IFSTL International Food Safety Training Laboratory | parera

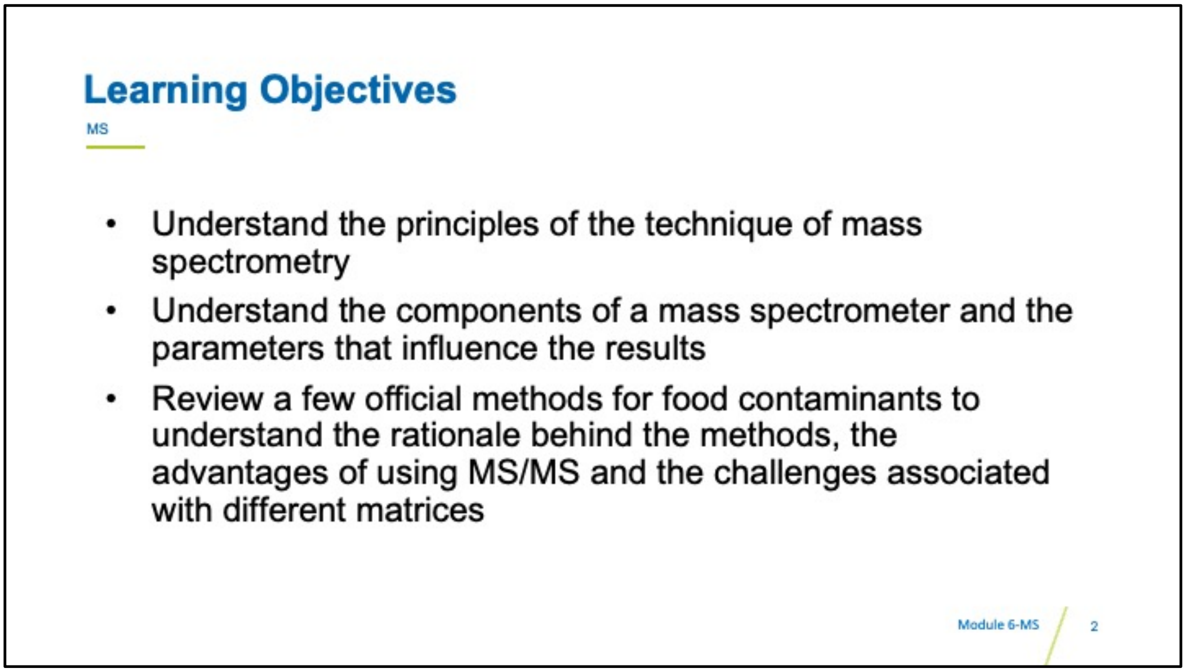
INAF Institute of Nutrition and Functional Foods

Université Laval
Faculty of Agriculture and Food Sciences

Confirmation Methods for Organic Chemical Contaminants

Module 6

Welcome to module 6 of this online training program on confirmation methods for organic chemical contaminants.

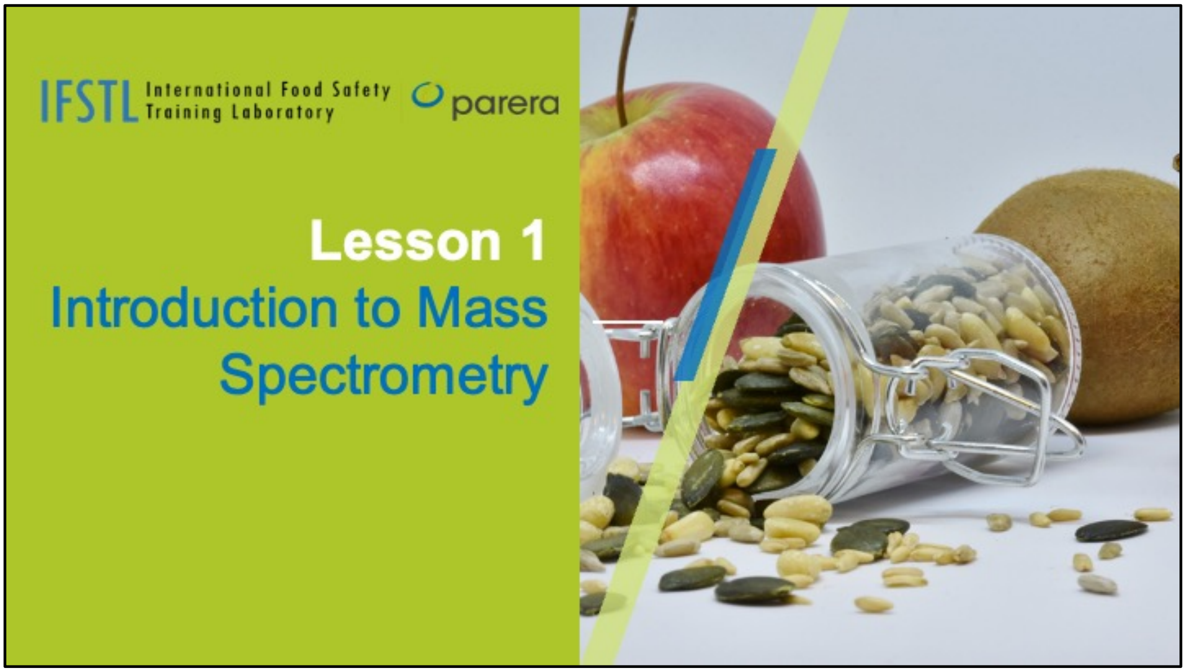


Learning Objectives

MS

- Understand the principles of the technique of mass spectrometry
- Understand the components of a mass spectrometer and the parameters that influence the results
- Review a few official methods for food contaminants to understand the rationale behind the methods, the advantages of using MS/MS and the challenges associated with different matrices

Module 6 is dedicated to mass spectrometry as a detection technique following chromatographic separation either by gas or liquid chromatography. The learning objectives of this module are to : Understand the principles of mass spectrometry as a measurement technique; to understand the components of the systems itself, the mass spectrometer, and the parameters that influence the results. The focus will be placed on a selection of parameters that are key elements of food safety methods. Finally, we will review a number of official methods for food contaminants with the objective to understand why mass spectrometry, both single quadrupole and tandem quadrupole, have become so important for the food safety laboratory.



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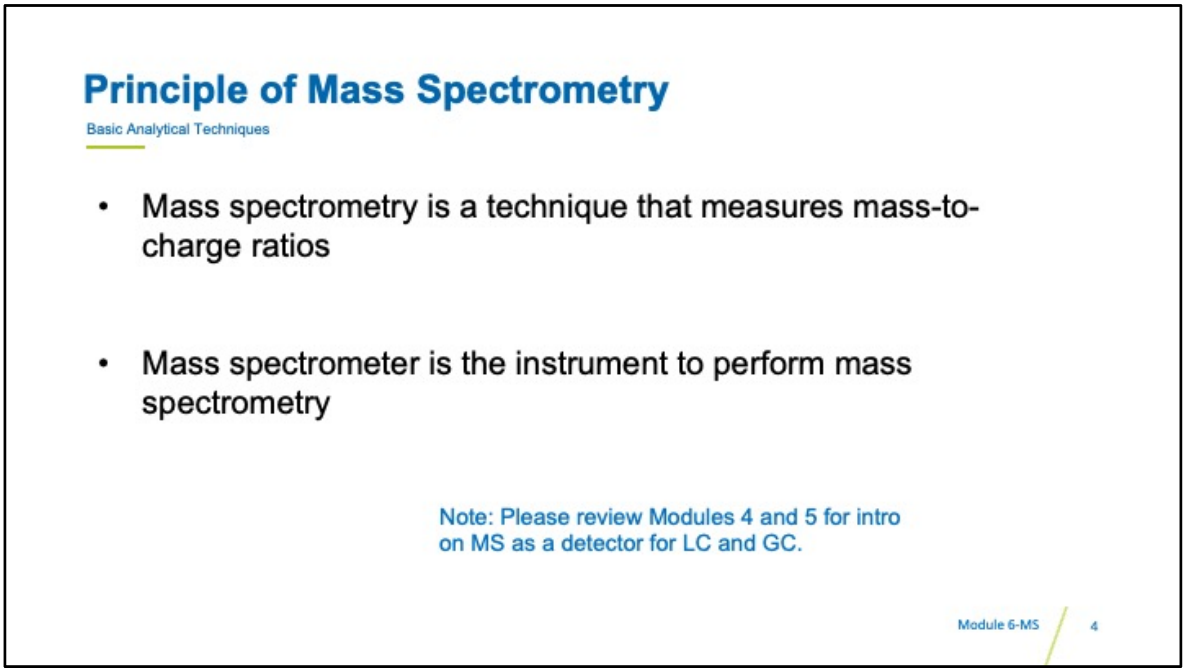
Lesson 1

Introduction to Mass Spectrometry

Lesson 1: Introduction to mass spectrometry.



