions at m/z 69 and m/z 105.¹⁰ Kanamori et al. reported the conserved nature of fragmentation pathways with 3-methylfentanyl analogs with all spectra having a base peak due to the benzyl cleavage.¹¹ The relative ion abundances were similar between compounds, with cis-isomers having larger abundances than trans-isomers, such as m/z 216, 203, 160, and 105 for cis-3-methylfentanyl relative to trans-3-methylfentanyl. However, some spectra, such as the diastereomers of β -hydroxy-cis-3-methylfentanyl, were too similar to differentiate. Ohta et al. have reported on the highly conserved nature of fentanyl analogs and called for forensic science laboratories to prepare for new designer drugs.¹² The conserved fragmentation pathways of these common structures has enabled National Institute of Standards and Technology (NIST) to develop an EI-MS mass spectral database and algorithm specifically designed to assist with the identification of novel opioids including fentanyl analogs.¹³

Whereas the use of tandem mass spectrometry is one of the most effective ways to identify fentanyl analogs, especially at trace levels in toxicological and seized drug samples,^{14,15} certain analogs are extremely difficult to distinguish. For example, Feasel et al. observed largely analogous collision-induced dissociation (CID) spectra between carfentanil and remifentanil.¹⁶ Caspar et al. discussed the value of having group-indicating ions that might help to identify novel compounds.¹⁷ They recommended HRMS to help identify the elemental compositions of different fragment ions, including the fragment at m/z 188.1439 in the case of fentanyl analogs.¹⁷ However,



FIGURE 1 Generic chemical structure of fentanyl highlighting regions of substitutions for fentanyl analogs⁸

Wichitnithad et al. observed at least two isobaric product ions with the same exact mass at m/z 188.1439.¹⁸ They were only able to distinguish the constitutional isomers using MS³ and deuterium labeling.¹⁸ Their observation further highlights the need for a better understanding of the fragmentation pathways of fentanyl and fentanyl analogs.

Two approaches that have demonstrated success in the identification of novel psychoactive substances (NPS) are the use of multi-stage mass spectrometry (MSⁿ)^{19,20} and the use of accurate mass measurements with high-resolution mass spectrometry (HRMS).^{21,22} The current work employs both of these tactics and isotopic labeling to elucidate additional fragmentation mechanisms of fentanyl and 4-ANPP. The discovery of three isobaric product ions at m/z 188 adds to the understanding of the fragmentation behavior of fentanyl analogs and helps to defend the use of transitions such as m/z 337 $\rightarrow m/z$ 188 for the quantitation and identification of fentanyl in seized drugs and biological fluids.^{8,23-25} The mechanism of formation of the new intermediate can be extrapolated to other fentanyl analogs to help explain some previously unidentified product ions in the spectra of existing and future fentanyl analogs.

2 | METHODS

2.1 | Sample preparation

The 4-ANPP fentanyl precursor, fentanyl, and fentanyl-d₅ (deuterated around the aniline) standards were purchased through Cayman Chemical (Ann Arbor, MI, USA). The 4-ANPP and fentanyl standards were dissolved in a solution of 48% HPLC grade methanol, 48% distilled water, and 2% acetic acid. The fentanyl-d₅ certified reference material (CRM) was left in the original methanol solvent to reduce the risk of hydrogen back exchange. The HPLC grade methanol was supplied by Fisher Scientific (Palo Alto, CA, USA) and the acetic acid was supplied by Acros Organics (Palo Alto, CA, USA).

2.2 | Instrumentation

2.2.1 | Thermo Scientific Velos Pro linear ion trap (LIT)

A Thermo Scientific Velos Pro linear ion trap (LIT) mass spectrometer was operated with a heated electrospray ionization (HESI) source. The HESI source was operated at 50°C with a spray voltage of 4000 V. The nitrogen sheath gas was operated at 8 arbitrary units with a nitrogen auxiliary gas flow of 5 arbitrary units. The mass spectrometer capillary temperature was 275°C. The scan range and normalized collision energies (NCEs) were compound specific and are labeled with each mass spectrum. An isolation width of 1 Da was used for all samples. Ultra-pure helium was used as the bath gas purchased through Matheson Tri-Gas (Fairmont, WV, USA).

