Online Training Program

Methods of Determination for Organic Contaminants

Principles of Mass Spectrometry

Module 6

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Lesson 1: Introduction to Mass Spectrometry

Module 6 is dedicated to mass spectrometry as a detection technique following chromatographic separation either by gas or liquid chromatography. The technology was briefly introduced in the detectors sections of Modules 4 and 5 and these basic concepts will only be reviewed briefly. The current module aims to develop an understanding of the main parameters that affect the measurements and they impact methods for the determination of specific types of food contaminants.

This module is designed as a part of an online and in-person training program and as such, contains information that is complementary to the video and in-person training. It should not be used as a stand-alone reference.

Learning Objectives

The learning objectives of this module are to:

- Understand the principles of mass spectrometry as a measurement technique;
- 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 important in food safety methods.
- Review a number of official methods for food contaminants with the objective of understanding why mass spectrometry, both single quadrupole and tandem quadrupole, have become so important for the food safety laboratory.

Mass Spectrometry

As the name suggests and introduced in Module 4, mass spectrometry is a technique that measures the spectrum of masses; however, the measurement is made possible by ionizing the

molecule of interest, *i.e.* giving them a positive or negative charge, because the mode of action of the instrument is driven by the mass-to-charge ratio (m/z). The instrument used for mass spectrometry is called a mass spectrometer.

In this module, we take a deeper dive into the components of the mass spectrometer in order to understand their applicability for food contaminants of interest. 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, or eluted from a GC or LC column, is introduced into the instrument and molecules of interest are charged. The ions then move to the mass analyzer, *i.e.* 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 systems 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 principle behind it is quite straightforward.

Ionization

Mass spectrometry can only be applied to analytes that can be ionized. There are many mechanisms to achieve this ionization, but it is an essential condition. The source is the chamber where molecules in solution, dry state or in a gas stream are ionized. The charge can be positive or negative. The equation that describes a molecule at this point is mass/charge, or

$$\frac{m}{z}$$

Where m = mass and z = charge

It may appear repetitive, but no measurement will be made if there is no charge (*i.e.* a charge of 0). We emphasize this basic principle because the absence of a band in a mass spectrum should normally indicate that the component of interest is absent, but as we just re-iterated here, the absence of a band could also mean that there was no ionization. This is the main reason why a flat

line is never considered as a demonstration of absence of a compound until a band is confirmed in the exact same conditions for the equivalent standard. This will be discussed further in Module 9 on quality assurance.

Ionization Source

There are three main types of ionization source used in the food laboratory: Atmospheric pressure ionization (API), matrix assisted laser desorption ionization (MALDI), and electron ionization (EI). The appropriate source is dependent on the sample itself, the compounds of interest and the concentration of these compounds.

The first group of sources is based on ionization performed in a chamber at atmospheric pressure. This 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.

MALDI is a technique that uses a laser beam to lift chemicals from a solid surface and produce ions. It is best suited for samples that either cannot be solubilized or vaporized, or where it is advantageous to control what is being extracted through adjustments of the laser.

Finally, electron ionization is based on the use of an electron beam to cause ionization. It requires the sample to be present in gas phase.

Electrospray Ionization

Electrospray ionization (ESI) is a system where the sample solution is sprayed through a heated capillary tube to which an electrical voltage is applied and surrounded by a stream of inert gas, typically nitrogen. As the solution approaches the tip of the capillary tube, its temperature increases and the combination of high temperature and the gas stream cause the formation of small droplets that immediately start to dry. The voltage applied at the tip of the capillary 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 solvent and split into smaller droplets, a process

called fission, until they become completely dry. In this kind of source, ions can be positively or negatively charged but whole. Ideally, we do not want to break up the molecule because we want to use the molecular ion for identification. When hydrogen is added to create that +1 charge, it creates a positively charged molecular ions. Conversely, in negative electrospray ionization, the negatively charged ion is produced by the removal of a proton (hydrogen ion) from the molecule. The molecular ion has the molecular weight of the compound of interest plus one or minus one. This means that no bonds were broken or formed other than the addition or removal of one proton.

A number of other ions may form during the process, which are useful under certain circumstances. For example, portions of the modifier used in the mobile phase (or added to the sample for direct injection) can be added to a molecule to cause it to become positively charged; the product of this addition is called an adduct. Adducts therefore weight the sum of the molecular masses of the original compound and the added compound, plus or minus 1 (if positively or negatively charged). 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 systems 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 as it enables more compounds to be included in the same method. In other words, we obtain more results from the same analysis, which saves time and money.

