Comparison of CID, AIF, and IS-CID Activation for the Identification of Nitazene Analogs Using DART Ionization and the NIST DIT

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INTRODUCTION

Direct analysis in real time-mass spectrometry (DART-MS) is a promising alternative rapid screening method for seized drug analysis [1]. However, given that predominantly protonated/deprotonated molecules are formed from DART ionization, collisional activation is required to gather structural information about the sample. Three common types of collisional activation coupled with DART ionization are collision-induced dissociation (CID), all ion fragmentation (AIF), and in-source CID (IS-CID). CID activation is commonly utilized with tandem mass spectrometry (MS/MS) for a targeted activation technique. In comparison, AIF and IS-CID are non-targeted activation techniques, with AIF being used with MS/MS and IS-CID being used with single-stage mass spectrometers to enhance the resulting degree of fragmentation [2].

Subtle differences in the resulting fragmentation from these three activation techniques can impact isomer differentiation, which has become an imperative aspect of screening. Particularly for classes of novel synthetic opioids (NSOs), such as nitazene analogs, which consist of a variety of isobaric analogs, isomer differentiation can be very challenging.

Therefore, this study evaluated CID, AIF, and IS-CID activation using 18 nitazene analogs. The National Institute of Standards and Technology Data Interpretation Tool (NIST DIT) was used to evaluate the mass spectra from each activation technique through comparison to the NIST DART-MS Forensics Database.

MATERIALS & METHODS

Chemicals and Sample Preparation

The 18 nitazene analogs analyzed in this study were purchased from Cayman Chemical or Cerilliant. All compounds were present in the NIST DART-MS Forensics Database. Each sample was prepared at approximately 30 ppm in LC-MS grade methanol, and 5 µL of sample was pipetted onto the closed end of glass capillaries.

Instrumentation

An Agilent Technologies 6530 quadrupole time-of-flight mass spectrometer was coupled to a DART JumpShot® ionization source, which was operated in positive ionization mode with a source gas temperature of 350 ˚C. For all three activation techniques, the drying gas was 300 ˚C, the sheath gas was 350 ˚C, and both had a flow rate of 8 L/min. The capillary voltage was 3500 V, the nozzle voltage was 600 V, and the skimmer was 65 V. Low, medium, and high energy spectra were collected for each activation technique (Table 1). The scan range was set to *m/z* 40-450. Glass capillaries were introduced to the DART source for approximately 10 seconds.

RESULTS & DISCUSSION

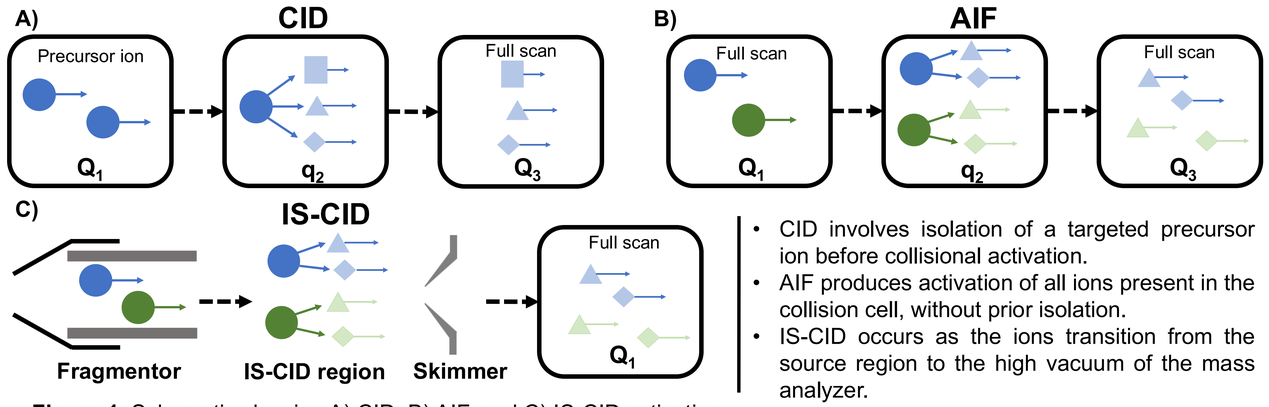

Figure 1. Schematic showing A) CID, B) AIF, and C) IS-CID activation.

