Identification of Novel Nitazene Analogs Using Electron Ionization-Mass Spectrometry and Electrospray Ionization-Tandem Mass Spectrometry

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INTRODUCTION

Nitazene analogs are some of the most potent novel synthetic opioids (NSOs) on the current illicit drug market (Figure 1). Due to their recent emergence and the continued proliferation of novel analogs, the identification of nitazene analogs in forensic laboratories is challenging. Our previous works have characterized a variety of nitazene analogs using electron ionization-mass spectrometry (EI-MS) [1] and electrospray ionization-tandem mass spectrometry (ESI-MS/MS) [2]. Although these works propose fragmentation mechanisms for the formation of the most common ions, there was no discussion about combining the information from each technique to identify novel nitazene analogs.

This work explores the information provided by both EI-MS and ESI-MS/MS for the analysis of nitazene analogs. Both techniques provide complementary information about nitazene analogs and can be helpful individually or in tandem to provide a more complete picture for identifying novel nitazene analogs.

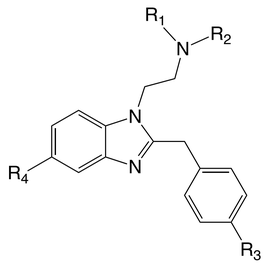

Figure 1. Core structure of nitazene analogs, highlighting four common locations of substitution.

MATERIALS & METHODS

A total of 42 nitazene analogs were analyzed for both EI-MS and ESI-MS/MS analyses. An Agilent 8890 GC-5977B MSD instrument was used for EI data collection. Samples were prepared at 100 ppm. The oven temperature was as follows: 150 °C for 1 min, 20 °C/min ramp to 250 °C for 1 min, 5 °C /min ramp to 300 °C for 15 min. The source temperature was 230 °C, the quadrupole temperature was 150 °C, the ionization energy was 70 eV, and the scan range was *m/z* 40-500.

RESULTS & DISCUSSION

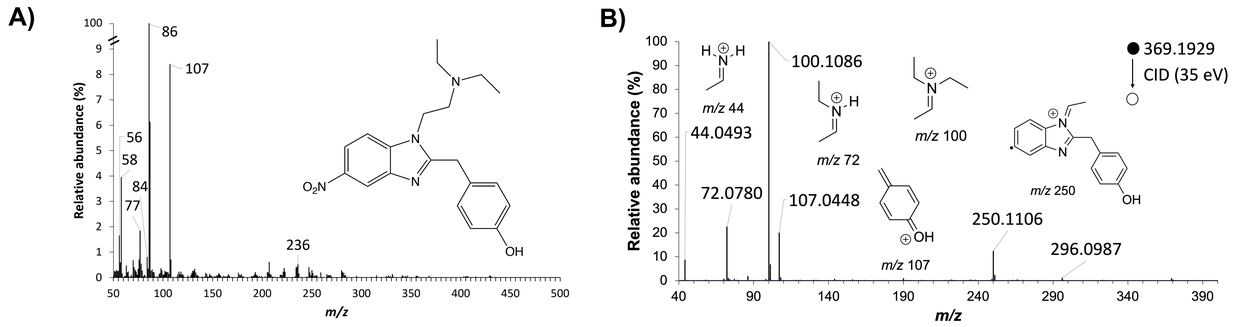

Figure 2. Exemplar 4'-hydroxy nitazene mass spectra collected using A) EI-MS and B) ESI-MS/MS.

- EI mass spectra contain lower abundance fragment ions than ESI-MS/MS product ion spectra.
- The ESI-MS/MS product ion spectra contained higher mass fragment ions and the protonated molecule.

