



MS: Elemental Composition Calculations and their Interpretation

Introduction

Accurate mass measurements are frequently used to determine the elemental composition for molecular and fragment ions. These measurements are normally obtained by acquiring a high resolution mass spectrum so that the masses are measured for a single species and not measured for unresolved or partially resolved peaks. A resolution of 10,000 (10% valley definition) is generally considered desirable for accurate mass measurements. The term "high resolution" is often used interchangeably with "accurate mass", but the terms really refer to two different things. Elemental composition calculations are used to calculate all possible elemental compositions that have calculated masses falling within a specified error tolerance of a measured mass. This calculation is usually (but not always) done for accurate masses obtained from a high-resolution mass spectrum. Accurate mass measurements are preferred over nominal mass measurements to limit the number of elemental compositions.

Limiting the number of compositions

The total number of possible elemental compositions increases with mass and can be very large even for modest masses. Therefore, the operator must define a set of constraints that place limits on the calculation. The constraints in the following list are described more fully later in this document:

- The measured mass or masses or a file name for a mass spectrum file.

- A set of allowed elements and their isotopes for the resulting compositions.
- An error tolerance for the calculated masses for the resulting compositions.
- Upper and lower bounds on the number of each element.
- Upper and lower bounds on the unsaturation for the resulting compositions.
- Choice of calculating compositions that correspond to odd-electron ions, even-electron ions, or both even and odd-electron ions.

Elemental data required by the calculations

The calculations use the following information for each element or isotope included in the elemental composition:

- Exact isotopic mass (required for the calculation).
- Valence of the element, e.g. 4 for carbon, 3 for nitrogen, 2 for sulfur (optional, used to calculate unsaturation).
- A minimum number of atoms of this element to be allowed in elemental composition (required, but a default value is suggested).
- A maximum number of atoms of this element to be allowed in elemental compositions (required, but a default value is suggested).
- Isotopic information about the atom (optional, used to calculate theoretical isotope ratios for a given composition).

The only required information is the exact isotopic mass.

Error limits

Error limits are used to limit the number of elemental compositions. The limits are defined in terms of millimass units (mmu),

unified atomic mass units (u), or parts-per-million (ppm). Parts-per-million error is defined as :

$$ppm = \frac{1.0 \times 10^{+6} (\textit{measured mass} - \textit{theoretical mass})}{\textit{theoretical mass}}$$

Clearly, errors defined in units of ppm are mass-dependent and get larger at low masses or smaller at high masses. To avoid the problems that the limit of the error tolerance approaches infinity as the mass approaches zero, or approaches zero as the mass becomes very large, it is necessary to place some additional limits on the errors at low and high mass. Therefore two extra parameters are needed when the error is defined in ppm:

- Low error bound (expressed in mmu): the error limit used for calculating elemental compositions for masses below a limiting mass calculated as described below.
- High error bound (expressed in mmu): the error limit used for calculating elemental compositions for masses above a limiting mass calculated as described below. The limiting mass for these bounds is given by:

$$\textit{Limiting mass} = \frac{1.0 \times 10^{+6} (\textit{mmu} * 1.0 \times 10^{-3})}{ppm}$$

where **mmu** refers to the low error bound or the high error bound, and **ppm** refers to the error limit expressed in parts-per-million. For example, if the error limit is defined as 10 ppm and the low error bound is 5 mmu and the high error bound is defined

as 20 mmu, then the calculation will use the following error limits:

- 5.0 mmu for masses less than 500 u
- 10.0 ppm error for masses between 500 and 2000 u
- 20.0 mmu for masses greater than 2000 u

If the error tolerance is expressed in u or mmu then that is the only parameter needed to define the error limits for the elemental composition calculation.