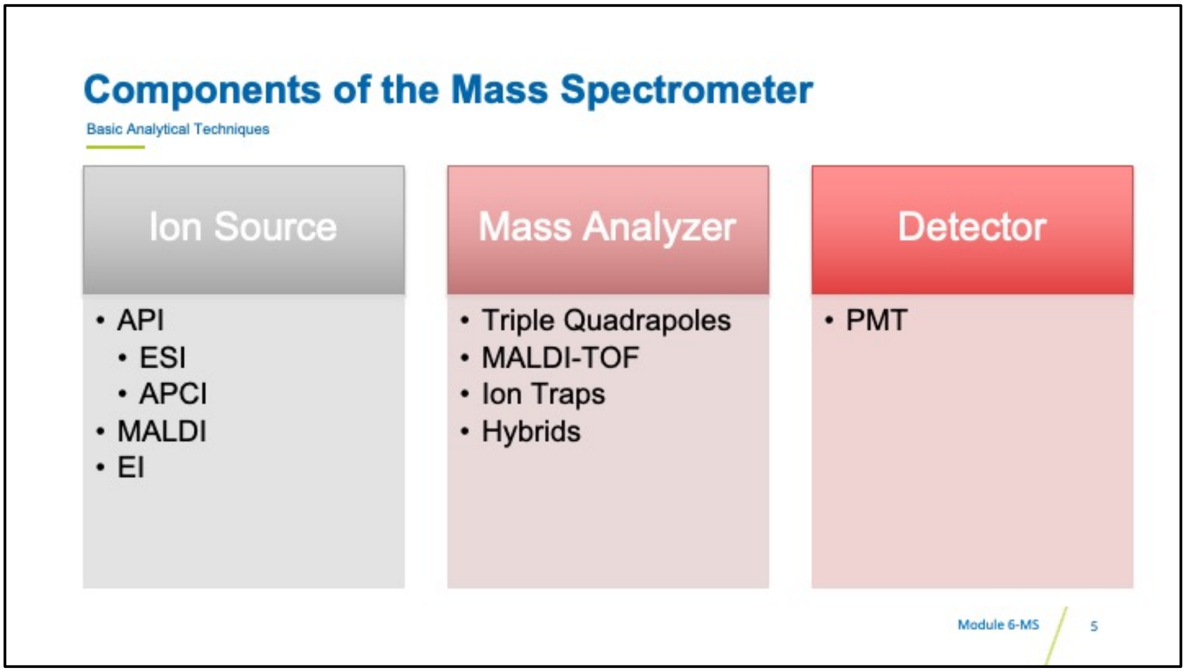
Principle of Mass Spectrometry

Basic Analytical Techniques

- Mass spectrometry is a technique that measures mass-to-charge ratios
- Mass spectrometer is the instrument to perform mass spectrometry

Note: Please review Modules 4 and 5 for intro on MS as a detector for LC and GC.

As the name suggests, mass spectrometry is a technique that uses mass; however, there would be no measurement possible if the molecule of interest was not charged, *i.e.* in ionic form. In reality, it is a measurement driven by mass-to-charge ratio. The instrument used for mass spectrometry is called a mass spectrometer. We briefly introduced the principles in modules 4 and 5.



Components of the Mass Spectrometer

Basic Analytical Techniques

Ion Source

- API
  - ESI
  - APCI
- MALDI
- EI

Mass Analyzer

- Triple Quadrapoles
- MALDI-TOF
- Ion Traps
- Hybrids

Detector

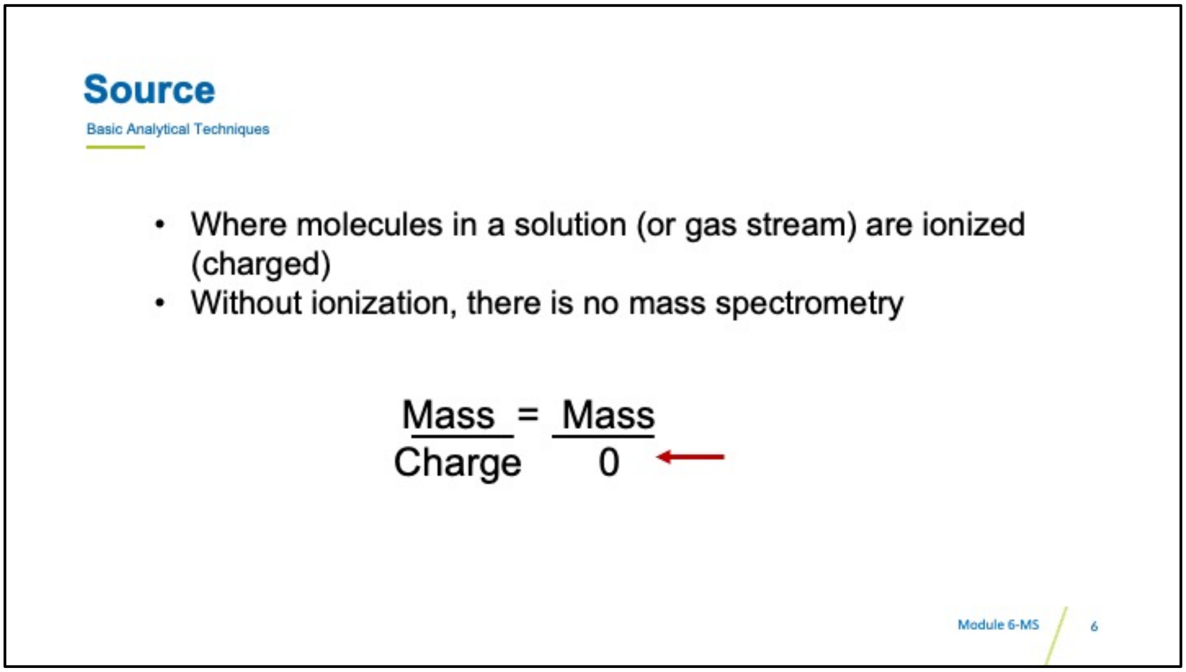
- PMT

There are three main components in the mass spectrometer. The ion source is where the solution containing the mixture of chemicals extracted from the matrix is introduced into the instrument and molecules of interest are charged.

The mass analyzer is the heart of the instrument, where the selection of ions is made by way of a movement of ions driven by a combination of electromagnetic current and radio waves. We will discuss different implementations of this system that provide certain advantages for the detection and quantitation of different types of chemicals in different concentrations.

Finally, a simple detector that counts particles without any type of selectivity is present at the end of the process to count whatever reaches it.

While the technology to achieve this process is very advanced and was only made possible by developments in electronics, fluid mechanics and software components, the principles behind it are quite straightforward.



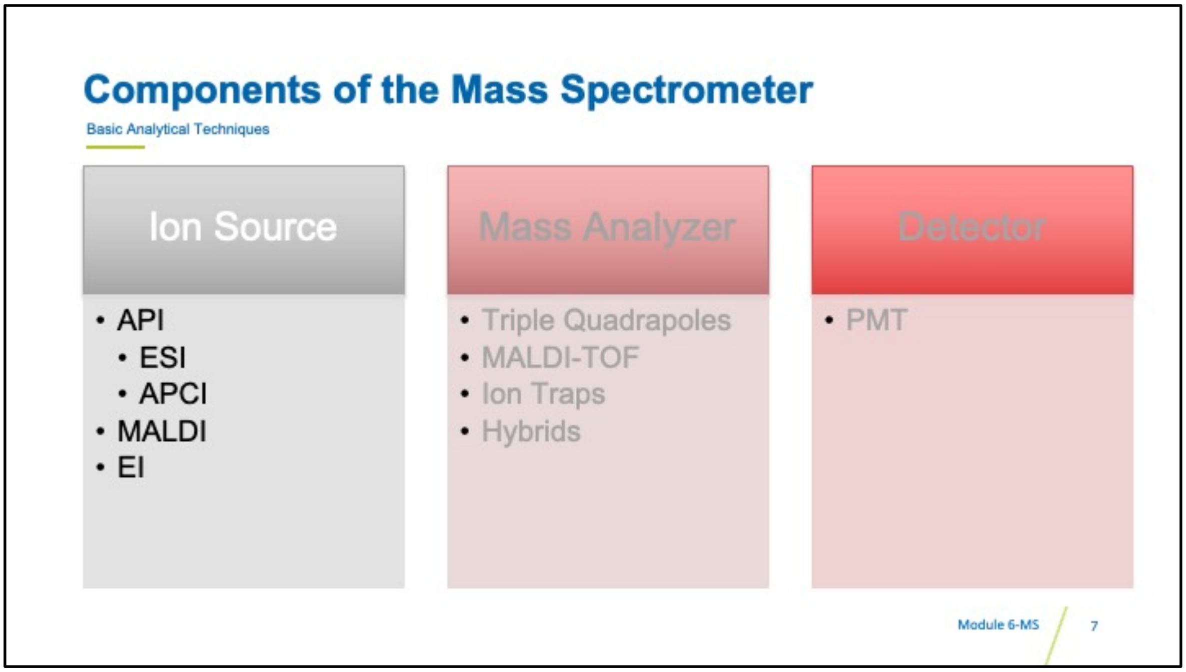
Source

Basic Analytical Techniques

- Where molecules in a solution (or gas stream) are ionized (charged)
- Without ionization, there is no mass spectrometry

$$\frac{\text{Mass}}{\text{Charge}} = \frac{\text{Mass}}{0} \longleftarrow$$

As mentioned before, the source is where the solution (or the gas stream coming out of a GC) enters the mass spectrometer and chemicals of interest are ionized or charged either negatively or positively. It is important to remember that without charge, there would be no mass spectrometry and while it looks simplistic, this formular reminds us. We will discuss this again when troubleshooting methods.



