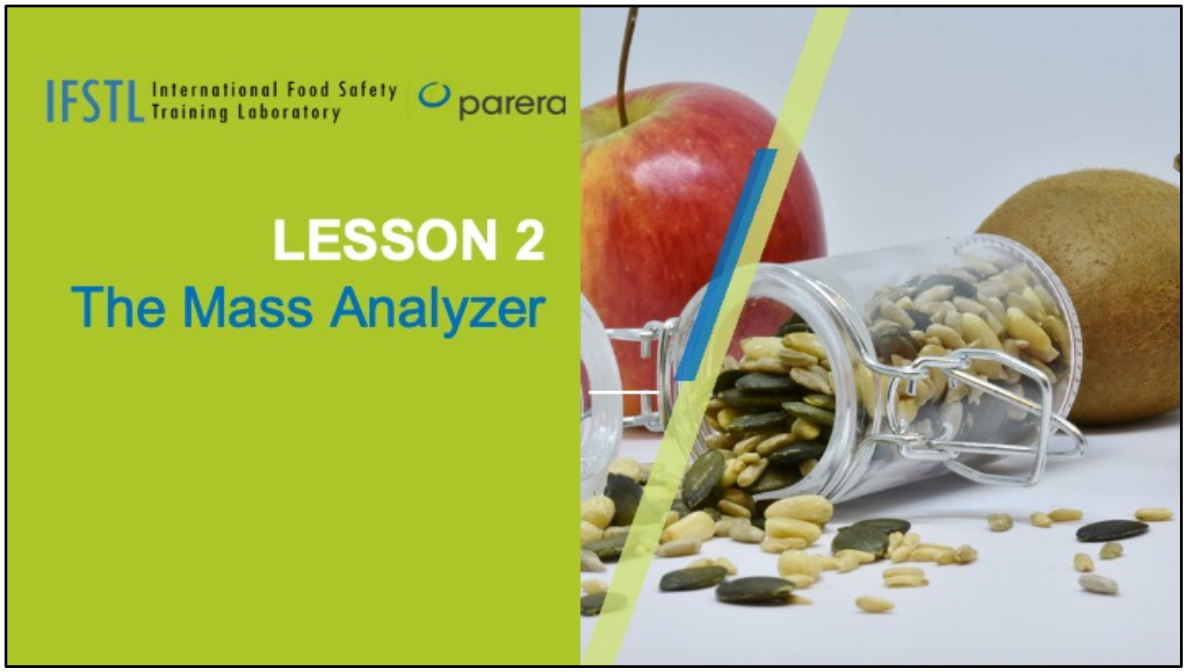
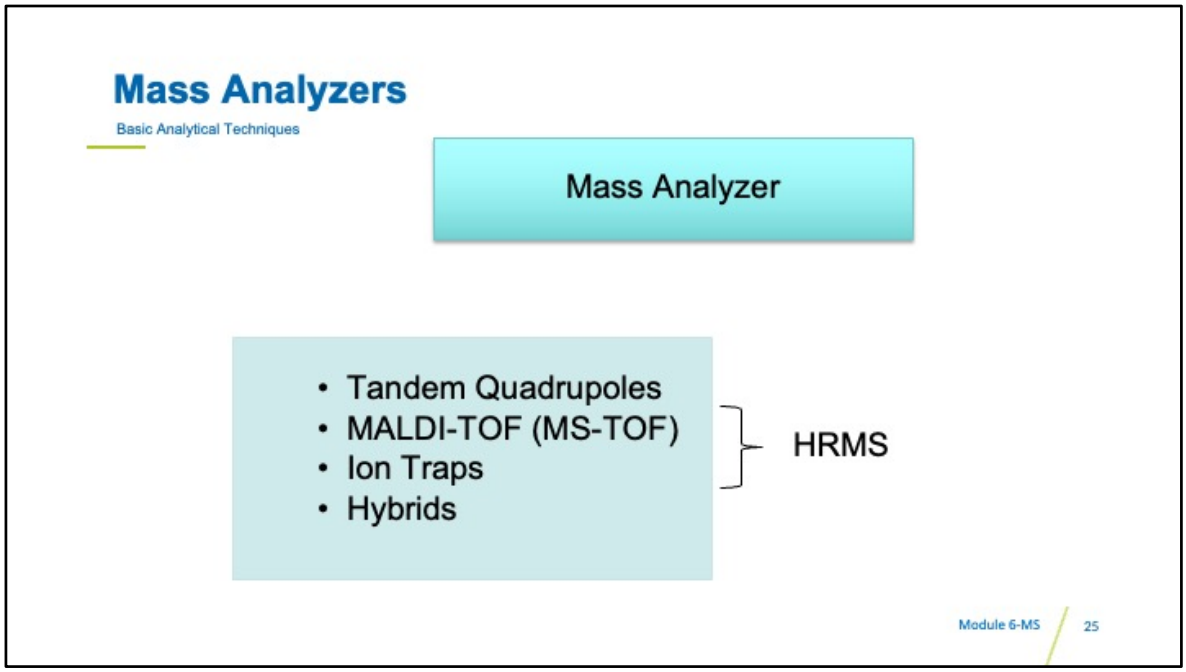