Unsaturation (double bond equivalents)

The total number of rings and sites of unsaturation can be calculated from any elemental composition by using the following formula:

$$D = 1 + \left[0.5 * \sum_i^{i_{\max}} N_i (V_i - 2) \right]$$

where **D** is the unsaturation, ***i*_{max}** is the total number of different elements in the composition, ***N*_{*i*}** the number of atoms of element ***i***, and ***V*_{*i*}** is the valence of atom ***i***. Each ring and each double bond counts as one site of unsaturation and each triple bond counts as two sites of unsaturation. Note that the calculation includes a division by two, so that the result can be either an exact integer, such as 4.0, or an integer with a remainder of 0.5. The remainder indicates whether a composition has an even-electron or odd-electron configuration (see the following discussion). An exact integer indicates an odd-electron ion, while a remainder of 0.5 indicates an even-electron ion. The minimum unsaturation value for an organic compound is -0.5 which corresponds to the unsaturation value for a protonated saturated compound (e.g. H₃O⁺).

Odd-electron and even-electron ions

A neutral, un-ionized compound will have an even number of electrons. Ions can have either an even or odd number of electrons. The electronic configuration can be calculated for any given elemental composition by calculating the unsaturation. This information can be used to identify molecular ions or fragment ions and to identify fragment ions that are likely to result from rearrangement reaction. Electron ionization (EI) produces a molecular ion that will have an odd number of electrons because one electron is lost during the ionization process. Most fragment ions in EI are formed by the loss of a radical, so most fragment ions have an even-electron configuration. Fragment ions that have an odd-electron configuration are usually the result of a rearrangement process, so it is useful to identify important odd-electron ions in a mass spectrum. Soft ionization methods such as FAB, CI, or electrospray ionization often produce species such as $[M+H]^+$ or $[M+Na]^+$ that have an even-electron configuration. The elemental composition calculation includes a parameter that specifies what kinds of elemental compositions are to be included in the report: EVEN-electron ions, ODD-electron ions, or BOTH. This can be used to limit the output. For example, an accurate mass measurement for an $[M+H]^+$ species produced by FAB should limit the possible elemental composition to EVEN-electron species. If you are looking for molecular ions in an accurate mass analysis of EI data, limit the output to ODD-electron ions.

The nitrogen rule

The nitrogen rule says that if an organic compound has an even molecular weight, then it must have an even number of nitrogens. If the compound has an odd molecular weight, then it must have an odd number of nitrogens. The nitrogen rule is taken into account by the unsaturation calculation. If you are looking for the elemental composition of a odd-mass molecular ion from an EI analysis and you limit the elemental compositions to those with an odd-electron configuration, then the only possible compositions will be those with an odd number of nitrogens. The

same will be true if you limit the compositions for a FAB analysis of a protonated molecular species to even-electron ions.

Isotope ratio measurements

Information about isotope ratios can also be used to limit the possible elemental compositions that correspond to a given measured mass. For example, a composition such as C_6H_5Cl cannot be a reasonable composition for an m/z 112 ion unless there are chlorine isotope peaks at m/z 112 and m/z 114 with a relative ratio of about 3:1. Of course, the presence of interference peaks may confuse the isotopic pattern. An interference at m/z 114 might cause the relative abundance of the m/z 114 peak to be larger than the expected 3:1 ratio, yet the m/z 112 peak could still correspond to C_6H_5Cl . Therefore caution and common sense should be used in making use of isotopic information to make elemental composition assignment. The elemental composition calculation is paired with theoretical isotope ratio calculations that allow the user to see what the expected isotopic pattern would be for any given elemental composition. This is very useful for the interpretation of the mass spectra of inorganic or organometallic compounds, because many of these compounds have distinctive isotope patterns that allow the chemist to determine how many metal atoms (for example) are present in a particular ionic species.

Examples

Methyl stearate

Suppose that you measure the mass for a molecular ion and find it to be 298.285189 u, and that you expect to find carbon, hydrogen, nitrogen, and oxygen to be possible elements for the composition. If the limits for the calculation are set as displayed below, only one composition is possible for that measured mass: $C_{19}H_{38}O_2$.

