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Mass Spectrometry

MS Interpretation

General Interpretation Strategies

i Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.

Aims and Objectives

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Aims

- Introduces students to the principles of mass spectral interpretation.
- Present fundamental concepts regarding mass spectral interpretation

Objectives

At the end of this Section you should be able to:

- List and explain the main characteristics of the mass spectrum
- Explain how mass spectrometric data can be used for structure analysis
- Explain why is important to optimise ESI/APCI parameters

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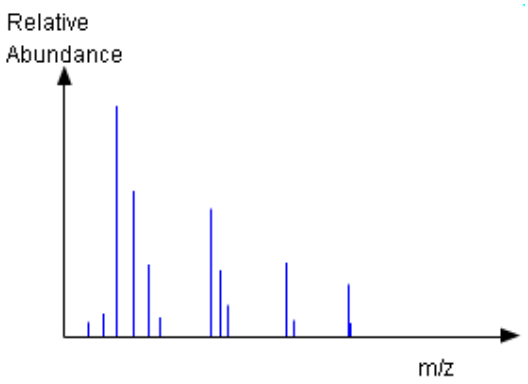
Introduction

Mass Spectrometry is a wide-ranging analytical technique. It relates to the production and subsequent separation and identification of charged species that are produced by a variety of ionisation methods.

The importance of learning how to identify mass spectra lies in the fact that we cannot create a standard library for the identification of any single chemical compound (the mass spectrum of a single compound could contain a huge amount of information and the number of chemical compounds is extremely huge). Nowadays standard libraries could include a few hundred thousands spectra.

A simple mass spectrum is represented by a two dimensional bar plot, the height of the bar (y component) is related to the intensity of the mass fragment ion and its location on the spectra (x component) is related to the charge to mass ratio.[1]



Mass Spectrum

Remember:

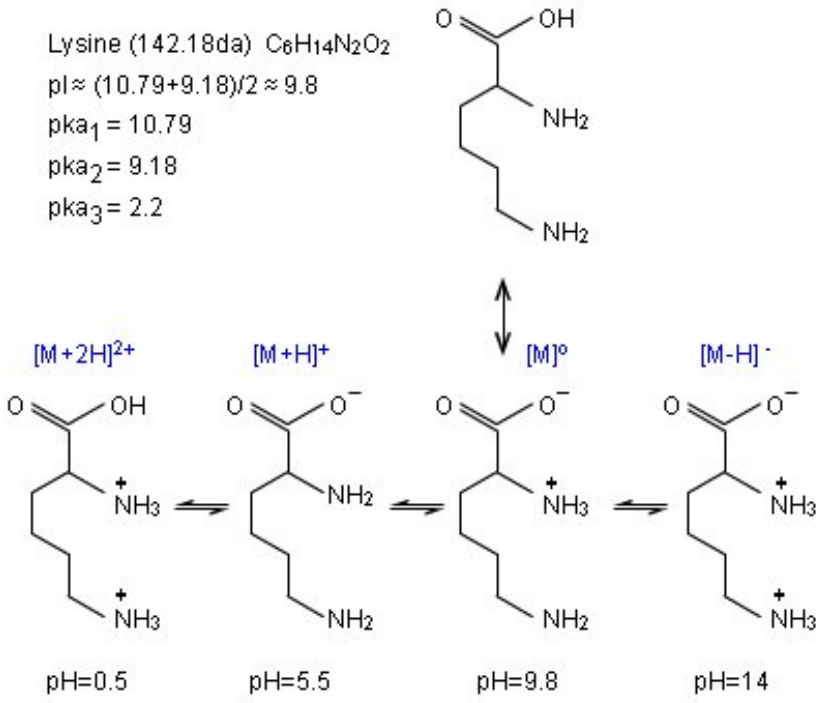
Ionisation is the process whereby electrons are either removed or added to atoms or molecules to produce ions. In LCMS, neutral molecules may be charged (producing cations in the positive ion mode or anions in the negative ion mode) by a number of different ways according to the selected ionisation interface.

Mass to Charge Ratio

The mass spectrum of an analyte species is represented by a bar graph that plots signal abundance of the ions against mass-to-charge (m/z). This represents the mass of a given particle (Da) to the number (z) of electrostatic charges (e) that the particle carries. The term m/z (measured in Da/e) is the parameter of the particle that is measured by the mass analyzer.

Prior to 1980 the mass-to-charge ratio was often incorrectly defined as m/e, where e is the charge on an electron (1.6×10^{-19} Coulomb). The mass of a given particle is equal to the sum of the atomic masses in daltons (Da) of all of the elements that compose it.[1,2,3]

Because many ions carry only one electrostatic charge the ratio m/z is frequently referred to as the particle 'mass'; however, this can be misleading when dealing with certain analyte species such as proteins which readily carry more than one fundamental charge.



Lysine acid-base equilibrium

Dalton:

One Dalton is the mass of a proton or neutron –an ethyl group has a mass of 27. The symbol for the mass unit is u, the use of u is no longer recommended by IUPAC (International Union of Pure and Applied Chemists) but Da.[2]

The symbol u corresponds to 1/12 the mass of ^{12}C, which is assigned the value 12.000000 under IUPAC convention. Naturally occurring carbon is 98.892% ^{12}C and 1.108% ^{13}C. The mass of ^{12}C is 12 Da, and that of ^{13}C is 13.00335 Da. Therefore, the average atomic mass of carbon is:

$(0.98892) \times (12\ Da) + (0.01108) \times (13.00335\ Da) \approx 12.01\ Da$

Monoisotopic, nominal and average mass calculations:

For the molecule of Butyric acid ($C_4H_8O_2$) calculate:

- Monoisotopic mass
- Nominal mass
- Average mass

Table 1. Properties of atoms in $CH_3CH_2CH_2COOH$.

Element	Molecular weight (Da)		
	Average	Lightest Isotope	Most abundant
H	1.00794	1.00783	1.00783
O	15.9994	15.9949	15.9949
C	12.0107	12	12

Monoisotopic mass: The mass of the ion or molecule calculated by summing the atomic masses of the lightest isotopes.

4×12+8×1.00783+2×15.9949 = 88.05244 Da

Nominal mass: (the mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value.

4×12 + 8×1 + 2×16 = 88 Da

Average mass: (the value calculated from the average masses of the elements, weighted for abundance)

4×12.0107+8×1.00794+2×15.9994 = 88.10512 Da

Mass Resolution

Mass resolution (R_m) is the ability of a mass analyser to separate two adjacent peaks. The resolving power of a mass analyser is described by the equation below:[5]

$R_m = m/\Delta m$

Or in terms of parts-per-million:

$ppm = 10^6 \times m/\Delta m = 10^6 \times R_m$

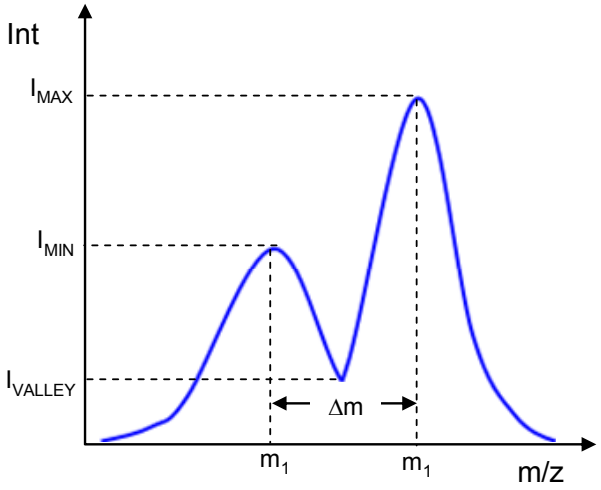
In practice the resolving power of a mass analyser is determined by one of the next two methods:[6]

- Doublet method
- Resolution of a single peak

Resolution is usually defined in one of two ways depending on the mass spectrometer being used.

The 10% valley (intensity) definition states that two peaks are considered to be resolved when they are separated by a valley, which is 10% of the height of each. This definition is used with magnetic sector instruments. The definition used with quadrupole, ion trap and time of flight mass spectrometers is based on a peak width measured at 50% peak height (full width half maximum or FWHM)

Doublet Method



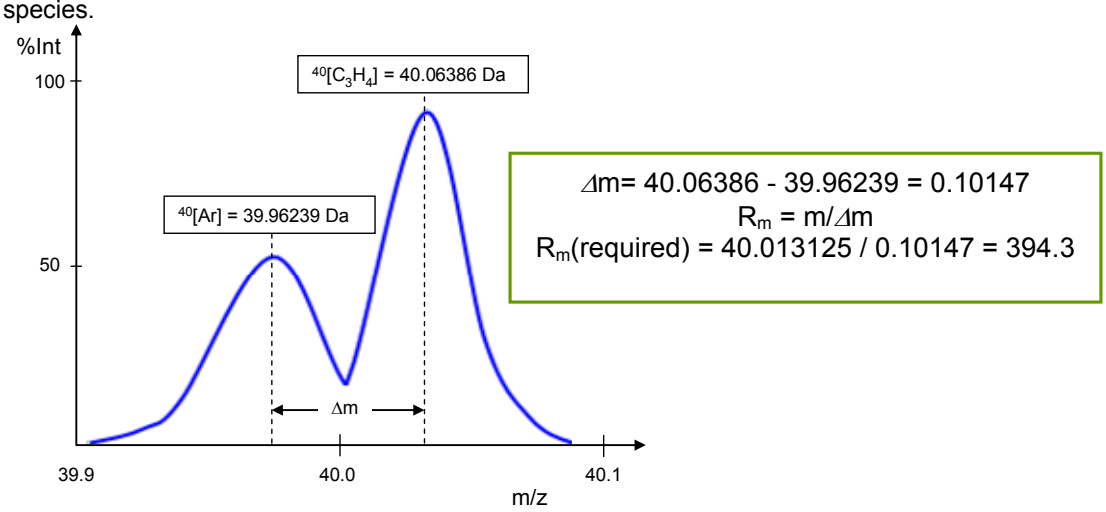
Resolution of two peaks

The doublet method states that the two presented peaks are resolved if:

1. I_{VALLEY} < 10% I_{MIN} (magnetic sector instruments).
2. I_{VALLEY} < 50% I_{MIN} (quadrupole, ion trap and time of flight instruments)

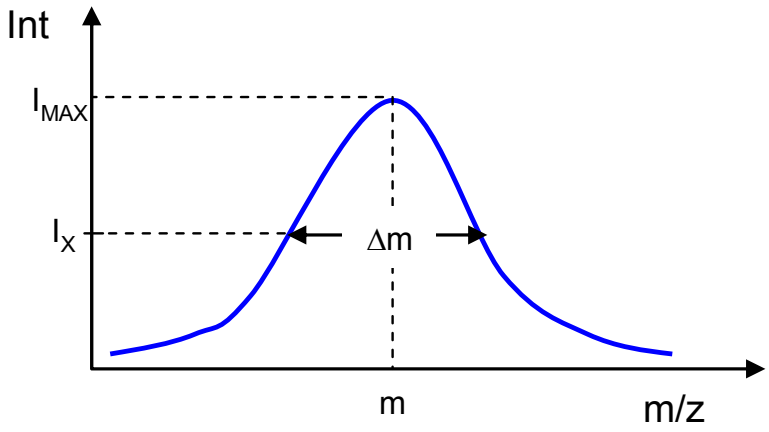
Here m is the measured or average mass and Δm is the mass difference between the two adjacent peaks. Note that resolution will have slightly different values depending on whether the FWHM, 10% or 50% valley definitions are used.

Two molecules of identical nominal (integral) mass and different elemental composition, such as Ar and C_3H_4 will differ significantly; e.g. 39.96239 and 40.06386. Any mass analyser which can operate with a resolving power of at least 394.3 will be able to distinguish between these two species.



Resolution of two molecules with the same nominal mass

Resolution of Single Peak

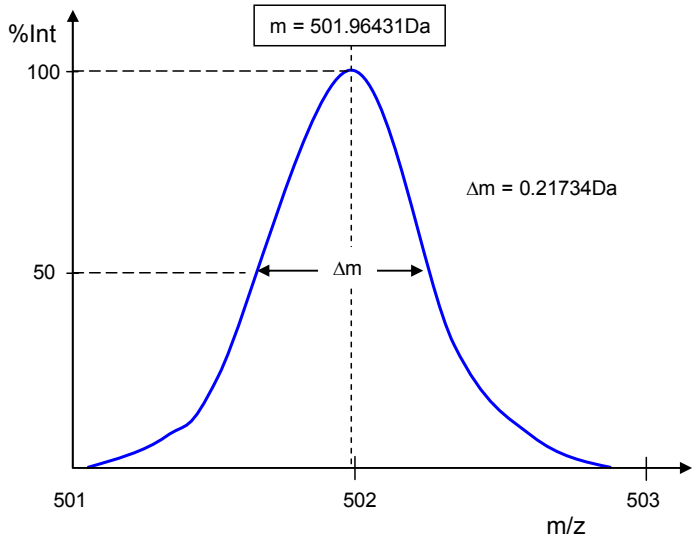


Resolution of single peak

If I_X = 50% I_{MAX}, then Δm is designated Full Width at Half Maximum (FWHM).

Usually Δm is taken to be at half of the peak height (50% of I_{MAX}) and Δm is designated Full Width at Half Maximum (FWHM). In some cases the peak width is measured at 5% of the peak height.[7]

Note that resolution will have different values depending on whether Δm is measured (5% or 50% of I_{MAX}). The resolving power required to resolve a peak can be easily calculated.



Resolution of a single peak

m = 501.96431Da
Δm = 0.21734Da
R_m = m/⊿m
R_m =501.96431/0.21734 = 2309.58089

High Mass Resolution

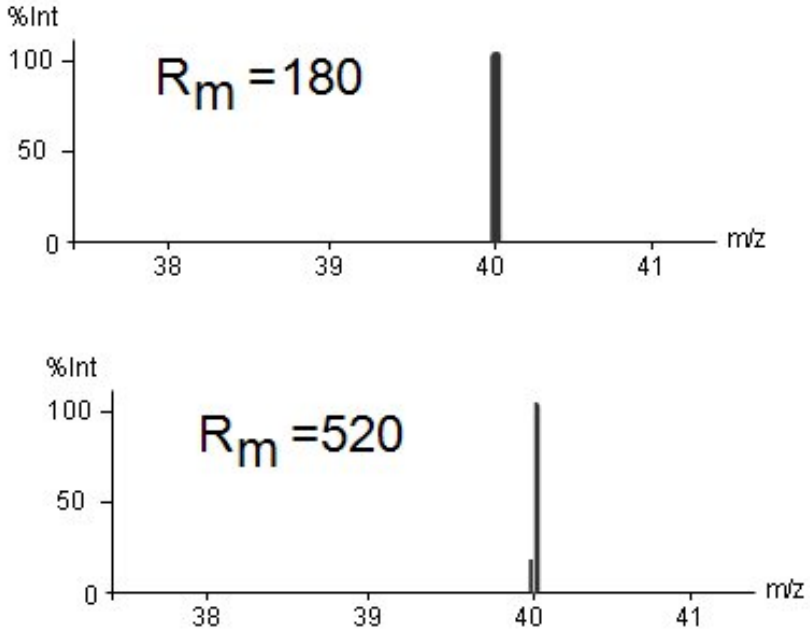
In order to elucidate or quantify analytes within organic mass spectrometry the mass analyser should be able to separate masses that differ by at least one mass unit. This is known as unit mass resolution.[7] Unit mass resolution is required when using spectral libraries for analyte identification.

High resolution instruments in combination with the ability to accurately measure mass are required to determine the elemental composition of analyte species.[8] High resolution mass analysers can be used for the removal of isobaric interferences (species with the same nominal mass), or increased selectivity in MS/MS experiments.

With ESI-MS and the trend towards analysis of heavier masses, higher resolution is required to isolate and identify analytes. High resolution mass analysers therefore have a greater role in high mass analysis applications where the resolving power is useful for the identification of the charge state of multiply-charged ions.

High resolution mass spectrometry has been used to determine atomic composition of ions. It is important to remember that different atoms present different molecular weights; therefore, different molecules presenting nominally the same mass but different atomic compositions should be differentiated in a mass spectrometer with sufficient resolving power.

In the previous section was stated that two molecules of identical nominal (integral) mass and different elemental composition, such as Ar and C_3H_4 will differ significantly; e.g. 39.96239 and 40.06386. Any mass analyser which can operate with a resolving power of at least 394.3 will be able to distinguish between these two species.

