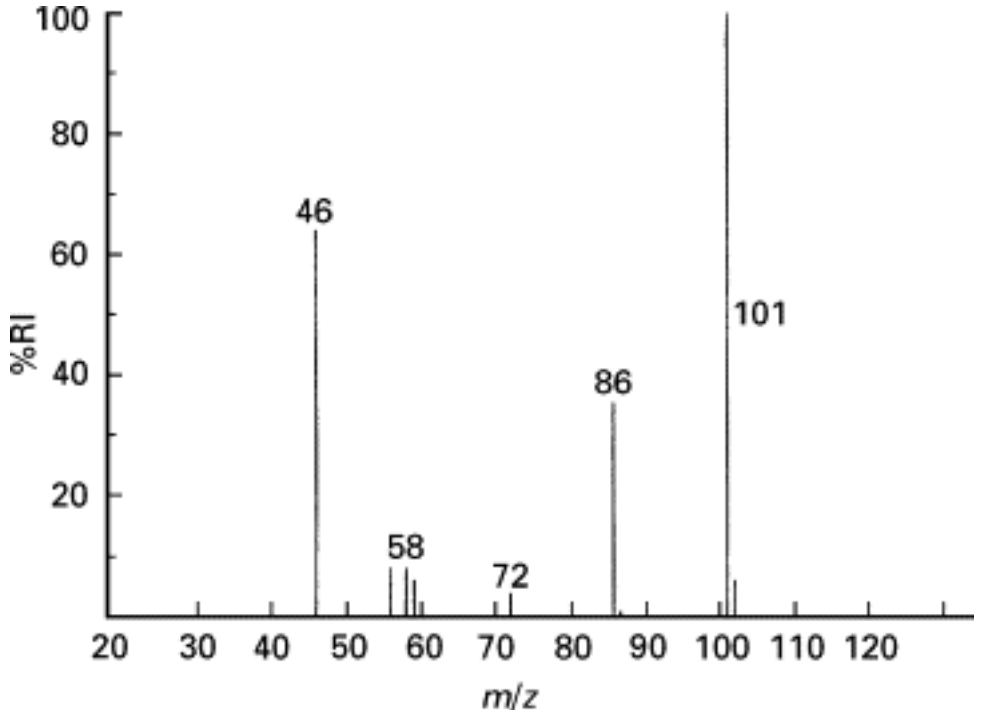
Mass Spectrometry

What is Mass Spectrometry?

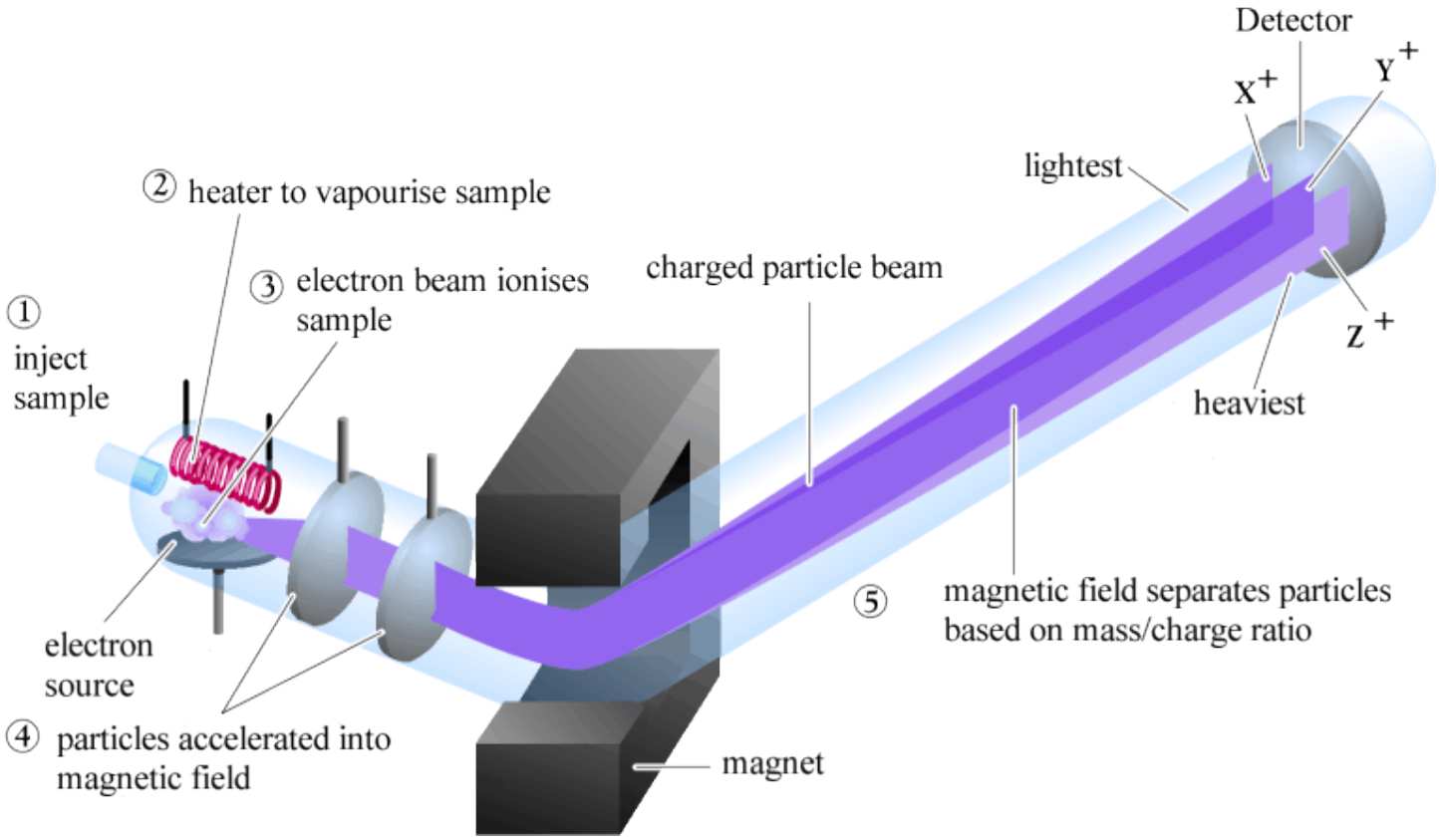
Mass spectrometry is an analytical technique that is used to determine the mass-to-charge ratio of molecules and ions. It involves **ionizing** a sample and then **separating** the resulting ions based on **their mass-to-charge ratio using electric and magnetic fields**. The separated ions are then detected and their relative abundance is measured, which can provide information about the chemical composition and structure of the sample.

Mass spectrometry is fundamentally different from spectroscopy. Spectroscopy involves the absorption (or emission) of light over a range of wavelengths. Whereas, mass spectrometry does **not use light at all.**



Mass Spectrometer

A mass spectrometer is an apparatus which proves to be essential, in order to configure the characteristics of individual particles; especially the masses of isotopes and molecules, by ionizing (converting particles to ions) so that they can be moved about and manipulated by external electric and magnetic fields. The measurements can then be derived from the trajectories present in the mass spectrometer.



Mass Spectrometry. Steps

Mass spectrometry involves several steps that are typically carried out in the following order:

Sample preparation: The sample is prepared by extracting the analytes of interest from a complex mixture or synthesizing them if they are not naturally occurring. The sample may also be purified or concentrated to increase the sensitivity of the analysis.

Ionization: The analytes are ionized, typically by one of several ionization techniques, such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), or atmospheric pressure chemical ionization (APCI). **FOLLOWING THIS STEP, MOLECULE FRAGMENTATION WILL OCCUR**

Ion separation: The ions are separated based on their mass-to-charge ratio (m/z) using a mass analyzer. There are several types of mass analyzers, including quadrupoles, time-of-flight (TOF) analyzers, magnetic sector analyzers, and ion trap analyzers.

Detection: The ions are detected by a detector, typically an electron multiplier, a Faraday cup, or a microchannel plate detector. The detector generates a signal proportional to the number of ions detected.

Data analysis: The signal generated by the detector is recorded as a mass spectrum, which is a plot of the relative abundance of ions as a function of their mass-to-charge ratio. The mass spectrum is analyzed to identify the analytes present in the sample and to determine their relative abundances.

Additional steps may be necessary depending on the specific application, such as fragmentation of ions in a tandem mass spectrometry (MS/MS) experiment to obtain structural information about the analytes

Mass Spectrometry. Steps: Sample preparation.

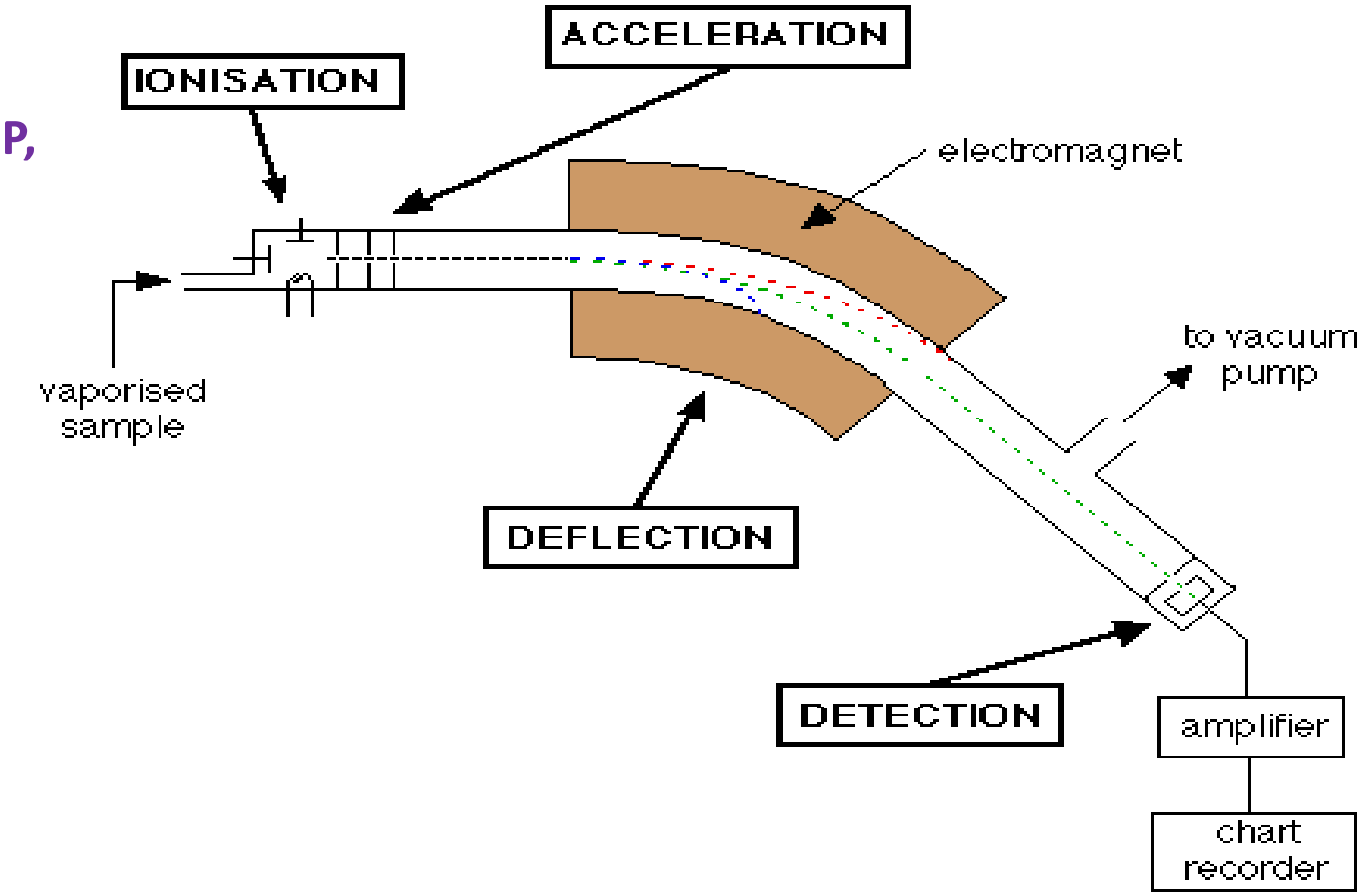
Sample preparation/ introduction. Sample is prepared for entering into the MS system. In all cases, in must be vaporized by heating/ laser desorption before ionization may occur. If coupled with GC, molecules are already in gas phase.

Ionisation. FOLLOWING THIS STEP, MOLECULE FRAGMENTATION OCCUR

Ion separation (Acceleration/ Deflection) (Mass Analyzer).

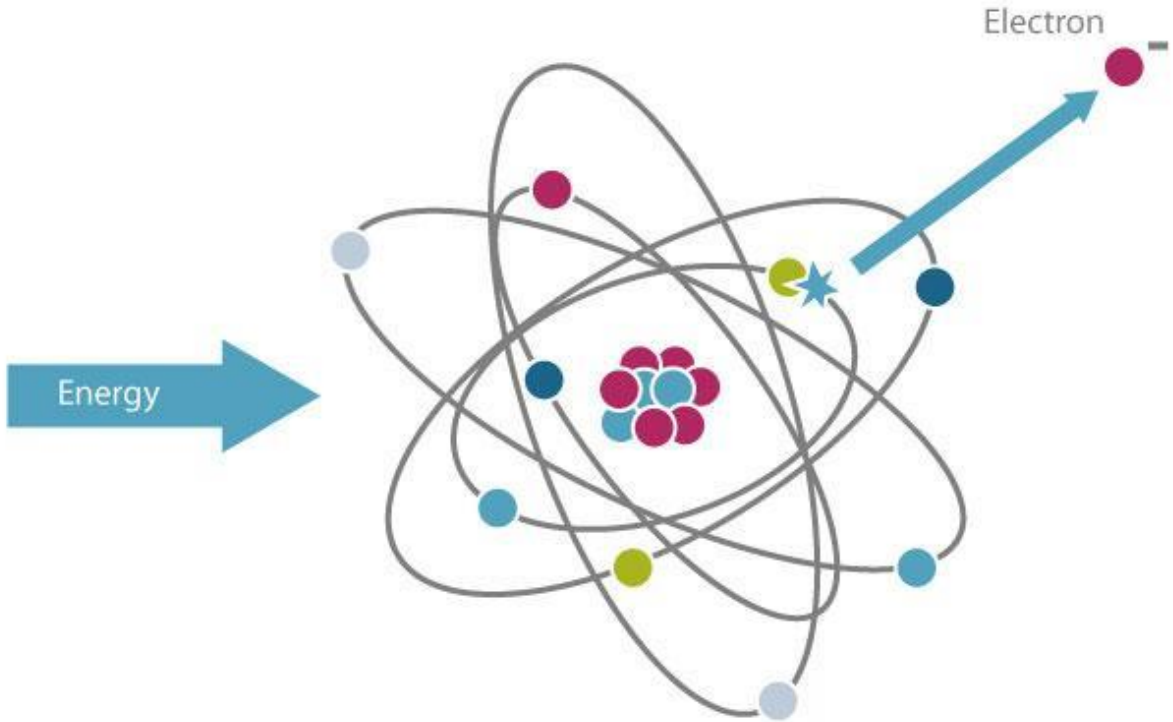
Detection.

Data analysis.



Ionization

Ionization: The atom or molecule is ionised by knocking one or more electrons off to give a **positive ion**. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Most mass spectrometers work with positive ions.



Ionization of water

$$H_2O_{(l)} \rightleftarrows H^+ + OH^-$$

$$Kw = [H^+][OH^-] = 1.0 \times 10^{-14}$$

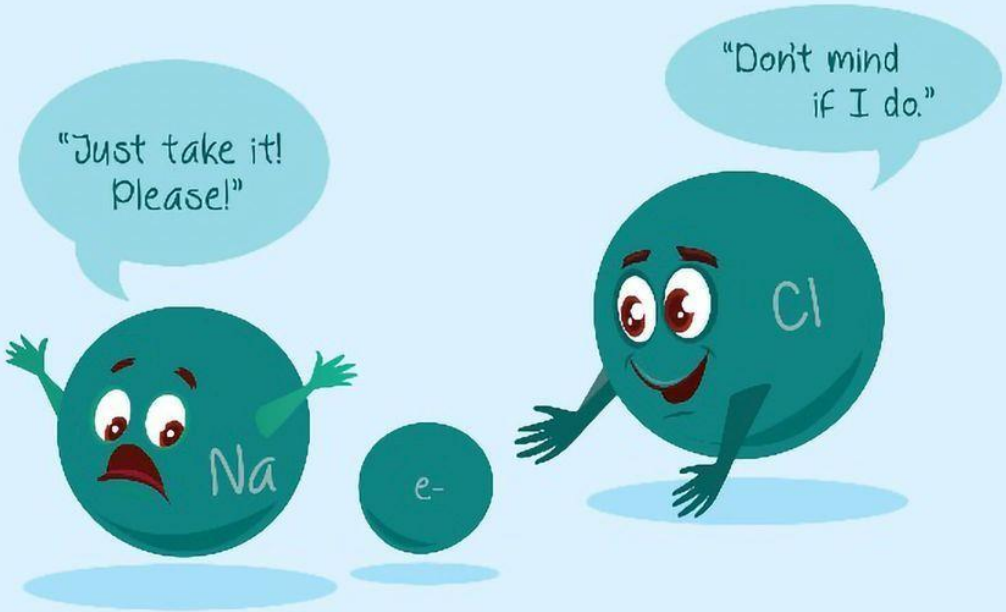
Always assume the temperature is 25 °C and that it is an aqueous solution to use this equation above.

Ion: Definition

An ion is an atom or molecule with a net electrical charge.

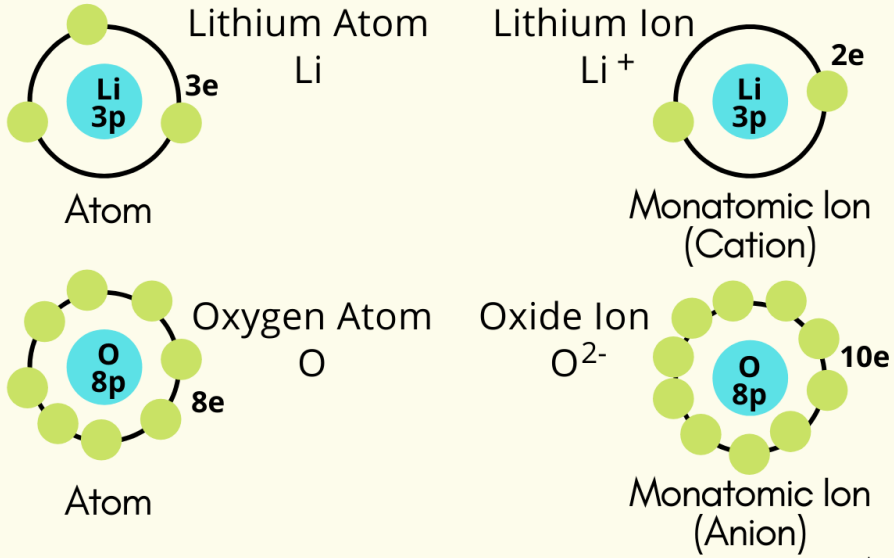
The charge of an electron is considered to be negative by convention and this charge is equal and opposite to the charge of a proton, which is considered to be positive by convention. **The net charge of an ion is not zero because its total number of electrons is unequal to its total number of protons.**

A cation is a positively charged ion with fewer electrons than protons while an anion is a negatively charged ion with more electrons than protons. Opposite electric charges are pulled towards one another by electrostatic force, so cations and anions attract each other and readily form ionic compounds.



Monatomic Ions

A monatomic ion is an ion consisting of one atom.
Find the charge by comparing the number of protons and electrons.



Ionization in MS (1)

There are several ionization methods used in mass spectrometry (MS), each with their own advantages and limitations. Some of the most common ionization methods include:

Electron Ionization (EI): It is a technique used in mass spectrometry to ionize molecules in the gas phase. In EI, a sample is bombarded with high-energy electrons, typically with energies in the range of 10-100 eV, which ionizes the sample molecules by ejecting one or more electrons.

Chemical Ionization (CI): This method uses a reagent gas to ionize the analyte, which is typically a small molecule. CI is often used to study the molecular structure of organic compounds.

Electrospray Ionization (ESI): This ionization method is widely used in MS and is especially useful for analyzing large molecules such as proteins and peptides. ESI uses an electric field to generate charged droplets of analyte solution, which then evaporate to produce gas-phase ions.

Matrix-Assisted Laser Desorption/Ionization (MALDI): This ionization method is often used for analyzing large biomolecules such as proteins and peptides. It involves embedding the analyte in a matrix material and then irradiating the sample with a laser to vaporize and ionize the analyte.