Components of the Mass Spectrometer

Basic Analytical Techniques

Ion Source

- API
  - ESI
  - APCI
- MALDI
- EI

Mass Analyzer

- Triple Quadrapoles
- MALDI-TOF
- Ion Traps
- Hybrids

Detector

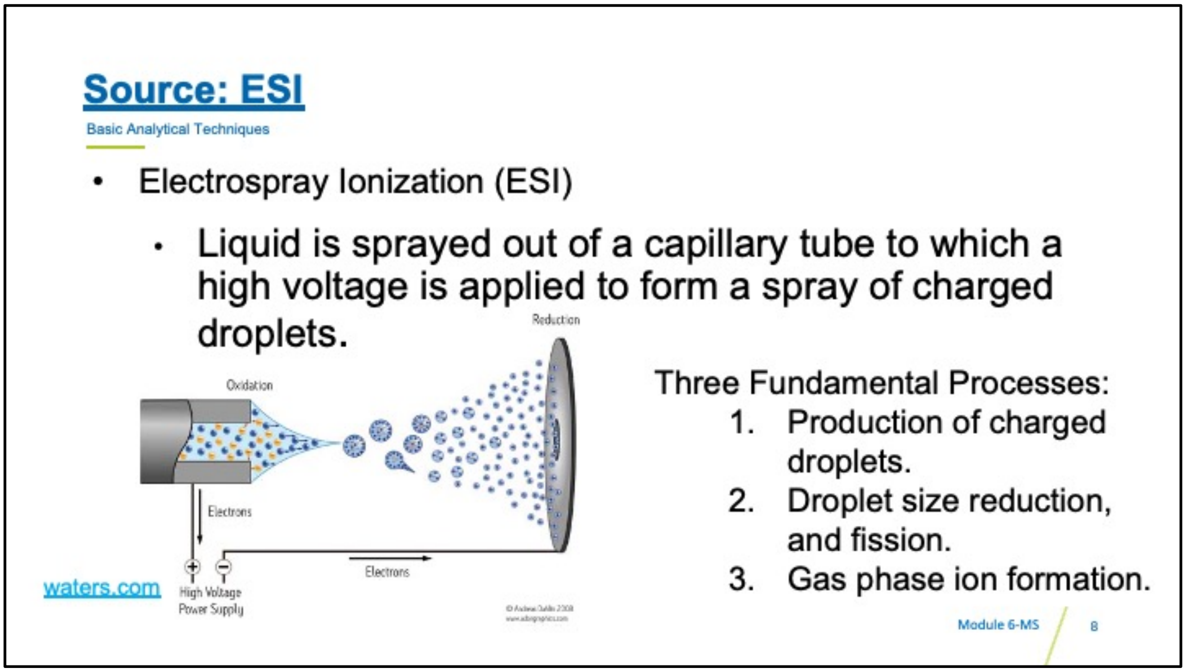
- PMT

There are different types of ion sources available and the selection of the appropriate one depends on the sample, the components we are trying to measure, and also the concentration at which they are present. The first group of sources is based on atmospheric pressure ionization (API). As the name indicates, this type of source operates at atmospheric pressure, which offers the advantage of avoiding a vacuum pump at this stage, making it a simpler system.

The most popular type of API is electrospray ionization (ESI), while atmospheric pressure chemical ionization offers some advantages for chemicals that are not easily charged.

Matrix-assisted laser desorption ionization (MALDI) is a technique that uses a laser beam to lift chemicals from a solid surface and produce ions.

Finally, electron ionization, EI, is based on the use of an electron beam to cause ionization. Let us review these techniques in some more details.



Source: ESI

Basic Analytical Techniques

- Electrospray Ionization (ESI)
  - Liquid is sprayed out of a capillary tube to which a high voltage is applied to form a spray of charged droplets.

Three Fundamental Processes:

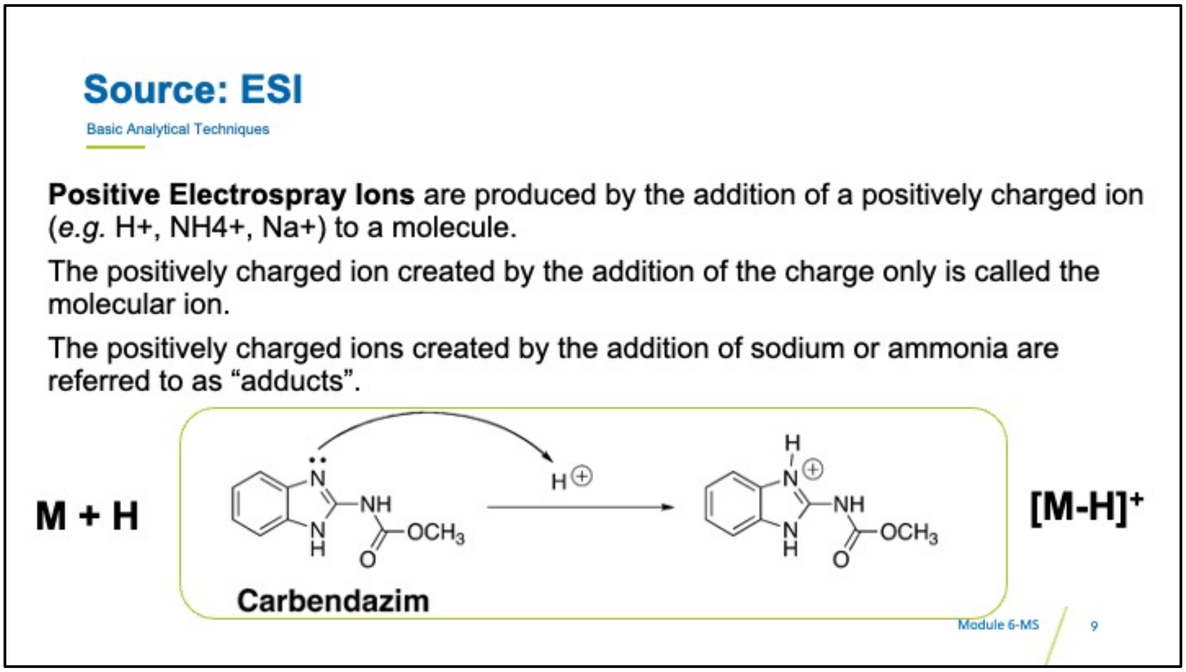
1. Production of charged droplets.
2. Droplet size reduction, and fission.
3. Gas phase ion formation.

waters.com

ESI, electrospray ionization, is essentially a system where the sample solution is sprayed through a heated tube to which an electrical voltage is applied and surrounded by a stream of inert gas (usually nitrogen). As the solution approaches the tip of the capillary tube, its temperature increases and when it leaves the tip, the spray effect, combined with the heat and the gas stream cause de formation of small droplets that immediately start to dry. The voltage applied at the tip of the capillary tube causes the droplets to be charged.

As the droplets proceed through the empty space between the source and the entrance of the mass analyzer, they go through more loss of the solution solvent and split up into smaller droplets. Ideally, they become completely dry, which leaves ions in gas phase to enter the mass analyzer.

The critical steps of this process are the addition of a charge to the droplets (negative or positive), fission into smaller droplets and evaporation of the sample's solvent. If any of these steps doesn't happen, there will not be ions entering the mass analyzer. Similarly, if any of these steps is only partially achieved, only a fraction of the expected quantity of ions will enter the mass analyzer.



Source: ESI

Basic Analytical Techniques

Positive Electrospray Ions are produced by the addition of a positively charged ion (*e.g.* H+, NH4+, Na+) to a molecule.

The positively charged ion created by the addition of the charge only is called the molecular ion.

The positively charged ions created by the addition of sodium or ammonia are referred to as “adducts”.

M + H

Carbendazim

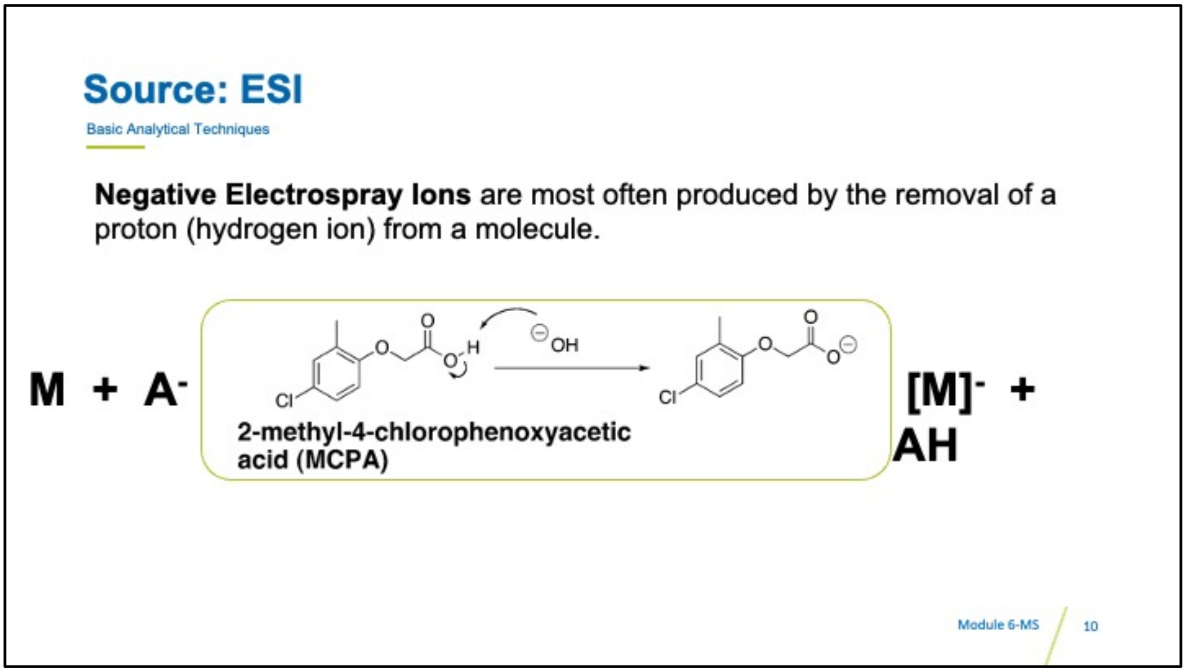
[M-H]$^+$

ESI can be used to create positively or negatively charged ions. When hydrogen is added to create a +1 charge, it creates what we call a molecular ion. A molecular ion can be positively or negatively charged, but the characteristic is that it is the original molecular weight of the compound of interest, plus one or minus one mass unit.