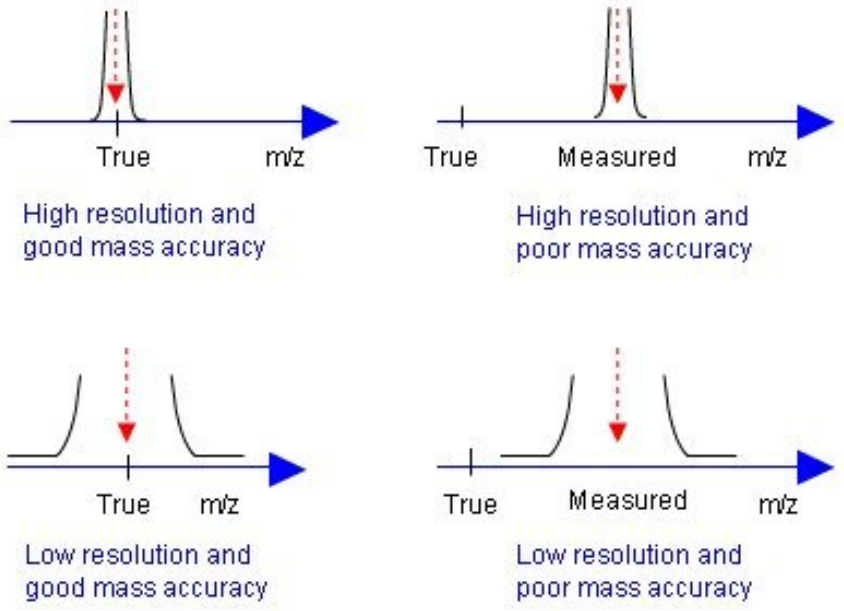


Low/high mass resolution

Mass Accuracy

Mass accuracy is the measurement of the closeness of the given measurement to the true mass of the analyte. In this instance Δ m accuracy denotes the difference between a measured value (mmeasured) and the true mass (mtrue) of a substance.[9]

Δ maccuracy = m_{true} — $m_{measured}$



In practice all mass analysers should be calibrated to the nearest mass unit and accurate assignment of mass is essential to virtually every aspect of mass analysis. Instruments should be evaluated to assess drift over extended operating periods with figures of around 0.1Da being reasonable.

Exact mass measurement is done with very high accuracy (ppm) and in practice this requires high resolution in conjunction with high mass accuracy in order to rule out the possibility of isobaric interferences.

High Mass Accuracy

Mass instability may arise from many sources including the stability of the instruments electronics, temperature fluctuations, tuning and contamination of the mass analyser etc. Mass accuracy must be maintained throughout the entire mass range and even small changes in mass accuracy can have an adverse effect on the interpretation of spectra, and quantitative results.

High mass accuracy is referred to as exact mass measurement, and when measuring the mass of a particular compound at the ppm level in mass accuracy, it is possible to unambiguously determine elemental composition. Knowledge of the elemental composition of a molecular or fragment ion is one of the most powerful analytical tools for identifying and elucidating the structure of unknowns. In practice, mass accuracy and high resolution are closely related.

To observe the isotopic profile of an ion, resolution must increase with m/z —a resolution of approximately twice the ion mass is required for isotopic resolution.

The table below presents typical resolution, mass range and accuracy for several types of mass analyser

Table 2 "resolution, mass range and accuracy for selected mass analyser types"

Instrument	Resolution	Mass Range	Mass Accuracy
Quadrupole	500-2000	2 – 2000Da	0.1Da
Ion Trap	500-2000	100-2000Da	0.1Da
Time of Flight	500-12,000	50 – 1x10^6Da	0.0001Da
Magnetic Sector	800-50,000	2 - 15,000Da	0.0001Da

Mass spectra of species with mass greater than 2000 Da can change as the isotopic mass distribution from the heavier isotopes shifts.[10] In general, high mass range is considered as any species with a mass of more than 2,000 Da.

Remember:

Accuracy: Degree of agreement between the best-estimated found value and its true value.

Precision: Is defined as the extent to which results correspond to each other.

Reproducibility: Refers to the agreement between analyses in different laboratories and include variations in equipments.

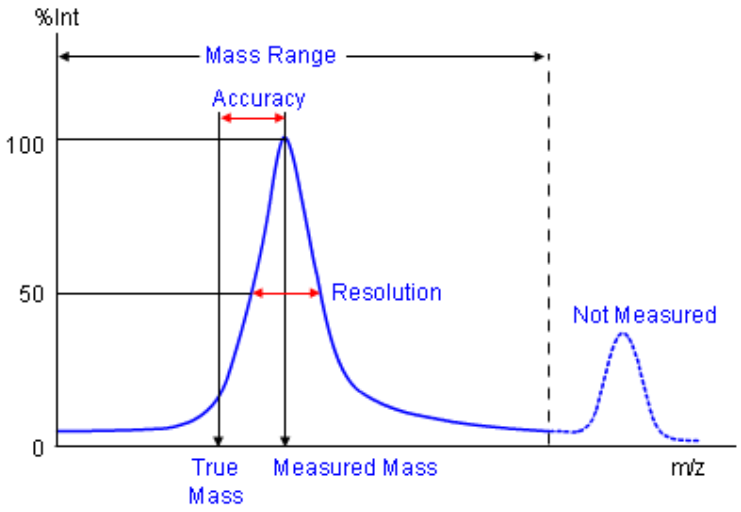
Mass Range

Mass analysers measure mass-to-charge ratio (m/z). The difference between the highest and lowest measurable m/z denotes the mass analyser range. The difference between mass resolution, mass accuracy and mass range is shown.

Electrospray ionisation allows more than one fundamental charge to reside on the gas phase molecule of interest (allowing the molecule to be measured at a lower m/z). An increase in z will extend the upper mass limit of the analyzer

Time-of-flight (TOF) mass analysers present no upper mass limit. Recent developments in rapid ion detection are promoting TOF to be the first choice for high mass analysis.

The mass range of any particular analyser is governed by the instrument configuration and its ability to generate gas phase ionic species.



Mass spectrum details

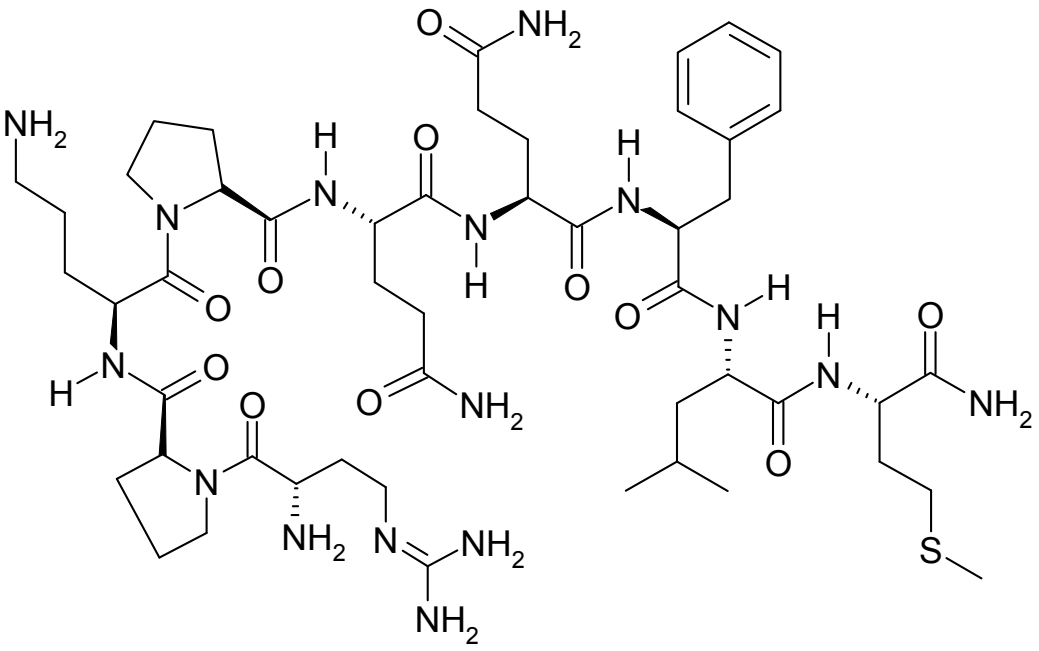
Multiply Charged Ions

It is possible that during the API process the analyte molecule will acquire two or more electrostatic charges, due to the molecular conformation, numbers of functional moieties capable of ionisation etc.

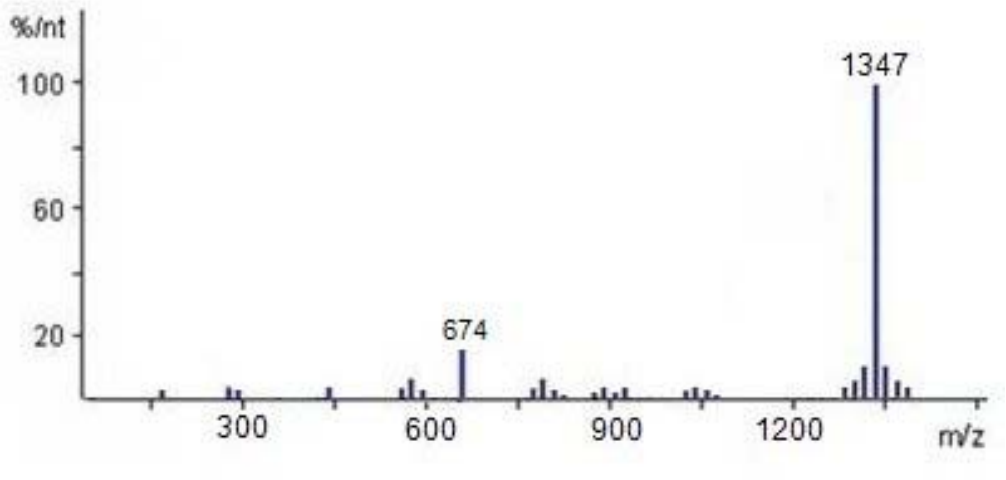
Doubly charged ions are produced when gaseous species acquire two electrostatic charges per molecule. Doubly charged ions present a peak in the mass spectrum at an m/z value exactly half of the mass of the molecular (singly charged) ion.

Some analyte species (high molecular weight proteins and peptides are good examples) may acquire many electrostatic charges during the API process. These species are known as 'multiply-charged' ions. Signals coming from multiply charged ions often need to be de-convoluted to establish the number of charges that the ion carries and to obtain information on its true molecular weight.[3]

Certain high molecular weights compounds presenting the ability of holding multiple charges can be analysed with traditional mass analysers. The m/z ratio (the property that is measured by the analyzer) decreases with the number of electrostatic charges held by the ion. Hence compounds that would otherwise be beyond the mass range of typical instruments can be analysed since the m/z ratio of the multiply-charged ion is within the mass range of the instrument.



Substance P ($C_{63}H_{98}N_{18}O_{13}S$, 1347.63 Da) is an 11-amino acid polypeptide with the sequence: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met.



ESI-MS mass spectrum of Substance P (oversimplified)

Nerve cells communicate with one another through neurotransmitters, Substance P is one such neurotransmitter. It is a protein found in the brain and spinal cord, and is associated with some inflammatory processes in the joints, its function is to cause pain.

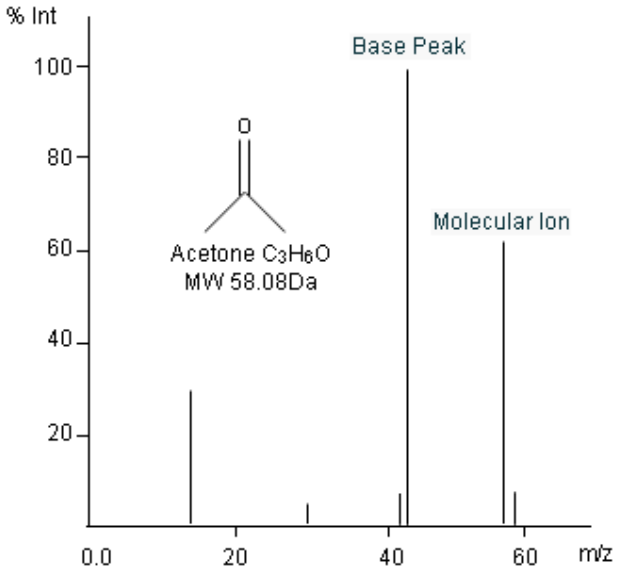
Spectral Features

Molecular ions represent intact species. They are precursors for all fragment ions that constitute the spectrum. The molecular-ion peak appears at an m/z value numerically equal to the nominal molecular weight of the compound (the weight calculated by summing the atomic masses of the most abundant isotope of each atom composing the molecule).

Very often in API-MS adducts ions are formed due to in-source gas phase reactions. Molecular species such as $[M+H]^+$ or $[M-H]^-$ appear at one mass unit above or below the nominal molecular mass of the analyte species.

The base peak refers to the most intense peak in the mass spectrum and is used as the reference peak when normalising all peaks within the spectrum.[4]

The relative intensity of a peak expresses its intensity relative to the base peak. The relative intensity of any peak is conventionally displayed as either Abundance or % on the y-axis of the mass spectrum.



Acetone mass spectrum (oversimplified)

Important:

Molecular Ion: The ionised form of the molecule

$$M^{\bullet}_{\bullet} \rightarrow M^{+\bullet} + e^{-}$$

The ion $M^{+\bullet}$ is known as the molecular ion.

An ion formed by the removal from (positive ions) or addition to (negative ions) a molecule of one electron without fragmentation of the molecular structure.

Base Peak: This peak presents the highest intensity in the spectrum

Isotopic abundances

Isotopes are atoms with the same atomic number but different atomic weight. The difference in weight between isotopes of the same element is caused by a difference in the number of neutrons. The relative abundance of an isotope in nature compared to other isotopes of the same element is relatively constant.[5, 6, 7, 8, 9, 10, 11]

The isotopic abundances of the elements can be classified into three general categories:

- "A": Elements with one natural isotope in appreciable abundance.
- "A+1": Elements with two natural isotopes, the second being one mass unit heavier than the most abundant isotope.
- "A+2": Elements with two natural isotopes, the second being two mass units heavier than the most abundant isotope.

The high mass region in some cases permits infer if "A+2" elements are present:[5]

Table 3. Relative abundances of selected isotopes

Atom	Mass	%R.A. "A"	%R.A. "A+1"	%R.A. "A+2"
Hydrogen	1.0078	100		
Oxygen	15.9949	100		
Fluorine	19.8884	100		
Phosphorous	30.9738	100		
Carbon	12.0000	100	1.1	
Nitrogen	14.0031	100	0.37	
Chlorine	34.9989	100		32.5
Bromine	78.9183	100		98
Iodine	126.9045	100		
Sulphur	31.9720	100	0.8	4.4

Where %R.A. is the percentage of relative abundance

Once the molecular ion is identified, then a normalization process must be done. Isotopic lines must be referred as a percentage of the molecular ion.

The halogens are particularly useful as their isotope patterns are very characteristic and easy to recognise.

- Compounds containing single chlorine exhibits a pair of lines separated 2 Da and in relative abundance of 3:1.
- Compounds containing single bromine exhibits a pair of lines separated 2 Da and in relative abundance of 1:1.
- Similar analysis can be done with elements as Si or S.
- If more than one "A+2" elements are present in a molecule, then the spectrum becomes complex.

The high mass region of the spectrum can be used to infer about the presence of certain isotopic elements.

1. Identify molecular ion and isotope cluster.
2. If A+2 abundance is lower than 98% of A abundance then NO bromines are present.
3. If A+2 abundance is lower than 33% of A abundance then NO chlorines are present.
4. If A+2 abundance is lower than 4.4% of A abundance then NO sulphur is present.
    - Sulphur's A+1 isotope will provide a 0.8% contribution to A+1 abundance.
5. If A+2 abundance is lower than 3.4% of A abundance then NO silicon is present.
    - Silicon's A+1 isotope will provide a 5.1% contribution to A+1 abundance.
    - This combination of A+1 and A+2 abundances make silicon easy to identify.
6. Multiple carbon atoms (and nitrogens) provide additional A+2 abundance that must be accounted for.

