

Automated Determination of Ion Compositions in Mass Spectra and Deconvolution of Composite Mass Spectra Using an Ion Correlation Program



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1. Introduction

For more than a decade in our laboratory, elemental compositions of ions in mass spectra have been routinely determined by measuring exact masses and relative isotopic abundances (RIAs) of ions in isotopic clusters using a GC coupled to a double focusing mass spectrometer. HPLC interfaced to both an accurate mass triple quadrupole and orthogonal acceleration, time-of-flight (oa-TOF) mass spectrometers provide larger mass and/or RIA errors than a double focusing mass spectrometer.^{2,3} To compensate, an Ion Correlation Program (ICP) was developed to provide molecular ion or precursor ion compositions, despite the larger measurement errors. In references 2 and 3, the ICP successfully correlated product and precursor ions and provided their compositions. In addition, the ICP provided ion non-correlations, so that a simulated composite mass spectrum could be deconvoluted.³ Manual input into the ICP of exact masses, RIAs, and error levels were required. This poster reports on an automated ICP that imports ASCII files of m/z ratios and ion chromatogram peak areas to yield ion compositions and to deconvolute mass spectra.

2. Ion Correlation and Non-correlation

Pairs of mass spectra acquired with low and high CID voltages for eight compounds by an Agilent orthogonal-acceleration, time-of-flight mass spectrometer are shown in Figure 1. The mass spectra are labeled with the possible precursor ion, product ion, and corresponding neutral loss compositions determined by the ICP. Precursor and product ions from all eight spectra are listed in Table 1 to simulate a composite mass spectrum from all eight compounds.

Table 2 illustrates the ion correlation testing sequence. Starting with the highest mass ion, each ion lower in mass was assumed to be a product ion. If this assumption was true, one or more possible compositions were found for both ions. If false, no compositions were found for either ion. Exact masses of correlated ions were re-entered for testing against the next lowest mass ion. This process was repeated until all ions had been tested for correlation. This procedure was then repeated for the m/z 285 ion, which was also a precursor ion. Likewise, each non-correlated, lower-mass ion was treated similarly as a precursor ion. These cycles required manual entry of exact masses for all ions and entry of RIAs for ions present in high abundance. Such tedious, repetitive tasks can be automated.

In Table 2, the four product ions typed in blue were correlated with the m/z 319 precursor ion, ions in red were not correlated, and the m/z 170 ion in green was falsely correlated because its composition (C₁₀H₁₂N₂) is a subset of the composition of the m/z 319 ion (C₁₇H₂₀N₂Cl). In Table 1, the same five ions are shown in blue, three product ions and the m/z 285 precursor ion are shown in purple, and four other precursor ions, [M+H]⁺, appear in other colors along with one product ion that each for three of them. Ions lower in mass than m/z 158 correlated with multiple precursor ions. For example, the ion C₈H₈⁺ (m/z 91) is a subset of seven precursor ion compositions and was correlated with all seven, even though this ion is prominent in the paired mass spectra in Figure 1 for only two of the compounds. The two precursor ions in red in Table 1 were compositional subsets of higher-mass precursor ions, and were falsely correlated with them.

High Analyte	Low Analyte	Correlation
219.080	170.062	Correlated
219.080	158.046	Correlated
219.080	146.030	Correlated
219.080	134.014	Correlated
219.080	122.000	Correlated
219.080	110.000	Correlated
219.080	98.000	Correlated
219.080	86.000	Correlated
219.080	74.000	Correlated
219.080	62.000	Correlated
219.080	50.000	Correlated
219.080	38.000	Correlated
219.080	26.000	Correlated
219.080	14.000	Correlated
219.080	2.000	Correlated
219.080	0.000	Correlated
219.080	170.062	Correlated
219.080	158.046	Correlated
219.080	146.030	Correlated
219.080	134.014	Correlated
219.080	122.000	Correlated
219.080	110.000	Correlated
219.080	98.000	Correlated
219.080	86.000	Correlated
219.080	74.000	Correlated
219.080	62.000	Correlated
219.080	50.000	Correlated
219.080	38.000	Correlated
219.080	26.000	Correlated
219.080	14.000	Correlated
219.080	2.000	Correlated
219.080	0.000	Correlated

Figure 1. Low and high fragmentor voltage mass spectra for eight analytes. Profile data are displayed. Mass peaks may appear to be one or two pixels wide.

3. Discerning Precursor Ions

To overcome the problem of mis-correlating low-mass precursor ions with higher-mass precursor ions, both low and high CID voltage mass spectra were considered. Within the low CID voltage mass spectra in Figure 1, precursor ions and Na-adduct ions are evident. Protonated dimer ions and a Na-dimer ion were observed at higher m/z ratios than those shown. But few product ions are seen, except for the product ion at m/z 148 in Figure 1a due to a facile loss of a water molecule from the m/z 166 ion. The high CID voltage mass spectra display numerous product ions and lower abundances of the precursor ions. Ions that lose ion abundance when the CID voltage is increased are precursor or adduct ions; those that increase in ion abundance are product ions. By considering the paired mass spectra in Figure 1, all precursor ions were identified.

