Welcome to module 5 of this training on confirmation methods for organic chemical contaminants. This module discusses the principles of gas chromatography.

Activities of Module 5

Work Plan

Learning Objectives	Content online	Content in-person	Lab
Understand the difference between LC and GC, describe the components of the GC system and principle of separation	Basic principles of gas chromatography	Examples of common GC applications in food safety	Compare modern LC and GC columns, learn to describe the column you need
Introduce GC detectors commonly used for food contaminants	Detectors for pesticides separated by GC	Discussion about pesticides amenable to both GC and LC	Understanding the selection of GC and LC

Welcome to the online portion of Module 5. The learning objectives of Module 5 are:

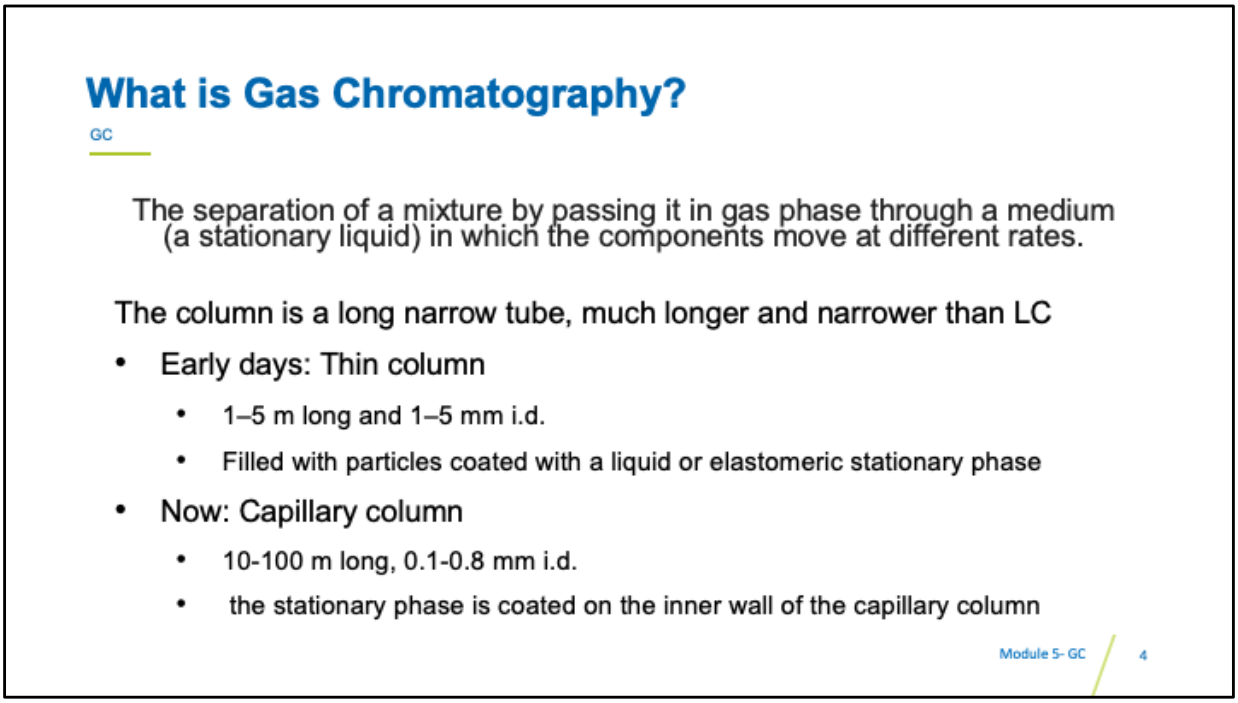
Section 1:

- To understand the difference between LC and GC
- To gain knowledge of the main components of the GC system
- to understand the principle of separation exploited in gas chromatography

In section 2, we introduce the n GC detectors commonly used for the determination of food contaminants

The online section of this class module focuses on the basic principles of the technique and the detectors typically used for the determination of pesticide residues in food. Pesticides residue determination is currently the most widespread application of GC in the food contaminants laboratory.

SECTION 1
Gas Chromatography



First and foremost, it is important to understand what Gas chromatography is and does. It is a separation techniques for mixtures of compatible chemicals, where the separation is achieved by passing this mixture in gas phase through a medium contained in a narrow column in which the components of the mixture move at different rates.

