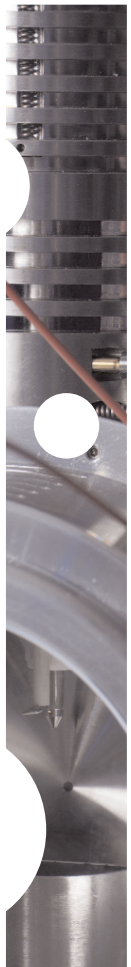
Best Practice Guide for Generating Mass Spectra



Best Practice Guide
for Generating Mass Spectra

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Ken Webb

LGC
Setting standards in analytical science

VAM
valid analytical measurement

dti
National Measurement System

BEST PRACTICE GUIDE FOR GENERATING MASS SPECTRA

December 2006

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Preface

This *Guide* was prepared as part of the Department of Trade and Industry's VAM Programme which forms part of the UK National Measurement System. The *Guide* arose from discussions held at the VAM Mass Spectrometry Working Group and was prepared by LGC in collaboration with the members. In addition to major contributions by the authors, other members of the Working Group provided suggestions and comments.

The idea for this work came about during preparation of an earlier guidance document* concerning accurate mass ("AccMass") applications of mass spectrometry. It became clear that users of mass spectrometry instrumentation or services, including both specialists and research chemists, frequently have little understanding of the instrumentation or the meaning of the spectra they produce. Often, they will obtain or request an accurate mass determination for confirmation of identity on the basis of spectra which are meaningless or which could not possibly have originated from the target molecule. Discussion of this problem highlighted the changes which have taken place in teaching chemistry and analytical science and the rapid expansion in the application of mass spectrometry. The latter has been fuelled by a number of factors, including advances in the automation and performance of instrumentation and recent rapid growth in the use of mass spectrometry for the biosciences. The outcome has been widespread use of complex instrumentation, often as a "walk up" service, by staff with little education or training relevant to the task.

The main aim of the *Guide* is to enable those unfamiliar with mass spectrometry to generate mass spectra that are fit for purpose, primarily for qualitative analysis of small molecules. We have done this by providing a clear and concise summary of the essential steps in obtaining reliable spectra. In addition, the reader should obtain a better understanding of the limitations of different types of spectrometer and the particular precautions which are necessary in setting up the instrument and acquiring a spectrum. Advice is also given on how to assess the quality of the spectrum from its appearance and locating the target molecular species within the spectrum. The emphasis is on giving practical advice which is specific, easy to follow and in a format which will encourage its use "on the job". With this in mind, we have set out the Guide in a number of short, targeted sections and made extensive use of bullet points, tables, illustrations and flow charts. We have also included a wide range of examples to illustrate key points and make it easier to identify

common problems. We hope that these features of the *Guide* will also facilitate its use for private study and training courses away from the laboratory. With this in mind, a glossary of the terms used has also been included.

Mike Sargent

Chairman, VAM Mass Spectrometry Working Group

LGC, September 2006

***** *Methodology for Accurate Mass Measurement of Small Molecules: Best Practice Guide*, Ken Webb, Tony Bristow, Mike Sargent and Bridget Stein (Coordinating Editors), LGC, November 2004. ISBN 0-948926-22-8. Available from www.vam.org.uk.

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Contents

1 INTRODUCTION 1
1.1 Aims of the guide 1
1.2 Examples of applications of mass spectrometry 1
1.3 Limitations of mass spectrometry 1

2 INSTRUMENT CONFIGURATION 2
2.1 Key components of a mass spectrometer 2
2.2 Analyser types 2

3 CHOICE OF IONISATION MODE 4

4 INSTRUMENT SET-UP: KEY DEFINITIONS 7
4.1 Peak shape 7
4.2 Mass resolution 7

5 ACQUIRING A MASS SPECTRUM 9
5.1 General sequence 9
5.2 Instrument tuning 9
5.3 Mass calibration 10
5.4 Background spectrum 10
5.5 Checking instrument performance 11

6 EVALUATING SPECTRUM APPEARANCE 13
6.1 What to look for in a good quality mass spectrum 13
6.2 Spectrum information 13

7 MOLECULAR SPECIES RECOGNITION 15
7.1 Definitions of molecular mass 15
7.2 Use of stable isotope information 16
7.3 How to find the molecular species 16
7.4 Questions to ask about the validity of the proposed molecular species 17
7.5 What if the expected molecular species is not visible in the spectrum? 17

8 FURTHER EXAMPLES OF MASS SPECTRA 18

9 COMMON BACKGROUND IONS 22
9.1 Electrospray ionisation 22
9.2 Electron ionisation 23

10 GLOSSARY OF TERMS 24

11 BIBLIOGRAPHY 27

1 INTRODUCTION

1.1 Aims of the guide

- To enable those unfamiliar with mass spectrometry to generate mass spectra that are fit for purpose.
- To help users recognise poor quality spectra and to understand some of the common causes for such spectra.
- To enable users to understand some of the limitations of mass spectrometry.
- Note that the guidance in the document is restricted to the qualitative analysis of 'small' molecules (*i.e.* larger molecules, such as biopolymers, are not covered).

1.2 Examples of applications of mass spectrometry

- Confirmation of identity of known compounds (*e.g.* compounds from target synthesis, metabolites, compounds extracted from a sample matrix).
- Identification of unknowns.
- Assessing the degree of isotope incorporation in labelling studies.
- As a detector linked to a chromatographic system for quantitative and qualitative analysis.
- Accurate mass measurements for the determination of molecular formulae.
- Fundamental studies of the physical chemistry of ions.

1.3 Limitations of mass spectrometry

- Not all compounds will give a mass spectrum as the compound of interest may not ionise.
- The relative abundances of ions in the mass spectrum obtained for a mixture does not necessarily reflect the proportions of the compounds in the mixture.
- It cannot be used directly to assess purity of a material since impurities may not ionise or may have different ionisation efficiencies.
- In general mass spectrometry cannot easily distinguish between isomers (unless it is coupled with a chromatographic technique).
- Spectra may be different on different types of instrument, or on different instruments of the same type, making library searching difficult.
- Poor quality spectra can mislead.
- Use of a second ionisation technique is often necessary to assist confirmation of identity. At all times other supporting analytical and chemical information should be considered.

2 INSTRUMENT CONFIGURATION

- A mass spectrometer is an instrument that operates under vacuum and separates charged gas phase species according to their mass and charge. A computer is used to control the mass spectrometer and for processing the data.
- Compounds introduced into the spectrometer produce a characteristic mass spectrum which can be used to obtain identity or structural information.

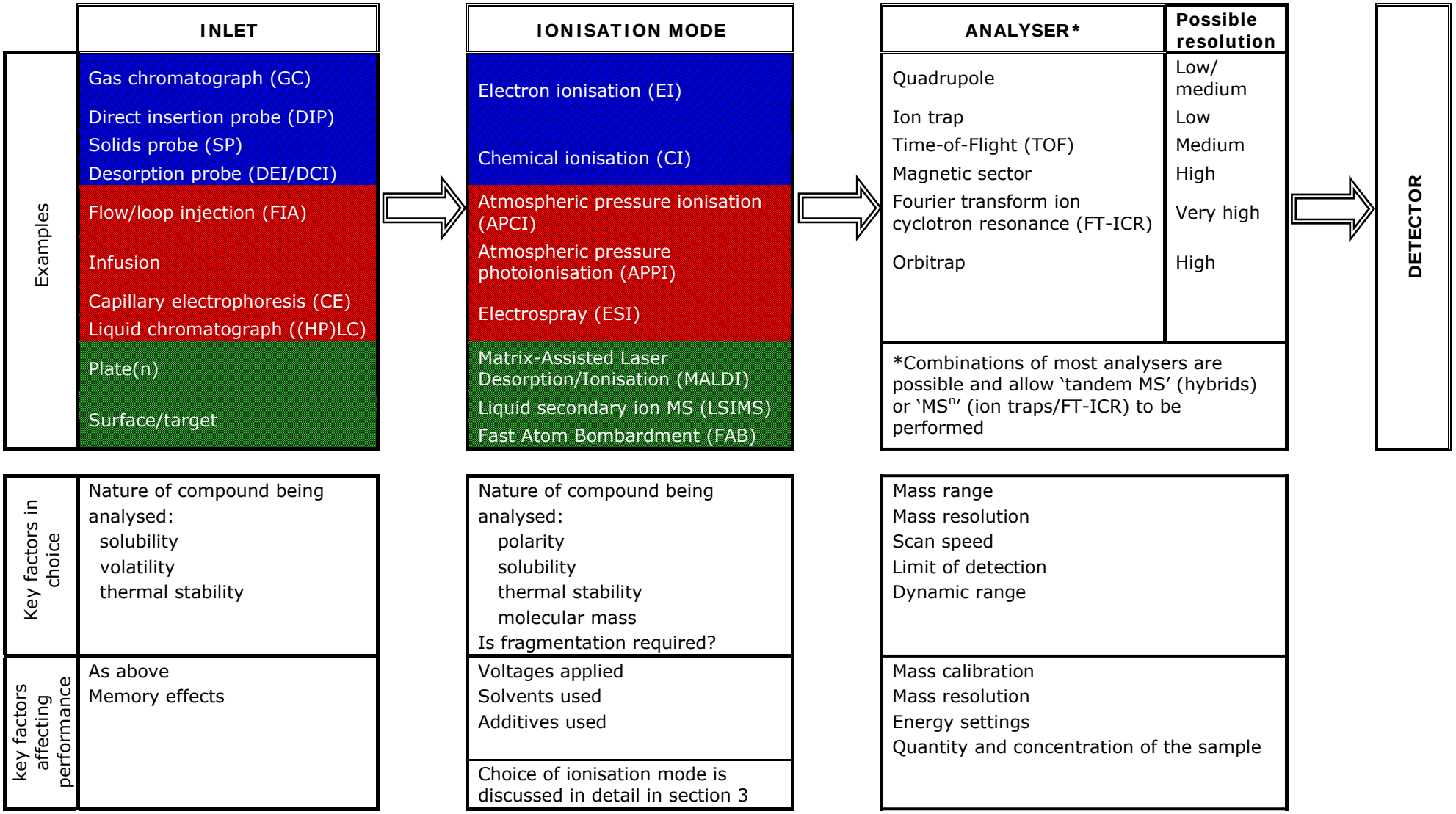
2.1 Key components of a mass spectrometer

- The key components of a mass spectrometer are shown in Figure 1.
- The analyst usually has to select the appropriate inlet, ionisation source and analyser. What is 'appropriate' will depend on the nature of the sample being analysed.
- The main types of inlet, ionisation mode and analyser are summarised in Figure 1. Different inlets are appropriate for different ionisation sources, as indicated by the colour-coding in Figure 1 (*e.g.* the inlets highlighted in blue can be coupled with the ionisation modes also highlighted in blue).

