Online Training Program

Methods of Analysis for Organic Contaminants in Food

Gas Chromatography

Module 5

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Introduction

This module focuses on gas chromatography. The first section of this module aims to understand the differences between LC and GC, to gain knowledge of the main components of the GC system, and the principle of separation exploited in gas chromatography. The second section introduces GC detectors commonly used for the determination of food contaminants. In a manner similar to the presentation of module 4 on liquid chromatography, we very specifically focus our attention on aspects of the technology that are most useful for laboratory analysts in food safety. Much greater detail is available in academic textbooks for those interested in technology development or advanced aspects of method development.

This chapter is designed as a part of a large training program with video and in-person components and as such, contains information that is complementary to the other portions of the training. It should not be used as a stand-alone reference.

Learning Objectives:

- Understand the principles of the analytical technique of gas chromatography
- Understand the difference between LC and GC
- Describe the components of the GC system
- Introduce GC detectors commonly used for food safety

Lesson 1: Gas Chromatography

The Technique

The technique of gas chromatography essentially separates chemicals present in gas phase. The separation of the mixture is done through a column containing a medium, in this case a stationary liquid, that has more or less retention effect on the components of the mixture. The gas chromatography column is much longer and narrower than the liquid chromatography column. Early columns were called thin and measured 1-5 meters long, with an internal diameter ranging from 1 to 5 millimeters. Originally, the columns were filled with particles coated with a liquid or elastomeric stationary phase. As the technology for the fabrication of capillary columns developed, it was applied to the manufacturing of GC columns and the technique benefited from these developments. Columns grew to lengths of 10 to 100 meters and the inside diameter shrunk to less than 1 millimeter, typically between 0.1 and 0.8 millimeter. The other major improvement of the technology was the replacement of the packing materials for the column, namely the stationary phase. Manufacturers stopped using particles packed in the column and replaced it with a coating on the inner wall of the column itself.

As introduced in module 4 on liquid chromatography, the column is the brain of a chromatograph. Consequently, much effort has been expensed on the development of the capillary columns and the stationary phase that they contain. One example of such development was the replacement of the stainless-steel column with fused silica. Glass is also an option but it doesn't offer the resilience of fused silica. While the metal columns worked very well because of their tolerance for high temperatures and the pressure developed as the temperature increases, the material limited the reduction of the internal diameter, which, as discussed in module 4, impacts peak width and peak height in the resulting chromatogram. High and narrow peaks enable the separation of a greater number of compounds, which is key for food safety applications often dealing with 10’s of potential contaminants. In addition, shorter and simpler sample preparation methods may lead to more components of the matrix being present in the sample portion injected in the GC, hence requiring the separation of more than just the contaminants of interest into distinct peaks. In summary, the combination of improvement in the fabrication of capillary columns and the move from packing beads to a liquid coating of the inner wall of the column have contributed to broaden the scope of application of gas chromatography in food safety.

GC is an essential technique for the measurement of a number of food contaminants that are volatile at relevant temperatures, and not soluble in typical LC-compatible solvents such as acetonitrile and water. In addition, the advent of coated capillary columns has led to highly effective separation, which is necessary for methods that, for example, separate ~50 pesticides or more. While many newer pesticides have been moved over to LC methods accommodating hundreds of compounds, hence providing a higher throughput of analysis, older pesticides require an analysis in gas phase. In addition, GC has not been replaced by LC for the pesticides compatible with both techniques in many laboratories for reasons of cost of the LC/MS/MS instrument and the difficulty hiring staff trained to use these instruments.

Components of the GC

The principle of operation of the gas chromatograph is straightforward. A sample is vaporized and injected into the head of a chromatography column. The sample is transported by a flow of mobile phase, which is an inert gas, and interacts with the liquid stationary phase coated on the inner wall of the column. A detector measures the quantity of sample coming out of the column in a selective or non-selective manner.

The mobile phase is an inert gas because it should not react with the sample or any of its components. Helium and nitrogen are the most common carrier gases, but argon, hydrogen and carbon dioxide can also be used. The two most popular gases, helium and nitrogen, have opposing advantages and disadvantages. Helium, while expensive, is safe and has a wide optimum linear velocity range, while nitrogen is cheap and safe, but has a narrow optimum linear velocity range and usually requires long analysis time. Regardless of the inert gas selected, it must be of the highest purity affordable by the laboratory, ideally >99.99% or even 99.995%, to minimize baseline noise.

