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BEST PRACTICE GUIDE FOR GENERATING MASS SPECTRA

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Setting standards
in analytical science

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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

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3 CHOICE OF IONISATION MODE

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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)*

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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.

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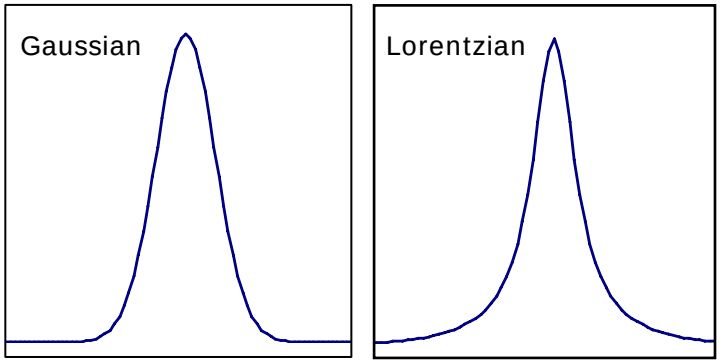
4 INSTRUMENT SET-UP: KEY DEFINITIONS

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.

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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. IncreasingD-0.56657 dostn4y/2e1m9c9.24aniM78t

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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

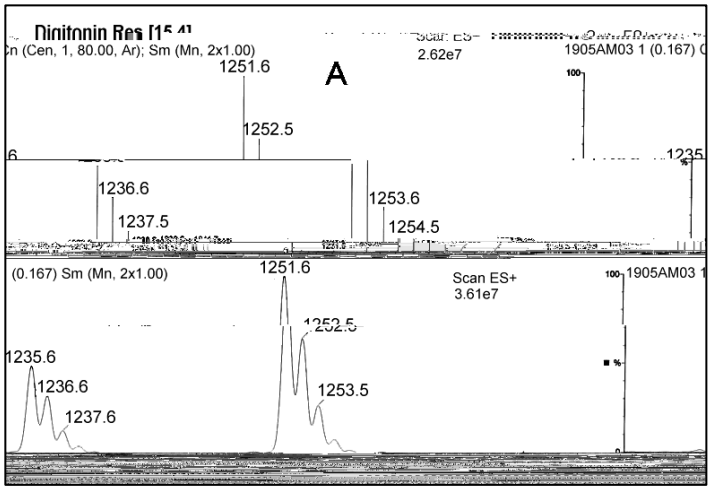

Figure 6a: Instrument correctly tuned

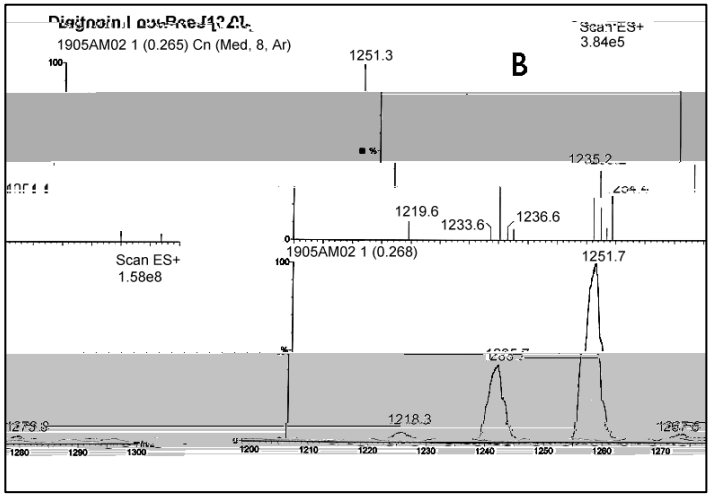

Figure 6b: Instrument incorrectly tuned

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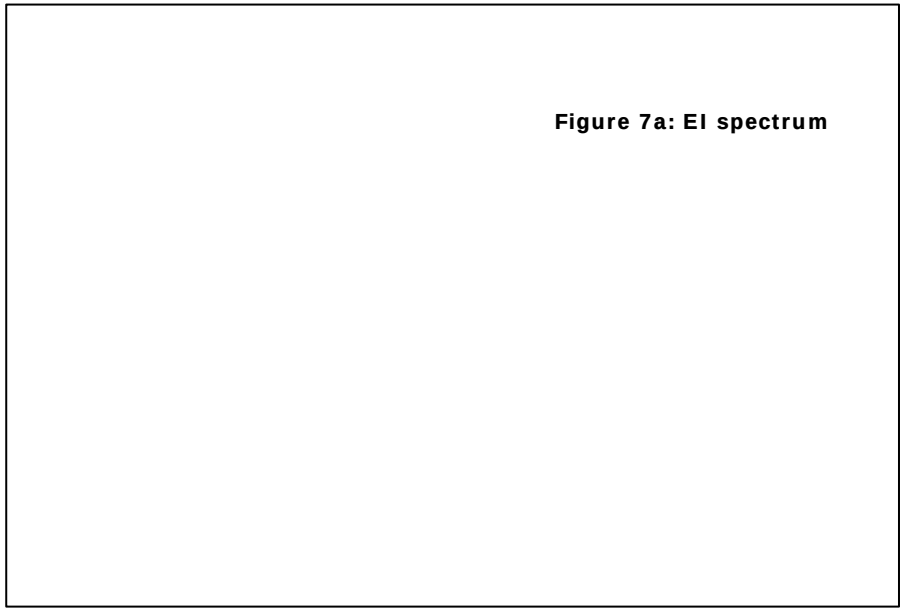
5.3 Mass calibration