The medium for the separation is called the stationary phase and it is coated in a very long and narrow tube. The column is much longer and narrower than in liquid chromatography. In the earlier days of GC, the column was 1 to 5 meters long, with and inside diameter of 1 to 5 mm. It was filled with particles coated with a liquid or a polymer. As the technology for the fabrication of capillary columns developed, the GC column became longer and narrower, now standing at 10 to 100 m in length with an inside diameter of 0.1 to 0.8 mm.

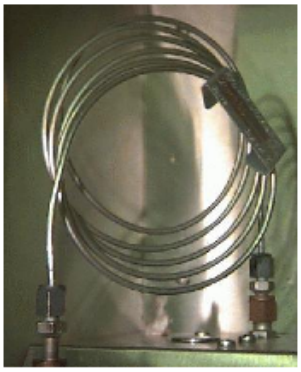
Besides the dimensions of the column, another important improvement brought about by technical development was the move from using particles packed in the column to coating the inner wall of the column itself with the stationary phase.

These and other changes contributed to the improvement of the separation

capabilities of GC.

Packed and Capillary Columns

Basic Analytical Techniques



Picture from chemistry.adelaide.edu.au

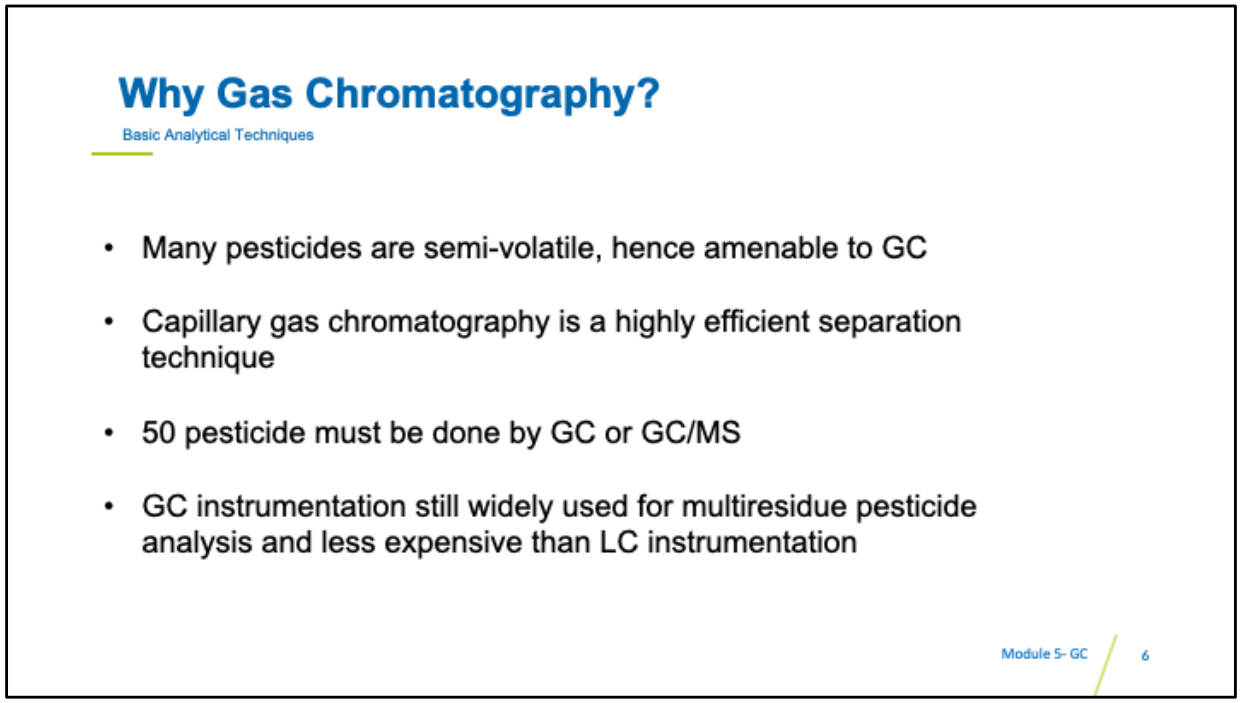
Packed column

Picture from huygensgcms.gsfc.nasa.gov

Capillary column

Another modification in the system that followed the development of materials science in the move from metal columns to fused silica. The metal columns were initially a great option because the metal was tolerant to the high temperatures used in GC and the pressure that could develop as the gas was trying to move through the packed particles.