Welcome to module 6 of this online training program on confirmation methods for organic chemical contaminants.




Welcome to section 2 focusing on the mass analyzer.



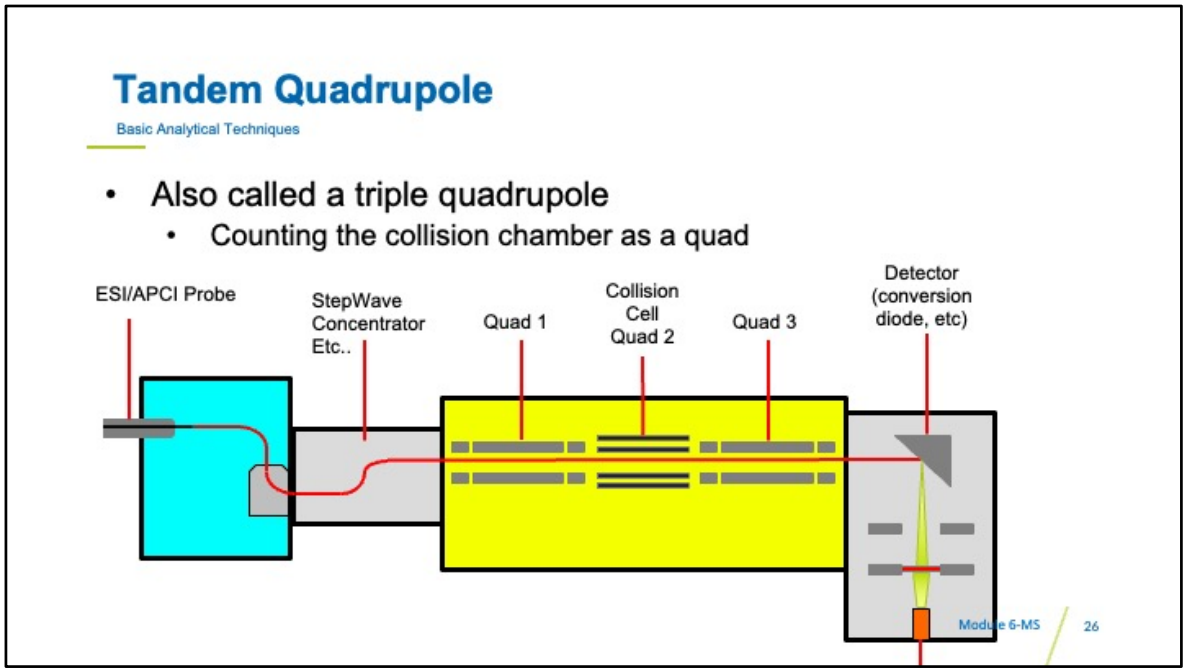

We briefly introduced the mass analyzer in modules 4 and 5 dedicated to HPLC and GC and some of the implementations of MS were discussed in section one of this module. In this section, we go deeper into the actual acquisition of data using the mass spectrometer.

The mass analyzer is the portion of the instrument where ions are selectively promoted through the system based on their mass-to-charge ratio. This is generally achieved by first passing a stream of ions moved under vacuum through a cleaning mechanism that varies slightly in different brands of instruments. While the precise process is typically proprietary, the objective is to focus the stream of ions and eliminate sources of problems such as any liquid droplets.

There are different types of mass analyzers used in the food safety laboratory. MS indicates a single quadrupole mass spectrometer, while MS/MS indicates the pairing of two quadrupoles with a collision chamber sandwiched between the two. TOF is a time-of-flight mass spectrometer, while Q-TOF is a quadrupole followed by a TOF and finally an ion trap is an instrument with a mechanism for concentrating ions prior to their detection.

There are other types, such a sector instruments and Fourier Transform Ion Cyclotron

Resonance Mass Spectrometers, but these are not used in the food safety laboratory and will not be covered here.

