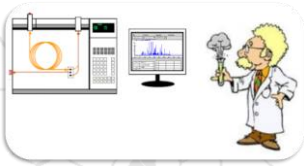
FE 315-INSTRUMENTAL ANALYSIS

Gas Chromatography-Flame Ionization Detector (GC-FID)



Dr. Derya KOÇAK YANIK

OUTLINE

- Gas Chromatography
- Components of a GC-FID system
- Operating principle of GC
- Basic Steps of a GC Analysis
- GC columns
- FID detector
- Aplication: Determination of fatty acid composition of olive oil

Gas chromatography is a chromatographic technique that can be used to separate and analyse the volatile organic compounds.

The flame ionization detector (FID) is the most sensitive, universal gas chromatographic detector for hydrocarbons.

How Does Chromatography Work?

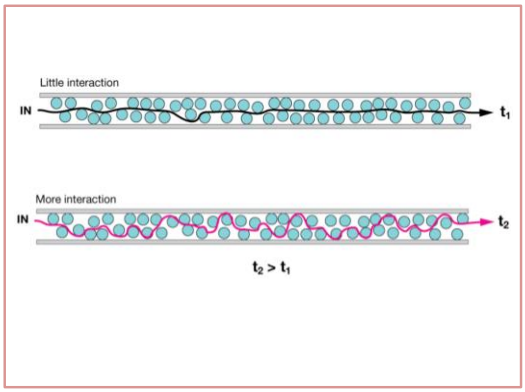
In all chromatographic separations, the sample is transported in a **mobile phase**. The mobile phase can be a gas, a liquid, or a supercritical fluid.

⇩

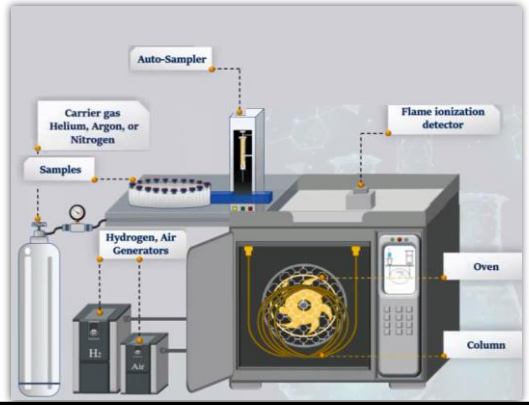
The mobile phase is then forced through a **stationary phase** held in a column or on a solid surface. The stationary phase needs to be something that **does not react** with the mobile phase or the sample.

⇩

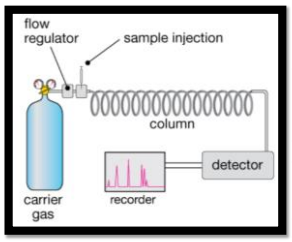
The **sample** then has the **opportunity to interact** with the stationary phase as it moves past it. Samples that interact greatly, then appear to move more slowly. Samples that interact weakly, then appear to move more quickly. Because of this difference in rates, the samples can then be **separated into their components**.



1. Components of a GC-FID system



Schematic of a typical gas chromatography setup



It consists of:

*a flowing mobile phase (**carrier gas** such as He, Argon and Nirogen) in a pressurized cylinder.

*a **flow regulator** to ensure a constant flow rate across the column,

*an **injection port** that allows the sample to be mixed into the flowing carrier gas in a very small volume

* **a stationary phase** that is called as **column** located in an oven with temperature to be controlled

* **a detector** that measure and analyse the gas stream eluted from the column

* **a recorder (PC)** to record the result.

Basic Steps of a GC Analysis

1. Sample Introduction

There are a number of options available for GC inlet systems; the most common being split-splitless injection.

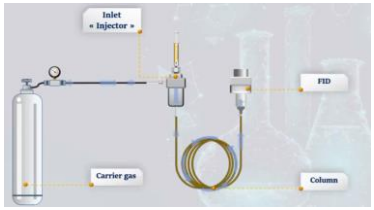
Liquid

Gas

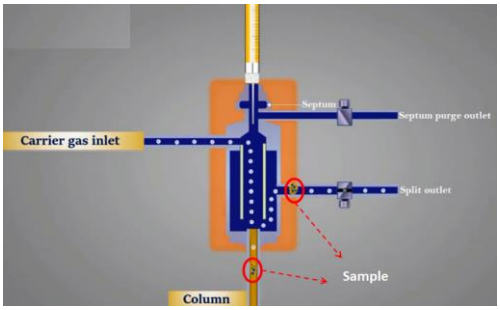
Solid

The most common injection method is where **a micro- syringe** is used to inject sample through a rubber septum into a flash vaporazer port at the head of the column. The sample to be separated is converted into the vapor, and mixed with carrier gas

