Addressing the Oxygen in the Room: A Novel Approach for the Identification of Nitazene Analogs by Oxygenated Species Using DART-MS

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ABSTRACT

Direct analysis in real time-mass spectrometry (DART-MS) has grown in popularity as a rapid screening technique for seized drug analysis due to the ability to quickly gain molecular weight information with only minimal sample preparation. However, in addition to protonation, adduct formation can readily occur with DART-MS, potentially complicating molecular weight determination. This study examines the presence of oxygenated species formed during DART ionization with both helium and nitrogen source gases for nitazene analogs and other common seized drug related compounds.

INTRODUCTION

Seized drug analysis is often comprised of a presumptive or screening test, followed by a confirmatory technique to verify a sample's identity. Common presumptive tests include color tests, thin-layer chromatography, or immunoassays. However, novel psychoactive substances (NPS) present a challenge for screening techniques. Often, there is no color test or immunoassay available due to the short life cycle of many NPS. In addition, reference standards for NPS may be difficult to acquire promptly. Therefore, other screening techniques are necessary to address NPS screening.

DART-MS is a screening technique that has gained popularity for seized drug analysis due to its rapid nature. Often, a full scan analysis can help determine the molecular weight of an unknown compound. However, adduct formation can complicate molecular weight determination. For example, the formation of oxygen adducts has been observed with DART-MS, particularly with nitrogen as the source gas [1-3].

This study explores the formation of oxygenated species for nitazene analogs, a relevant class of NPS, formed during DART ionization. Although this phenomenon was first observed in nitazene analog data, a few other seized drug related substances were subsequently analyzed, and oxygenated species were present as well. Tandem mass spectrometry (MS/MS) experiments were performed to characterize the oxygenated species.

MATERIALS & METHODS

Chemicals & Sample Preparation

A total of 18 nitazene analogs were analyzed in this study, with varying substitutions to the core structure. The analogs include 4'-hydroxy nitazene, 5-aminoisotonitazene, 5-methyl etodesnitazene, butonitazene, etodesnitazene, etonitazene, isotodesnitazene, isotonitazene, menitazene, metodesnitazene, metonitazene, N-desethyl etonitazene, N-desethyl isotonitazene, N-piperidinyl etonitazene, N-pyrrolidino etonitazene, propylnitazene, protodesnitazene, and protonitazene. Additionally, heroin, fentanyl, PCP, and tetracaine were analyzed. All samples were prepared at 25 ppm in methanol for helium analysis, and the nitazene analogs were also prepared at 100 ppm in methanol for nitrogen analysis.

RESULTS & DISCUSSION

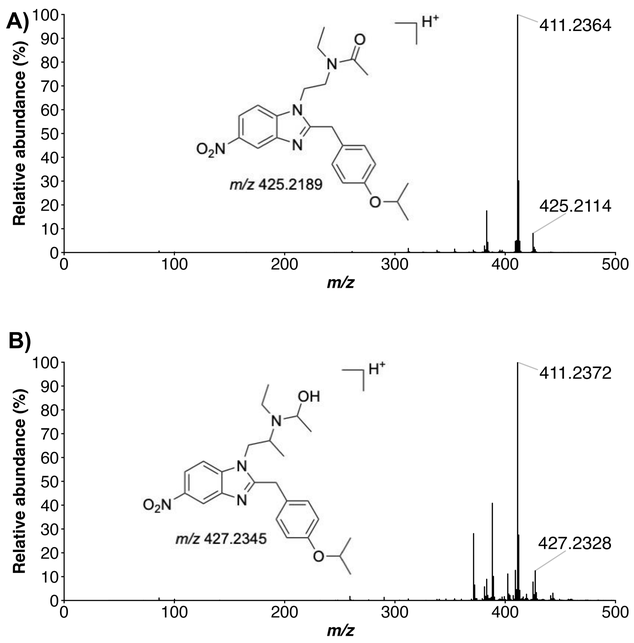

Figure 1. Exemplar full scan mass spectrum of isotonitazene with A) helium, and B) nitrogen.