Adducts are extremely important for confirmation of identity when using a single quadrupole mass spectrometer because their formation is both predictable and reproducible. This will be discussed further during one of the practical laboratory sessions. When MS/MS is available, we favor the use of the molecular ion because it is more selective and we have the ability to create other ions (product ions) for the confirmation of identity using the second MS. We will discuss this further in the upcoming section on the mass analyzer.

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 chromatographic separation, we have

to ensure that the eluent from the chromatographic column is compatible with the source, ESI being the most common source following LC, and will generate the positive and/or negative molecular ions from 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 addition or subtraction of a proton. 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 chromatographic 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.

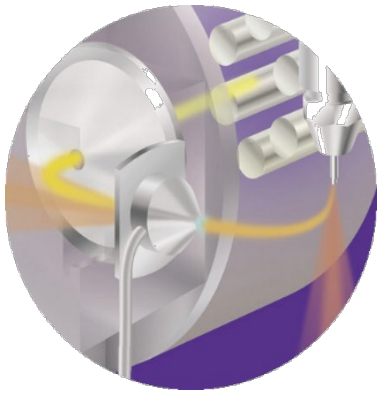

Figure 1: Schematic of an ESI source (reproduced with permission from ww.waters.com)

Figure 1 is a schematic from the Waters Corporation that 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 typical cone of droplets produced by a spray nozzle. The yellow stream represents the droplets that become small enough, through the drying process called desolvation and fission, aided by the nitrogen gas flow and the high temperature, to be attracted by the vacuum coming from the horizontal 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.

The source chamber will be examined in the practical laboratory portion of this course. In addition, some troubleshooting discussion related to poor results from methods will be discussed in Module 8, which includes a poor source performance. For example, failing to optimize the source parameters can result in 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.

Atmospheric Pressure Chemical Ionization

The atmospheric pressure chemical ionization (APCI) source works in a manner very similar to ESI, and usually in the same chamber, but there is no voltage applied on 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 the spray is subjected to a high voltage delivered by a needle called the corona needle or corona pin. This in turn creates a plasma, where analyte ions and electrons are produced. While the sample can be in liquid phase, the actual chemical ionization occurs in the gas phase. Protonated and other molecular adducts are produced, but as expected from the heat generated by a high voltage, there can be some degradation of heat sensitive compounds. Overall, APCI shows a very efficient ionization, which improves the sensitivity of a method, it is also rugged and highly reproducible. The charge 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 as noted above, this is a much higher charge than delivered at the tip of the capillary in ESI, which limits its use to thermally stable molecules. In addition, both ESI and APCI work well for “small molecules”, *i.e.* molecules weighing less than ~1500 daltons.

MALDI

Matrix-assisted laser desorption ionization is a completely different mode of ionization than ESI and APCI. MALDI is a technique that removes the need for a sample to be in gas phase (or volatilized) to create ions. Briefly, a laser pulse is used to ionize analytes without fragmentation (*i.e.* it is a soft ionization technique like ESI and APCI) by taking advantage of the absorption of the UV laser energy by matrix molecules. Matrix molecules are typically small and characterized by a conjugated pi system, *i.e.* with alternating single and multiple bonds. This type of molecule is very stable due to the delocalized electrons, but also shows strong resonance absorption of energy. The process starts with a sample in solution with a suitable matrix dried onto a stainless-steel plate. When the pulsed 337 nm laser (*i.e.* ultraviolet) hits the mixture of matrix and sample, the matrix absorbs energy, which in turn causes the dried mixture to volatilize. This is known as the ablation process. Ionization occurs at this stage through proton transfer with the matrix molecules; this typically results in ions with a +1 charge.

The main advantage of MALDI over the ionization techniques presented before is the ability to ionize larger molecules, such as proteins and biomolecules, and samples that either cannot or should not be volatilized via a solution. In addition, the pulsed nature of the laser works well with time-of-flight mass spectrometry (TOF). MALDI-TOF is used in food safety for the identification of bacteria, while MALDI-MS imaging shows promise for the elucidation of the chemical composition of spatially organized systems. In the latter case, the sample (a tissue for example) is raster-scanned with the laser and the chemical composition of each spot is determined by mass spectrometry. While very appealing for biological applications and some functional food systems, MALDI-MS imaging is typically not a technique of the food safety laboratory. MALDI-TOF will be described further in the next section of this module.

