Nitazene Analog Identification Using a Combination of Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS)

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ABSTRACT

The identification of nitazene analogs in seized drug material has become an increasing problem with the forensic science community. The similarity of their electron ionization (EI) mass spectra and similar chromatographic behavior can require comparison of multiple nitazene analogs, increasing the time and cost associated with analysis. This study utilized the combination of liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) to develop an analytical scheme for the identification of nitazene analogs.

INTRODUCTION

In the past year, the Texas Department of Public Safety (DPS) Crime Laboratory in Houston has seen an increase in submitted tablet evidence containing nitazenes, also known as 2-benzylbenzimidazoles. Typically, these compounds are analyzed using GC-MS and identified using their retention time and structural information from their EI mass spectra. However, due to the structural similarities of nitazene analogs, these compounds are challenging to correctly identify[1]. Nitazene analogs typically produce highly similar EI mass spectra and can have very similar retention times[2]. Given these similarities, laboratories may need to purchase and analyze multiple nitazene standards to confidently identify an unknown compound, which is costly and inefficient.

This research proposes an alternative analysis scheme for nitazene analog identification that combines GC-MS retention times and the $[M+H]^+$ protonated molecule obtained from LC-MS to improve nitazene analog identification. The necessary instrumentation is already readily available in many crime laboratories and reduces the need to analyze multiple analytical standards to correctly identify nitazene analogs in forensic casework.

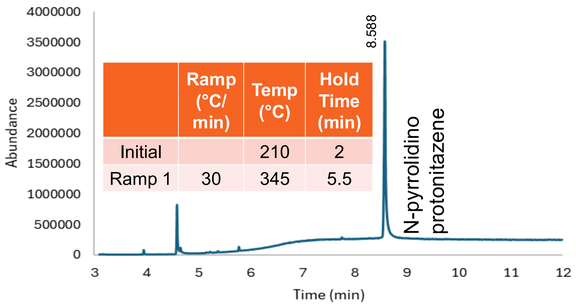
MATERIALS & METHODS

This study examined 15 nitazene analogs: butonitazene, clonitazene, etonitazene, isotonitazene, metonitazene, protodesnitazene, protonitazene, N-desethyl isotonitazene, N-desethyl protonitazene, N-pyrrolidino etodesnitazene, N-pyrrolidino etonitazene, N-pyrrolidino isotonitazene, N-pyrrolidino metonitazene, N-pyrrolidino protodesnitazene, and N-pyrrolidino protonitazene.

For LC-MS analysis, the standards were prepared individually at 1.0 mg/mL in LC-MS grade methanol, filtered, and then 300 µL of the solution was combined with 200 µL of methanol in a 500 µL LC-MS reduced vial. Samples were prepared at 1.0 mg/mL in methanol and placed into inserts for GC-MS analysis.

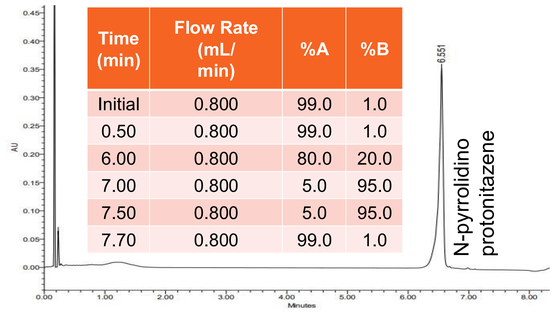
Experimental parameters for both the LC-MS and GC-MS were validated and currently used in casework[3]. For the GC-MS, a ZB-5MSi column was used with a 1.5 mL/min helium carrier gas flow rate operated in pulsed split mode at a 50:1 split ratio.

RESULTS & DISCUSSION



	Ramp (°C/min)	Temp (°C)	Hold Time (min)
Initial		210	2
Ramp 1	30	345	5.5

Figure 1: Chromatogram of the N-pyrrolidino protonitazene standard at 1 mg/mL using a TX DPS-approved GC-MS method.



Time (min)	Flow Rate (mL/min)	%A	%B
Initial	0.800	99.0	1.0
0.50	0.800	99.0	1.0
6.00	0.800	80.0	20.0
7.00	0.800	5.0	95.0
7.50	0.800	5.0	95.0
7.70	0.800	99.0	1.0

Figure 2: Chromatogram of the N-pyrrolidino protonitazene standard at 1 mg/mL using the TX DPS-approved LC-MS method.

- The GC-MS and LC-MS retention times provide two means of comparison between known and unknown nitazene analogs.