4. Selecting Mass Spectra

The user must select appropriate mass spectra at both CID voltages before creating an ASCII file of the m/z ratios, mass peak heights, and mass peak areas. Masses accurate to within 2 mmu are obtained when the ion abundance is less than half of the saturated level. Moving off the maximum in the chromatographic peak of the base ion, mass spectra were examined until this level was found. This strategy overcame the limitation of the limited dynamic range associated with time digital converters. For the mass spectra used, up to one-half of the dynamic range was utilized, so that RIAs of 1% or less were measurable. ASCII files for mass spectra obtained at both low and high CID voltages including all ions with abundances greater than 10,000 or 5000 counts, respectively, were saved.

5. Automating the ICP

Working with low and high CID voltage pairs of mass spectra for the eight standards, strategies and error limits to be used in an automated fashion evolved until unique compositions were obtained for nearly all of the precursor and product ions from all eight standards. Additional data tests will be included in the automated ICP as it is applied to other analytes. Each failure to provide any or sensible compositions for ions must be understood and where possible, corrected by modified or additional code. The strategies, assumptions, and tests currently employed are:

The elements C, H, N, O, S, Cl, and Br are normally considered. The user can add P and/or F.

The first of three procedures searches ions in the low CID voltage mass spectrum from low to high masses. To be considered a precursor ion, an ion's abundance must exceed 200,000 counts, which corresponds to the red line in the low CID spectra in Figure 1. The precursor ion cannot be an ammonium, sodium, or potassium adduct, a protonated or sodiated dimer, an isotopic mass peak of a previously examined monoisotopic ion, or be the product ion of a heavier ion that has lost a water molecule. The +1 ion must have an abundance less than the number of Si atoms possible multiplied by the isotopic abundance of Si plus the number of C atoms possible for the remaining mass of the ion multiplied by the isotopic abundance of C. Otherwise, the ion under consideration is a precursor ion that has lost an H atom. If a suspected precursor ion is found in the high CID voltage product ion spectrum, it must have a lower ion abundance than in the low CID voltage spectrum.

After the precursor ions have been located, the product ions are collected from the higher CID voltage mass spectrum. Only a narrow mass range based on measurement error and the mass differences among isotopes is acceptable for an ion to be selected as a +1 or +2 isotopic mass peak for both precursor or product ions. Files containing up to eight times the number of precursor ions, the precursor ions, and adduct ions are created and saved. Examples corresponding to Figure 1g are provided in Table 3.

Table 3. ASCII Files Generated from the Low and High CID Voltage Mass Spectra in Figure 1g.

Precursor and Product Ions		Precursor Ions	Adduct Ions	
Exact Mass	%1 RIA	%2 RIA	Exact Mass	
284.9618	6.717775	95.89635	284.9618	301.9868 Am
223.1567	0	51.93771	306.943 Na	306.943 Na
222.9685	3.941114	61.29501	568.9146 D	590.896 NaD
186.9921	0	25.59148		
160.9761	0	29.12837		
124.9993	0	0		
98.98725	0	0		
80.97341	0	0		

File 285ICP contains the precursor ion, and the seven most abundant lower-mass ions possibly produced from it. Exact masses are listed for each ion, but RIAs are only present when the ion abundance of the +1 or +2 ion exceeded the 5000 count or 10,000 count thresholds used to prepare the ASCII file. In Figure 1g, fewer than seven product ions are labeled with their masses because not all of the seven most abundant ions were correlated with the precursor ion (m/z 223.1567 was not).

These files are imported by a second procedure, which prepares sets of ions and error limits to be sent to a third procedure where ion compositions for each exact mass are found that are consistent with RIAs, if present, and where ion correlation is tested. In the second procedure, elemental limits based on the largest number of atoms of each element from one or more possible compositions for the precursor ion are determined. Tight exact mass and RIA error limits are used (2 mmu and 20%) and sent to the third procedure, which returns the possible compositions of the precursor ion. If no compositions are found, the error limits are increased to 4 mmu and 20%, then 4 mmu and 20% for the +1 RIA with no consideration of the +2 RIA, and finally to 4 mmu and 1E6% (i.e., both RIAs are ignored).

Having established elemental limits, each product ion is checked for correlation using error limits of 2 mmu and 20%. These narrow error limits minimize the number of possible compositions for each product ion. However, some product ions may have larger errors and will not be correlated. Hence, product ions are again correlated using error limits of 5 mmu and 1E6%. RIAs are more often distorted by interferences than are exact masses. When additional product ion correlations are found, their possible compositions are added to the list of correlated ions, but the additional compositions found for previously correlated ions are not.

