Practical Experiments in the Fundamentals of Mass Spectrometry

[Laboratory Instructor Handbook – Abridged Example]

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Revision A

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Contents [of the full version]

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Introduction

This handbook was prepared as a learning and teaching resource to support undergraduate students in developing an understanding of theoretical and practical fundamentals of mass spectrometry. The ACQUITY™ QDa™ Detector is a robust and reliable bench-top mass spectrometer that, due to its ease of use, is ideal for teaching students fundamental principles of mass spectrometry.

The experiments in this handbook are presented in a specific order to support incrementally building the student's knowledge of mass spectrometry. This includes the basic operation of the ACQUITY QDa, the generation and interpretation of spectra using MassLynx™ Software, and the interpretation of isotope patterns, polarity switching, analysis of simple mixtures, polymers and fragmentation analysis.

This handbook includes additional background information for the Laboratory Demonstrator, model answers for the experiment questions, and is supplemented by multimedia content to enrich the teaching of this series of practical experiments.

Aims

The information and practical experiments detailed within the handbook will support students in gaining a practical understanding of the fundamentals of mass spectrometry: the instrumentation and its component parts, instrument operation, acquisition and spectral interpretation and the generation of characteristic mass data as the basis of modern compound identification.

Upon completion, the following topics will have been covered:

- Instrumentation: inlet, ion source, ionization techniques, mass analyzer, ion detection, and data acquisition.
- Instrument operation: sample introduction, data acquisition, polarity switching (positive and negative modes), full scan versus selected ion monitoring, and continuum versus centroid data.
- Interpretation of mass spectra: *m/z*, monoisotopic mass versus average mass, isotope patterns, adducts, fragmentation, multiple charges, and the nitrogen rule.

Transferable Skills

- Problem solving
- Laboratory skills
- Numerical and mathematical skills
- Data analysis
- Effective communication

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Experiment 1 - Interpreting mass spectra

Aim

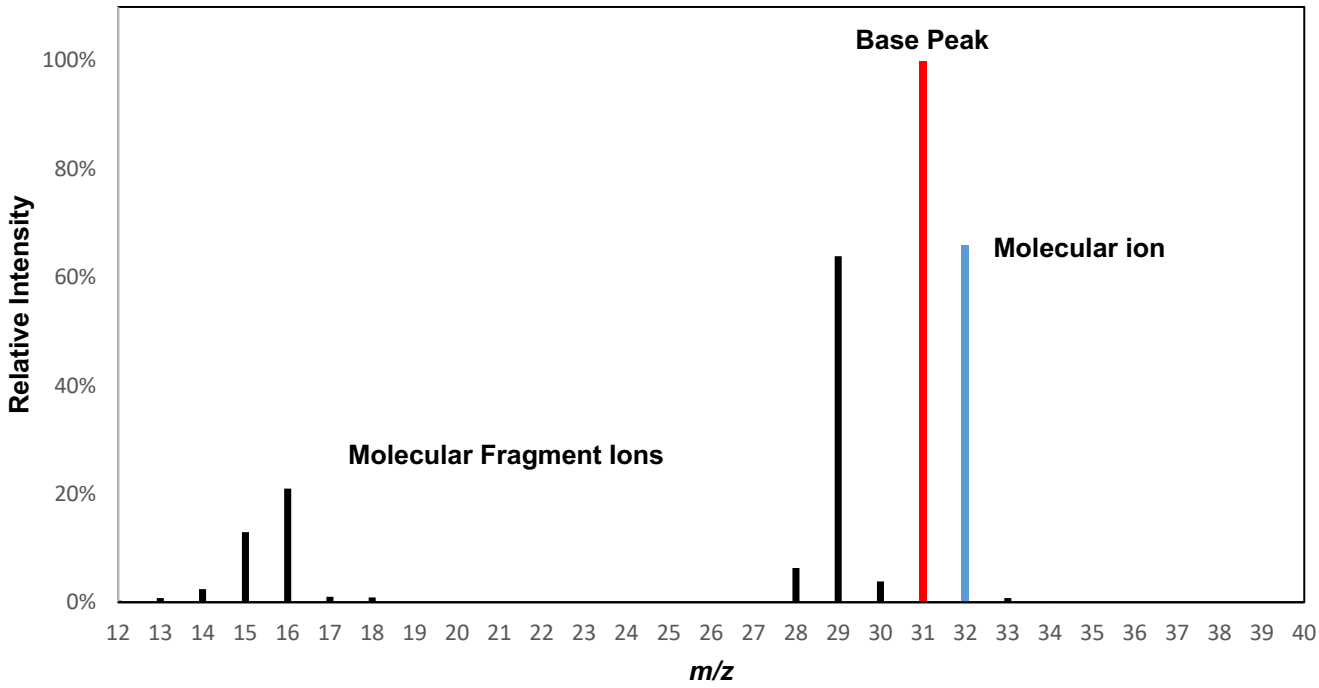
During this experiment you will learn how to prepare samples for analysis, introduce samples into the ACQUITY QDa, and acquire and interpret data using MassLynx Software.