When sodium or ammonia ions are added to a molecule to cause it to become positively charged, we call the product of this addition an adduct. Adducts therefore weight the sum of the molecular masses of the original compound and the added compound, plus or minus 1.

Adducts are extremely important for confirmation of identity when using a single quadrupole mass spectrometer. This will be discussed further during our practical laboratory sessions. When MS/MS is available, we favor the use of the molecular ions since these are more specific and we have the ability to create other elements for the confirmation of identity using the second mass spectrometer. We will discuss this further in the upcoming section on the mass analyzer.

The figure above shows one example of the creation of a molecular ion for carbendazim through the addition of a hydrogen, which creates a charge of +1 and a molecular weight equal to carbendazim +1. Carbendazim is a pesticide contained in the official method discussed in section three of this module.



Source: ESI

Basic Analytical Techniques

Negative Electrospray Ions are most often produced by the removal of a proton (hydrogen ion) from a molecule.

M + A⁻ → **[M]⁻ + AH**

2-methyl-4-chlorophenoxyacetic acid (MCPA)

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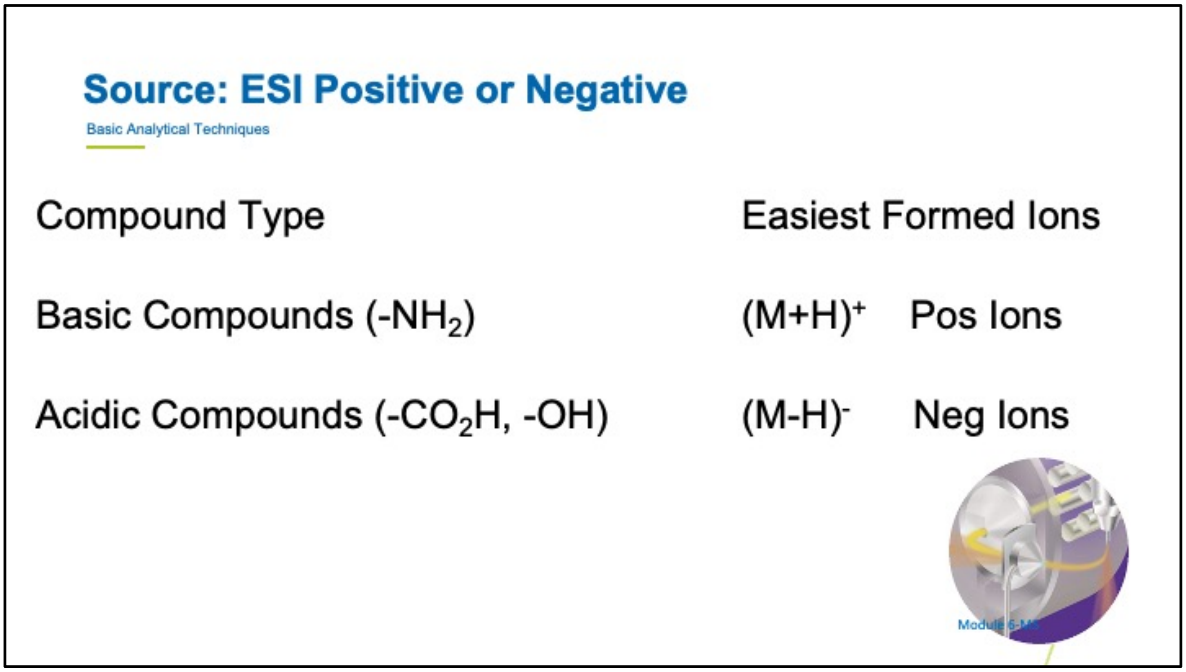
The same principle of action is used to create negatively charged molecules in negative electrospray ionization. In this case, the conditions favor the removal of a proton, which causes the resulting ion to be negatively charged. Similarly to positive electrospray, we favor the formation of the negative molecular ion in tandem quadrupole MS, and adducts when needing a confirmation of identity using a single MS.



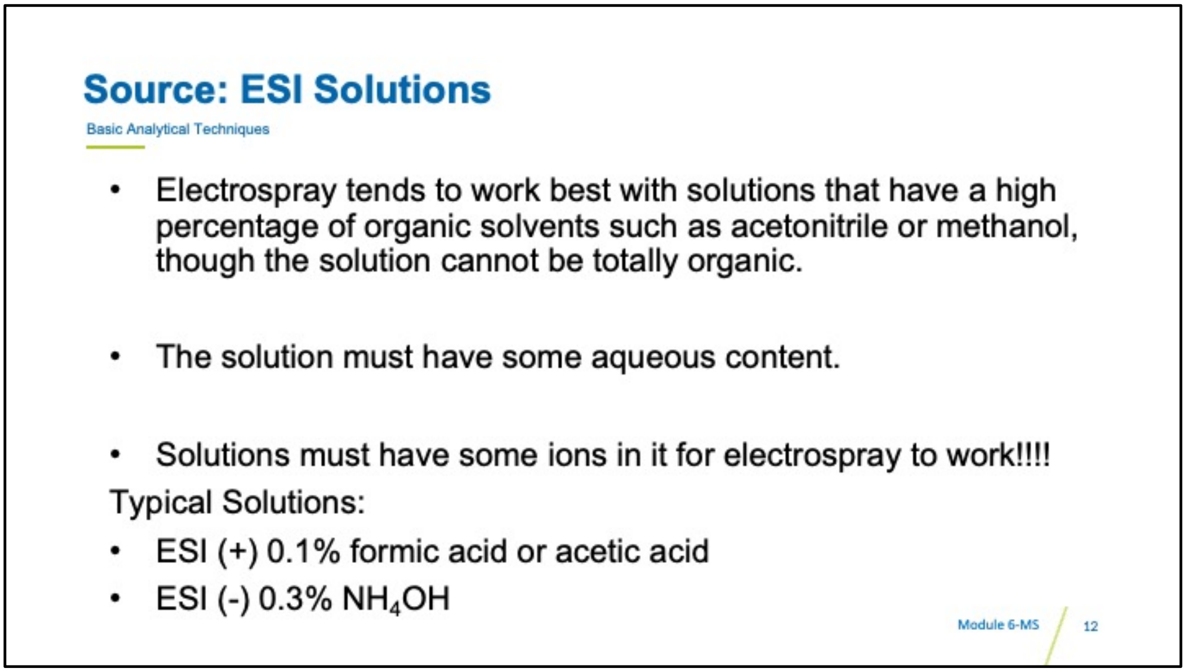
Source: ESI Positive or Negative

Basic Analytical Techniques

Compound Type	Easiest Formed Ions	
Basic Compounds (-NH_2)	$(M+H)^+$	Pos Ions
Acidic Compounds (-CO_2H, -OH)	$(M-H)^-$	Neg Ions

Chemical compounds are by their nature better suited for positive or negative electrospray ionization. Basic compounds more easily form positive molecular ions, while acidic compounds more readily form negative molecular ions.

Modern mass spectrometry system have the ability to switch between negative and positive electrospray ionization within the same run. This is an important advantage in food safety where we are often working to quantify large numbers of contaminants at once and it enables more compounds to be included in the same method. In other words, we obtain more results for the same analysis, which saves time and money.



Source: ESI Solutions

Basic Analytical Techniques

- Electrospray tends to work best with solutions that have a high percentage of organic solvents such as acetonitrile or methanol, though the solution cannot be totally organic.

- The solution must have some aqueous content.

- Solutions must have some ions in it for electrospray to work!!!!

Typical Solutions:

- ESI (+) 0.1% formic acid or acetic acid
- ESI (-) 0.3% NH_4OH

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Sample preparation must always match the type of source we intend to use on our instrumentation. In the case of mass spectrometry used as a detector following chromatography separation, we have to ensure that the eluent from the chromatography column is compatible with ESI and with the positive and/or negative charge that we want on the compounds of interest.