Advances in the manufacturing of capillary columns initially using different metals and eventually adopting fused silica led to the biggest step in the improvement of separation capabilities. Indeed, it is the combination of the move from particles packed into the column and a small internal diameter, to a sub-millimeter inner diameter with the stationary phase coated onto the wall gave GC the separation capabilities required for mixed samples containing tens of contaminants such as extracts from food matrices.



So why did gas chromatography find its place in the food safety laboratory? After all, since food is consumed in liquid and solid forms, one may not expect a gas analysis to be relevant…

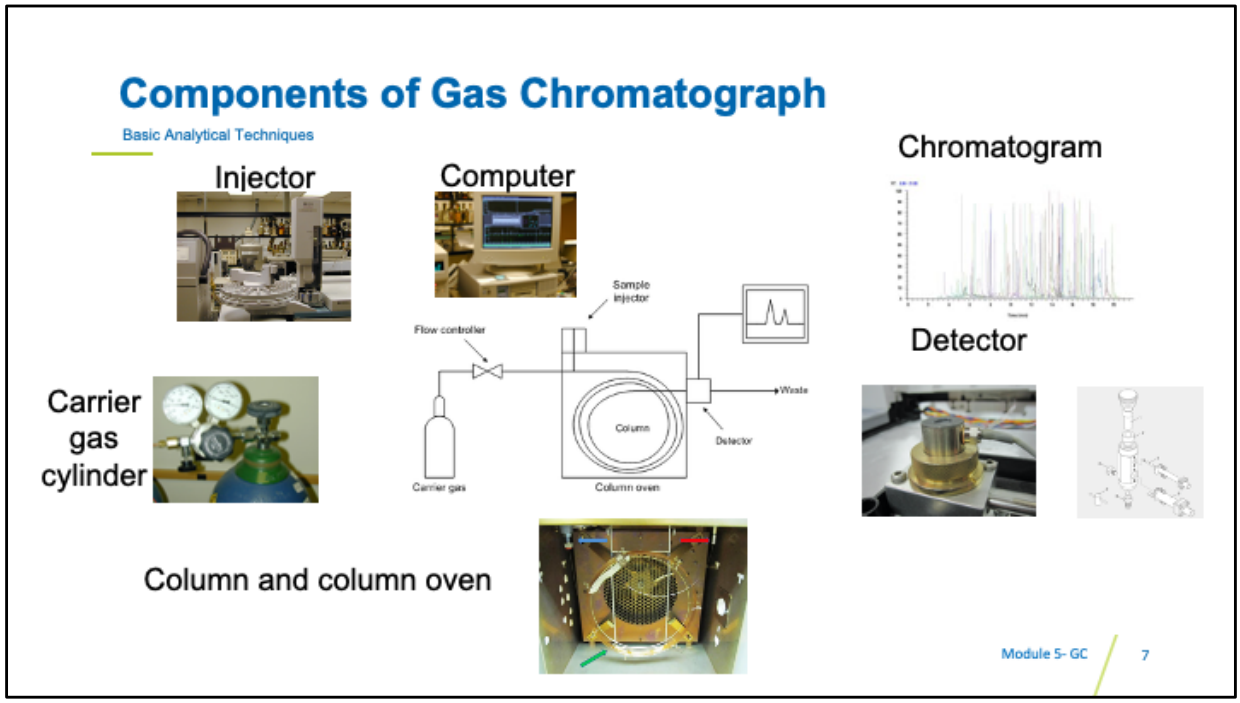
First and foremost, we must remember that gas chromatography is based on the separation of gases, which only means that the components of interest must be volatilized for analysis. They do not need to be in gas phase in their natural state, or specifically in this scenario, in the food. The chemicals are brought to gas phase through the use of heat and pressure changes. So, the only relevant criteria are whether a chemical can be volatilized to move through the capillary column without decomposing, whether is can be extracted (or separated) from other matrix components that would prevent it from vaporizing, and whether a suitable detection mechanism exists to measure its presence and/or quantity in gas.