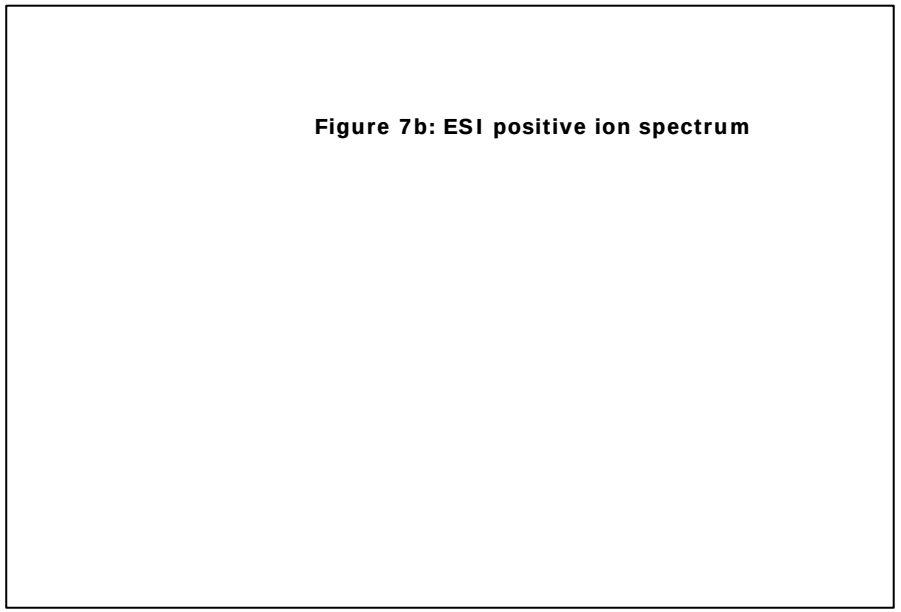
- Calibration of the *m/z* scale of the mass spectrometer is an important step in obtaining reliable mass spectra.
- Calibration typically involves analysing a calibration compound which yields ions of known *m/z*. The *m/z* scale is then adjusted to give the correct values for the calibration peaks.
- 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





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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

Is sensitivity/response satisfactory?

If no peaks are observed, check the following:

- Is the vacuum system OK? – check gauges
- Is the isolation valve open? (if there is one)
- Are all the electrical signals present? – check the readbacks
- Is the ion source contaminated? – visually check and clean if needed
-

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Figure 8 continued

Is mass resolution satisfactory? (see section 4.2)

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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

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7 MOLECULAR SPECIES RECOGNITION

7.1 Definitions of molecular mass

There are different appr

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7.2 Use of stable isotope information

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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}

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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

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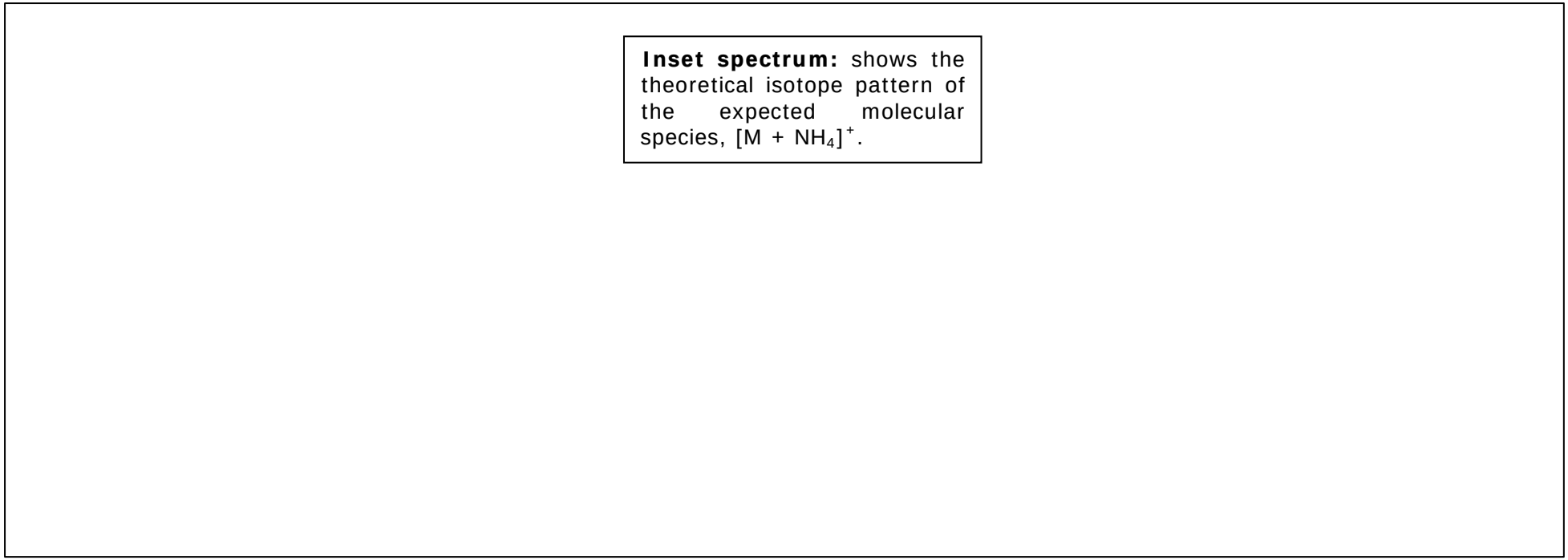
Figure 13b: Improved mass spectrum of $C_{15}H_{23}OSiBr$



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 $[M + NH_4]^+$.

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Figure 14: Poor quality mass spectrum of $C_{25}H$

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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

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[redacted]

[redacted]ion

m/z	Ion	Compound
35/37	M^-	chloride

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 samp

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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

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