Demonstrator: Refer the students to the supporting videos; "MS Fundamentals: Resolution" and "MS Fundamentals: Isotopes" for further background.

Theory

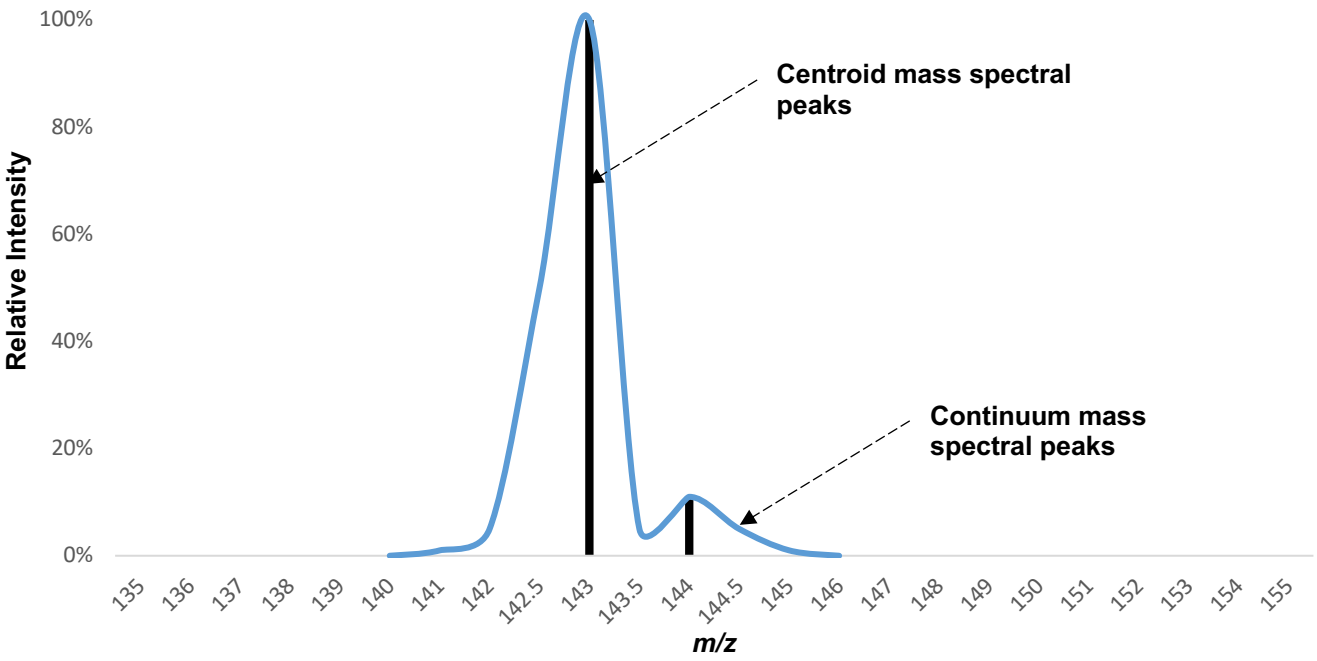
A mass spectrum is a record of the *m/z* values and abundances of the ions that are formed when a sample molecule is ionized. The *x-axis* represents the *m/z* (historically referred to as mass-to-charge ratio) where *m* is the molecular mass of the ion and *z* is the number of charges on the ion. The *y-axis* represents the relative intensity of the ions in relation to the base peak, which is the most intense peak.