- CID involves isolation of a targeted precursor ion before collisional activation.
- AIF produces activation of all ions present in the collision cell, without prior isolation.
- IS-CID occurs as the ions transition from the source region to the high vacuum of the mass analyzer.

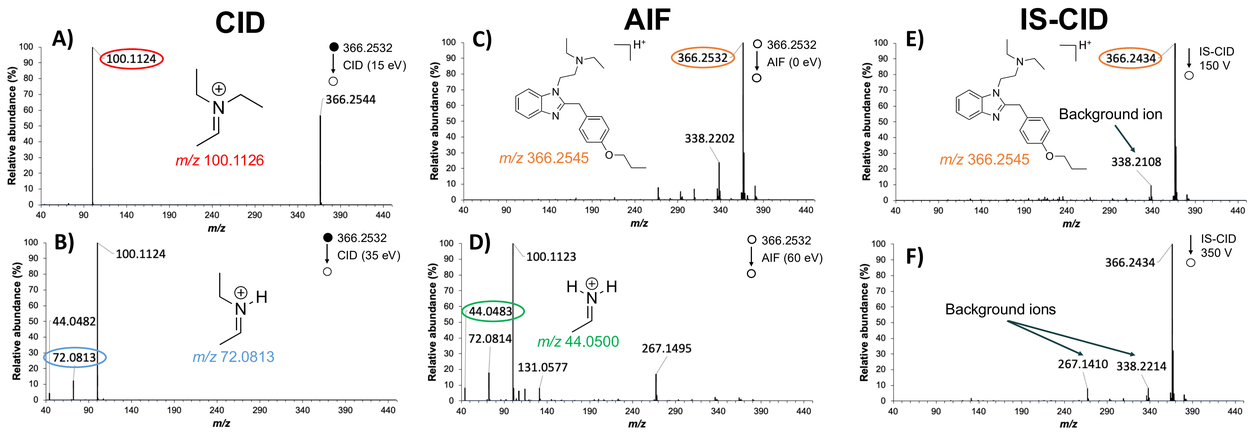

Figure 2. DART mass spectra of protodesnitazene for A) low energy CID, B) high energy CID, C) low energy AIF, D) high energy AIF, E) low energy IS-CID, and F) high energy IS-CID activation.

- AIF tends to have more fragment ions than CID or IS-CID, especially at higher energies.
- IS-CID spectra exhibited minimal fragmentation across all activation energies, resulting in higher mass product ions.

Table 2. NIST DIT classification for the 18 compounds analyzed in this study. Green indicates a correct identification and red indicates an incorrect identification based on the top result as sorted by the reverse match factor.

Compound	CID	AIF	IS-CID	Compound	CID	AIF	IS-CID
4'-OH nitazene				Metodesnitazene			
5-aminoisotonitazene				Metonitazene			
5-methyl etodesnitazene				N-desethyl etonitazene			
Butonitazene				N-desethyl isotonitazene			
Etodesnitazene				N-piperidinyl etonitazene			
Etonitazene				N-pyrrolidino etonitazene			
Isotodesnitazene				Propylnitazene			
Isotonitazene				Protodesnitazene			
Menitazene				Protonitazene			

- The NIST DIT had the most correct identifications when utilizing AIF (14/18) compared to CID and IS-CID (13/18).

MATERIALS & METHODS

Table 1. Summary of the energies for the low, medium, and high activation conditions for each technique.

Activation Energy	CID	AIF	IS-CID
Low	15 eV	0 eV	150 V
Medium	25 eV	30 eV	250 V
High	35 eV	60 eV	350 V

Data Analysis

The low, medium, and high energy spectra were compared to the NIST DIT, and identification criteria were established. A correct identification was determined when the top result listed by the reverse match factor for the protonated molecule of the compound of interest was the correct compound.

CONCLUSIONS

- ❖ All three activation techniques produced varying degrees of fragmentation.
- ❖ AIF produced more fragmentation than either CID or IS-CID.
- ❖ IS-CID tended to show product ions at a higher *m/z* than either AIF or IS-CID, even at the high activation energy, likely due to the mass spectrometer configuration.
- ❖ The NIST DIT correctly identified 13/18 compounds for both CID and IS-CID, and 14/18 compounds for AIF.
- ❖ All incorrect identifications were due to an isomer being the top match.
- ❖ The NIST DIT struggles with isomer differentiation, which is of concern particularly for nitazene analogs and other NSOs.

REFERENCES

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