2.2.2 | Agilent Technologies 6538 UHD Accurate-Mass quadrupole time-of-flight (Q-TOF)

An Agilent Technologies 6538 UHD Accurate-Mass quadrupole timeof-flight (Q-TOF) mass spectrometer was operated with dual electrospray ionization (ESI) with a spray voltage of 3500 V. The nitrogen gas was set to 300°C with a drying gas flow of 5 L/min and a nebulizer flow of 30 psig. The MS fragmentor and skimmer voltages were operated at 225 V and 65 V, respectively. The scan range and collisions energies were specific to each compound and are labeled in each mass spectrum. An isolation width of 1.3 Da was used for all samples. Ultra-pure nitrogen was used for the collision gas purchased through Matheson Tri-Gas (Fairmont, WV, USA).

2.3 | Data analysis

Xcalibur 2.0.0.48 software was used for the Velos Pro LIT data analysis, whereas MassHunter Qualitative Analysis B.05.00 was used for the Agilent Q-TOF data analysis. Microsoft Excel version 14 (Microsoft, Redmond, WA, USA) and ChemDraw 16.0 (PerkinElmer, Waltham, MA, USA) were used for the mass spectral plots and mass spectral fragmentation mechanisms.

2.3.1 | Mass spectral interpretation and mechanisms

The fragmentation mechanisms for each compound are based on MSⁿ analyses, accurate mass measurements, and prevailing electron pushing conventions. The proposed mechanisms follow the expected lowest energy pathways.²⁶ Whereas the identification of the exact hydrogen(s) involved in a specific rearrangement was not possible in this study, we could exclude certain hydrogen atoms by using perdeuterated analogs. Examples include MSⁿ of fentanyl that was perdeuterated on the aniline ring. MSⁿ resolved the relationships between primary, secondary, and tertiary product ions in a variety of pathways, and even though the exact atoms could not always be resolved in a rearrangement, the results provided a deeper level of understanding than the present status.

3 | RESULTS AND DISCUSSION

Figure 2 shows an example of the product ion spectrum generated from the fragmentation of the $[M+H]^+$ protonated precursor of fentanyl observed at m/z 337 with the major structural fragments embedded. The base peak in this spectrum is observed at m/z 188, which has been identified as a characteristic ion for the identification of fentanyl derivatives.¹⁷ However, based on the competing mechanisms for the loss of the *N*-phenylpropanamide neutral, previous literature has demonstrated that this peak must contain at least two isobaric product ions with different constitutional structures.¹⁸



FIGURE 2 Tandem mass spectrum of protonated fentanyl using CID in a linear ion trap mass spectrometer (35% NCE)

Other ions of significant abundance are observed at m/z 281 and m/z 216, of which the ion at m/z 281 has been shown to fragment into m/z 188.^{15,19,27} Figure 3 shows the MS³ product ion spectrum from the isolation and fragmentation of the intermediate product ion at m/z 281 (Figure 3A) and the MS⁴ product ion spectrum of the isolation and fragmentation of the intermediate product ion at m/z 188 from Figure 3A. These spectra contain the major structural fragments for each MS-level. The distribution of product ions in Figure 3B is in agreement with the work of Wichitnithad et al.¹⁸

As described by Wichitnithad et al.,¹⁸ the intermediate product ion at m/z 188 forms through a 6-centered rearrangement resulting in the loss of *N*-phenylpropanamide. However, based on our MSⁿ studies, we have identified the intermediate product ion at m/z 281 as an intermediate between the [M+H]⁺ precursor and the fragment at m/z188. Our proposed mechanism involves a 4-center-elimination of the methylketene from the *N*-phenylpropanamide moiety, as shown at the top of Figure 4. Figure 4 also shows the two proposed fragmentation pathways that explain the experimentally observed MS⁴ mass



