Identifying the Similarity Between In-Source Collision-Induced Dissociation (IS-CID) Fragment Ion Spectra and Tandem Mass Spectrometry (MS/MS) Product Ion Spectra for Seized Drug Identifications

Jared Sharp, BS* and J. Tyler Davidson, PhD

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

MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

ABSTRACT

Currently, only limited information exists regarding the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra of forensically relevant compounds, which limits the applicability of IS-CID for seized drug identifications. However, when utilizing optimal conditions, IS-CID fragment ion spectra and MS/MS product ion spectra can be highly similar. This study provides a comprehensive assessment of the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra for a series of representative compounds commonly encountered within forensic laboratories.

INTRODUCTION

Soft ionization sources, such as electrospray ionization (ESI), have traditionally been combined with tandem mass spectrometry (MS/MS) to induce fragmentation in a process known as collision-induced dissociation (CID). However, because CID requires a tandem mass spectrometer, which increases the cost and required analyst expertise, an alternative collisional activation technique known as in-source CID (IS-CID) has grown in popularity [1]. IS-CID is achieved by controlling the voltages that guide the ions between the ionization source, which is at atmospheric pressure, and the high vacuum of the mass analyzer. Figure 1 provides a general schematic of the IS-CID region that is located between the capillary and skimmer. IS-CID is induced on this instrumental setup based on the voltages applied to the fragmentor and skimmer, respectively.

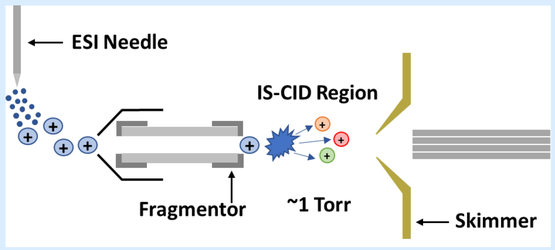

Figure 1. Generalized IS-CID schematic.

In recent years, IS-CID has grown in popularity within the seized drug community, but without a thorough assessment of the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra [2]. As the seized drug community continues to shift towards the use of IS-CID with single-stage mass spectrometers, there is a growing need for the identification of the spectral similarity between IS-CID fragment ion spectra and MS/MS product ion spectra. This study provides a comprehensive assessment of the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra using breakdown curves, Pearson-product moment correlations (PPMCs), and simple match factors (SMFs). Identifying the similarity between IS-CID fragment ion spectra and MS/MS product ion spectra will assist the seized drug community by furthering our understanding of the applicability of IS-CID on single-stage mass spectrometers for seized drug identifications.