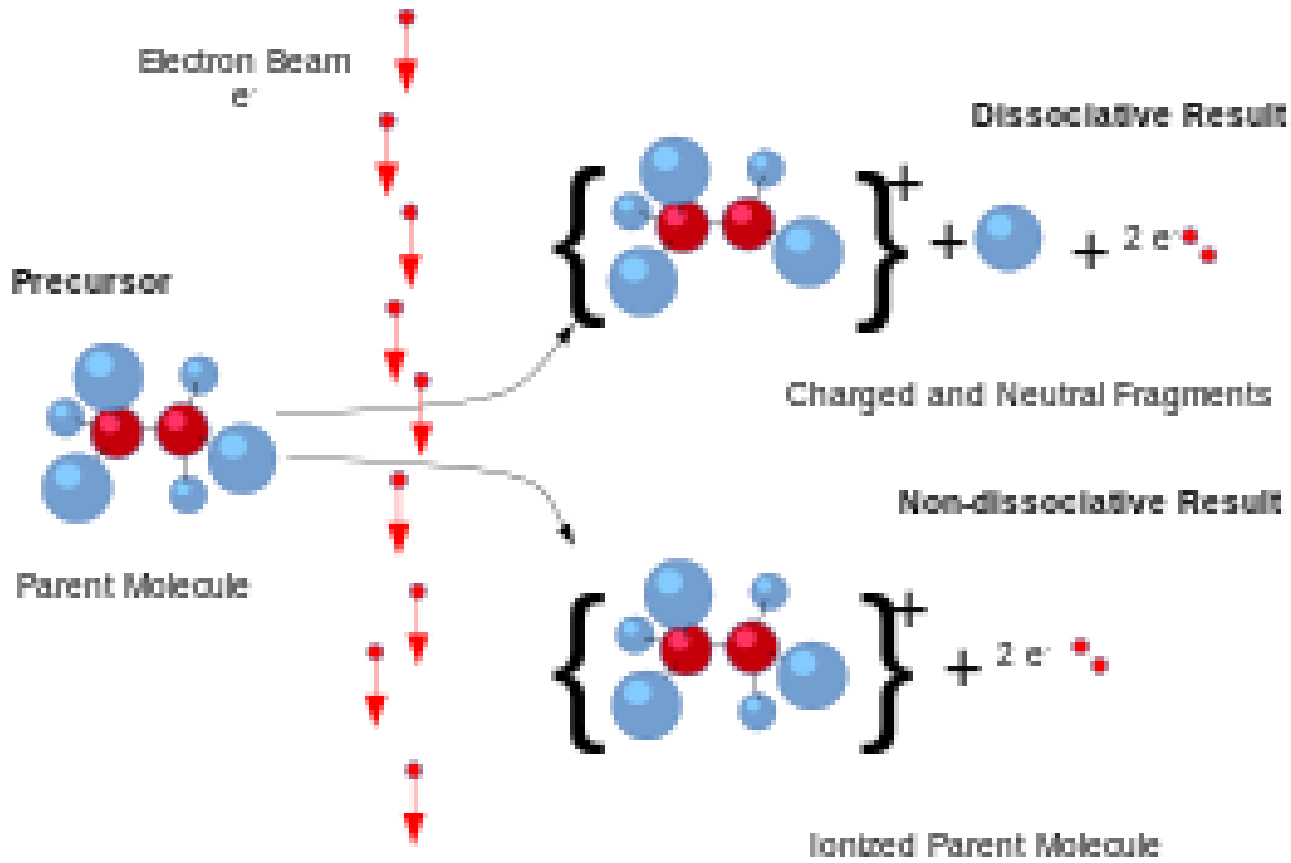
Ionization in MS (6)

Electron Ionization (EI) is a technique used in mass spectrometry to ionize molecules in the gas phase. In EI, a sample is bombarded with high-energy electrons, typically with energies in the range of 10-100 eV, which ionizes the sample molecules by **ejecting one or more electrons.**

The resulting gas-phase ions are then analyzed by a mass spectrometer, which separates the ions based on their mass-to-charge ratio.

EI is particularly useful for analyzing small and volatile molecules, such as organic compounds, due to its ability to produce highly reproducible fragmentation patterns that can be used to identify the molecule's structure.

EI can cause significant fragmentation of the sample molecules.

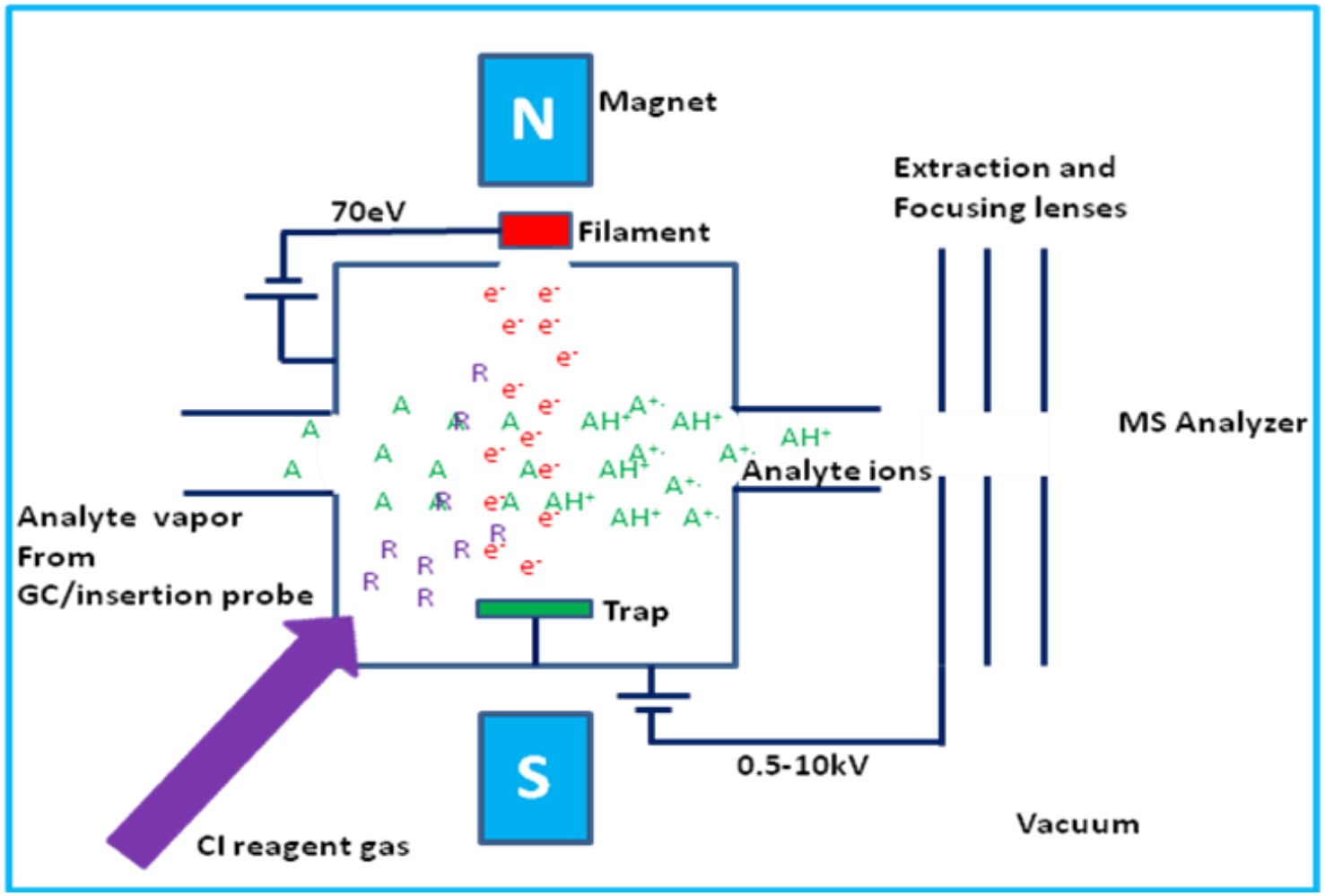


Ionization in MS (5)

Chemical ionization (CI) is a technique used in mass spectrometry that involves the use of a reagent gas to ionize molecules.

In CI, the reagent gas is typically methane, isobutane or ammonia, and is introduced into the mass spectrometer chamber where it is ionized by an electron source, typically a filament or electron gun. The resulting ions are then accelerated towards the sample, where they react with the molecules of interest, forming protonated or ammoniated ions.

The ion-molecule reactions that occur in CI can produce less fragmentation than other ionization techniques such as electron ionization (EI), allowing for the detection of intact molecular ions and providing more information about the structure of the molecule.



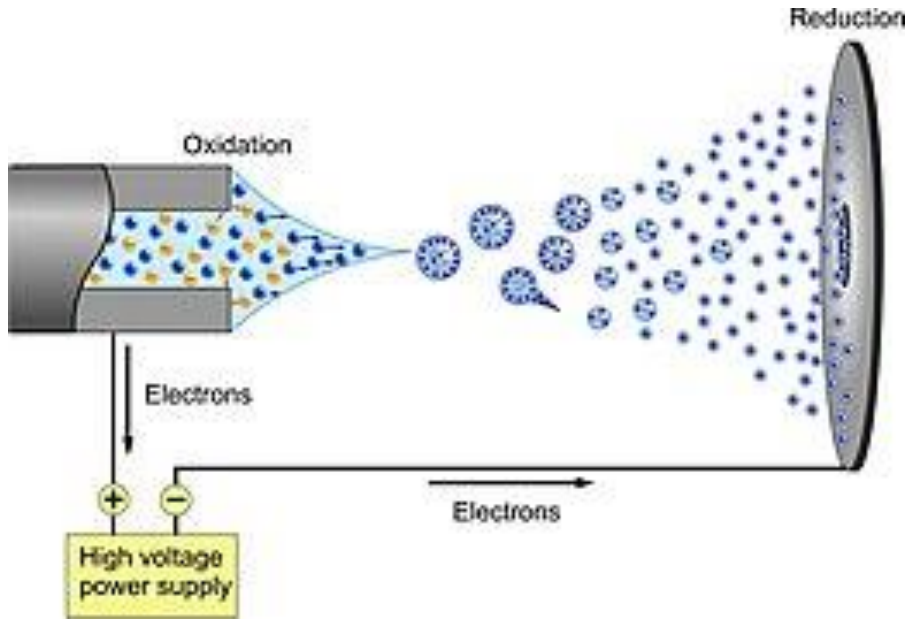
Ionization in MS (3)

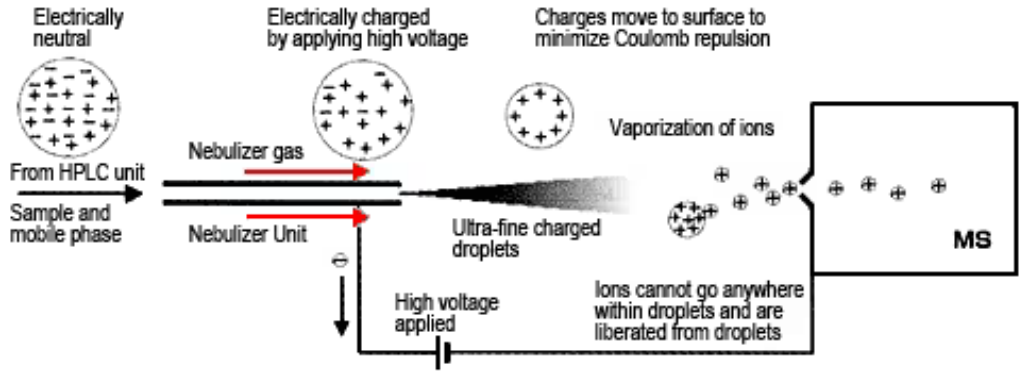
Electrospray ionization (ESI) is a technique used in mass spectrometry to ionize molecules in the gas phase. It works by creating a **high electric field** that is used to generate charged droplets from a liquid sample containing the molecules of interest. These charged droplets are then desolvated and the resulting gas-phase ions are analyzed by a mass spectrometer.

In ESI, the sample solution is typically pumped through a small, thin capillary under high voltage. As the solution emerges from the capillary, the electric field at the tip causes the formation of a Taylor cone, which emits a fine spray of droplets. The droplets contain both charged and neutral molecules from the sample solution.

As the droplets move through the atmosphere in the mass spectrometer, the solvent evaporates, leaving behind a gas-phase cloud of charged molecules. These ions are then separated by mass-to-charge ratio and detected by the mass spectrometer.

ESI is widely used in many areas of chemistry and biochemistry, including **proteomics, metabolomics,** and small molecule analysis, due to its ability to ionize a wide range of molecules with minimal fragmentation.





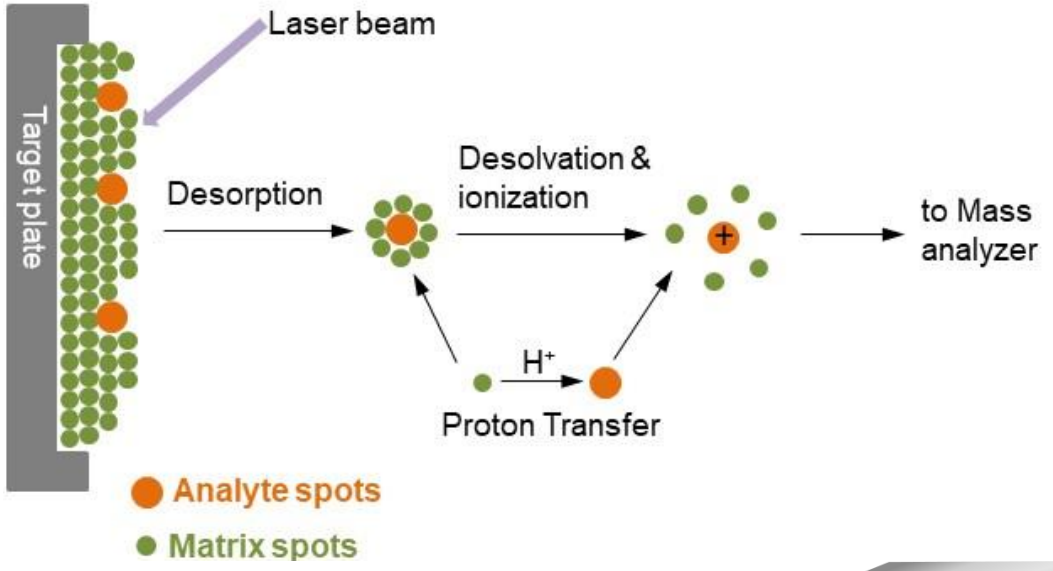
Ionization in MS (4)

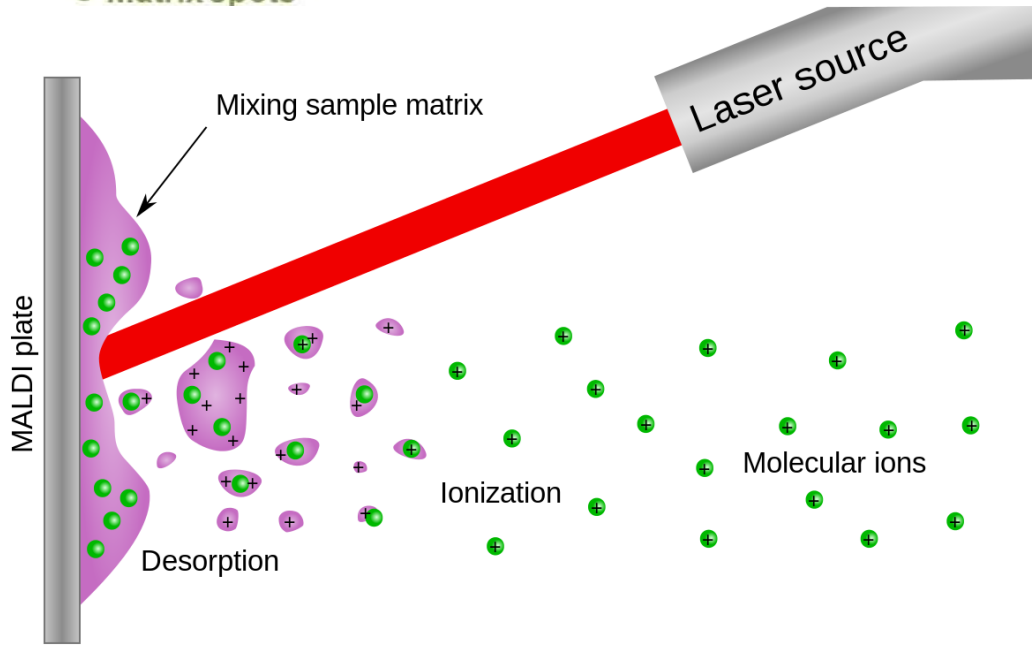
MALDI (Matrix-Assisted Laser Desorption/Ionization) is a technique used in mass spectrometry to ionize large biomolecules such as proteins, peptides, and nucleic acids.

In MALDI, a sample is mixed with a **matrix** material, typically a small organic molecule such as sinapinic acid or α-cyano-4-hydroxycinnamic acid, **which absorbs the laser energy and helps to vaporize and ionize** the sample molecules. The matrix material also serves to protect the sample molecules from degradation during ionization.

The sample-matrix mixture is then spotted onto a metal plate and dried. A laser beam is then directed onto the sample, which causes the matrix to absorb the energy and vaporize, along with the sample molecules. This produces a plume of ionized molecules that can be analyzed by a mass spectrometer.

MALDI is particularly useful for **analyzing large biomolecules** that are difficult to ionize using other ionization techniques, such as electrospray ionization (ESI). It is widely used in many areas of biology and biochemistry, including proteomics, metabolomics, and lipidomics, for the identification and characterization of biomolecules.





Molecular fragmentation (will get into this later on)

Molecule fragmentation occurs in a mass spectrometer (MS) when a sample molecule is ionized and then undergoes collision-induced dissociation (CID) or fragmentation, which results in the production of smaller fragments.

In EI (electron ionization), for example, the sample molecule is ionized by the bombardment of high-energy electrons, which typically have an energy of 70 eV. This process causes the molecule to break apart into a series of smaller fragments, which are then detected by the mass spectrometer. The fragmentation occurs through the cleavage of chemical bonds in the molecule, resulting in the generation of a series of fragment ions with varying masses and charges.

In other ionization methods, such as CI (chemical ionization) and ESI (electrospray ionization), the fragmentation can be less extensive than in EI. This is because these methods typically produce softer ions with less energy, resulting in less fragmentation and more intact molecular ions being observed in the mass spectrum.

The fragmentation pattern of a molecule in a mass spectrometer is highly dependent on the specific ionization method used, as well as the structure and composition of the molecule itself. By analyzing the fragmentation pattern of a molecule, researchers can identify the molecular structure of the original compound, as well as determine the presence of specific functional groups and other chemical characteristics.

Ionization in MS (2)

Atmospheric Pressure Chemical Ionization (APCI): This method uses a corona discharge to ionize the analyte in the presence of a reagent gas. APCI is commonly used to analyze small molecules such as drugs and metabolites.

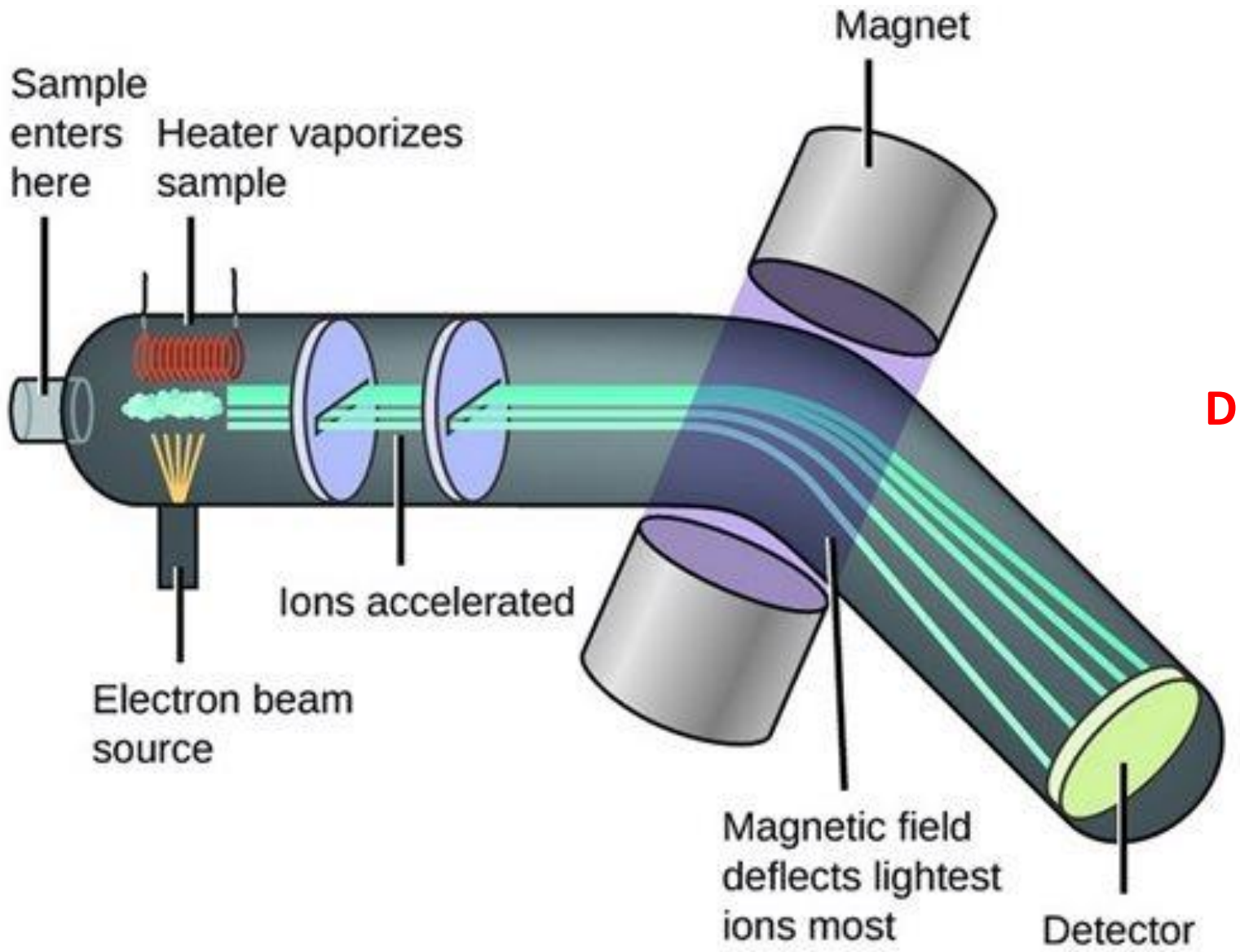
Electrospray Ionization Atmospheric Pressure Chemical Ionization (ESI-APCI): This method combines the benefits of both ESI and APCI, allowing for the analysis of a wide range of analytes including small and large molecules.

Surface-Assisted Laser Desorption/Ionization (SALDI): This method is a variant of MALDI that involves depositing the analyte onto a rough or porous surface before laser desorption and ionization.

There are also other ionization methods used in MS, including **Fast Atom Bombardment (FAB**), **Desorption Electrospray Ionization (DESI**), and **Direct Analysis in Real Time (DART**). The choice of ionization method depends on the type of analyte, the required sensitivity and specificity, and the instrumentation available.