FIGURE 3 Product ion mass spectra of protonated fentanyl collected under different conditions: (A) MS³ product ion spectrum for the transition m/z 337 \rightarrow 281 \rightarrow at 35% NCE, and (B) MS⁴ product ion spectrum for the transition m/z 337 \rightarrow 281 \rightarrow 188 \rightarrow at 33% NCE

FIGURE 4 Proposed mechanisms for the formation of different product ions from two isobaric intermediates at m/z 188.¹⁸ Pathway (A) leads to the formation of product ions at m/z 134, 160, and 120, whereas pathway (B) leads to the formation of product ions at m/z 146, 132, and 105 [Colour figure can be viewed at wileyonlinelibrary.com]



spectrum (Figure 3B). Pathway (A) describes the formation of the product ion at m/z 134 through a retro-Diels-Alder reaction as well as the formation of the intermediate product ion at m/z 160 and ultimately, the product ion at m/z 120. Pathway (B) describes the formation of the product ions at m/z 146, 132, and 105 from the second isobaric intermediate product ion at m/z 188.

In addition to the two isomers at m/z 188 that form from the intermediate at m/z 281, a third isobar at the nominal mass of m/z188 forms via fragmentation of the intermediate product ion at m/z216 (Figure 5A). However, fragmentation of this particular isomer at the MS^4 level only forms a product ion at m/z 132 (Figure 5B). The product ion at m/z 188 formed through the intermediate at m/z216 fragments differently from either of the two isomers at m/z188 identified by Wichitnithad et al.¹⁸ Supplemental Figure 1 shows product ion spectra collected in pseudo-MS³ mode on the Q-TOF instrument with proposed structures of the major fragments embedded. In these spectra, in-source CID of the protonated molecular ion generated pseudo-MS² primary fragments, which were then isolated at the MS^2 level and fragmented in the collision cell to generate pseudo-MS³ spectra. CID of the fragment at m/z 216.1300 gave many ions at the MS^3 level, one of which has an accurate mass of m/z188.1086. The accurate mass for the new product ion is consistent with an elemental composition of $C_{12}H_{14}NO^{+}$, which has an exact mass of 188.1075 Da (6 ppm error). In contrast, the isomers formed via the intermediate at m/z 281.2020 have an accurate mass of m/z188.1465. This product is consistent with an elemental composition of $C_{13}H_{18}N^+$, which has an exact mass of 188.1439 Da (14 ppm error). The measured masses are different by almost 200 ppm, so there can



FIGURE 5 Product ion mass spectra of protonated fentanyl collected under different conditions: (A) MS³ product ion spectrum for the transition m/z 337 \rightarrow 216 \rightarrow at 35% NCE, and (B) MS⁴ product ion spectrum for the transition m/z 337 \rightarrow 216 \rightarrow 188 \rightarrow at 25% NCE

be no confusion between the different elemental compositions of the isobars formed via the two different pathways.

As shown in Figure 6, the intermediate product ion at m/z 216 most likely forms through the opening of the piperidine ring and charge stabilization on a tertiary carbocation as described by Thevis



FIGURE 6 Proposed mechanisms for the formation of the novel product ion at m/z 188 from the intermediate product ion at m/z 216 [Colour figure can be viewed at wileyonlinelibrary.com]

et al.¹⁵ From the structure at m/z 216, the intermediate product ion at m/z 188 can then form through a 4-center-elimination at the carbocation. The product ion at m/z 132 can be formed via two different pathways, one through the intermediate at m/z 188 and the other through the intermediate at m/z 160. Formation of m/z 132 from the intermediate at m/z 188 occurs through a 4-center-elimination of propenone. Formation of m/z 132 from m/z 160 can occur via the 4-center-elimination of ethene. Either way, the resulting product at m/z 132 contains the original aniline moiety rather than the previously described phenyl moiety. This newly identified pathway provides a third alternative structure for the isobars at the nominal mass of m/z188 in the tandem mass spectra of fentanyl.

