HPTLC- High Performance Thin Layer Chromatography is a sophisticated and automated form of TLC.

Main Difference of HPTLC and TLC - Particle and Pore size of Sorbents.

The other differences are

	HPTLC	TLC
Layer of Sorbent	• *100µm*	• 250µm
Efficiency	• High due to smaller particle size generated	• Less
Separations	• 3 - 5 cm	• 10 - 15 cm
Analysis Time	• Shorter migration distance and the analysis time is greatly reduced	• Slower
Solid support	• Wide choice of stationary phases like silica gel for normal phase and C8 , C18 for reversed phase modes	• Silica gel , Alumina & Kiesulguhr
Development chamber	• New type that require less amount of mobile phase	• More amount
Sample spotting	• Auto sampler	• Manual spotting
Scanning	• Use of UV/ Visible/ Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer	• Not possible

Features of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard
2. Several analysts work simultaneously
3. Lower analysis time and less cost per analysis
4. Low maintenance cost
5. Simple sample preparation - handle samples of divergent nature
6. No prior treatment for solvents like filtration and degassing
7. Low mobile phase consumption per sample
8. No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination
9. Visual detection possible - open system
10. Non UV absorbing compounds detected by post-chromatographic derivatization

Steps involved in HPTLC

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning
9. Documentation of chromatic plate

Selection of chromatographic layer

· Precoated plates - different support materials - different Sorbents available

· 80% of analysis - silica gel GF · Basic substances, alkaloids and steroids - Aluminum oxide

· Amino acids, dipeptides, sugars and alkaloids - cellulose

· Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18

· Preservatives, barbiturates, analgesic and phenothiazines- Hybrid plates-RPWF254s

Sample and Standard Preparation

To avoid interference from impurities and water vapours

Low signal to noise ratio - Straight base line- Improvement of LOD

Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:!0:1), Methylene chloride : Methanol (1:1), 1%

Ammonia or 1% Acetic acid

Dry the plates and store in dust free atmosphere

Activation of pre-coated plates

Freshly open box of plates do not require activation

Plates exposed to high humidity or kept on hand for long time to be activated

By placing in an oven at 110-120ºc for 30′ prior to spotting

Aluminum sheets should be kept in between two glass plates and placing in oven at 110-120ºc for 15 minutes.

Application of sample and standard

· Usual concentration range is 0.1-1μg / μl

· Above this causes poor separation

· Linomat IV (automatic applicator) - nitrogen gas sprays sample and standard from syringe on TLC plates as bands

· Band wise application - better separation - high response to densitometer

Selection of mobile phase

- Trial and error
- one's own experience and Literature
- Normal phase
- Stationary phase is polar
- Mobile phase is non polar
- Non-polar compounds eluted first because of lower affinity with stationary phase
- Polar compounds retained because of higher affinity with the stationary phase

· Reversed phase

- Stationary phase is non polar
- Mobile phase is polar
- Polar compounds eluted first because of lower affinity with stationary phase
- Non-Polar compounds retained because of higher affinity with the stationary phase
- 3 - 4 component mobile phase should be avoided
- Multi component mobile phase once used not recommended for further use and solvent composition is expressed by volumes (v/v) and sum of volumes is usually 100
- Twin trough chambers are used only 10 -15 ml of mobile phase is required

-· Components of mobile phase should be mixed introduced into the twin - trough chamber

Pre- conditioning (Chamber saturation)

· Un- saturated chamber causes high Rf values

· Saturated chamber by lining with filter paper for 30 minutes prior to development - uniform distribution of solvent vapours - less solvent for the sample to travel - lower Rf values.

Chromatographic development and drying

· After development, remove the plate and mobile phase is removed from the plate - to avoid contamination of lab atmosphere

· Dry in vacuum desiccator - avoid hair drier - essential oil components may evaporate

Detection and visualization

· Detection under UV light is first choice - non destructive

· Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length)

· Spots of non fluorescent compounds can be seen - fluorescent stationary phase is used - silica gel GF

· Non UV absorbing compounds like ethambutol, dicylomine etc - dipping the plates in 0.1% iodine solution

· When individual component does not respond to UV - derivatisation required for detection

Quantification

· Sample and standard should be chromatographed on same plate - after development chromatogram is scanned

· Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode - scanning speed is selectable up to 100 mm/s - spectra recording is fast - 36 tracks with up to 100 peak windows can be evaluated

· Calibration of single and multiple levels with linear or non-linear regressions are possible

· When target values are to be verified such as stability testing and dissolution profile single level calibration is suitable

· Statistics such as RSD or CI report automatically

· Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors

Documentation

E - Merck introduced plates with imprinted identification code - supplier name. Item number, batch number and individual plate number - Avoid manipulation of data at any stage - coding automatically get recorded during photo documentation

Validation of analytical method

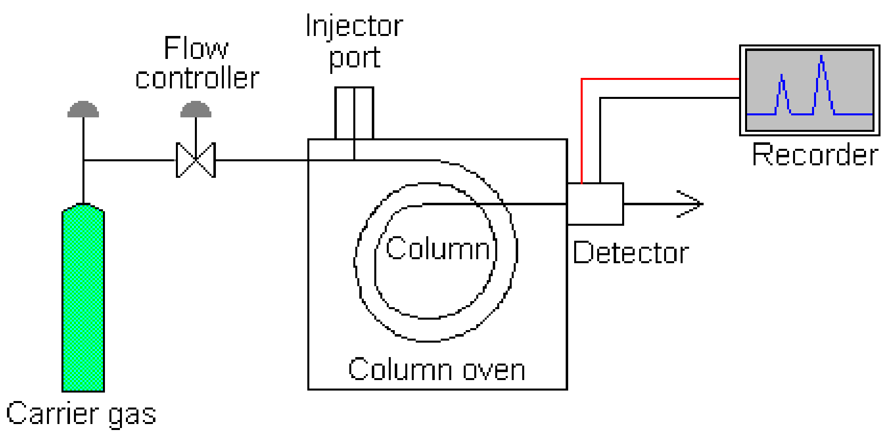
All validation parameters such as precision, accuracy, LOD, LOQ, Ruggedness, Robustness can be performed