Ion separation (Mass analyzer) (What is it?)

ACCELERATION



DEFLECTION

Ion separation (Mass analyzer) (1)

There are several types of ion separation techniques used in mass spectrometry. The most common types of ion separation include:

Quadrupole: A quadrupole mass analyzer separates ions based on their mass-to-charge ratio (m/z) by selectively filtering ions that pass through a set of four parallel rods that create a radiofrequency (RF) electric field. The RF voltage is applied to the rods in a specific sequence to create a potential energy barrier that traps the ions, allowing for their separation based on their m/z.

Time-of-flight (TOF): In a TOF mass analyzer, ions are accelerated by a high voltage electric field and their m/z is determined based on the time it takes them to travel down a flight tube to a detector. The time it takes for ions to reach the detector is proportional to their m/z.

Magnetic sector: A magnetic sector mass analyzer separates ions based on their m/z by deflecting them onto a curved path using a magnetic field. The degree of deflection is proportional to the m/z of the ions, allowing for their separation.

Ion separation (Mass analyzer) (2)

Ion trap: In an ion trap mass analyzer, ions are trapped in a three-dimensional space by a combination of electric and magnetic fields. The ions are selectively ejected based on their m/z by varying the voltages applied to the electrodes.

Orbitrap: An Orbitrap mass analyzer operates on the principles of a Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer. Ions are trapped in a cylindrical electrostatic field and their frequencies of motion are detected by measuring the current induced in the outer electrode of the trap. The ion current signal is then Fourier transformed to obtain a mass spectrum.

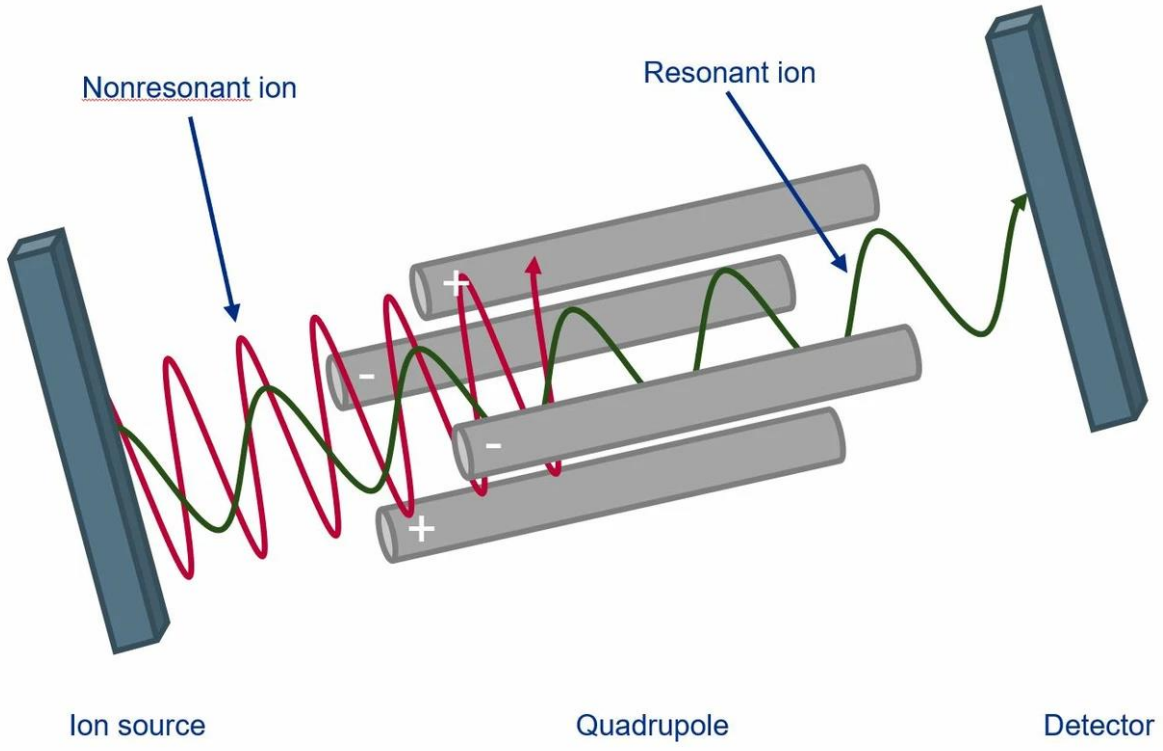
Fourier Transform Ion Cyclotron Resonance (FT-ICR): In an FT-ICR mass analyzer, ions are trapped in a magnetic field and their m/z is determined based on the frequency of their motion in the field. The ion current signal is Fourier transformed to obtain a mass spectrum. FT-ICR is a high-resolution technique that can separate ions with very similar m/z values.

Mass analyzer: Quadrupole (1)

A **quadrupole mass analyzer** works by using a combination of electric and magnetic fields to selectively separate ions based on their mass-to-charge ratio.

After the sample is ionized, the resulting ions are accelerated into the quadrupole mass filter. The quadrupole mass filter consists of four parallel metal rods that are arranged in a square pattern and are held at a specific voltage. A radiofrequency voltage is also applied to the rods, which creates a quadrupolar electric field.

As the ions enter the quadrupole mass filter, they are subject to the combined electric and magnetic fields. **Only ions with a specific mass-to-charge ratio will have a stable trajectory** and be transmitted through the quadrupole to the detector. Ions with other mass-to-charge ratios will have unstable trajectories and will collide with the rods or be lost.

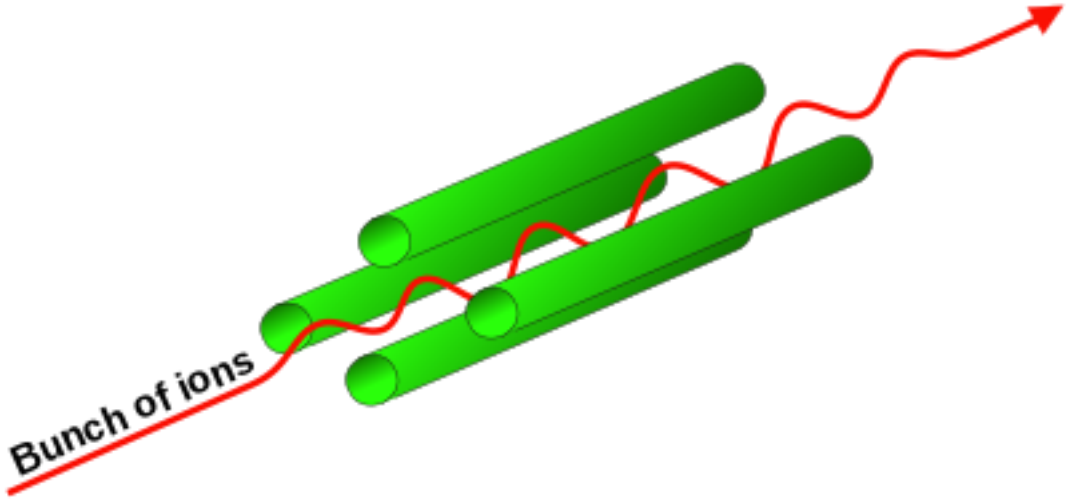


Mass analyzer: Quadrupole (2)

By **varying the voltage** applied to the quadrupole rods, the mass-to-charge ratio of the ions that can pass through the filter can be selectively changed, allowing for the detection and quantification of different ion species in the sample.

The detector at the end of the quadrupole mass filter detects the ions that pass through the filter and produces an electrical signal that is proportional to the number of ions detected. The signal is then recorded and used to construct a mass spectrum, which shows the relative abundance of ions as a function of their mass-to-charge ratio.

Quadrupole mass spectrometry is a versatile technique that can be used for both qualitative and quantitative analysis of small molecules, proteins, and other biomolecules. It is commonly used in pharmaceuticals, environmental monitoring, and other fields to identify and quantify trace amounts of molecules in complex mixtures.

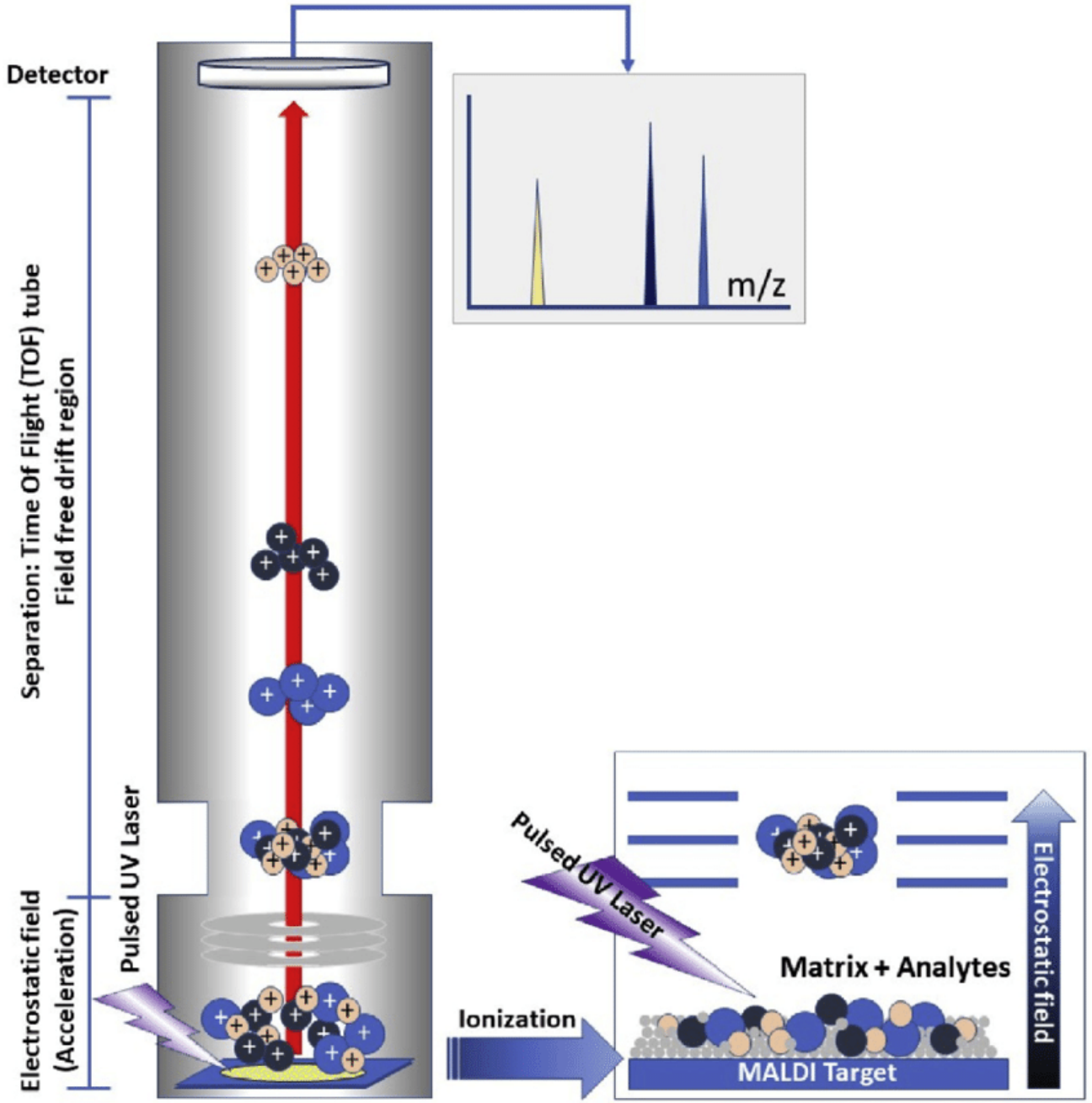


Mass analyzer: TOF (1)

A time-of-flight (TOF) mass analyzer works by accelerating ions through a vacuum chamber and measuring the time it takes for the ions to travel a specific distance. **The time of flight is proportional to the mass-to-charge ratio of the ion**, so by measuring the time of flight, the mass-to-charge ratio of the ion can be determined.

The basic components of a TOF mass spectrometer include an ion source, an **acceleration region, a drift region,** and a detector. In the ion source, the sample is ionized, and the resulting ions are extracted and accelerated by an electric field into the acceleration region.

Once the ions are accelerated, they enter the drift region, which is typically a long, evacuated tube with a known length. As the ions travel through the drift region, they experience a constant electric field, which causes them to continue accelerating.

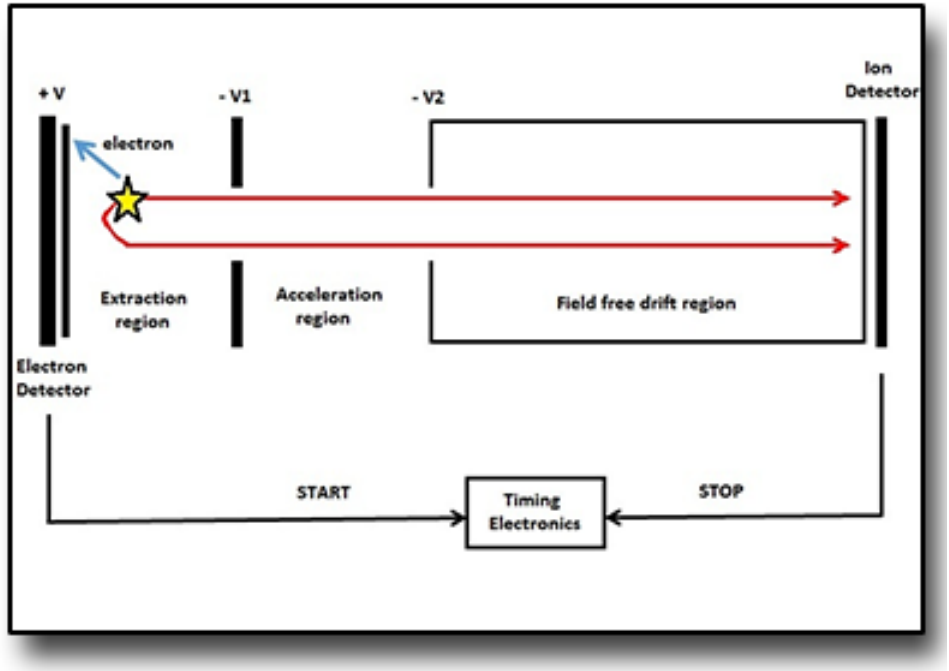


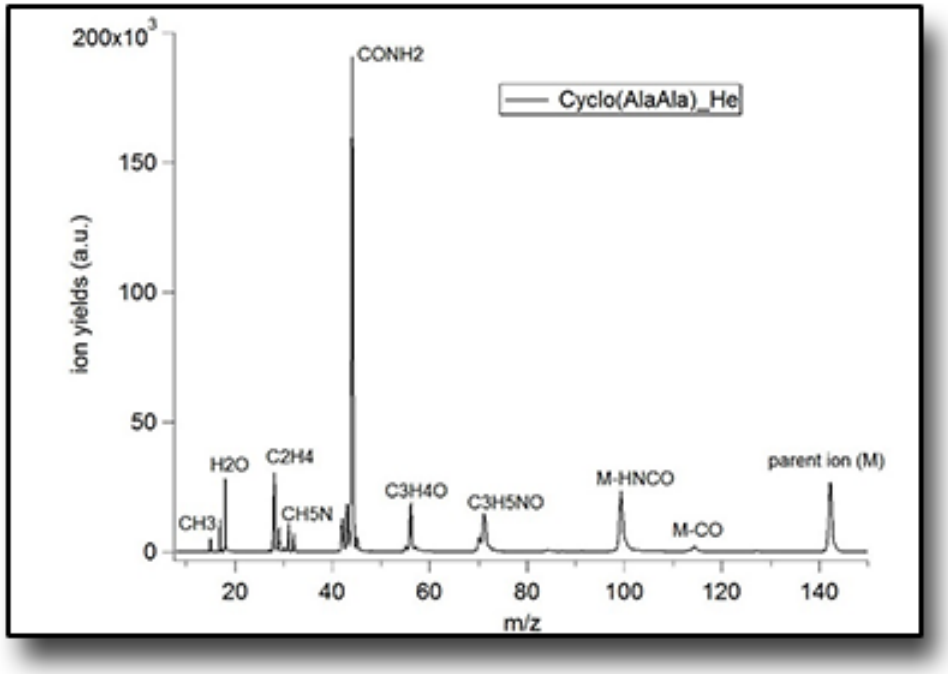
Mass analyzer: TOF (2)

The time it takes for the ions to travel through the drift region is measured using a detector at the end of the tube. The detector produces an electrical signal that is proportional to the number of ions that reach it, and the signal is recorded over a specific time period.

The mass-to-charge ratio of the ions is then calculated by dividing the length of the drift region by the time it takes for the ions to reach the detector. By measuring the time of flight of the ions, the TOF mass spectrometer can determine the mass-to-charge ratio of the ions, which can be used to identify and quantify the ions present in the sample.

TOF mass spectrometry is a powerful analytical technique because it can rapidly measure the mass-to-charge ratio of ions over a wide mass range with high sensitivity and resolution. It is commonly used in proteomics, metabolomics, and other fields to identify and quantify molecules in complex mixtures.



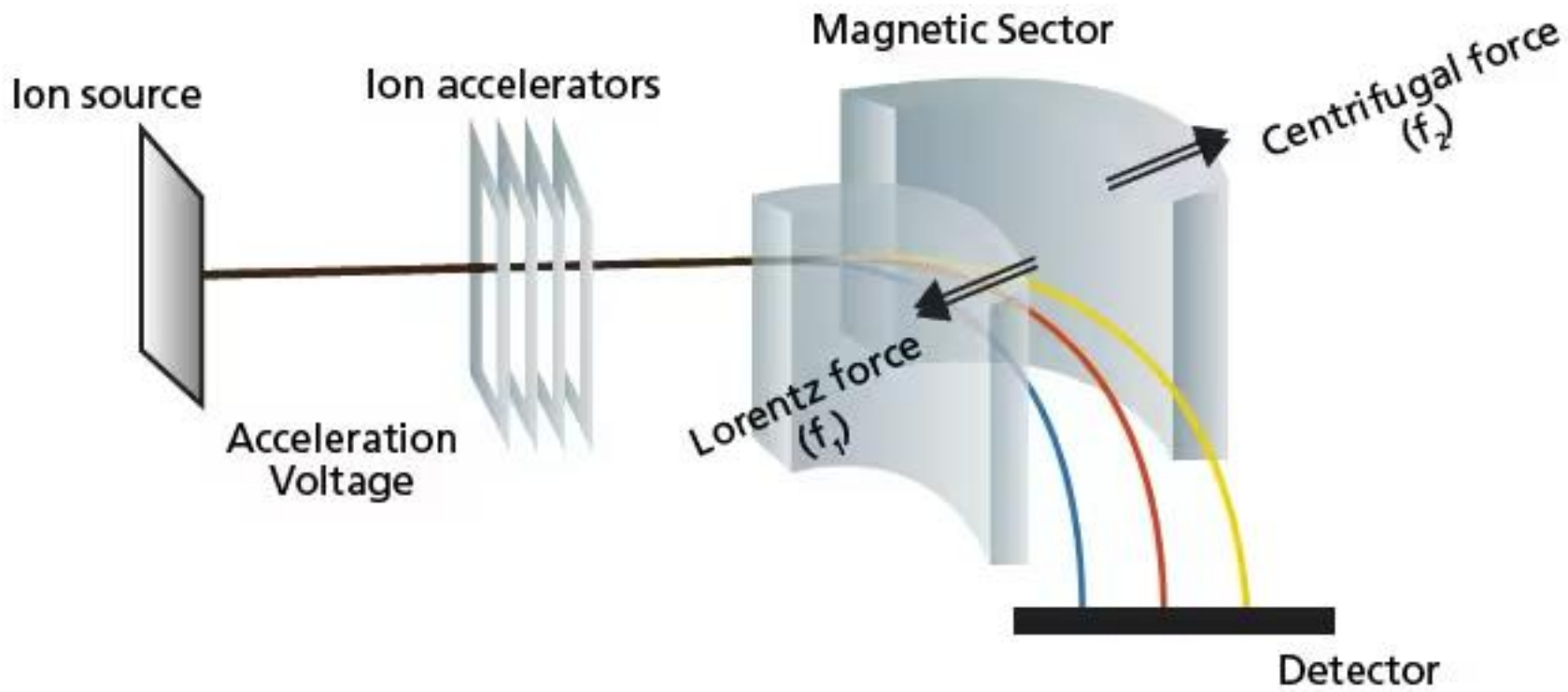


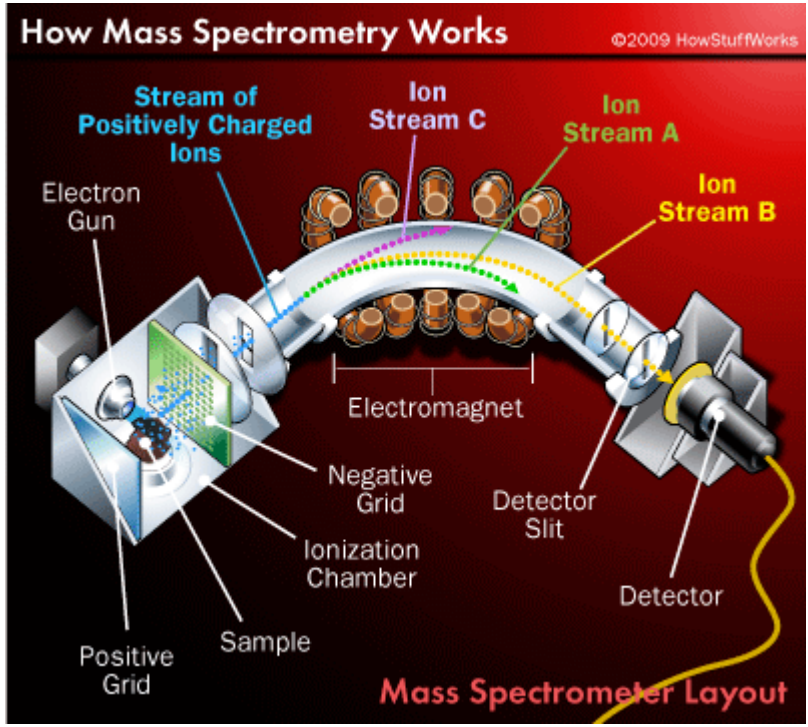
Mass analyzer: Magnetic sector (1)

A **magnetic sector mass analyzer uses a magnetic field** to selectively separate ions based on their mass-to-charge ratio.