Element Limits: C 5/50 H 10/100 N 0/2 O 0/4
 Tolerance: 10.00 PPM
 Low error bound (mmu): 5.0 (for masses < 500)
 High error bound (mmu): 20.0 (for masses < 2000)
 Even or odd electron ion or both: BOTH
 Minimum unsaturation: -0.5
 Maximum unsaturation: 10.0

Meas. mass u	Abund.	Diff. ppm	Unsat.	Compositions
298.28519	0.00	-6.75	1.0	C19 H38 N0 O2

Chlorpyrifos

If there are too many possible heteroatoms, the number of possible elemental compositions may be very large and it may be difficult to determine which is correct without additional information. An example is the insecticide chlorpyrifos which has the composition C₉H₁₁Cl₃NO₃PS. If we assume a large range for the elemental limits, then the number of compositions is very large:

Element Limits:
 C 5/20 H 5/42 N 0/5 O 0/10 Cl 1/4 P 0/5 S 0/5
 Tolerance: 5.00 PPM
 Low error bound (mmu): 5.0 (for masses < 1000)
 High error bound (mmu): 20.0 (for masses < 4000)
 Even or odd electron ion or both: ODD
 Minimum unsaturation: -0.5
 Maximum unsaturation: 10.0
 Rel. abundance cutoff (percent): 0.000

Meas. mass u	Abund.	Diff. ppm	Unsat.	Compositions
348.924988	0.00	-10.58	5.0	C5 H5 N5 O7 Cl2 P0 S1
		-5.07	5.0	C5 H6 N3 O9
		13.03	5.0	C11 P2 S0 C5 H6 N5 O5

C12 P1 S1	-14.17	5.0	C5 H7 N5 O5
C12 P2 S0	9.36	5.0	C5 H8 N5 O3
C12 P3 S0	-12.59	4.0	C5 H9 N5 O3
C11 P1 S3	11.02	4.0	C5 H10 N5 O1
C11 P2 S3	-10.85	0.0	C5 H11 N1 O8
C13 P1 S0	7.26	0.0	C5 H11 N3 O4
C14 P0 S1	12.77	0.0	C5 H12 N1 O6
C13 P2 S0	3.67	0.0	C5 H13 N3 O2
C14 P2 S0	-5.60	5.0	C6 H6 N3 O8
C12 P1 S0	12.51	5.0	C6 H6 N5 O4
C13 P0 S1	-4.02	4.0	C6 H8 N3 O6
C11 P0 S3	8.92	5.0	C6 H8 N5 O2
C13 P2 S0	-13.12	4.0	C6 H9 N5 O2
C12 P0 S3	-7.70	4.0	C6 H10 N3 O4
C11 P2 S2	10.67	4.0	C6 H10 N5 O0
C12 P1 S3	-11.37	0.0	C6 H11 N1 O7
C14 P0 S0	12.24	0.0	C6 H12 N1 O5
C14 P1 S0	-11.28	4.0	C6 H12 N3 O2
C11 P4 S1	12.33	4.0	C6 H13 N3 O0

C11 P5 S1	-6.12	5.0	C7 H6 N3 O7
C13 P0 S0	8.40	5.0	C7 H8 N5 O1
C14 P1 S0	0.87	4.0	C7 H9 N1 O7
C11 P1 S2	-8.13	4.0	C7 H10 N3 O3
C12 P1 S2	-2.71	4.0	C7 H11 N1 O5
C11 P3 S1	-6.47	3.0	C7 H12 N3 O1
C11 P0 S5	-11.81	4.0	C7 H12 N3 O1
C12 P3 S1	-6.30	4.0	C7 H13 N1 O3
C11 P5 S0	-2.89	9.0	C8 H5 N5 O3
C11 P1 S2	-6.56	9.0	C8 H7 N5 O1
C11 P3 S1	0.35	4.0	C8 H9 N1 O6
C12 P0 S2	-8.66	4.0	C8 H10 N3 O2
C13 P0 S2	-3.24	4.0	C8 H11 N1 O4
C12 P2 S1	-12.33	4.0	C8 H12 N3 O0
C13 P2 S1	-1.57	3.0	C8 H13 N1 O2
C11 P1 S4	-6.91	4.0	C8 H13 N1 O2
C12 P4 S0	-5.25	3.0	C8 H15 N1 O0
C11 P3 S3	-3.41	9.0	C9 H5 N5 O2
C12 P0 S2	2.01	9.0	C9 H6 N3 O4