2.2 Analyser types

- **Magnetic sectors** – generally use a combination of electrostatic and magnetic fields and can operate as low and high resolution instruments. Sector instruments operate with high voltages on the ion source (typically 5 - 10 kV).
- **Quadrupoles** – use a combination of rf (radio frequency) and dc (direct current) voltages, are generally low resolution instruments and operate at relatively low voltages (<500V). Capable of fast scanning hence are ideally suited to interfacing to GC, HPLC, CEC and CE inlets.
- **Time-of-Flight** (TOF) – measures the time an ion takes to travel a specific distance after acceleration by a high potential. Different mass ions travel at different speeds. Reflectron systems produce higher resolution than linear systems. ES-TOF, MALDI-TOF, GC-TOF and hybrid instruments are now available.
- **Ion traps** – usually the term 'Ion Trap' is used to refer to the Quadrupole Ion Trap (QIT). In this analyser, ions are produced and stored in the trap and progressively ejected by increasing the rf voltage. The trapped ions can be induced to undergo dissociation and hence generate a product ion spectrum within the one analyser. The majority of ion traps are bench-top instruments used for routine analysis. The **FT-ICR MS** is a type of ion trap where the frequencies of the circulating ions are measured and a Fourier transform is performed to give the mass spectrum. Unlike a conventional bench-top ion trap, it is capable of very high mass resolution. Another (new) type of ion trap is the Orbitrap.

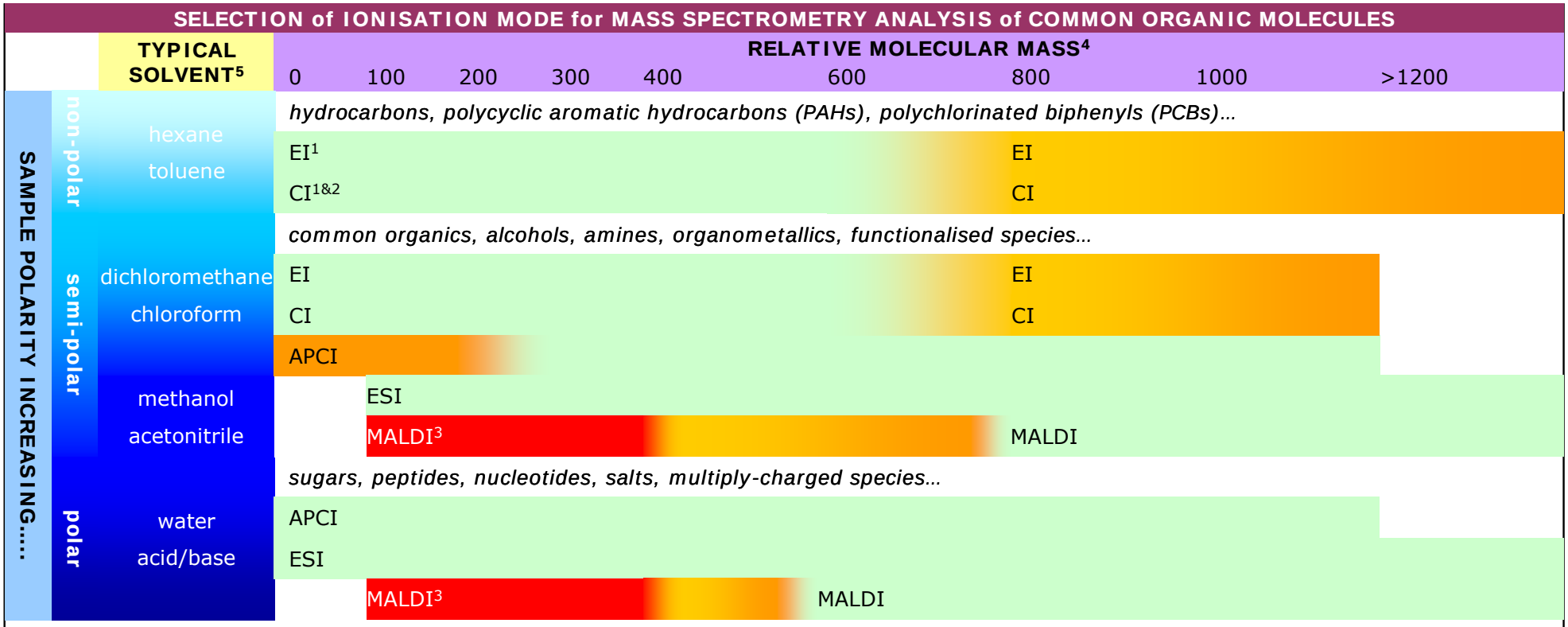
Figure 1: Components of a mass spectrometer



3 CHOICE OF IONISATION MODE

- The principal factors that affect the choice of ionisation mode are the polarity, molecular mass and thermal stability of the compound being analysed.
- If an inappropriate ionisation mode is selected then no spectrum, or a poor quality spectrum, will be obtained.
- Most samples will be initially analysed to obtain positive ion spectra, however, negative ion spectra can be useful where the negatively charged molecule may be more stable than the corresponding positively charged one and may also provide complementary structural information through different fragmentation processes.
- In general a 'hard' ionisation process, such as electron ionisation, can produce many fragment ions and possibly poor abundance of the molecular species; a 'soft' ionisation process, such as electrospray, produces few fragment ions with abundant molecular species.
- Use the chart shown in Figure 2 and the information in Table 1 to help you select the most appropriate method of ionisation for your sample – this will enable you to obtain the most useful spectrum to assist in compound identification.
- Figure 2 shows the polarity of the sample increasing down the y axis - sample types can range from, *e.g.* aromatic compounds (non-polar) to salts and peptides (polar). Typical solvents for the polarity range are also shown. The polarity of the sample can be assessed by investigating which solvents it will dissolve in.
- Where a choice of ionisation methods is indicated those coloured green are the ideal first choice, those coloured red could be used if there is no other choice.
- As an example, consider toluene (C_7H_8) – this is a non-polar molecule with a relative molecular mass of 92. Using Figure 2 the ionisation method of first choice would be EI.
- As a further example, consider the complex vitamin folic acid ($C_{19}H_{19}N_7O_6$) – this is a polar molecule with a relative molecular mass of 441, which is insoluble in most organic solvents but slightly soluble in water. So from the chart the most appropriate ionisation method would be ESI or APCI.
- Other methods not shown in Figure 2 or Table 1 include fast atom bombardment (FAB), liquid secondary-ion mass spectrometry (LSIMS) (both similar to MALDI), field desorption/field ionisation (FD/FI) used for non-volatile molecules instead of EI/CI.

Figure 2: Chart to assist with ionisation mode selection *(reproduced from EPSRC National Mass Spectrometry Service Centre Summer School, B K Stein, 2006, with permission from EPSRC National Mass Spectrometry Service Centre)*



KEY: *Examples of compounds of differing polarity are shown in italics*

Primary technique (most likely to work well)
Secondary technique (should work and will give complementary information to the primary technique)
Tertiary technique (try it if you have no choice)

NOTES:

1 For all EI and CI a suitable inlet will also be required to match sample volatility (*e.g.* GC or solids probe or desorption probe).

2 CI suitability will depend on gas selection (*e.g.* ammonia is unsuitable for hydrocarbons which will need, *e.g.* methane).

3 MALDI matrix ions cause interference at lower *m/z* [so MALDI without matrix (LDI) or surface assisted (SALDI) can be used more readily at lower mass].

4 Mass range also depends on mass analyser and *m/z* of ion produced.

5 The polarity of the sample material can be assessed by investigating which solvents it will dissolve in (*e.g.* a material that dissolves in hexane but not in water is non-polar). The solvents listed are for assessment of sample polarity only – they are not suggested solvents for dissolution of the sample for analysis by mass spectrometry.

Table 1: Summary of ionisation modes

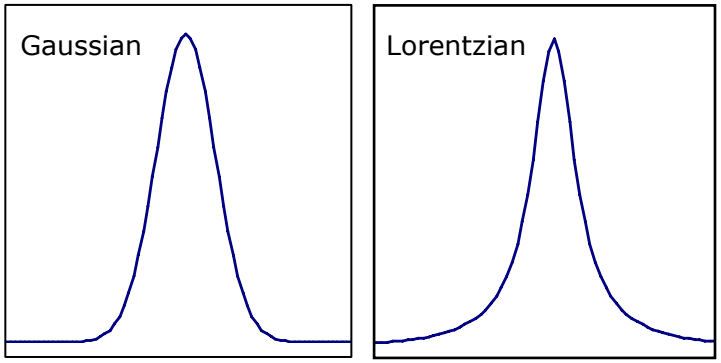
	Ions formed in a vacuum		Ions formed at atmospheric pressure			Ions formed in vacuum or atmospheric pressure
Ionisation mode	**Electron Ionisation (EI)**	**Chemical Ionisation (CI)**	**Electrospray Ionisation (ESI)**	**Atmospheric Pressure CI (APCI)**	**Atmospheric Pressure Photoionisation (APPI)**	**Matrix-Assisted Laser Desorption/Ionisation (MALDI)**
Types of compound	Non-polar, and moderately polar species, *e.g.* hydrocarbons, aromatics *etc*. Molecule must be volatile and thermally stable.	As for EI. Increased chance of detecting a molecular ion. Appropriate choice of reagent gas is required.	Any compound sufficiently basic (in gas phase) to accept a proton or other cation (positive mode), or sufficiently acidic to lose a proton (negative mode).	Many compounds which will not ionise by ESI will be protonated by APCI as stronger gas phase acids are present in source.	Optimised for non-polar compounds. New technique – range of applications being evaluated.	Wide range, from non-polar to ionic, can be analysed. Good for large molecules.
Nature of ionising mechanisms	Loss of electron leads to radical cation. Excess internal energy may result in significant fragmentation.	+ve ion: reaction with ionised reagent gas (*e.g.* ammonia or methane). Ionisation mostly by cation attachment. -ve ion: electron capture or anion attachment.	+ve ion: addition of cation (*e.g.* H^+, Na^+, NH_4^+). -ve ion: loss of proton or anion attachment. Molecular clusters are common.	+ve ion: addition of proton most common. -ve ion: electron capture.	+ve ion: addition of proton. -ve ion: electron capture.	+ve ion: radical cation or addition of proton. Molecular clusters also formed. -ve ion: electron capture or loss of proton.
Typical ions observed	+ve ion: $M^{+}\cdot$, $[M - H]^+$ -ve ion: EI not effective in negative mode	+ve ion: $[M + H]^+$, $[M + NH_4]^+$ -ve ion: $M^{-}\cdot$	+ve ion: $[M + H]^+$, $[M + Na]^+$, $[M + nH]^{n+}$ -ve ion: $[M - H]^-$, $[M + X]^-$	+ve ion: $[M + H]^+$ -ve ion: $M^{-}\cdot$, $[M - H]^-$	+ve ion: $M^{+}\cdot$, $[M + H]^+$ -ve ion: $M^{-}\cdot$	+ve ion: $M^{+}\cdot$, $[M + H]^+$, $[M_2 + H]^+$, $[M + 2H]^{2+}$ -ve ion: $M^{-}\cdot$,$[M - H]^-$
Fragmentation	Significant fragmentation. Very informative about structure of molecule.	Much less fragmentation than with EI; more likely to observe an ion closely related to the original molecule.	Low energy process; few fragments. Greater fragmentation by MS-MS or increased source voltages.	As for ESI.	As for ESI.	Little or no fragmentation. Greater fragmentation requires MS-MS.
Sample introduction	Directly from a temperature controlled probe or *via* a GC column.	As for EI.	Sample must be dissolved in an appropriate solvent.	As for ESI.	As for ESI.	Sample needs to be applied in an appropriate matrix.
Typical solvent	GCMS requires volatile non-polar solvent. GC injection temperature can dictate choice. Insoluble samples can be introduced as a solid if compound is sufficiently volatile and thermally stable.	As for EI.	Mixture of water/organic solvent with optional addition of electrolyte (*e.g.* formic acid or ammonium acetate); frequently typical reversed phase HPLC gradient mixtures. Solvent choice may be critical.	As for ESI. Also, hydrocarbon/alcohol mixtures as for normal phase HPLC.	As for APCI.	Solvent from which sample will form crystalline mixture with matrix. Choice of matrix and sample preparation can be critical.

