Investigating the Effect of Substitution Location on Fentanyl Analog Identification for Methyl-Substituted Fentanyl Analogs Using GC-EI-MS

Alexis Pollard, BS* and J. Tyler Davidson, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

ABSTRACT

Gas chromatography-electron ionization-mass spectrometry (GC-EI-MS) is one of the most widely employed analytical techniques for the identification of controlled substances. When it comes to the identification of fentanyl analogs, analysts rely on the presence of unique fragment ions and mass spectral search algorithms. Understanding the effect that substitution location has on the observed fragmentation and successful identification using mass spectral search algorithms is essential for the identification of novel fentanyl analogs. This study explores the use of unique EI fragmentation and the NIST Similarity Search (SSS) and Hybrid Similarity Search (HSS) algorithms to understand how substitution location affects fentanyl analog identification.

INTRODUCTION

Fentanyl is a Schedule II synthetic opioid that provides substantial pain relief but is also abused recreationally[1]. The Drug Enforcement Administration (DEA) is responsible for scheduling new compounds and temporarily placed all fentanyl-related substances into Schedule I in February 2018[2]. However, the continued emergence of new fentanyl analogs necessitates research regarding the identification of novel fentanyl analogs using instrumentation widely available in forensic laboratories. GC-EI-MS is the current gold standard for seized drug identification despite electron ionization being a hard ionization source that often results in the absence of molecular ions for many seized drugs[3]. Therefore, fentanyl analogs must be differentiated based on only subtle differences in their EI mass spectra. Mass spectral libraries play an important role in the identification of fentanyl analogs but are limited by the size of the reference database and the performance of the mass spectral search algorithm.

This study investigates the effect of substitution location on the ability to identify fentanyl analogs through the analysis of a series of methyl-substituted fentanyl analogs using GC-EI-MS. In addition to identifying conserved fragmentation behavior enabling differentiation of methyl-substituted fentanyl analogs, this study also assesses the performance of the NIST SSS and HSS for the identification of methyl-substituted fentanyl analogs that may be present or absent from the reference database.

MATERIALS & METHODS

Sample Preparation

Each fentanyl analog was diluted with methanol to prepare working solutions at 10 µg/mL. After GC-MS method optimization, two mixtures were prepared with each fentanyl analog at 10 µg/mL by splitting the 11 fentanyl analogs into two groups. Mixture 1 was composed of isobutyryl fentanyl, 4-methyl fentanyl, fentanyl, ortho-methyl acetyl fentanyl, α-methyl fentanyl, and butyryl fentanyl. Mixture 2 was composed of β-methyl fentanyl, α-methyl acetyl fentanyl, trans-3-methyl fentanyl, ortho-methyl fentanyl, and 4'-methyl fentanyl.

RESULTS & DISCUSSION

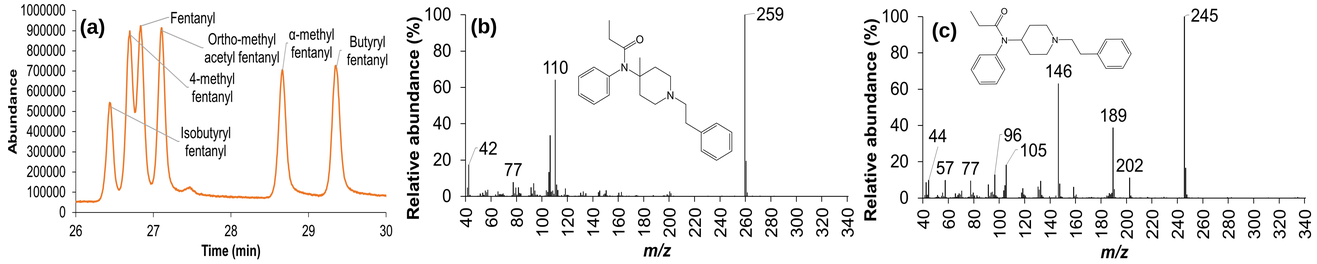

Figure 1. Truncated total ion chromatogram (TIC) of mixture 1 on the Agilent 8890 GC-5977B MS (a), exemplar EI mass spectrum of 4-methyl fentanyl (b), exemplar EI mass spectrum of fentanyl (c), and the corresponding chemical structures.

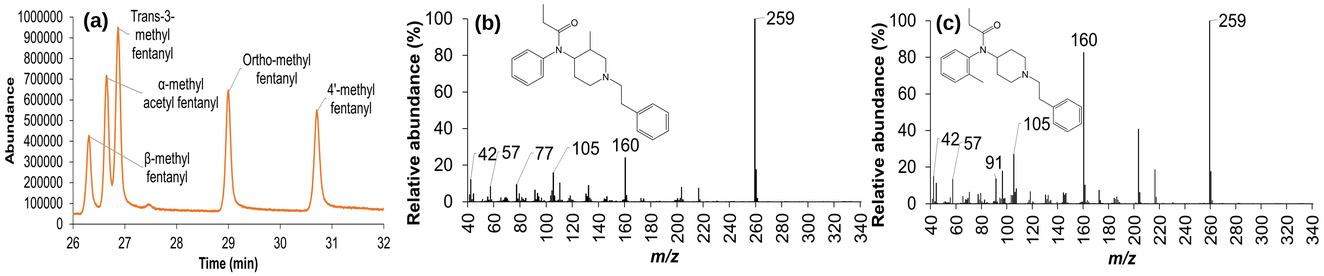

Figure 2. Truncated TIC of mixture 2 on the Agilent 8890 GC-5977B MS (a), exemplar EI mass spectrum of trans-3-methyl fentanyl (b), exemplar EI mass spectrum of ortho-methyl fentanyl (c), and the corresponding chemical structures.