Table 4. A+2 Intensities

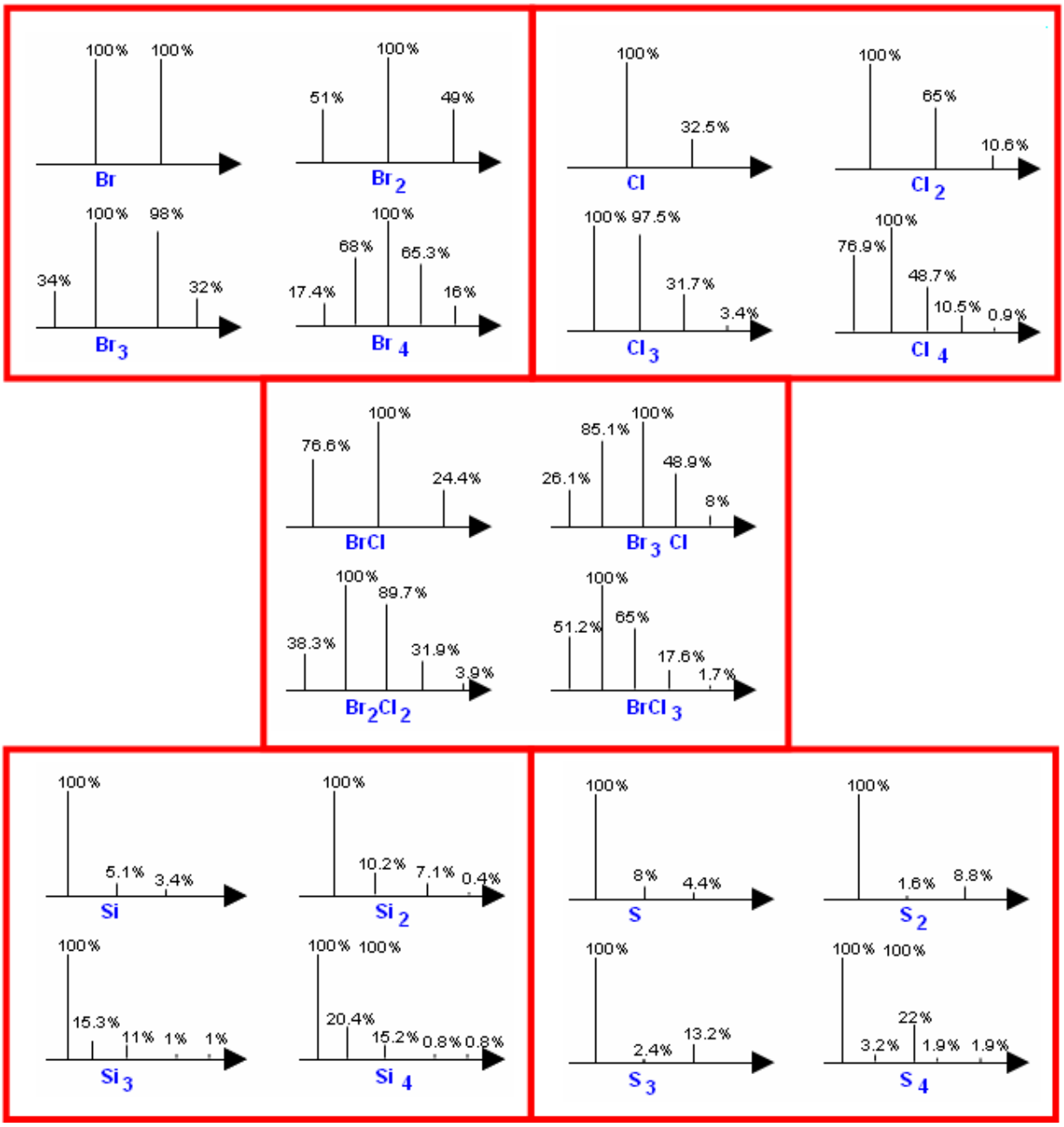
Intensity of "A+2" peak lower than	Sample
98% A abundance	Bromine is not present
33% A abundance	Chlorine is not present
4.4% A abundance	Sulphur is not present
3.4% A abundance	Silicon is not present

Important:

The high mass region of the spectrum in some cases permits infer if "A+2" elements are present:[5]

- Compounds containing single chlorine exhibits a pair of lines separated 2 Da and in relative abundance of 3:1.
- Compounds containing single bromine exhibits a pair of lines separated 2 Da and in relative abundance of 1:1.
- Similar analysis can be done with elements as Si or S.
- If more than one "A+2" elements are present in a molecule, then the spectrum becomes complex.

Characteristic high mass region spectrums for samples containing selected "A+2" elements are presented below.



High mass region of samples containing seleceted “A+2” elements

Table 5. Relative abundances of selected isotopes

Name	Symbol	Mass	*Abund.*	Symbol	Mass	Abund.	Symbol	Mass	Abund.	Symbol	Mass	Abund.
Aluminum.	Al(27)	26.98154	*100*									
Antimony.	Sb(121)	120.9038	*57.3*	Sb(123)	122.9042	*42.7*						
Argon.	Ar(36)	35.96755	0.34	Ar(38)	37.96273	0.063	Ar(40)	39.96238	99.6			
Arsenic.	As(75)	74.9216	100									
Barium.	Ba(130)	129.9063	0.11	Ba(132)	131.905	0.1	Ba(134)	133.9045	2.42			
	Ba(135)	134.9057	6.59	Ba(136)	135.9046	7.85	Ba(137)	136.9058	11.23	Ba(138)	137.9052	71.7
Beryllium	Be(9)	9.012183	100									
Bismuth	Bi(209)	208.9804	100									
Boron	B(10)	10.01294	19.8	B(11)	11.00931	80.2						
Bromine	Br(79)	78.91834	50.69	Br(81)	80.91629	49.31						
Cadmium	Cd(106)	105.9065	1.25	Cd(110)	109.903	12.49	Cd(111)	110.9042	12.8	Cd(112)	111.9028	24.13
	Cd(113)	112.9044	12.22	Cd(114)	113.9034	28.73	Cd(116)	115.9048	7.49			
Calcium	Ca(40)	39.96259	96.95	Ca(42)	41.95862	0.65	Ca(43)	42.95877	0.14	Ca(44)	43.95549	2.086
	Ca(46)	45.95369	0.004	Ca(48)	47.95253	0.19						
Carbon	C(12)	12	98.9	C(13)	13.00336	1.1						
Cerium	Ce(136)	135.9071	0.19	Ce(138)	137.906	0.25	Ce(140)	139.9054	88.48	Ce(142)	141.9092	11.08
Cesium	Cs(133)	132.9054	100									
Chlorine	Cl(35)	34.96885	75.77	Cl(37)	36.9659	24.23						
Chromium	Cr(50)	49.94605	4.35	Cr(52)	51.94051	83.79	Cr(53)	52.94065	9.5	Cr(54)	53.93888	2.36
Cobalt	Co(59)	58.9332	100									
Copper	Cu(63)	62.9296	69.17	Cu(65)	64.92779	30.83						
Dysprosium	Dy(156)	155.9243	0.06	Dy(158)	157.9244	0.1	Dy(160)	159.9252	2.34			
	Dy(161)	160.9269	18.9	Dy(162)	161.9268	25.5	Dy(163)	162.9287	24.9	Dy(164)	163.9292	28.2
Erbium	Er(162)	161.9288	0.14	Er(164)	163.9292	1.61	Er(166)	165.9303	33.6	Er(167)	166.9321	22.95
	Er(168)	167.9324	26.8	Er(170)	169.9355	14.9						
Europium	Eu(151)	150.9199	47.8	Eu(153)	152.9212	52.2						
Fluorine	F(19)	18.9984	100									

Table 5. Relative abundances of selected isotopes (cont.)

Name	Symbol	Mass	*Abund.*	Symbol	Mass	Abund.	Symbol	Mass	Abund.	Symbol	Mass	Abund.
Gallium	Ga(69)	68.92558	60.1	Ga(71)	70.9247	39.9						
Gadolinium	Gd(152)	151.9198	0.2	Gd(154)	153.9209	2.18	Gd(155)	154.8226	14.8	Gd(156)	155.9221	20.47
	Gd(157)	156.924	15.65	Gd(158)	157.9241	24.84	Gd(160)	159.9271	21.86			
Germanium	Ge(70)	69.92425	20.5	Ge(72)	71.92208	27.4	Ge(73)	72.92346	7.8	Ge(74)	73.92118	36.5
	Ge(76)	75.9214	7.8									
Gold	Au(197)	196.9666	100									
Hafnium	Hf(174)	173.9401	0.16	Hf(176)	175.9414	5.2	Hf(177)	176.9432	18.6	Hf(178)	177.9437	27.1
	Hf(179)	178.9458	13.74	Hf(180)	179.9466	35.2						
Helium	He(3)	3.016029	0.0001	He(4)	4.002603	100						
Holmium	Ho(165)	164.9303	100									
Hydrogen	H(1)	1.007825	99.99	H(2)	2.014102	0.015						
Indium	In(113)	112.9041	4.3	In(115)	114.9039	95.7						
Iodine	I(127)	126.9045	100									
Iridium	Ir(191)	190.9606	37.3	Ir(193)	192.9629	62.7						
Iron	Fe(54)	53.93961	5.8	Fe(56)	55.93494	91.72	Fe(57)	56.9354	2.2	Fe(58)	57.93328	0.28
Krypton	Kr(78)	77.9204	0.35	Kr(80)	79.91638	2.25	Kr(82)	81.91348	11.6	Kr(83)	82.91413	11.5
	Kr(84)	83.91151	57	Kr(86)	85.91061	17.3						
Lanthanum	La(138)	137.9071	0.09	La(139)	138.9064	99.91						
Lead	Pb(204)	203.973	1.4	Pb(206)	205.9745	24.1	Pb(207)	206.9759	22.1	Pb(208)	207.9766	52.4
Lithium	Li(6)	6.015123	7.42	Li(7)	7.016005	92.58						
Lutetium	Lu(175)	174.9408	97.4	Lu(176)	175.9427	2.6						
Magnesium	Mg(24)	23.98505	78.9	Mg(25)	24.98584	10	Mg(26)	25.9826	11.1			
Manganese	Mn(55)	54.93805	100									
Mercury	Hg(196)	195.9658	0.15	Hg(198)	197.9668	10.1	Hg(199)	198.9683	17	Hg(200)	199.9683	23.1
	Hg(201)	200.9703	13.2	Hg(202)	201.9706	29.65	Hg(204)	203.9735	6.8			
Molybdenum	Mo(92)	91.90681	14.84	Mo(94)	93.90509	9.25	Mo(95)	94.90584	15.92			
	Mo(96)	95.90468	16.68	Mo(97)	96.90602	9.55	Mo(98)	97.90541	24.13	Mo(100)	99.90747	9.63
Neodymium	Nd(142)	141.9077	27.13	Nd(143)	142.9098	12.18	Nd(144)	143.9101	23.8	Nd(145)	144.9126	8.3
	Nd(146)	145.9131	17.19	Nd(148)	147.9169	5.76	Nd(150)	149.9209	5.64			

Table 5. Relative abundances of selected isotopes (cont.)

Name	Symbol	Mass	*Abund.*	Symbol	Mass	Abund.	Symbol	Mass	Abund.	Symbol	Mass	Abund.
Neon	Ne(20)	19.99244	90.6	Ne(21)	20.99385	0.26	Ne(22)	21.99138	9.2			
Nickel	Ni(58)	57.93535	68.27	Ni(60)	59.93079	26.1	Ni(61)	60.93106	1.13	Ni(62)	61.92835	3.59
	Ni(64)	63.92797	0.91									
Niobium	Nb(93)	92.90638	100									
Nitrogen	N(14)	14.00307	99.63	N(15)	15.00011	0.37						
Osmium	Os(184)	183.9525	0.02	Os(186)	185.9539	1.58	Os(187)	186.9558	1.6	Os(188)	187.9559	13.3
	Os(189)	188.9582	16.1	Os(190)	189.9585	26.4	Os(192)	191.9615	41			
Oxygen	O(16)	15.99492	99.76	O(17)	16.99913	0.038	O(18)	17.99916	0.2			
Palladium	Pd(102)	101.9056	1.02	Pd(104)	103.904	11.14	Pd(105)	104.9051	22.33	Pd(106)	105.9035	27.33
	Pd(108)	107.9039	26.46	Pd(110)	109.9052	11.72						
Phosphorus	P(31)	30.97376	100									
Platinum	Pt(190)	189.9599	0.01	Pt(192)	191.961	0.79	Pt(194)	193.9627	32.9	Pt(195)	194.9648	33.8
	Pt(196)	195.9649	25.3	Pt(198)	197.9679	7.2						
Potassium	K(39)	38.96371	93.2	K(40)	39.964	0.012	K(41)	40.96183	6.73			
Praseodymium	Pr(141)	140.9077	100									
Rhenium	Re(185)	184.953	37.4	Re(187)	186.9558	62.6						
Rhodium	Rh(103)	102.9055	100									
Rubidium	Rb(85)	84.9118	72.17	Rb(87)	86.90918	27.84						
Ruthenium	Ru(96)	95.9076	5.52	Ru(98)	97.90529	1.88	Ru(99)	98.90594	12.7	Ru(100)	99.90422	12.6
	Ru(101)	100.9056	17	Ru(102)	101.9043	31.6	Ru(104)	103.9054	18.7			
Samarium	Sm(144)	143.912	3.1	Sm(147)	146.9149	15	Sm(148)	147.9148	11.3			
	Sm(149)	148.9172	13.8	Sm(150)	149.9173	7.4	Sm(152)	151.9197	26.7	Sm(154)	153.9222	22.7
Scandium	Sc(45)	44.95591	100									
Selenium	Se(74)	73.92248	0.9	Se(76)	75.91921	9	Se(77)	76.91991	7.6	Se(78)	77.9173	23.5
	Se(80)	79.91652	49.6	Se(82)	81.91671	9.4						
Silicon	Si(28)	27.97693	92.23	Si(29)	28.9765	4.67	Si(30)	29.97377	3.1			
Silver	Ag(107)	106.9051	51.84	Ag(109)	108.9048	48.16						
Sodium	Na(23)	22.98977	100									
Strontium	Sr(84)	83.91343	0.56	Sr(86)	85.90927	9.86	Sr(87)	86.9089	7	Sr(88)	87.90563	82.58

Table 5. Relative abundances of selected isotopes (cont.)

Name	Symbol	Mass	*Abund.*	Symbol	Mass	Abund.	Symbol	Mass	Abund.	Symbol	Mass	Abund.
Sulfur	S(32)	31.97207	95.02	S(33)	32.97146	0.75	S(34)	33.96787	4.21	S(36)	35.96708	0.02
Tantalum	Ta(180)	179.9475	0.012	Ta(181)	180.948	99.99						
Tellurium	Te(122)	121.9031	2.6	Te(123)	122.9043	0.91	Te(124)	123.9028	4.82	Te(125)	124.9044	7.14
	Te(126)	125.9033	18.95	Te(128)	127.9045	31.69	Te(130)	129.9062	33.8			
Terbium	Tb(159)	158.9254	100									
Thallium	Tl(203)	202.9723	29.52	Tl(205)	204.9744	70.48						
Thorium	Th(232)	232.0381	100									
Thulium	Tm(169)	168.9342	100									
Tin	Sn(112)	111.9048	0.97	Sn(114)	113.9028	0.65	Sn(115)	114.9033	0.36	Sn(116)	115.9017	14.7
	Sn(117)	116.903	7.7	Sn(118)	117.9016	24.3	Sn(119)	118.9033	8.6	Sn(120)	119.9022	32.4
	Sn(122)	121.9034	4.6	Sn(124)	123.9053	5.6						
Titanium	Ti(46)	45.95263	8	Ti(47)	46.95177	7.3	Ti(48)	47.94795	73.8	Ti(49)	48.94787	5.5
	Ti(50)	49.94479	5.4									
Tungsten	W(180)	179.9467	0.13	W(182)	181.9482	26.3	W(183)	182.9502	14.3	W(184)	183.951	30.67
	W(186)	185.9544	28.6									
Uranium	U(234)	234.0409	0.006	U(235)	235.0439	0.72	U(238)	238.0508	99.27			
Vanadium	V(50)	49.94716	0.25	V(51)	50.94396	99.75						
Xenon	Xe(124)	123.9059	0.1	X(126)	125.9043	0.09	Xe(128)	127.9035	1.91	Xe(129)	128.9048	26.4
	Xe(130)	129.9035	4.1	Xe(131)	130.9051	21.2	Xe(132)	131.9041	26.9	Xe(134)	133.9054	10.4
	Xe(136)	135.9072	8.9									
Ytterbium	Yb(168)	167.9339	0.13	Yb(170)	169.9348	3.05	Yb(171)	170.9363	14.3			
	Yb(172)	171.9364	21.9	Yb(173)	172.9382	16.12	Yb(174)	173.9389	31.8	Yb(176)	175.9426	12.7
Yttrium	Y(89)	88.90586	100									
Zinc	Zn(64)	63.92915	48.6	Zn(66)	65.92604	27.9	Zn(67)	66.92713	4.1	Zn(68)	67.92485	18.8
	Zn(70)	69.92533	0.6									
Zirconium	Zr(90)	89.90471	51.45	Zr(91)	90.90564	11.27	Zr(92)	91.90504	17.17	Zr(94)	93.90632	17.33
	Zr(96)	95.90827	2.78									

High Mass Region –Brominated Sample

In the present example we are going to explore the high mass region of a bromobenzene sample.

As was explained before, in some cases, the high mass region (of a mass spectrum) permits infer if "A+2" elements are present.

For practical purposes we are going to consider that our presented spectrum was obtained under the right conditions (all parameters optimised).