4 INSTRUMENT SET-UP: KEY DEFINITIONS

4.1 Peak shape

- Each instrument will have its own 'optimum' peak shape. In order to achieve an accurate peak centroid and reproducible intensity measurements, a peak without any spikes or shoulders on the sides is essential. For all systems excluding TOFs the peak should be symmetrical about the centroid. Two common 'ideal' peak shapes are Gaussian and Lorentzian, shown in Figure 3. Note that peaks produced by TOF analysers tend to be asymmetric, but the asymmetry should be minimised.
- Poor peak shapes are often due to poor tuning and will result in incorrect identification of the peak centroid. This can lead to errors in mass assignment which could result in misidentification of samples. Poor peak shapes will also cause problems with mass resolution and sensitivity problems can arise.

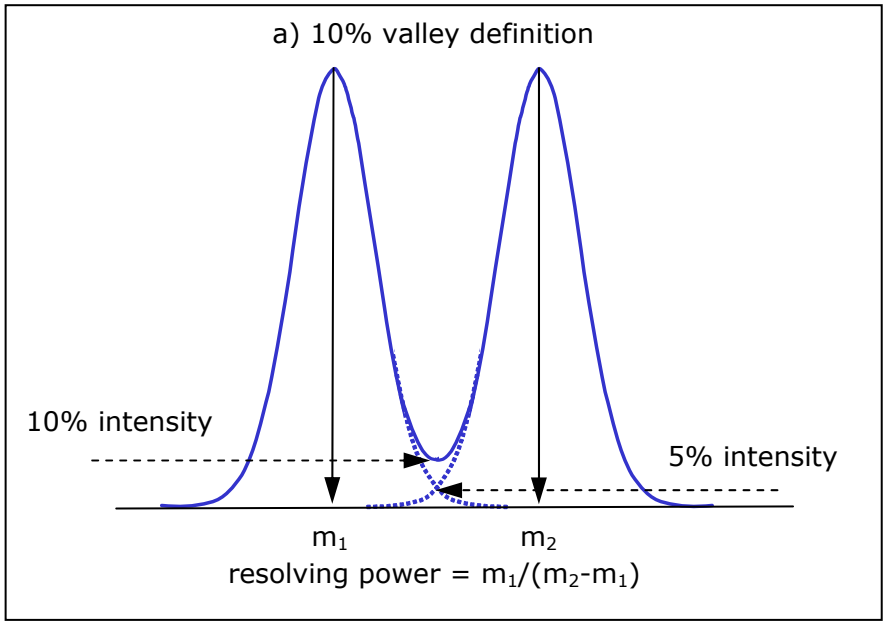
Figure 3: Examples of ideal peak shapes

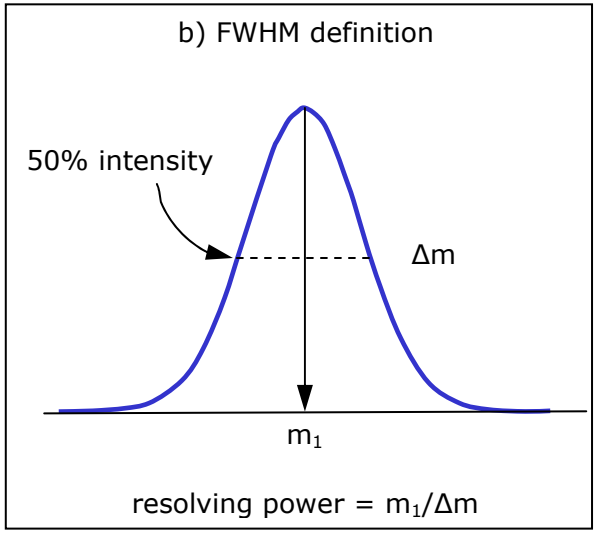


4.2 Mass resolution

- The resolving power is the ability of a mass spectrometer to separate ions of two different *m/z* values. It is defined as m/Δm, where m is the *m/z* value of a single-charged ion and Δm is the difference between m and the next highest *m/z* value ion that can be separated from m.
- Two different approaches to calculating the resolving power are used routinely, depending on the type of instrument employed. These are illustrated in Figure 4.
- The term 'mass resolution' is also used. This is defined as the smallest mass difference (Δm) between two equal magnitude peaks, such that the valley between them is a specified fraction of the peak height.
- The mass resolution should be correct for the analytical requirements of the sample. For instruments where mechanical slits are used (*e.g.* sector instruments) there is a trade-off between sensitivity and resolution - as resolution is increased the sensitivity decreases so a compromise often must be sought.

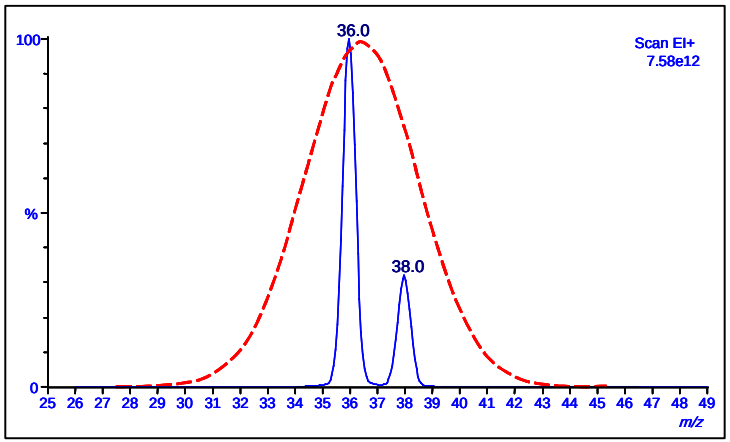
Figure 4: Definitions of resolving power





- ***10% valley definition***: this is useful only for instruments giving Gaussian peaks. Two peaks of equal intensity are considered to be resolved when they are separated by a valley which is 10% of the height of each peak (made up from a 5% contribution from each component) (Figure 4a). In practice, by this definition a resolving power of 1000 means that peaks at *m/z* 1000 and *m/z* 1001 have a 10% valley between them.
- ***Full width half maximum (FWHM) definition:*** the quadrupole, FT-ICR MS, ion trap and TOF definition is based on a peak width (Δm) measured at 50% peak height (Figure 4b).

Figure 5: Mass spectrum of HCl at low (broken line) and higher (solid line) resolution



- The effect of increasing mass resolution can be seen in the example in Figure 5. At low mass resolution the two peaks for the ions at *m/z* 36 and *m/z* 38 merge into one peak. Increasing the mass resolution enables both ions to be observed.

5 ACQUIRING A MASS SPECTRUM

5.1 General sequence

- The general sequence of actions when acquiring a mass spectrum is as follows: tune instrument, mass calibrate, acquire a background spectrum, analyse a test compound, analyse the sample. This sequence requires a number of essential checks on aspects of instrument performance before acquiring a mass spectrum, to ensure that spectra obtained for samples will be of acceptable quality. Initial instrument performance should be checked by acquiring the mass spectrum of a test compound using a defined protocol.

5.2 Instrument tuning

- In order to obtain an acceptable quality spectrum, the instrument must be tuned according to the instrument protocol to ensure good sensitivity and peak shape and to ensure that the mass resolution is appropriate for the analytical requirements of the sample.
- Figure 6 shows electrospray spectra of a sample containing two triterpene glucosides of relative molecular mass 1228.6 and 1212.6. Each figure shows the centroid spectrum (top) and continuum spectrum (bottom). The ions at *m/z* 1251.6 and *m/z* 1235.6 in Figure 6a are the sodiated adducts $[M + Na]^+$. The spectrum in Figure 6b was obtained for the same sample using an instrument that was badly tuned and gave a non-symmetric peak shape and low mass resolution.
- In Figure 6a, the centroid spectrum shows the full expected range of the ^{13}C isotope peaks for the molecular ions, but in Figure 6b these are absent. Note also that the values of the centroids in Figure 6b differ from those in Figure 6a.
- After the instrument has been tuned, check that the peak shape is satisfactory (see section 4.1).

