Characterizing Product Ions in a Reference Tandem Mass Spectral Library

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➢ **Overview:** Fragmentation paths for product ions were examined for building a high quality and comprehensive reference tandem mass spectral library.

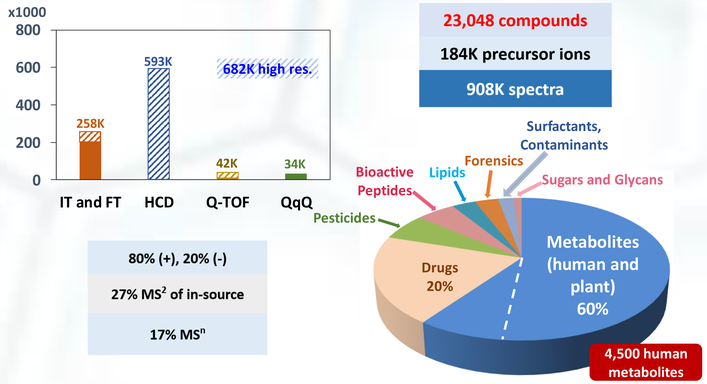


Figure 1. Types of Mass Spectra and Compounds in the NIST Tandem Mass Spectral Library

➢ **Introduction:** Tandem mass spectral library searching has been proven to be a fast and reliable data analysis technique for identifying metabolites in complex mixtures from LC/MS/MS data. Product ion characterization is critical to ensure the spectral correctness in building a high quality reference tandem mass spectral library.

➢ **Methods:** The peaks in a high resolution spectrum were first assigned with chemical formula(s) and then validated with possible product ion structures using MS Interpreter (free download from **https://chemdata.nist.gov**) and Mass Frontier.

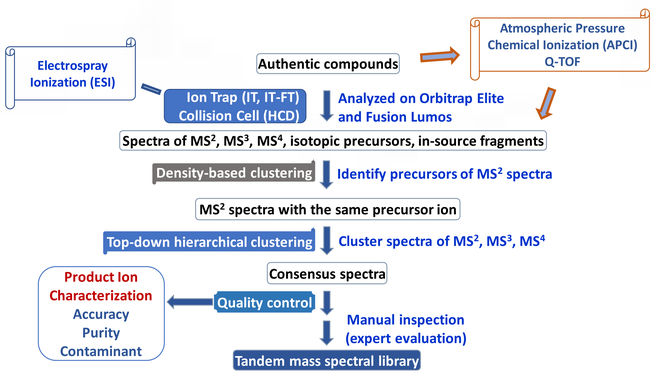


Figure 2. Procedure of Building the NIST Tandem Mass Spectral Library

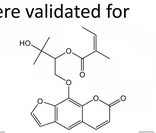
➢ **Results:** Major product ions (losses from precursors and fragments, observed with >100 compounds with relative abundance >5%) were examined by using the high resolution (HCD and FT-IT) mass spectra of authentic compounds in the library. These product ions associated with compounds are particularly useful for finding structures for more complex and less common group rearrangement.

- 110,465 spectra of 8,396 compounds, $[M+H]^+$
- 510 major neutral losses, 730 fragments
- 47,377 spectra of 3,601 compounds $[M-H]^-$
- 173 major losses, 225 fragments

	positive loss from precursor	num of compounds	positive fragments	num of compounds	negative loss from precursor	num of compounds	negative fragments	num of compounds
1	p-H2O	3283	C6H5	4649	p-H2O	1179	C6H5O	875
2	p-CH2O2	1408	C5H5	4496	p-CO2	1143	C2H3O2	655
3	p-CO	1123	C4H3	4402	p-CH2O3	539	C8H7O	584
4	p-NH3	1031	C7H7	4162	p-CH2O2	502	C8H5O	582
5	p-C2H4O2	1010	C6H7O	4140	p-CH3	463	C3H3O2	575
6	p-2H2O	935	C5H3	3932	p-CO	436	C3H5O	550
7	p-C3H6O2	782	C7H5	3869	p-C2H4O2	430	C5H5	541
8	p-CH3	737	C4H2	3681	p-C2H4O	410	C4HO	528
9	p-C2H4O	730	C6H5N2	3500	p-C3H4O2	354	C5H5O	526
10	p-C2H6O	712	C6H7	3405	p-2H2O	343	C6H7O	517
11	p-C4H4O	688	C9H7	3350	p-C3H6O2	319	C4H5O	508
12	p-C2H2O	671	C4H5	3300	p-CH4	285	C7H7O	506
13	p-C3H6O	669	C8H7	3073	p-C3H6O	268	C7H5O2	506
14	p-C3H4O2	645	C6H6	3009	p-C3H6O3	267	C6H4O2	506
15	p-C4H10O	631	C6H3	2949	p-C2H3O	264	C4H3O	435
16	p-C2H6O2	627	C8H6	2834	p-C2H2O	250	C6H3O	426
17	p-C4H8	615	C10H8	2340	p-C2O3	246	C5H7O	426
18	p-C4H6O3	607	C5H2	2187	p-C2H3O2	239	C10H7O	415
19	p-C3H6O3	585	C5H7	2138	p-C3H4O3	238	C5H3O2	401
20	p-C4H6O2	584	C10H7	2087	p-CH2O	236	C7H6O	398
21	p-C3H8O2	571	C6H2	2060	p-H2	229	C8H5O2	396
22	p-C3H8O	568	C8H9	2053	p-C4H6O2	228	C7H7	380
23	p-C3H4O3	561	C7H6	1942	p-CH4O	222	C12H7O	373
24	p-C4H8O2	555	C3H3O	1901	p-C2H6	221	C4H5O2	371
25	p-C4H8O3	522	C6H4	1866	p-CH4O4	216	C6H5O2	370
26	p-C5H10O2	515	C4H7	1851	p-C4H6O3	207	C9H7O	363
27	p-C2H2O3	504	C10H6	1826	p-C2H2O3	200	C7H7O2	362
28	p-C2H5O2	501	C7H7O	1812	p-C2H6O2	197	C9H5O2	360
29	p-C2H6O3	500	C12H8	1759	p-C5H8O3	191	C9H7	356
30	p-C2H3O	492	C7H9	1721	p-H	182	C12H7O2	336
31	p-CH4O3	481	C11H7	1687	p-C6H10O5	172	C7H9O	333
32	p-C5H6O3	479	C5H5O	1686	p-C4H4O3	171	C9H7O2	330

