Differentiation of Isobaric Methyl-Substituted Fentanyl Analogs Using Direct Analysis in Real Time Mass Spectrometry (DART-MS) and All Ion Fragmentation (AIF)

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ABSTRACT

One potential solution to address the growing backlogs in seized drug laboratories is the incorporation of rapid seized drug screening using direct analysis in real time mass spectrometry (DART-MS), an ambient ionization technique that enables the analysis of samples with only minimal sample preparation. The combination of DART-MS with AIF activation enables the collection of fragment ions for all precursor ions present without prior knowledge regarding the sample composition. This study explores the characteristic differences in the AIF data of isobaric methyl-substituted fentanyl analogs and how the location of the methyl substitution impacts the formation of diagnostic fragment ions under AIF conditions.

INTRODUCTION

Given the ongoing opioid epidemic and the abundance of fentanyl analogs in seized drugs casework, the reliable identification of fentanyl analogs is critical for forensic laboratories [1]. However, the differentiation of isobaric methyl-substituted fentanyl analogs is quite challenging, especially when present in mixtures. Unfortunately, because DART is a soft ionization technique that produces predominantly protonated/deprotonated molecules, DART-MS cannot readily distinguish between isomers [2]. Tandem mass spectrometry (MS/MS) can be used to generate product ions through collision-induced dissociation (CID) that may help differentiate isomers; however, targeted MS/MS analysis requires knowledge about potential compounds of interest [3]. In comparison, AIF enables CID activation of all precursor ions present without prior knowledge regarding the sample composition. This study explores the combination of AIF with DART-MS for the differentiation of isobaric methyl-substituted fentanyl analogs, even when present in mixtures, based on differences in fragmentation derived from the location of the methyl substitution to the core fentanyl structure.

MATERIALS & METHODS

Chemicals and Materials

Eight methyl-substituted fentanyl analogs representative of different R-group substitutions were analyzed in this study.

Table 1. Summary of the isobaric methyl-substituted fentanyl analogs utilized in this study.

R Group	Methyl-Substituted Fentanyl Analog
R_1	Ortho-methyl fentanyl
R_2	Butyryl fentanyl Isobutyryl fentanyl
R_3	Trans-3-methyl fentanyl 4-methyl fentanyl
R_4	α-methyl fentanyl β-methyl fentanyl
R_5	4'-methyl fentanyl

RESULTS & DISCUSSION

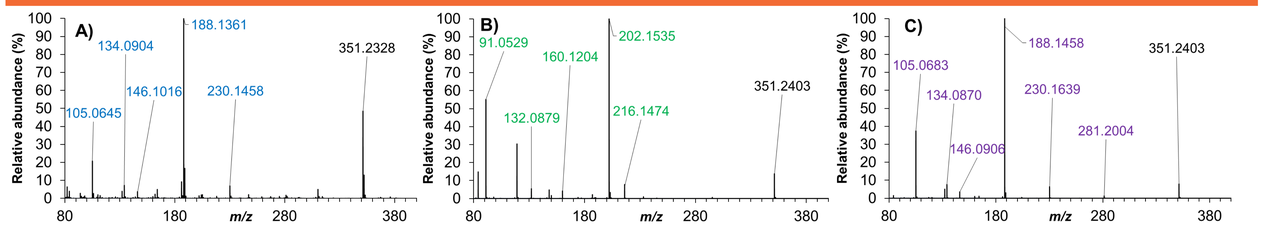

Figure 1. Exemplar product ion spectra of pure, methyl-substituted fentanyl analogs at different R-group locations: A) ortho-methyl fentanyl (R_1), B) β-methyl fentanyl (R_4), and C) butyryl fentanyl (R_2).

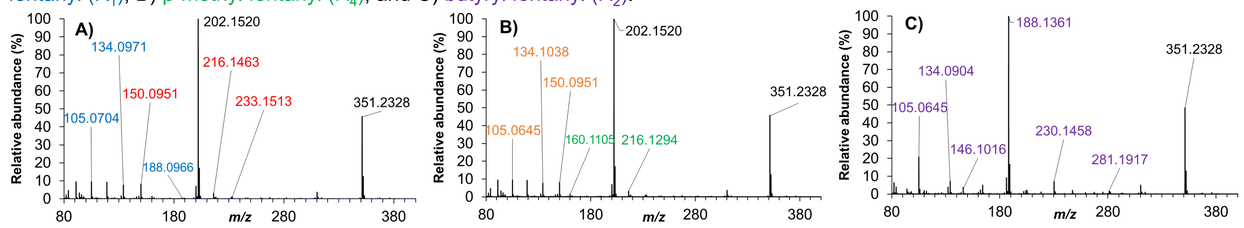

Figure 2. Exemplar product ion spectra of two-component mixtures: A) ortho-methyl fentanyl (R_1) and α-methyl fentanyl (R_4), B) 4-methyl fentanyl (R_3) and β-methyl fentanyl (R_4), and C) butyryl fentanyl (R_2) and isobutyryl fentanyl (R_2).