MSⁿ-level analysis of protonated fentanyl also provided evidence for a fourth fragmentation pathway for a product with a nominal mass of *m*/*z* 188. This fourth pathway has a measured accurate mass of *m*/*z* 188.1465, which is within 10 ppm of the exact mass of $C_{13}H_{18}N^+$. This minor pathway, which occurs at approximately 0.5% of the abundance of *m*/*z* 188 in Figure 2, involves an R-group transfer of the propionaldehyde moiety of the molecular ion to form the intermediate product ion at *m*/*z* 244. Figure 7A shows that although the formation of the product at *m*/*z* 190 is the preferred product from the activation of the intermediate fragment at *m*/*z* 244, the product ion at *m*/*z* 188 is still observable at the MS⁴ level. Figure 7B shows that MS⁴level fragmentation via the sequence *m*/*z* 337 \rightarrow 244 \rightarrow 188 \rightarrow results in product ions at *m*/*z* 120 and *m*/*z* 134. Such fragmentation was not observed by Wichitnithad et al.,¹⁸ presumably because this pathway is negligible in abundance relative to the other pathways.

Figure 8 shows that the proposed R-group transfer to form the product ion at m/z 244 involves transferring the propionaldehyde group from the aniline moiety to the piperidine nitrogen via



FIGURE 7 Product ion mass spectra of protonated fentanyl collected under different conditions: (A) MS³ product ion spectrum for the transition m/z 337 \rightarrow 244 \rightarrow at 25% NCE, and (B) MS⁴ product ion spectrum for the transition m/z 337 \rightarrow 244 \rightarrow 188 \rightarrow at 25% NCE

nucleophilic attack of the carbonyl carbon by the lone pair on the piperidine nitrogen atom. Transfer to the nitrogen atom is more consistent with both MS⁴-level spectra of fentanyl and MS³-level spectra of other fentanyl analogs. This mechanism is more easily visualized when the piperidine ring adopts a boat configuration. In the boat configuration, the propionaldehyde transfer to the nitrogen atom can occur via a sterically favored 6-center rearrangement. Transfer of the propionaldehyde group to the carbon atoms of the piperidine ring would involve sterically unfavorable rearrangements and weaker nucleophilic attack.

After transfer of the propionaldehyde group, the intermediate at m/z 244 forms via a 4-center-elimination of aniline from the rearranged precursor. The intermediate product ion at m/z 188 forms from a 4-center elimination of methylketene, which can then undergo a retro-Diels-Alder reaction to produce the product ion at m/z 134. Fragmentation at the MS³ level shows that the product ion observed at m/z 134 can also form through the intermediate product ion at m/z 190, which is formed through the loss of cyclobutene from the intermediate product ion at m/z 190 was identified through the conserved loss of 54 Da from a variety of fentanyl analogs (unpublished data) and the accurate mass measurements using the Q-TOF HRMS instrument (Supplemental Figure 2). The measured accurate mass of m/z 190.1249 is less than 9 ppm different than the exact mass for C₁₂H₁₆ON⁺.

The analysis of the d_5 version of fentanyl, which is deuterated around the aniline moiety, provides support for the three proposed mechanisms. Figure 9A shows the tandem mass spectrum of protonated fentanyl- d_5 observed at m/z 342 with the structures of the major fragments embedded. The incorporation of 5 deuterium atoms instead of 5 hydrogen atoms onto the aniline ring allows this moiety to be



FIGURE 8 Proposed mechanisms for the formation of the product ion at *m*/*z* 188 from the intermediate product ion at *m*/*z* 244 [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 9 Product ion mass spectra of protonated fentanyl-d₅ collected under different conditions: (A) MS² product ion spectrum of the precursor at *m/z* 342 at 35% NCE, (B) MS³ product ion spectrum for the transition *m/z* 337 \rightarrow 286 \rightarrow at 25% NCE, and (C) MS³ product ion spectrum for the transition *m/z* 337 \rightarrow 221 \rightarrow at 25% NCE

traced down each fragmentation pathway. The observed product ions at m/z 286 and m/z 221 confirm that the deuterated aniline moiety is present, whereas the product ion at m/z 244 indicates the deuterated aniline moiety has been lost. The proposed R-group transfer in Figure 8 is further supported by the observation of the product ion at m/z 244 from both fentanyl and fentanyl-d₅. The fragment at m/z 244 occurs through the loss of 93 Da from fentanyl and 98 Da from fentanyl-d₅.