Just a couple of decades ago, we also had to worry about whether a chemical of interest was different enough to be separated from other chemicals in a sample. At the time, the detectors did not offer much specificity themselves so the measurement of a chemical was possible or impossible solely based on

the separation achieved in the column. The advent of highly selective detectors, such as mass spectrometers, vastly increased the applications of chromatography.

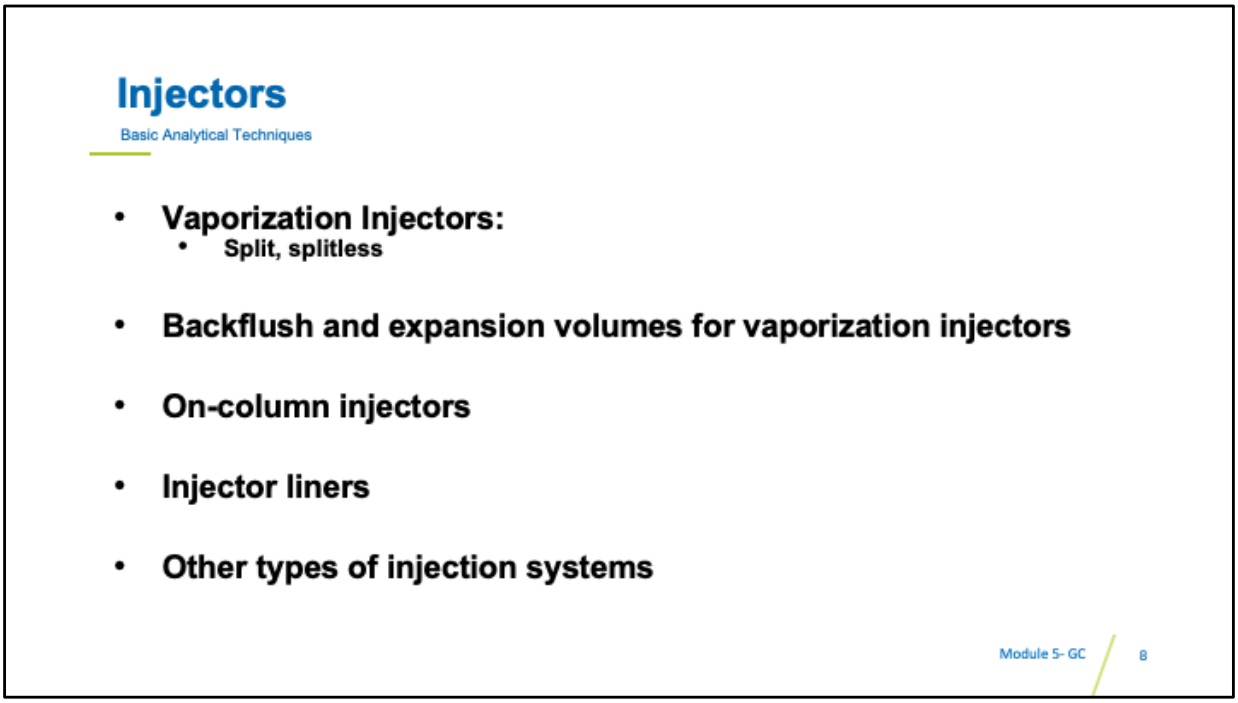
Pesticides were among the first chemical residues of interest in food safety. Pesticides, or at least older pesticides, are semi-volatile, meaning that they can be volatilized at high temperature even though they are liquid at room temperature. They are also stable at high temperature. This makes them well-suited for GC. With such chemicals, capillary columns deliver very effective separation. As a result, it was possible to separate, detect and quantify, at least at the concentration levels of interest, relatively large numbers of pesticides.

While there was a trend to create very large methods, or methods that targeted the separation of a large number of pesticides (as in upwards of 100), this trend was reversed by the advent of newer pesticides that are less or not volatile, also in combination with modern liquid chromatography equipped with mass spectrometry. LC is generally thought to be easier to use and more reproducible in the hands of multiple analysts, which resulted in many pesticides suitable for both LC and GC being moved over to LC methods. However, there are still around 50 pesticides that are not amenable to LC and measured by GC even in the most modern food safety laboratory. GC can be less expensive to use, which is a reason some laboratories are keeping more pesticides in their GC methods.