RESULTS AND DISCUSSION

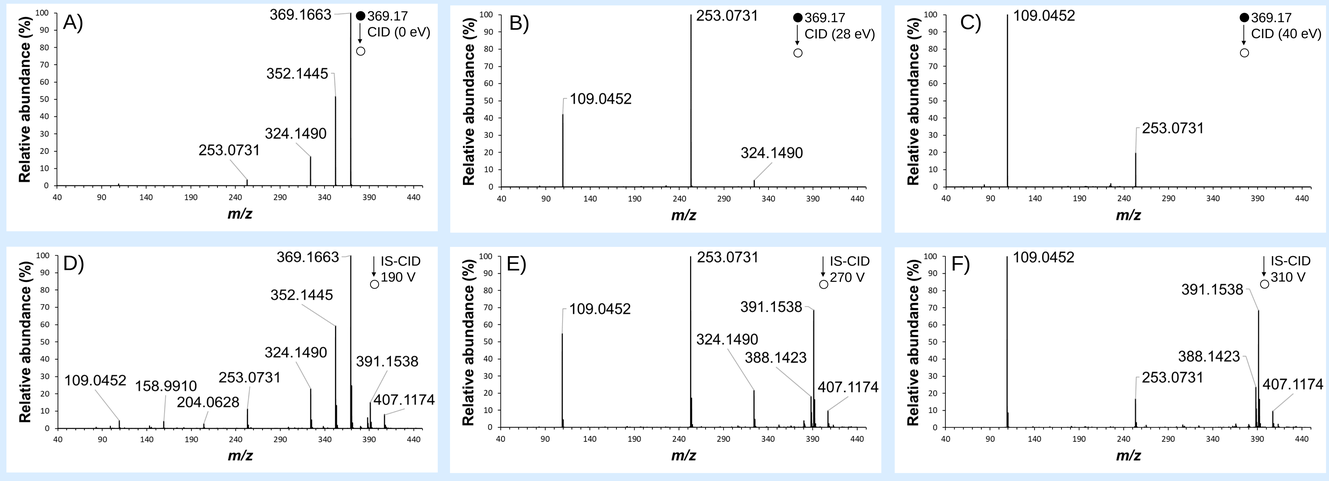

Figure 2. Comparison of MS/MS (top) and IS-CID (bottom) mass spectra for AB-FUBINACA under the following conditions: A) 0 eV, B) 28 eV, C) 40 eV, D) 190 V, E) 270 V, F) 310 V.

- Similar distribution of ions under low, medium, and high activation conditions between MS/MS and IS-CID except for the presence of sodiated and potassiated adducts at nominal *m/z* 391 and *m/z* 407, respectively.

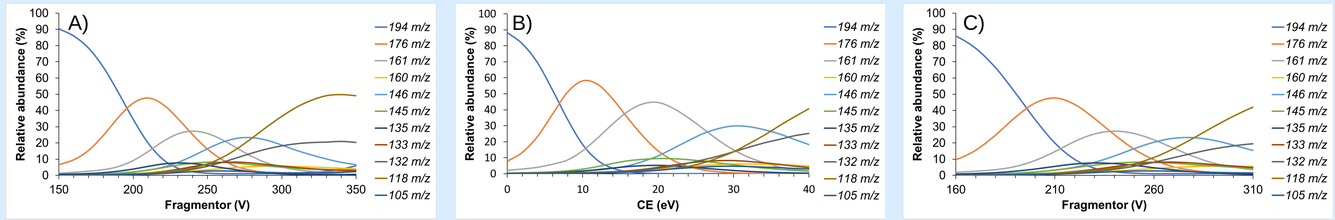

Figure 3. Breakdown curves of 3-methoxymethcathinone: A) IS-CID, B) MS/MS, and C) IS-CID truncated to 160-310 V.

- Breakdown curves demonstrate similar overall shape and slope highlighting the similar fragmentation behavior associated with the selected ions, particularly when truncating the IS-CID data to the optimum comparison region.