Remember: When analysing high mass region signals, you should:

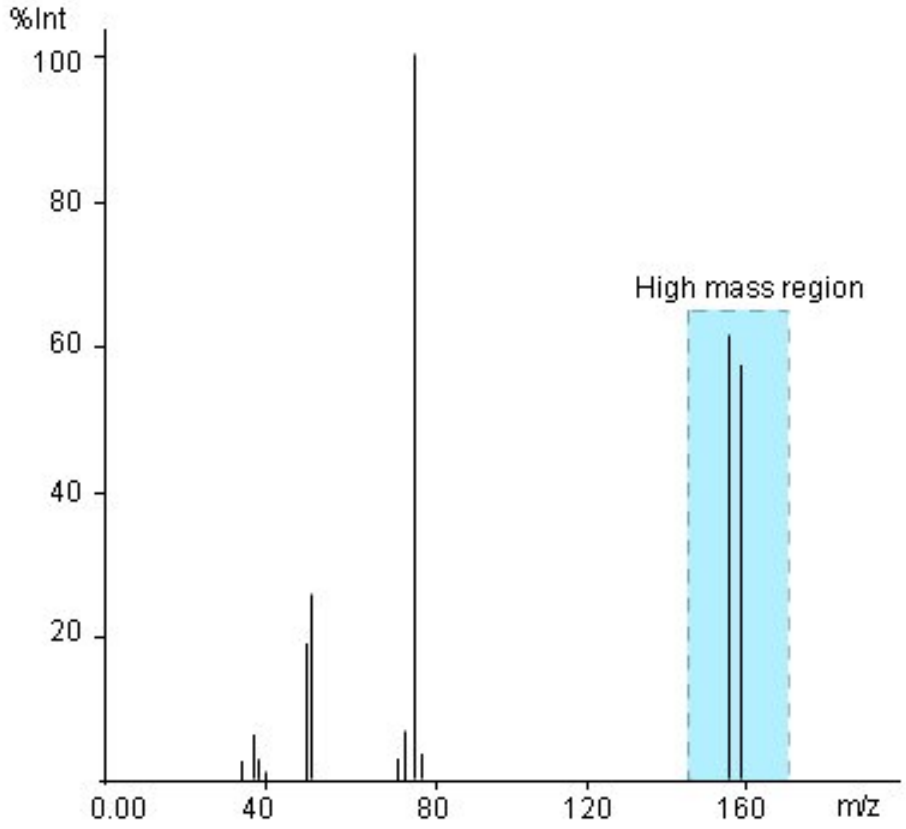
1. Identify the molecular Ion ("A" peak)

2. Calculate the Normalising Factor (NF), according to the formula:

$$NF = \frac{100}{Intensity\ Molecular\ Ion}$$

3. Normalise high mass region signals (multiply all intensities by NF)

4. Compare your spectrum (if possible) with generic high mass spectrums



Bromobenzene mass spectrum (oversimplified)

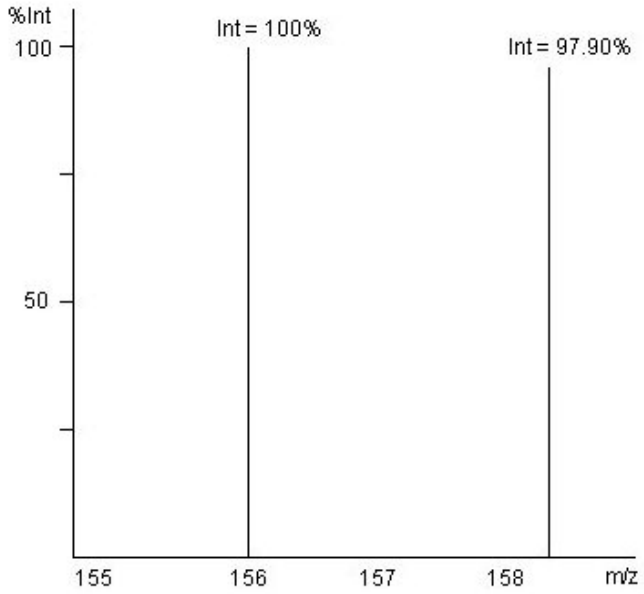
1. Molecular Ion ("A" peak):
   - Located at m/z = 156.
   - Intensity 61.9

2. NF = 100/(Intensity Molecular Ion) = 100/61.9

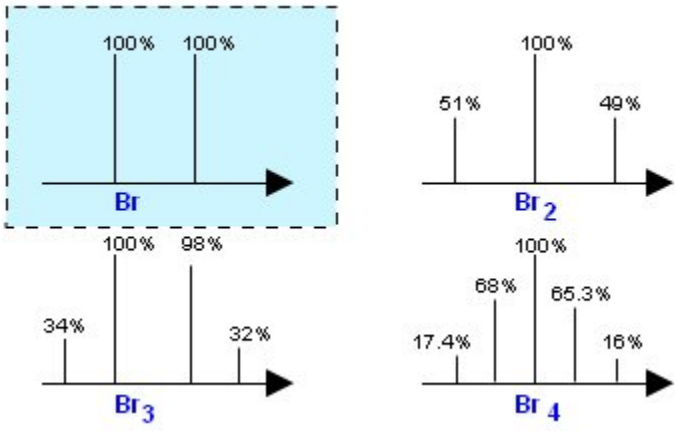
3. Normalise signals (multiply intensities by NF)

Table 6. Normalisation process

m/z signal	Intensity	Normalised Intensity
156	61.9	61.9 × 100 / 61.9 = 100
158	60.9	60.9 × 100 / 61.9 = 97.9



4. Compare with generic spectrums



Note that the general appearance of the high mass region closely matches the generic spectrum for compounds possessing one bromine atom per structure.

The Nitrogen Rule

The "Nitrogen Rule" states that:[13]

"A compound with an odd molecular weight will have an odd number of nitrogen's. Compounds with an even molecular weight will have either no nitrogen or an even number of nitrogen atoms".

Therefore if you have identified the molecular ion and it is odd you can assume an odd number of nitrogen atoms are present.

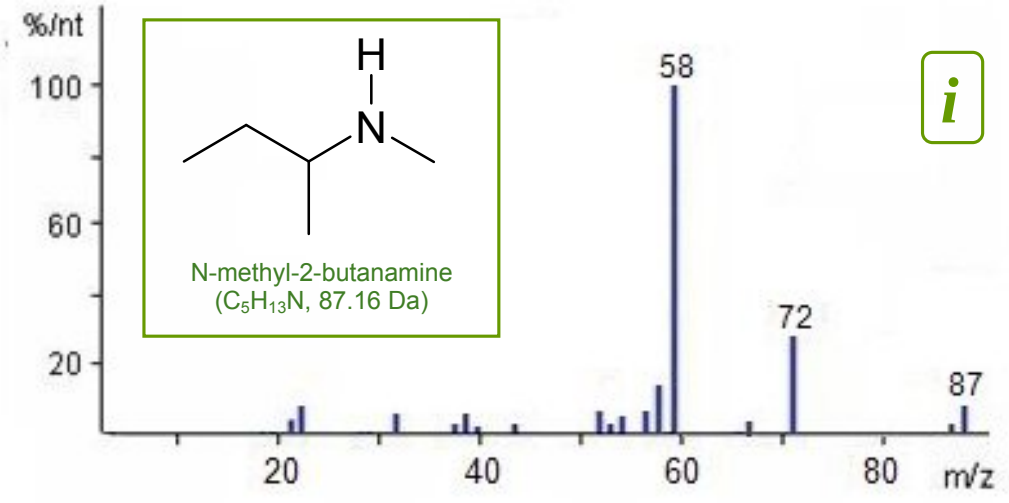
Molecules with an ODD number of nitrogen atoms have ODD masses. Ions which result from simple fragmentation have EVEN masses.

Molecules with an EVEN number of nitrogen atoms (cero is an even number) have EVEN molecular weights. Ions which result from simple fragmentation have ODD masses.

Important:

Molecules with an ODD number of nitrogen atoms have ODD masses. Ions which result from simple fragmentation have EVEN masses.

Molecules with an EVEN number of nitrogen atoms (cero is an even number) have EVEN molecular weights. Ions which result from simple fragmentation have ODD masses.



N-methyl-2-butanamine ($C_5H_{13}N$, 87.16 Da) mass spectrum (oversimplified)

Interpretation Strategy

1. Ionisation type. Consider what type of ion source was used to produce the MS spectrum (soft like ESI or harsh like electron impact).

2. General inspection of the spectrum. Does it have many lines (easily broken bonds) or does it have relatively few lines (stable ions). Information that needs to be considered from the mass spectrum:

- Mass of fragments
- Presence of isotopes lines
- Gaps between lines

3. Find the molecular ion if possible. Molecular ions are not always present in the spectrum. In some cases higher mass adduct ions can be formed making difficult the molecular ion identification. The presence of the molecular ion can be confirmed by the use of "logical and illogical losses"

4. Identify the highest m/z fragment. Is this fragment the molecular ion? If this fragment has an odd m/z value and effectively is the molecular ion, then the presence of an odd number of nitrogen atoms in the molecule can be expected (the nitrogen rule).[13]

5. Try to find "A+2" elements. By inspection (high mass region of the spectrum), try to find the number and the kind(s) of "A+2" elements.

6. Propose possible structures. Use all of the information available from the spectrum plus other available techniques.

Logical Losses

Logical losses are used to confirm the molecular ion. The use of the logical losses should only be considered as losses from the molecular ion.

Table 7. Some important logical looses

From Bond Cleavage		From rearrangements	
Fragment	Mass	Neutral Molecule	Mass
H	1	H_2	2
CH_3	15	NH_3	17
NH_2 or O (from NO)	16	H_2O	18
OH	17	C_2H_2	26
F	19	HCN	27
CN	26	CO or C_2H_4	28
C_2H_3	27	CH_2O	30
C_2H_5 or CHO	29	CH_3OH	32
NO	30	H_2S	34
OCH_3 or CH_2OH	31	C_3H_6 or C_2H_2O	42
SH or H_2O+CH_3	33	CO_2	44
Cl	35	C_2H_5OH	46
C_3H_7 or CH_3CO	43	C_3H_7OH or CH_3CO_2H	60
NO_2	46	C_6H_6	78
C_4H_9	57	HBr	80

1. Losses of neutral molecules can occur from both radicals and cations.
2. Loss of a neutral molecule results in a fragment which is an odd electron ion.
3. The loss of a neutral molecule can only occur through "concerted" intramolecular atom transfer and elimination. This is a less likely process than simple bond fragmentation.
4. The loss of a neutral molecule from an even mass molecular ion usually results in an even fragment in the mass spectrum.

Illogical losses

Illogical losses can be used to confirm the molecular ion.

In compounds containing C, H, N, O it is virtually impossible to lose 5-13 Daltons

Similarly losses of 21-25 Daltons are also virtually impossible

Table 8. Some illogical looses

Fragment	Mass	Neutral Molecule	Mass
H_3	3	B	11
He	4	BH or C	12
HeH	5	CH	13
½ C	6	N or CH_2	14
Li	7	NeH	21
LiH or He_2	8	B_2	22
Be	9	BC	23
BeH	10	C_2	24

There are no possible losses from the molecular ion that would correspond to the masses presented in the previous table.

These "rules" apply to losses from either the molecular ion or fragment ions, but only losses from the molecular ion are easy to identify.

If you see ions in these regions from your proposed molecular ion then it isn't the molecular ion!!

Fragmentation in API

ESI, APCI and APPI are soft ionisation techniques, i.e. the registered spectra tend to show very little fragmentation. That is during the formation of ions, the analyte molecules do not receive enough energy to break the intra-molecular bonds. However, under certain conditions these techniques can produce considerable fragmentation (especially with labile molecules or when API parameters are not optimised).

The major problem with these ionisation techniques is that fragmentation and ion clustering can occur if the parameters are not optimised.

An abundant molecular ion with low intensity fragment ions indicates a stable molecular structure e.g. aromatic, cyclic, and conjugated.

A low intensity molecular ion with many intense fragment ions indicates a weak molecular structure e.g. aliphatic, acyclic, saturated, etc.

The next table presents some typical fragments produced from common organic compounds.

Table 9. Typical fragments on selected organic compounds

Compound type	Formula	Typical Fragments
Alkanes	$C_nH^+_{2n+1}$	15, 29, 43, 57, 71, 85…
Alkenes, Cycloalkanes	$C_nH^+_{2n}$	28, 42, 56, 70, 84…
Cycloalkenes	$C_nH^+_{2n-1}$	27, 41, 55, 69, 83…
Aldehydes, Ketones	$C_nH_{2n-1}O^+$	29, 41, 55, 69, 83…
Amines	$C_nH_{2n+2}N^+$	30, 44, 58, 72, 86…
Alcohols, Ethers	$C_nH_{2n+1}O^+$	31, 45, 59, 73, 87…
Acids, Esters	$C_nH_{2n-1}O^+$	45, 59, 73, 87…
Aromatic compounds		91, 77

Rings and Insaturations

The number of rings and insaturations (very useful for structural confirmation) can be calculated according to the expression:

Rings + Insaturations = X – ½ (Y) + ½ (Z) + 1

X = Carbon and Silicon
Y = Hydrogen, Chlorine, Fluorine and Iodine
Z = Nitrogen and Phosphorous

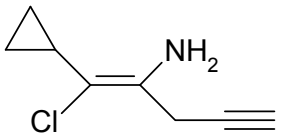
Table 10. Rings + Insaturations values

If the structure presents	Rings + Insaturations
One ring	1
One double bond	1
One triple bond	2

When applied to suspected molecular ion, It provides two pieces of information:

- If the answer is an integer then the proposed formula is an odd electron molecule and maybe the molecular ion
- If the answer is a non-integer then it is NOT the molecular ion

Example $C_8H_{10}NCl$

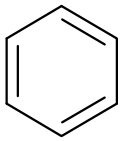


Rings + Insaturations = X – ½ (Y) + ½ (Z) + 1
Rings + Insaturations = 8 – ½ (10+1) + ½ (1) + 1 = 4

Table 11. Rings + Unsaturations of $C_8H_{10}NCl$

Elements	Incidence	Rings + Unsaturations
Rings	1	1×1 = 1
Double bonds	1	1×1 = 1
Triple bonds	1	1×2 = 2
Total		4

Example C_6H_6



Benzene (C_6H_6, 78 Da)

Rings + Unsaturations = X – ½ (Y) + ½ (Z) + 1
Rings + Unsaturations = 6 – ½ (6) + ½ (0) + 1 = 4

Table 12. Rings + Unsaturations C_6H_6

Elements	Incidence	Rings + Unsaturations
Rings	1	1×1 = 1
Double bonds	3	3×1 = 3
Triple bonds	0	0×2 = 0
Total		4

Number of Carbons

Having identified the molecular ion we can use the isotope pattern to estimate the number of carbon atoms in the molecule by performing a normalisation of the high mass signals with respect to the molecular ion.

In the first approximation (and if no other information is available), the number of carbons in a molecule can be estimated with the intensity of the ^{13}C isotope (A+1) signal (considering no other contributions).

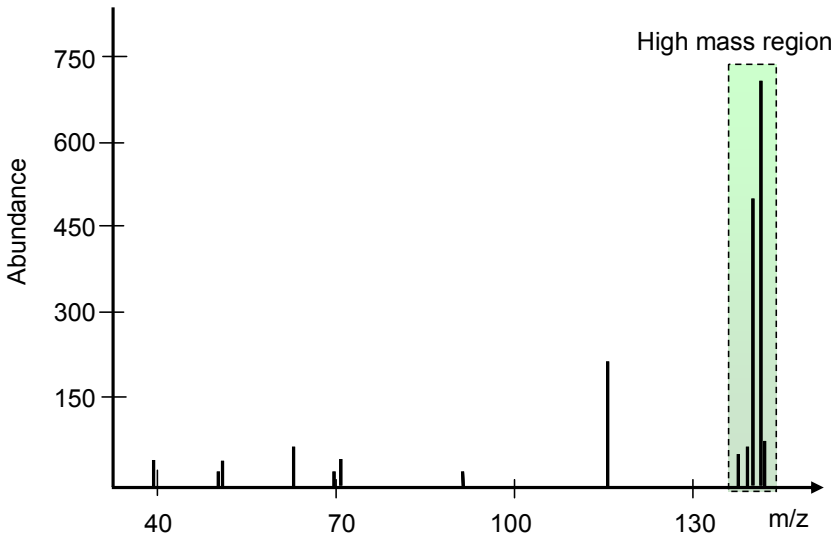
If the presence of nitrogen is suspected, then 0.37 should be subtracted from the A+1 signal. Fortunately the ^{15}N contribution to the "A+1" signal is usually small (0.37%) so unless there are a large number of nitrogens present then it will not seriously affect the calculation of the number of carbons.

Halogens and their distinctive patterns were already covered; now, we are going to consider mainly sulphur and silicon:

- Sulphur has isotopes at 32, 34 (4.4%) and a small 33 (0.8%)
- Silicon has isotopes at 28, 29 (5.1%) and 30 (3.4%)

The presence of these elements WILL affect the normalisation calculation and we need to predetermine the presence of these elements. This can be done by looking at the "A+2" mass intensity and at the general spectrum.

A sample containing only carbon and hydrogen was analysed under ESI/MS^2 conditions (all parameters were optimised), giving the next spectrum. The molecular ion was identified to be located at m/z = 142. With the information provided, estimate the number of carbons present in the molecule.



Hydrocarbon MS/MS mass spectrum

In order to estimate the number of carbons in the molecule, we have to centre our attention into the high mass region of the spectrum.