Figure 1-A. Typical Electron Ionisation (EI) Spectrum of Methanol



The MassLynx software is used to control the ACQUITY QDa data acquisition and processing, and aids in data interpretation. The software digitizes the electrical signal from the detector, usually comprising a number of data points per peak, and stores the resulting data. The data is displayed as a continuum spectrum that represents the analog signal produced by the ions. The continuum spectrum can be converted to a centroid spectrum, which is a bar chart representing the line through the median centre, top, or percentage of the top, of each continuum mass spectral peak.

Figure 1-B. Continuum Spectra vs Centroid Spectra



The ability of a mass spectrometer to separate closely spaced ions, such as isotopes, is called resolution (***R***). Within a mass spectrum this is the observed *m/z* value divided by the smallest difference Δ(*m/z*) for two ions that can be separated: (*m/z*)/Δ(*m/z*). High resolution mass spectrometers, including time-of-flight mass spectrometers and magnetic sectors, are typically able to detect ions to multiple decimal places and can differentiate between ions with the same nominal mass. Therefore such instruments can report a resolution value in the order of tens of thousands or higher. A larger resolution value indicates a better separation of mass spectral peaks. The ACQUITY QDa is not designed for structural elucidation or identification of unknowns, where high resolution may be required, and the quadrupole mass analyzer provides significantly lower resolution than is achievable with high resolution mass spectrometers. The ACQUITY QDa demonstrates unit mass resolution, such that it is possible to clearly distinguish a peak corresponding to a singly charged ion from its neighbors 1 u away.

$$R = (m/z)/\Delta(m/z)$$

There are different approaches to measure the resolution of mass spectrometer, and therefore it is very important to disclose the methodology used. Where a single mass spectral peak is used to state the resolution, Δ(*m/z*) is typically determined using the *peak width definition* of resolution, where the width of the spectral peak is measured at a specified percentage of peak height. This is commonly performed at 50% of the peak height (Full Width Half Maximum) though other specified percentages such as 5 or 0.5% can be used.

For two adjacent mass spectral peaks of equivalent heights it is also possible to use the *10% valley definition of resolution*. Specifically, this is value of (*m/z*)/Δ(*m/z*) measured for two peaks of equal height in a mass spectrum at *m/z* and *m/z* ± Δ(*m/z)* that are separated by a valley which at its lowest point is 10 % of the height of either peak.

Figure 1-C. Peak width definition of resolution (measured at 50% peak height)