Generally, ESI works best with solutions that have a high organic content. For example, it works best with a high acetonitrile or methanol content in HPLC. However, we need to have at least some aqueous phase with a modifier, such as acid or ammonia, because we need a source for the H+ or OH- that give or take the charge to form a molecular ion. Typical acids that promote ionization in positive mode are acetic and formic acid, while ammonia is a usual modifier for ESI in negative mode.

It is important to note that these modifiers serve a function of helping the ionization, but they must also help, or at least not hinder, the chromatography separation that precedes mass spectrometry. Other modifiers are available and useful in various applications, but for the new analysts in the food safety laboratory, remembering these three will help understand most of the relevant methods.

Source: ESI

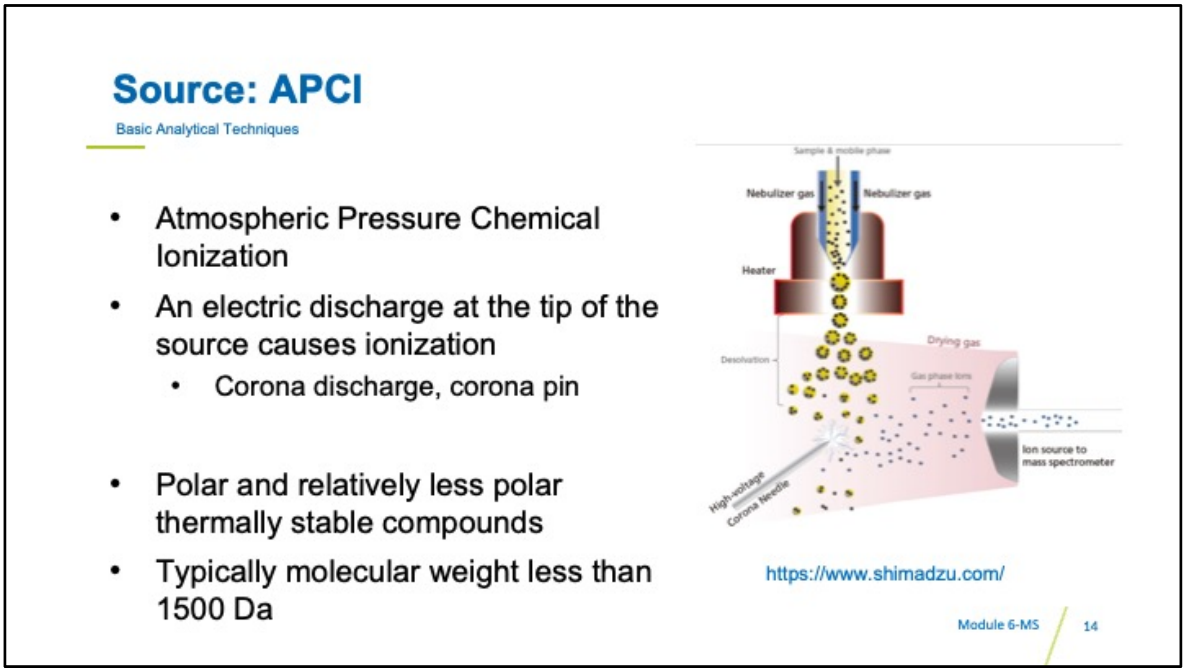
Basic Analytical Techniques

www.waters.com

This schematic from the Waters corporation shows the position and the actual shape of the different parts of the ESI source. The capillary tube is seen on the right, where an orange stream of sample is sprayed and forms the usual cone seen from a spray nozzle. The yellow stream represents the droplets that become small enough, through the drying process called desolvation, aided by the nitrogen gas flow and the high temperature, to be attracted by the vacuum coming from the cone located in the middle of the diagram. This angled design ensures that large droplets that still contain a lot of solvent do not enter the mass analyzer, which can only move gases through the quadrupoles. The wet stream will simply land on the floor of the source.

We will look at this source chamber in the practical laboratory portion of this course. At this time, we will start introducing some reasons for failed measurements, or in other words, discuss troubleshooting poor results. An example of a poor result that could be due to a problem in the source is a very low signal intensity. Looking back at the critical steps of the ESI process, we will discuss how an inadequate solvent composition at the outlet of the HPLC, or a low temperature or wrong voltage at the tip of the capillary tube, or a wrong angle between the capillary and the inlet cone could all result in few or no ions making it into the mass analyzer.

The ESI source is used for pesticide residues, veterinary drug residues, mycotoxins and many other food contaminants analyzed by LC-MS/MS. As such, it is by far the most common source in the food safety laboratory.



Source: APCI

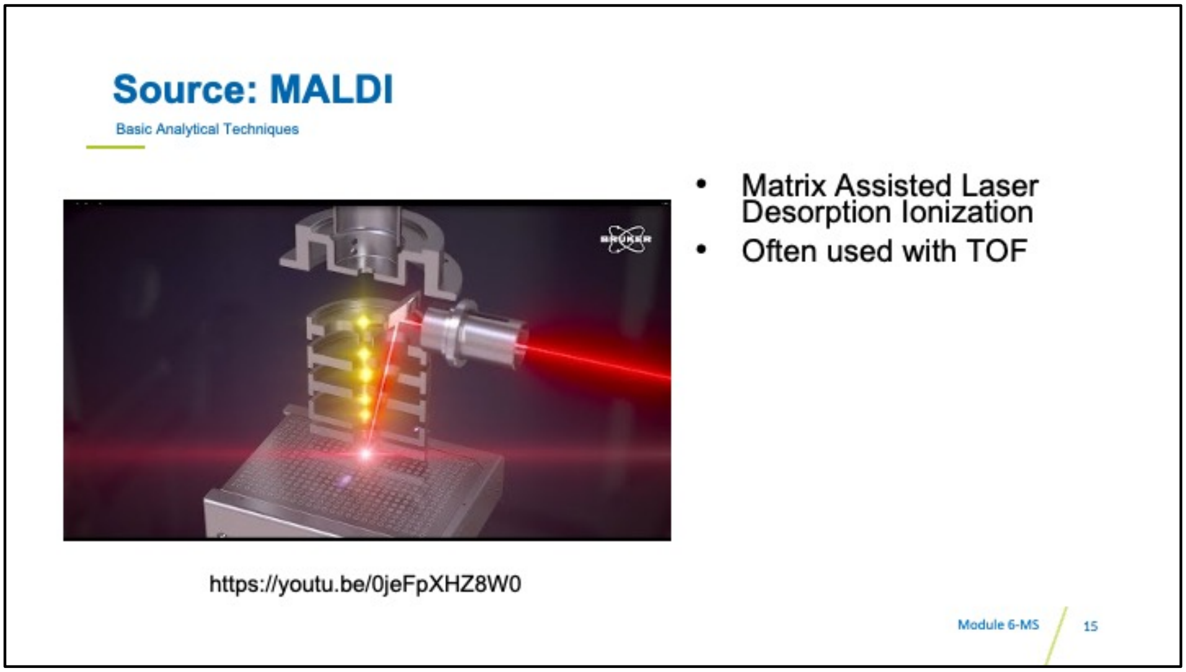
Basic Analytical Techniques

- Atmospheric Pressure Chemical Ionization
- An electric discharge at the tip of the source causes ionization
  - Corona discharge, corona pin
- Polar and relatively less polar thermally stable compounds
- Typically molecular weight less than 1500 Da

https://www.shimadzu.com/

The atmospheric pressure chemical ionization (APCI) source works in a manner very similar to ESI, except that there is no voltage at the tip of the capillary tube. The solution containing the sample is sprayed out of the capillary tube at high temperature, to aid the desolvation, and then it is subjected to a high voltage delivered by a needle called the corona needle or corona pin. The charged ions now present in gas phase are pulled into the sample cone by the same vacuum as in ESI.

The electric discharge from the corona pin causes the ionization, but we should note that this is a much higher charge than delivered at the tip of the capillary tube in ESI, which limits its use to thermally stable molecules. In addition, both ESI and APCI work well for “small molecules”, molecules are those that weigh less than 1500 daltons.