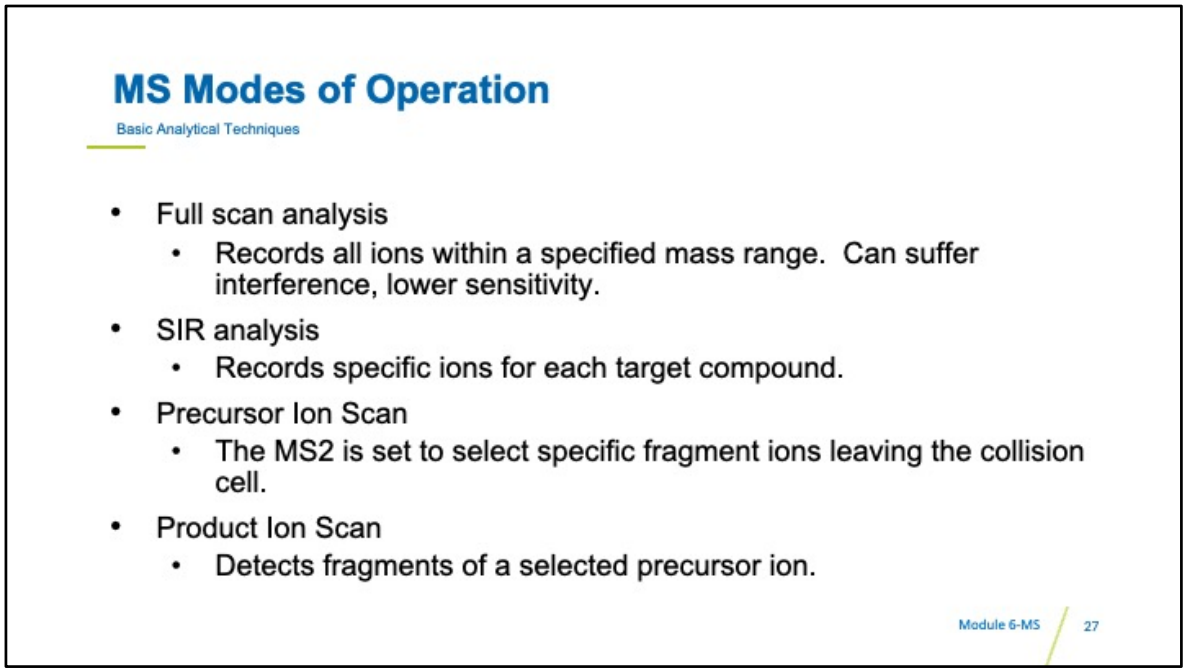



The most widespread implementation for the analysis of food contaminants by LC with a mass spectrometer is the tandem quadrupole. As mentioned earlier, some vendors call it a triple quadrupole as they count the collision chamber as quadrupole 2. It is also not uncommon for these to be called quads, rather than quadrupoles.

The sample is represented in this schematic with a red line. It enters the instrument at the source, most commonly one of the atmospheric pressure sources (electrospray ionization or atmospheric pressure chemical ionization). The sample, now in gas form, travels through a proprietary chamber that can concentrate and clean up the sample somewhat before it reaches the actual mass analyzer. The purpose of this step is primarily to avoid any droplets from entering the mass spectrometer, which would require more frequent (expensive) cleaning. Modern concentrators or indicated in this one as a StepWave or any of the commercial names given to this chamber are essential to prepare a steady, dry sample stream in a quantity optimized for the quadrupoles.

The sample then enters the first quad, also called MS1 in tandem mass spectrometry, where a precursor ion is selected and passed on to the collision

chamber. All other ions are discarded. The selection can be static, i.e. a single precursor ion is selected during the entire experiment, or it can be dynamic, where MS1 is modulated to select different precursor ions in sequence. This dynamic application is the most common mode of operation in the food contaminants laboratory as we typically are looking to identify and measure numerous chemicals in one experiment. This modulation can be synchronized with the elution of the chromatography column in multi-residue analysis (using multi-reaction monitoring), or it can be sequential to acquire a mass spectrum.




The quadrupole mass spectrometer can be operated in a number of different modes depending on the objective of the analysis. The ability to vary the operational parameters enables this option. It is important to know that vendors of instrumentation may use different names for the acquisition modes in their software, so we will focus on using descriptions of the modse rather than the wording of a particular software in this text. The hands-on portion of the workshop will also be tailored to use both the Waters-specific software nomenclature and the descriptions.