Let's have a look at the components of a typical GC. The gas chromatograph is composed of an injector, where the liquid sample is converted to a gas phase, followed by a column, where the separation actually happens, and a detector that measures the presence of a signal in general or a signal arising from a particular compound depending on its specificity.

The main differences from the HPLC reviewed in the previous module are the carrier gas, and the size of the oven for the column. While HPLCs are typically equipped with a small heated chamber or housing for the column, the GC requires a large oven to accommodate the coiled column.



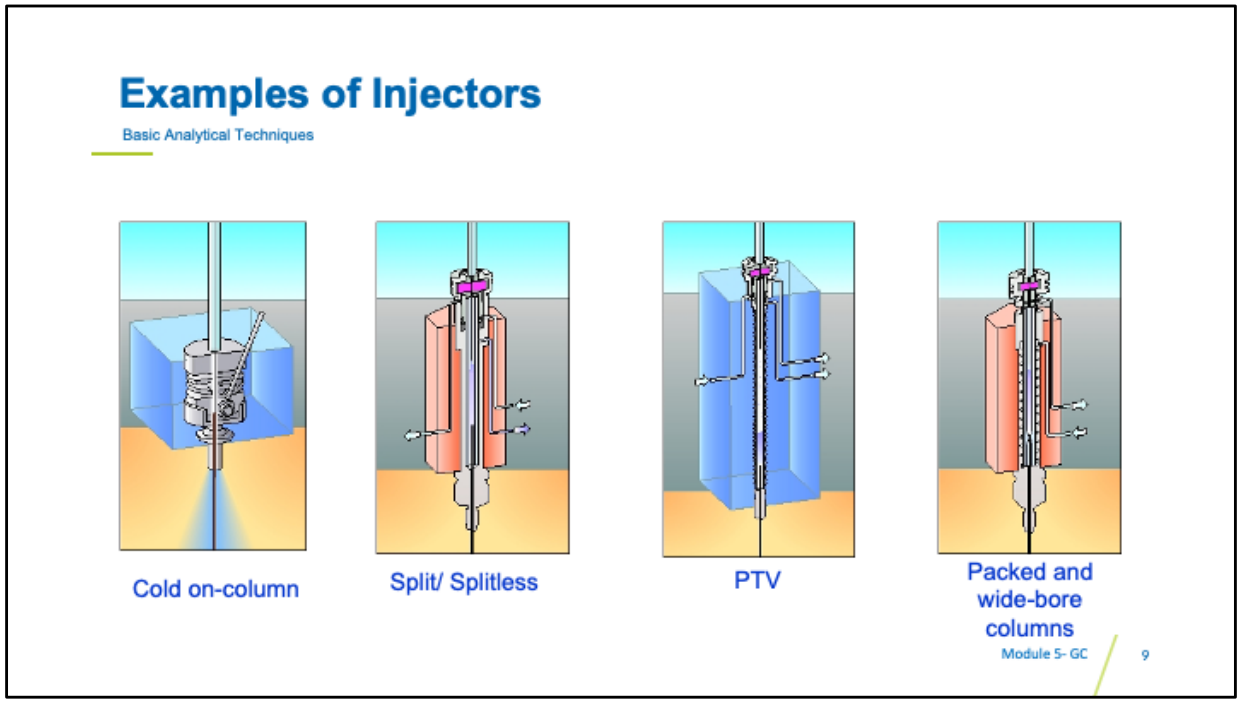
The injector plays a critical role in gas chromatography. Obviously, its function is to introduce the sample into the column. But there is more than one way to achieve this and the manner selected impacts the separation efficiency of the system.

Vaporization injectors can simply be described as those that have a chamber for the vaporization of the sample characterized by an expansion volume. They can force the entire sample into the column, those are called splitless, or be equipped with a mechanism to inject only a portion of it (split). “Splitting” the sample benefits from on-board control of the amount of sample reaching the column, something that otherwise needs to be entirely achieved through sample preparation and the selection of the injection volume. These injectors often benefit from backflush capabilities as well that can reduce carry-over between samples, a common source of contamination in sequential analyses.