Figure 2 illustrates that an additional ion (m/z 239) is correlated after the larger error limits are used for the analyte from Figure 1h. Notice that only one composition was possible for the m/z 214 ion when correlated with the precursor ion using the smaller error limits.

Table 4a. Correlations found for the three individual analytes.

Possible Precursor Ion Compositions	Possible Product Ion Compositions	Possible Neutral Loss Compositions
319.1042 C17H20N2S CL (8.5)	274.0468 C15H13N S CL (9.5)	45.0574 C2H7 N (0.0)
274.0468 C15H13N S CL (9.5)	246.0154 C13H9N S CL (9.5)	73.0888 C4H11 N (0.0)
246.0154 C13H9N S CL (9.5)	239.0779 C15H13N S (10.0)	80.0263 C2H7 N CL (-0.5)
239.0779 C15H13N S (10.0)	233.0077 C12H8N S CL (9.0)	86.0965 C5H12 N (0.5)
233.0077 C12H8N S CL (9.0)	214.0432 C13H9N CL (9.5)	105.061 C4H11 N S (0.0)
214.0432 C13H9N CL (9.5)	86.09792 C5H12 N (0.5)	233.0063 C12H8 N S CL (9.0)

Possible Precursor Ion Compositions	Possible Product Ion Compositions	Possible Neutral Loss Compositions
192.1389 C12H18 N O (4.5)	119.0505 C8H7 O (5.5)	170.0975 C12H12 N (7.5)
119.0505 C8H7 O (5.5)	91.0542 C7H7 (4.5)	169.089 C12H18 (8.0)
91.0542 C7H7 (4.5)	86.0979 C5H12 N (0.5)	153.07 C12H9 (8.5)
86.0979 C5H12 N (0.5)	80.0263 C2H7 N (0.0)	152.0623 C12H8 (9.0)
80.0263 C2H7 N (0.0)	106.0431 C7H9 O (5.0)	101.0859 C5H11 N O (1.0)
106.0431 C7H9 O (5.0)	134.0748 C9H10 O (5.0)	106.0431 C7H9 O (5.0)

Possible Precursor Ion Compositions	Possible Product Ion Compositions	Possible Neutral Loss Compositions
170.0975 C12H12 N (7.5)	169.089 C12H18 (8.0)	1.0085 H (0.5)
169.089 C12H18 (8.0)	153.07 C12H9 (8.5)	17.0275 N H3 (0.0)
153.07 C12H9 (8.5)	152.0623 C12H8 (9.0)	18.0352 N H4 (-0.5)
152.0623 C12H8 (9.0)	101.0859 C5H11 N O (1.0)	27.0142 C N H (2.0)
101.0859 C5H11 N O (1.0)	106.0431 C7H9 O (5.0)	79.04335 C5 H5 H (4.0)
106.0431 C7H9 O (5.0)	134.0748 C9H10 O (5.0)	112.0313 C9 H4 (8.0)

Possible Precursor Ion Compositions	Possible Product Ion Compositions	Possible Neutral Loss Compositions
170.0975 C12H12 N (7.5)	169.089 C12H18 (8.0)	1.0085 H (0.5)
169.089 C12H18 (8.0)	153.07 C12H9 (8.5)	17.0275 N H3 (0.0)
153.07 C12H9 (8.5)	152.0623 C12H8 (9.0)	18.0352 N H4 (-0.5)
152.0623 C12H8 (9.0)	101.0859 C5H11 N O (1.0)	27.0142 C N H (2.0)
101.0859 C5H11 N O (1.0)	106.0431 C7H9 O (5.0)	79.04335 C5 H5 H (4.0)
106.0431 C7H9 O (5.0)	134.0748 C9H10 O (5.0)	112.0313 C9 H4 (8.0)

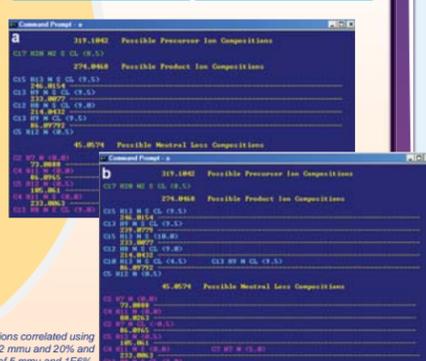


Figure 2. (a) ions correlated using error limits of 2 mmu and 20%, and (b) error limits of 5 mmu and 1E6%.