C11 P2 S1			
	-7.08	9.0	C9 H7 N5 O0
C12 P2 S1			
	-1.57	9.0	C9 H8 N3 O2
C11 P4 S0			
---CORRECT---->	-3.67	4.0	C9 H11 N1 O3
C13 P1 S1			
	-2.10	3.0	C9 H13 N1 O1
C12 P0 S4			
	-7.43	4.0	C9 H13 N1 O1
C13 P3 S0			
	10.67	9.0	C10 H5 N1 O7
C11 P1 S1			
	1.57	9.0	C10 H6 N3 O3
C12 P1 S1			
	6.91	9.0	C10 H7 N1 O5
C11 P3 S0			
	3.15	8.0	C10 H8 N3 O1
C11 P0 S4			
	-2.19	9.0	C10 H8 N3 O1
C12 P3 S0			
	-4.29	4.0	C10 H11 N1 O2
C14 P0 S1			
	-7.87	4.0	C10 H13 N1 O0
C14 P2 S0			
	10.06	9.0	C11 H5 N1 O6
C12 P0 S1			
	1.05	9.0	C11 H6 N3 O2
C13 P0 S1			
	6.47	9.0	C11 H7 N1 O4
C12 P2 S0			
	-2.62	9.0	C11 H8 N3 O0
C13 P2 S0			
	8.05	8.0	C11 H9 N1 O2
C11 P1 S3			
	4.46	8.0	C11 H11 N1 O0
C11 P3 S2			
	5.95	9.0	C12 H7 N1 O3

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C13 P1 S0
          7.70      8.0    C12 H9 N1 O1
C12 P0 S3
          5.42      9.0    C13 H7 N1 O2
    
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If we look at the measured isotope ratios, we can probably put some more reasonable limits on the elements to be included. Suppose that a low resolution mass spectrum shows the following (ignoring any interferences or overlapping losses that might be found in a real mass spectrum):

m/z	Rel. abundance %
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349	98.5
350	11.3
351	100
352	11.5
353	35.7
354	4.1
355	4.9
356	0.4
357	0.2

From this, we might conclude that there are between 8 and 11 carbons (because the ^{13}C peak should be 1.1% times the number of carbons) and three chlorines (because of the 349/351/353 isotope peaks). The maximum number of hydrogens is estimated as twice the number of carbons plus 2. These assumptions will drastically reduce the number of possible compositions:

Element Limits:

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C 8/11   H 5/24   N 0/5   O 0/10   Cl 3/3   P 0/5   S
0/5
    
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Tolerance: 5.00 PPM

Low error bound (mmu): 5.0 (for masses < 1000)

High error bound (mmu): 20.0 (for masses < 4000)

Even or odd electron ion or both: ODD

Minimum unsaturation: -0.5

Maximum unsaturation: 10.0

Rel. abundance cutoff (percent): 0.000

Meas. mass u	Abund.	Diff. ppm	Unsat.	Compositions
348.924988 C13 P0 S2	0.00	-8.66	4.0	C8 H10 N3 O2
C13 P2 S1		-12.33	4.0	C8 H12 N3 O0
C13 P1 S1 <--		-3.67	4.0	C9 H11 N1 O3
C13 P3 S0		-7.43	4.0	C9 H13 N1 O1
C13 P0 S1		1.05	9.0	C11 H6 N3 O2
C13 P2 S0		-2.62	9.0	C11 H8 N3 O0

There is still no easy way to tell the correct composition. If we have better mass accuracy, say, a 5 ppm error tolerance, then there are three compositions that are possible:

Meas. mass u	Abund.	Diff. ppm	Unsat.	Compositions
348.924988 C13 P1 S1 <--	0.00	-3.67	4.0	C9 H11 N1 O3
C13 P0 S1		1.05	9.0	C11 H6 N3 O2
C13 P2 S0		-2.62	9.0	C11 H8 N3 O0

The final decision about the correct composition will depend on whether we know something about the unsaturation (4 or 9 rings and/or sites of unsaturation) and whether we know anything about the number of nitrogens, sulfurs, oxygens, or phosphorus atoms.