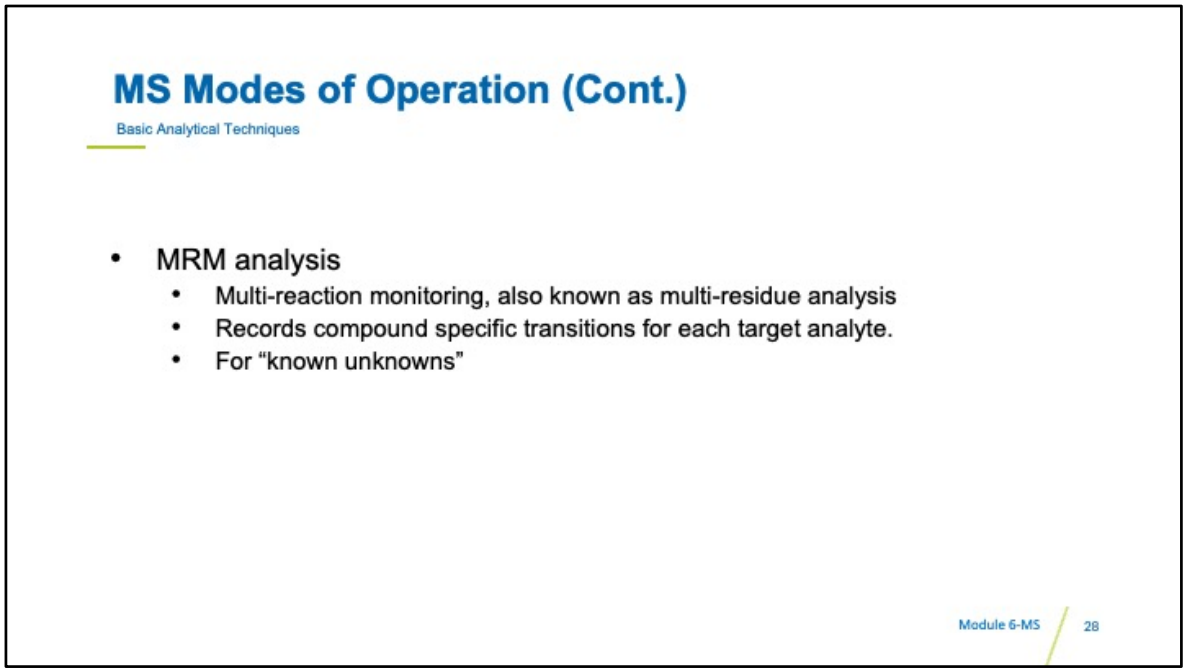
A full scan analysis, sometimes simply referred to as a scan, is an experiment where the instrument records all ions within a specified mass range. The disadvantage is that it can suffer from interference and result in a lower sensitivity than a more targeted analysis. The advantage is to provide a picture of all the ions that are present. For experiments aiming to detect food contaminants in an extract injected directly into the mass spectrometer, this type of experiment provides a snapshot, or an indication, of which contaminants may be present. It is more difficult to use this mode of operation following chromatographic separation, *i.e.* LC-MS or GC-MS, because of the time restrictions imposed by the flow rate of the chromatograph. Put simply, the mass analyzer can only find a compound during the elution of its chromatographic peak and this time window can be very small in UHPLC. This concept will be discussed and demonstrated during the hands-on portion of this

training.

Single ion monitoring, or SIM analysis, records specific ions for each target compound in a sample. Once again, the applicability of this mode of operation varies based on the set up in place. For example, it can look for the molecular ion and a selection of adducts promoted through the quadrupole, or for a number of precursor ions for a mixed sample. When applied in a combination instrument with chromatography, the number of ions that can be monitored is again limited by the width of the elution peak. This mode of operation is generally more sensitive than full scan because the instrument parameters can be adjusted to accumulate the signal for a specific ion for a longer period of time, hence improving the signal to noise ratio.

When the instrument includes a second quadrupole, or what some vendors called a triple quad instrument or a tandem quadrupole instrument, it can be operated in additional modes because the precursor ions and product ions are created in two separate spaces and are analyzed by two separate quadrupoles. The tandem mass spectrometer can be operated in full scan, single ion monitoring, MS/MS product ion mode and MS/MS single reaction monitoring mode.

