**FE 315 INSTRUMENTAL ANALYSIS**

**Gas Chromatography (GC)**

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Chromatography is a series of seperation techniques which provide the seperation and determination of different components in complex matrices. In all of the chromatographic applications there is a stationary phase and mobile phase. The components of a mixture is carried with mobile phase throughout the stationary phase. Meanwhile the diffetent components of mixture separates from each other due to the different migration speed on mobile phase. So the components of mixture is seperated from each other.

In food analysis, gas chromatography (GC) represents one of the key separation techniques for many groups of (semi)volatile compounds. The high separation power of GC in a combination with a wide range of the detectors makes GC an important tool in the determination of various components that may occur in such complex matrices as food crops and products.

**Basic Components of a typical GC-FID**



The process of gas chromatography is carried out in a specially designed instrument, called a gas chromatograph. A very small amount of liquid mixture is injected through a rubber septum into the instrument and is volatilized in a hot injection chamber. Then, it is swept by a stream of carrier gas through a heated column which contains the stationary, high-boiling liquid. As the mixture travels through this column, its components go back and forth at different rates between the gas phase and dissolution in the high-boiling liquid, and thus separate into pure components. Just before each compound exits the instrument, it passes through a detector. When the detector sees a compound, it sends an electronic message to the recorder, which responds by plotting a peak. For similar compounds, the area under a GC peak is roughly proportional to the amount of compound injected. If a two-component mixture gives relative areas of 75:25, you may conclude that the mixture contains approximately 75% of one component and 25% of the other.

**Basic Steps of a GC Analysis**

**1. Sample Introduction**

There are a number of options available for GC inlet systems; the most common (characterizedbelow) being split=splitless, programmed temperature vaporizer, and cold on-column (COC)injector. The choice of an optimum sample introduction strategy depends mainly on the concentrationrange of target analytes, their physico-chemical properties, and the amount and nature of matrixco-extracts present in the sample.

In a split injection mode, typically small volume of sample extract (0.1–2 mL) is rapidlydelivered into a heated glass liner followed by its splitting into two streams: the larger part isvented, while the smaller part is transferred onto the column.



**2. Sample Separation**

To be amenable for the GC analysis, an analyte should possess not only appreciable volatility attemperatures below 3508C–4008C, but also must be able to withstand relative high temperatureswithout degradation and reaction with other compounds present in the GC system.With regard to a typically complex mixture of matrix components occurring in food extracts(often even after its purification), the optimization of GC separation requires careful attention to anumber of important variables and their interaction. Both physical (column length, internal diameter,and stationary phase including its film thickness), and parametric (temperature and flowvelocity) column variables affect the separation process.



### *Retention Time (RT)*

The retention time, RT, is the time it takes for a compound to travel from the injection port to the detector; it is reported in minutes on our GCs. 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. If you do not press start at the same time you inject your sample, the RT values will not be consistent from run to run.

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.

Generally the number one factor to consider in separation of compounds on the GCs in the teaching labs is the **boiling points of the different components**. A difference in polarity of the compounds is only important if you are separating a mixture of compounds which have widely different polarities. Column temperature, the polarity of the column, flow rate, and length of a column are constant in GC runs. For each planned GC experiment, these factors should be optimized to separate your compounds.

**3. Sample Detection**

Depending upon the type of food compounds being measured several different detectors areavailable for this purpose (Table), each with its own advantages and drawbacks. The followingsections briefly introduce various GC detectors most commonly in use today.



***Flame Ionization Detector***

Flame ionization detector (FID) represents one of the most widely used detectors. The effluent froman analytical column is mixed with hydrogen and air, and is directed into a flame, which breaksdown organic molecules and produces ions. A voltage potential is applied across the gap betweenthe burner tip and an electrode located just above the flame. The resulting current is then measuredand is proportional to the concentration of the components present.



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***Thermal Conductivity Detector***

Thermal conductivity detector (TCD) consists of an electrically heated wire or thermistor. Thetemperature of the sensing element depends on the thermal conductivity of the gas flowing around it.Changes in thermal conductivity cause a temperature rise in the element, which is sensed as achange in resistance.

***Electron Capture Detector***

In ECD, the sample is introduced into the detector through an analytical column and passes over a63Ni radioactive source emitting b particles, which causes ionization of the carrier gas and thesubsequent release of electrons. When organic molecules containing electronegative functionalatoms or groups pass by the detector, they capture some of the electrons and reduce the currentmeasured between the electrodes.

***Mass Spectrometric Detector***

The mass spectrometer (MS) is by far the most powerful and flexible of the detectors used in theanalysis of GC-amenable food components today. The advantage over all GC detectors describedabove is a possibility to obtain, in addition to selective detection of analyte eluted at certain retentiontime, also structural information, enabling either confirmation of target compound or identificationof unknown species. The character of data obtained largely depends on the type of mass analyzeremployed.

**Food Analysis Applications of GC**

Since a large range of food compounds are (semi)volatile compounds, the GC is widely used for theirdetermination. The choice of an optimal GC setup depends on the requirements for the performancecharacteristics of methods used, cost, speed, and several other factors. In Table 7.7, the current GCmethods for several groups of food constituents are summarized with special attention paid toapplicability of recent advances in the field of this technique for their analysis.







**PROCEDURE**

**Determination of fatty acid composition of olive oil**

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.

**1. Methylation of fatty acids:**

FAMES are typically produced by an alkali-catalyzed reaction between fats and methanol in the presence of base such as sodium hydroxide or potassium hydroxide

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**Trans- esterification with cold methanolic solution of potassium hydroxide:**

Approximately weight 0.1 g of oil sample in a 5 mL screw-top test tube. Add 2 mL of heptane and shake. Add 0.2 mL of 2 N methanolic potassium hydroxide solution. Tighten the cap and shake vigorously for 30 seconds. Leave to stratify until the upper solution becomes clear. Decant the upper layer containing the methyl esters. The heptane solution is suitable for injection into the GC. It is advisable to keep the solution in the refrigerator until GC analysis. Storage of the solution for more than 12 hours is not recommended.

**2. GC –FID analysis**

After methylation, the fatty acid composition was determined with an Agilent 7890A gas  chromatography (Agilent Technologies, USA) equipped with a flame ionization detector,  a split/splitless injector and a HP-88 capillary column (88 % Cianopropylaryl 100 m x  0.250 mm ID x 0.20 µm) following the method suggested by Çiftçi and others (2009). The  temperature of the injector was 250ºC and the split ratio was 50:1. The oven was kept  at 120ºC for 1 min, then the temperature was increased from 120ºC to 175ºC at a rate  of 10 ºC/min and then from 175 to 210ºC at a rate of 5ºC/min, followed by increase  5ºC/min to 230ºC and held at this temperature for 5 min.  The detector was set at 260ºC.  Helium was used as the carrier gas, flowing at a rate of 15 mL/min. FAMEs were identified by comparison with the relative retention times of standard mixtures.