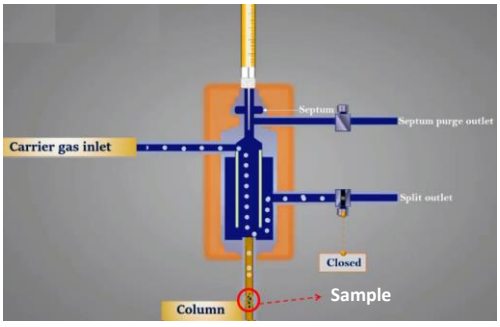
!! The temperature of the sample port usually about 50 °C higher than the boiling point of the least volatile component of the sample.



Split Mode



Splitless Mode



Manual Injection & Automatic Injection

- Automation
- Up to 150 samples
- Instantaneous injection
- Same amount of sample injected every time

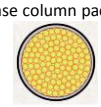
2. Separation of Components

Column is the heart of the GC. Separation of sample take place in it.

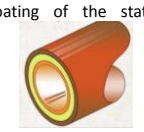
According to column types there are two main types of GC!!!!!!

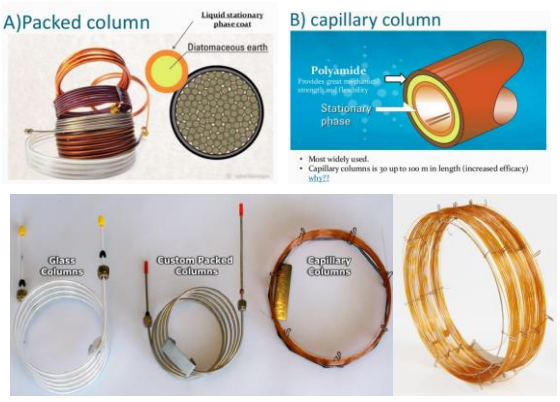
Gas-Solid Chromatography (GSC)	Gas-Liquid Chromatography (GLC)
– Has a solid stationary phase that physically adsorbs the compounds leading to their retention on the column – Has a limited use due to long retention of active or polar molecules and severe tailing of elution peaks – Real application is for specific low MW compounds	– Has a thin layer of a liquid stationary phase immobilised inside the column and the compounds partition between the gaseous mobile phase and the liquid phase. – Widely adopted method and is now known as ***Gas Chromatography (GC)***

The various components of the gaseous mobile phase are separated by differential **adsorption** on that stationary phase column packing.



In this case, the separation is performed by **partitioning** of gases between the mobile phase and the liquid coating of the stationary phase.



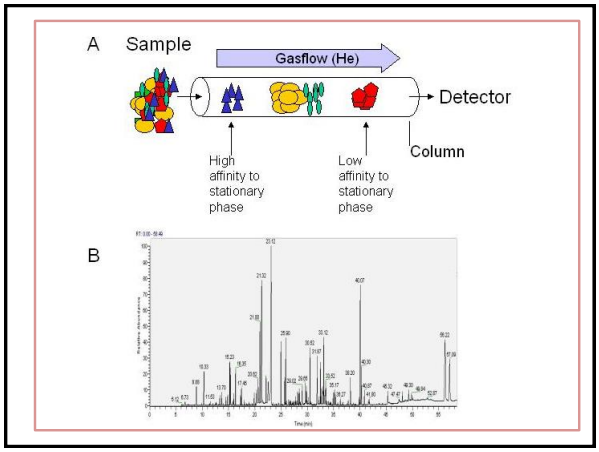


Important Attributes of Stationary Phase

1. Low volatility (boiling point at least 100 °C higher than max. column operating temperature)
2. Thermo stability (wide temperature operating range)
3. Chemical inertness (non-reactive to both solutes and carrier gas)
4. Solvent characteristics (differential solvent for different components

Commonly recommended bonded-phases:

- Dimethylpolysiloxane
- Methyl(phenyl)polysiloxane
- Polyethlene glycol (Carbıwax 20 M)
- Trifluoropropylpolysiloxane
- Cyanopropylpolysiloxane

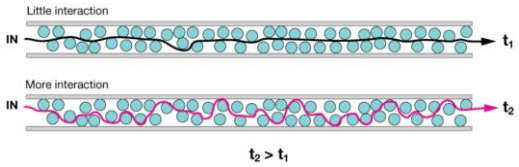


Retention time:

The retention time, RT, is the time it takes for a compound to travel from the injection port to the detector.