Electron Ionization

Electron ionization (EI) is a hard ionization technique, meaning that it results in fragmentation of the analytes. In the process of EI following LC, solvent elimination (*i.e.* vaporization) and ionization happen in sequential steps as opposed to the simultaneous process in ESI and APCI. In this source, the sample in gas phase (out of the GC or once volatilized following LC) is bombarded with an electron

beam, which causes fragmentation resulting in the liberation of charged and neutral fragments, or only charged analyte, plus electrons. The fragmentation into a charged and a neutral fragment is known as dissociative ionization, while the simple loss of an electron to form a positively charged molecular ion is known as non-dissociative. This is the most typical source used in GC-MS because the sample coming out of the GC is in gas phase in a nitrogen stream and ready for ionization, and non-dissociative ionization produces a positively charge molecular ion, which is highly specific for identification.

At this time, it is recommended to review the video portion of this training material as it contains animations that illustrate the processes described above.

Lesson 2: The Mass Analyzer

The mass analyzer is the portion of the instrument where ions are selectively promoted through the systems based on their mass-to-charge ratio. This is generally achieved by first passing the 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 them. 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 sectors and Fourier Transform Ion Cyclotron Resonance Mass Spectrometer, but these are not used in the food safety laboratory and will not be covered here.

Definitions

Mass to charge ratio (m/z): 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, often but not always whole, numbers such as 250 or 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 module

when we review the different way to express 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.

Mass Accuracy: 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.

Precursor (Parent) Ion: The ion produced by the ionization source.

Product (Daughter) Ion: Ions that are formed from a precursor ion after fragmentation in the collision chamber.

Collision Chamber: A component in an MS/MS where a precursor ion collides with an inert gas to create product ions.

Collision Induced Dissociation (CID): The collision of a molecular ion with a gas molecule creates fragment ions.

Collision Gas: An inert gas used in the collision cell typically argon or helium. An inert gas is required to avoid chemical reactions.

Collision Energy: The energy used to optimize precursor ion fragmentation in a collision cell.

Quadrupole

Most mass spectrometers found in regulatory and industry food safety laboratories use the quadrupole technology. The main advantages are ease of use, fit-for-purpose mass range, linearity in the concentrations range relevant for food safety and appropriate mass resolution.

A quadrupole is simply a set of four metallic rods arranged at equal distances from each other, but operating in pairs. One pair of rods is at a positive electrical potential (known as the high mass filter), while the other is at a negative potential (the low mass filter). A combination of direct current (dc) and

radio frequency (rf) voltages are applied to each pair and the combination acts as a filter that only allows a very specific m/z to progress through the length of the metallic rods. Put simply, only the ions that resonate at a particular amplitude of the dc and rf can “float” and progress through the chamber, while all other ions will be destabilized and “fall” on the metallic rods or on the walls.

The quadrupole can be used as a single mass analyzer, or paired with a collision chamber and a second quadrupole in the variant called MS/MS.

Modes of Operation

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 mode 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, especially in UHPLC. This concept will be discussed and demonstrated during the hands-on portion of this training.

Single ion monitoring, SIM analysis (SIR in the Waters software), 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 different molecular ions for a mixed sample. When applied in a combination instrument with chromatographic separation, the number of ions that can be monitored is again limited by the width or duration of the eluted 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, the instrument 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 second quadrupole. As mentioned in the video portion of this workshop, some vendors call the second quadrupole “quad 3” because the collision chamber is named “quad 2”. There is no physical difference between these instruments, it is only a difference in nomenclature.

One important advantage of using a “triple quad” instrument (i.e. tandem quadrupole or MS/MS) is the ability to study the fragmentation of a precursor ion. the fragmentation of the molecular ion in particular is more specific to a chemical compound then this study of adducts like would be required for confirmation of identity with a single mass spectrometer. A second advantage of the tandem mass spectrometer is that the first quadrupole serves as a filter for the precursor ions, in some cases compensating for imperfect separation in chromatography. This can provide a significant time saving on the chromatographic separation as well as render methods much more broadly applicable.