Figure 9B shows the MS³-level fragmentation of the intermediate at m/z 286. The base peak at m/z 188 indicates that the deuterated aniline moiety is lost, which agrees with the proposed pathways in Figure 4. The most important discovery from the analysis of fentanyld₅ is that the intermediate product at m/z 221 fragments into product ions at m/z 193, 165, and 137 (Figure 9C). The product ion at m/z193 is consistent with the deuterated version of the product ion at m/z 188, which is described in Figure 6.

Deuterium labeling provides definitive proof that the aromatic ring that is ultimately incorporated into the product ion at m/z



FIGURE 10 Product ion mass spectra of protonated 4-ANPP: (A) MS² product ion spectrum of protonated precursor ion at m/z 281 (35% NCE) and (B) MS³ product ion spectrum for the transition m/z 281 \rightarrow 188 \rightarrow at 35% NCE

188 from the intermediate product ion at *m/z* 216 pathway must be from the aniline moiety and not from the phenyl moiety. In contrast, the phenyl moiety is retained in the more-dominant pathways to *m/z* 188 that occur through intermediates at *m/z* 281 and *m/z* 244. Also, observation of product ions at *m/z* 165 and *m/z* 137 in the deuterated fentanyl sample (Figure 9C) provides support for the mechanisms presented in Figure 6 through the incorporation of five deuterium into the proposed product ions at *m/z* 160 and *m/z* 132. Finally, Supplemental Figure 3 contains the accurate mass measurements for the analysis of in-source CID generated *m/z* 221.1700 for fentanyl-d₅ collected on the Q-TOF instrument. The incorporation of the deuterated aniline moiety is observed at every step along the proposed fragmentation pathway presented in Figure 6.

MSⁿ analysis of 4-ANPP enabled the identification of similar fragmentation pathways as fentanyl. For example, Figure 10A shows the tandem mass spectrum of the [M+H]⁺ precursor of 4-ANPP at m/z 281. The MS² product ion spectrum shows the characteristic base peak observed at m/z 188, which is present in most fentalogs. Product ions of other significance are observed at m/z 146, 134, and 105, which are all product ions of the dominant product ion at m/z 188 in Figure 4. Figure 10B shows that MS³-level fragmentation of the intermediate at m/z 188. The product ion distribution is identical to that of fentanyl and is consistent with the proposed mechanisms in Figure 4. The two new pathways to the formation of isobars at m/z 188 for fentanyl are not possible for 4-ANPP. However, tandem mass spectrometry analysis of a variety of other fentalogs also show R-group transfers that are consistent with the mechanism shown in Figure 8, the results of which are the topic of ongoing work.

4 | CONCLUSIONS

This manuscript demonstrates the use of multi-stage mass spectrometry (MSⁿ), accurate mass measurements with HRMS and isotopic labeling for the elucidation of the fragmentation mechanisms for fentanyl and 4-ANPP. Specifically, this manuscript establishes the identification of three isobaric fentanyl product ions at m/z 188 including a novel product ion formed through the intermediate product ion at m/z 216. This realization has a potential impact on product ion selection for quantitative analyses. The product ion at m/z 188 is commonly reported in literature as a product ion used for quantification based on monitoring the transition from m/z 337 \rightarrow 188. However, there are at least three isobaric fentanyl product ions at m/z 188 which have different rates and energies of formation. These differences can lead to variation in ion abundances, which can affect the accuracy and precision of quantitative analyses,¹⁸ but recognition of these different pathways can also help to identify similar mechanisms in emerging fentanyl analogs. As new fentanyl analogs enter the drug market our ability to identify characteristic fragmentation pathways and conserved fragmentation mechanisms can assist medical examiners, toxicologists, and seized drug analysts with the identification of novel fentanyl related compounds.