Molecular Ion ("A" peak):

- Located at m/z = 142.
- Intensity 740

NF = 100/(Intensity Molecular Ion) = 100/740

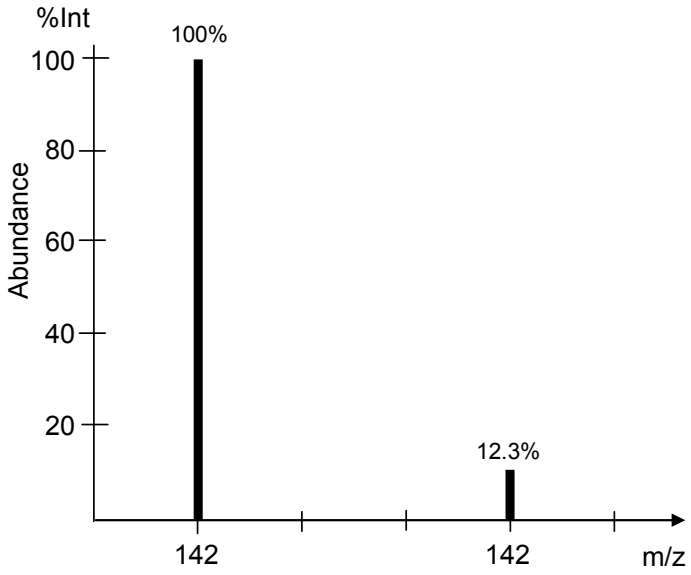
Table 13. Normalise signals (multiply intensities by NF)

m/z signal	Intensity	Normalised Intensity
139	74	74 × 100/740 = 10
140	74	74 × 100/740 = 10
141	518.7	581.7 × 100/740 = 70.1
142	740	740 × 100/740 = 100
143	91	91 × 100/740 = 12.3

The total number of carbon atoms in the molecule is given by the normalised abundance of the "A+1" signal divided by the isotopic abundance of ^{13}C that is 1.1:

Carbon atoms = 12.3/1.1 = 11.2 (±10% for statistical errors)

Carbon atoms = 11.2 ± 1.12 = 10.1 to 12.3 = 10 to 12



Hydrocarbon MS/MS High mass region

Table 14. Possible structures

N° Carbon atoms	N° Hydrogen atoms	Possibility
10	142 - 12×10 = 22	Yes
11	142 – 11×12 = 10	Yes
12	142 – 12×12 = -2	No

- Alkane $C_{10}H_{22}$ (zero "Rings + Unsaturations")
- Aromatic $C_{11}H_{10}$ (seven "Ring + Unsaturations").

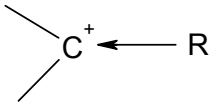
At this point, it is not possible to confirm which structure is the one responsible for the spectrum; however, alkanes produce a lot of fragmentation, the aromatic option is the most reasonable answer.

Cleavages –Ion Abundance

Ion abundance is a function of its stability. This is related to how easy it is for a charge to be delocalised through the ion.

The general rule of thumb is that if a charge is very localised (all concentrated on one atom) the ion is much less stable than if the charge is spread out over several atoms.

The cation stability is affected by the presence of hetero-atoms or functional groups. Alkyl groups present electron donating (stabilizing) effect.



However certain groups withdraw electrons and destabilize the cation.

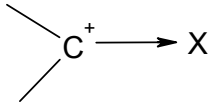
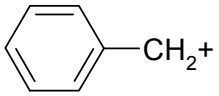
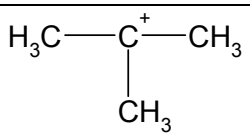
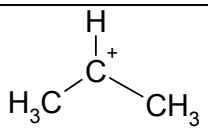


Table 14. Inductive effect of selected groups

Electron Donating	Electron Withdrawing
CH_3-	-NO_2
CH_3-CH_2-	-CN
CH_3-CH-CH_3	-COOH

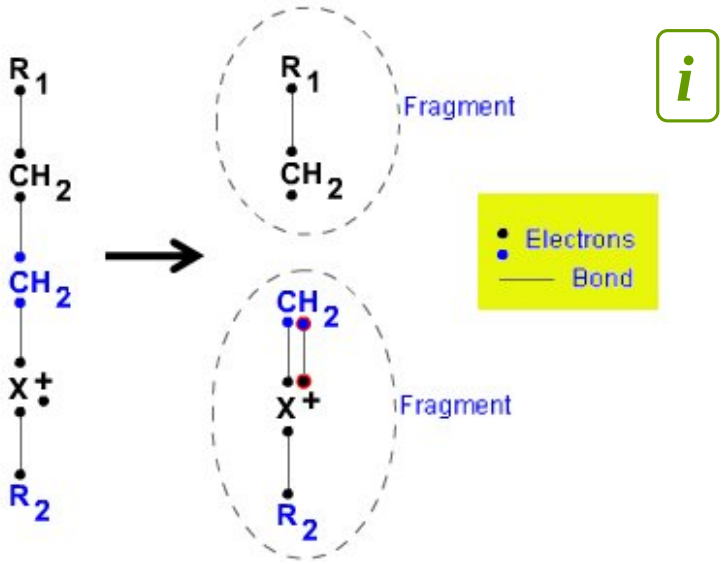
Important: Ion fragments can experience further fragmentations!!!

Table 15. Ion stability

Ion	Name	Increasing Stability
	Benzyl	
CH_2=CH-CH_2+	Allyl	
	Tertiary	
	Secondary	
CH_3CH_2+	Primary	
CH_3+	Methyl	

Cleavages –Simple Mechanisms

An alpha cleavage is a mechanism which involves 2 one electron transfer processes. This results in a bond cleavage one bond away from the ionisation site (α position) whilst the charge remains at the site of ionisation.



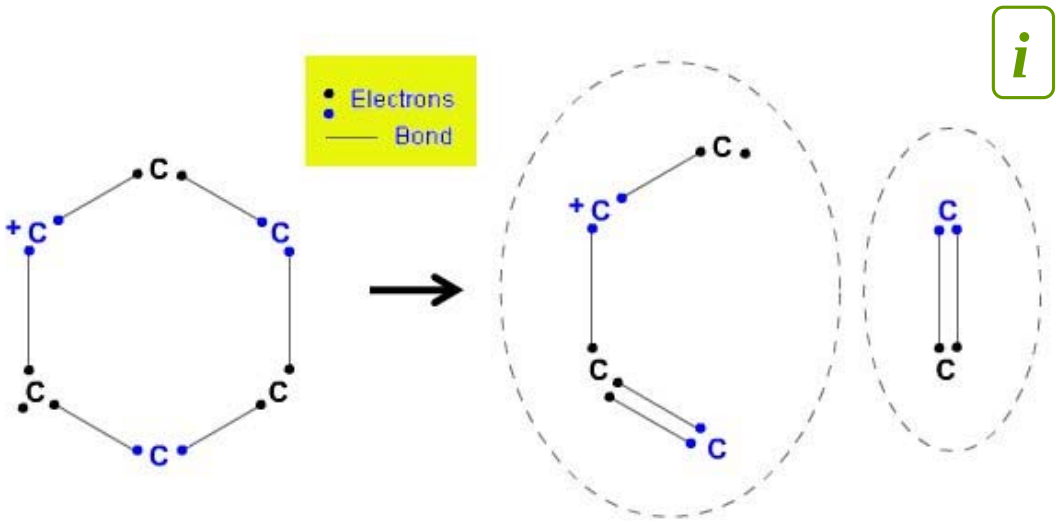
The process is radical initiated and can occur as a sequential fragmentation with cyclic compounds. Typical ionisation sites which can undergo this type of fragmentation include:

- Nitrogen
- Phosphorous
- Oxygen
- Sulphur
- C=C bonds

When alkenes fragment, they tend to give series of ions corresponding to allylic fragmentations (ß- to the double bond).

It is well known that during the fragmentation of cyclo-alkanes olefins are eliminated.

Cyclo-alkenes presenting a six member ring do show a characteristic retro Diels-Alder fragmentation.

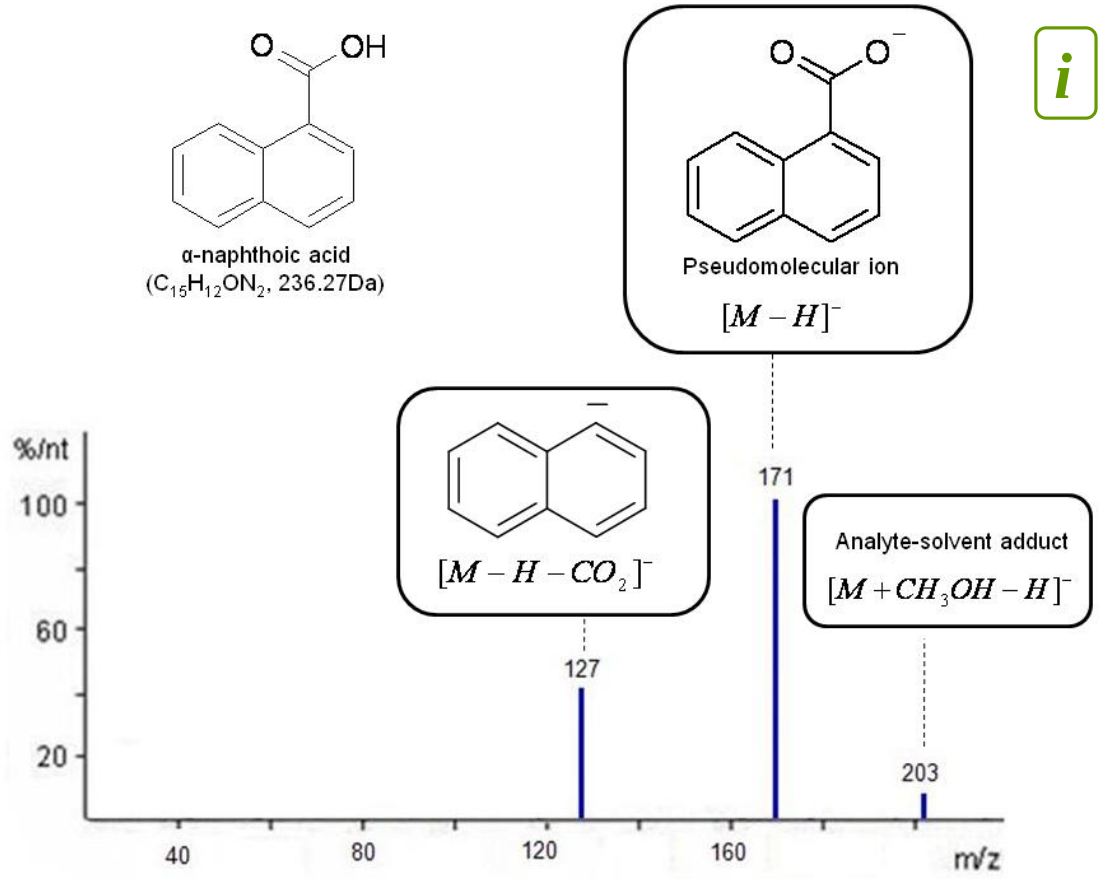


Electrospray Ionisation

Electrospray ionization (ESI) has emerged as a very important ionisation technique capable of producing either singly as multiply charged ions (according to the chemical nature of the analyte and eluent system). It provides a rapid and accurate means of analysing a wide range of polar and ionisable molecules.

Electrospray tend to show very little fragmentation due to the 'soft' nature of the processes. Analyte molecules do not receive enough energy to break the intra-molecular bonds.

In general ESI produces protonated $[M+H]^+$ in positive ion mode and deprotonated $[M-H]^-$ in negative ion mode. Comparing the registered ESI mass spectra in positive and negative ion mode should be done whenever possible; because, it could reveals the nature of the molecular ion.



ESI(-) mass spectra of α-naphthoic acid (20% methanol in water)

Under ESI conditions, some molecules produce abundant adduct ions like $[M+Na]^+$ or $[M+NH_4]^+$.

Analysis conditions must be optimised and it is important NOT to assume that the highest recorded ion peak corresponds to an M+1 or M-1 ion.

Polar molecules with low tendency to form $[M+H]^+$ and/or $[M-H]^-$ can be charged with ESI through the formation of ion adducts.

Table 16. Adduct Ions Positive ESI.[14]

Observed	Explanation	Mass
$[M+H]^+$	Protonation.	M + 1
$[M+H+NH_4]^+$	Mainly seen when using CH_3NH_4	M + 18
$[M+H+nH_2O]^+$	M + H_2O adduct	M + 1 + 18×n
$[M+H+H_2O]^+$	M + H_2O adduct	M + 19
$[M+H+Na]^+$	M + Na adduct	M + 24
$[M+H+K]^+$	M + K adduct.	M + 40
$[2M+H]^+$	Analyte dimerisation.	2×M + 1
$[M+H+CH_3CN]^+$	In presence of CH_3CN	M + 42
$[M+H+CH_3CN+nH_2O]^+$	Adduct of CH_3CN and H_2O	M + 42 + 18×n

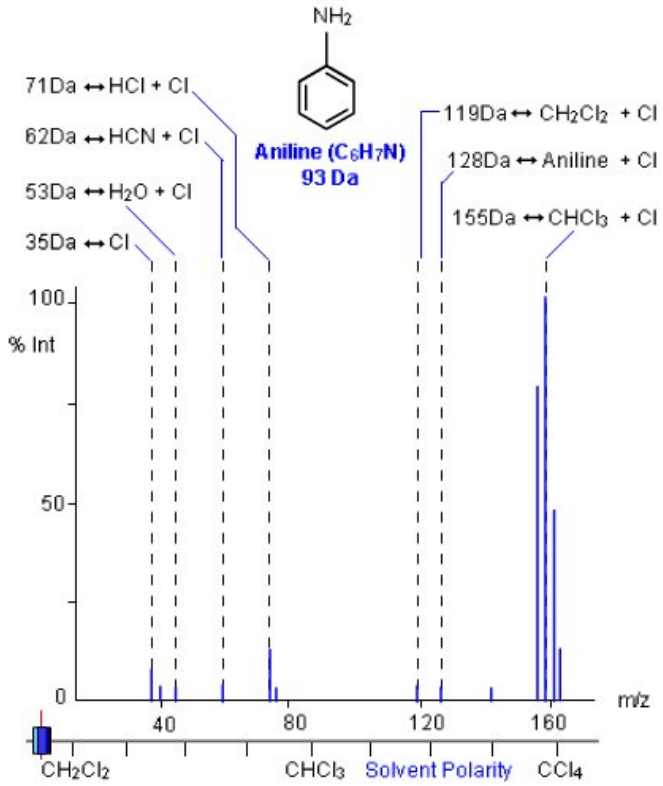
Table 17. Adduct Ions Negative ESI.[14,15,16]

Observed	Explanation	Mass
$[M-H]^-$	Deprotonation.	M - 1
$[M-H-nH_2O]^-$	Deprotonation and water	M – 1 - 18×n
$[M+Cl]^-$	Ion attachment.	M + Cl
$[M-2H+Na]^-$	M + Na adduct	M + 21
$[M-H-CO_2]^-$	Carbon dioxide looses.	M - 45

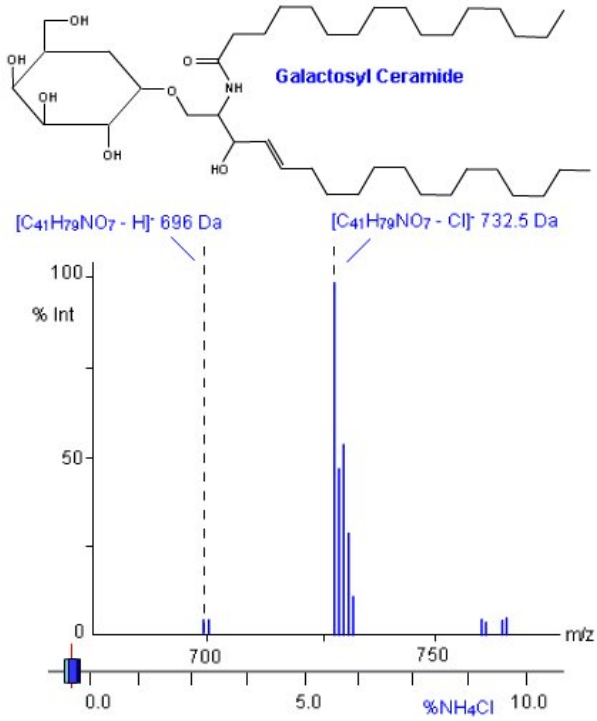
ESI Considerations

In mass spectral interpretation it is very useful to compare the positive and negative ion spectra, as $[M+H]^+$ and $[M-H]^-$ will appear 2Da apart.

Solvent polarity influence the predominant form of analyte anions appearing the ESI mass spectrum. When a competition exits between deprotonation $[M-H]^-$ and anion attachment $[M+Cl]^-$, polar solvents favour mainly the deprotonation.[17,18]



ESI(-) of 1.0% aniline in different solvents (93 Da Da)



ESI(-) of 0.1gL/mL of galactosyl ceramide (697 Da)

Care should be taken in the interpretation of electrospray spectra when the analysis has taken place in the presence of additives or contaminants (such as ammonium or sodium ions).