In the ion source, the sample is ionized, and the resulting ions are accelerated into the analyzer magnet. **The analyzer magnet consists of a curved magnetic field that causes ions with different mass-to-charge ratios to follow different paths through the magnet.**

As the ions enter the magnetic field, they are subject to a force that is perpendicular to both their velocity and the magnetic field. This force causes the ions to follow a curved path through the magnet. The radius of curvature is proportional to the mass-to-charge ratio of the ion, so ions with different mass-to-charge ratios follow different paths.



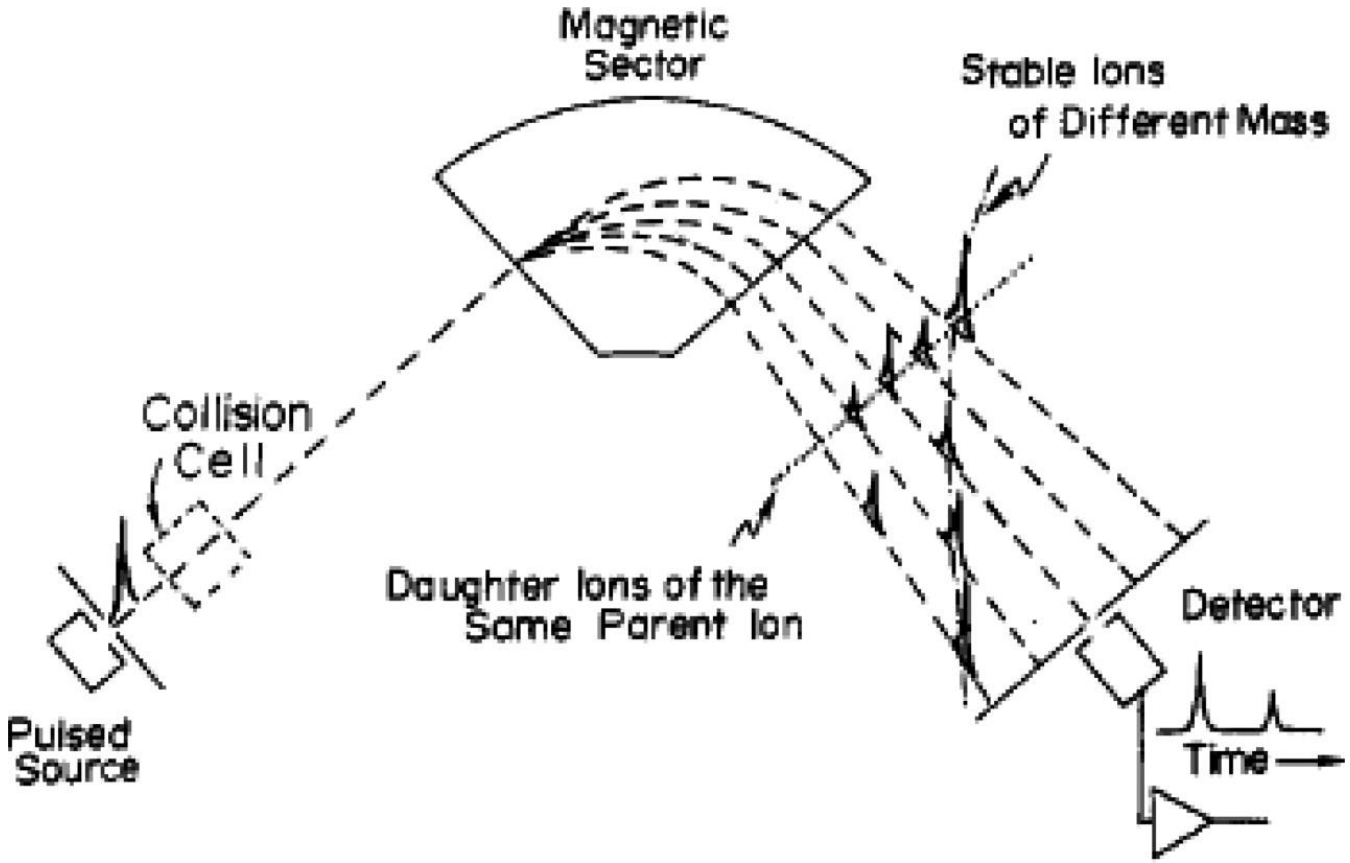


Mass analyzer: Magnetic sector (2)

Only ions with a specific mass-to-charge ratio will follow a path that leads them to the detector at the end of the analyzer magnet. Other ions will follow paths that cause them to collide with the walls of the magnet or be lost.

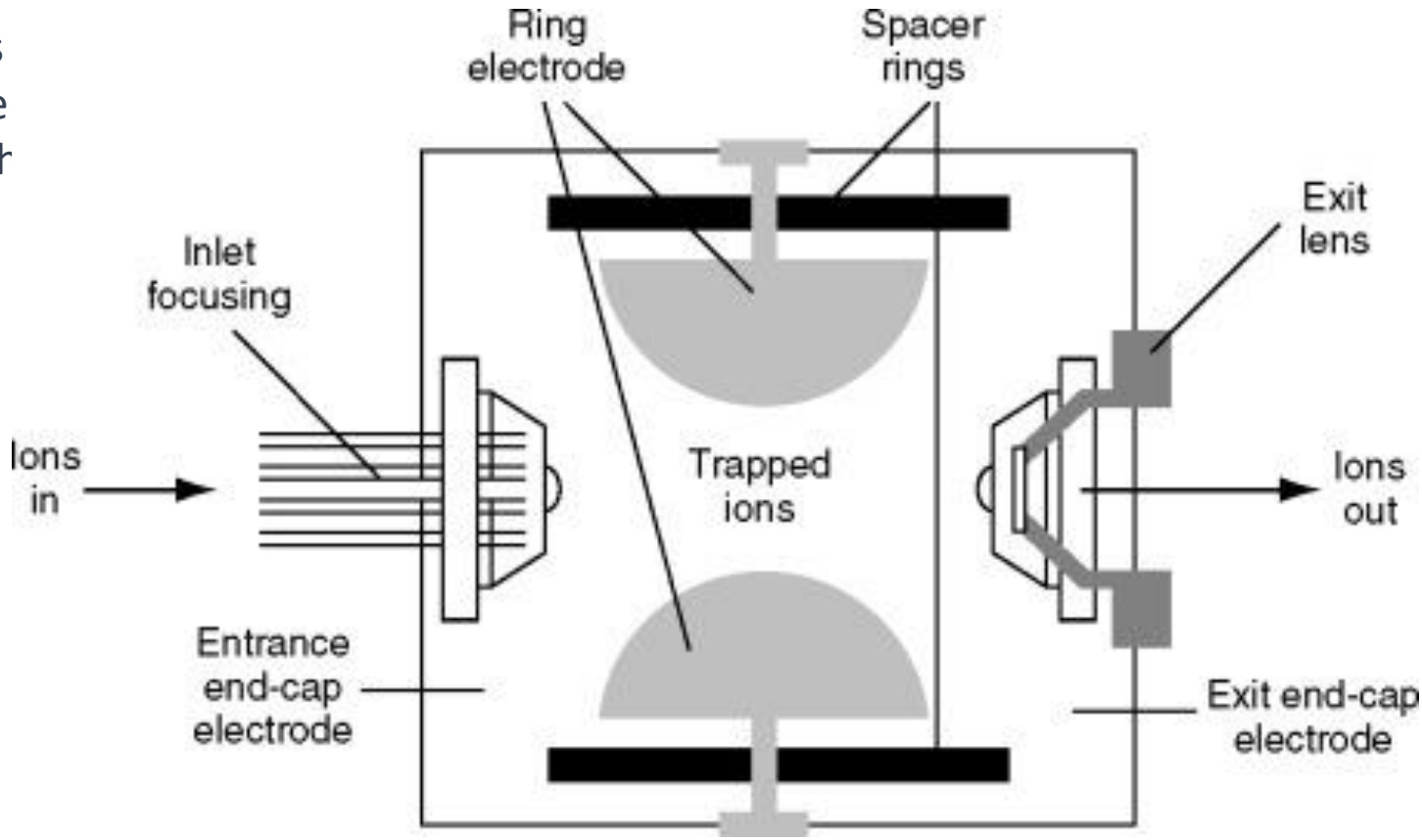
The detector at the end of the analyzer magnet detects the ions that follow the correct path and produces an electrical signal that is proportional to the number of ions detected. The signal is then recorded and used to construct a mass spectrum, which shows the relative abundance of ions as a function of their mass-to-charge ratio.

Magnetic sector mass spectrometry is a powerful technique that can be used for both qualitative and quantitative analysis of small molecules, proteins, and other biomolecules. It is commonly used in environmental monitoring, forensic analysis, and other fields to identify and quantify trace amounts of molecules in complex mixtures.



Mass analyzer: Ion trap

An ion trap mass spectrometer (ITMS) is a type of mass spectrometer that uses an electrostatic field to trap ions in a three-dimensional space. In an ion trap MS, ions are first ionized by one of several ionization techniques, such as electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The ions are then introduced into the ion trap where they are trapped by a combination of radiofrequency (RF) and direct current (DC) electric fields.



The basic design of an ion trap consists of two endcap electrodes and a ring electrode. The endcap electrodes are typically held at a positive or negative DC voltage, while the ring electrode is held at a higher RF voltage. The combination of the RF and DC voltages creates a potential energy well that traps ions in the center of the trap.

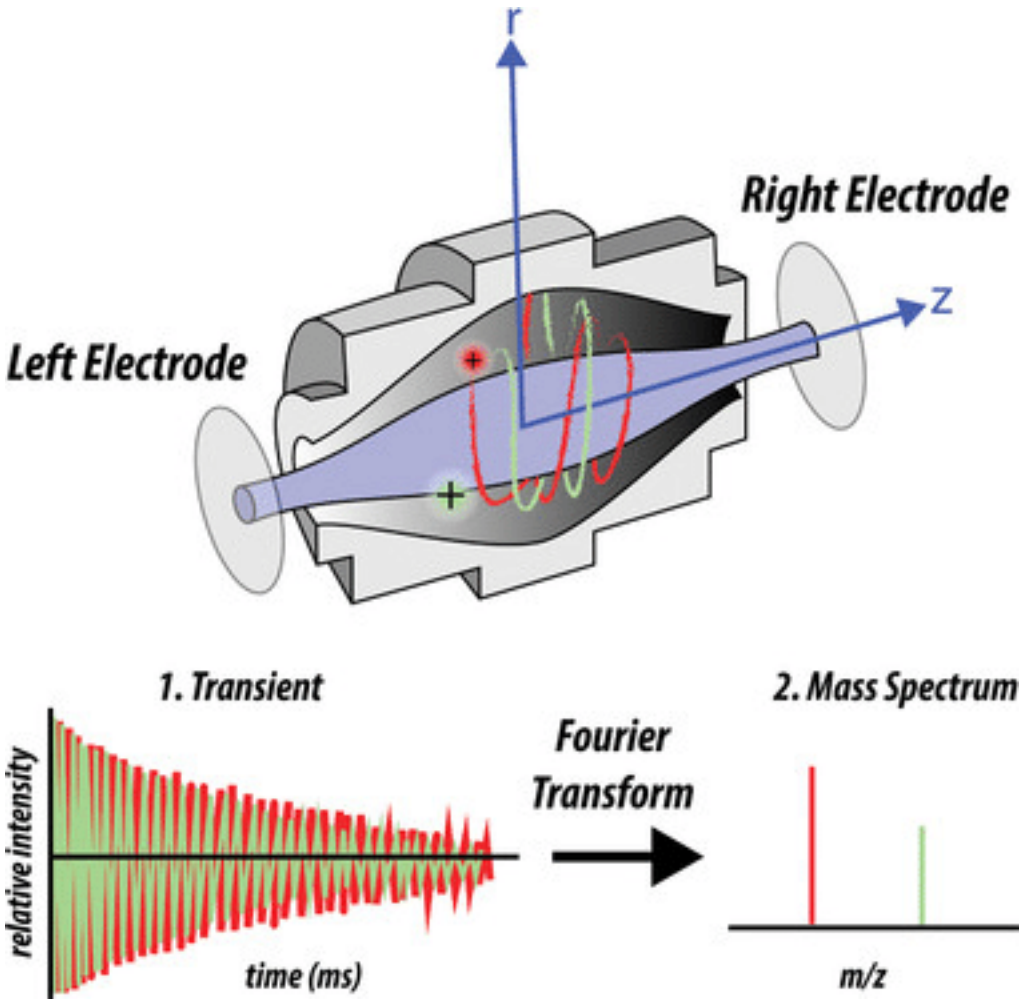
Once the ions are trapped in the ion trap, they can be analyzed using several techniques, including mass selective ejection, collision-induced dissociation (CID), and resonant ejection. **In mass selective ejection, the RF and DC voltages are adjusted to selectively eject ions based on their mass-to-charge ratio (m/z) through one of the endcap electrodes.** In CID, the trapped ions are subjected to collisions with a neutral gas, which can cause them to fragment and provide structural information. **In resonant ejection, the RF voltage is adjusted to resonantly excite the ions, causing them to be ejected from the trap.**

Mass analyzer: Orbitrap

In **the Orbitrap mass analyzer**, ions are trapped in a radial electrostatic field between the outer barrel-shaped electrode and the inner spindle-shaped electrode. **When a voltage is applied to the electrodes, the ions oscillate back and forth along the central spindle electrode, generating an image current that is detected by electrodes positioned at the ends of the spindle.** The frequency of the ion motion is inversely proportional to the ion's mass-to-charge ratio (m/z).

The image current is digitized and Fourier-transformed to obtain a mass spectrum. The resolving power of the Orbitrap analyzer is extremely high, typically in the range of 100,000 to 1,000,000, which allows for the separation of closely spaced mass peaks and the detection of low-abundance ions.

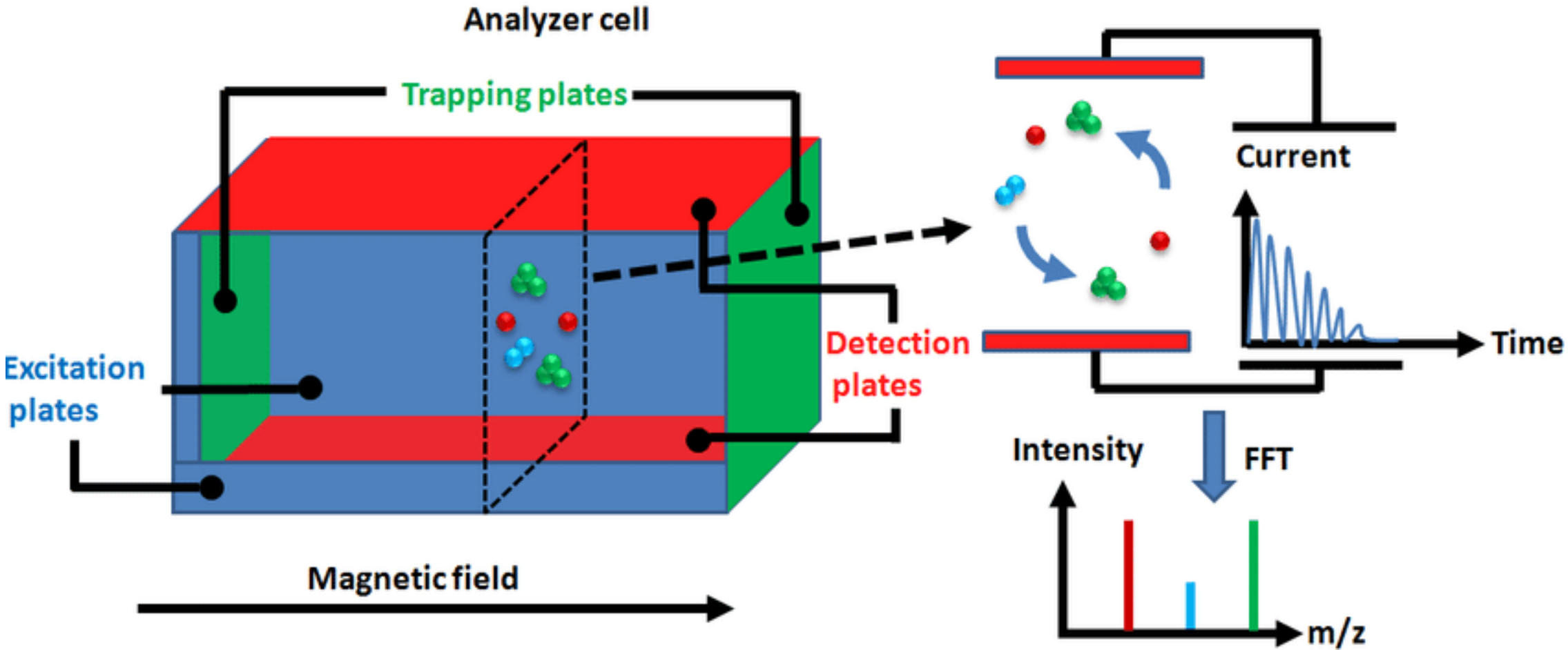
Orbitrap mass spectrometry is widely used in many areas of research, including proteomics, metabolomics, lipidomics, and small molecule analysis. It is particularly useful for the identification and quantification of complex mixtures, such as those found in biological samples, and for the analysis of post-translational modifications in proteins.



Mass analyzer: FT-ICR (1)

A Fourier transform ion cyclotron resonance (FT-ICR) mass analyzer, ions are accelerated into the ion trap, which consists of a combination of magnetic and electric fields. **The magnetic field confines the ions to a circular orbit around the center of the trap**, while the electric field applies a radial force that balances the centrifugal force and keeps the ions in a stable orbit.

Once the ions are trapped, an external radiofrequency (RF) electric field is applied to the ion trap. This causes the ions to oscillate around their stable orbits at a characteristic frequency, known as the cyclotron frequency, which is proportional to the ion's mass-to-charge ratio.

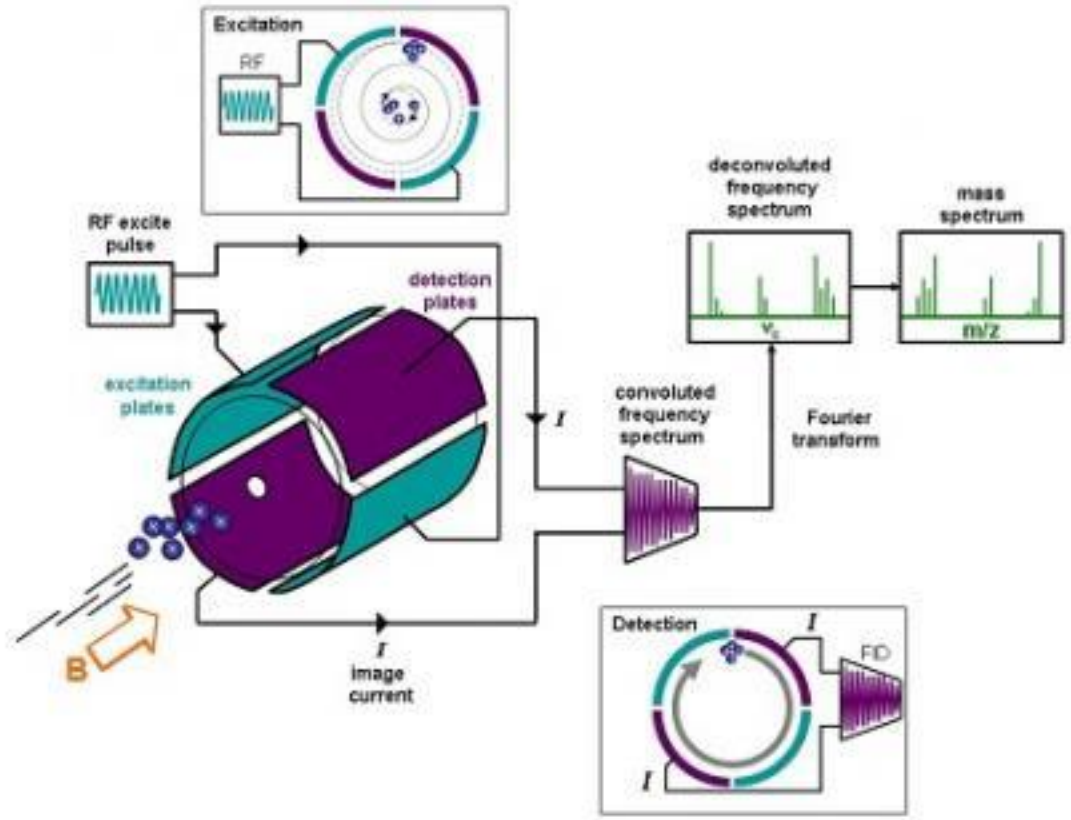


Mass analyzer: FT-ICR (2)

The ions' motion induces an image current in the detector plates outside the ion trap, which is recorded over time as a time-domain signal. This signal contains information about the ions' cyclotron frequencies, which can be transformed mathematically into a frequency-domain mass spectrum.

The Fourier transform of the time-domain signal reveals the specific mass-to-charge ratios of the ions, with very high resolution and mass accuracy. The resolution of FT-ICR mass spectrometry is limited only by the duration of the signal acquisition, while the mass accuracy is typically in the parts-per-million range.

FT-ICR mass spectrometry is a powerful analytical technique that can be used to analyze complex mixtures of small molecules, proteins, and other biomolecules, with high sensitivity and resolution. It is commonly used in proteomics, metabolomics, and other fields to identify and quantify molecules in complex mixtures.