Each of the quadrupoles of the tandem quad instruments can be used in the modes mentioned above. In an MS1 scan the first quadrupole is scanning and the collision cell and 2nd quadrupole do not play any role. In an MS2 scan the first quadrupole only serves as a guide for ions the collision chamber is inactive and the second quadruple scans. In a single ion reaction or single ion monitoring, the first

quadrupole is used as a mass filter sequentially passing ions of interest through, and the collision chamber and second quadrupole do not play a role. In a product ion scan, formerly known as a daughter scan, the first quadrupole serves as a mass filter, the collision chamber is activated to cause fragmentation and the second quadrupole is used in scan mode. This is used to determine the most appropriate product ions for confirmation of identity. In a precursor ion scan the first quadrupole is used in scan mode the collision chamber is activated and promoting fragmentation and the second quadrupole is used as a mass filter. This mode is used for identification, meaning qualitative analysis, and can be used for quantitation.

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 by 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, is to enable the analysis of multiple components in the same run to save time, and the ability to measure a number of product ions, which serve as confirmation of identity for an analyte. The number of product ions that must be measured will be discussed later on.

Two additional modes of operation are available in the tandem quadrupole set up. Neutral lots analysis uses both quadrupoles in scan mode and the collision chamber is activated to promote fragmentation. The same happens in neutral gain analysis. Neutral loss can be used in for quantitative analysis, while neutral gain analysis is strictly qualitative. These modes of operation are not used in the typical routine food safety contaminant laboratory.

In samples where the concentration of analytes of interest is very small, the single quadrupole or triple quadruple may not be sensitive enough to detect or quantitate the analytes. An ion trap-equipped analyzer can be used in such circumstances. As the name indicates, that principle is to store the ions and accumulate them ahead of the detection. The typical quadrupole ion trap analyzer contains a ring electrode and two end cap electrodes where the ions are stabilized through the application of an rf voltage in a chamber filled with an inert gas, typically high purity helium. This setup

enables additional modes of operation for the single quadrupole instrument. For example, the trap can be set up to keep only one ion with a particular mass to charge ratio in the track, and consequently act as a filter. It can also be used like a collision chamber by inducing vibrations that will cause fragmentation. In this case, this is equivalent to MS/MS, but differentiated in terminology by calling it MS/MS "in time". When a single ion is kept in the trap and the fragmentation energy is progressively changed, this type of MS/MS is called MS/MS^n. 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, as it is becoming more widely available.

High Resolution Mass Spectrometers

High resolution mass spectrometers are gaining traction in the food safety laboratory. The most widespread instrument is the time of flight mass spectrometer, known as QTOF, where ions are formed in a source, filtered through a quadrupole, possibly fragmented in a collision cell and accelerated by an electric field into a long chamber that can include a number of reflections, or bounces, until it reaches the detector. In the time of flight instruments, the velocity reached by an ion is inversely proportional to its mass. Since the distance between the entrance and the detector is fixed, the time required by an ion to travel this fixed distance is a characteristic of an ion of a particular mass to charge ratio.

There are some advantages that justify the interest in the time of flight instrument for food safety. The very wide applicable mass range, the high mass resolution and mass accuracy provide a level of certainty for the identification of compounds that is greater than that obtained from a single mass spectrometer an equivalent or superior to that of the tandem mass spectrometer. The very high mass accuracy also permits the use of spectral library search as a means of presumptive identification for unknown compounds alleviating the need to run standards of all possible unknowns. This is often referred to as the "unknown unknown" in contrast to the "known unknowns" analyzed by MS/MS and for which we must run a standard to compare precursor and product ion presence and ratio for identification.

The time of flight instrument can be used following MALDI ionization and in this circumstance, the system must measure the travel time within the chamber for ions originating from a single pulse of the laser. In this case, the laser intensity can be used to selectively ionize certain analytes, thereby serving to a degree as a first filter like the quadrupole did in the previous implementation. MALDI-TOF is particularly useful for larger molecules, such as biomolecules, which are typically of lesser interest in the routine food safety laboratory.

High resolution mass spectrometer is measured mass to charge ratios to four or more decimal places. This is known as the exact mass in contrast with the nominal mass measured by lower resolution spectrometers such as tandem quadruples which measure to the nearest whole number.

