Quantifying the Mass Spectral Similarity Between IS-CID Fragment Ion Spectra and MS/MS Product Ion Spectra for Seized Drug Identifications

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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 for the comparison of these two activation techniques.

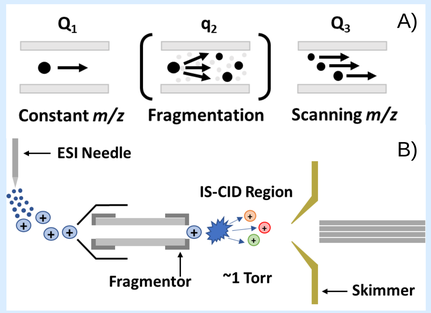

Figure 1. General schematic for CID (A) and IS-CID (B).

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. 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).

MATERIALS AND METHODS

Sample Preparation

AB-FUBINACA, AB-PINACA, AM-2201, MAB-CHMINACA, 3-methoxymethcathinone (3-MeOMC), 4-chloroethcathinone (4-CEC), α-PVP, and pentylone were analyzed as 50 ppm solutions in 49.9:49.9:0.2% MeOH:H_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.

RESULTS AND DISCUSSION

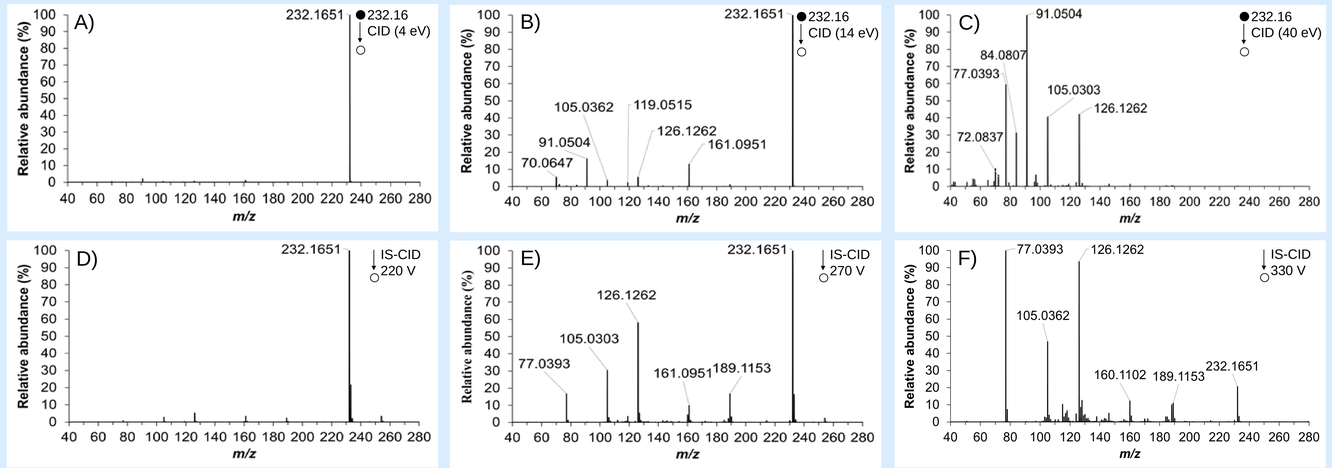

Figure 2. Comparison of MS/MS (top) and IS-CID (bottom) mass spectra for α-PVP under the following conditions: A) 4 eV, B) 14 eV, C) 40 eV, D) 220 V, E) 270 V, F) 330 V.

- Spectral comparisons were generally similar; however, differences in energy deposition can lead to deviations in observed fragment ions [2].

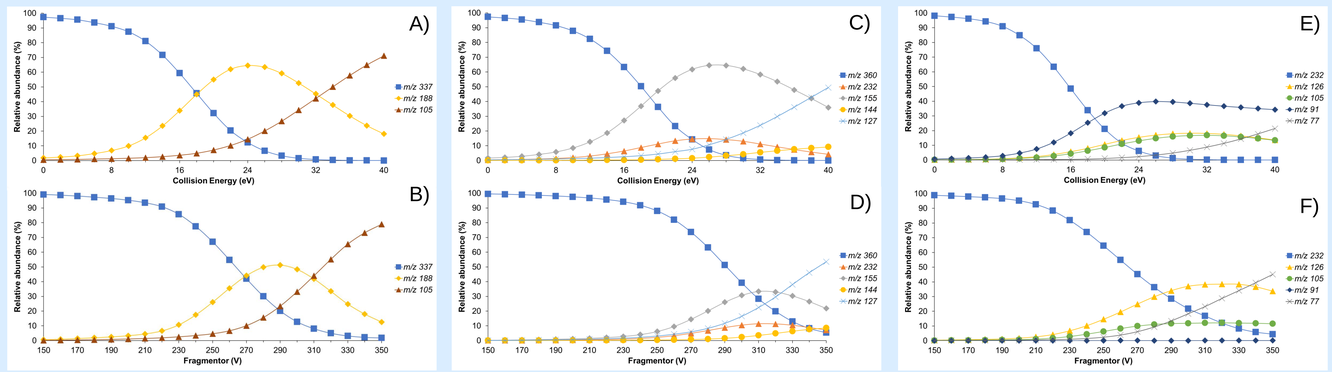

Figure 3. Comparison of breakdown curves for MS/MS (top) and IS-CID (bottom) for fentanyl (A & B), AM-2201 (C & D), and α-PVP (E & F).