Figure 6: Errors arising from poorly tuned instruments

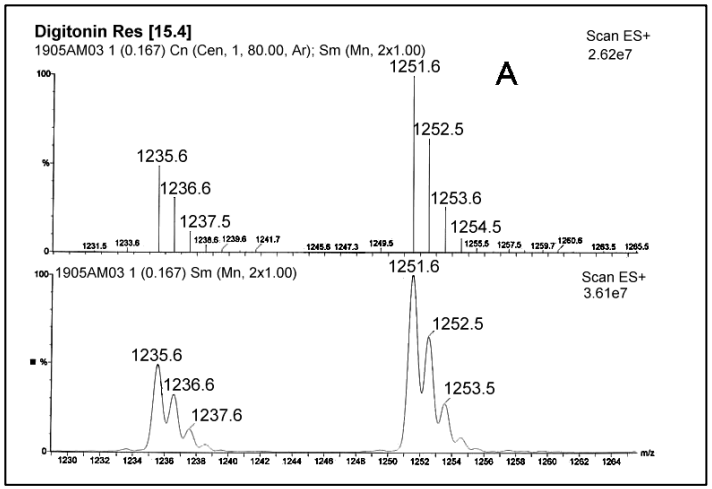


Figure 6a: Instrument correctly tuned

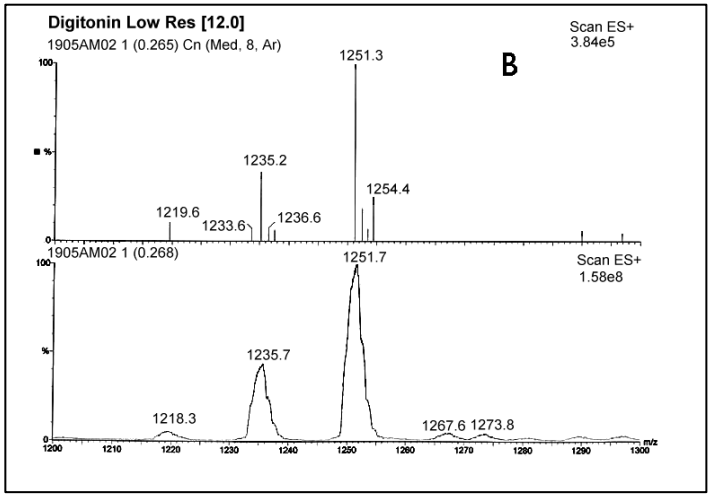


Figure 6b: Instrument incorrectly tuned

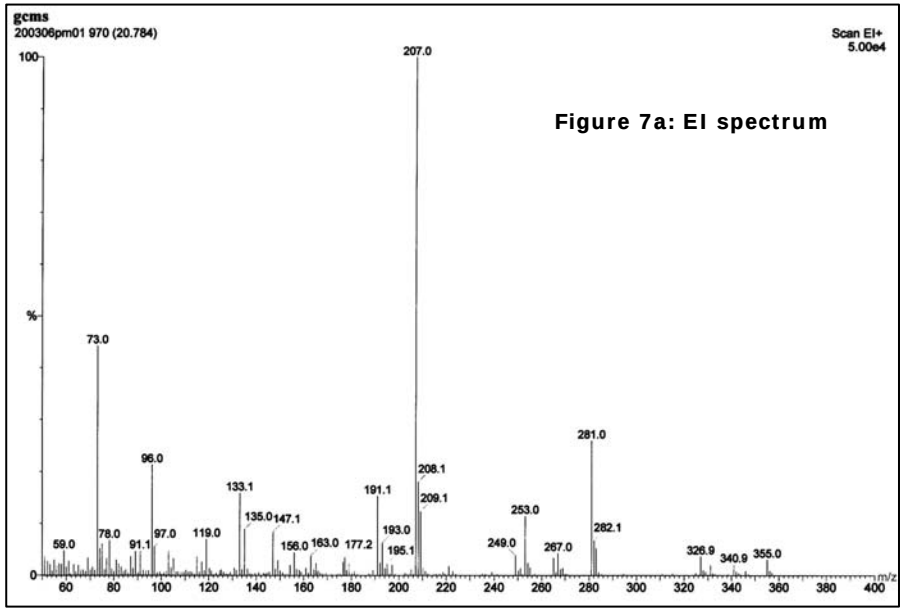
5.3 Mass calibration

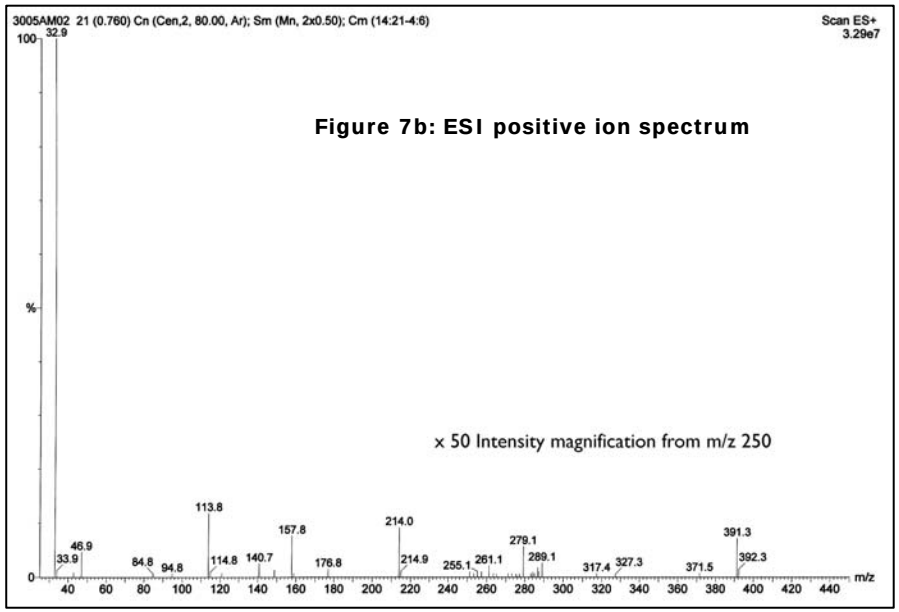
- Calibration of the *m/z* scale of the mass spectrometer is an important step in obtaining reliable mass spectra.
- Calibration typically involves analysing a calibration compound which yields ions of known *m/z*. The *m/z* scale is then adjusted to give the correct values for the calibration peaks.
- The exact calibration protocol, including the calibration compound to be used, will vary with the instrument and the ionisation mode – consult the instrument manual.
- Frequency of calibration will depend on the instrument and the reason for acquiring the mass spectrum. For example, mass calibration is one of the most critical parameters when undertaking accurate mass measurements.
- The mass calibration should cover the complete range of analyte masses.

5.4 Background spectrum

- Acquire a background spectrum before analysing the sample to check for contaminants that may be present in the instrument (see section 9 for a list of common background ions).
- Figure 7a shows an EI spectrum of typical 'bleed' from a non-polar GC column. Most of the signals arise from the methyl silicone compounds bonded in the column. Figure 7b is the ESI positive ion spectrum for a 1:1 v/v mixture of water and methanol with 0.05% formic acid, being introduced at 10 μL/min. Apart from solvent clusters a number of ions from plasticisers can be identified.

Figure 7: Typical background spectra





5.5 Checking instrument performance

Figure 8 summarises the aspects of instrument performance that should be checked and lists some of the common causes of problems.

Figure 8: Instrument performance troubleshooting

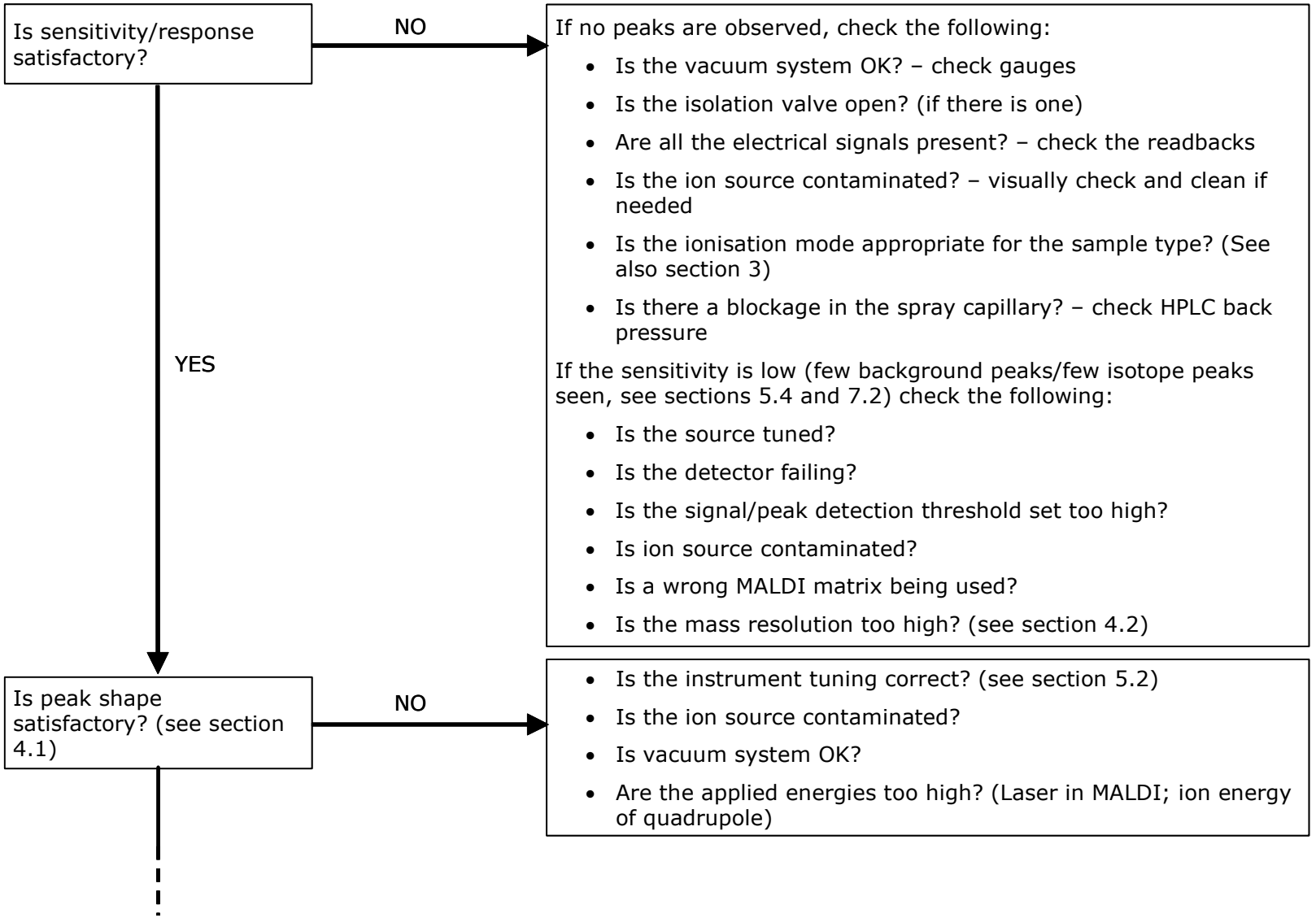
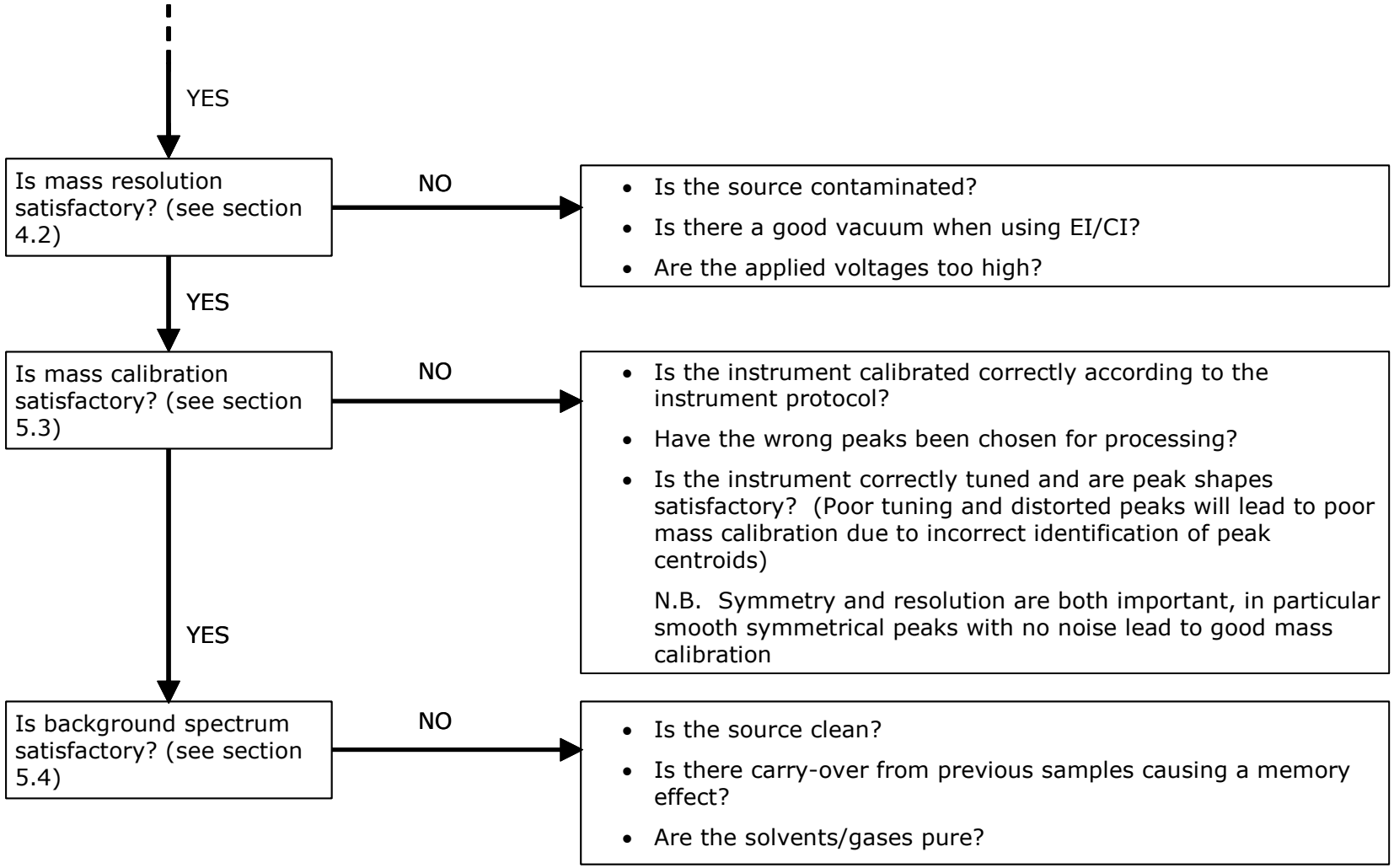