Source: MALDI

Basic Analytical Techniques

- Matrix Assisted Laser Desorption Ionization
- Often used with TOF

https://youtu.be/0jeFpXHZ8W0

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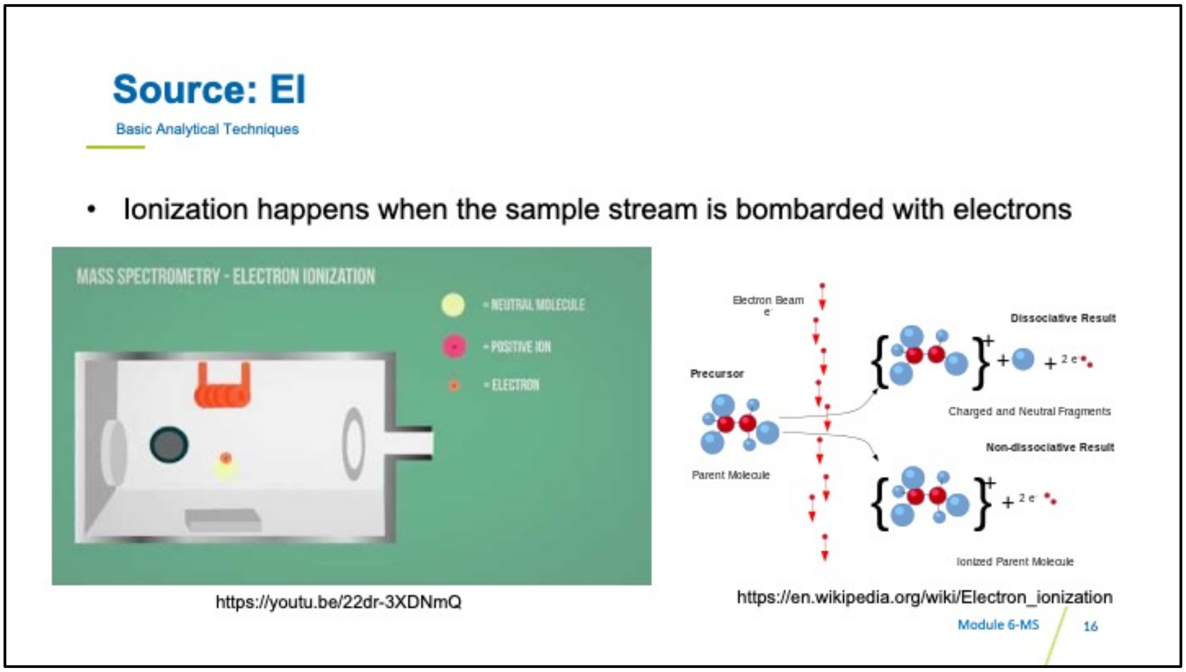
MALDI, or matrix-assisted laser desorption ionization is a completely different mode of ionization. First and foremost, the sample has to be deposited on a solid surface in a mixture oof particles that can absorb a lot of energy. This is called the matrix. The mixture is allowed to dry and is then hit with a UV laser beam that causes the matrix to absorb energy, and eventually explode off the metal holder. This vaporization in a high energy environment also favors the exchange of a proton between the matrix and the analytes, which causes them to become ions with a charge of +1.

MALDI is most commonly seen in the food sector in tandem with time of flight mass spectrometry (TOF), or for imaging purposes. In imaging, a spatially organized system is “raster-scanned” with the laser and the chemical composition is elucidated for each position. This is typically not used in food safety.

Here is a promotional video from Bruker that explains MALDI-TOF.



Source: EI

Basic Analytical Techniques

- Ionization happens when the sample stream is bombarded with electrons

https://youtu.be/22dr-3XDNmQ

https://en.wikipedia.org/wiki/Electron_ionization

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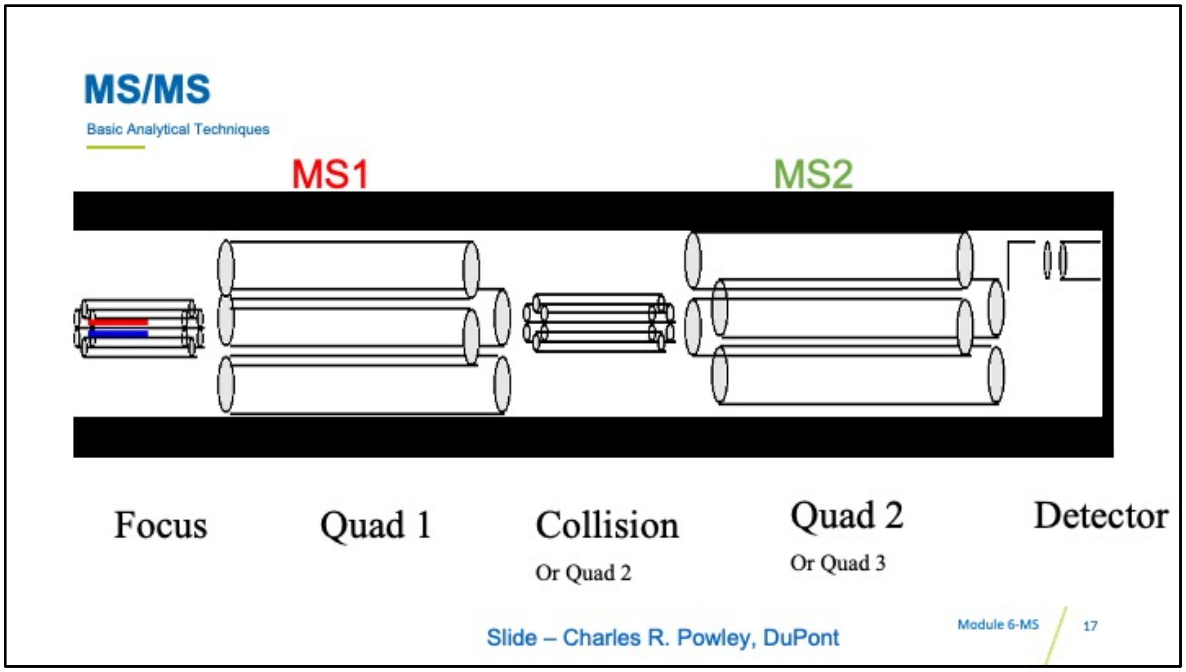
Electron ionization (EI) happens when a sample stream is bombarded with electrons. This can produce both dissociative and non-dissociative ions. EI is the most common source of ionization in GC-MS for food safety.

This time, we watch a video from Simple Science to help us understand Electron ionization.



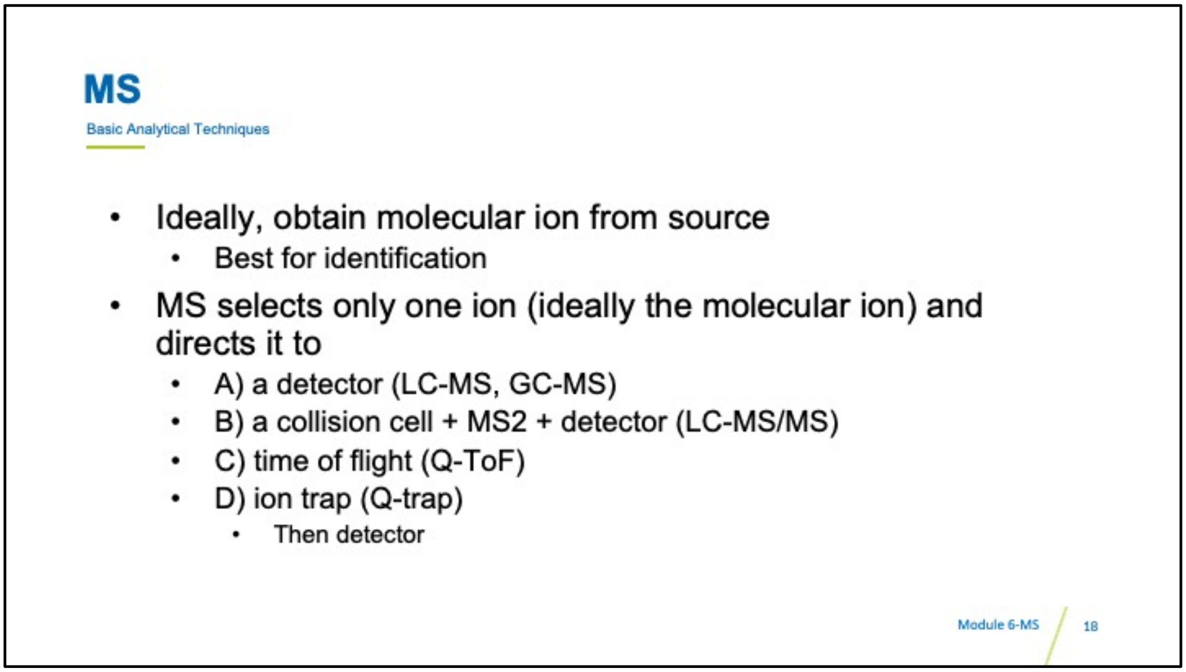
MS/MS

Basic Analytical Techniques

Slide – Charles R. Powley, DuPont

MS/MS is quite simply the combination of two quadrupoles in series. The first quadrupole receives the sample from the source, usually cleaned up or concentrated using a proprietary design, and selectively allows the ions meeting the target mass/charge ratio through and to the collision chamber. These ions are are known as the precursor ions. They used to be called parent ion, but the term has been replaced with precursor. The collision chamber, which is called the second quadrupole by some vendors, is a cell filled with argon gas where the ions that made it through the first quadrupole are subjected to a high level of collision energy. The parent ion breaks and forms a number of different product ions (previously known as daughter ions). These products can be close in M/Z if the portion that was knocked off is small, or they can be very small. Ideally, we select larger product ions for confirmation purposes as smaller ions can be produced by breaking too many different types parent ions. They consequently lack specificity.

In summary the ideal precursor ion is the molecular ion in LC-MS produced by the sources, i.e. the molecular weight of the compound of interest plus or minus one, divided by a charge of 1, and the ideal product ions are those that keep most of the chemical structure of the parent and are thereby not easily formed by many different parent ions.