Analytes with high molecular masses (typically up to 200,000Da) may acquire many electrostatic charges during the ESI process. A series of multiply charged species are thus produced.

Multiply charged ions can be processed by the data system to give a molecular weight profile with a mass accuracy of ±0.01% (100ppm). This process is termed deconvolution.

APCI Considerations

APCI spectra tend to show very little fragmentation due to the 'soft' nature of the ionisation processes. That is during the formation of ions, the analyte molecules do not receive enough energy to break the intra-molecular bonds.

The major problem with APCI is fragmentation and ion clustering that can occur if the parameters are not optimised.

When using air the primary ions produced in the APCI interface are often limited to $[H^+(H_2O)_n]$ and $[O_2^-(H_2O)_n]$.[20]

Depending on the reactant gas, hydrogen transfer may not occur and instead secondary ions react with analyte molecules to give adducts rather than protonated molecules.[21,22,23]

Unlike ESI, APCI does not produce a multiply charged series, and so is unsuitable for the analysis of high molecular weight compounds such as proteins and polymers.

Table 18. Adduct Ions Positive APCI.[20,21,22,23]

Observed	Explanation	Mass
$[M+H]^+$	Protonation.	M + 1
$[M+H+nH_2O]^+$	M + H_2O adduct	M + 1 + 18×n
$[M+H+H_2O]^+$	M + H_2O adduct	M + 19
$[M+H+CH_3CN]^+$	In presence of CH_3CN	M + 42
$[M+H+CH_3CN+nH_2O]^+$	Adduct of CH_3CN and H_2O	M +43 + 18×n
$[M-R]^+$	Aliphatic or organic looses.	M - R
$[M+NH_4]^+$	Ammonium adduct.	M + 18
$[M+NH_4-R]^+$	Ammonium adduct.	M + 18 - R

Table 19. Adduct Ions Negative APCI.[20,21,22,23]

Observed	Explanation	Mass
$[M-H]^-$	Deprotonation.	M - 1
$[2M-H]^-$	Analyte dimerisation.	2×M – 1
$[M+Cl]^-$	Ion attachment.	M + Cl

APPI Considerations

The basic mechanism of photoionization is:

$$M + h\nu \rightarrow M^+ + e^-$$

In some instances, which seem to correlate with molecules of low proton affinity, the molecular ion M^+ is observed; however, the predominant ion observed in APPI is $[M+H]^+$ which suggests the possible involvement of a chemical reaction with the solvent S:

$$M^+ + S \rightarrow [M+H]^+ + [S-H]$$

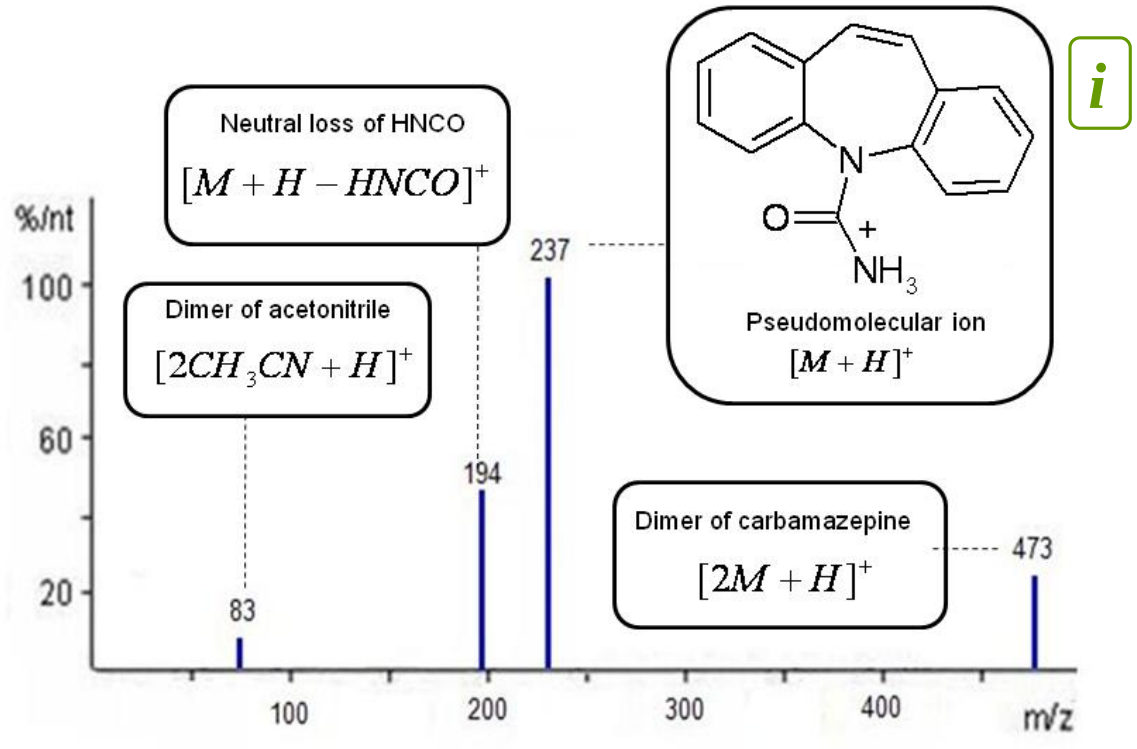
However there is not certain if this process dominates at atmospheric pressure. In the presence of easily photo-ionised molecules (like toluene or acetone) that are called dopants the analyte of interest is indirectly ionised:

$$M + D^+ \rightarrow [M+H]^+ + [D-H]$$

Here D represents the dopant.

When the last mechanism dominates the process is termed dopant assisted APP

APPI is not limited to the positive ion mode. In negative ion mode, low energy electrons (produced from dopant molecules) can be absorbed by other species.



APPI(+) mass spectra of carbamazepine (in acetonitrile)

Solvent molecules and other species present in the eluent system can form adduct ions with the analyte.

Table 20. Selected common ions in APPI positive ion mode

Specie	Mass
M^+	M
$[M+H]^+$	M + 1
$[2M+H]^+$	2 × M + 1
$[M+Na]^+$	M + Na
$[M+H-nH_2O]^+$	M + 1 – 18 × n
$[M+H-R]^+$	M + 1 - R
$[M+H+nS]^+$	M + 1 – S × n

Solvent molecules and other species present in the eluent system can form adduct ions with the analyte.

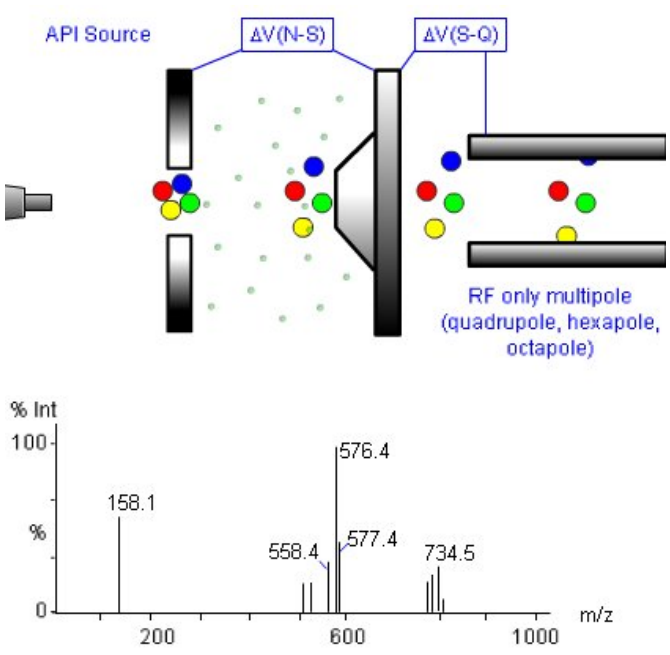
Table 21. Selected common ions in APPI negative ion mode

Specie	Mass
M^-	M
$[M-H]^-$	M - 1
$[2M-H]^-$	2 × M - 1
$[M-H-R]^-$	M - 1 + R
$[M-H+S]^-$	M – 1 + S
$[M-H+S-R]^-$	M – 1 + S - R
$[M+Cl]^-$	M + Cl

LC-MS Structural Information Modes

As was pointed out API spectra tend to show very little fragmentation due to the 'soft' nature of the ionisation processes. That is during the formation of ions, the analyte molecules do not receive enough energy to break the intra-molecular bonds.

By increasing the voltage difference ΔV(S-Q) or ΔV(N-S)], the frequency and energy of collisions imparted to the analyte molecule increase causing intra-molecular bonds to be broken and fragmentation to occur.[24,25]



Erithromycin CID experiment

MS/MS Overview

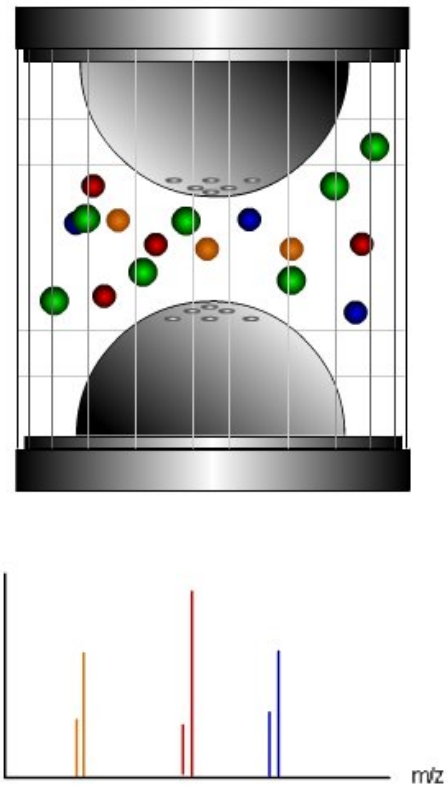
API ionisation techniques (ESI, APCI, APPI) are soft ionisation techniques that produce spectra with little or no fragmentation, this simplifies the spectra considerably; however, this also means less structural information available (no fragmentation pattern). Fragmentation pattern is important when undertaken:

- Structure elucidation, the fragmentation pattern of any molecule is a very powerful tool to elucidate its structure.
- Identity confirmation, when preliminary information about existence of certain compound is available then its confirmation based on raw fragmentation data can be achieved.

Tandem mass spectrometry (or MS/MS analysis) is used to regain structural information by fragmenting ions (that in this case were produced in the API interface). The hardware used to perform MS/MS experiments usually includes a combination of two mass analysers and a collision cell:

- First mass analyser, used to select the initial ion(s) of interest.
- Collision cell, where the ion(s) of interest receive enough collisional energy to fragment.
- Second mass analyser, used to collect and measure the fragment ions of interest.

MS/MS can also be performed in a single specially designed analyser (like ion trap mass analyser).



MS/MS experiments in an ion trap mass analyser

MS/MS Experiments

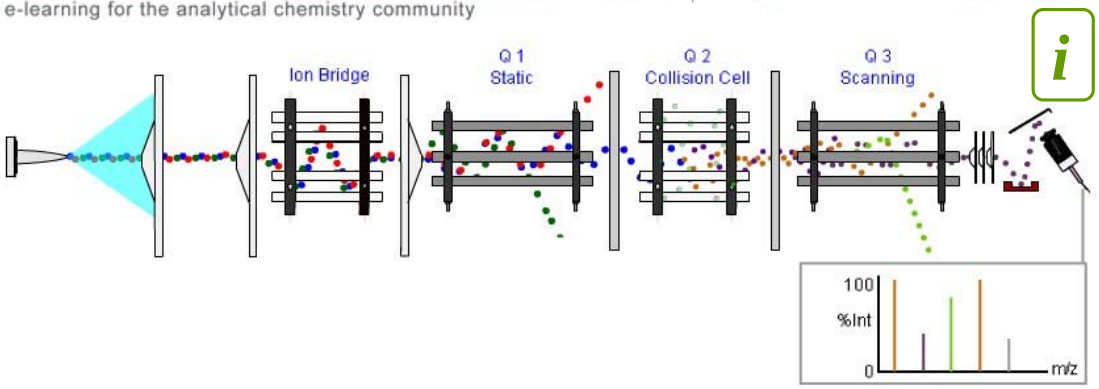
Tandem mass spectrometry (or MS/MS analysis) is used to produce structural information for analyte elucidation or to follow specific fragmentation reactions in order to greatly improve the specificity of the detection technique for analytes in complicated matrices. These advantages can be achieved by fragmenting specific sample ions within the mass spectrometer and identifying either the precursor or product ions.

Several common MS experiments have emerged as being of great value when extra specificity or structural information is required and these include:

- Product Ion Scanning
- Precursor Ion Scanning
- Constant Neutral Loss Scanning
- Single / Multiple Reaction Monitoring

To illustrate the use of various modes of MS/MS some application examples can be found elsewhere.[26, 27, 28, 29, 30, 31, 32, 33]

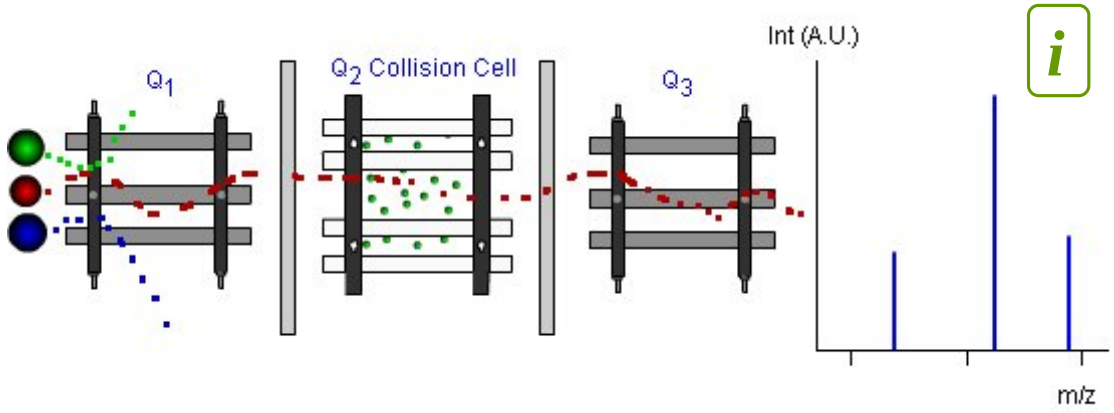
MS/MS is the combination of two or more MS experiments. The aim is either to get structural information by fragmenting the ions isolated during the first experiment, and/or to achieve better selectivity and sensitivity for quantitative analysis. MS^n (should read MS to the n) is an acronym that refers to multiple ion filtering within a single instrument. MS/MS (or MS^2) is usually termed product ion scanning.



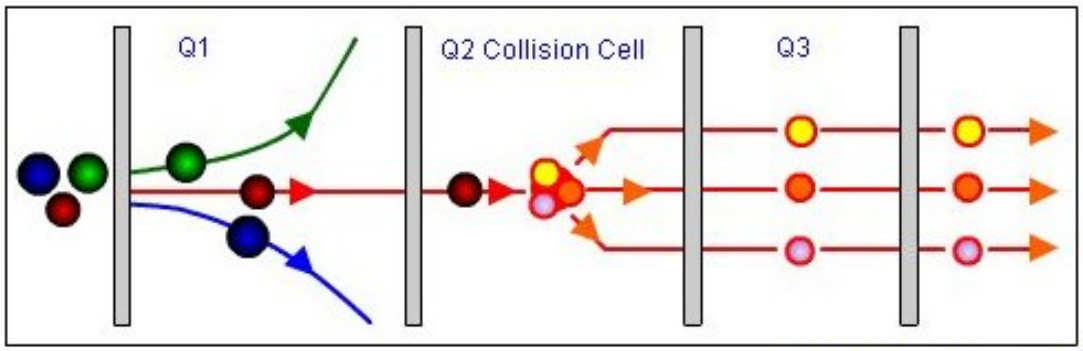
Product Ion Scanning

Overview

The first mass analyser is set to transmit a specific ion (usually the pseudomolecular ion) to the collision cell where its fragmentation takes place. The fragment ions thus produced are then transmitted to the last mass analyser where structural information is gathered. This mode gives molecular weight and structural information about the specific ion selected.



In product ion scanning, the precursor ion (ion of interest) is focussed in Q_1 and transferred to fragment into Q_2. All fragment ions are then measured by scanning Q_3 and structural information of original ion is gathered.



Product Ion Scanning

This method is useful to identify compounds.

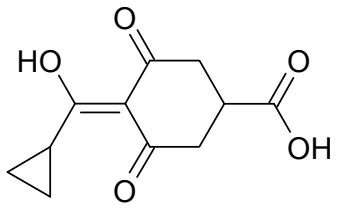
Application

Product Ion Scanning can be used as a confirmatory method for the presence certain analyte.

In the present application, a wheat sample is going to be tested for the presence of **trinexapac acid**. The wheat sample is first extracted with acetonitrile in aqueous phosphate buffer and then injected to the analyser.