Figure 8 continued



6 EVALUATING SPECTRUM APPEARANCE

- In a mass spectrum the x-axis represents the mass-to-charge ratio (*m/z*) and the y-axis ion abundance, usually shown as 0 to 100%. There are two popular forms of mass spectrum. In one, the continuum or analogue spectrum, the full profile of each ion peak is shown. Such data recording takes up a lot of storage space and is commonly simplified as the centroid (histogram or 'stick') spectrum. Here only two pieces of data are recorded, the peak centroid (*i.e.* the *m/z* value) and the maximum intensity (*i.e.* the ion abundance). These are plotted with the ion abundance of the most intense peak (base peak) normalised to 100%. The two types of spectrum are shown in Figure 10.

6.1 What to look for in a good quality mass spectrum

In a valid and good quality mass spectrum the following should all be apparent:

- Significant peaks mass-labelled to an appropriate number of decimal places (*i.e.* appropriate to the mass resolution of the instrument, but to at least one decimal place for ions >*m/z* 500).
- Mass differences of adjacent related ions consistently equal to 1 Da/n (for ions of charge = n). N.B. Da (Dalton) is a non-SI unit of mass and is the term used to describe a mass unit.
- Mass peaks appear as resolved isotope clusters [exceptions: ion is multiply charged and instrument is low resolution; the species is 100% mono-isotopic (very unusual)].
- Continuum data show adequate resolution of singly charged ions.
- Spectrum abundance is normalised to the most abundant ion.
- Abundance of largest peak(s) does not saturate detector (peaks are not 'off-scale').

6.2 Spectrum information

- Should **include at least:** Ionisation mode and polarity used.
- **Good laboratory practice would also require:**

  Date, instrument name/type, additives (solvents, matrices, reagents, *etc.*).

  All post-acquisition data processing (*e.g.* details of smoothing).

  Labelling of magnified areas (with degree of magnification and range to which applied).

- **Additional useful information**

  Sample concentration.

  Location/name of data file.

Figure 9: Mass spectrum (centroid) for naphthalene ($C_{10}H_8$)

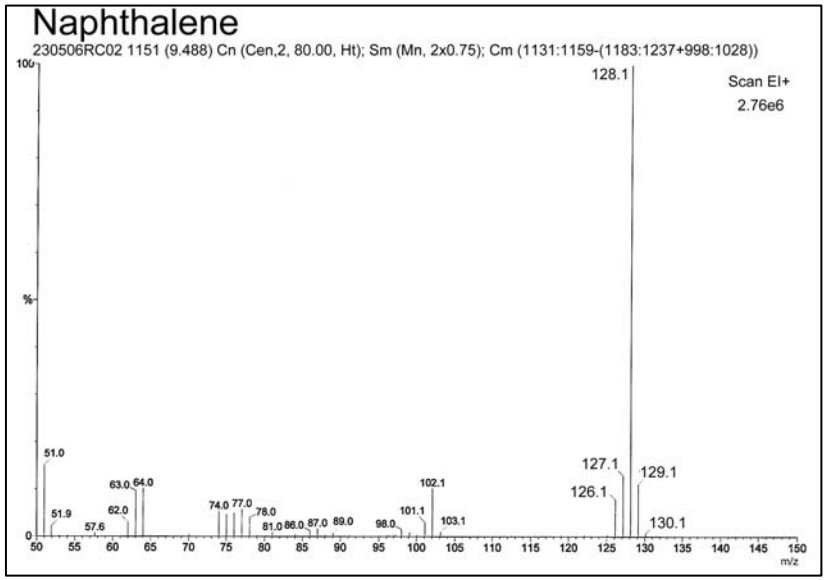


Figure 9 features of note:

- The mass calibration is good:
  - the measured mass of the molecular ion ($M^{+\cdot}$) at *m/z* 128.1 is consistent with the molecular mass for naphthalene.
  - significant peaks are mass-labelled to an appropriate number of decimal places.
- The mass resolution is good: the molecular ion is clearly visible together with its ^{13}C isotope peak at the correct ratio for this compound. The molecular ion $^{13}C_2$ isotope peak is also present (at *m/z* 130).
- Ionisation mode and polarity are shown (EI positive).

Figure 10: Molecular ion region of mass spectrum for ketoconazole (centroid and continuum)

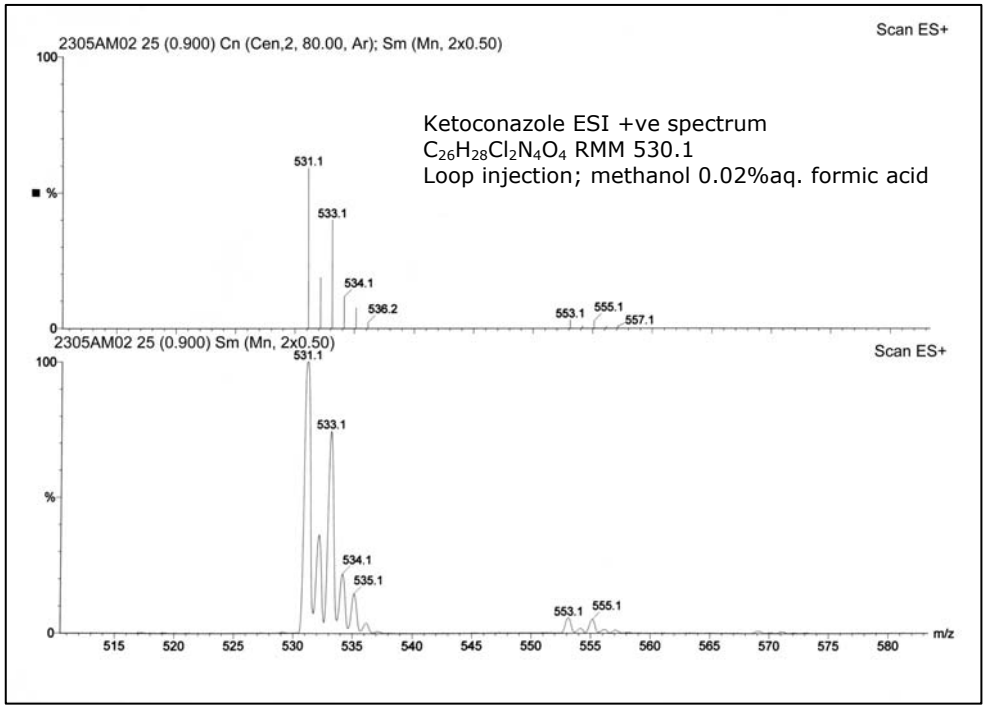


Figure 10 features of note:

- The mass calibration is good:
  - the measured mass of the $[M + H]^+$ ion at *m/z* 531.1 is consistent with the expected molecular mass for ketoconazole.
  - significant peaks are mass-labelled to an appropriate number of decimal places.
- The mass resolution is good: mass peaks appear as resolved isotope clusters. The ions at *m/z* 531.1 and *m/z* 533.1 represent the isotope pattern for a molecule containing two chlorine atoms (see section 7.2). (The ions at *m/z* 553.1 and *m/z* 555.1 are sodium adducts $[M + Na]^+$).
- Ionisation mode and polarity are shown (ESI positive).

7 MOLECULAR SPECIES RECOGNITION

7.1 Definitions of molecular mass

There are different approaches to calculating the molecular mass:

- **Average** – calculated using the average mass of each element weighted for its natural isotopic abundance. This is the value chemists usually use when calculating molecular masses.
- **Nominal** – calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value.
- **Monoisotopic** – calculated using the mass of the most abundant isotope of each element.
- **Exact** – calculated using the exact mass of a single isotope (most frequently the lightest isotope) of each element present in the molecule.
- Note that in each case it is actually the *relative* molecular mass that is being calculated, using the appropriate relative masses of the elements that make up the molecule. The relative mass of an element is defined as the mass of one atom relative to one-twelfth of the mass of one atom of ^{12}C. Relative masses therefore have no units.
- As an example, consider a compound of formula $C_{33}H_{40}N_2O_9$:

Using Average Atomic Masses:	**Using Nominal Masses:**	**Using Monoisotopic Masses:**
C: 33 x 12.011 = 396.363	C: 33 x 12 = 396	C: 33 x 12.0000 = 396.0000
H: 40 x 1.0079 = 40.316	H: 40 x 1 = 40	H: 40 x 1.0078 = 40.3120
N: 2 x 14.0067 = 28.013	N: 2 x 14 = 28	N: 2 x 14.0031 = 28.0062
O: 9 x 15.9994 = 143.995	O: 9 x 16 = 144	O: 9 x 15.9949 = 143.9542
Average molecular mass = 608.687	Nominal molecular mass = 608	Monoisotopic molecular mass = 608.2723

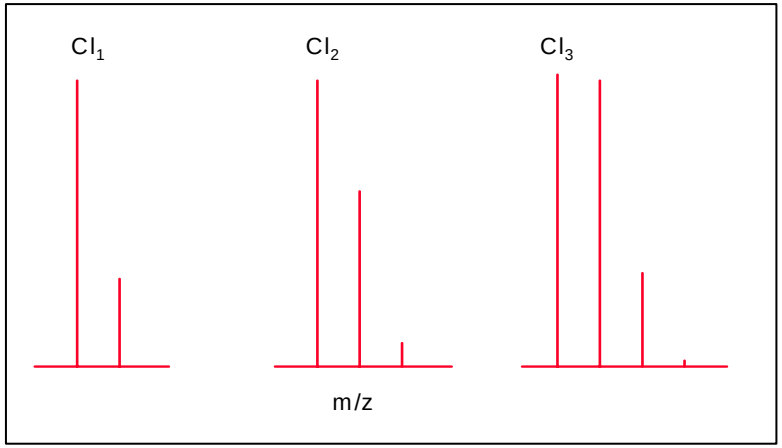
- Note that different calculations for molecular mass are used by mass spectrometrists and other chemists. Average molecular masses are generally used by chemists to calculate molecular masses, nominal molecular masses are used by mass spectrometrists analysing small molecules (<800 Da) on low resolution instruments and exact molecular masses are used by mass spectrometrists using high resolution instruments.
- The term 'accurate mass' is also used. This refers to an experimentally determined mass of an ion, measured with high precision and accuracy, generally to determine an elemental formula. For accurate mass measurement, the lowest mass isotope is measured whenever possible, to avoid measuring isotopically mixed species. However, when the intensity of the lowest mass peak is too low, the monoisotopic or another peak of the cluster must be measured, and account taken of the mixed nature of this peak.