The rising interest in the use of high-resolution mass spectrometry for regulatory purposes has prompted regulatory agencies to issue guidance on the acceptance criteria for the confirmation of identity of chemical contaminants using exact mass. For example, the FDA food and veterinary medicine program has released guidance[1] which we reviewed in the video portion of this training module. This and other guidance documents will be reviewed in greater detail during the in-person portion of this training.

Detectors

There are essentially three types of detectors coupled with mass spectrometers. The quadrupole and ion trap instruments typically use electron multipliers, where a conversion dynode is used to converge positively and negatively charged ions into electrons. Following amplification, the current detected is proportional to the quantity of ions present. The photomultiplier detector, or dynolyte photomultiplier, uses a conversion dynode to convert the ions exiting the quadrupole into electrons. These electrons continue on to a phosphor, which gets excited and emits photons. The photons then strike a photocathode at the front of the photo multiplier, which converts them back to electrons; the

[1]Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data for the FDA Foods and Veterinary Medicine Program. Available at: https://www.fda.gov/media/96499/download. Accessed 11/10/2020

photomultiplier serves to amplify the signal. This type of detector it is known for its durability. Finally, microchannel plate can be used, typically with a time of flight spectrometer. The advantage of the microchannel plate is the possibility to detect many ions at the same time in their individual channels.

Conclusions

There are many variations on the setup of mass spectrometry instrumentation. The appropriate set up is selected based on the objectives of the analysis. In the food safety laboratory focused on regulatory analysis, there is a great need for fast analysis of a large number of chemical contaminants. Therefore, instruments that deliver speed while maintaining the required level of accuracy and reproducibility are favored, which means the tandem quadrupole mass spectrometer is often the best choice. The increasing availability of high resolution mass spectrometry is slowly changing the landscape where regulatory agencies who can afford the technology use the advantage of being able to detect unknown unknowns. This is especially useful in environments where intentional contamination may be suspected or there is an ill defined environmental contamination landscape.

Lesson 3: Applications of MS for Food Contaminants.

This last section of module 6 is dedicated to the examination of some of the most popular applications of mass spectrometry for the analysis of food contaminants. It is not meant to be a complete review of all applications but rather a selection of examples that promote the understanding of the principles explained before as well as the impact of a number of instrumental parameters on the measurements.

Pesticide Residues

Pesticide residue analysis was the first application broadly deployed for food contaminants. Mass spectrometry provided a solution to many of the challenges presented by pesticide residues. For example, there are thousands of pesticides and degradation products that may need to be measured. These compounds are typically found at trace levels and, in fewer cases in greater amounts, such as parts per million. Most pesticides are synthetic chemicals and they are therefore known; their measurement corresponds to the “known unknown” introduced previously, which means that we have information about which standards to include in a residue method. One of the challenges of the food matrix is its extreme complexity and the potential for some of the matrix components to affect the measurement or the extractability of the components for chromatography. This is where the additional filtering capability of mass spectrometry comes in handy. The short shelf life of fruits and vegetables is another challenge that translates in the requirement for fast analysis for pesticide residues as many circumstances require reporting of the results within one or two days of receipt of the samples. In addition, regulatory and private laboratories analyzing for pesticides typically work on a large number of samples, which also requires speed.

It was mentioned above that the food matrix is a complex one. First and foremost, we are dealing with a broad range of agricultural commodities, which means fruits vegetables and grains, in addition to the occasional meat and fish sample. Moreover, some laboratories must make the measurements in prepared products that contain a large number of added ingredients. These products can also be in a form that renders them more difficult such as prepared foods containing

high amounts of sugar or fat. Finally, the technology is also applied in measuring pesticides and decomposition products in other matrices such as water and soil.

Using HPLC or GC-MS/MS enables the measurement of hundreds of pesticides in a single experiment. The source for the HPLC-MS experiment is typically ESI, while EI is used for GC-MS. The triple quadrupole instrument is used in multi-reaction monitoring mode (MRM) and the sample preparation is optimized to be as simple as possible while providing the level of separation in chromatography that will, in addition to the filtering ability of the first quadrupole, enable the identification and quantification of pesticides. In the regulatory laboratory, there generally is not a need for further concentration of the sample beyond sample preparation using a set up like an ion trap. However, if the data is acquired for risk assessment and consequently evaluating concentrations in the low ppt range, it may be advantageous to select options for low concentration during sample preparation and instrumental analysis.