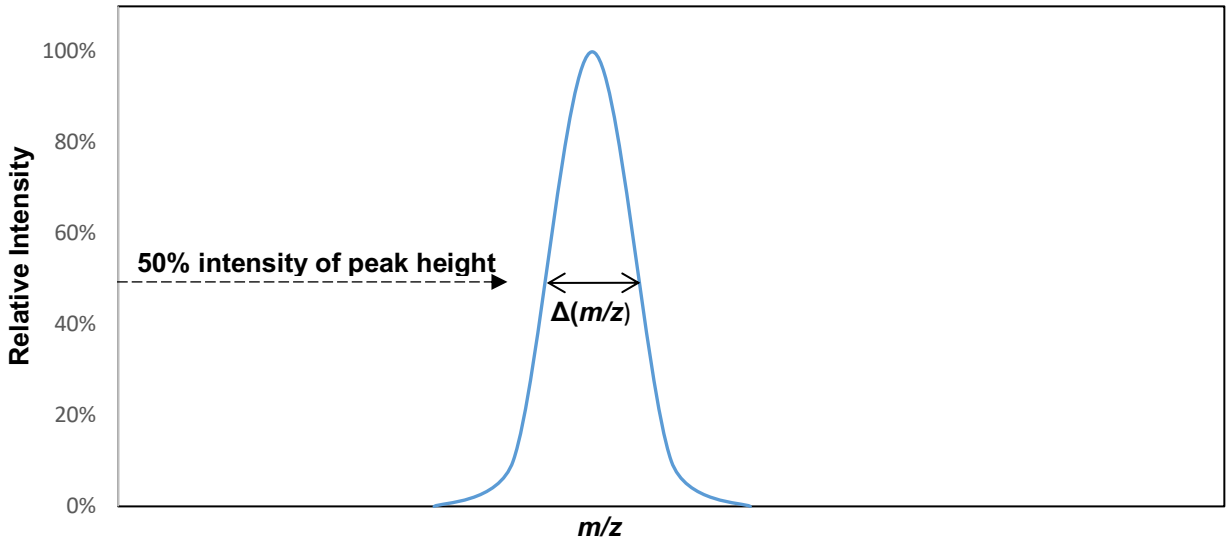
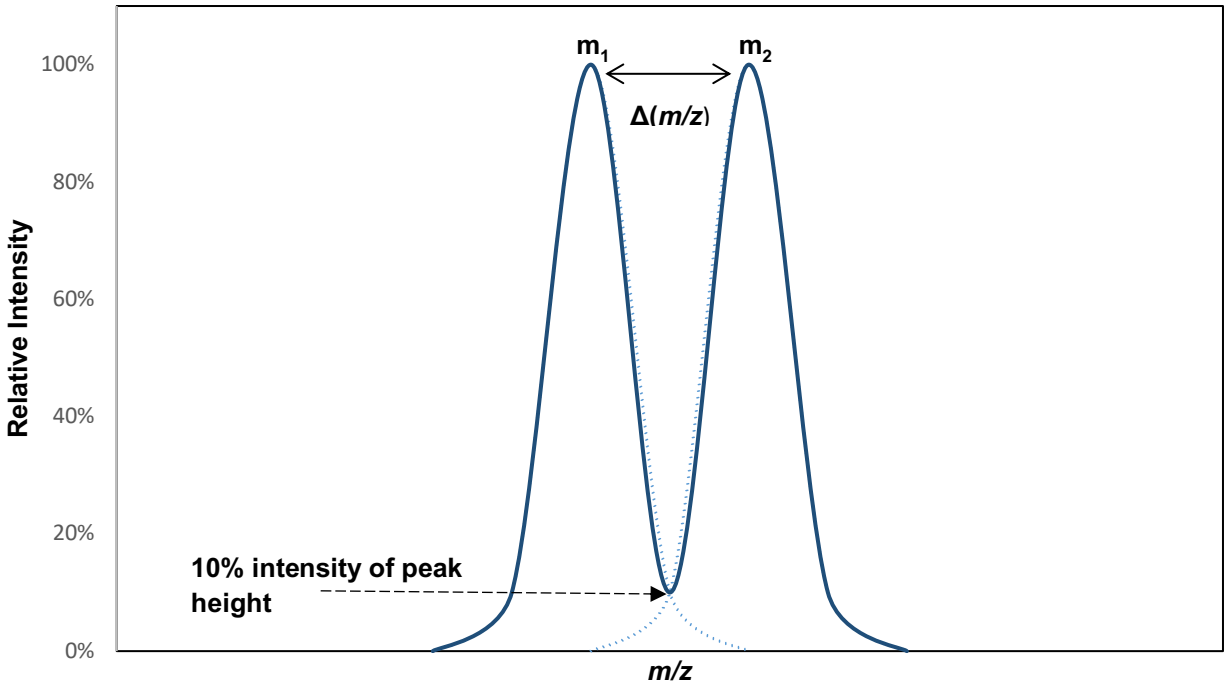
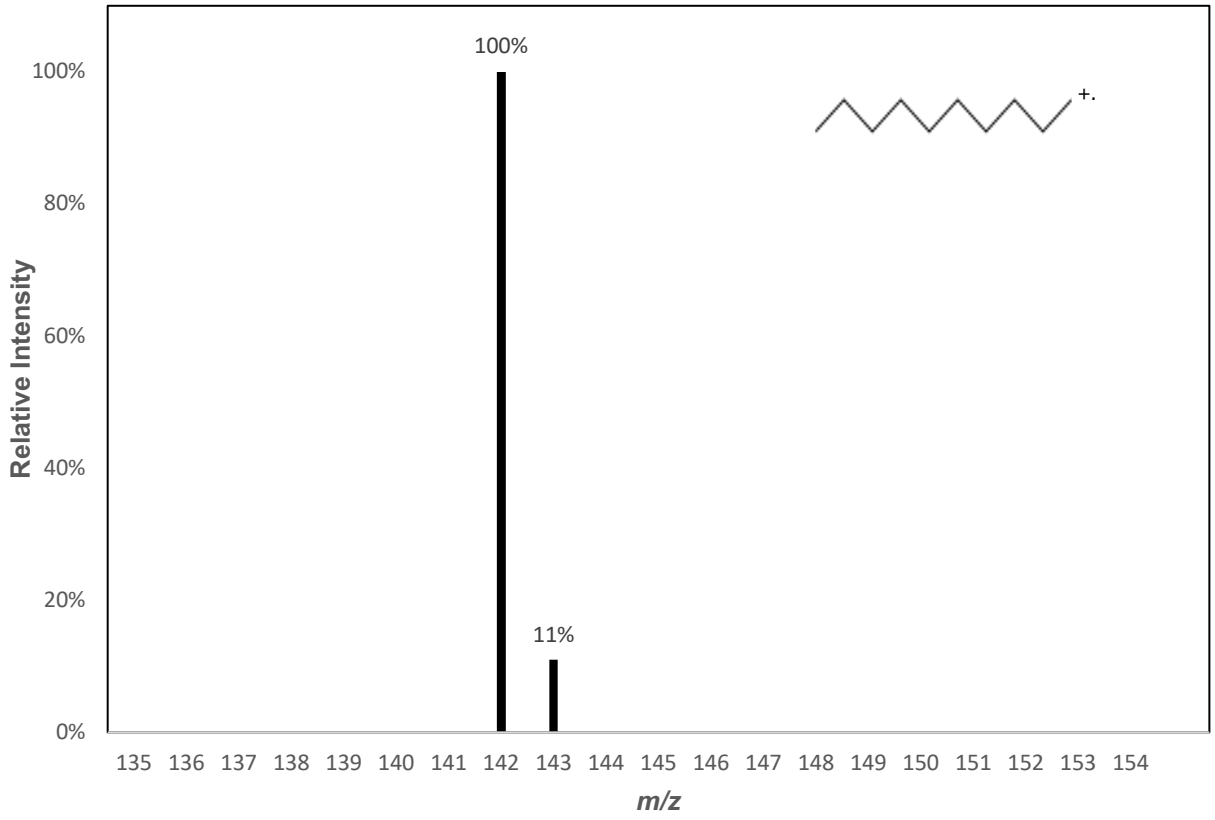