7.2 Use of stable isotope information

Naturally occurring carbon exists principally as a mixture of two isotopes, ^{12}C (98.9%) and ^{13}C (1.1%). Hence for every carbon atom present there is a 1.1% chance that it will be ^{13}C not ^{12}C.

- A spectrum of a compound containing 10 carbon atoms will show a signal with an abundance of 11% of that of the molecular ion at one *m/z* higher.

Figure 11: Isotope patterns for 1, 2 and 3 chlorine atoms



- Elements with distinctive isotopes will manifest themselves by the patterns of the isotope clusters in a spectrum (*e.g.* ^{35}Cl and ^{37}Cl) as shown in Figure 11. Naturally occurring chlorine is a mixture of two isotopes ^{35}Cl (75.5%) and ^{37}Cl (24.5%). Thus a compound containing one chorine atom will display two isotope peaks (^{35}Cl and ^{37}Cl), a compound containing two chlorine atoms will display three isotope peaks ($^{35}Cl_2$, $^{35}Cl^{37}Cl$ and $^{37}Cl_2$) and a compound containing three chlorine atoms will display four isotope peaks ($^{35}Cl_3$, $^{35}Cl_2{}^{37}Cl$, $^{35}Cl^{37}Cl_2$ and $^{37}Cl_3$).

7.3 How to find the molecular species

In this document, 'molecular species' refers to the molecular ion or an adduct ion containing the intact molecule. The molecular species observed will depend on the ionisation mode used (see Table 1), for example $M^{+\cdot}$ in EI (see spectrum of naphthalene in Figure 9), $[M + H]^+$ in ESI (see spectrum of ketoconazole in Figure 10).

To find the molecular species:

- Calculate the nominal molecular mass of the analyte (see 7.1).
- In MALDI and atmospheric pressure ionisation methods check for cation adducts, $[M + Na]^+$, $[M + Solvent + H]^+$ *etc.*, $[2M + H]^+$ and $[M + nH]^{n+}$; or anions if using negative ion mode. For example, in the ESI spectrum of ketoconazole (Figure 10) the peaks at *m/z* 553.1 and *m/z* 555.1 represent sodium adduct ions.
- Apply the Nitrogen Rule: A compound containing C, H, O, N, S, P, or a halogen will have an *odd* numbered molecular mass if it contains an *odd* number of nitrogen atoms. The molecular mass will be an *even* number if there is *zero* or an *even* number of nitrogen atoms.
- Look for the peak with highest *m/z* value, allowing for isotope patterns.
- The *m/z* value of the molecular ion should be accurate to better than ±0.2 *m/z* units.
- In EI check that fragment ions have possible *m/z* values (*i.e.* not differing by 3-13 and 21-25 units). Be prepared for a vanishingly small signal with increasing mass, *e.g.* hydrocarbons.
- Note that for EI mass spectra some compounds do not show a molecular ion at all *e.g.* many phthalate esters.

7.4 Questions to ask about the validity of the proposed molecular species

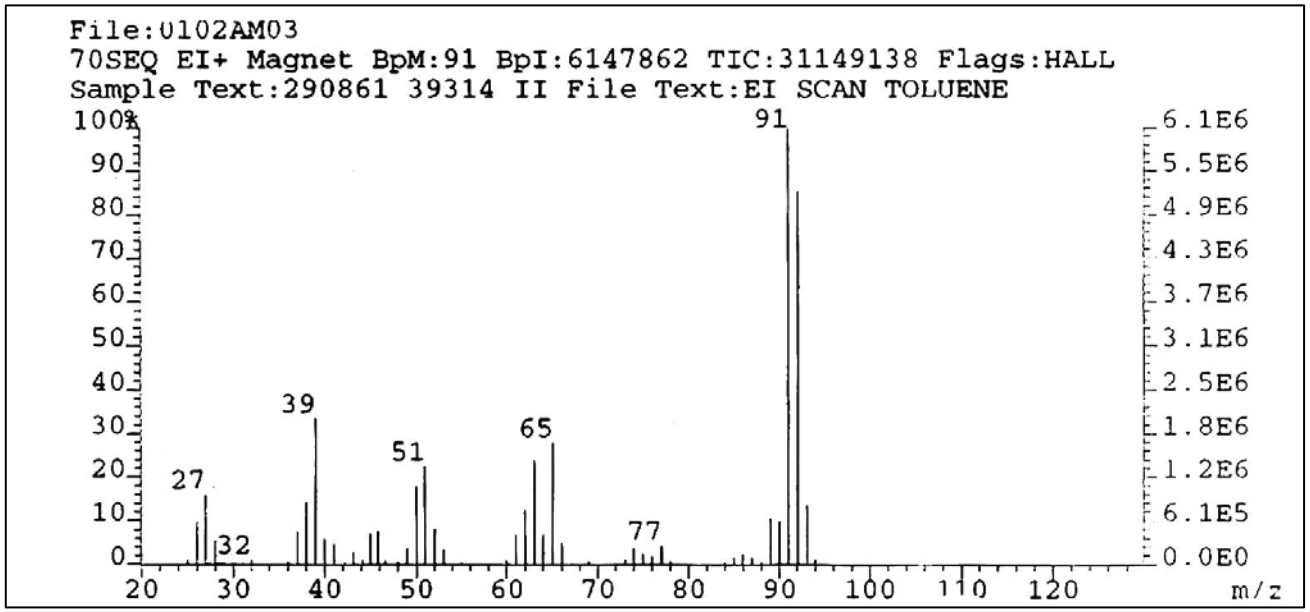
- Does the observed isotope pattern match the theoretical one? *E.g.* in the ketoconazole spectrum (Figure 10) the ion at *m/z* 531.1 is the $[M + H]^+$ (^{35}Cl). The presence of the ion at *m/z* 533.1, and its abundance, is consistent with the $^{35}Cl^{37}Cl$ isotope peak for a molecule containing two chlorine atoms.
- Is the measured mass of the molecular ion consistent with the expected mass (within the expected precision and mass calibration of the instrument)?
- Is the signal/noise (S/N) ratio of major isotopes at least 3:1?
- Are the proposed molecular species the highest *m/z* species in the spectrum? [Possible exceptions: (predictable) adducts and/or dimer and fragments of dimer may occur with soft ionisation such as CI, ESI, APCI, APPI and MALDI where little or no fragmentation occurs]. In the ketoconazole spectrum the ions at *m/z* 553.1 and *m/z* 555.1 are sodiated adducts.
- Was the scan range to a sufficiently high mass to allow the point above to be properly answered? (100 *m/z* units above the highest observed mass should generally be adequate).
- Are the proposed molecular species separated from other (lower *m/z*) ions by masses corresponding to feasible chemical entities? In the spectrum of toluene (Figure 12) the loss of one *m/z* unit from the molecular ion is acceptable ($H^\bullet$ loss) and the next fragment ion 15 *m/z* units below 92 *m/z* is also reasonable ($CH_3^\bullet$ loss).

7.5 What if the expected molecular species is not visible in the spectrum?

- Has the correct sample been analysed?
- Are expectations wrong? [Check the molecular formula and calculated mass! Is the Nitrogen Rule obeyed?]
- Has an inappropriate ionisation mode been used?
- Has the wrong polarity (positive or negative ionisation) been applied?
- Are the MS ionisation conditions too harsh, thus causing significant fragmentation (*e.g.* cone voltage in ESI; use of 'hard' ionisation, like EI)?
- Are the MS physical conditions causing sample decomposition? (*e.g.* source temperature in EI/CI)
- Are analyte additives (*e.g.* solvent, acid) causing sample decomposition or other changes?
- Is the sample not reaching the ionisation source? (*e.g.* too volatile for probe analysis; insoluble for ESI)
- Is ionisation of the expected molecular species being suppressed by other species present (*e.g.* because the sample is a mixture)?
- Has an appropriate amount of sample been used for the analysis? (Remember too much sample can be an issue)
- Are 'memory' effects being observed (due to 'carry-over' of previous sample(s) analysed)?
- Was the spectrometer performing to specification at the time of measurement?
- Was the mass calibration correct?

8 FURTHER EXAMPLES OF MASS SPECTRA

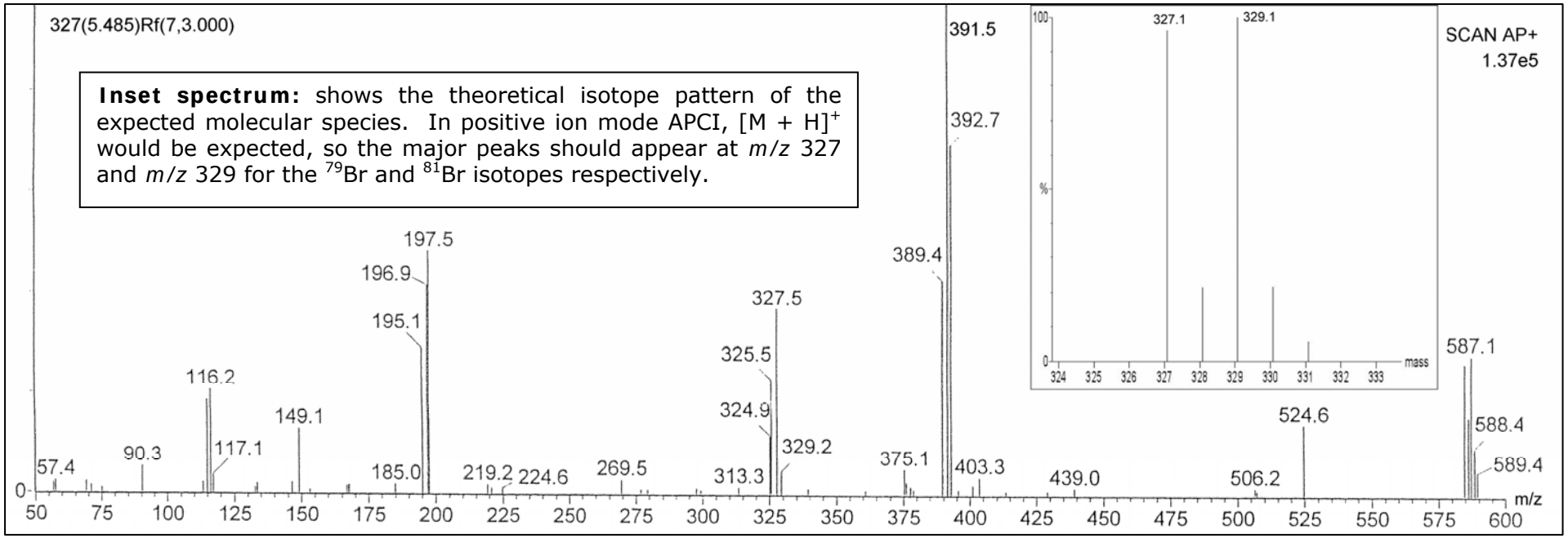
Figure 12: Good quality mass spectrum of toluene (C_7H_8)



This spectrum of toluene is good because:

- The mass calibration is good. Significant peaks are mass labelled to an appropriate number of decimal places.
- The measured mass of the molecular ion ($M^{+\cdot}$) at *m/z* 92 is consistent with the expected molecular mass for toluene.
- Mass difference of adjacent related ions consistently equal to 1 *m/z* unit.
- The mass resolution is good. Mass peaks appear as resolved isotope clusters, the ions at *m/z* 93 and *m/z* 94 are the ^{13}C containing isotope ions.
- Ionisation mode and polarity are shown (EI positive).
- In the case of electron ionisation, the molecular ion cluster is the highest *m/z* species in the spectrum.
- The molecular ion cluster is separated from other, lower *m/z* ions by masses corresponding to feasible chemical entities.