A method that uses gas chromatography will always specify the flow control mode. The gas flow can be a constant pressure mode, where the head pressure is fixed. As the sample travels along the length of the column, it is subject to a decreasing amount of pressure which will cause peak broadening at longer retention times. This is especially significant with capillary columns that are very long. In constant flow mode, expressed in volume per second, the flow is maintained through the application of increased pressure as the sample progresses through the length of the column. The last option, constant linear velocity mode, expressed in distance per second, also maintains the sample flow through the

application of increased pressure, however a compensation or correction is performed to correct for the expansion of the column as the temperature increases. During a temperature gradient run this effect will be more important at the longer retention times and therefore the difference between the constant flow mode and the constant linear velocity mode will be greater; in this instance, the constant linear velocity mode would be a better choice. It is also a better option when trying to match retention times between instruments operating with detectors under vacuum versus atmospheric pressure.

Injectors

The injection port is a critical part of the gas chromatograph because it is where the sample is vaporized or converted from a liquid to a gas phase. In some cases, the injection port also serves to limit the quantity of sample that reaches the column in order to avoid overloading it. As discussed previously, an excess of sample in a chromatography column results in band broadening and poor resolution. The simplest form of injector is a micro syringe plunged through a rubber septum sitting on the top of a small chamber where vaporization occurs. To ensure complete volatilization of the sample, the temperature of the injection port typically needs to be at least 50 degrees Celsius higher than the boiling point of the compound of interest. As we deal with complicated samples in food safety which often contain multiple components of interest as well as matrix components, the injection port temperature needs to be set 50 degrees higher than the compound with the lowest boiling point. The chamber is typically made of glass called the glass liner and it is one of the components of a GC that require the most maintenance. Luckily, this maintenance is very easy and performed by the instrument operator.

Injectors for capillary column GC can be split or splitless. The word split is used to describe the division of a sample into two fractions; one fraction is injected in the column and the second fraction is discarded. This “split” of the sample is required to reduce the total volume injected in the collar. Packed columns used a sample volume ranging from tenths of a microliter up to approximately 20 microliters. Being much smaller, capillary columns use smaller volumes of samples, typically hundredths of a milliliter (10^{-3} ml). If the injector is used in splitless mode, the entire sample introduced into the chamber is passed onto the column.

Controlling the volume of injection is very important. The need for high throughput in the modern food safety laboratory has prompted the acquisition of automatic sample injectors integrated into the instrument. While it is obvious that the automatic injector serves the purpose of running multiple samples without operator intervention, the other important advantage is that the sample volume is controlled by computer and consequently very precise, even at very small volumes.

Columns

The food safety laboratory measuring contaminants typically uses a capillary column in the GC instruments. The internal diameter of these columns is very small, typically less than 500 micrometers (0.5 mm). The stationary phase can be coated directly on the inner wall of the column, and these columns are called wall coated open tabular (WCOT) columns. The stationary phase may alternatively be coated onto a support material adhered to the inner wall; this is known as support coated open tabular (SCOT) column. Most of the columns used in food safety are wall coated open tubular columns (WCOT) where the stationary phase is chemically bonded to the inside wall of the fused silica tube. A 30-meter-long column with an internal diameter of 0.25 mm is quite typical in the food safety laboratory.

The stationary phase chemically bonded to the inner wall of the capillary column can be nonpolar, log polar, medium polarity, and high polarity. Nonpolar columns typically contain dimethylpolysiloxane as the liquid phase. The polarity is increased by introducing a second material in the coating, such as phenyl, diphenyl or cyanopropylphenyl. Polyethyleneglycol or trifuoropropylmethylpolysiloxane is also used for high polarity stationary phases.

In gas chromatography, the temperature of the column is critical for the elution of the sample and the production of narrow bands. As mentioned earlier, the temperature of the column depends on the boiling point of the sample. We try to use the lowest temperatures that provide good elution in our applications because the resolution is better at lower temperatures. However, the use of a lower temperature increases the runtime. In the case of samples containing a larger number of components of interest, and consequently a broader range of boiling points, it is often a good solution to use a temperature gradient during the run. The temperature gradient acts in a manner similar to the mobile phase gradient in liquid chromatography, where the change of solvents creates an increasing pull on

the molecules that were more attracted to the stationary phase with the initial mobile face conditions. In GC, increasing the temperature progressively promotes the elution of compounds with increasing boiling points, while also weakening affinity bonds.