Detection

A detector (in MS) is a device that measures the ions produced by ionization of a sample in the mass spectrometer. The detector is responsible for detecting and recording the mass-to-charge ratio (m/z) of the ions produced. The choice of detector depends on the specific application and the type of mass spectrometer being used. There are several types of detectors used in mass spectrometry, including:

Faraday detector: This detector measures the total charge produced by the ion beam as it hits a metal plate. It is a simple and robust detector that is widely used in mass spectrometry.

Electron multiplier: This detector uses a series of electron multiplication stages to amplify the signal produced by the ion beam. It is a more sensitive detector than the Faraday detector, but is more complex and can be easily damaged by high-energy ions.

Time-of-flight detector: This detector measures the time it takes for the ions to travel a known distance. The time-of-flight detector is capable of high mass resolution and can be used in combination with other types of detectors.

Ion trap detector: This detector traps the ions in a small space using electromagnetic fields and then releases them to be detected. It is a versatile detector that can be used for both mass analysis and tandem mass spectrometry.

Mass Spectrometer. Parts (Quadrupole)

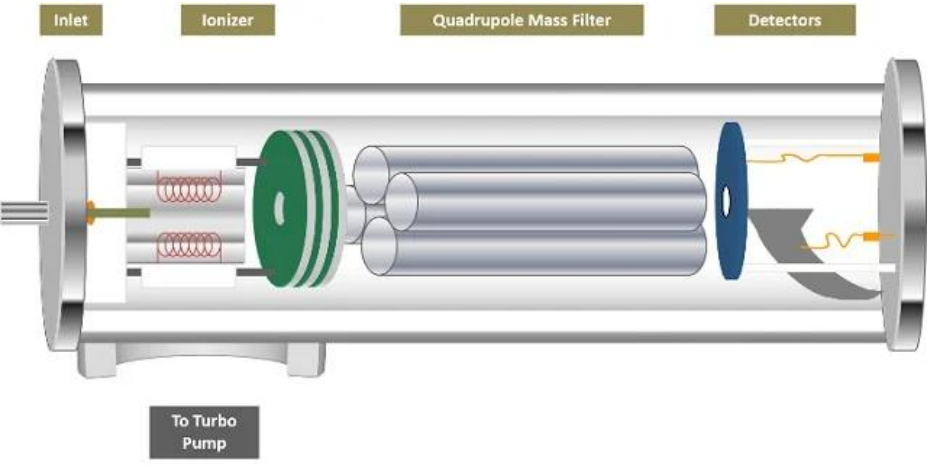
Ion source: This is where the sample is ionized, typically by electron ionization (EI) or electrospray ionization (ESI), and the resulting ions are extracted and focused into a beam.

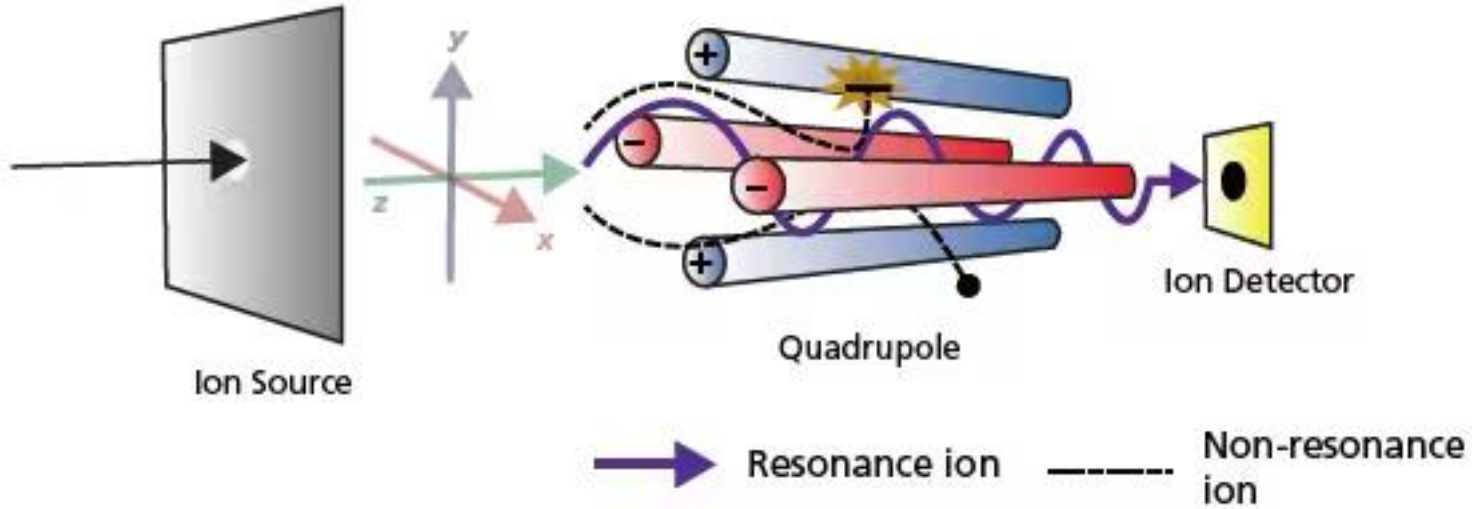
Quadrupole rods: These are a set of four parallel metal rods arranged in a square or hyperbolic pattern. The rods are typically made of stainless steel and are held at a specific voltage that generates a radio frequency (RF) electric field.

RF generator: This provides the RF voltage that is applied to the quadrupole rods. The frequency of the RF voltage determines the mass range that the instrument can analyze. Mass analyzer: The quadrupole rods act as a mass analyzer, selectively transmitting ions of a specific mass-to-charge ratio while filtering out other ions. The mass-to-charge ratio of the transmitted ions can be changed by adjusting the RF voltage or the DC voltage applied to the quadrupole rods.

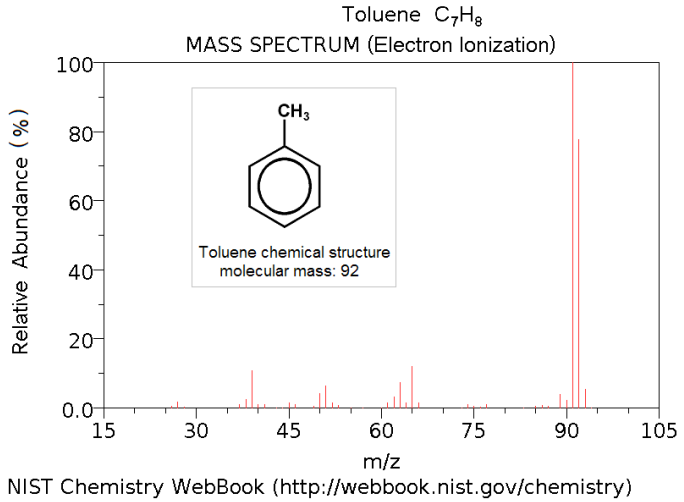
Detector: The transmitted ions are detected by a detector, which can be a Faraday cup, an electron multiplier, or another type of ion detector. The detector measures the ion current as a function of time, allowing the mass spectrum of the sample to be obtained.

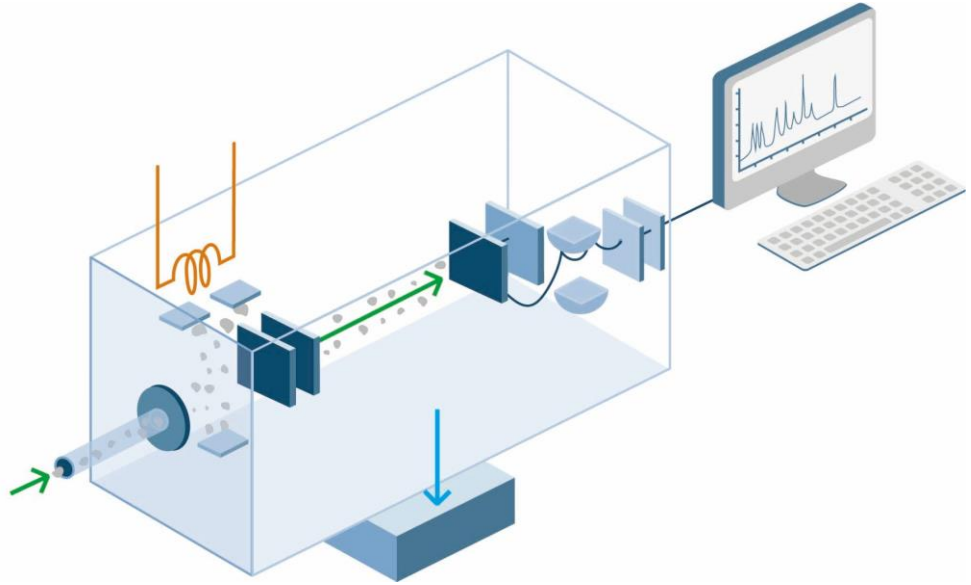
Data system: The signals from the detector are processed and analyzed by a computer system, which generates the mass spectrum and provides other information about the sample, such as the relative abundance of each ion and the molecular formula of the compounds in the sample.

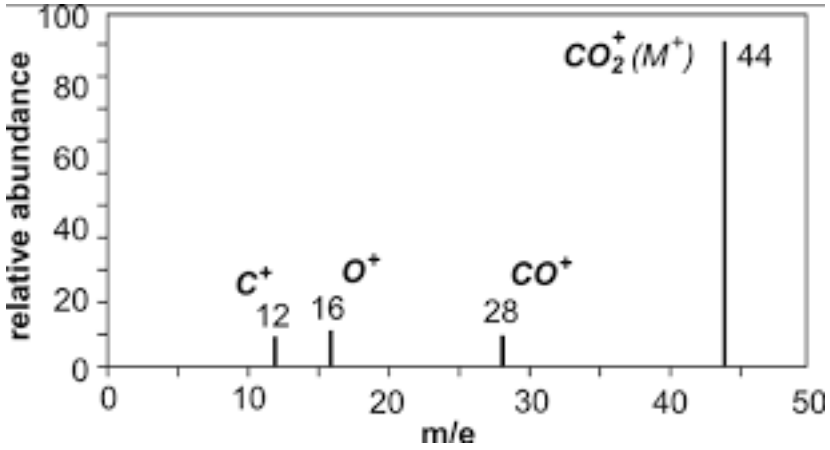




Ok, we ionized the sample, separated a bunch of ions according to their m/z (which effectively, in many cases such as EI-Quadrupole MS means MASS)… NOW WHAT?





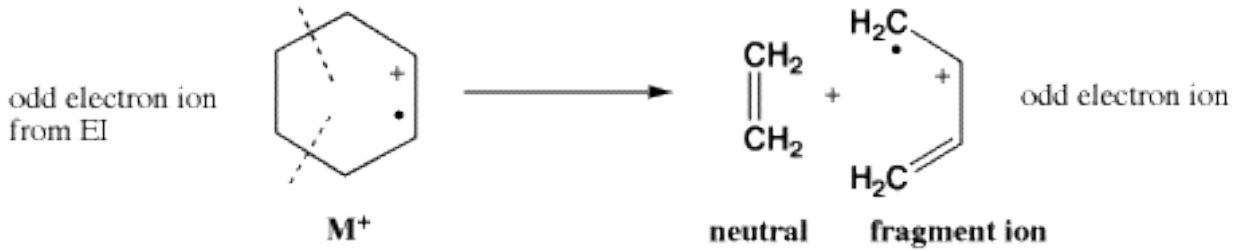


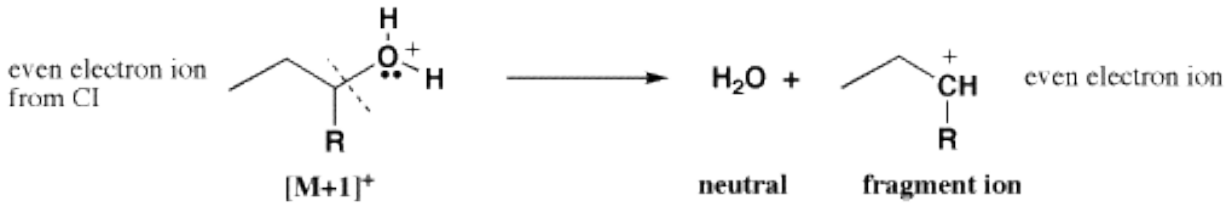
MOLECULAR FRAGMENTATION

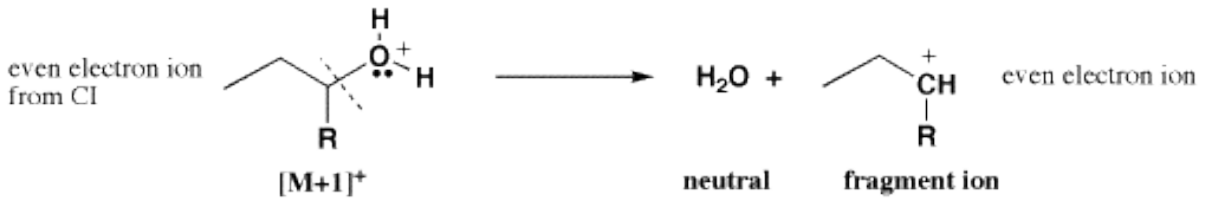
Molecule fragmentation (in MS) **is the process by which a molecule is broken apart into smaller fragments in a controlled manner** using high-energy collisions with gas molecules, electrons, or photons. The resulting fragments are then separated and detected by the mass spectrometer, **providing information about the molecular structure and composition of the original molecule.**

There are several types of molecule fragmentation techniques used in mass spectrometry, including collision-induced dissociation (CID), electron capture dissociation (ECD), electron transfer dissociation (ETD), and photodissociation (PD). These techniques can be used to generate different types of fragment ions, such as radical ions, cations, and anions, which can be analyzed to obtain information about the chemical bonds, functional groups, and isotopic composition of the original molecule.

Molecule fragmentation is an important tool in the field of analytical chemistry, as it allows for the **identification and characterization of unknown compounds, as well as the study of chemical reactions and molecular dynamics**.







What does molecular fragmentation depend upon?

Energy input: The energy of the collision, electron or photon that interacts with the molecule is the primary factor controlling fragmentation. High-energy collisions can break chemical bonds and cause the molecule to fragment into smaller ions.

Molecular structure: The structure of the molecule, including the types of chemical bonds and functional groups present, determines the likelihood and types of fragmentation that can occur. Some bonds are more easily broken than others, and some functional groups may stabilize or destabilize ions.

Mass-to-charge ratio (m/z) of the ion: The mass-to-charge ratio of the ion being analyzed affects the fragmentation process. Different ions may fragment differently due to differences in their m/z values.

Collision gas: In collision-induced dissociation (CID) fragmentation, the choice of collision gas used can affect the fragmentation pattern of the ion. The type and pressure of the gas can determine the types of collisions that occur, leading to different fragmentation pathways.

Instrumental parameters: The instrumental parameters of the mass spectrometer, such as the voltage applied to the ionization source and the mass analyzer, can also affect fragmentation. Adjusting these parameters can change the type and amount of fragmentation that occurs.

Molecule fragmentation: Uses

Structural analysis: Molecule fragmentation can provide information about the structural characteristics of a molecule, such as the presence of functional groups and the location of double bonds or other chemical bonds.

Identification of unknown compounds: Molecule fragmentation can be used to identify the chemical structure and composition of unknown compounds, by comparing the mass spectra of the fragments with those in databases of known compounds.

Confirmation of synthesis products: Molecule fragmentation can be used to confirm the success of a chemical synthesis reaction by comparing the mass spectra of the product with those of the starting materials.

Study of chemical reactions and mechanisms: Molecule fragmentation can provide information about the chemical reactions and mechanisms that lead to the formation of the fragments, by analyzing the patterns and abundances of the ions produced.

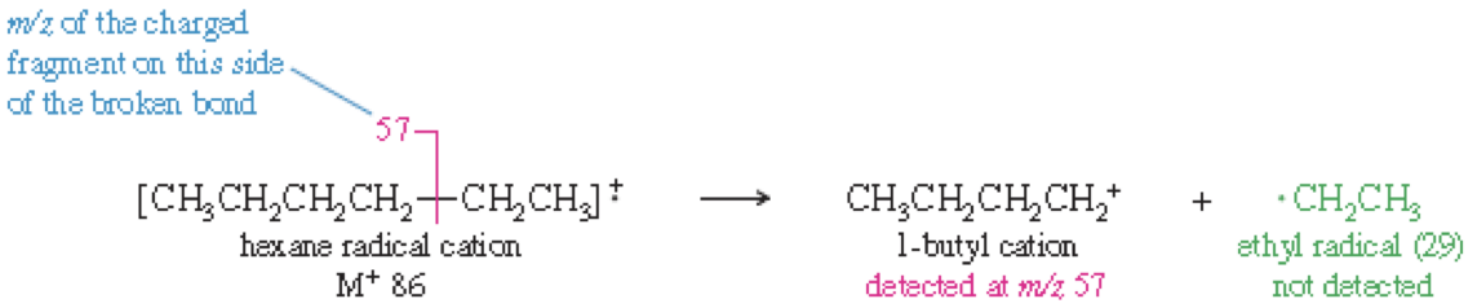
Quantitative analysis: Molecule fragmentation can also be used for quantitative analysis, by measuring the intensity of the fragment ions produced and using this information to determine the abundance of the original molecule.

Molecule fragmentation: Uses. Structural analysis (Spectral interpretation and assambling the partner molecule as if it is a maze)

Molecule structure controls fragmentation in mass spectrometry by affecting the **types of bonds** that can be broken and the stability of the resulting fragments. **Some types of bonds**, such as weak bonds or bonds that are easily broken, **are more likely to break during fragmentation**. Other **bonds may require more energy** or specific types of interactions in order to break.

Additionally, the presence of functional groups or other structural features can affect fragmentation patterns. For example, certain functional groups such as carboxylates, sulfates, and phosphates can stabilize negatively charged ions and may therefore appear more frequently as fragment ions. Other groups, such as aromatic rings, may stabilize cationic species.

The structure of the molecule can also influence the order and probability of bond cleavage during fragmentation. Certain bonds may be more likely to break than others, leading to specific fragmentation pathways. The position of functional groups and substituents on the molecule can also affect the order and types of



Example of molecule fragmentation: Ethanol

In electron ionization (EI) mass spectrometry, a molecule is ionized by high-energy electrons, resulting in the formation of a series of fragment ions. The major ions expected in the EI mass spectrum of ethanol (CH3-CH2-OH) are:

Molecular ion (M+): The molecular ion is the ion that is formed by the addition of an electron to the neutral molecule, resulting in the preservation of the molecular mass. For ethanol, the molecular ion has a mass-to-charge ratio (m/z) of **46**, corresponding to the molecular formula [CH3-CH2-OH]+. (And **45** after the loss of a proton)

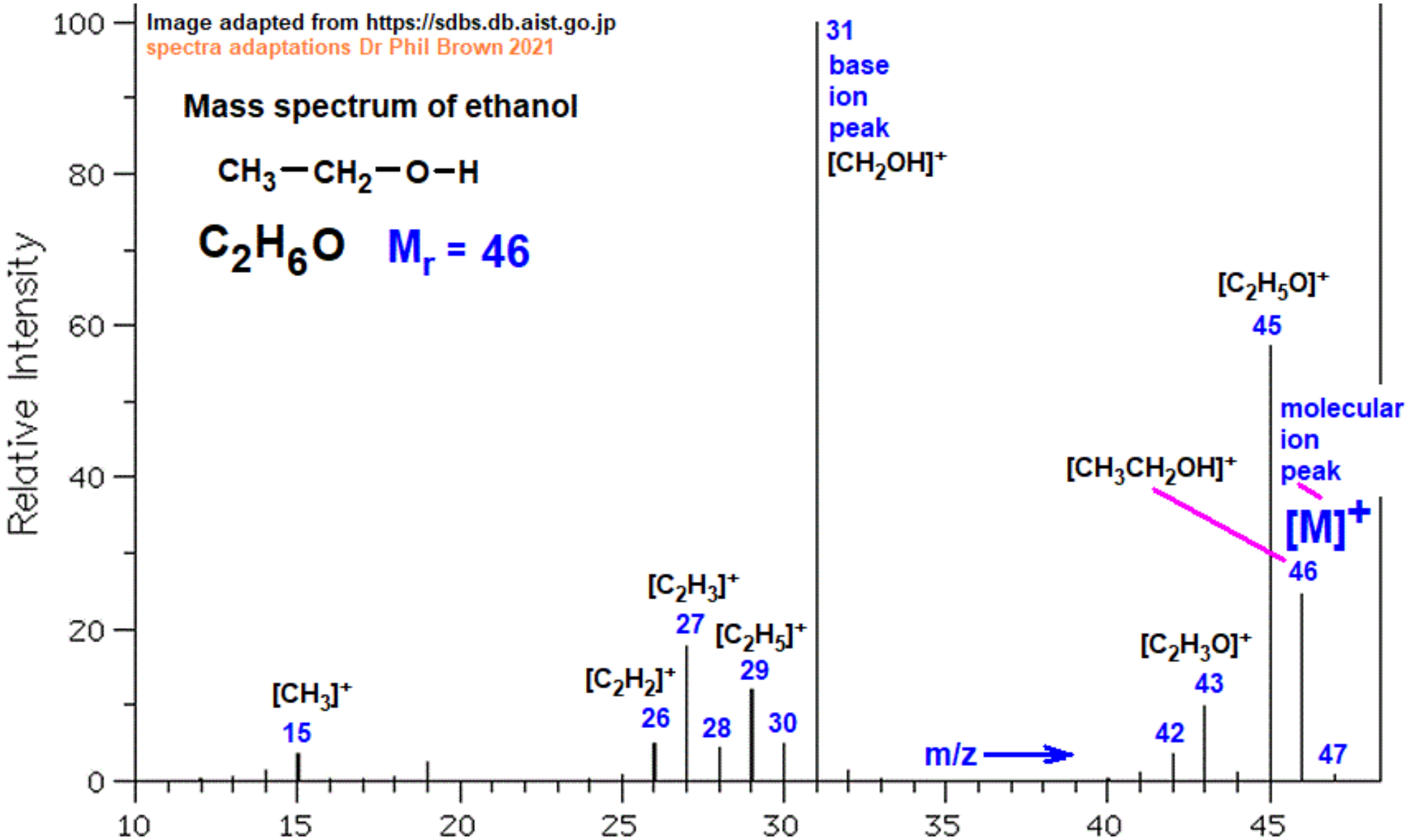
Ethene ion (M-29): The loss of a hydroxyl radical (OH) from the molecular ion results in the formation of an ethene ion, which has an m/z of **29**.