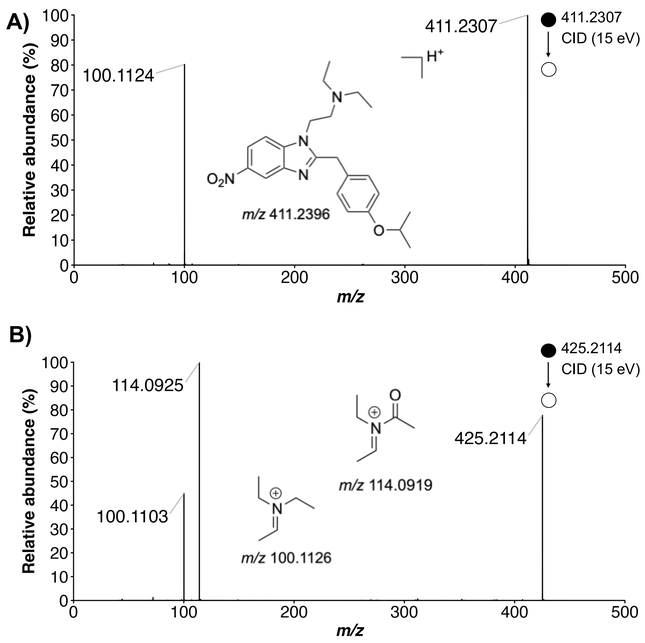

Figure 2. Exemplar 15-eV CID mass spectrum of isotonitazene under helium for A) the protonated molecule, and B) the oxygenated species.

- With helium, the oxygenated species observed was 13.9750 Da greater than the protonated molecule.
- The $\Delta m/z$ between the accurate masses of the protonated molecule and oxygenated species indicates the addition of an O atom and the loss of H_2 ($C_{23}H_{29}N_4O_4^+$, -7.5 mDa mass error).
- With nitrogen, the oxygenated species observed was 15.9956 Da greater than the protonated molecule.
- The $\Delta m/z$ between the accurate masses of the protonated molecule and oxygenated species indicates the addition of only an O atom ($C_{23}H_{31}N_4O_4^+$, -1.7 mDa mass error).

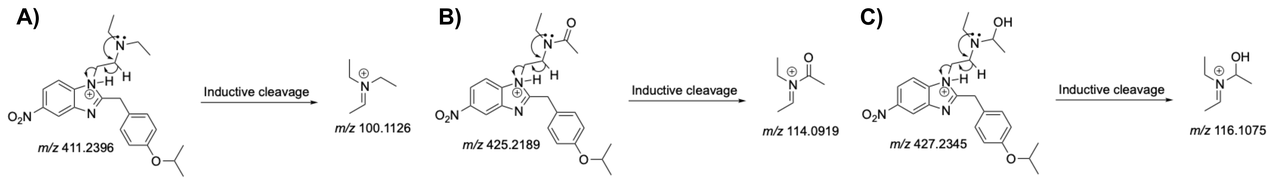

Figure 3. Proposed fragmentation pathways for the formation of A) the *m/z* 100 base peak, B) the *m/z* 114 base peak, and C) the *m/z* 116 base peak upon CID activation.

Table 1. Accurate mass table for fentanyl, heroin, PCP, and tetracaine.

Compound	Protonated Molecule (*m/z*)	Oxygenated Species Accurate Mass (*m/z*)	Δ*m/z*	Oxygenated Species Theoretical Mass (*m/z*)	Elemental Composition	Mass Error (mDa)
Fentanyl	337.2170	351.2099	13.9929	351.2073	$C_{22}H_{27}N_2O_2$	+2.65
Heroin	370.1678	384.1432	13.9754	384.1447	$C_{21}H_{22}NO_6$	-1.51
PCP	244.2057	258.1834	13.9777	258.1858	$C_{17}H_{24}NO$	-2.39
Tetracaine	265.1869	281.1811	15.9942	281.1865	$C_{15}H_{25}N_2O_3$	-5.42

MATERIALS & METHODS

Instrumentation & Data Analysis

An Agilent 6530 Q-TOF mass spectrometer was coupled with a DART JumpShot® ionization source for this study. The source temperature was 350 °C. The grid electrode was set to 350 V. The fragmentor voltage was 150 V and the skimmer voltage was 65 V. All spectra were collected in positive ionization mode. A targeted MS/MS method was used to acquire data, with a 15-eV collision energy for both helium and nitrogen analysis. The scan range for all analyses was *m/z* 40-500.

For sample introduction, 5 µL of sample was pipetted onto the closed end of a glass capillary and left to dry before being inserted into the DART source for ~10 seconds.

Data was processed using Agilent MassHunter Analysis version 10.0 and exported to Microsoft Excel. ChemDraw was used to create all structures and proposed fragmentation pathways.

CONCLUSIONS

- Oxygenated species can occur with helium or nitrogen as the source gas, with an average of 5.3% relative abundance for helium, and 9.8% relative abundance for nitrogen.
- For nitazene analog analysis, ions 14 Da greater than the protonated molecule are likely secondary ketones that have bonded at the amine moiety.
- Similarly, ions 16 Da greater than the protonated molecule, observed mainly with nitrogen, are likely secondary alcohols that have bonded at the amine moiety.
- Other seized drug compounds, like fentanyl, also displayed oxygenated species in their full scan mass spectra when analyzed with both helium and nitrogen.

REFERENCES

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