- In general, breakdown curves support similar fragmentation behavior based on the overall shape, slope, and order of fragment ion formation.

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	**0.9609**	**0.9623**	**0.9642**	**0.9645**	**0.9548**	0.9183	0.8466	0.7513	0.6596	0.5879	0.5329	0.4807	0.4208	0.3532	0.2842	0.2186	0.1617	0.1133	0.0783	0.0559	0.0428
MS/MS 2 eV	0.9253	0.9287	0.9345	0.9429	0.9483	**0.9338**	0.8831	0.7989	0.7059	0.6254	0.5599	0.4987	0.4326	0.3612	0.2899	0.2230	0.1653	0.1163	0.0808	0.0581	0.0447
MS/MS 4 eV	0.8642	0.8697	0.8795	0.8965	0.9184	0.9293	0.9070	0.8436	0.7576	0.6729	0.5975	0.5262	0.4524	0.3758	0.3012	0.2320	0.1725	0.1221	0.0855	0.0619	0.0478
MS/MS 6 eV	0.7971	0.8046	0.8182	0.8430	0.8803	0.9167	0.9263	0.8916	0.8208	0.7367	0.6523	0.5691	0.4848	0.4006	0.3206	0.2473	0.1848	0.1318	0.0931	0.0678	0.0524
MS/MS 8 eV	0.7248	0.7337	0.7498	0.7799	0.8285	0.8866	**0.9283**	0.9281	0.8819	0.8067	0.7182	0.6249	0.5299	0.4368	0.3497	0.2708	0.2035	0.1462	0.1041	0.0761	0.0586
MS/MS 10 eV	0.6407	0.6503	0.6672	0.6994	0.7538	0.8266	0.8965	**0.9344**	0.9233	0.8697	0.7886	0.6933	0.5917	0.4909	0.3957	0.3089	0.2344	0.1704	0.1226	0.0900	0.0687
MS/MS 12 eV	0.5658	0.5753	0.5918	0.6232	0.6773	0.7547	0.8404	0.9066	**0.9290**	0.9044	0.8426	0.7574	0.6587	0.5561	0.4561	0.3622	0.2793	0.2066	0.1508	0.1116	0.0849
MS/MS 14 eV	0.5022	0.5114	0.5270	0.5561	0.6067	0.6826	0.7745	0.8593	0.9101	**0.9164**	0.8817	0.8163	0.7299	0.6329	0.5327	0.4334	0.3419	0.2588	0.1927	0.1446	0.1104
MS/MS 16 eV	0.4379	0.4466	0.4608	0.4869	0.5321	0.6024	0.6938	0.7893	0.8634	0.9011	**0.8990**	0.8622	0.7980	0.7155	0.6215	0.5210	0.4225	0.3289	0.2510	0.1919	0.1476
MS/MS 18 eV	0.3592	0.3671	0.3798	0.4023	0.4410	0.5029	0.5884	0.6872	0.7778	0.8434	0.8752	**0.8724**	0.8388	0.7799	0.7010	0.6071	0.5078	0.4080	0.3202	0.2500	0.1944
MS/MS 20 eV	0.2636	0.2706	0.2817	0.3007	0.3331	0.3861	0.4628	0.5589	0.6585	0.7453	0.8074	0.8387	**0.8393**	**0.8104**	0.7547	0.6764	0.5847	0.4860	0.3934	0.3148	0.2485
MS/MS 22 eV	0.1717	0.1779	0.1875	0.2037	0.2309	0.2758	0.3432	0.4332	0.5348	0.6343	0.7182	0.7774	0.8083	0.8085	0.7777	0.7194	0.6412	0.5500	0.4584	0.3758	0.3021
MS/MS 24 eV	0.1057	0.1112	0.1196	0.1336	0.1568	0.1954	0.2547	0.3376	0.4373	0.5421	0.6393	0.7177	0.7710	0.7940	**0.7843**	0.7443	0.6802	0.5985	0.5113	0.4284	0.3509
MS/MS 26 eV	0.0603	0.0653	0.0729	0.0852	0.1056	0.1393	0.1918	0.2676	0.3629	0.4686	0.5729	0.6642	0.7344	0.7757	0.7842	0.7611	0.7111	0.6400	0.5592	0.4788	0.4004
MS/MS 28 eV	0.0338	0.0383	0.0452	0.0564	0.0746	0.1045	0.1513	0.2204	0.3099	0.4130	0.5198	0.6189	0.7012	0.7576	0.7824	0.7754	0.7398	0.6803	0.6075	0.5314	0.4546
MS/MS 30 eV	0.0198	0.0239	0.0303	0.0406	0.0571	0.0840	0.1261	0.1888	0.2718	0.3703	0.4760	0.5786	0.6694	0.7383	0.7786	0.7879	0.7674	0.7210	0.6578	0.5876	0.5140
MS/MS 32 eV	0.0126	0.0165	0.0224	0.0318	0.0470	0.0713	0.1092	0.1659	0.2420	0.3341	0.4358	0.5386	0.6346	0.7142	0.7696	**0.7961**	0.7922	0.7606	0.7087	0.6461	0.5772
MS/MS 34 eV	0.0089	0.0124	0.0178	0.0265	0.0403	0.0623	0.0962	0.1468	0.2153	0.2996	0.3950	0.4950	0.5934	0.6815	0.7511	0.7950	0.8091	0.7940	0.7555	0.7022	0.6394
MS/MS 36 eV	0.0069	0.0100	0.0149	0.0227	0.0351	0.0547	0.0847	0.1294	0.1900	0.2655	0.3533	0.4483	0.5464	0.6405	0.7223	0.7827	**0.8146**	0.8168	0.7929	0.7504	0.6950
MS/MS 38 eV	0.0056	0.0084	0.0127	0.0196	0.0306	0.0479	0.0742	0.1130	0.1659	0.2327	0.3121	0.4010	0.4968	0.5943	0.6856	0.7602	0.8085	**0.8276**	0.8186	0.7878	0.7408
MS/MS 40 eV	0.0046	0.0070	0.0108	0.0168	0.0263	0.0414	0.0641	0.0974	0.1428	0.2011	0.2722	0.3543	0.4466	0.5453	0.6432	0.7292	0.7917	0.8265	**0.8323**	**0.8136**	**0.7757**