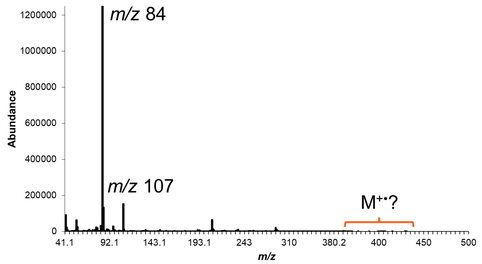


Figure 3: EI mass spectrum of the N-pyrrolidino protonitazene standard at 1 mg/mL using a TX DPS-approved GC-MS method.

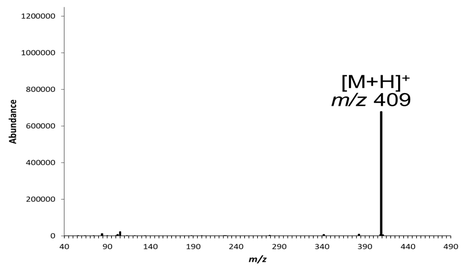


Figure 4: 5 eV cone voltage ESI mass spectrum of the N-pyrrolidino protonitazene standard at 1 mg/mL using the TX DPS-approved LC-MS method.

- The sparce fragmentation and lack of distinctive ions with EI mass spectra make it challenging to compare nitazene analogs.
- The $[M+H]^+$ protonated molecule provides molecular weight information due to ionization through the addition of a hydrogen atom.
- However, the $[M+H]^+$ protonated molecule does not enable the differentiation of structural isomers, which have the same elemental composition but a different arrangement of the atoms.

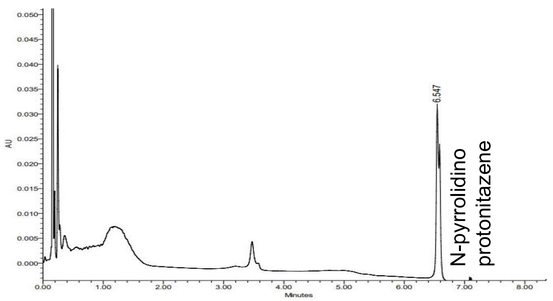


Figure 5: Chromatogram of an authentic tablet sample using the approved LC-MS method, showing the presence of N-pyrrolidino protonitazene

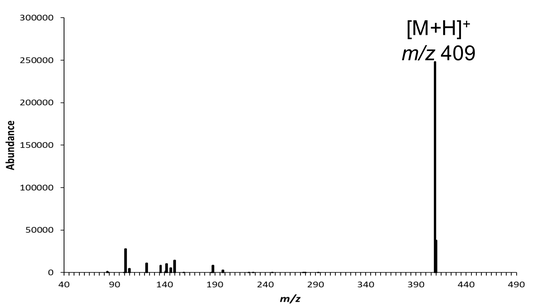


Figure 6: ESI mass spectrum from the peak apex of the authentic tablet sample using the proposed LC-MS method, collected with a 5 eV cone voltage.

- The combination of retention times with the $[M+H]^+$ protonated molecule enables improved identification of nitazene analogs.

MATERIALS & METHODS

The GC temperature program is provided in as an insert in Figure 1. The mass spectrometer scan range was *m/z* 40-500. A 70 eV ionization energy was used for all analyses.

For the LC-MS, a Cortecs 50 mm length x 2.1 mm ID x 1.6 µm particle size C18 column was used with a 0.8 mL/min flow rate. The mobile phase gradient is provided as an inset in Figure 2, with mobile phase A being 0.1% formic acid in water and mobile phase B being acetonitrile. The QDa data was collected in positive ionization mode with a 5 and 55 eV cone voltages and a scan range of *m/z* 40-500.

Retention time studies were also performed on the LC-MS and GC-MS, consisting of N-pyrrolidino protonitazene, N-pyrrolidino etonitazene, and N-pyrrolidino isotonitazene at low (0.2 mg/mL), medium (0.5 mg/mL), and high (1.0 mg/mL) concentrations, analyzed in quadruplicate.

CONCLUSIONS

- ❖ The proposed analysis scheme using the GC-MS retention time information and the $[M+H]^+$ protonated molecule from the LC-MS aided in the identification of nitazene analogs.
- ❖ The $[M+H]^+$ ion was found to be extremely beneficial for the differentiation of nitazene analogs, excluding structural isomers.
- ❖ The proposed analysis scheme was tested on an authentic casework sample that consisted of a tablet containing N-pyrrolidino protonitazene.
- ❖ This study demonstrates a cost-effective and readily implementable alternative analysis scheme for the identification of nitazene analogs in forensic casework.

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