On-column injectors can be described simply as a port and an injection gap where a needle can be inserted to push the liquid sample. This can be done "cold" or at a temperature below the lowest boiling point. They are the simplest form of injectors and there is usually one of these on each GCs, regardless of assurance activities where it is important to isolate the contributions of

various parts of a system to verify their performance.

Injector liners are specific to GC and no equivalent exists in LC. The liner is quite simply a glass tube forming a chamber for the vaporization. The liner is probably the part of the GC that requires the most maintenance as it gets dirty over time. Using a liner, which is removable and an inexpensive part, prevent the accumulation of residues in other parts of the instrument that would be harder and more expensive to clean. It is not a disposable item *per se* because it can be cleaned, but it also needs replacing when it is either damaged by the cleaning or no longer possible to clean.



Other types of injectors include PTV and packed wide bore columns. The PTV, or programmed temperature vaporization, is simply an on-column injector where the temperature can be raised in a controlled manner to vaporize the desired fraction of the sample. These also find applications in large-volume injections where a larger sample can be accommodated in the gap and released through a program of temperature rise.

The packed and wide-bore columns are best suited for larger columns as they don't provide the pressure normally used with capillary columns. We do not see these last injectors much in the food safety laboratory where we work almost exclusively with capillary columns.

Expansion Volume for Liquid

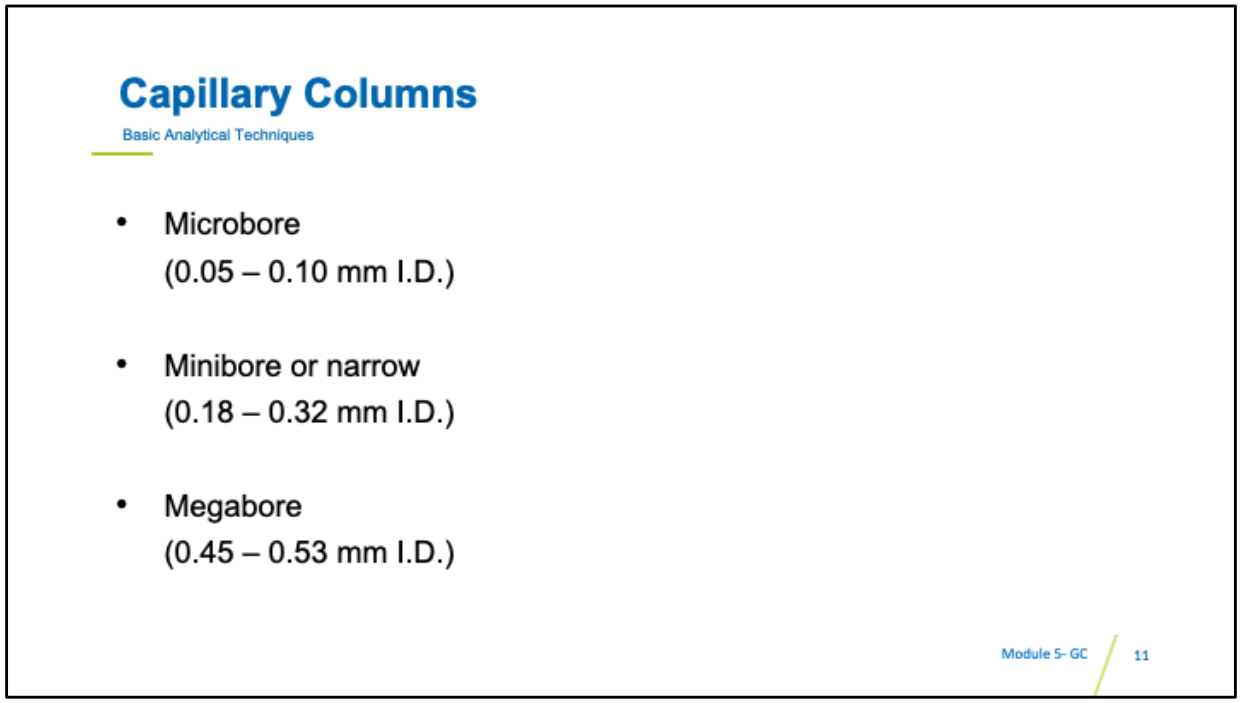
Basic Analytical Techniques

Injection volume = 1 µL

Solvent	Boiling Point (°C)	Expansion Volume (µL)
Acetone	56	290
Acetonitrile	85	405
Carbon disulfide	46	355
Ethyl acetate	77	215
Isooctane	99	130
Methanol	65	525
Methylene chloride	40	330
n-Hexane	69	165
Toluene	111	200
Water	100	1180