The retention time is measured by the recorder as the time between the moment you press start and the time the detector sees a peak.

The components more interect with stationary phase travels slower, while less interacting ones travels faster.



Efficient separation of compounds in GC is dependent on the compounds traveling through the column at different rates. The rate at which a compound travels through a particular GC system depends on the factors listed below:

Volatility of compound: Low boiling (volatile) components will travel faster through the column than will high boiling components
Polarity of compounds: Polar compounds will move more slowly, especially if the column is polar.
Column temperature: Raising the column temperature speeds up all the compounds in a mixture.
Column packing polarity: Usually, all compounds will move slower on polar columns, but polar compounds will show a larger effect.
Flow rate of the gas through the column: Speeding up the carrier gas flow increases the speed with which all compounds move through the column.
Length of the column: The longer the column, the longer it will take all compounds to elute. Longer columns are employed to obtain better separation.

3. Detecting and Recording Results

After the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column.

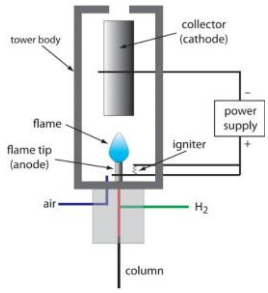
Typical GC Detectors

Table 15.5 Properties of Selected Gas Chromatography Detectors

Type	Approximate limit of detection (g s^{-1})	Approximate linear range	Comments
Thermal conductivity (TCD)	10^{-5}–10^{-8}	10^{5}–10^{7}	Universal detector Measures changes in heat conduction
Flame ionization (FID)	10^{-12}	10^{6}–10^{7}	Universal detector Measures ion currents from pyrolysis
Electron capture (EC or ECD)	10^{-14}	10^{2}–10^{5}	Selective detector for compounds containing atoms with high electron affinities
Flame photometric (FPD)	10^{-13}	10^{3}	Selective detector for compounds containing S, P
Nitrogen-phosphorus	10^{-9}–10^{-14}	10^{5}–10^{7}	Selective for N, P containing compounds
Photoionization (PID)	10^{-9}–10^{-12}	10^{5}	Universal (some selectivity due to to identity of gas in lamp)
Hall Detector	10^{-13}	10^{5}	Specific detector for compounds which contain halogen, S, or N
Mass spectrometer (MS)	10^{-12}	*a*	Universal detector
Fourier-transform infrared (FTIR)	10^{-10}	10^{3}	Polar molecules

a. Varies, depending on the type of mass spectrometer as well as the kinds of compounds being analyzed.

Flame Ionization Detector (FID)

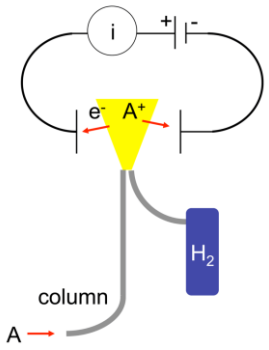


In a FID sample and carrier gas from the column pass through a hydrogen-air flame.

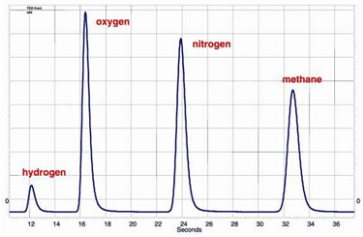
The hydrogen-air flame alone creates few ion, but when an organic compound is burned there is an increase in ions produced.

A polarizing voltage attracts these ions to a collector located near the flame.

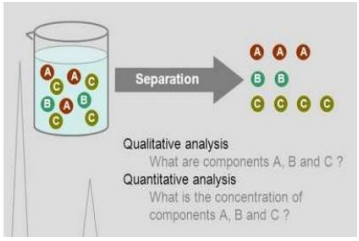
The current produced is proportional to the amount of sample being burned. This current is sensed by an electrometer, converted to digital form, and sent to an output device.



Example: GC-FID analysis gas mixture of hydrogen, oxygen, nitrogen and methane



4. Data analysis



Information Obtained from the Analysis Results ?????

- Retention time → What is it → Qualitative analysis
- Area or height of the peak → How much is there → Quantitative anaysis

Qualitative analysis

1. If the sample components are known and peaks need to be assigned!

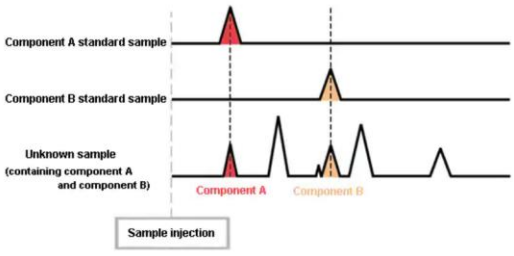
By injecting standards of pure compound assign the peaks in the chromatogram based on the retention time of the standard.