Veterinary Drugs

Animal entering the food supply may have been treated with veterinary medicines to cure or prevent diseases. These pharmaceutical compounds may be present in the edible portion of the animals along with some of their metabolites. In order to ensure the safety of consumers and the availability of antibiotics for the treatment of diseases in humans, regulations are in place to limit the amounts of residues of these veterinary drugs that may be present in animal meat. Veterinary drugs are typically present in very low concentrations from ppt to ppm, but we generally know what we are testing for; they are therefore known unknowns. Meat, fish, seafood and meat product, such as sausage, are all very complex matrices. In addition, these foods have a relatively short shelf life if they are not frozen and therefore must be analyzed as rapidly as possible. While most regulatory laboratories that analyze a broad range of commodities may not see as many requests for analysis for veterinary drug residues as they see pesticides, some specialized laboratories must analyze upwards of a hundred samples per day.

One element that is very different between pesticides and veterinary drugs is that veterinary drugs are almost all heat sensitive and consequently not amenable for analysis by gas chromatography.

Due to the fact that they are used either orally or through injection, veterinary drugs are typically soluble in aqueous solutions, which makes them good candidates for liquid chromatography. They are also largely friendly to analysis by mass spectrometry. Analysts must pay attention to thermal sensitivity in the selection of the temperature in the ionization source; it is indeed possible to thermally degrade the analyte. Once again, this is a reminder of the importance of confirming the validity of the analysis through the use of standards in the same instrumental conditions, and on the same day. As noted before instrumental conditions includes the necessity to use the same instrument in addition to setting the same parameters

Mycotoxins

Mass spectrometry is also increasingly used for the determination of mycotoxins. While the focus was placed on aflatoxins in the past, there is growing interest to analyze up to 20 different mycotoxins. It is possible to analyze them individually or in small groups using less expensive and resource intensive instrumentation, but the advantage of using mass spectrometry is to develop a single method that applies to a large number of commodities. The real advantage of using a single method is that the requirement for validation is then limited to one method. One important disadvantage is that mass spectrometry technology requires expensive infrastructure because the instrument needs to be operated in a temperature-controlled laboratory with low humidity and a stable source of electricity. Notwithstanding the barrier caused by the infrastructure requirement the technology also imposes a large barrier to implementation in regions where few laboratory analysts are trained specifically for the application of mass spectrometry. Unfortunately, these regions often correspond to where the risk of mycotoxins contamination is greatest.

Nevertheless, mass spectrometry is gaining popularity for mycotoxins, especially in the field of risk assessment. In this situation the quantitation must be performed at ppt levels, which are not compatible with rapid techniques. Mycotoxins are known unknowns and their standards are commercially available. Much like pesticides, mycotoxins must be extracted from complex matrices and in the case of milk, results are expected within one day of the admission of the sample. Grains and pulses benefit from longer shelf life and typically can be held for testing for a longer period.

Mycotoxins are compatible with mass spectrometry because they are small molecules that are easily transferred into solution and ionized. Mycotoxins are generally heat sensitive but soluble in aqueous solutions or weak organic solvents, which makes them compatible with liquid chromatography. While a fluorescence detector attached to an HPLC is sufficient for the determination of aflatoxins due to their natural fluorescence, it is not possible to analyze other mycotoxins that do not display fluorescence using this instrumentation. Multi-residue methods using a mass spectrometer coupled to an HPLC or a UHPLC have a broad scope of application.

Industrial Contaminants

The list of industrial contaminants of interest is growing. In addition, metabolites are increasingly important for risk assessments. These contaminants are typically measured at trace level and up to ppb levels. In this case, some measurements qualify as known unknowns while others are unknown unknowns. The first is true in a regulatory laboratory that is measuring to verify compliance with maximum residue levels, while health research, risk assessment, and some pre-regulatory data acquisition programs may not know the breath of contaminants that should be investigated in a particular food and/or region. For the unknown unknowns, high resolution mass spectrometry is typically the preferred tool, especially in the research laboratory. The tandem quadrupole instrument operated in multi residue mode is the norm in regulatory monitoring laboratories.

Industrial contaminants our analyzed primarily in foods that are known to concentrate residues. for example, fish may concentrate industrial contaminants along their food chain, while root vegetables may take up contaminants from the soil. Many industrial contaminants are volatile and can be analyzed with gas chromatography, but others are either compatible for both HPLC and GC or better suited for liquid chromatography. Therefore, both chromatographic techniques are used ahead of the mass spectrometer.