MS

Basic Analytical Techniques

- Ideally, obtain molecular ion from source
  - Best for identification
- MS selects only one ion (ideally the molecular ion) and directs it to
  - A) a detector (LC-MS, GC-MS)
  - B) a collision cell + MS2 + detector (LC-MS/MS)
  - C) time of flight (Q-ToF)
  - D) ion trap (Q-trap)
    - Then detector

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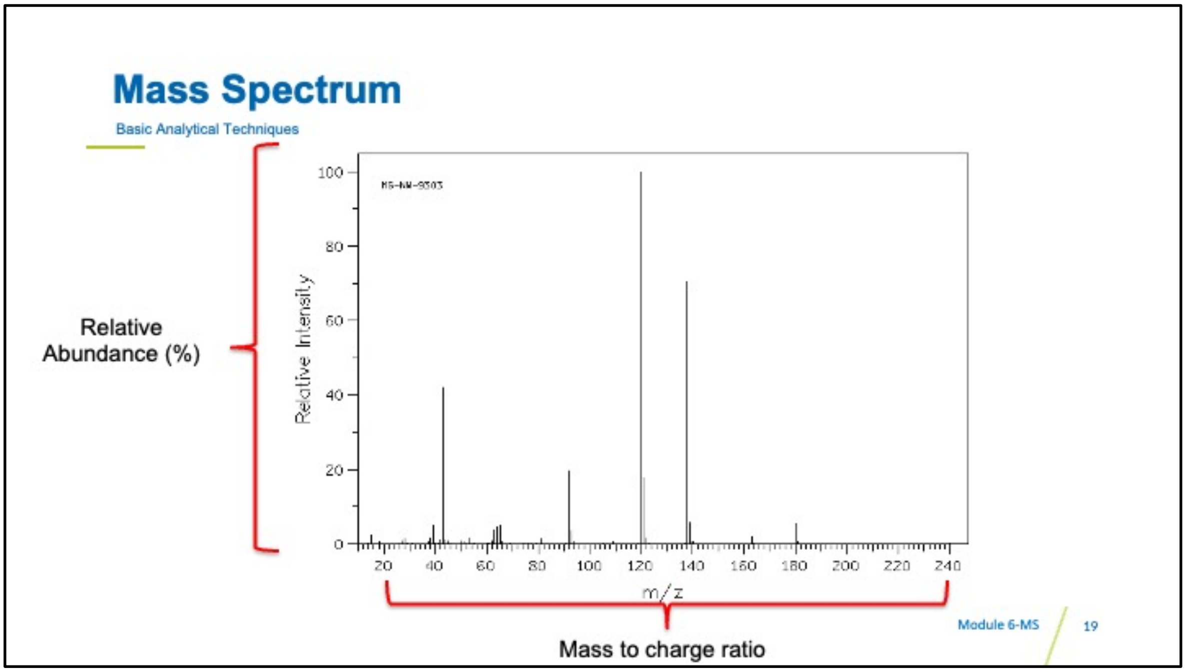
When using single MS, we ideally want to obtain the molecular ion as the first criterial for identification, and then adducts that maintain as much of the chemical structure of the parent ion as these are more specific. In all cases, it is important to be able to justify the formation of the adducts from the parent chemically to avoid analyzing ions that may originate from contaminants or compounds originating from the sample matrix that were not removed during sample preparation.

When only one MS is used and the ion goes to the detector, the instrument is known as just MS. But this is not the only option. The ion can be directed to a time-of-flight chamber (MS-Q-ToF) for even more specific selectivity before ions reach the detection. The ion can go to a collision chamber and a second quadrupole as seen before, for MS/MS, also to increase selectivity ahead of the detector. Alternatively, the ion can be directed to an ion trap for concentration, which would allow the system to reach much lower limits of quantitation and detection (LOQ and LOD).

A number of hybrid systems exists on the market for various applications, but GC-MS and LC-MS/MS are the most broadly used, while LC-Q-ToF and LC/Q-Trap are growing as we seek to measure lower concentrations of contaminants at higher mass resolution, especially for the purpose of risk assessment.



Mass Spectrum

Basic Analytical Techniques

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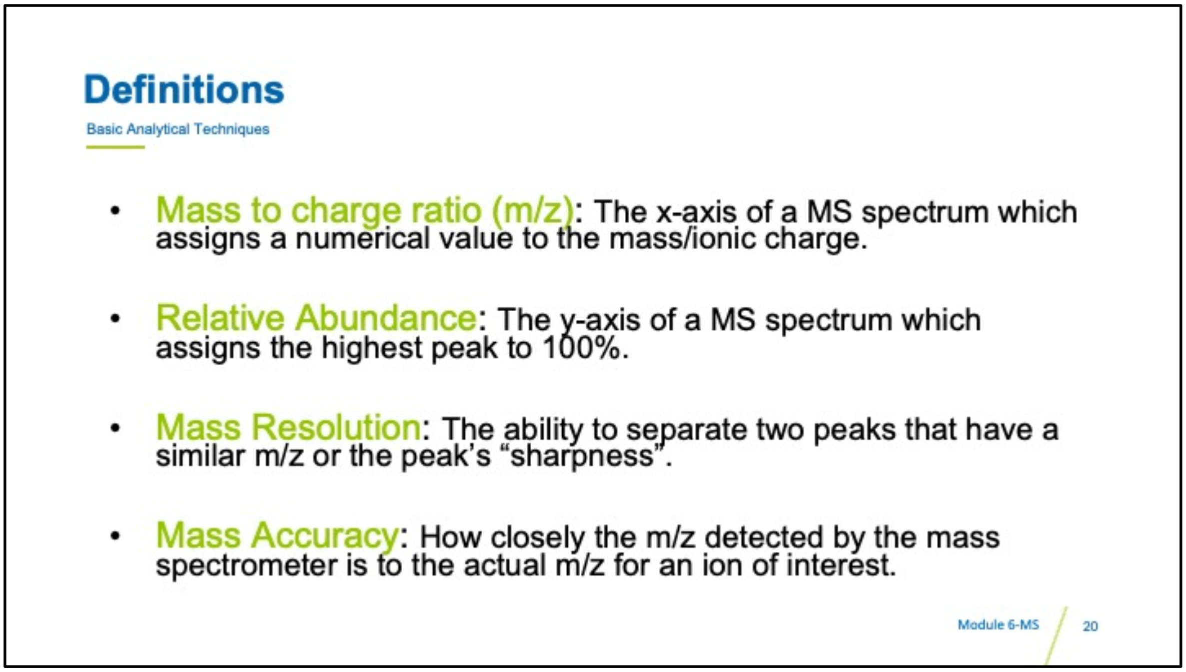
The mass spectrum is a graphical representation of the ions, as represented by their mass-to-charge ratio, produced by the source. The y-axis of the graphic represents the relative abundance of each ion. Mass spectra are characterized by the “stick graph” because the mass to charge ratio is a single number and there is no relation between two M/Z, unlike chromatograms where molecules elute during a certain period of time, which creates a normal distribution around a maximum intensity. It is this maximum intensity that we call the retention time, despite the fact that it is really a retention window.

While this may appear too basic for a trained spectroscopist, the characteristic graphical representations of the mass spectrum and chromatograms help the novice users navigate the software of their instrument…



Definitions

Basic Analytical Techniques

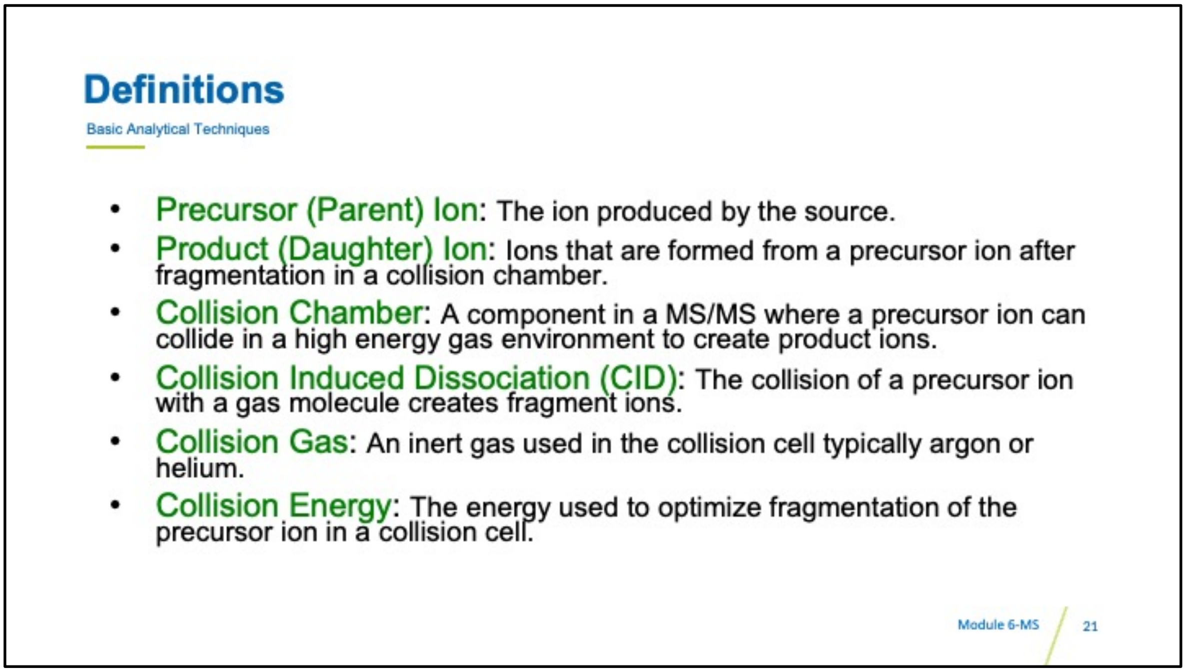
- **Mass to charge ratio (m/z)**: The x-axis of a MS spectrum which assigns a numerical value to the mass/ionic charge.
- **Relative Abundance**: The y-axis of a MS spectrum which assigns the highest peak to 100%.
- **Mass Resolution**: The ability to separate two peaks that have a similar m/z or the peak's "sharpness".
- **Mass Accuracy**: How closely the m/z detected by the mass spectrometer is to the actual m/z for an ion of interest.