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REFERENCES

- Schueler HE. Emerging synthetic fentanyl analogs. Acad Forensic Pathol. 2017;7(1):36-40. https://doi.org/10.23907/2017.004
- Drugs of Abuse. A DEA Resource Guide. 2017, DEA: U.S. Department of Justice Drug Enforcement Administration pp. 40-41.
- Armenian P, Vo KT, Barr-Walker J, Lynch KL. Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review. *Neuropharmacology*. 2018;134(Pt A):121-132. https://doi.org/10. 1016/j.neuropharm.2017.10.016
- NFLIS-Drug 2017 Annual Report. 2017, U.S. Department of Justice Drug Enforcement Administration: National Forensic Laboratory Information System (NFLIS).
- Control of immediate precursor used in the illicit manufacture of fentanyl as a schedule II controlled substance. *Fed Regist*. 2010;75(124): 37295-37299.
- Suzuki J, El-Haddad S. A review: fentanyl and non-pharmaceutical fentanyls. Drug Alcohol Depend. 2017;171:107-116. https://doi.org/ 10.1016/j.drugalcdep.2016.11.033
- Recommended methods for the Identification and Analysis of Fentanyl and its Analogues in Biological Specimens. 2017: United Nations Office on Drugs and Crime. pp. 5–11.
- Lurie IS, lio R. Use of multiple-reaction monitoring ratios for identifying incompletely resolved fentanyl homologs and analogs via ultrahigh-pressure liquid chromatography-tandem mass spectrometry. *J Chromatogr A*. 2009;1216(9):1515-1519. https://doi.org/10.1016/j. chroma.2008.12.097
- Mallette JR, Casale JF, Toske SG, Hays PA. Characterization of (2R,4S)- and (2R,4R)-2-methylfentanyl and their differentiation from cis- and trans-3-methylfentanyl. *Forens Chem.* 2018;8:64-71. https:// doi.org/10.1016/j.forc.2018.02.001
- Mallette JR, Casale JF, Hays PA. Characterization and differentiation of cyclopropylfentanyl from E-crotonylfentanyl, Z-crotonylfentanyl, and 3-butenylfentanyl. *Sci Justice*. 2018;59(1):67-74.
- Kanamori T, Iwata YT, Segawa H, et al. Characterization and differentiation of geometric isomers of 3-methylfentanyl analogs by gas chromatography/mass spectrometry, liquid chromatography/mass spectrometry, and nuclear magnetic resonance spectroscopy. *J Forensic Sci.* 2017;62(6):1472-1478. https://doi.org/10.1111/1556-4029.13395