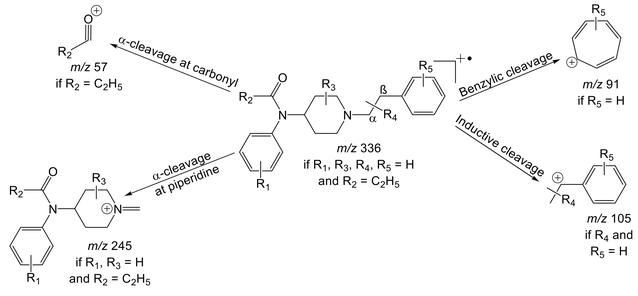

Figure 3. General fragmentation pathways for fentanyl analogs observed with EI fragmentation using fentanyl as an exemplar.

Table 2. Comparison of SSS and HSS identification results for 6 replicates; positive identification is reported as the percentage that the correct compound was in the top 5 results and the average match score.

	SSS (NIST 20)	HSS	SSS (NIST 05)	HSS
R_1 (N=2)	100% (933)	42% (941)	100% (830)	25% (939)
R_2 (N=3)	100% (976)	28% (895)	0%	0%
R_3 (N=2)	50% (976)	50% (976)	50% (890)	50% (977)
R_4 (N=3)	100% (870)	61% (867)	100% (853)	50% (881)
R_5 (N=1)	100% (923)	0%	100% (908)	0%

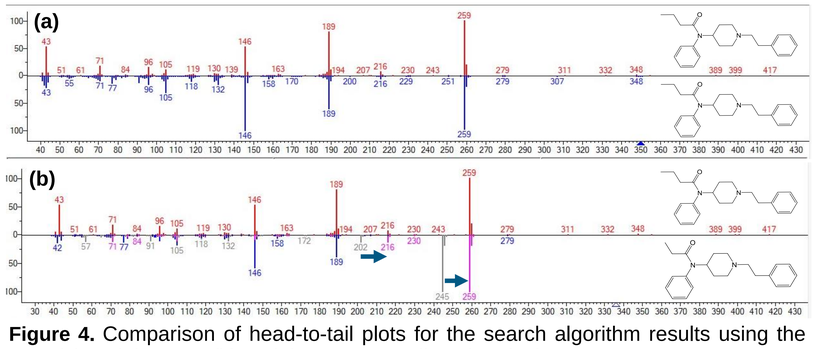

Figure 4. Comparison of head-to-tail plots for the search algorithm results using the SSS (a) and the HSS (b) and the corresponding chemical structures.

- Fentanyl analogs can be differentiated based on observable differences in the conserved EI fragmentation behavior.
- The HSS exhibits a shift in the mass axis to indicate possible substitution, which may allow for the identification of compounds absent from the mass spectral library.
- Library size and spectral quality effect the SSS and HSS algorithm performance.
- The HSS performance also depends on the provided input information, such as the presence of the molecular ion in the spectra or the compound's molecular weight.

MATERIALS & METHODS

Instrumentation

Table 1. Summary of key GC-MS method parameters.

	Agilent 8890 GC-5977B MS	Agilent 7890A GC-5975C MS
Temperature programming	100 °C, 20 °C/min to 200 °C, 2 °C/min to 240 °C, hold 7 min	100 °C, 20 °C/min to 200 °C, 2 °C/min to 240 °C, hold 9 min
Injection type	Pulsed Splitless	Pulsed Splitless
Pulse pressure	45 psi until 0.3 min	45 psi until 0.7 min
Purge flow to split vent	50 mL/min to 0.2 min	50 mL/min to 0.6 min

Data Analysis

The raw data from ChemStation was searched against the National Institute of Standards and Technology (NIST) EI mass spectral library available on each instrument. Comparisons between the effectiveness of each NIST library and search algorithm were also performed.

CONCLUSIONS

- A GC-EI-MS method was developed for the separation of 11 methyl-substituted fentanyl analogs.
- Differences in the EI mass spectra are observed due to the location of substitution to the core fentanyl structure.
- Primary fragmentation pathways differentiate fentanyl analogs with substitutions at locations R_2, R_4, and R_5.
- Secondary fragmentation pathways differentiate fentanyl analogs with substitutions at locations R_1 and R_3.
- NIST 20 SSS performed better than NIST 05 SSS based on the library size and reference fentanyl analogs present.
- The HSS provides useful compound class information but struggles with specific identification.
- The HSS and SSS can both successfully identify fentanyl analogs, but to varying extents depending on the library size and chosen algorithm.

REFERENCES

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