PPMC	150 V	160 V	170 V	180 V	190 V	200 V	210 V	220 V	230 V	240 V	250 V	260 V	270 V	280 V	290 V	300 V	310 V	320 V	330 V	340 V	350 V
MS/MS 0 eV	0.9620	0.9627	0.9620	0.9622	0.9627	0.9623	0.9631	0.9634	0.9623	0.9562	0.9302	0.8613	0.7308	0.5581	0.3929	0.2622	0.1704	0.1034	0.0614	0.0382	0.0255
MS/MS 2 eV	0.9636	0.9643	0.9635	0.9636	0.9640	0.9634	0.9641	0.9639	0.9620	0.9543	0.9256	0.8532	0.7193	0.5442	0.3781	0.2483	0.1587	0.0948	0.0553	0.0338	0.0224
MS/MS 4 eV	0.9633	0.9640	0.9633	0.9634	0.9639	0.9633	0.9641	0.9641	0.9626	0.9557	0.9283	0.8577	0.7256	0.5519	0.3865	0.2565	0.1660	0.1006	0.0597	0.0371	0.0248
MS/MS 6 eV	0.9625	0.9633	0.9626	0.9628	0.9634	0.9630	0.9639	0.9642	0.9634	0.9577	0.9324	0.8647	0.7357	0.5645	0.4001	0.2699	0.1779	0.1101	0.0667	0.0422	0.0285
MS/MS 8 eV	0.9611	0.9619	0.9613	0.9616	0.9623	0.9620	0.9632	0.9640	0.9642	0.9605	0.9385	0.8753	0.7513	0.5838	0.4212	0.2905	0.1962	0.1247	0.0776	0.0500	0.0341
MS/MS 10 eV	0.9569	0.9578	0.9574	0.9579	0.9587	0.9588	0.9604	0.9621	0.9641	0.9639	0.9476	0.8924	0.7771	0.6165	0.4572	0.3260	0.2276	0.1497	0.0959	0.0631	0.0433
MS/MS 12 eV	0.9449	0.9460	0.9458	0.9466	0.9479	0.9484	0.9508	0.9541	0.9592	0.9649	0.9589	0.9180	0.8186	0.6707	0.5178	0.3863	0.2813	0.1924	0.1271	0.0851	0.0586
MS/MS 14 eV	0.9091	0.9106	0.9108	0.9122	0.9141	0.9156	0.9194	0.9253	0.9360	0.9527	0.9653	0.9510	0.8822	0.7597	0.6208	0.4905	0.3749	0.2672	0.1819	0.1239	0.0856
MS/MS 16 eV	0.8175	0.8194	0.8204	0.8226	0.8255	0.8284	0.8342	0.8440	0.8632	0.8962	0.9375	0.9653	0.9469	0.8691	0.7577	0.6345	0.5071	0.3747	0.2618	0.1812	0.1261
MS/MS 18 eV	0.6492	0.6515	0.6533	0.6565	0.6604	0.6648	0.6727	0.6869	0.7153	0.7665	0.8408	0.9192	0.9647	0.9479	0.8783	0.7729	0.6399	0.4864	0.3475	0.2449	0.1730
MS/MS 20 eV	0.4439	0.4464	0.4490	0.4528	0.4575	0.4631	0.4725	0.4900	0.5253	0.5905	0.6915	0.8131	0.9171	0.9605	0.9372	0.8578	0.7306	0.5694	0.4165	0.3005	0.2174
MS/MS 22 eV	0.2657	0.2684	0.2714	0.2756	0.2807	0.2870	0.2971	0.3164	0.3555	0.4285	0.5454	0.6949	0.8397	0.9291	0.9452	0.8934	0.7809	0.6251	0.4709	0.3511	0.2632
MS/MS 24 eV	0.1493	0.1519	0.1552	0.1596	0.1648	0.1715	0.1818	0.2018	0.2424	0.3185	0.4426	0.6061	0.7738	0.8919	0.9360	0.9083	0.8140	0.6705	0.5226	0.4046	0.3155
MS/MS 26 eV	0.0794	0.0820	0.0854	0.0898	0.0950	0.1019	0.1123	0.1323	0.1732	0.2497	0.3759	0.5450	0.7245	0.8607	0.9265	0.9217	0.8492	0.7238	0.5874	0.4747	0.3860
MS/MS 28 eV	0.0418	0.0443	0.0477	0.0521	0.0573	0.0642	0.0744	0.0942	0.1343	0.2092	0.3334	0.5018	0.6852	0.8323	0.9161	0.9355	0.8894	0.7884	0.6687	0.5646	0.4779
MS/MS 30 eV	0.0221	0.0245	0.0278	0.0320	0.0371	0.0438	0.0537	0.0727	0.1108	0.1817	0.2996	0.4611	0.6411	0.7937	0.8940	0.9400	0.9264	0.8570	0.7602	0.6688	0.5863
MS/MS 32 eV	0.0120	0.0142	0.0173	0.0213	0.0260	0.0324	0.0416	0.0591	0.0940	0.1583	0.2655	0.4137	0.5833	0.7357	0.8498	0.9231	0.9453	0.9127	0.8440	0.7691	0.6934
MS/MS 34 eV	0.0070	0.0090	0.0118	0.0154	0.0196	0.0254	0.0337	0.0492	0.0795	0.1349	0.2274	0.3567	0.5093	0.6558	0.7801	0.8794	0.9383	0.9451	0.9078	0.8526	0.7865
MS/MS 36 eV	0.0046	0.0063	0.0088	0.0118	0.0156	0.0207	0.0279	0.0411	0.0666	0.1123	0.1890	0.2977	0.4309	0.5682	0.6985	0.8187	0.9091	0.9511	0.9432	0.9069	0.8510
MS/MS 38 eV	0.0033	0.0048	0.0069	0.0095	0.0128	0.0172	0.0234	0.0345	0.0554	0.0925	0.1547	0.2445	0.3590	0.4861	0.6187	0.7538	0.8685	0.9386	0.9547	0.9347	0.8883
MS/MS 40 eV	0.0026	0.0039	0.0057	0.0079	0.0107	0.0146	0.0200	0.0294	0.0466	0.0766	0.1271	0.2014	0.3005	0.4183	0.5514	0.6963	0.8284	0.9191	0.9534	0.9461	0.9076