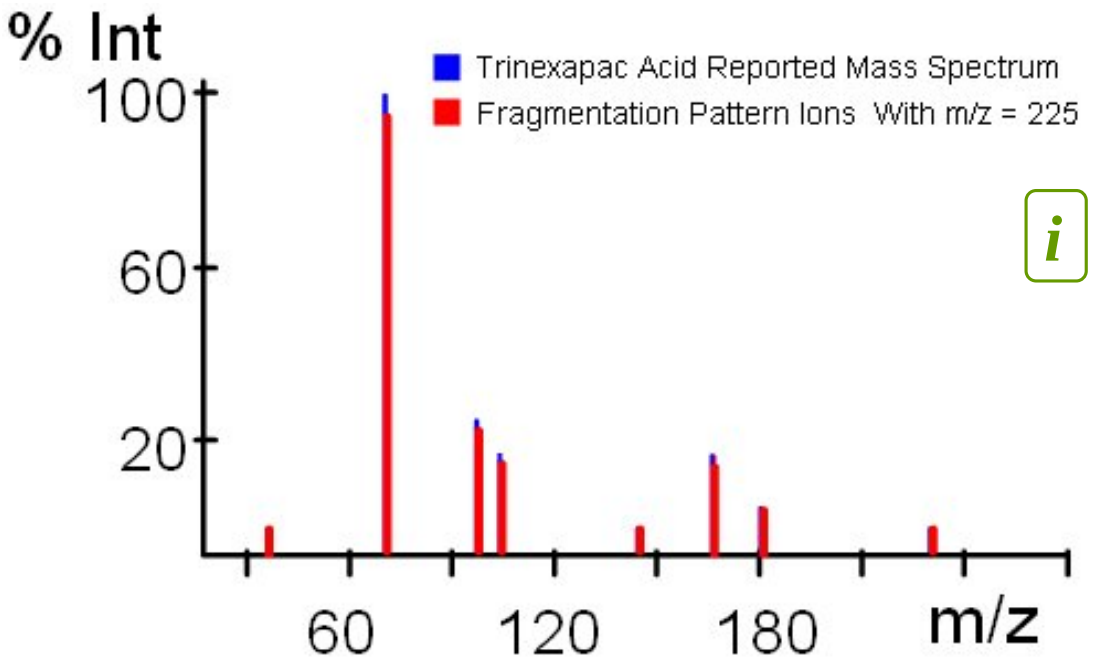
Trinexapac acid:

Trinexapac acid is used to improve seed quality and shoot counts in some cultivars. Due regulations, its content in human food needs to be controlled.



Trinexapac acid ($C_{11}H_{12}O_5$, 224 Da)

If ions of m/z = 225 (ESI positive ion mode) are detected from the sample, then pseudomolecular ions $[Trinexapac\ Acid + H]^+$ can be suspected. The fragmentation pattern of these ions can be used to confirm identity.



Only ions of m/z = 225 are allowed to pass through Q_1, then they fragment in Q_2 and all fragments pass through Q_3 (see previous page for clarification on the meaning of Q's).

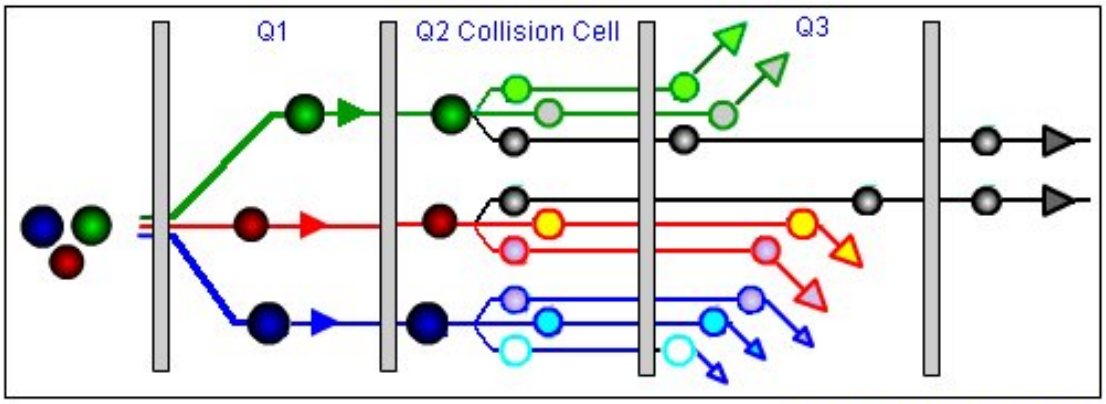
The fragmentation pattern of ions with m/z = 225 clearly matches the mass spectrum reported for trinexapac acid (regression coefficient of 99.9%). The sample contains trinexapac acid.

Precursor Ion Scanning

Overview

In this mode, the first analyser is set to scan across all ions in the sample; these ions are transmitted (one at a time) to the collision cell, where fragmentation takes place. All fragmented ions are transmitted to the last mass analyser, which is held to measure the occurrence of a particular fragment ion (or more if needed). This mode identifies all ions capable of producing the specific fragment.

In the example below, you can scan the first mass analyser (note that in real life you can programme your equipment to do it automatically) across the masses m_1, m_2 and m_3 (allowing them to pass one at a time), the last analyser is set to measure only one fragment of interest (or more if needed). Consider that you are interested in identify alkyl-benzene compounds from certain sample, only ions that yield the characteristic tropylium ion (m/z = 91) would be of interest.



Precursor Ion Scanning

In precursor ion scanning, Q_1 is scanned to transmit ions to fragment into Q_2. Fragmented ions are transmitted to Q_3 which is fixed to transmit a specific fragment ion (or more if needed). This method is useful to identify compounds.

Application

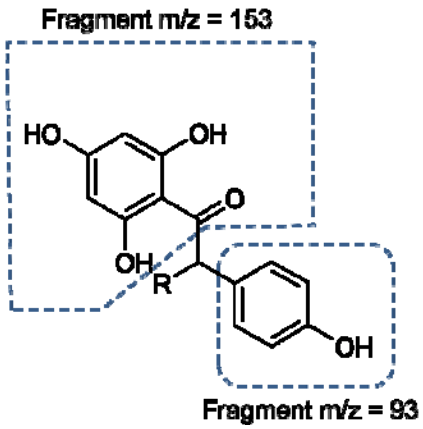
Precursor Ion Scanning can be used to quickly identify molecules that probably belong to a chemical family (or with certain molecular structure).

An alcoholic extract of certain plant was tested for the presence of **chalcones**. A preliminary experiment had revealed the presence of ions with m/z ration of 320, 343 and 551 (m/z scan range from 250 to 1000). Which of these three signals could be due to a chalcone?

Chalcones:

Chalcones belong to a group of polyphenolic compounds called flavoniods with multiple biochemical applications.

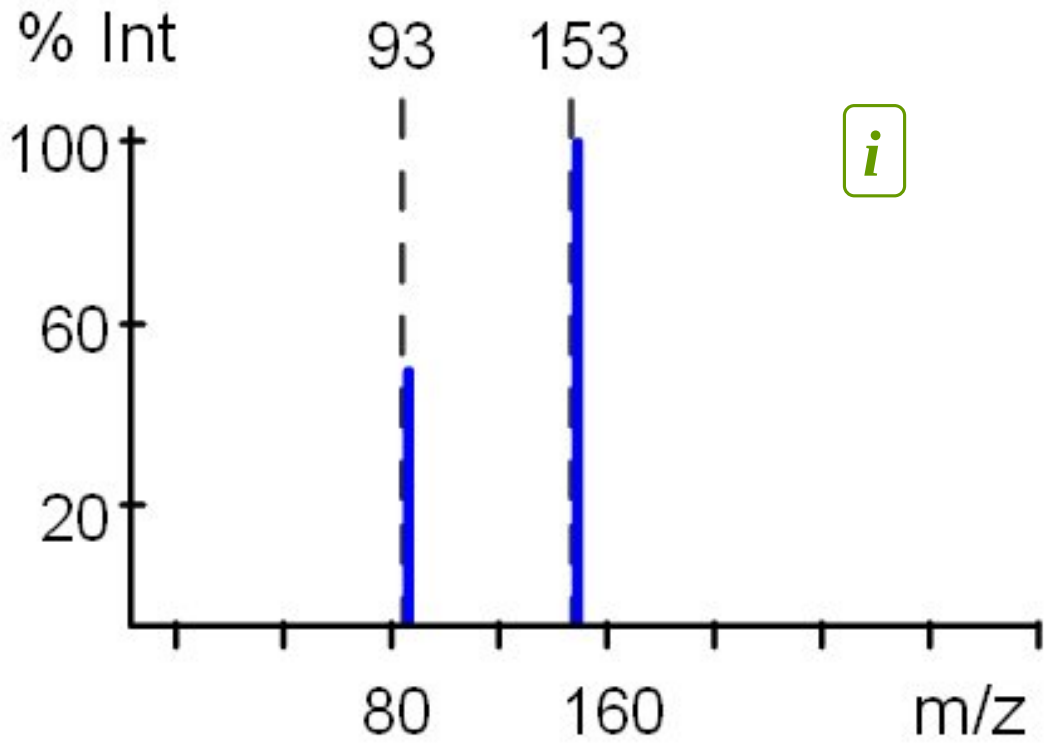
Important: Compounds belonging to the chalcones group will fragment producing signals at m/z = 93 and 153 (see opposite).



Chalcone important fragments

Q1 is programmed to allow to transmit only one type of ion at a time (320, 343 or 551).
Q3 is programmed to allow to transmit only fragments of m/z ratio 93 AND 153.

See previous page for clarification on the meaning of Q's.



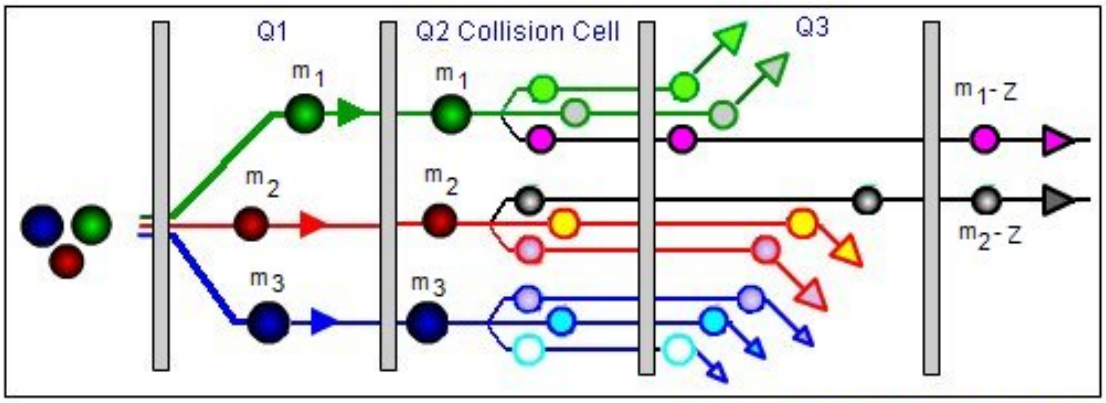
In the example ions of m/z = 343 would probably correspond to a chalcone (note the produced fragments at m/z = 93 and 153).

Constant Neutral Loss Scanning

Overview

In this mode, the first analyser is set to scan across all ions in the sample; these ions are transmitted to the collision cell, where fragmentation takes place. All fragmented ions are transmitted to the last mass analyser which in turn is scanned to produce a spectrum of precursor ions that undergo a particular neutral loss.

In the example below, you can scan the first mass analyser (note that in real life you can programme your equipment to do it automatically) across the masses m_1, m_2 and m_3, the last analyser is set to measure only ions with masses (m_1-Z), (m_2-Z) and (m_3-Z), where the parameter Z is defined by the user. Consider that you are interested in identify primary alcohols from certain sample, only ions that present a characteristic loss of water (M - 18) would be of interest.



Constant Neutral Loss Scanning

In constant neutral loss scanning, Q_1 is scanned to transmit ions to fragment into Q_2. Fragmented ions are transmitted to Q_3 which is also scanned for ions that had experienced certain neutral loss.

This method is useful to identify compounds.

Application

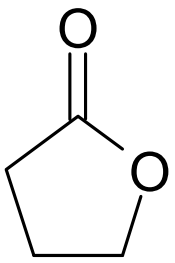
Constant Neutral Loss Scanning can be used to quickly identify molecules that could fragment releasing neutral molecules.

An alcoholic wood extract was obtained from milled oak wood and was tested for the presence of **lactones**. A preliminary experiment had revealed the presence of ions with m/z ration of 150, 157 and 171 (m/z scan range from 85 to 500). Which of these three signals could be due to a lactone?

Lactone:

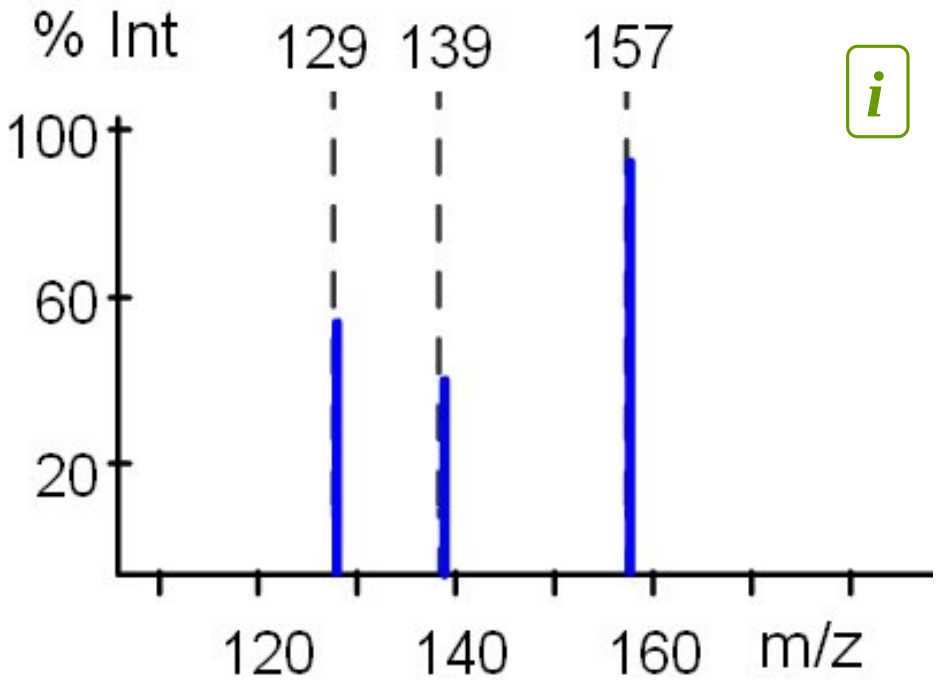
Lactone: is an organic cyclic ester. These compounds have an economic impact as food flavourings and in medicinal activities.

Important: Lactones will release carbon monoxide and water (neutral losses of 28 and/or 18 respectively).



Lactone

Q1 is programmed to allow to transmit only one type of ion at a time (150, 157 and 171). Q3 is programmed to allow to transmit not only the same masses as in Q1 but also these masses minus 18 and minus 28 (three signals at a time). See previous page for clarification on the meaning of Q's.

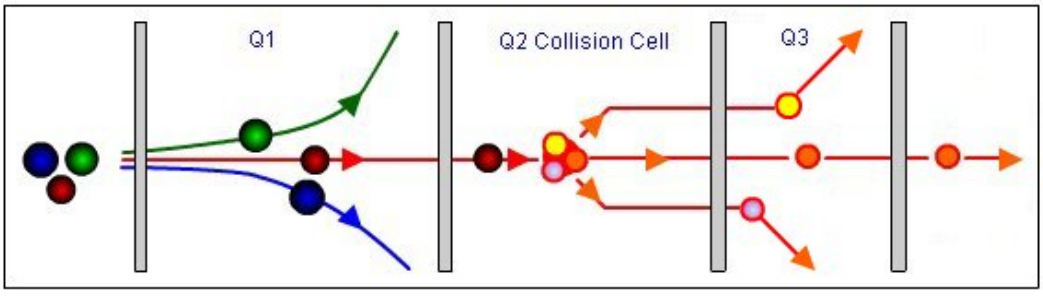


In the example ions of m/z = 157 would probably correspond to a lactone (pseudomolecular ion m/z =157, neutral loss of water = 157 – 18 = 139 and neutral loss of CO = 157 – 28 = 129).

Single/Multiple Reaction Monitoring

Overview

The first mass analyser is set to transmit a specific ion which is fragmented in the collision cell. The second analyser is set to transmit one specific fragment (or more if needed). Useful for identifying target compounds.



Single/Multiple Reaction Monitoring

In single/multiple reaction monitoring, Q_1 is fixed to transmit a specific precursor ion and Q_3 to transmit a specific product ion.

The method also allows quantitation to be done effectively. The method can be more sensitive than other MS/MS methods, as both of the analytical quadrupoles are static at fixed m/z, rather than scanning.

Application

The next mixture of **lipids** can be analysed by for the APPI(+)-MS/MS.