Figure 13a: Poor quality mass spectrum of $C_{15}H_{23}OSiBr$ (nominal molecular mass = 326 using ^{79}Br)



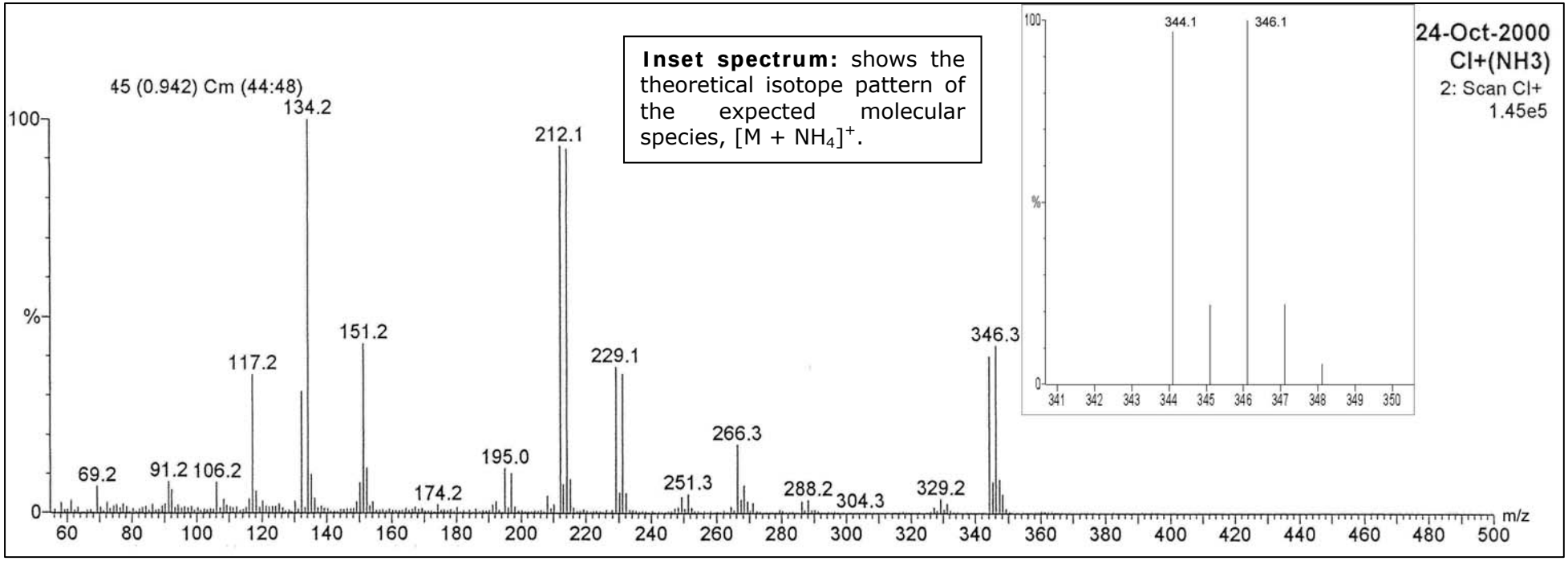
Good points of Spectrum 13a:

- Significant peaks are mass-labelled to an appropriate number of decimal places.
- Header information includes ionisation mode and polarity used (AP+ = APCI +ve ion) and post acquisition processing information [Rf(7,3.000)].
- Signal/noise ratio of major isotopes is at least 3:1.

Bad points of Spectrum 13a:

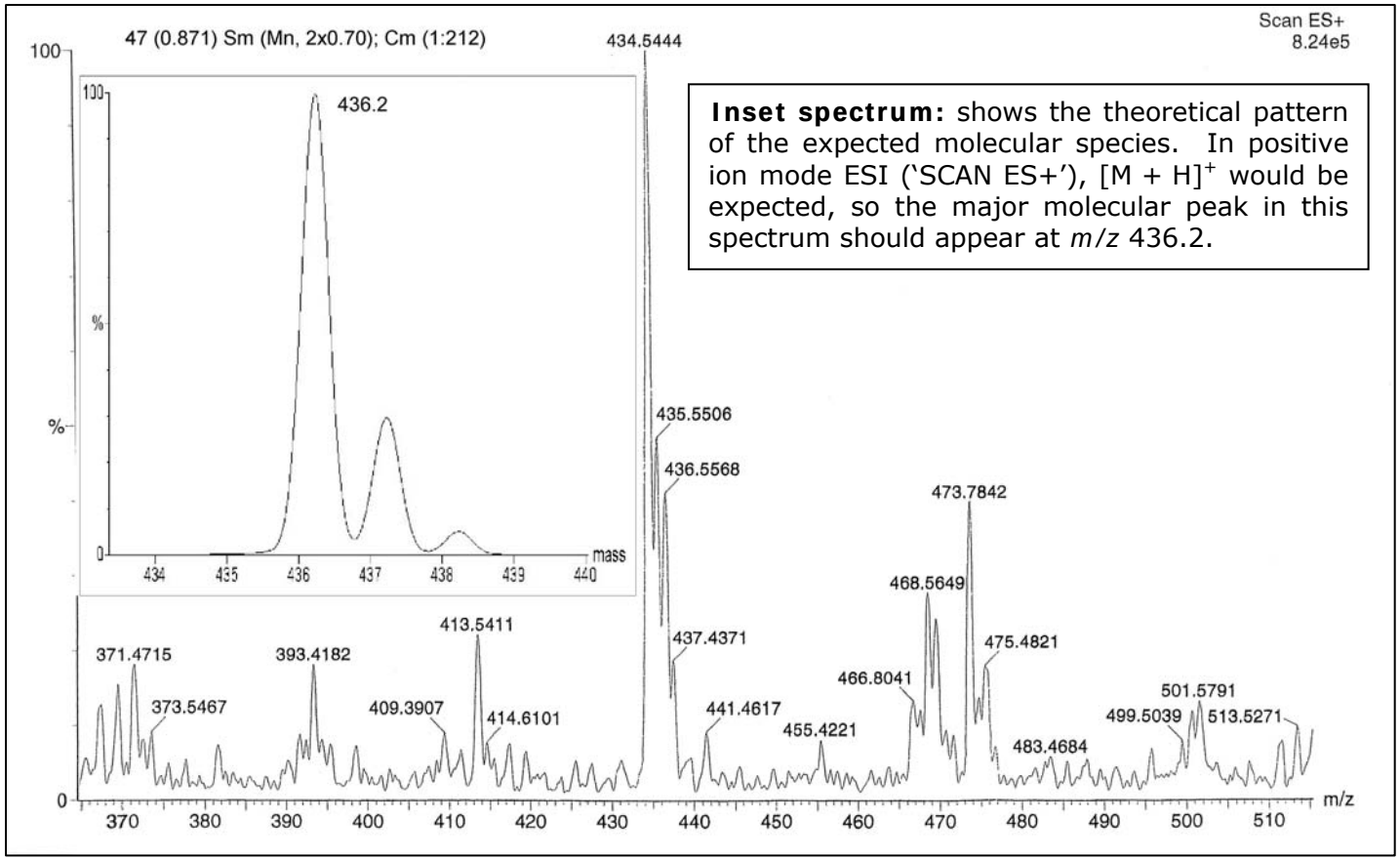
- Mass difference of adjacent related ions is not consistently equal to 1 *m/z* unit, *e.g.* peaks at *m/z* 389.4, 391.5, 392.7; peaks at 195.1, 196.9, 197.5.
- Isotope clusters are not resolved, *e.g. m/z* 524.6 has no isotope peaks.
- There is no information given on date of analysis or solvents/additives.
- Isotope pattern incorrect: isotope ratio should be *ca.*1:1 at *m/z* 327 and 329 ([M + H]+ of ^{79}Br and ^{81}Br isotopes respectively). Also, the measured masses of these two ions are not consistent with the expected masses.
- Scan range used was not sufficiently high: higher *m/z* ions are evident, and, although the mass range is sufficiently above the expected molecular ion, because the limit is close to the highest observed ions, at *m/z* 585/587, it is not possible to know whether these ions represent molecular or fragment species (if this occurs and there is ambiguity, analysis should be repeated to a higher *m/z*).

Figure 13b: Improved mass spectrum of $C_{15}H_{23}OSiBr$



This is an example of a better quality mass spectrum of the same sample used for Figure 13a, obtained using a different ionisation mode (*i.e.* ammonia CI rather than APCI). Note how the higher *m/z* species are no longer present, indicating that they were artefacts, and thus show another symptom of a poor quality spectrum. The molecular species observed is $[M + NH_4]^+$.

Figure 14: Poor quality mass spectrum of $C_{25}H_{29}N_3O_4$ (nominal molecular mass = 435)



Inset spectrum: shows the theoretical pattern of the expected molecular species. In positive ion mode ESI ('SCAN ES+'), $[M + H]^+$ would be expected, so the major molecular peak in this spectrum should appear at *m/z* 436.2.

Bad points of Spectrum 14:

- Mass accuracy is poor: the mass of the expected molecular ion (monoisotopic) is *m/z* 436.2 (inset spectrum) whereas the ^{12}C peak is observed at *m/z* 434.5.
- Because of the lack of mass accuracy, the number of decimal places used in peak labelling (four) is inappropriate.
- Inadequate mass resolution: The isotope peaks are not completely resolved.
- No information is given on date of analysis or solvents/additives used.
- A wider scan range should have been used, considering the fact that higher *m/z* ions are present.

9 COMMON BACKGROUND IONS

- The peaks you observe in your spectrum may not be from your compound of interest, they can arise from impurities in your sample (*e.g.* residual solvent, phthalate plasticisers) or may be present in the spectrometer (*e.g.* contamination of the instrument from the analysis of previous samples) – acquire a background spectrum before running your sample (see section 5.4).