Methanol ion (M-31): The loss of a methyl group (CH3) from the molecular ion results in the formation of a methanol ion, which has an m/z of **31**.

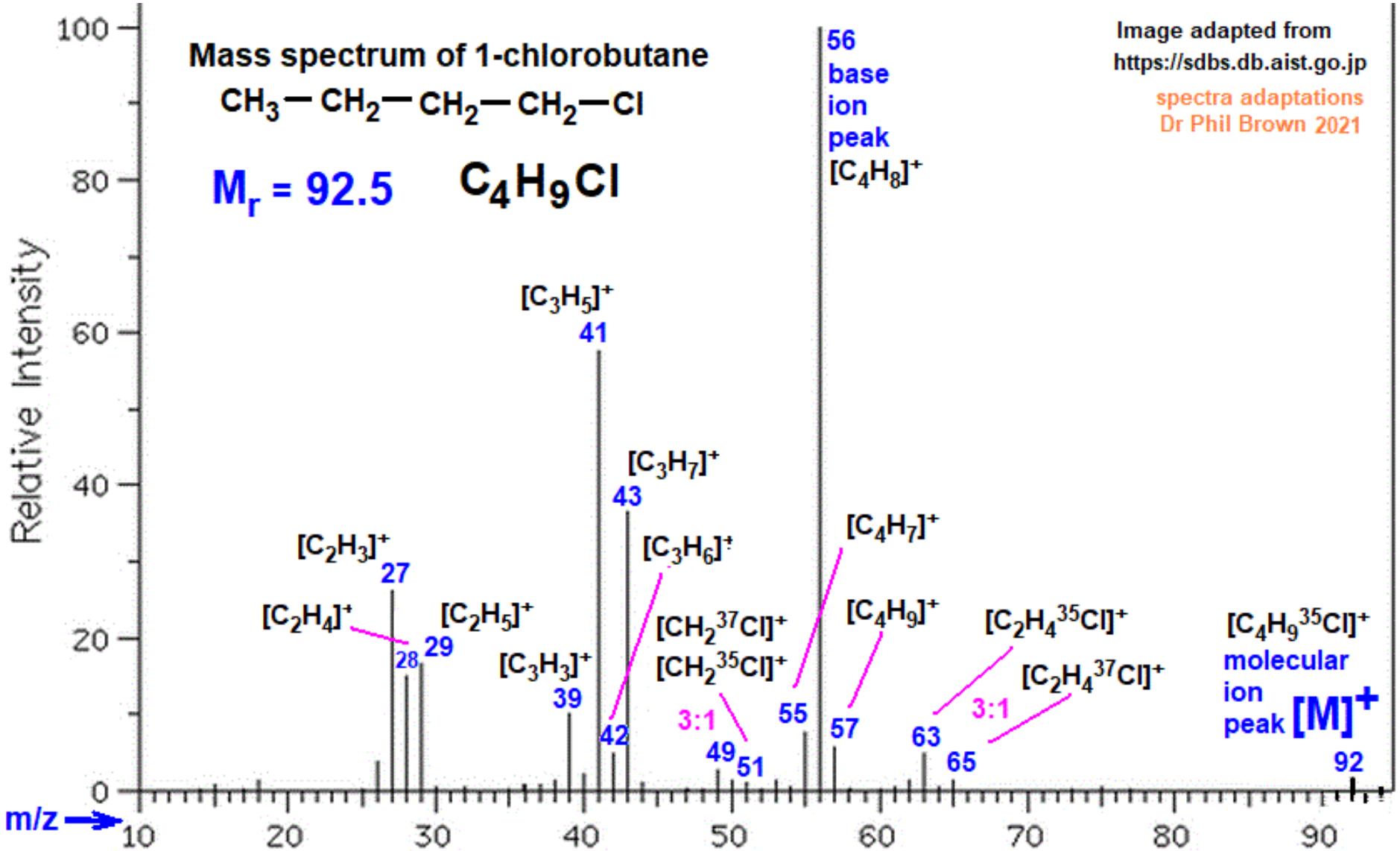
Ethyl ion (M-15): The loss of a methoxy radical (CH3O) from the molecular ion results in the formation of a methyl ion, which has an m/z of **15**.

Other minor fragment ions may also be observed in the mass spectrum, depending on the EI conditions and the structure of the molecule. For example, the loss of a hydrogen radical from the molecular ion can result in the formation of an [CH3-CH-CH2]+ ion, which has an m/z of 43. However, the four ions listed above are typically the most abundant in the EI mass spectrum of ethanol.

Example of molecule fragmentation: Ethanol



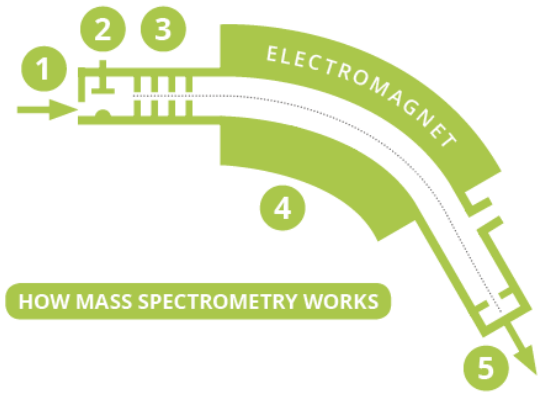
Example of molecule fragmentation: 1-Chlorobutane



Molecule fragmentation: Uses. Structural analysis (Spectral interpretation and assambling the partner molecule as if it is a maze)(2)

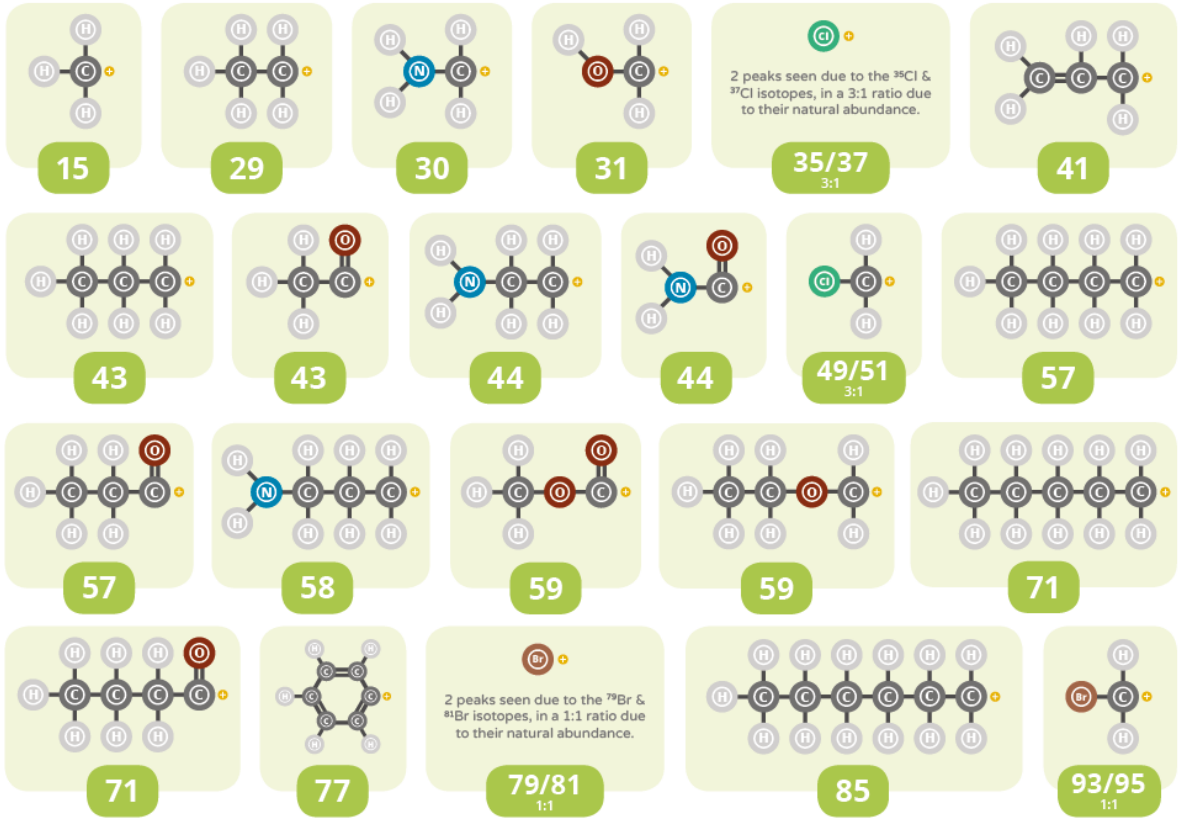
A GUIDE TO INTERPRETING MASS SPECTRA

Mass spectrometry is an analytical technique that allows us to measure the masses of atoms and molecules. The most important peak in a mass spectrum is the molecular ion peak, which can be used to determine the mass of the molecule, but fragment ions can also provide information on chemical structure.



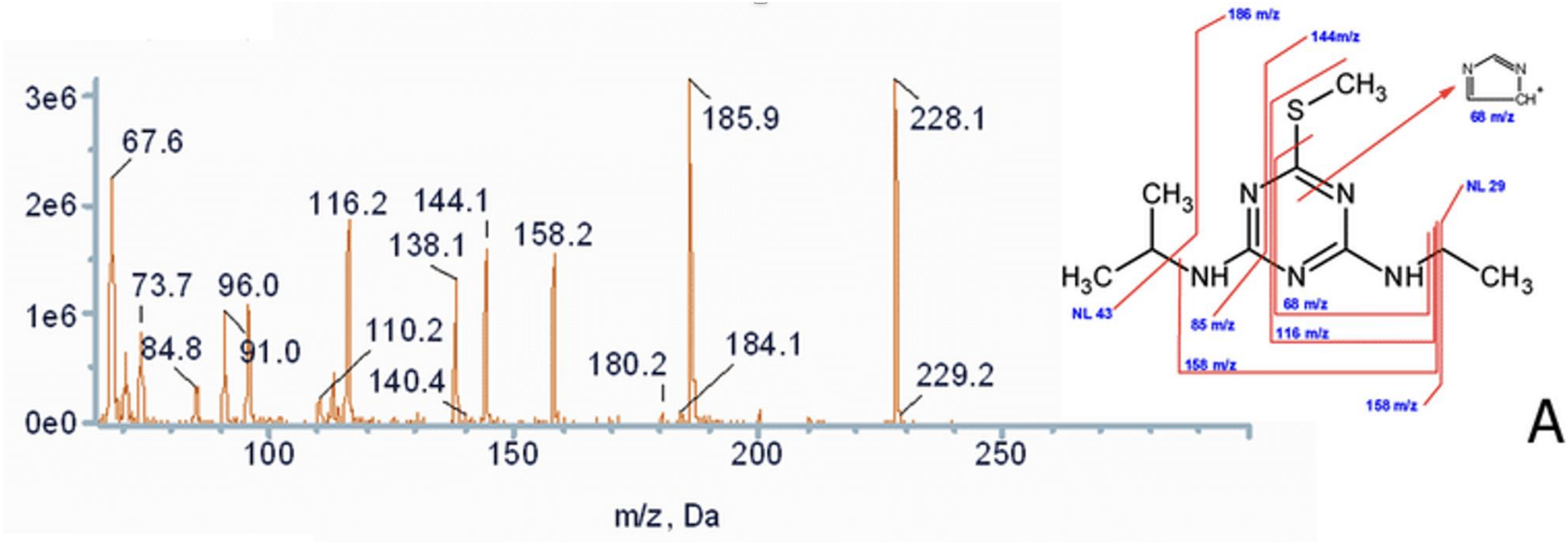
HOW MASS SPECTROMETRY WORKS

1. The sample is introduced to the mass spectrometer. Only very small samples are required. A heater is often present to vapourise the sample.
2. An electron gun ionises molecules in the sample by knocking out electrons, producing positive ions. Some molecules break into smaller ions & fragments.
3. The positive ions generated are passed through an electric field which accelerates them into a magnetic field generated by an electromagnet.
4. As the positive ions pass through the magnetic field, they are deflected. Lighter ions are deflected more than heavier ions, as are those with higher charges.
5. The positive ions hit a charged plate & accept electrons, creating a signal. The more ions that hit, the greater the signal. The output is a complex stick diagram.

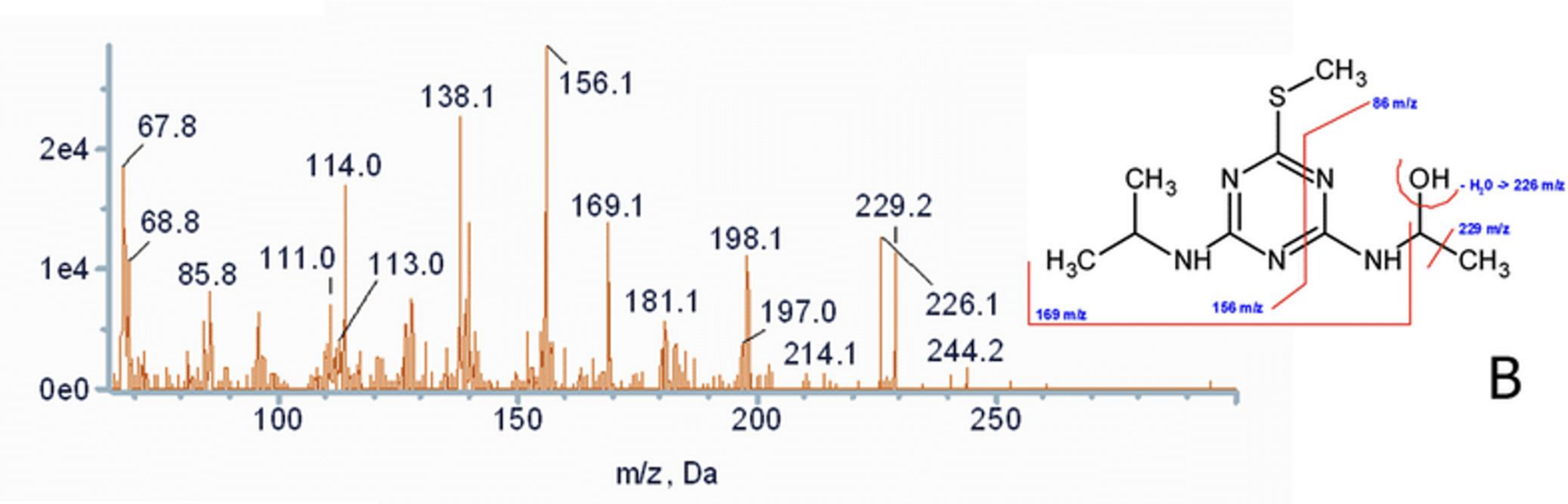


Above are shown a selection of common fragment ions seen in mass spectra, along with their masses. Note that the structures shown are general representations, and it can also be possible for isomeric structures (those with the same constituent atoms, but a different structure) to cause the peaks in spectra. There are also many more fragments possible than those shown, but knowledge of these should suffice to interpret spectra of most simple molecules.