Figure 1-D. Resolution of two peaks at 10% valley



The isotope profile of a simple compound containing only C, H, N, O, S and P elements can be used to determine the number of carbon atoms present. The natural abundance of carbon-13 (^{13}C) is 1.1% so there is a 1.1% chance that any given carbon in a compound will be a ^{13}C. For example an intensity of 11% for ^{13}C indicates the presence of 10 carbon atoms in the compound.

Figure 1-E. Spectrum of decane showing relative intensities of Carbon-12 and Carbon-13 peaks



Procedure (see *Getting Started* section on page 11 for more detail)

Prepare Sample and System

1. Pipette 1 mL of the 1 mg/mL caffeine stock solution into a 10 mL volumetric flask and make up with the Sample Diluent to a concentration of 100 µg/mL. Label your flask.
2. In MassLynx set up a sample list as appropriate, selecting the Experiment 1 MS File.

Spectrum Chromatogram Map Edit Samples

	File Name	File Text	MS File
1	Date_Sample_001	Interpreting a mass spectrum, Blank	Experiment 1
2	Date_Sample_002	Interpreting a mass spectrum, Caffeine	Experiment 1

Run Blank Injection

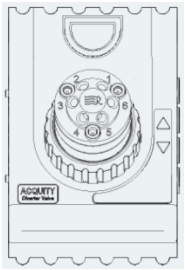
3. Ensure that the Diverter Valve is in load position 1 (one). If this is not the case, firmly press the down arrow on the valve. Fill the syringe with your Sample Diluent and inject approximately 30 µL (to ensure the loop is filled with sample) into the Diverter Valve needle port on the side of the ACQUITY QDa. Demonstrator: remind students of the importance of running blanks to identify any contaminants in their solvents which may interfere with the measurement of the analyte.

Manually actuate the ACQUITY Diverter Valve by pressing the front-panel buttons.

Digital display – Shows the valve position number (1 or 2).

Up arrow – Valve moves to, or stays in, position 2.

Down arrow – Valve moves to, or stays in, position 1.



4. In MassLynx enter appropriate *File Name* and *File Text* details for the sample, highlight the entry (by left clicking on the row number) and begin the data acquisition process by clicking on the Play button (►) within the main MassLynx toolbar. Firmly press the up arrow on the Diverter Valve so the valve moves to position 2 (two) to deliver the sample. Remove the syringe, empty any remaining Sample Diluent into the appropriate waste solvent bottle and flush the syringe with methanol.
5. Acquire two minutes of experimental data, as specified in the MS File method. This will be sufficient data to process after acquisition.
6. Firmly press the down arrow to return the Diverter Valve to position 1 (one).