- Characteristic product ions enable analogs with different R-group substitution locations to be distinguishable.

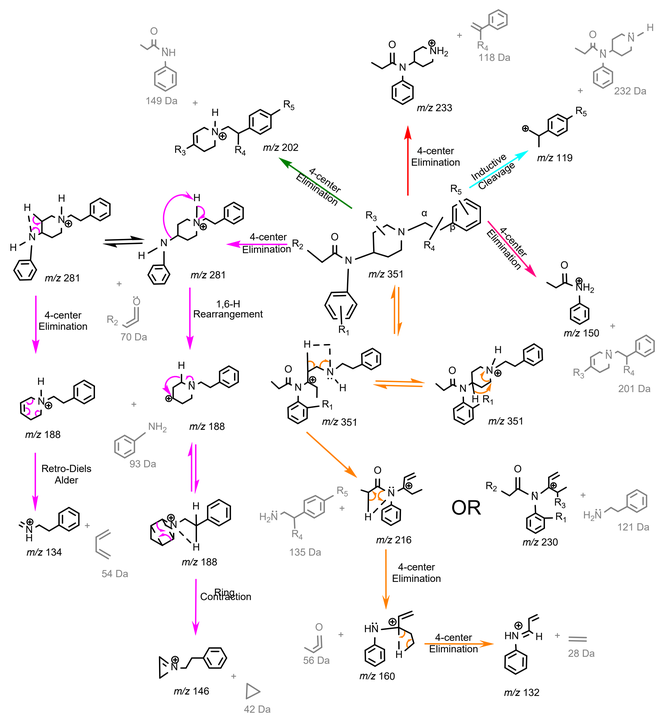

Figure 3. Overview of the proposed fragmentation pathways for the diagnostic product ions *(m/z)* used for differentiating the isobaric methyl-substituted fentanyl analogs.

Table 2. Diagnostic ions for analog differentiation.

Methyl-Substituted Fentanyl Analog	Diagnostic Ions *(m/z)*
Ortho-methyl fentanyl	230, 188, 146, 134
Butyryl fentanyl	281, 230, 188, 146
Isobutyryl fentanyl	281, 230, 188, 146
Trans-3-methyl fentanyl	230, 202, 160, 146
4-methyl fentanyl	202, 150, 134, 105
α-methyl fentanyl	233, 216, 202, 150
β-methyl fentanyl	216, 202, 160, 132
4'-methyl fentanyl	216, 202, 160, 119

MATERIALS & METHODS

Sample Preparation

All standards were prepared at a concentration of 10 ppm in methanol. Two-component mixtures of methyl-substituted fentanyl analogs were also prepared at a total concentration of 20 ppm in methanol. All analogs were purchased as Certified Reference Materials from Cayman Chemical or Cerilliant.

Instrumentation

A DART JumpShot® ionization source coupled to an Agilent 6530 quadrupole-time-of flight (Q-TOF) mass spectrometer was utilized in this study. Samples were introduced to the DART ionization source by depositing 5 µL of sample onto a QuickStrip™, which was allowed to dry before analysis. All analyses were completed in positive ionization mode using helium as the source gas heated to 350 °C. AIF was used to obtain low (i.e., 0 eV), medium (i.e., 30 eV), and high (i.e., 60 eV) activation energy spectra.

CONCLUSIONS

- The fragment ion mass spectra of eight methyl-substituted fentanyl analogs were characterized using DART-MS and AIF.
- Except for butyryl fentanyl and isobutyryl fentanyl, all methyl-substituted fentanyl analogs produced either a unique combination of fragment ions or distinct differences in ion abundance ratios that enabled differentiation.
- Due to the location of the methyl substitution on the amide moiety, the mass spectra for butyryl fentanyl and isobutyryl fentanyl remained indistinguishable even under AIF conditions.

REFERENCES

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