Gas Chromatography

Introduction

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vapourised and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

Have a look at this schematic diagram of a gas chromatograph:



Instrumental components

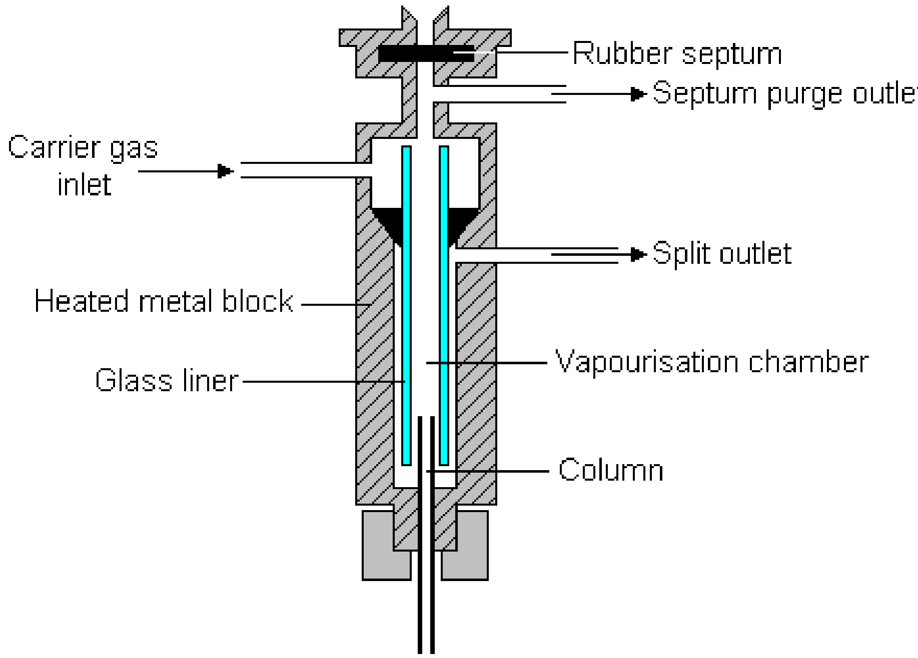
Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependant upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapouriser port at the head of the column. The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample. For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} µL. For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;

The split / splitless injector



The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vapourises to form a mixture of carrier gas, vapourised solvent and vapourised solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

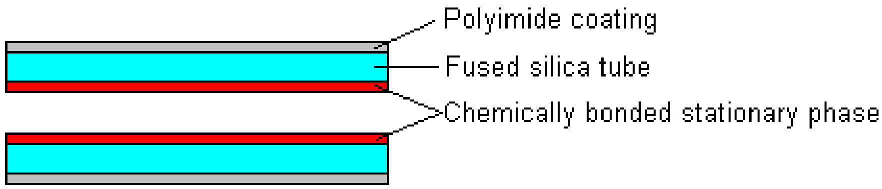
Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 - 10m in length and have an internal diameter of 2 - 4mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT). Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

In 1979, a new type of WCOT column was devised - the *Fused Silica Open Tubular* (FSOT) column;

Cross section of a Fused Silica Open Tubular Column



These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependant upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds.

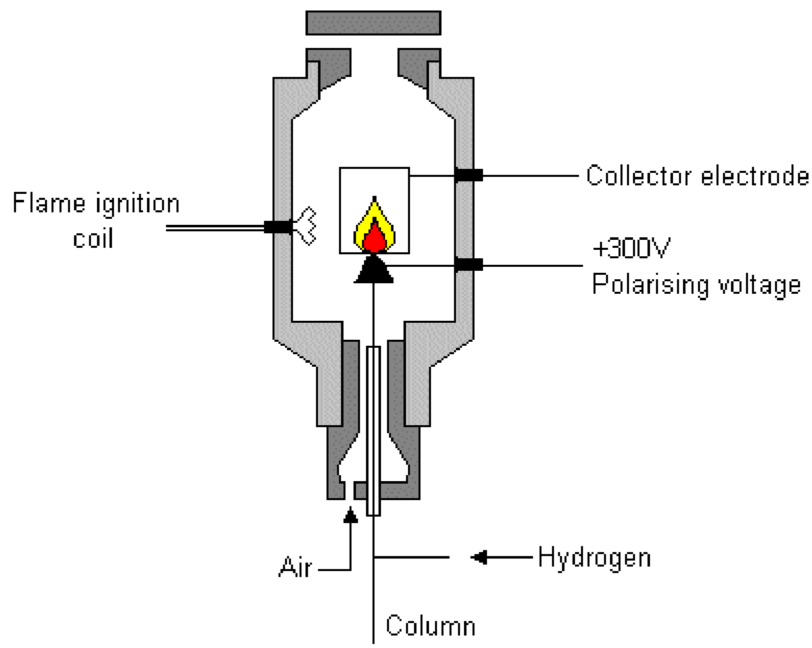
Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound. Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common GC detectors:

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic cpds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5

Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

The Flame Ionisation Detector



The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.

Review your learning

You should be aware of how a GC instrument works and the principles behind the operation of the major instrumental components, including injectors, columns and detectors.

Chromatography - Introductory Theory Quiz	**Gas Chromatography**	Gas Chromatography - Quiz

Back to index of topics

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