Run Sample Injection

7. Once the analysis of the Sample Diluent has completed, fill the syringe with the 100 µg/mL caffeine solution and inject approximately 30 µL (to ensure the loop is filled with sample) into the Diverter Valve needle port on the side of the ACQUITY QDa by following steps 3 to 6 described above.
8. In MassLynx open the Chromatogram window for this experiment, look for the most intense peak and select *Process* then *Combine* from the toolbar, right-click and drag across the width of the peak and then right-click and drag across an area adjacent to the peak. This will subtract background ions from the combined sample spectrum (see *Getting Started* section for more detail).
9. A new window will open showing a continuum data mass spectrum. Before converting the data to centroid data, by selecting *Process* then *Smooth* and *Process* then *Center* in turn, ensure that the highlighted icon below is **not** selected. This ensures the original continuum data mass spectrum is retained and not replaced by the centroid data mass spectrum (see *Getting Started* section for more detail).

10. When the experiment is complete firstly turn off the flow from the Reagent Manager (by pressing *Run/Stop*). Secondly, place the ACQUITY QDa into *Standby* via the ACQUITY Console.

Results

Complete the table by noting the *m/z* and relative intensity of the most intense peaks*:*

Peak	*m/z*	Relative Intensity / %
1	e.g. 195.1	100
2	196.0	10
3	197.1	<1
4		

Questions

Note*:* The mass accuracy of the ACQUITY QDa is ±0.2 Da across the mass range. Therefore measured *m/z* values will deviate from the expected value by up to ±0.2 Da.

Discuss the following questions within the group and note down answers on these sheets.

1. What is the mass-to-charge ratio of the base peak?

   m/z 195.1

2. What is the absolute intensity of the base peak?

   In the range $1x10^{-5}$ – $1x10^{-7}$ a.u.

3. Calculate the average molecular mass of caffeine ($C_8H_{10}N_4O_2$) to one decimal place.

Element	Average Mass
Hydrogen	1.008
Carbon	12.011
Nitrogen	14.007
Oxygen	15.999

194.2 Da

4. Explain why the measured *m/z* 195.1 is different to the calculated mass 194.2Da for Caffeine?

   Electrospray Ionization is a soft ionization mode that typically produces $[M+H]^+$ ions. The addition of a proton increases the mass by approximately 1 Da. In addition, the base peak in the spectrum is the monoisotopic ion and therefore it's *m/z* is not influenced by natural, less abundant isotopes (e.g. Carbon-13) whereas the average mass is the mass weighted for its natural isotopic abundance.

5. Why is there a peak at *m/z* 196.0?

   The peak at *m/z* 195.1 is the monoisotopic peak and contains only the first isotopes of each element. The peak at *m/z* 196.0 contains a carbon-13 isotope and, as its mass is different, it has a different *m/z*. Carbon-13 has a natural abundance of 1.1% and so, with eight carbons in the molecule, the peak at *m/z* 196.1 should be at approximately 8.8% the intensity of the peak at *m/z* 195.1.

6. Using the peak at *m/z* 195.1 calculate the mass resolution (peak width definition at 50% of the peak height). *[NOTE: the data must be shown in continuum mode to enable a measurement across the mass spectral peak.]*

   $R = (m/z)/\Delta(m/z)$, therefore: 195.1/~0.7 = ~279

7. Explain why it's important to take measurement across a chromatographic peak, rather than from a single point within the width of the chromatographic peak.

   Taking a measurement across the peak of the chromatogram provides an opportunity to sample multiple data points and provide an averaged spectrum which should provide a more representative view of the peak, rather than producing a spectrum from a single data point within the peak. This will provide more accurate results.

8. Explain the differences between centroid and continuum mode.

   The continuum mode spectrum is a continuous analogue signal recorded by the detector. When the spectrum is drawn graphically it is created by joining each data point to the adjacent point, which produces a smooth curve. This approach provides a large amount of data, including mass spectral peak shape.

   The centroid mode spectrum is a digital representation in the form of a bar graph obtained by plotting a line through the center of a mass spectral peak's distribution.

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