6. Ion Pair Correlations

Finally, possible neutral losses corresponding to mass differences of product ions adjacent in mass are determined when only one possible composition was found for one ion, but multiple compositions were found for the other. Figure 3 illustrates that all but one of the multiple compositions can often be eliminated when the lower-mass product ion is a compositional subset of the higher-mass product ion. A single composition was found for the m/z 223 ion and two for the m/z 187 ion. By treating the m/z 223 ion as a precursor ion for the m/z 187 product ion, the unique composition for the m/z 187 ion was found.

Similarly, the correct composition for the m/z 125 ion was found based on its mass difference with the m/z 161 ion. The single remaining composition for the m/z 125 ion became the precursor ion for the m/z 99 ion, and finally the single composition remaining for the m/z 99 ion became the precursor for the m/z 81 ion. For each of the four ions with two possible compositions, considering ion pairs eliminated the erroneous composition. After the unique product ion compositions were known, the correct compositions of the corresponding neutral losses were determined by subtracting the product ion compositions from the single m/z 285 precursor ion composition.

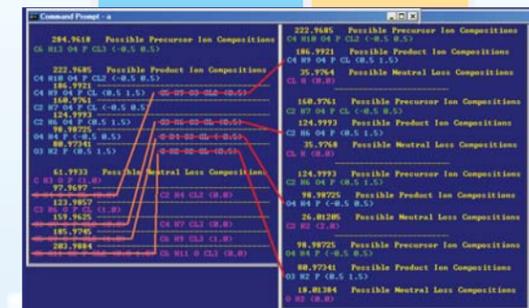


Figure 3. Two possible ion compositions were initially found for four ions and their corresponding neutral losses. The correct compositions were then found by treating the next highest-mass product ion with a single composition as the precursor ion. This was done sequentially from the highest to the lowest-mass pairs of ions. The unique compositions of the product ions subtracted from the m/z 285 ion composition provided the composite neutral losses.

7. Composite Mass Spectra

When multiple precursor ions are found for a composite mass spectrum, this entire process is automatically repeated for each precursor ion in order to deconvolute the individual mass spectra. While the precursor ion for larger-mass ions is usually determined, often the origin of small-mass ions is not. Tables 4a and 4b provide the ion correlations obtained for ion lists from three individual analytes and from a combined list of the ions. The m/z 91 and 58 ions were compositional subsets of both the m/z 192 and 170 precursor ions. A unique origin for these small-mass ions was not revealed from the simulated composite mass spectrum. The m/z 86 ion, a compositional subset of both the m/z 319 and 192 ions, was produced from the 319 ion, not from the m/z 192 ion.

A limitation of the ICP is that only up to seven product ions can be determined for a precursor ion due to memory limitations associated with QuickBASIC. Hence, its true origin was not revealed from the composite mass spectrum. The precursor ions for ions lower in mass than 100 Da are generally not discernable. However, the unique compositions obtained for the three precursor ions and one or more product ions from each still provide important information for determining the analytes' identities. Notice that use of two CID voltages revealed that the m/z 170 ion was a precursor ion even though it is a compositional subset of the m/z 319 ion.

8. ICP Details

- All possible compositions having at least -0.5 rings and double bonds (RDB) consistent with the elemental limits considered, exact masses and mass error limit, and RIA and RIA tolerance set by the user are calculated for the precursor and product ions and stored for further processing. The precursor ion compositions are calculated first to establish upper elemental limits for the subsequent calculation of the possible product ion compositions.
- All possible neutral loss compositions are calculated based on the mass differences between the precursor ion and all product ions. The formulas characterized by an RDB value of no less than -2.0 are saved and stored for further processing.
- Those precursor ion compositions are rejected which cannot be derived by the summation of the number of atoms of each element in a product ion-neutral loss pair. This formula discrimination criterion is applied for each product ion exact mass.
- Product ion compositions that do not provide a remaining possible precursor ion when summed with a corresponding neutral loss composition are rejected, as are neutral loss compositions that do not provide a remaining possible precursor ion when summed with a corresponding product ion composition.

Also in the automated ICP version:

- Compositions for the mass difference between adjacent product ions are calculated when there is one composition for one and multiple compositions for the other. Often, only one composition corresponds to the mass difference and all but one of the multiple compositions are discarded.

9. Conclusions

- The correct compositions of the precursor ion and unique compositions for the product ions were found for all eight analytes from their individual mass spectra by the automated Ion Correlation Program (ICP).
- Use of both low and high CID voltage mass spectra provides the precursor ions in composite mass spectra, even if a low-mass precursor ion is a compositional subset of a larger-mass precursor ion.
- The ICP provided three precursor ions and correlated at least one fragment ion with each from a simulated, three-component composite mass spectrum.
- Ions with masses less than 100 Da cannot be reliably correlated with precursor ions in composite mass spectra, since they are often compositional subsets of multiple precursor ions.

10. References

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