Figure 4. PPMC comparisons between IS-CID and MS/MS conditions for crotonyl fentanyl across five replicate analyses. Conditional formatting is used to highlight stronger correlation in green and weaker correlation in red. The average highest PPMC was 0.9565 +/- 0.0063.

- The average (N = 5) PPMC for the largest correlation for each pairwise comparison was 0.8963 +/- 0.0261 for synthetic cathinones, 0.9537 +/- 0.0067 for synthetic cannabinoids, and 0.9382 +/- 0.0150 for fentanyl analogs, indicating a strong correlation.
- The average (N =5) for the highest SMF for each pairwise comparison was 766 +/- 29 for synthetic cathinones, 787 +/- 15 for synthetic cannabinoids, and 767 +/- 21 for fentanyl analogs, indicating a fair correlation.

MATERIALS AND METHODS

Sample Preparation

AB-FUBINACA, AB-PINACA, AM-2201, MAB-CHMINACA, 3-methoxymethcathinone, 4-chloroethcathinone, α-PVP, and pentylone were analyzed as 50 ppm solutions in 49.9:49.9:0.2% MeOH:dH_2O:CH_3COOH. Alfentanil, α-methyl acetyl fentanyl, crotonyl fentanyl, and fentanyl were analyzed as 10 ppm solutions, due to increased potencies and associated handling risks, in the same solvent.

Instrumentation and Data Analysis

An Agilent Technologies 6530 quadrupole time-of-flight (Q-TOF) mass spectrometer was used for the collection of MS/MS and IS-CID data. The MS/MS activation data was collected with a fragmentor voltage of 150 V and a skimmer voltage of 65 V with collision energies ranging from 0 eV to 40 eV in 2 eV increments. The IS-CID activation data was collected with the fragmentor voltage ranging from 150 V to 350 V in 10 V increments with a constant skimmer voltage of 65 V. Data was extracted through MassHunter Qualitative Analysis version 10.0 and exported to Microsoft Excel for similarity assessment.

CONCLUSIONS

- Spectral similarity observed based on breakdown curves, PPMCs, and SMFs.
- General optimum comparison range between 150-280 V and 0-22 eV.
- Subtle differences in fragmentation behavior based on breakdown curve analyses.
- No obvious trends in the PPMCs and SMFs between compounds within a given class.
- Reproducible results across five replicate data sets.
- The 95% CI average PPMC and SMF for the highest similarity for each pairwise comparison for all 12 compounds were 0.9294 +/- 0.0106 and 773 +/- 13, respectively.
- Numerical support for the use of IS-CID fragment ion spectra for seized drug identifications with pure compounds.

REFERENCES

[1] Buré, C. and Lange, C. Comparison of Dissociation of Ions in an Electrospray Source, or a Collision Cell in Tandem Mass Spectrometry. *Curr. Org. Chem.*, **2003**. 7: 1613-1624.

[2] Davidson, J.T., Sasiene, Z.J., and Jackson, G.P. Comparison of in-source collision-induced dissociation and beam-type collision-induced dissociation of emerging synthetic drugs using a high-resolution quadrupole time-of-flight mass spectrometer. *J. Mass Spectrom.*, **2021**. 56(2): e4679.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Forensic Science at Sam Houston State University for providing the resources necessary to conduct this research and also thank Daniel Do for his assistance with this project.