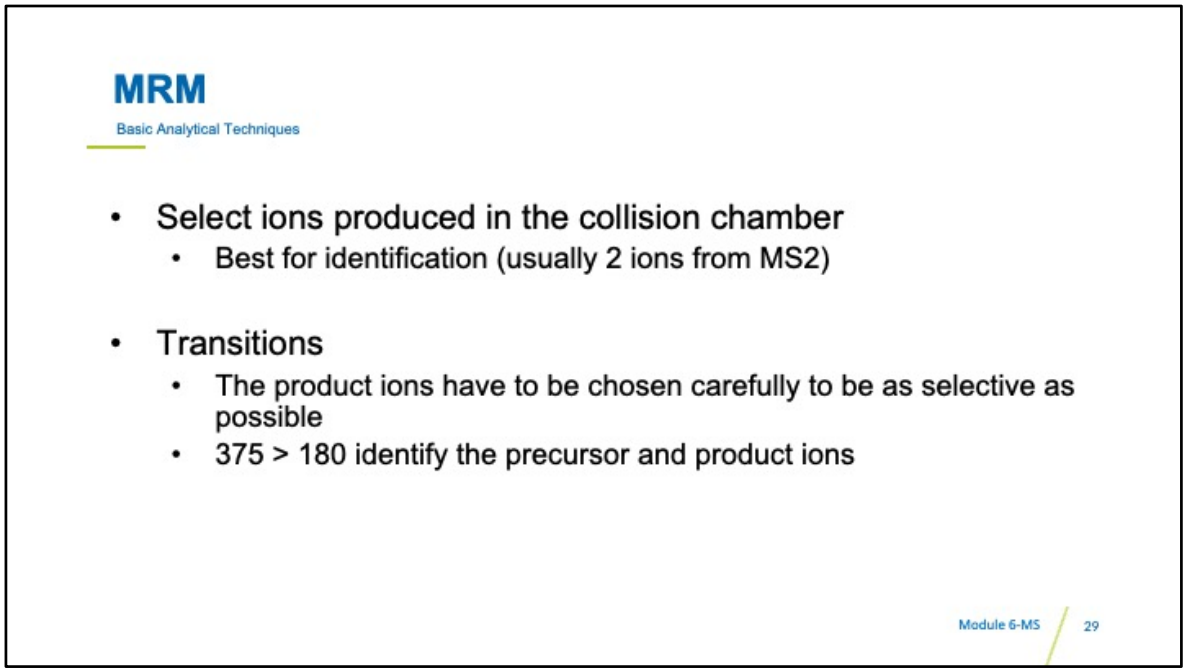
The first quadrupole is used to select the precursor iron which is promoted through the first quadrupole into the collision chamber where it is fragmented. The level of energy applied in the collision chamber affects the fragmentation and therefore the fragment ions that can be promoted through the 2nd quadrupole.




In multiple reaction monitoring (MRM), both quadrupoles are used as mass filters and the collision chamber is energized to cause fragmentation. This mode is typically used for quantitative analysis and is the most widely deployed mode for food safety contaminants analysis.

The use of multiple reaction monitoring enables the analysis of a large number of analytes in a single experiment by dividing the time spent for each quadrupole to perform tasks enabling the identification and quantification of multiple ions in sequence.

Multiple reaction monitoring is also known as multi residue monitoring, especially in the field of pesticide residue analysis. The main advantages of MRM, as mentioned before, are to enable the analysis of multiple components in the same run, which saves time, and the ability to measure product ions in sufficient number for confirmation analysis.




We will use MRMs in our hands-on training session, but briefly, the advantage is that we can produce multiple product ions by changing the energy in the collision chamber and letting select ions through MS 2 to reach the detector. Most MRMs use 2 product ions, but some use three when needing more specificity.

The combination of the precursor ion and product ion is called a transition. It is written as the two ions with an arrow between them, sometimes a full arrow and sometimes just an arrowhead.