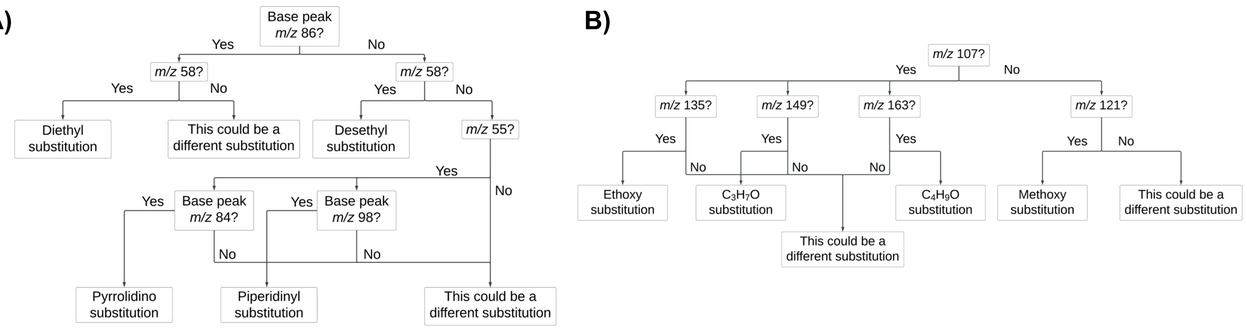

Figure 3. Decision trees for A) the R_1/R_2 group and B) the R_3 group, based on observed EI fragmentation.

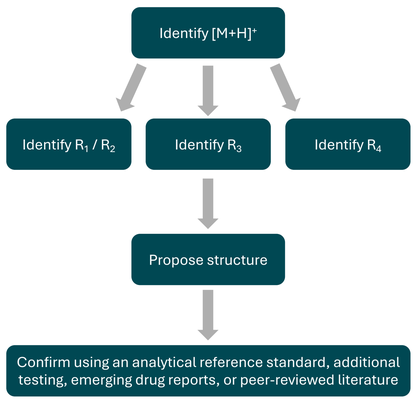

Figure 4. Workflow for structural elucidation using ESI-MS/MS product ion spectra.

- The base peak in both the EI-MS and ESI-MS/MS mass spectra indicates the R_1/R_2 substitution.
- Certain fragment ions in the EI-MS and ESI-MS/MS mass spectra indicate the R_3 substitutions.
- R_4 substitutions can be identified with the presence or absence of the molecular ion in EI-MS spectra, or the presence or absence of a doubly charged ion in the ESI-MS/MS full scan spectra.
- ESI-MS/MS mass spectra often provide molecular weight information that is lost with EI-MS.
- EI-MS mass spectra contain fragment ions that enable the identification of all four R groups in one spectrum, whereas identification using ESI-MS/MS requires the use of full scan, low, and high energy spectra.

MATERIALS & METHODS

An Agilent 6530 Q-TOF mass spectrometer was used for ESI-MS/MS analysis. The samples were prepared at 10 ppm in 49.9%:49.9%:0.2% methanol:DI water:formic acid and were introduced to the ESI source via direct infusion. Three replicates of each analog were collected using collision energies of 15, 25, 35, and 45 eV.

Fragmentation pathways were determined by using isotopically labeled compounds and the lowest expected energy pathways.

CONCLUSIONS

- ❖ Both EI-MS and ESI-MS/MS spectra show key ions that reveal information about the different R-group substitutions.
- ❖ ESI-MS/MS provides molecular weight information via the protonated molecule, whereas many of the nitazene analogs do not have a molecular ion in the EI-MS spectra.
- ❖ EI-MS spectra provide fragment ions, though at low abundances, that enable the identification of R_1/R_2, R_3, and R_4 groups from one spectrum.
- ❖ The provided decision trees and workflow can assist analysts with structural elucidation, regardless of the technique utilized.

REFERENCES

[1] Hardwick EK, Davidson JT. Structural characterization of nitazene analogs using electron ionization-mass spectrometry (EI-MS). *Forensic Chem*. 2024;40.

[2] Hardwick EK, Davidson JT. Structural Characterization of Nitazene Analogs Using Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS). *Drug Test. Anal*. 2025.

ACKNOWLEDGEMENTS

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