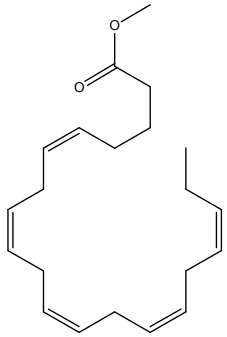
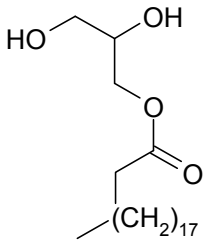
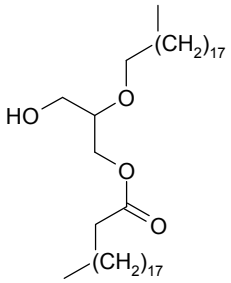
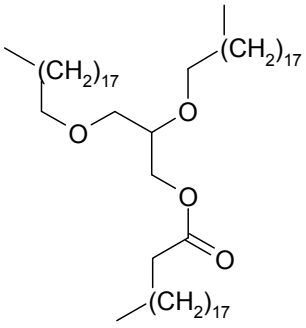
- Eicosapentaenoic acid methyl ester
- Monoarachidin
- Diarachidin
- Trielaidin

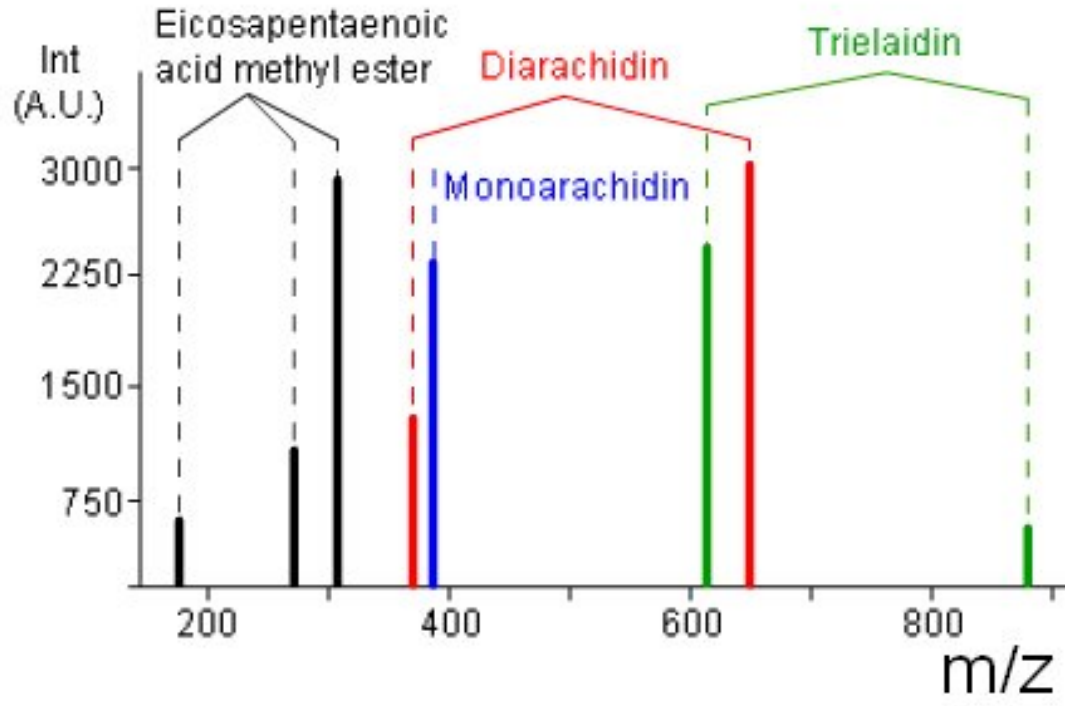
Look at the APPI/MS/MS spectrum, the most intense signals of each analyte compound can be used in Single/Multiple Reaction Monitoring for quantitative purposes.

Lipids:

Lipids comprise a family of biomolecules that play prominent roles in many critical metabolic and biochemical processes such as energy production and storage, the formation and functioning of cellular membranes, signal transduction, etcetera.

Table 22. Analysis of lipids

Analyte/Structure	Analyte/Structure
Eicosapentaenoic acid methyl ester 	Monoarachidin 
Diarachidin 	Trielaidin 



Q1 is programmed to allow to transmit several ions at a time (317, 387, 681 and 885). Q3 is programmed to allow to transmit eight signals simultaneously: three signals for the acid (267, 285 and 317); one for monoarachidin (387); two for diarachidin (369 and 663) and two for trielaidin (603 and 885). See previous page for clarification on the meaning of Q's.

MS/MS Quantitative Considerations

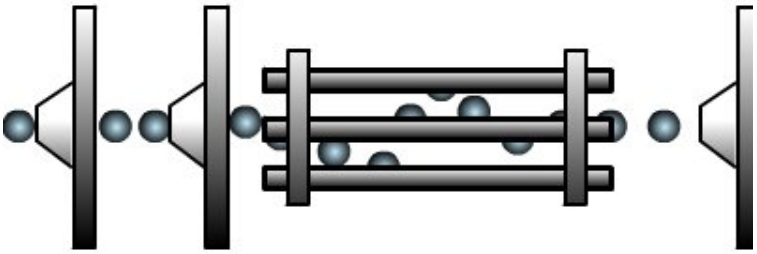
The rate of scanning over the mass range has an important effect on the instrument sensitivity; basically, slower scan rates result in improved sensitivity (the instrument takes longer collecting data for each ion).

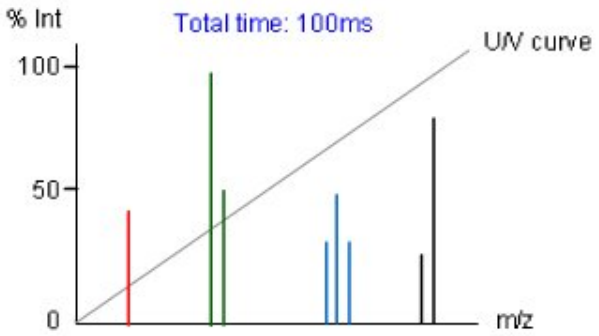
For quantitative analysis of target compounds instead of scanning the entire mass range, only specific ions that have been chosen in advance, and that are characteristic of the target compounds should be monitored.

According to the ionisation type (soft or hard) used to produce ions, different ion signals should be considered; in general, for quantitative porpoises, when using:

- Soft ionisation techniques, only the pseudo-molecular ions of interest are chosen.
- Strong ionisation techniques, it is highly recommended to use at least three characteristic fragment ions (usually the most stable ones) coming from each compound of interest.

Finally, you have to consider that in API, acids ionise best in negative ion mode than in positive, whilst bases do the opposite. Avoid agents that can jeopardise your analysis; in positive ion mode signal suppression can arise when using certain additives (like DEA), whilst the same phenomenon could happen in negative ion mode if using acidic compounds (like TFA or HFBA).





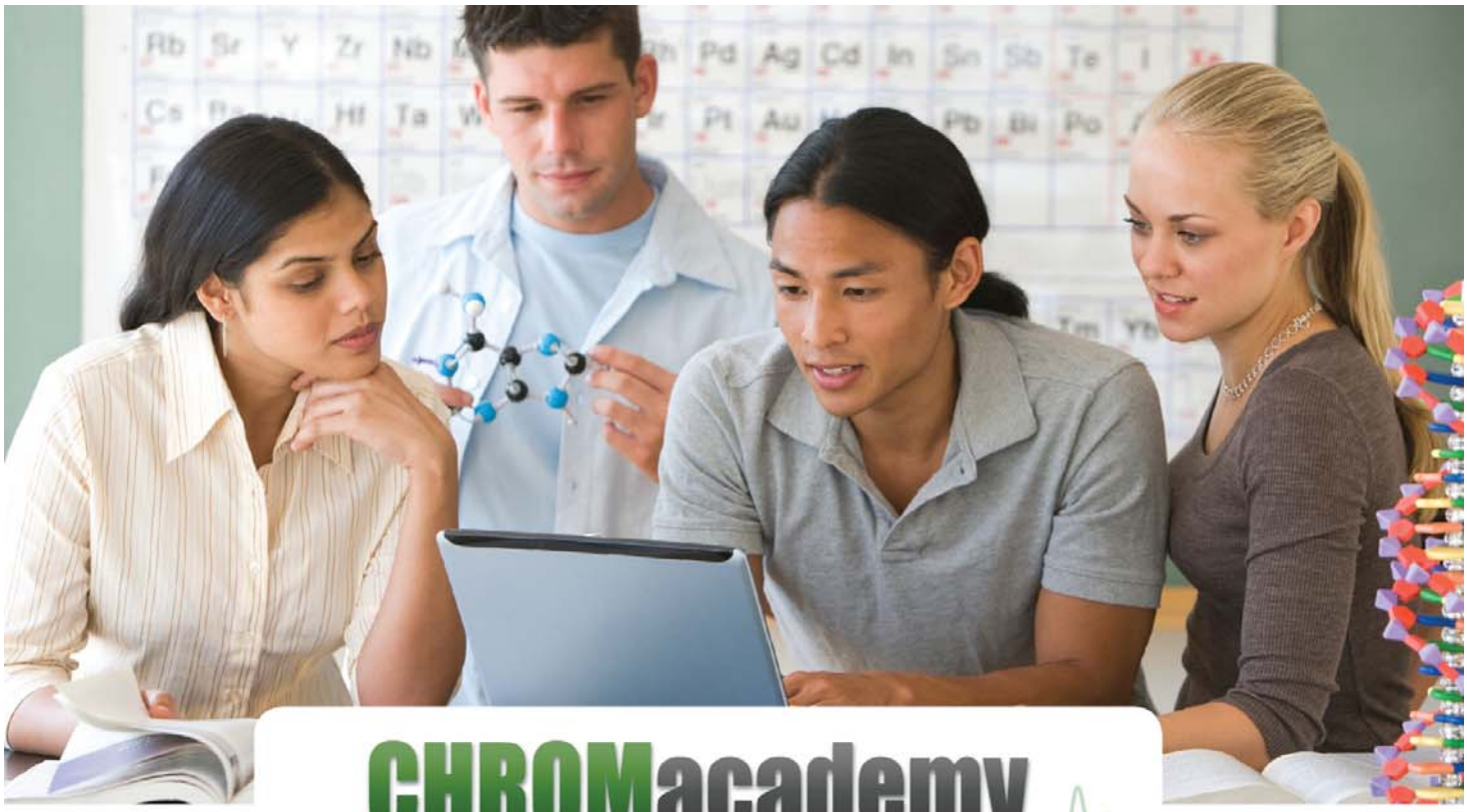
Important:

For LC-MS, the ions being monitored can be changed during the course of the separation (using the expected retention time as part of the detection criteria) collecting more data for the ions of interest and increasing sensitivity. The major drawback with using this technique is that information on compounds other than the target ones is lost.

References

1. Pure & Appl. Chem., 1999, 71, 1594-1607.
2. G. Zerbi and G. H. Benyon. IUPAC Symbolism and nomenclature for MS. Org. Mass Spectrom. 12, (1977), 115-118.
3. Terrence A. Lee. "A Beginner's Guide to Mass Spectral Interpretation." John Wiley and Sons 1998, 1-20.
4. J. T. Watson. "Introduction to Mass Spectrmetry." Chapter 2. Lippincott-Raven, Pa, USA, (1997), 16.
5. James Barker. "Mass Spectrometry" John Wiley and Sons 1999, 117-147.
6. E. De Hoffmann, J. Charette, and V. Stroobant. "Mass Spectrometry –Principles and Applications." John Wiley and Sons 1996, 39-48.
7. F. W. McLafferty. "Interpretation of Mass Spectra." 3rd Edition. University Science Books: Mill Valley, (1980), 15.
8. M. E. Bier and J. E. Schwartz in "Electrospray Ionisation Mass Spectrometry." R. B. Cole [ed.], John Wiley and Sons Inc, (1997), 263.
9. E. Sheehan and S. Mitrovich in "A Global View of LC/MS", 1st edition, (Appendix D), Global Publishing, PA, USA, (1998), 106.
10. J. Yergy, D. Heller, G. Hansen, R. J. Cotter, C. Fenselau. "Isotopic Distributions in MS of Large Molecules." Anal. Chem. 55, (1983), 353-356.
11. Ross Willoughby, Ed Sheehan, Sam Mitrovich. "A Global View of LC/MS" Global View Publishing. 1998, 297-324
12. **http://www.sisweb.com/referenc/source/exactmaa.htm**
13. Reg Davis, Martin Frearson. "Mass Spectrometry" John Wiley and Sons 1990, 15-22
14. Susana Grimalt, Óscar J. Pozo, Jose M. Marín, Juan V. Sancho, and Félix Hernández. "Evaluation of Different Quantitative Approaches for the Determination of Noneasily Ionizable Molecules by Different Atmospheric Pressure Interfaces Used in Liquid Chromatography Tandem Mass Spectrometry: Abamectin as Case of Study." J. Am. Soc Mass Spectrom. 2005, 16, 1619–1630.

15. Nicolas Fabre, Isabelle Rustan, Edmond de Hoffmann, Joëlle Quetin-Leclercq. "Determination of Flavone, Flavonol, and Flavanone Aglycones by Negative Ion Liquid Chromatography Electrospray Ion Trap Mass Spectrometry" J Am Soc Mass Spectrom. 12, (2001), 707–715.
16. Helena Keski-Hynnilä, Leena Luukkanen, Jyrki Taskinen, and Risto Kostiainen. "Mass Spectrometric and Tandem Mass Spectrometric Behavior of Nitrocatechol Glucuronides: A Comparison of Atmospheric Pressure Chemical Ionization and Electrospray Ionization" J Am Soc Mass Spectrom. 10, (1999), 537–545.
17. Richard B. Cole and A. Kamel Harrata. "Solvent effect on analyte charge state, signal intensity, and stability in negative ion electrospray mass spectrometry; implications for the mechanism of negative ion formation" J Am Soc Mass Spectrom. 4, (1993), 546-556.
18. Cole RB, Zhu J. Rapid Commun. Mass Spectrom. 1999; 13:607.
19. Junhua Zhu and Richard B. Cole. "Formation and Decompositions of Chloride Adduct Ions, $[M + Cl]^-$, in Negative Ion Electrospray Ionization Mass Spectrometry" J Am Soc Mass Spectrom. 11, (2000), 932–941.
20. Christophe Guimbaud, Thorsten Bartels-Rausch, Markus Ammann. "An atmospheric pressure chemical ionization mass spectrometer (APCI-MS) combined with a chromatographic technique to measure the adsorption enthalpy of acetone on ice." International Journal of Mass Spectrometry. 226, (2003) 279–290.
21. G. A. Eiceman, J. F. Bergloff, J. E. Rodriguez, W. Munro, and Z. Karpas. "Atmospheric Pressure Chemical Ionization of Fluorinated Phenols in Atmospheric Pressure Chemical Ionization Mass Spectrometry, Tandem Mass Spectrometry, and Ion Mobility Spectrometry" J Am Soc Mass Spectrom 1999, 10, 1157–1165.
22. Beata M. Kolakowski, J. Stuart Grossert, and Louis Ramaley. "Studies on the Positive-Ion Mass Spectra from Atmospheric Pressure Chemical Ionization of Gases and Solvents Used in Liquid Chromatography and Direct Liquid Injection" J Am Soc Mass Spectrom 2004, 15, 311–324.
23. Tiia Kuuranne, Mikko Vahermo, Antti Leinonen, Risto Kostiainen. "Electrospray and Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometric Behavior of Eight Anabolic Steroid Glucuronides" J Am Soc Mass Spectrom 2000, 11, 722–730.
24. R. D. Smith, J. A. Loo, C. J. Barinaga, C. G. Edmonds and H. R. Hudspeth. J. Am. Soc. Mass Spectrom. 1, (1990), 53.
25. R. D. Smith and C. J. Barinaga. Rapid Commun. Mass Spectrom. 4, (1990), 54.
26. **http://webbook.nist.gov/chemistry/**
27. Robert A. W. Johnstone and Malcolm E. Rose. "Mass spectrometry for chemists and biochemists". Cambridge Univeristy Press. 1996.
28. Dynamic mass spectrometry, volume I. Edited by D. Price and J. E. Williams. Heyden and Son Ltd, London, US distribution: Sadtler Research Labs. Inc, Philadelphia, March 1970.
39. Dynamic mass spectrometry, volume II. Edited by D. Price and J. E. Williams. Heyden and Son Ltd, London, US distribution: Sadtler Research Labs. Inc, Philadelphia, March 1970
30. A. D. Postle. "The analysis of lung surfactant phospholipid by electrospray ionisation mass spectrometry –Applications to disease states." Appl. Cardio. Path. 9, (2000), 286-289.
31. P. L. Haslam, K. Raymondas and C. S. Baker. "Measurement of pulmonary surfactant components and function in bronchoalveolar lavage fluid." Eur. Respir. Rev. 9(66), (1999), 43-69.
32. **http://www.soton.ac.uk/~msweb/**



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