## Summary of Modes for Tandem Quad

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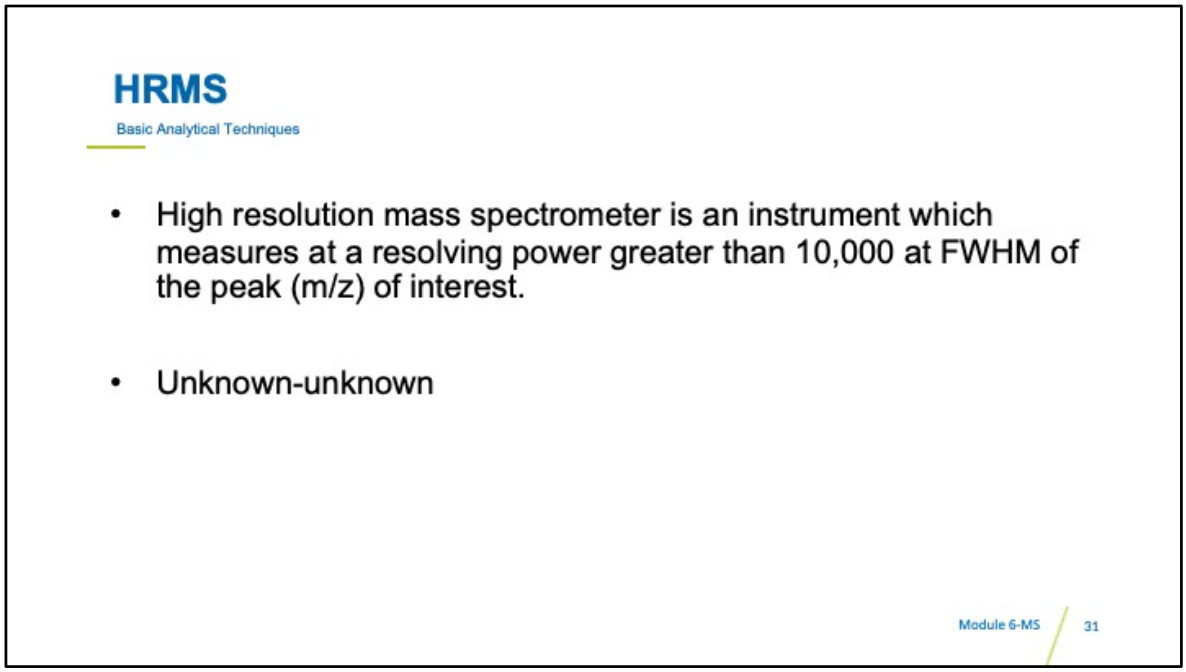
| Scan type | Instrument component: MS1 | Instrument component: Collision cell | Instrument component: MS2 | Data type |
|---|---|---|---|---|
| MS scan | Scan | Inactive | RF only (acts as ion guide) | Qualitative |
| MS2 scan | RF only (acts as ion guide) | Inactive | Scan | Qualitative |
| SIR | Mass filter | Inactive | RF only (acts as ion guide) | Quanitative |
| Product ion (daughter) scan | Mass filter | Active | Scan | Qualitative |
| Precursor ion (parent) scan | Scan | Active | Mass filter | Usually Qualitative, sometimes Quanitative |
| Multiple reaction monitoring (MRM) | Mass filter | Active | Mass filter | Quanitative |
| Neutral Loss Analysis | Scan | Active | Scan | Usually Qualitative, sometimes Quanitative |
| Neutral Gain Analysis | Scan | Active | Scan | Qualitative |

waters.com



This slide summarizes what is achieved in each quadrupole during the different modes of operation.




A high resolution mass spectrometer is an instrument that measures at a resolving power greater than 10,000 at full-width at half-max of the peak (m/z) of interest.

The main benefit of high resolution MS is that the high mass resolution enables the differentiation of compounds even when their masses are vey close, meaning smaller than one or even 1 tenth or 1/100$^{th}$ or less of a mass unit.

This type of instrument is typically used for unknown unknowns; we will discuss these further in module 8.

## Ion Trap

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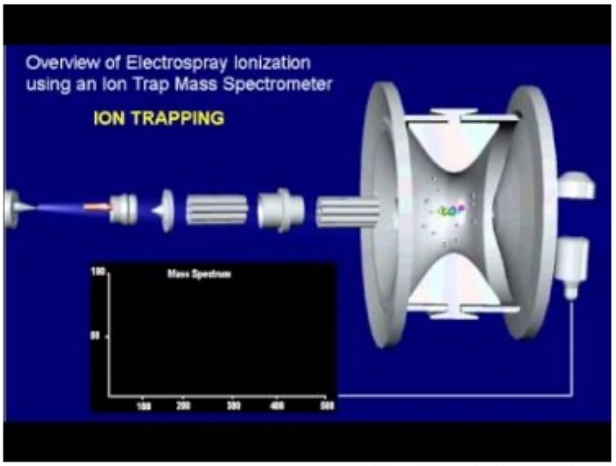


https://youtu.be/3uUwa1DDoHQ



Ion trap is another implementation that differs from the quadrupole and serves a purpose.

The ion trap mass spectrometer instrument is quite specialized and more often found in research laboratories than routine testing laboratories. It is however increasingly investigated as a tool for food contaminants, especially those present at very low concentrations, and as it is becoming more widely available.

Let's look at this video.

## Instrumentation in High Resolution MS

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- QTOF



For those who have never seen them, this is what the time of flight instrument looks like. These instruments are characterized by a long chamber, either vertical or horizontal, where the ions take flight.