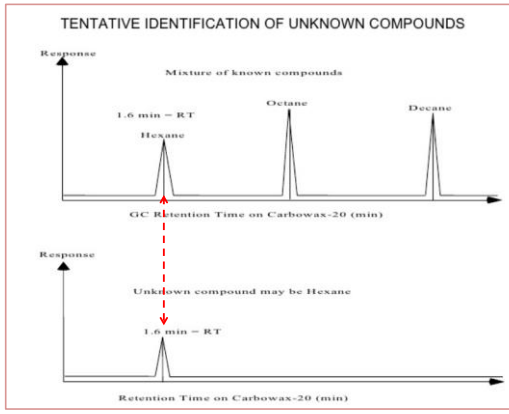
Inject standard solutions under identical conditions and then identify the components by comparing the retention time of the peak in the chromatogram of the standard solution with the sample.

In other words, when the same component is analyzed under the same conditions, a peak is confirmed at the same time.

For example, imagine an unknown sample known to contain **component A** and **component B**. The chromatogram obtained from the unknown sample looks as follows. It is not possible to know which peak is component A, and which peak is component B.

However, if **standard samples of A** and **B** are prepared, and are analyzed under the same conditions, the retention times for **A** and **B** become evident. By comparing these chromatograms, the peaks for **A** and **B** in the chromatogram of the unknown sample can be determined.





2. If the sample is a complete unknown!

Need to use detectors that can be used to aid in identification, such as mass spectrometer.

It may also be necessary to collect the elluent for characterization using infra-red or Nuclear Magnetic Resonance Spectroscopy (NMR)

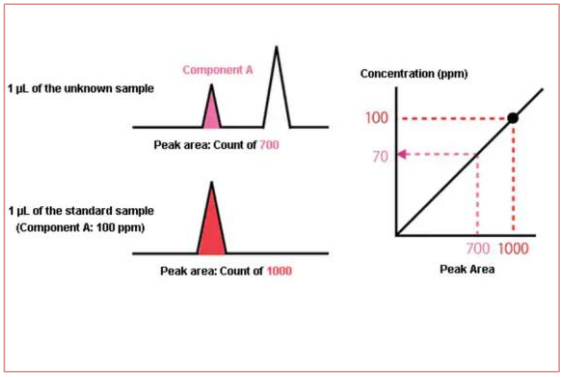
Quantitative Analysis

In a GC chromatogram, the size and area of the peaks are proportional to the amount of the component reaching the detector.

Here, we describe a quantitative analysis investigating the concentration of component A in an unknown sample.
First, 1 µL of the unknown sample is analyzed, and the area of the peak for component A in the chromatogram obtained has a count of 700.

Next, a standard sample is prepared with a concentration of component A of 100 ppm. 1 µL of this is analyzed under the same conditions, and a count of 1000 is obtained as the peak area.

The peak area is proportional to the amount of the component, so if a 100 ppm concentration has a count of 1000, a 700 count means a 70 ppm concentration.
As with qualitative analysis, one could say that a standard sample is also required for quantitative analysis.



Applications:

Criteria of compounds to be analysed by GC

1. Volatility
2. Thermostability

Examples:

- Aromatics (benzene, toluene, …)
- Hydrocarbons
- Flavors and Fragrances
- Permanent gases (H_2, N_2, O_2, Ar, CO_2, CH_4)
- Pesticides, Herbicides

Disadvantages:

Material has to be volatilized at 250 °C without decomposition. If not the analyte has to be derivatized.

Example:

Fatty acids → methyl esters

Phenols → Silanized

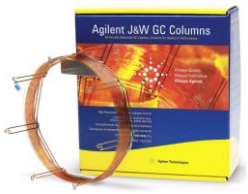
Sugars → Silanized

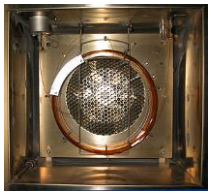
Determination of fatty acid composition of olive oil by using GC-FID

The GC-FID at Food Engineering Department

Agilent 7890 A Gas Chromatograph with Flame Ionization Detector

Column for FAMEs Analysis in our GC-FID system





HP-88 is a high-polarity **column** designed for the separation of fatty acid methyl esters (FAMEs).