Mass to charge ratio (represented by m/z) is the x-axis of a MS spectrum which assigns a numerical value to the mass/ionic charge. As discussed before, M/Z is not unique to a molecule, for example M/Z of 250 could be M=250 and charge = 1 or M= 500 and charge = 2...

Relative Abundance: The y-axis of a MS spectrum which assigns the highest peak to 100%. All other peaks are represented as their relative height (*i.e.* abundance) to the highest peak. It is important to note that this is **not** a percentage so the sum of the abundances in not 100.

Mass Resolution: The ability to separate two peaks that have a similar m/z or the peak's "sharpness". We represent M/Z using discrete, mostly whole, numbers such as 2 50 0r 500. As the instrumentation was developed over the years, it became possible to differentiate ions that are closer in M/Z, i.e. less than one mass unit. This concept will be revisited in the next section of this chapter when we review the different types of mass that we can use to identify an ion. For now, the take-home message is that there are instruments with higher and some with lower mass resolution, or ability to differentiate mass/charge ratios.

Finally, Mass Accuracy means how closely the m/z detected by the mass spectrometer is to the actual m/z for an ion of interest. Mass spectrometers are calibrated to provide a specific mass accuracy. However, the instrument can change over time and depart gradually from its calibrated state, or they may simply be less accurate by virtue of their design (and intended function). A good mass accuracy is important for multi-residue methods in food safety.

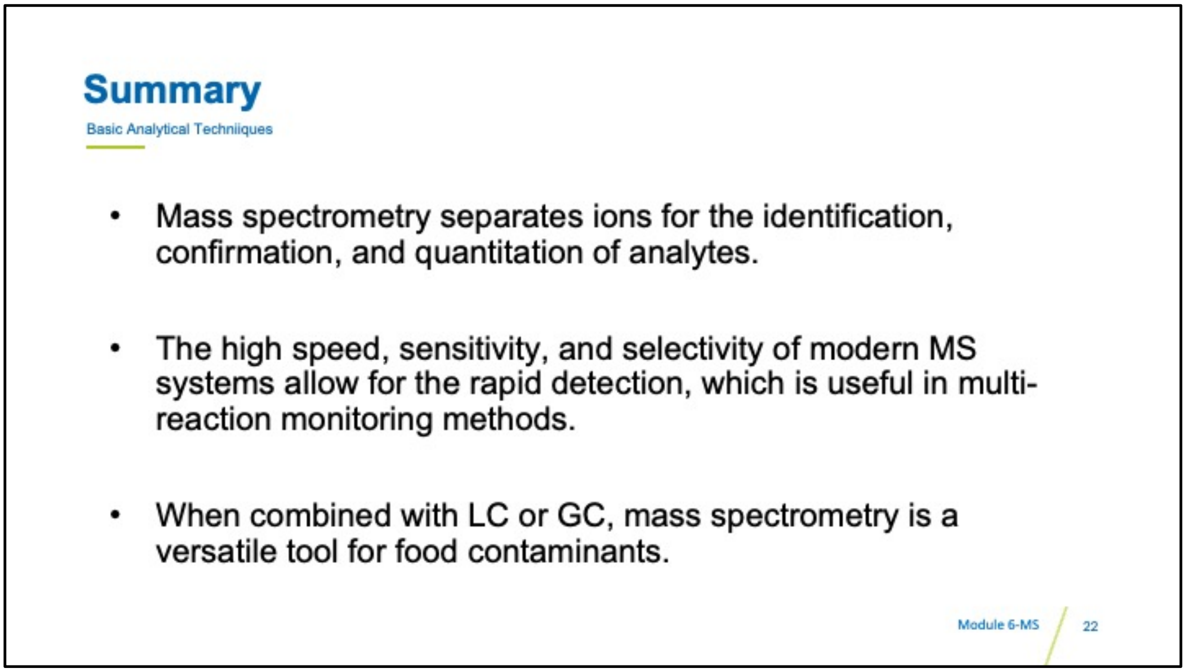


Definitions

Basic Analytical Techniques

- **Precursor (Parent) Ion**: The ion produced by the source.
- **Product (Daughter) Ion**: Ions that are formed from a precursor ion after fragmentation in a collision chamber.
- **Collision Chamber**: A component in a MS/MS where a precursor ion can collide in a high energy gas environment to create product ions.
- **Collision Induced Dissociation (CID)**: The collision of a precursor ion with a gas molecule creates fragment ions.
- **Collision Gas**: An inert gas used in the collision cell typically argon or helium.
- **Collision Energy**: The energy used to optimize fragmentation of the precursor ion in a collision cell.

Precursor (previously known as the parent) Ion is the ion produced by the source.
Product (previously known as daughter) ion is formed from a precursor ion when fragmentation occurs in the collision chamber.
Collision Chamber is a component in a MS/MS where a precursor ion can collide in a high energy gas environment to create product ions.
Collision Induced Dissociation (CID) is the collision of a precursor ion with a gas molecule, which creates fragment ions.
Collision Gas is an inert gas used in the collision cell and is typically either argon or helium.
Collision Energy is the energy we apply to optimize the fragmentation of the precursor ion in the collision cell.



Summary

Basic Analytical Techniiques

- Mass spectrometry separates ions for the identification, confirmation, and quantitation of analytes.
- The high speed, sensitivity, and selectivity of modern MS systems allow for the rapid detection, which is useful in multi-reaction monitoring methods.
- When combined with LC or GC, mass spectrometry is a versatile tool for food contaminants.

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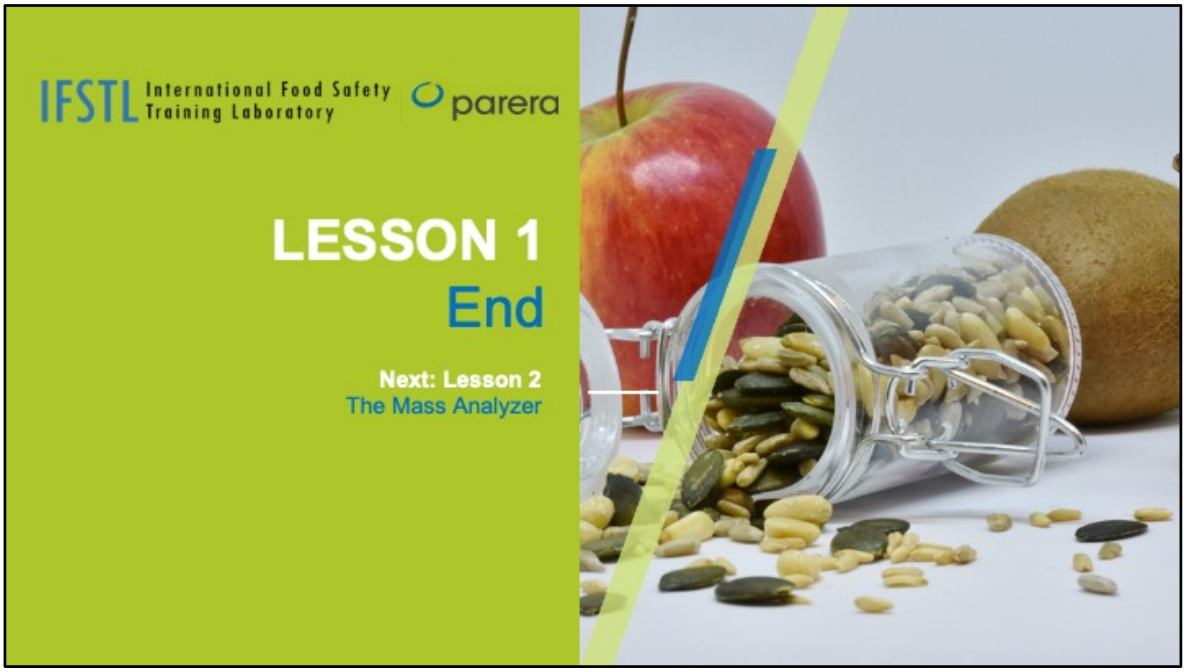
In summary for section 1, mass spectrometry is a tool to separate ions for the purpose of identification, quantification and sometimes confirmation. The ions are produced by a source that is selected based on the thermal stability of the compounds of interest, the ease of producing charged ions and the subsequent steps that lead to detection.

The versatility of the instrumentation, its high speed, sensitivity, and selectivity allow for the rapid detection, which is useful in multi-reaction monitoring methods.

Finally, when combined with chromatographic separation, the mass spectrometer becomes one of the most versatile tools for the determination of large numbers of residues in a single or few analyses, which is the reason GC/MS and LC/MS/MS are so broadly deployed in food safety laboratories.



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LESSON 1

End

Next: Lesson 2

The Mass Analyzer

You have reached the end of lesson 1. In lesson 2, we focus on the mass analyzer.