9.1 Electrospray ionisation

9.1.1 Positive ion

m/z	Ion	Compound	*m/z*	Ion	Compound
42	$(M + H)^+$	acetonitrile	236	$(M + Na)^+$	N-butyl benzenesulfonamide
59	$(M + NH_4)^+$	acetonitrile	239/241	$[(M.HCl)_2\text{-}Cl]^+$	triethylamine
64	$(M + Na)^+$	acetonitrile	242	M^+	tetrabutylammonium $(C_4H_9)_4N^+$
79	$(M + H)^+$	dimethyl sulfoxide (DMSO)	243	M^+	trityl cation
85	$(M + H)^+$	d_6-DMSO	257	$(3M + Na)^+$	DMSO
101	$(M + Na)^+$	DMSO	268	$(M + Na + MeOH)^+$	N-butyl benzenesulfonamide
102	$(M + H)^+$	triethylamine (TEA)	273	M^+	monomethoxytrityl cation (MMT)
104/106	$(M + Cu)^+$	acetonitrile	303	M^+	dimethoxytrityl cation (DMT)
122	$(M + H)^+$	tris buffer (tris(hydroxymethyl)-aminomehtane	338	$(M + H)^+$	erucamide
123	$(M + H)^+$	dimethylaminopyridine (DMAP)	360	$(M + Na)^+$	erucamide
130	$(M + H)^+$	diisopropylethylamine (DIPEA)	391	$(M + H)^+$	diisooctyl phthalate (plasticiser)
144	$(M + H)^+$	tripropylamine (TPA)	409	$(M + Na)^+$	slip agent from plastic tips
145/147	$(2M + Cu)^+$	acetonitrile	413	$(M + Na)^+$	diisooctyl phthalate (plasticiser)
153	$(M + H)^+$	1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)	429	$(M + K)^+$	diisooctyl phthalate (plasticiser)
157	$(2M + H)^+$	DMSO	449	$(2M + H)^+$	dicyclohexyl urea (DCU)
169	$(2M + H)^+$	d_6-DMSO	454	$(M + Na + CH_3CN)^+$	diisooctyl phthalate (plasticiser)
179	$(2M + Na)^+$	DMSO	798	$(2M + NH_4)^+$	diisooctyl phthalate (plasticiser)
214	$(M + H)^+$	N-butyl benzenesulfonamide	803	$(2M + Na)^+$	diisooctyl phthalate (plasticiser)
225	$(M + H)^+$	dicyclohexyl urea (DCU)	819	$(2M + K)^+$	diisooctyl phthalate (plasticiser)

9.1.2 Negative ion

m/z	Ion	Compound
35/37	M^-	chloride
45	$(M - H)^-$	formic acid
59	$(M - H)^-$	ethanoic acid
79/81	M^-	bromide
97	$(M - H)^-$	phosphoric acid
97	$(M - H)^-$	sulfuric acid
97	M^-	phosphate $(H_2PO_4)^-$
113	$(M - H)^-$	trifluoroacetic acid
113	$(2M - Na)^-$	sodium formate (see cluster series in 9.1.3)
127	M^-	iodide
212	$(M - H)^-$	N-butyl benzenesulfonamide
227	$(2M - H)^-$	trifluoroacetic acid
249	$(2M - Na)^-$	sodium trifluoroacetate (see cluster series in 9.1.3)

9.1.3 Oligomeric series/cluster ions (positive or negative ionisation)

44 *m/z* units apart	ethoxylate
58 *m/z* units apart	propoxylate
68 *m/z* units apart	sodium formate
74 *m/z* units apart	dimethylsiloxane (*e.g.* from septa)
114 *m/z* units apart	trifluoroacetic acid
136 *m/z* units apart	sodium trifluoroacetate

9.2 Electron ionisation

m/z	Ion	Compound	*m/z*	Ion	Compound
18	$M^{+\cdot}$	water	167	F^+	phthalate
28	$M^{+\cdot}$	nitrogen	205	F^+	butylated hydroxytoluene (BHT)
32	$M^{+\cdot}$	oxygen	220	$F^{+\cdot}$	butylated hydroxytoluene (BHT)
40	$M^{+\cdot}$	argon	207	F^+	polydimethylsiloxane
44	$M^{+\cdot}$	carbon dioxide	281	F^+	polydimethylsiloxane
73	F^+ (fragment)	trimethylsilyl (TMS)	355	F^+	polydimethylsiloxane
147	F^+	polydimethylsiloxane	429	F^+	polydimethylsiloxane
149	F^+	phthalate $[C_6H_4(CO)_2OH]^+$	446	$F^{+\cdot}$	polyphenol ether diffusion pump oil

9.2.1 Oligomeric series

74 *m/z* units apart	dimethylsiloxane (*e.g.* from septa)

10 GLOSSARY OF TERMS

Accurate mass	Experimentally determined mass of an ion used for the determination of an elemental formula.
Analyser	See mass analyser.
APCI	Atmospheric pressure chemical ionisation: chemical ionisation that takes place using a nebulised liquid and atmospheric pressure corona discharge (c.f. chemical ionisation which takes place at reduced pressure).
API	Atmospheric pressure ionisation: any ionisation process in which ions are formed in the gas phase at atmospheric pressure.
APPI	Atmospheric pressure photoionisation: atmospheric pressure chemical ionisation in which the reactant ions are generated by photoionisation.
CE	Capillary electrophoresis: separation of dissolved ionic species by migration under the influence of a voltage gradient in a capillary containing a buffer.
CEC	Capillary electrochromatography: separation as for CE but using a capillary packed with an HPLC stationary phase with the added impetus of a flowing buffer.
Centroid	The centre of mass of a peak. It is the point at which the *m/z* for the peak is measured. A centroid (histogram or 'stick') spectrum shows the *m/z* value (x-axis) and the ion abundance (y-axis).
CI	Chemical ionisation: Formation of a new ion in the gas phase by the reaction of a neutral species with an ion. The process may involve the transfer of an electron, a proton or other charged species between the reactants.
Da	Dalton: non-SI unit of mass equal to the unified atomic mass unit, *u* (*u* = one-twelfth of the mass of one atom of ^{12}C).
DCI	Desorption chemical ionisation: chemical ionisation of a solid sample by vaporisation from a conductive filament in the presence of a reagent gas.
DEI	Desorption electron ionisation: electron ionisation of a solid sample by vaporisation from a conductive filament in the presence of an electron beam.
Detector	That part of a mass spectrometer that detects the ions after mass separation.
DIP	Direct insertion probe: a system for introducing a single sample of a solid or a liquid (usually contained in a quartz or other non-reactive holder) into a mass spectrometer ion source.
EI	Electron ionisation: ionisation of an atom or molecule by electrons that are typically accelerated to energies between 10 and 150 electron volts (eV) in order to remove one or more electrons from the molecule; 70eV is the accepted norm for acquisition of EI spectra.
ES	Electrospray: see ESI.

ESI	Electrospray ionisation: a process in which ionised species in the gas phase are produced from a solution *via* highly charged fine droplets, by means of spraying the solution from a narrow-bore needle tip at atmospheric pressure in the presence of a high electric field (1000 to 10000 eV).
FAB	Fast atom bombardment: the ionisation of any species by the interaction of a focused beam of neutral atoms, having a translational energy of several thousand eV, with a sample that is typically dissolved in a solvent matrix.
FD	Field desorption: the formation of gas-phase ions from a material deposited on a solid surface.
FI	Field ionisation: ionisation by the removal of electrons from any species, usually in the gas phase, by interaction with a high electric field.
FIA	Flow injection analysis: a sample introduction system by which a sample is injected into a continuous liquid flow that enters into the mass spectrometer.
FT-ICR	Fourier transform ion cyclotron resonance: a type of ion trap mass analyser based on the principle of ion cyclotron resonance in which an ion in a (very strong) magnetic field moves in a circular orbit at a frequency characteristic of its *m/z* value.
GC	Gas chromatograph: a system for separating the volatile components of a mixture (after conversion to the gas phase) using a suitable column as the stationary phase and a carrier gas as the mobile phase. When used with a mass spectrometer the carrier gas is usually helium.
HPLC	High performance liquid chromatography: a system for separating the components of a solution or liquid mixture, using a suitable solvent as , the mobile phase, and chemically coated material packed into a tube ("column") as the stationary phase.
Inlet	The sample introduction system of a mass spectrometer.
Ionisation mode	The method of ionisation used to produce sample ions for separation in the mass spectrometer analyser.
Ion trap	Usually this term refers to a Quadrupole Ion Trap (q.v.). The FT-ICR (q.v.) and Orbitrap (q.v.) are also types of ion trap.
Isotopes	Atoms of the same element that have the same number of protons but a different number of neutrons in the nucleus and consequently have different masses (*e.g.* ^{12}C and ^{13}C – both have 6 protons in the nucleus but ^{12}C has 6 neutrons whereas ^{13}C has 7).
LDI	Laser desorption/ionisation: formation of gas phase ions by the interaction of photons from a pulsed laser with a solid or liquid material.
LSI	Liquid secondary ionisation: the ionisation of any species by the interaction of a focused beam of ions with a sample that is dissolved in a solvent matrix.
Magnetic sector	A type of mass analyser which accelerates ions through a perpendicular magnetic field in a curved flight path to separate ions of different mass (momentum). For accurate mass measurement a "double focussing" mass spectrometer is used where the magnetic sector is coupled with an electric sector to minimise energy dispersion and thus achieve higher resolution. The term "magnetic sector" is often used when actually referring to a "double focussing" mass spectrometer.

MALDI	Matrix-assisted laser desorption/ionisation: formation of gas-phase ions from molecules that are present in a solid or liquid matrix that is irradiated with a pulsed laser.
Mass analyser	That part of a mass spectrometer that separates a mixture of ions according to their mass-to-charge ratio through the application of electric and magnetic fields.
Mass resolution	The smallest mass difference (Δm) between two equal magnitude peaks, such that the valley between them is a specified fraction of the peak height.
Mass resolving power	In a mass spectrum, this is the observed mass divided by the difference between two masses that can be separated (m/Δm).
Mass spectrum	Plot of the relative abundance of a beam or other collection of ions as a function of their *m/z* values.
Molecular ion	An ion formed by the removal of one or more electrons to form a positive ion or the addition of one or more electrons to form a negative ion.
Nitrogen rule	The rule stating that an organic molecule containing the elements C, H, O, S, P, or a halogen has an odd nominal mass if it contains an odd number of nitrogen atoms.
Orbitrap	A type of ion trap. Ions are orbitally trapped and oscillate harmonically along the trap axis. The oscillation frequency is inversely proportional to the square root of *m/z*.
Quadrupole	A type of mass analyser, consisting of four parallel rods whose centres form the corners of a square and whose opposing poles are connected, that separates ions based on oscillations in an electric field (the quadrupole field) created with the use of radio frequency and direct current voltages.
Quadrupole ion trap	(Often called just "ion trap") A type of mass spectrometer analyser that confines ions using electric and/or magnetic fields and then selectively ejects ions of different *m/z* by ramping the rf voltage. The ion trap is frequently used for high order fragmentation studies ("MS^n") because it allows successive series of trapping and fragmentation.
Relative molecular mass	The mass of one molecule of a compound, with specified isotopic composition, relative to one-twelfth of the mass of one atom of ^{12}C.
SALDI	Surface-assisted laser desorption/ionisation: a type of MALDI using a specially prepared surface to take the role of the matrix.
SFC	Supercritical fluid chromatography: chromatographic separation in packed capillary HPLC or GC columns using a supercritical fluid as the mobile phase. A pressure reduction device is required at the exit of the column.
S/N	Signal-to-noise ratio: a measure of a signal (peak) intensity relative to the baseline ("noise") level
SP	Solids probe, see DIP.
TOF	Time-of-flight: a mass analyser that separates ions of different *m/z* by their time of travel between the ion source and detector, through a field-free region after acceleration by a constant voltage in the source. The ions will have differing velocities depending on their mass.

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