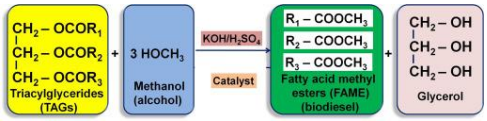
(88% Cyanopropy)aryl-polysiloxane

250/260 °C upper temperature limits

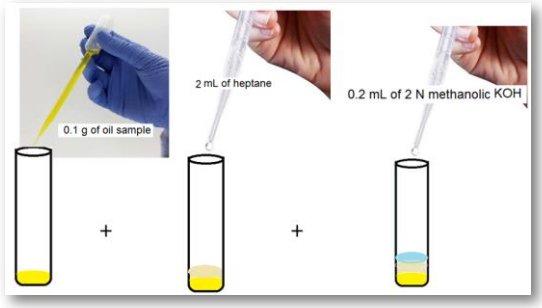
1. Sample Preparation

Short chain fatty acids, volatile fatty acids, are analyzed in their free acid form using GC. However, larger (C8-C24+) fatty acids typically converted to fatty acid methyl esters (FAMEs). These volatile derivatives are then introduced into a GC.

Trans- esterification with cold methanolic solution of potassium hydroxide:

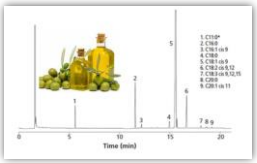


Trans- esterification Procedure:



2. Injection to GC-FID system

3. Data acquisition



Watch Video

Watch Video

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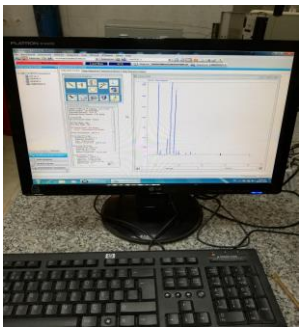
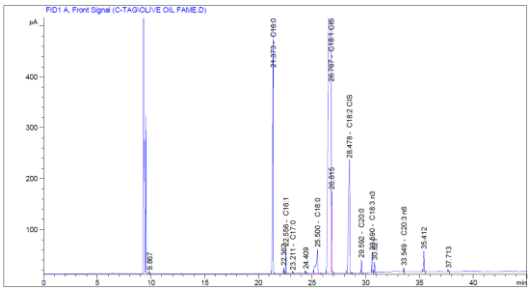


RESULT:

CHROMATOGRAM

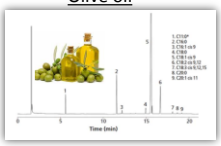


Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Area %	Name
10	19.081		0.0000	0.00000	0.00000	C14:1
11	19.503		0.0000	0.00000	0.00000	C15:0
12	21.000		0.0000	0.00000	0.00000	C15:1
13	21.373	BB	0.0708	2475.06860	11.86205	C16:0
14	22.362	BB	0.0468	34.73328	0.16646	?
15	22.556	BB	0.0444	141.67294	0.67898	C16:1
16	23.211	BB	0.0554	8.46999	0.04059	C17:0
17	24.409	BB	0.0501	13.15028	0.06302	?
18	24.672		0.0000	0.00000	0.00000	C17:1
19	25.500	BB	0.1384	496.08170	2.37753	C18:0
20	26.167		0.0000	0.00000	0.00000	C18:1 TRANS
21	26.767	BV	0.1695	1.49849e4	71.81671	C18:1 CIS
22	26.815	VB	0.0321	330.26093	1.58281	?
23	27.498		0.0000	0.00000	0.00000	C18:2 TRANS
24	28.478	BB	0.1117	1841.87781	8.82741	C18:2 CIS
25	29.592	BB	0.0520	85.20665	0.40836	C20:0
26	29.865		0.0000	0.00000	0.00000	C18:3 n6
27	30.590	BB	0.0589	148.39659	0.71121	C18:3 n3
28	30.821	BB	0.0489	64.19354	0.30765	?

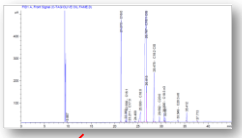
Fatty acids	**%**
Palmitic acid	11.8
Stearic acid	2.3
Oleic acid	71.8
Linoleic acid	8.8

DATA FOR STUDENTS !!!!

Olive oil



Unknown oil



Compare the results and decide whether the unknown oil is olive oil?

For GC-FID Lab Report

- Title page (2.5 points)
- Purpose (10 points)
- Theory (20 points)
- ~~Material & Method~~
- Results (20 points)
- Discussion (45 points)
- References (2.5 points)


Your questions.