At 250° C at 15 psig, Source: J & W Scientific Catalog, 1998 edition

The injector is the component of the instrument where the sample goes from a liquid to a gas phase. Consequently, it expands. The expansion factor and the boiling point, or the vaporization point, of the sample solvent plays a critical role in the efficiency of injection, and overall, in the separation. However, the boiling point is not the only factor. Compatibility with the chemicals of interest, non-reactivity and expansion volume must also be considered. Indeed, the solvent must not cause a chemical reaction with the sample or any of the chemicals of interest when the desired outcome is to measure those chemicals. Measuring degradation products or by-products of a reaction is typically not favored as many reactions could produce the same products and therefore either complicate or completely prevent the quantification of the contaminant of interest. As a general rule, it is best to measure an unreacted chemical compound.



The column, much like discussed in the module on HPLC, is the heart of the separation. Diameter, or more precisely the inside diameter, is a critical factor in GC, even more so than in LC since there are no longer particles in the GC columns and we rely on a coating of the column wall. Consequently, narrower columns mean that the gas carried in the column more intimately interacts more with the wall coating and there is very little room for a stream of gas in the center of the column that just flows without interaction.

We recall that older GC instruments had columns with internal diameters of 1 to 5 mm, but we also recall that they were packed with beads. The newer columns, the capillary columns, fall into three categories.

The megabore columns have an internal diameter around half of one millimeter. Minibore columns are one fifth to one third of a millimeter in diameter, while microbore columns fall between one tenth and one twentieth of a millimeter.

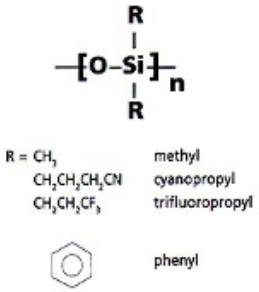
While narrower columns generally yield better separation, they are not always the best choice for reasons such as the availability of desired coating, the speed of analysis and the backpressure that they generate causing a need for more advanced pumping systems which also mean and added costs.

Column Stationary Phase

GC

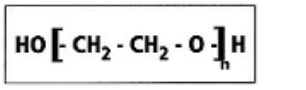
Non-polar stationary phases

- Dimethylpolysiloxane
- (5%-Phenyl)-methylpolysiloxane
- (50%-Phenyl)-methylpolysiloxane



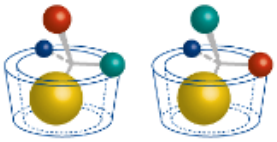
Polar stationary phases

- Polyethylene Glycol



Specific stationary phases

- Chiral columns



Module 5- GC 12

The stationary phase coated on the inside wall of the capillary column plays the same role as the packed beads in the HPLC. It interacts with the chemicals in the sample and causes them to move more of less rapidly through the column, or in other words, to separate.

There are many different types of column chemistry available on the market and the selection is directly linked wth the types of chemicals one is separating and the goals. For example, a GC instrument could be used for the purification of a compound in synthetic chemistry, in which case isolation of this one compound is the only criteria in the selection of the stationary phase. It is also easy to understand that the petrochemical industry doesn't need to separate the same compounds as the pharmaceutical or as the food industries.

So focusing on food safety needs greatly narrows down the types of stationary phases that can achieve our objectives. As a reminder, our objectives are to separate a relatively large number of chemicals from a single sample and to obtain a separation that is clear enough to quantify these chemicals. Let's not forget that this must be achieved in a manner that produces a stream at the end of the column that is compatible with a detector providing a desired level

of selectivity.

We will see in thein-person and practical sessions of this course that we have options for column chemistries that have been developed to satisfy the needs of the food safety laboratory.

You have reached the end of the online portion of Module 5, section 1. In section 2, we discuss detectors for GC.