Intentional Adulteration

Intentional is alteration is a relatively new concern in the regulatory laboratory. While the phenomenon is not new, the very large supply chains associated with international trade and consequent risk to large populations make it more critical today. In this case, we don't know what we should be looking for and therefore are definitely looking for unknown unknowns. The first large scale crisis related to intentional adulteration was the addition of melamine in milk by unscrupulous producers who were aiming to trick the protein content test. Briefly, protein is typically tested by measuring the amount of nitrogen present in the milk. Consequently, adding any source of nitrogen will increase the perceived protein content. Intentional adulteration can use volatile and non-volatile molecules and there is essentially an infinite number of possibilities. However, the most likely scenarios are the use of an unapproved additive, color, preservative or flavor due to a simple lack of understanding of what is approved or not. There have been very few situations where adulteration involved the purposeful use of a toxic substance to increase the economic value of a commodity or product. Nevertheless, these would be unexpected and consequently are considered unknown unknowns.

Advantages and Disadvantages

Mass spectrometry has become an important tool in the food safety laboratory in large part due to the ability to perform multi residue testing, which provides a large time advantage. The possibility to use the 1st quadrupole as a filter, which in turn allows for simple sample preparation, combined with the relative ease to compensate for matrix effects contribute further to this speed of analysis. Indeed, it is quite common for a laboratory to analyze a few dozen samples in a day for hundreds of pesticide residues; translated into equivalent single residue analysis, this corresponds to thousands of tests in a single day.

For regulatory testing, it is essential to confirm the identity of the analytes being quantified. Confirmation is typically defined to mean the agreement of two independent analyses. The tandem quadrupole instrument combined with chromatography provides a number of different identification points that combined build confidence in the confirmation of identity. For example, if an analyte elutes at the same time as the standard and the parent ion fragments into the same two product

ions in MS/MS with the same ratio of intensity, there is sufficient information to confirm identity. It bears repeating that comparison of the chromatograms and MS/MS profiles must always be verified with the standard ran on the same instrument and under the same conditions. At this point in time, a spectral library is only acceptable for the identification of compounds using high revolution mass spectrometry.

The disadvantages or weaknesses of using mass spectrometry for contaminants in food safety are largely related to the matrix effects in atmospheric pressure ionization. When matrix components are eluted alongside contaminants, their presence can affect the ionization efficiency of the target analytes in the source. This can lead to matrix-dependent signal suppression or enhancement. This phenomenon is well-known and compensation techniques are typically employed. For example, stable isotope internal standards can be used to calculate the matrix effects, but their optimal use is very expensive because, in theory, each compound should be measured alongside its stable isotope. This is actually rarely applied because these standards are too expensive or are simply not available. Matrix-matched calibrations often are used to compensate for matrix effects because they involve a smaller expense, but there is a price to pay in the amount of time necessary to prepare the matrix-matched calibration and such calibration must be prepared for each matrix or group of matrices that show a similar behavior.

Another solution to matrix effects is simply to dilute the sample until the effect is no longer observed. Of course, this solution can only be used if the limit of quantitation is not an issue. Finally, optimizing sample preparation by adding steps that focus on the removal of the compounds causing the effects is another possible option. The disadvantage of increasing the number of steps in the sample preparation is the time cost, the cost for consumables and the fact that it may limit which matrices are adequate for this new sample preparation. For example, one would need to ensure that the additional cleaning steps do not remove some of the other analytes of interest.

Conclusions

In summary, while mass spectrometry is a versatile analytical technique for the food safety laboratory, it is not the solution for all contaminants in all foods. The speed brought about by parallel analysis of hundreds of pesticides, for example, and the simple sample preparation procedures developed to produce sufficiently cleaned samples for MS/MS are the main reasons for the popularity of the technique. Combining the strength of HPLC or GC to separate large numbers of analytes of interest, and the identification and quantification capabilities of mass spectrometry instruments has dramatically changed the expectations in the food safety laboratory. Very large numbers of samples are expected to be analyzed each day and for an equivalently large number of contaminants. In spite of the relative ease of use of chromatography and mass spectrometry-based methods, it is imperative that each laboratory utilizing these tools have the expertise required to understand the potential sources of errors as well as the situations where the instrumentation is not appropriate.