Figure 4. Average PPMC comparisons for all 12 compounds across five replicate analyses for each pairwise comparison. Conditional formatting is used to highlight higher comparisons in green and lower comparisons in red. The 95% confidence interval average highest PPMC was 0.9565 +/- 0.0063.

- Conserved trends in 95% confidence interval PPMCs and SMFs for each comparison metric: synthetic cathinones (0.8963 ± 0.0261 & 766 ± 29), fentanyl analogs (0.9387 ± 0.0150 & 767 ± 21) and synthetic cannabinoids (0.9537 ± 0.067 & 787 ± 15).
- The optimum comparison range was between 0-22 eV and 150-280 V based on 0.8000 PPMC and 700 SMF threshold values.

Table 1. Average repeatability across five replicate analyses using an expanded uncertainty of two times the relative standard deviation (2σ) of the relative ion abundances for any ion above a 5% relative ion abundance threshold.

Compound	MS/MS (%)	IS-CID (%)
Fentanyl	27.6	21.0
Crotonyl fentanyl	25.7	19.8
α-Methyl acetyl fentanyl	28.4	21.8
Alfentanil	26.2	8.9
3-MeOMC	8.4	7.8
4-CEC	7.5	11.2
α-PVP	8.1	16.6
Pentylone	9.7	13.0
AB-FUBINACA	14.3	14.4
AB-PINACA	10.3	12.5
AM-2201	5.5	13.7
MAB-CHIMINACA	11.0	15.9
Overall average	15.2	14.7

- Repeatability of simulated mixtures (2-4 compounds) was also assessed, but results were skewed by competitive ionization.
  - ESI-MS/MS = 24.7%
  - ESI-IS-CID = 11.0%

CONCLUSIONS

- Considerable spectral similarity observed based on breakdown curves, PPMCs, and SMFs.
- Subtle differences in fragmentation behavior based on spectral comparisons and breakdown curves.
- Related to differences in energy deposition between CID and IS-CID activation.
- Conserved trends in PPMCs and SMF comparisons between compound classes possibly due to the generation of direct cleavages rather than consecutive neutral loses.
- General optimum comparison range between 0-22 eV and 150-280 V.
- No discernable differences in repeatability of relative ion abundances for ions above a 5% threshold between MS/MS and IS-CID activation.
- Numerical support for the use of IS-CID fragment ion spectra for seized drug identifications with pure compounds.
- Caution with the use of IS-CID with mixtures without chromatography or deconvolution software.

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