Lesson 2: Detectors

Finally, the compounds contained in the eluant go to a detector for detection and quantitation. The technical description of each detector is covered in the online presentation for this lesson. Many detectors are available and they are best suited for different kinds of compounds. The flame ionization detector, FID, is applicable for most organic compounds. It is thereby one of the most common detectors in the food safety laboratory.

The electron capture detector, ECD, is very useful with halides, nitrates, nitriles, peroxides, anhydrides and organometallics. It is used for pesticide residues and environmental contaminants in food safety as many of these compounds are electrophilic. Briefly, the nitrogen carrier gas is ionized by a beta ray and the electrons released are captured by the electrophilic compounds that results in a negative ion. These ions are conductive and the change in the current produces a peak.

Flame photometric detectors, FPD, are good for sulphur, phosphorus, tin and a few other elements that burn to specific colors (wavelengths) in a hydrogen flame. FPD is highly sensitive and selective which makes it well suited for food contaminants and their usually low concentrations. In addition, it is a very stable detector. It is especially useful in the food safety laboratory to measure phosphorus pesticides.

The flame thermionic detector (FTD), also known as NPD –Nitrogen, Phosphorus Detector, operate using the same principle as FPD. It detects organic nitrogen, organic and inorganic phosphorus. It is however less selective to phosphorus compounds than FPD. It can be applied for the analysis of drugs nitrogen pesticides and phosphorus pesticides.

The mass spectrometer, MS, is an increasingly popular detector for gas chromatography. It works using the exact same principle as described in Module 4 on HPLC detector. Briefly compounds exiting the GC column enter the source of the mass spectrometer in gas phase and are converted to ions that are

passed on to a single or tandem quadrupole system. The advantages of the mass spectrometer are high selectivity and high sensitivity. The main disadvantages are the cost, maintenance and the level of expertise associated with MS. Despite these disadvantages mass spectrometers are increasingly found in the food safety laboratory using gas chromatography because they offer the level of selectivity and very low limits of detection required for regulatory analysis.

The source in the mass spectrometer coupled to a GC uses one of three ionization modes: electron ionization (formerly electron impact ionization), positive chemical ionization and negative chemical ionization. Electron ionization, EI, is the most widely used mode of ionization in GC-MS because it works with most compounds that are amenable to GC. It causes a high level of fragmentation of a molecule and these fragments are used for the identification and quantification of the compounds. The chemical bonds in the molecule are broken at the weaker bonds and the analysis of the fragments through the spectrum provides information about the chemical structure of the compound. This technique is highly reproducible because the fragmentation is consistent when performed under the same analytical conditions. Electron ionization is known as a hard ionization as opposed to the electrospray organization, which is known as a soft ionization.

Chemical ionization starts with the ionization of a gas molecule, usually methane, to protonate the analyte. An excess amount of gas, at the level of 1000 time the concentration of the analyte molecules, is ionized by an electron beam. This technique produces quasimolecular ions $[M+H]^+$ in positive mode, and other ions in smaller quantities such as $[M+CH_5]^+$ $[M+C_2H_5]^+$. The main advantage of chemical ionization is that a quasimolecular ion is obtained, which provides a more reliable identification of a particular compound.

In negative chemical ionization, a heated metal filament is used to produce free electrons in a chamber filled with a gas such as methane, isobutane or carbon dioxide. The purpose of the gas is to slow down the electrons emitted by the metal filament. While almost all neutral analytes can form positive ions through chemical ionization, much fewer analytes can produce stable negative ions, which makes this ionization source more selective than electron impact or positive chemical ionization. It is particularly useful in food safety for environmental contaminants such as PCBs, fire retardants, and pesticides. If the concentrations are very low, an electron capture detector may be needed.

Conclusion

The gas chromatograph is an important instrument in the food safety laboratory because, equipped with appropriate detectors, it enables the analysis of a large number of semi volatile compounds for which regulations exist. Liquid chromatography and gas chromatography are complementary techniques in the sense that they target molecules that are nonvolatile for LC, and semi volatile or volatile for GC. The selection of the detector is critical and as described in this module, many detectors exist that are suitable for specific chemical contaminants of food. However, mass spectrometry in the form of a single or tandem quadrupole is playing an increasingly important role for the identification and quantification of a wide variety of chemical contaminants. However, the high cost this type of detector, the higher cost of maintenance, and most importantly the need for a specialized workforce mean that there will be a need for the other detectors for many years to come.