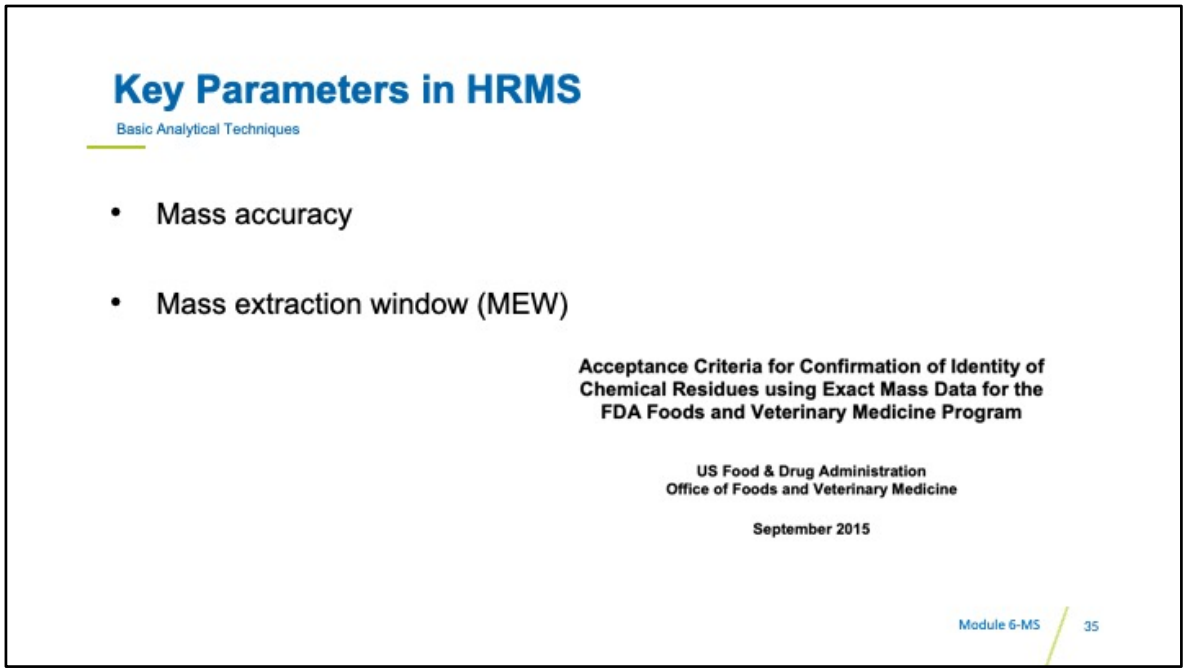
## QTOF

Basic Analytical Techniques


https://youtu.be/W-DRL-V2Rkg



Let's look at this video from Agilent explaining the TOF instrument. It also provides a great summary, wrapped up in marketing of course, but nevertheless a great summary of the various parts of the mass spectrometer.




There are two key parameters that define a high resolution mass spectrometer. The mass accuracy and the mass extraction window.
Acceptance criteria are now published for methods using HRMS. This is the example of the document published by the US FDA and we will use it to illustrates some of the important concepts.

## Exact Mass

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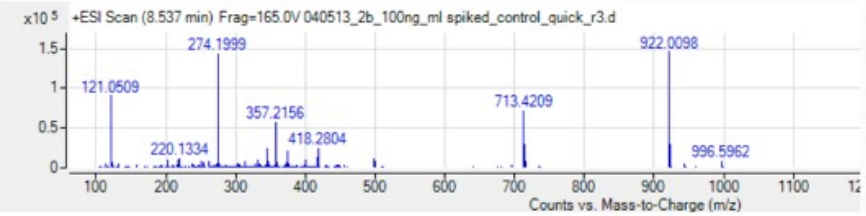
- High resolution mass spectrometers measure m/z ratios to four (or more) decimal places: Exact mass




- Low resolution mass spectrometers such as tandem quads report m/z values to the nearest whole number : Nominal mass

https://www.fda.gov/food/science-research-food/laboratory-methods-food



High resolution mass spectrometers measure m/z ratios to four (or more) decimal places: This is called the exact mass. The nominal mass produced by lower resolution implementations, such as the tandem quads we use for MRM, is reported in whole numbers, sometimes with one decimal.