- 12. Ohta H, Suzuki S, Ogasawara K. Studies on fentanyl and related compounds IV. Chromatographic and spectrometric discrimination of fentanyl and its derivatives. *J Anal Toxicol.* 1999;23:280-285.
- Moorthy AS, Wallace WE, Kearsley AJ, Tchekhovskoi DV, Stein SE. Combining fragment-ion and neutral-loss matching during mass spectral library searching: a new general purpose algorithm applicable to illicit drug identification. *Anal Chem.* 2017;89(24):13261-13268. https://doi.org/10.1021/acs.analchem.7b03320
- Strayer KE, Antonides HM, Juhascik MP, Daniulaityte R, Sizemore IE. LC-MS/MS-based method for the multiplex detection of 24 fentanyl analogues and metabolites in whole blood at sub ng mL⁻¹ concentrations. ACS Omega. 2018;3(1):514-523. https://doi.org/10.1021/ acsomega.7b01536
- Thevis M, Geyer H, Bahr D, Schanzer W. Identification of fentanyl, alfentanil, sufentanil, remifentanil and their major metabolites in human urine by liquid chromatography/tandem mass spectrometry for doping control purposes. *Eur J Mass Spectrom*. 2005;11(4):419-427. https://doi.org/10.1255/ejms.761
- Feasel MG, Wohlfarth A, Nilles JM, Pang S, Kristovich RL, Huestis MA. Metabolism of carfentanil, an ultra-potent opioid, in human liver microsomes and human hepatocytes by high-resolution mass spectrometry. AAPS J. 2016;18(6):1489-1499. https://doi.org/ 10.1208/s12248-016-9963-5
- Caspar AT, Kollas AB, Maurer HH, Meyer MR. Development of a quantitative approach in blood plasma for low-dosed hallucinogens and opioids using LC-high resolution mass spectrometry. *Talanta*. 2018;176:635-645. https://doi.org/10.1016/j.talanta.2017.08.063
- Wichitnithad W, McManus TJ, Callery PS. 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):2547-2553. https://doi.org/10.1002/ rcm.4673
- Shoff EN, Zaney ME, Kahl JH, Hime GW, Boland DM. Qualitative identification of fentanyl analogs and other opioids in postmortem cases by UHPLC-ion trap-MSⁿ. J Anal Toxicol. 2017;41(6):484-492. https://doi.org/10.1093/jat/bkx041
- Rittgen J, Putz M, Zimmermann R. Identification of fentanyl derivatives at trace levels with nonaqueous capillary electrophoresiselectrospray-tandem mass spectrometry (MSⁿ, n = 2, 3): analytical method and forensic applications. *Electrophoresis*. 2012;33(11):1595-1605. https://doi.org/10.1002/elps.201100655
- 21. Breindahl T, Kimergard A, Andreasen MF, Pedersen DS. Identification of a new psychoactive substance in seized material: the synthetic

opioid N-phenyl-N-[1-(2-phenethyl)piperidin-4-yl]prop-2-enamide (Acrylfentanyl). *Drug Test Anal*. 2017;9(3):415-422. https://doi.org/ 10.1002/dta.2046

- Noble C, Weihe Dalsgaard P, Stybe Johansen S, Linnet K. Application of a screening method for fentanyl and its analogues using UHPLC-QTOF-MS with data-independent acquisition (DIA) in MS^E mode and retrospective analysis of authentic forensic blood samples. *Drug Test Anal.* 2018;10(4):651-662. https://doi.org/10.1002/dta.2263
- 23. Koch DE, Isaza R, Carpenter JW, Hunter RP. Simultaneous extraction and quantitation of fentanyl and norfentanyl from primate plasma with LC/MS detection. *J Pharm Biomed Anal.* 2004;34(3):577-584. https://doi.org/10.1016/s0731-7085(03)00652-6
- Pihlainen K, Grigoras K, Franssila S, Ketola R, Kotiaho T, Kostiainen R. Analysis of amphetamines and fentanyls by atmospheric pressure desorption/ionization on silicon mass spectrometry and matrixassisted laser desorption/ionization mass spectrometry and its application to forensic analysis of drug seizures. J Mass Spectrom. 2005;40 (4):539-545. https://doi.org/10.1002/jms.831
- Takashina Y, Naito T, Mino Y, Kagawa Y, Kawakami J. Validated LC coupled to ESI-MS/MS analysis for fentanyl in human plasma and UV analysis in applied reservoir transdermal patches using a simple and rapid procedure. J Clin Pharm Ther. 2009;34(5):523-529. https://doi. org/10.1111/j.1365-2710.2009.01033.x
- Sleno L, Volmer D. Ion activation methods for tandem mass spectrometry. J Mass Spectrom. 2004;39(10):1091-1112.
- Grzeskowiak T, Zgola-Grzeskowiak A, Rusinska-Roszak D, Zaporowska-Stachowiak I, Jeszka-Skowron M. Fragmentation studies of selected drugs utilized in palliative care. *Eur J Mass Spectrom*. 2018; 420-436. https://doi.org/10.1177/1469066718812459

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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