Figure 3. Major losses and fragments from high resolution spectra in the library

➢ **High Resolution HCD MS^2 and FT-IT MS^n:** Product ions were validated for accurate compound identification.



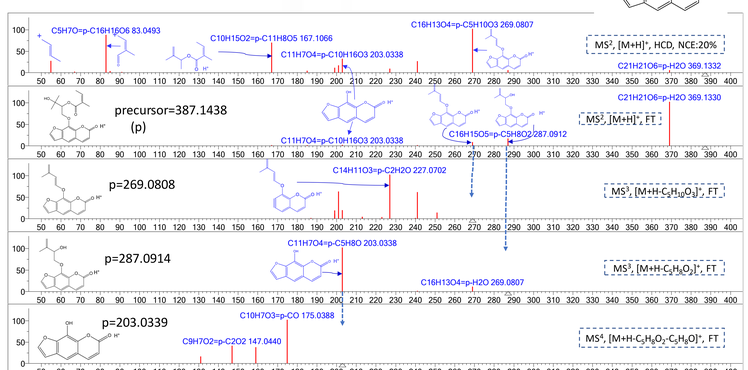


Figure 4. An example of product ions characterized in high resolution spectra

➢ **Distinguishing Isomers:** By examining the characterized product ions, >5,000 isomers were distinguished with MS^2, MS^3, MS^4 spectra in the library. For example, 10 out of 12 compounds with molecular formula $C_{10}H_{13}NO_2$, were distinguished by using their unique feature product ion peaks (▭ in Figure 5).

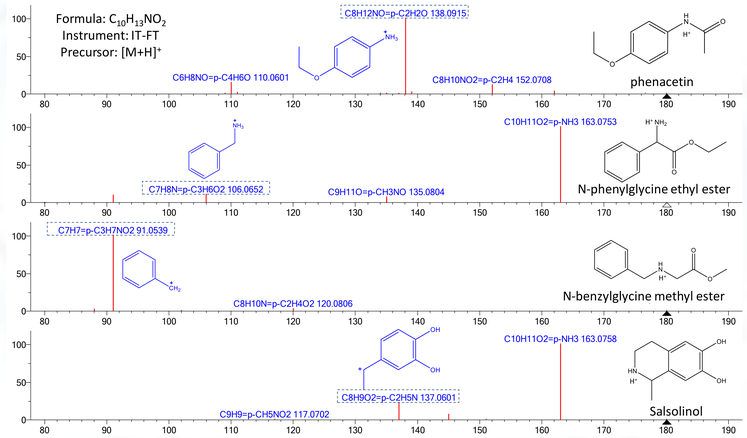


Figure 5. An example of 4 isomers distinguished by characterized product ions

➢ **Library Application:** This comprehensive reference library with >23,000 compounds and >908,000 spectra including 4,500 human metabolites has been applied in metabolite identification in human plasma, urine, and milk samples.

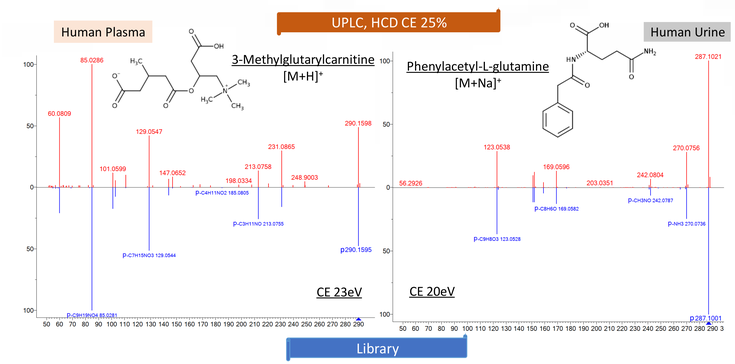


Figure 6. An example of human metabolites identified by searching the library

➢ **Please visit booth #616 for more info about NIST MS libraries and related software**