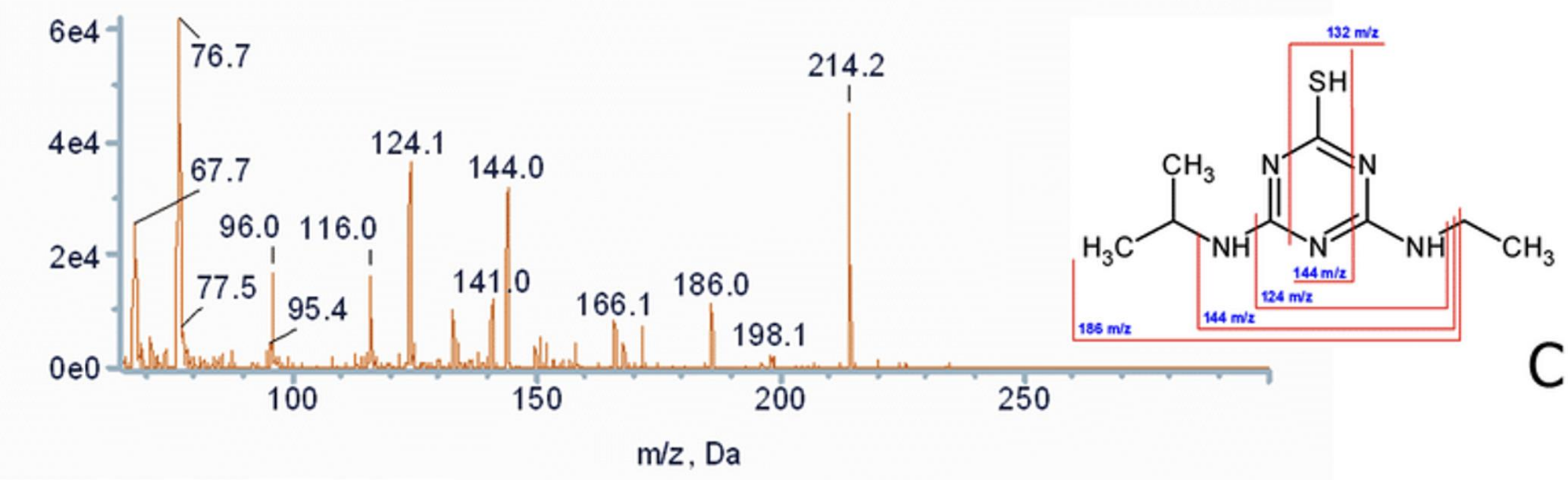
Molecule as a maze



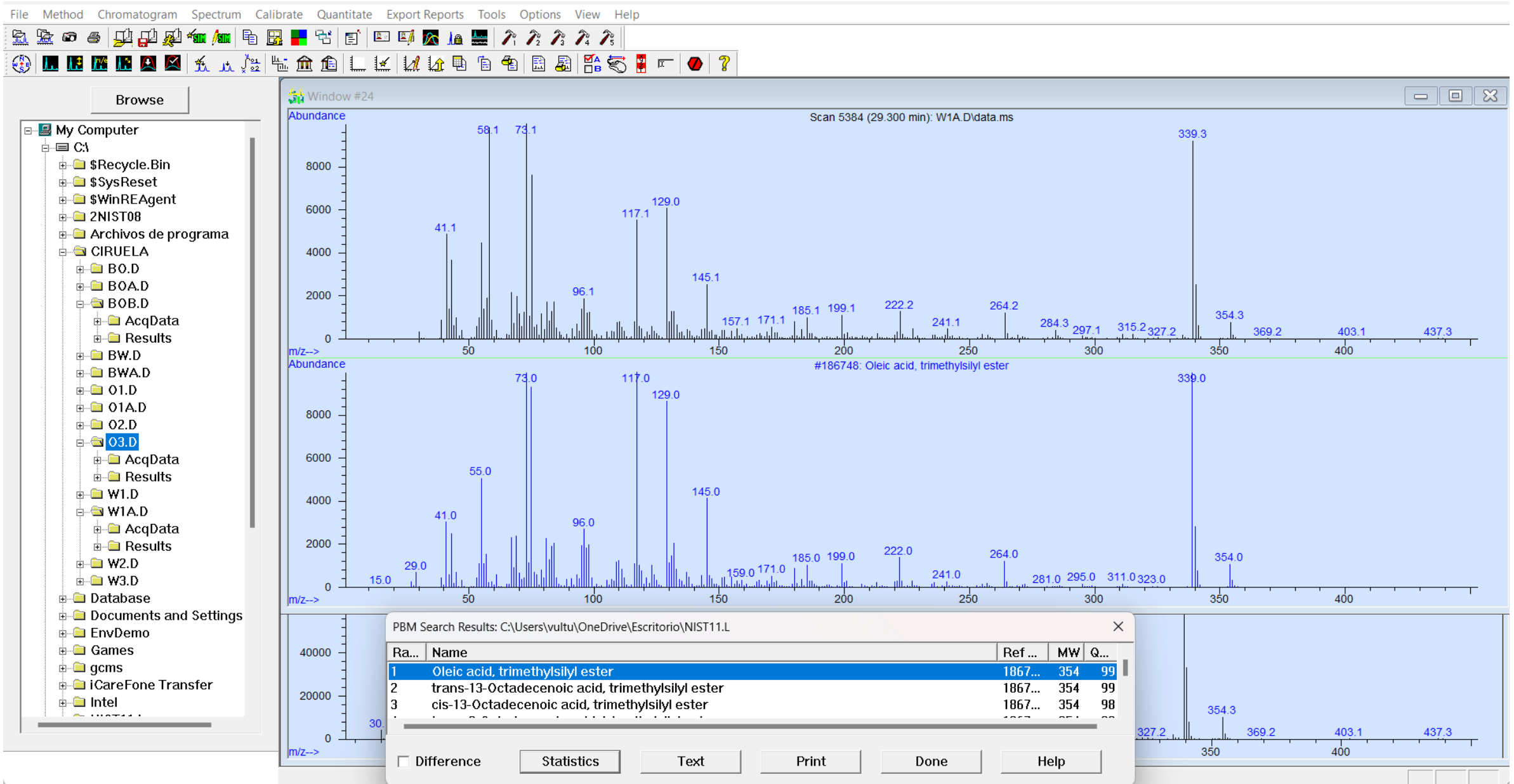
Molecule as a maze



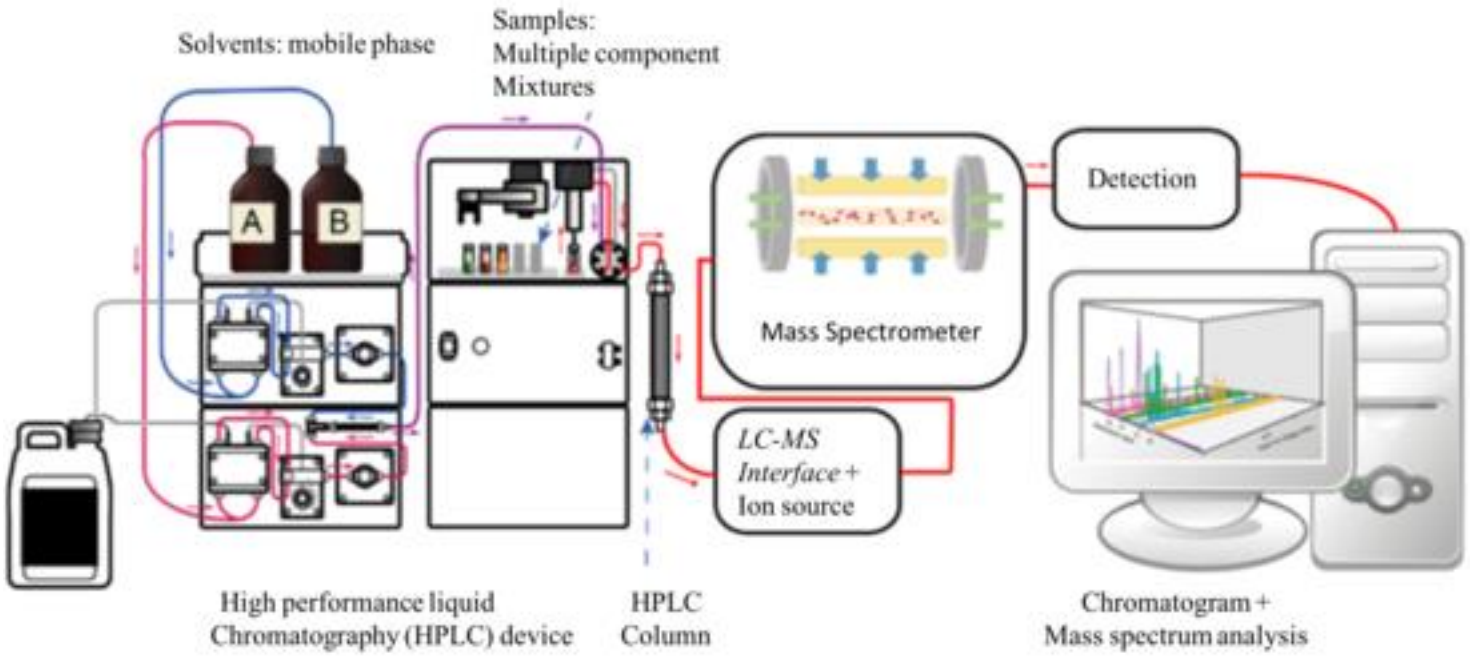
Molecule as a maze

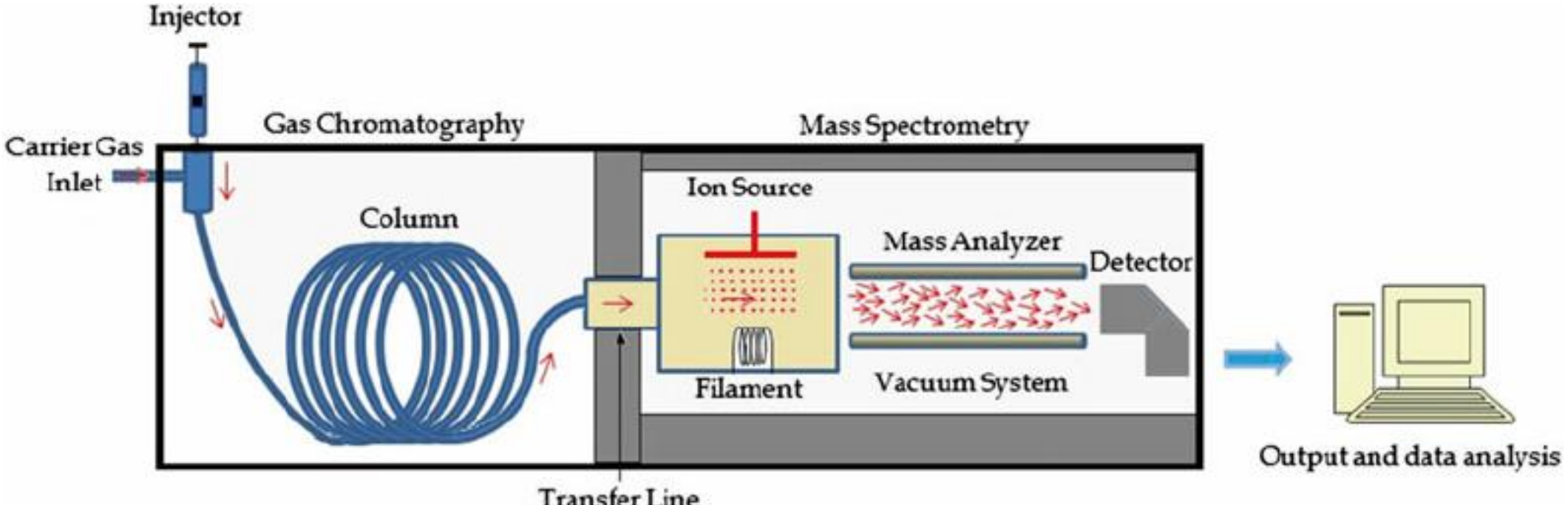


Molecule fragmentation: Uses. Identification of unknown compounds: Library comparisson

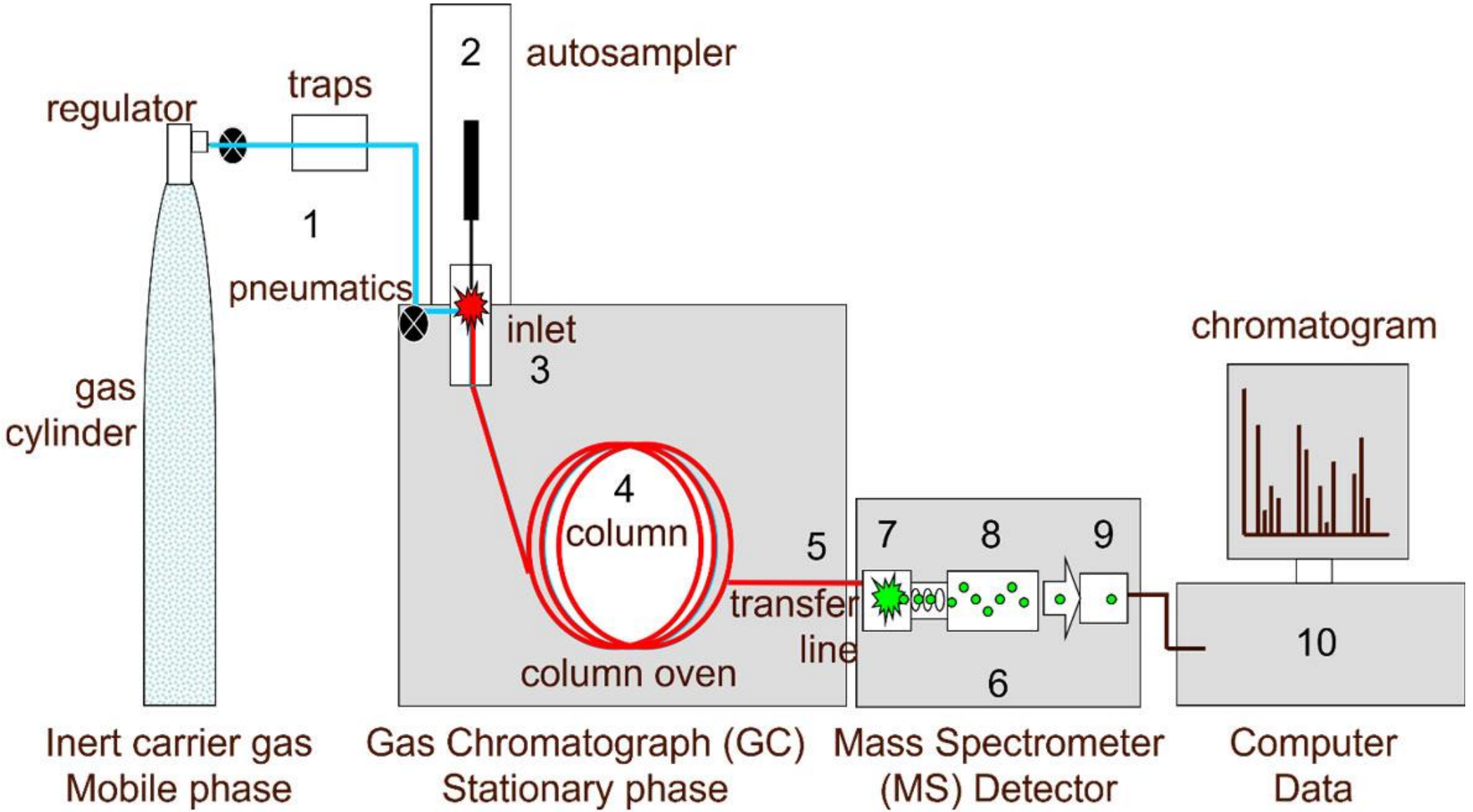


CHROMATOGRAPHY – MASS SPECTROMETRY

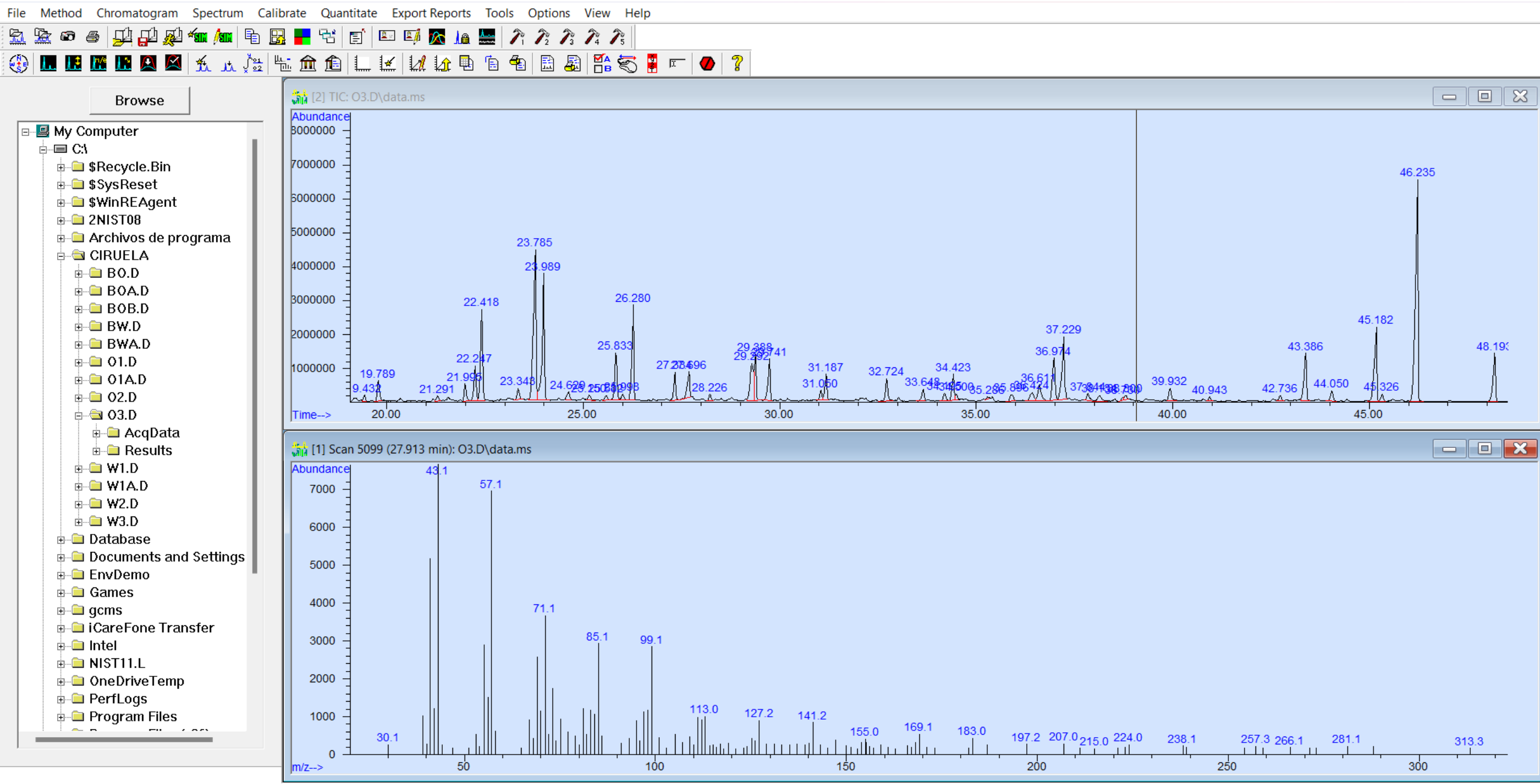




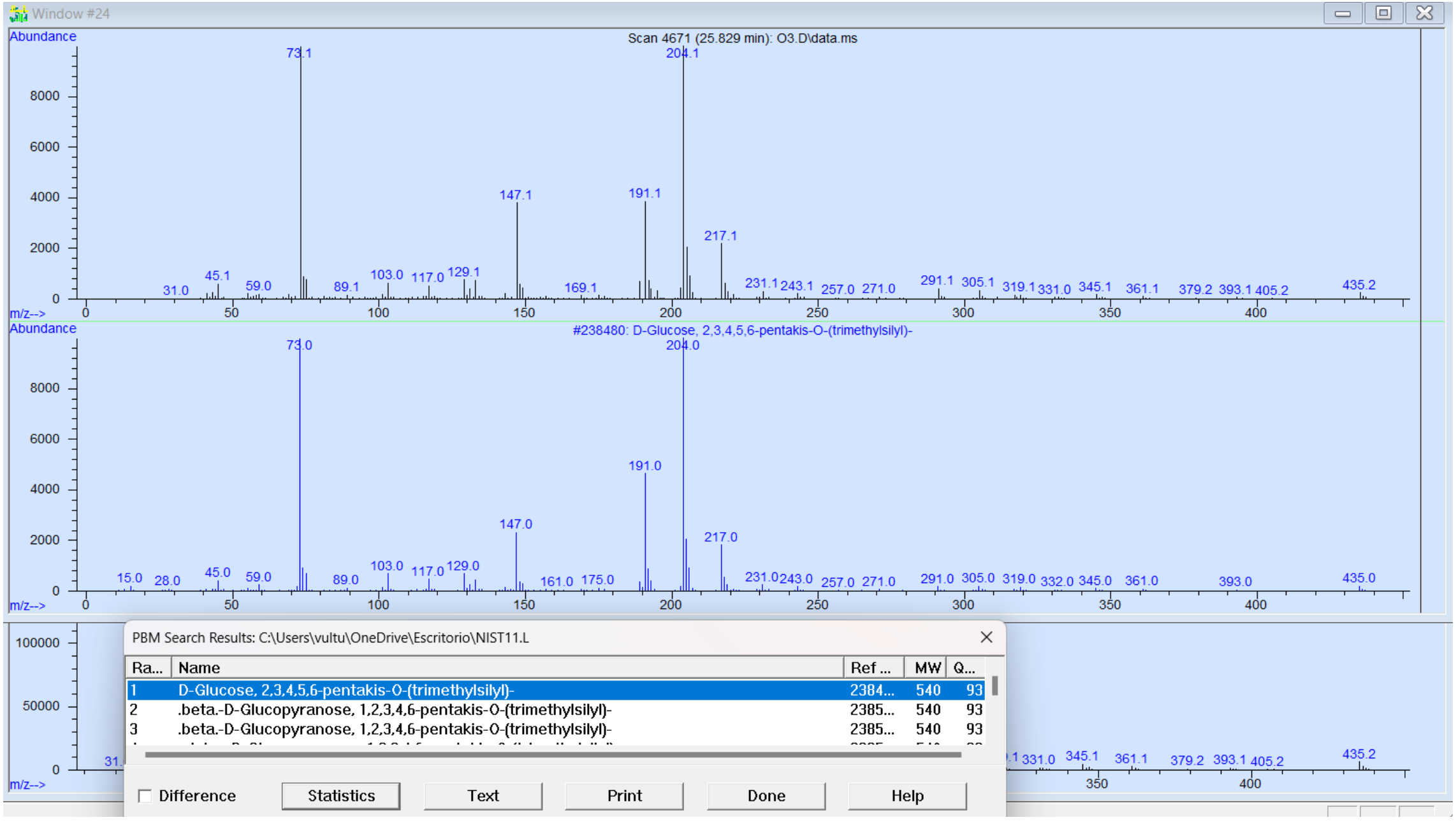
GAS CHROMATOGRAPHY – MASS SPECTROMETRY



GC-MS Chromatogram



Library comparisson

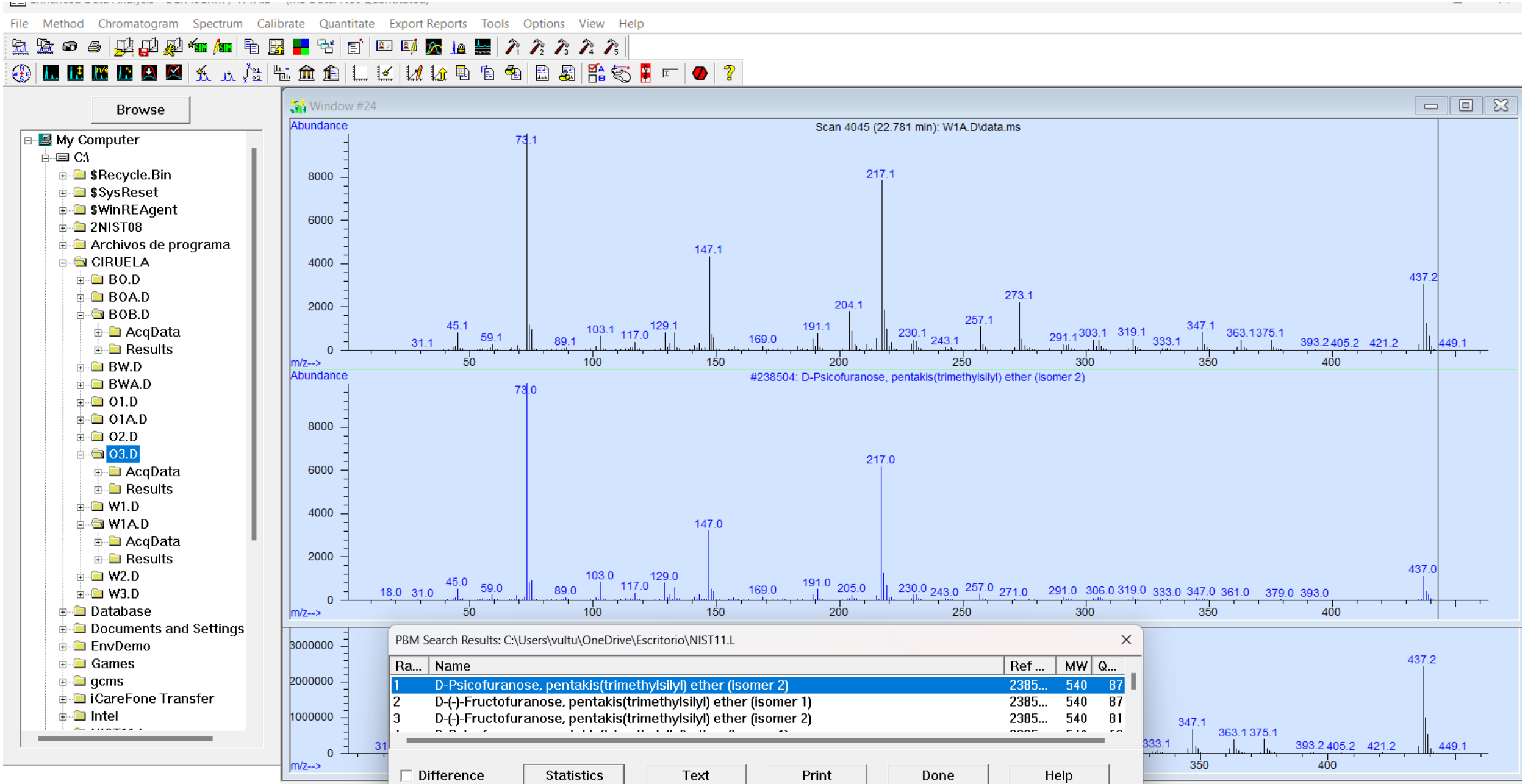


PBM Search Results: C:\Users\vultu\OneDrive\Escritorio\NIST11.L

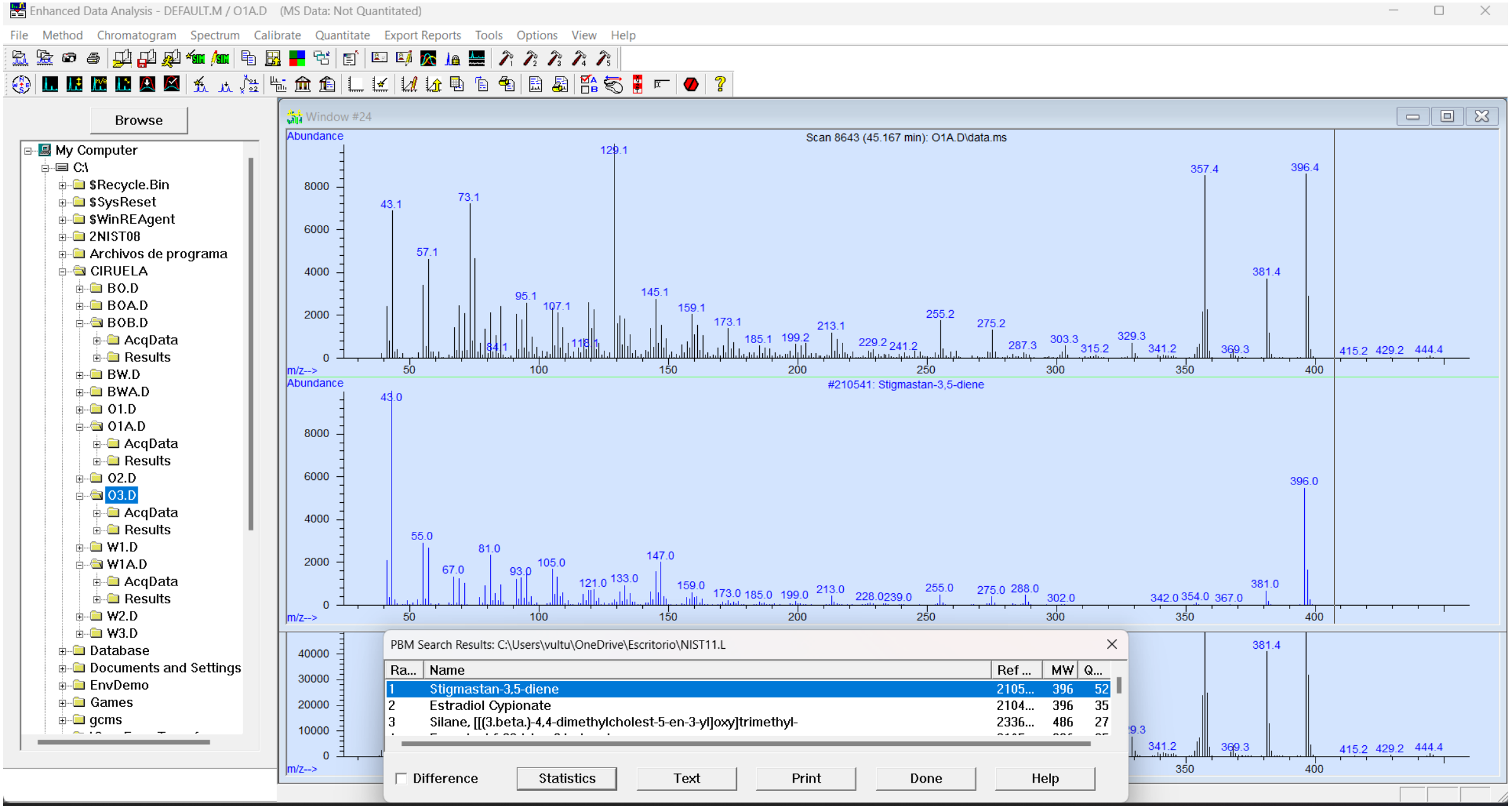
Ra...	Name	Ref ...	MW	Q...
1	D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-	2384...	540	93
2	.beta.-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	2385...	540	93
3	.beta.-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	2385...	540	93