## FDA Acceptance Criteria

Basic Analytical Techniques

**Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data for the FDA FVM Program**

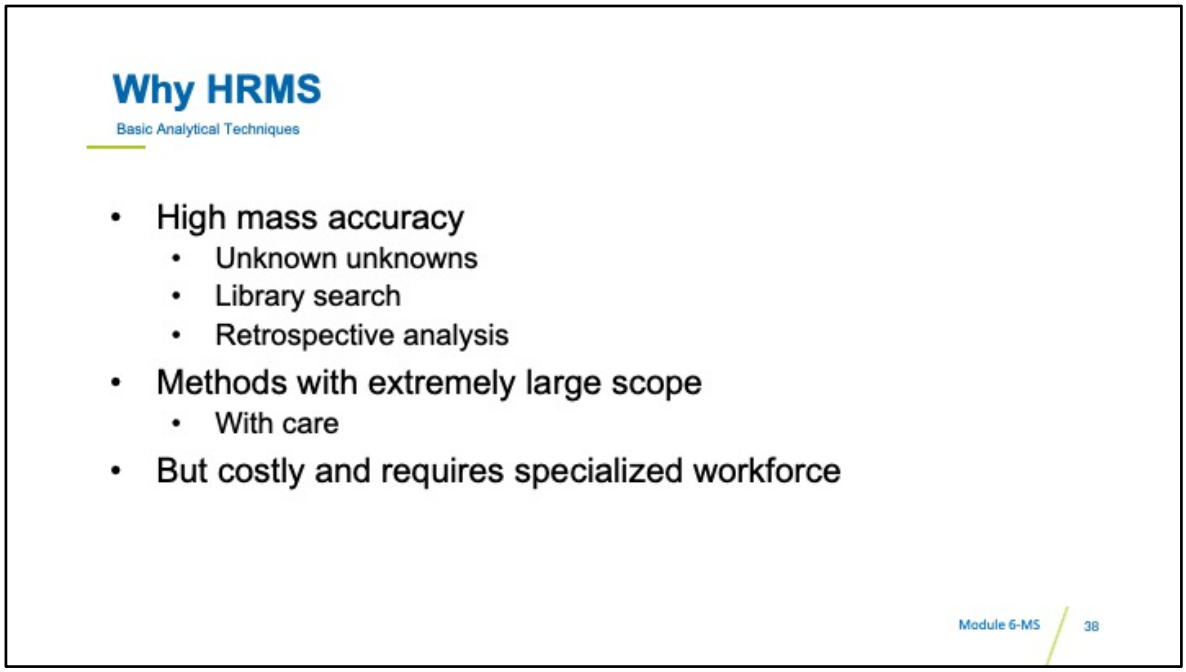
Table 1. Summarized Requirements for Confirmation of Identity

| MS mode | MS[1] | MS/MS | MS[1] and MS/MS |
|---|---|---|---|
| EIC: signal requirement (absolute) | A criterion to be set by one of the following methods:<br>(1) a S/N threshold ≥ 3;<br>(2) an intensity ratio relative to the comparison standard equal or above a preset threshold | | |
| EIC: retention time, relative to comparison standard | A criterion to be set by one of the following methods:<br>(1) ≤ 0.2 min, or<br>(2) within ±2.5%, not to exceed 0.5 min, or<br>(3) within an established error range, not to exceed 0.5 min | | |
| MS: number of structurally significant ions | Minimum 2 | Minimum 2 | Minimum 2 combined |
| MS: mass accuracy | ≤ 5 ppm | ≤ 10 ppm | MS[1]: ≤ 5 ppm; MS/MS: ≤ 10 ppm |



Regulatory agencies are issuing their acceptance criteria for the use of high-resolution mass spec in confirmation analysis. This table shows the criteria set by FDA.

The criteria are typically not very different from MS/MS, except of course for the accuracy of the mass data.




There is growing interest for high resolution mass spectrometry, especially in laboratories interested in the unknown unknowns. Because the instrument can use a library search for preliminary identification, it makes it possible to detect contaminants that we did not expect and would consequently not have included in the calibration of a tandem quad method.

Another important advantage is the ability to perform retroactive analysis of a sample's dataset. Indeed, the entire mass spectra are saved, so it is possible to run the data against new libraries, and while paying attention to specific contaminants that were not of interest at the time of data acquisition. For example, one could look for the prevalence of a certain mycotoxin that is not currently regulated but can be investigated using stored data.

We are seeing laboratories develop gigantic methods that are meant to screen for seemingly everything under the sun! These types of methods should be applied with great care. HRMS is powerful, but it is still expensive and one of the biggest hurdles for deployment is the need for highly specialized analysts.


We are already at the end of lesson 2… In the next lesson, we move on to the applications of mass spectrometry in our labs. See you soon!