Difference | Statistics | Text | Print | Done | Help

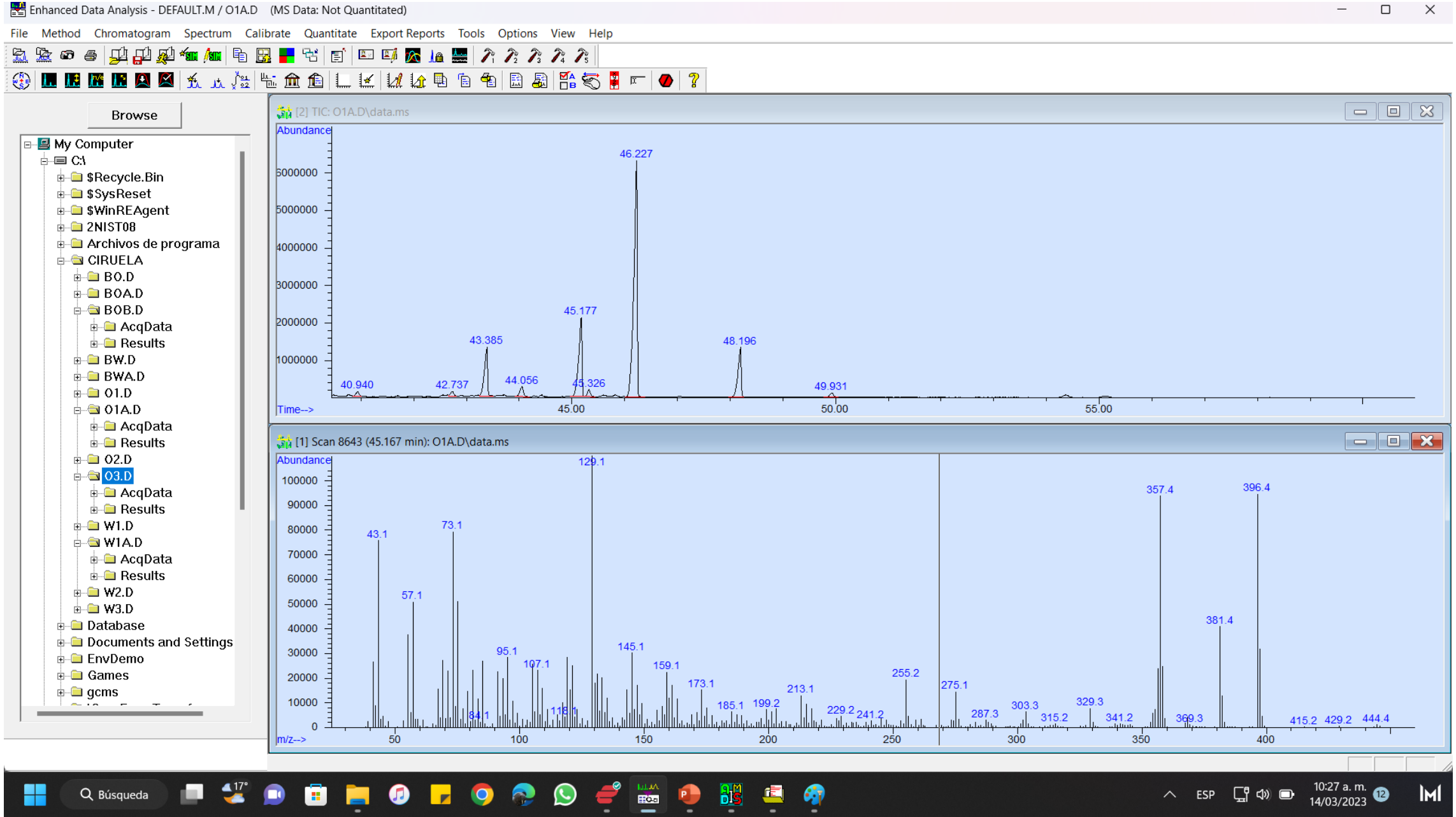
Library comparisson



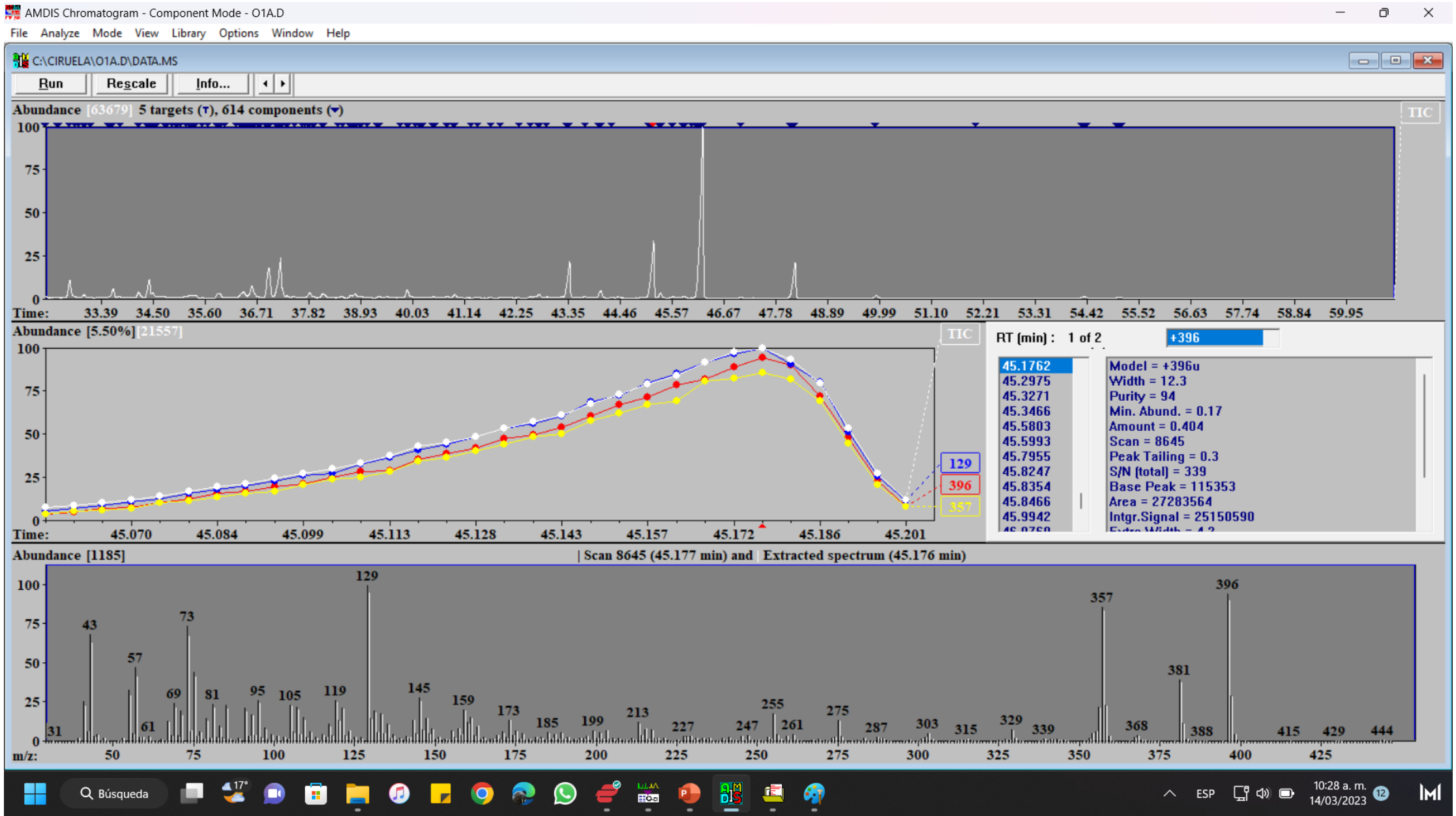
Sometimes they don´t match



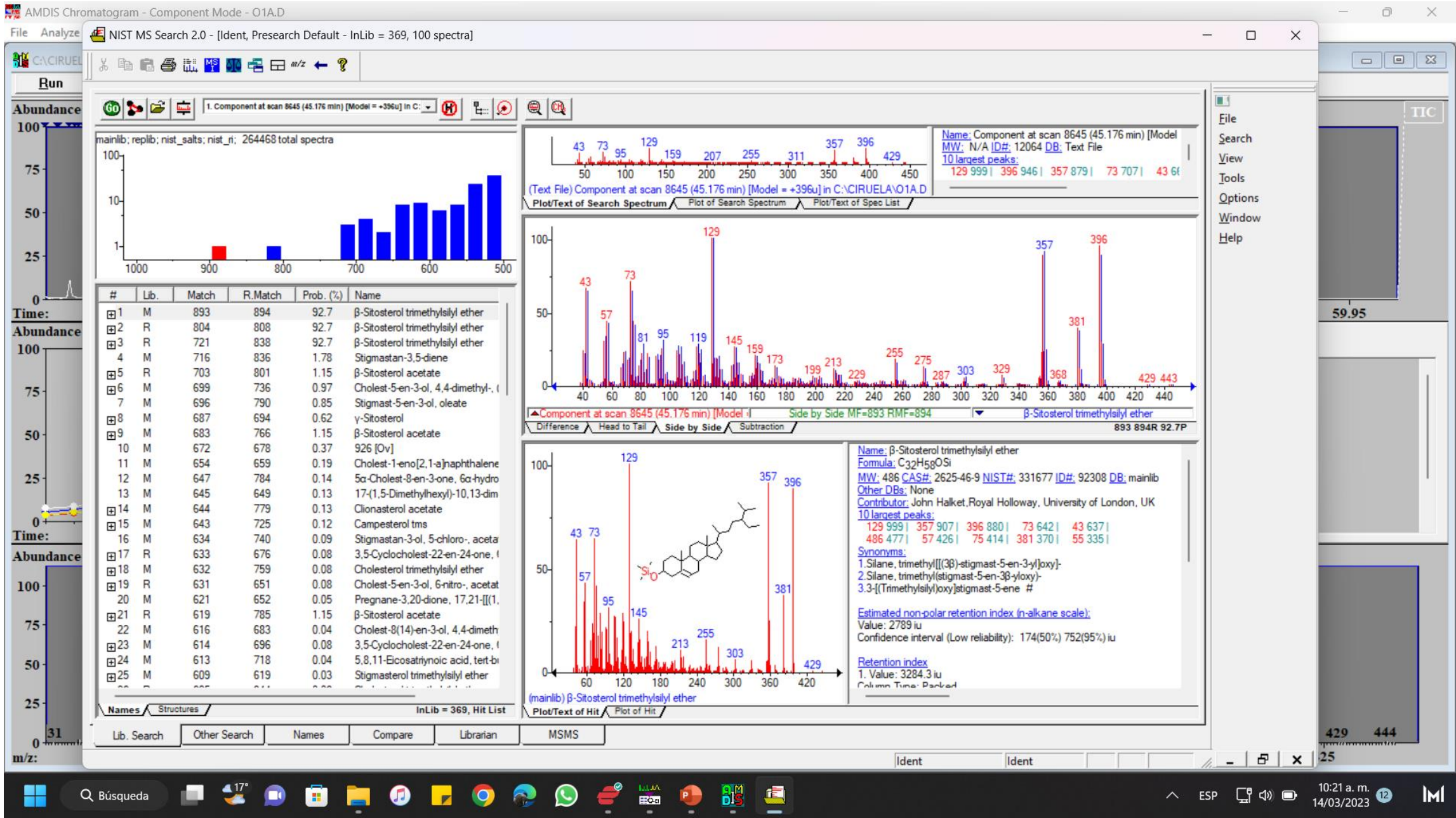
Chromatogram where compound doesn´t match



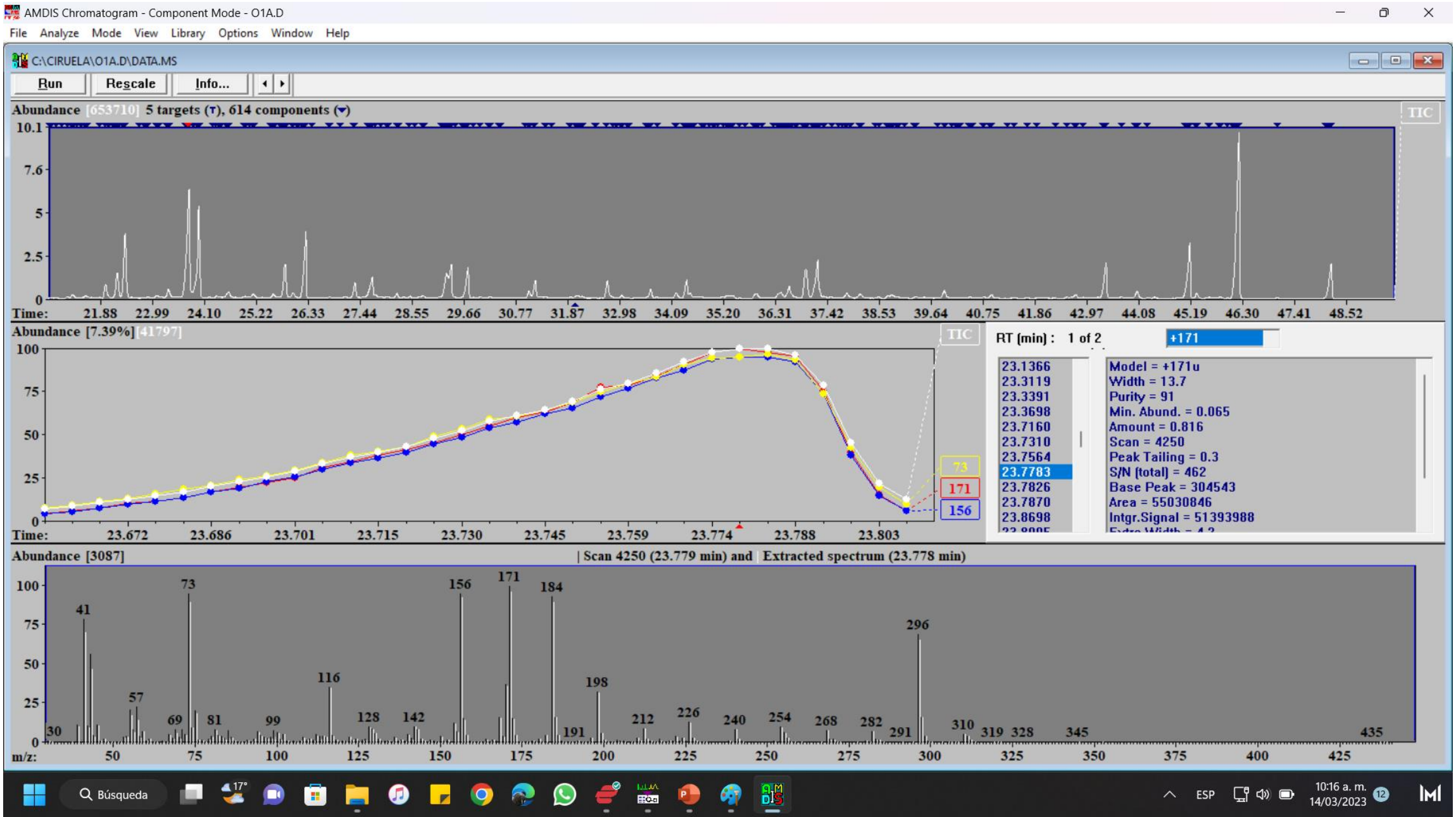
Bring chromatogram to deconvolution software (AMDIS)



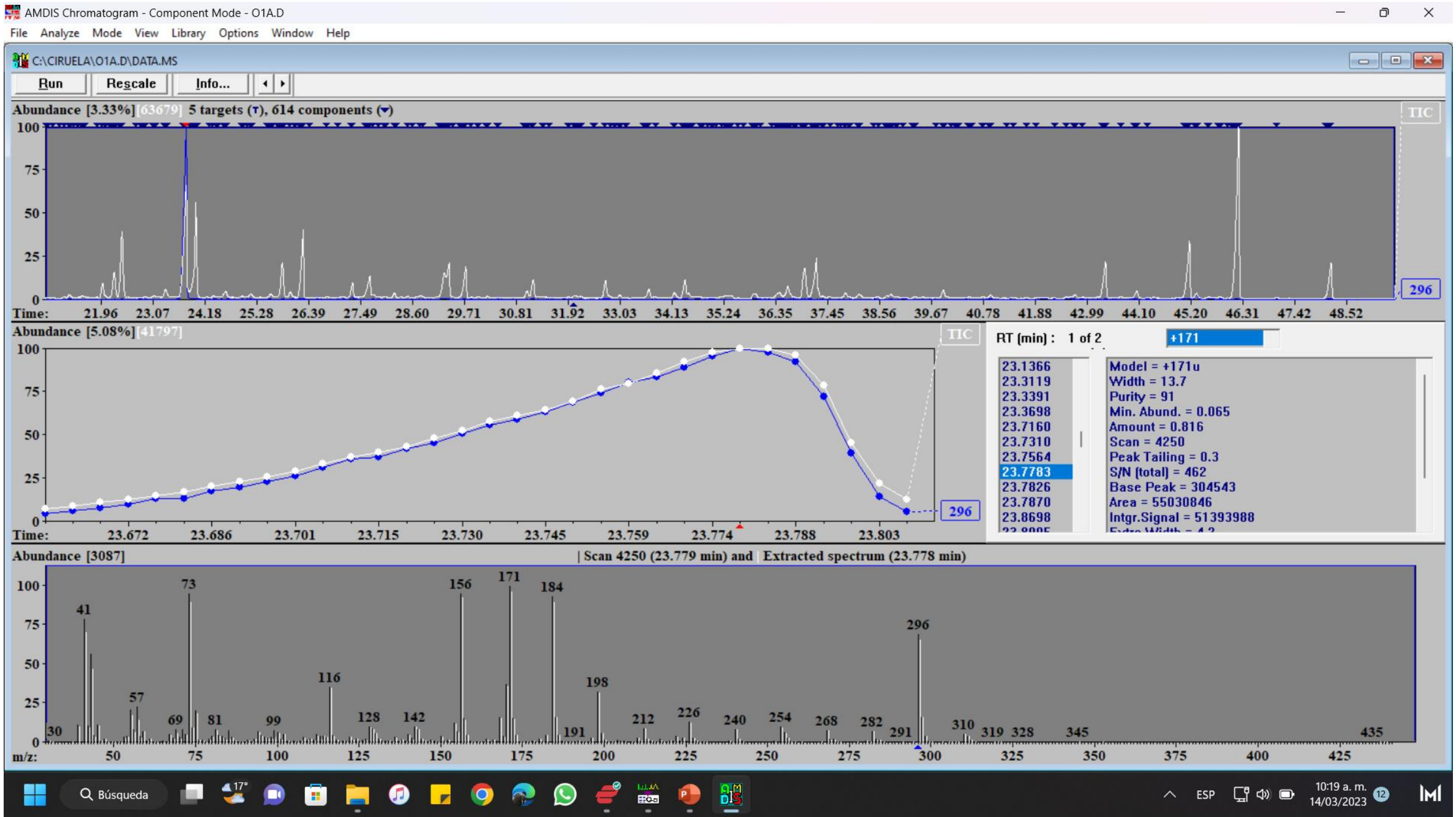
Get a better spectrum. Use tools. Library search. Success



Example of deconvolution



Deconvolution: “Distillation” of ions (1)



Deconvolution: “Distillation” of ions (2)