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Review Article

Advancements in column chromatography: A review

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ABSTRACT

Chromatography is a method used for separating organic and inorganic compounds. The word chromatography means "color writing". Chromatography is based on differential migration. The mobile phase moves through the stationary phase(column) picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. The classical preparative chromatography column, is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m. Thus, 25 g of adsorbent is required to provide a better separation in a 1 cm diameter column than in a 2 cm diameter column. As the technique column chromatography is more productive in qualitative and quantitative aspects scientists thought to improve its application in many fields. They worked on the technique to reduce the disadvantages by changing the column size, particle size of the stationary phase, changing the composition of the mobile phases etc. In the advancements of their works they developed the techniques like Flash chromatography, High Performance Liquid Chromatography, Gas Chromatography, Ultra Performance Liquid Chromatography and Ultra Performance Convergence Chromatography (Super Critical chromatography). The aim of the article is to specify the changes that the scientists have made in each and every step to improve the technique (column chromatography). Now over 60% of chemical analysis worldwide is currently done with chromatography.

Key Words: Chromatography, Mobile Phase, Elution, Estimation, Separation

INTRODUCTION

The word chromatography means "color writing". Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied. Chromatography is a great physical method for observing mixtures and solvents. Chromatography is such an important technique that two Nobel prizes have been awarded to chromatographers. Over 60% of chemical analysis worldwide is currently done with chromatography. [1] Chromatography is based on differential migration. In all chromatography there is a mobile phase and a stationary phase. The mobile phase moves through the stationary phase picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. At different points in the stationary phase the different components of the compound are going to be absorbed and are going to stop moving with the mobile phase. This is how the results of any chromatography are gotten, from the point at which the different components of the compound stop moving and separate from the other components.

Estimation of Drugs in Dosage Forms: Administration of two or more drugs at a time becomes imperative for several therapeutic reasons. The multi-component formulations have gained lot of importance now a days due to greater patient acceptability, increased potency, multiple action, fewer side effects and quicker relief. [2,3]
Analytical methods for the drugs in combined dosage forms includes:

- ✓ Non-instrumental methods of analysis
- ✓ Instrumental methods of analysis

i. Electro analytical methods
ii. Physical methods
iii. Spectral methods
iv. Chromatographic methods

Chromatography: Modern pharmaceutical formulations are complex mixtures including in addition to one or more therapeutically active ingredients, a number of inert materials such as

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diluents, disintegrants, colors and flavors. In order to ensure quality and stability of the final product, the pharmaceutical analyst must be able to separate the mixtures into individual components prior to quantitative analysis. Amongst the most powerful techniques available to the analyst for the separation of these mixtures are group of highly efficient methods collectively called chromatography. It is a group of technique for the separation of compounds of mixture that depends on the affinities of the solutes between two immiscible phases. One of the phases is affixed bed of large surface area, while the other is a fluid which moves through the surface of the fixed phase. The fixed phase is called stationary phase and the other is termed as the mobile phase. [4]
Based on the type of stationary material used for separation, it is of two types:

Normal phase: Stationary phase (Polar)
Mobile phase (Non-polar)
Non polar Compounds Elutes first.
Reverse phase: Stationary Phase (Non-polar)
Mobile phase (Polar)
Polar compounds Elutes first.

In most of the analysis, Reverse Phase is used as many of the drugs are polar in nature.
Depending on the type of chromatography employed, the mobile phase may be a pure liquid or a mixture of solutions (eg: Buffer etc) or it may be gas (pure or homogenous mixture).
Chromatographic methods can be classified according to the nature of the stationary and mobile phases.

The different types of Chromatography are

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Size exclusion or gel permeation chromatography.

The modern instrumental techniques of GLC and HPLC provide excellent separation and allow accurate assay of very low concentrations of wide variety of substance in complex mixtures.

Adsorption Chromatography: In adsorption chromatography, the mobile phase containing the dissolved solutes passes over the surface of the stationary phase. Retention of the component and their consequent separation depends on the ability of the atoms on the surface to remove the solutes from the mobile phase and adsorb them temporarily by means of electrostatic forces. Usually silica or alumina is utilized as the adsorbent with relatively non polar solvents such as hexane as the mobile phase in normal phase adsorption whereas in reversed phase adsorption non polymer beds with relative polar solvents such as water, acetonitrile methanol as mobile phase.

Partition Chromatography: In partition chromatography an inert solid material such as silica gel or diatomaceous earth serves to support a thin layer of liquid which is the effective stationary phase. As the mobile phase containing the solutes passes in close proximity to this liquid phase, retention and separation occur due to the solubility of the analytes in the two fluids as determined by their partition coefficients. The method suffers from disadvantage due to some solubility of stationary phase in the mobile phase. Hence precautions must be taken to limit dissolution of stationary phase.

Ion Exchange Chromatography: In ion exchange chromatography, the stationary phase consists of a polymeric resin matrix on the surface of which ionic functional groups, eg., carboxylic acids or quaternary amines, have been bonded chemically. As the mobile phase passes over this surface, ionic solutes are retained by forming electrostatic chemical bonds with the functional groups. The mobile phase used in this type is always liquid.

Size Exclusion Chromatography: In size exclusion chromatography the stationary phase is a polymeric substance containing numerous pores of molecular dimensions. Solutes whose molecular size is sufficiently small will leave the mobile phase to diffuse into the pores. Large molecules which will not fit into the pores remain in the mobile phase and are not retained. This method is mostly suitable for the separation of mixtures in which the solutes vary considerably in molecular size. The mobile phase in this type may be either liquid or gaseous.

COLUMN CHROMATOGRAPHY: [5,6]
Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The classical preparative chromatography column, is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom. Two methods are generally used to prepare a column: the dry method, and the wet method. The technique typically requires mesh 70 – 230 (63 – 200 µm) silica gel.
Columns: The columns available in the department are simple glass tubes, varying in length and diameter. They usually have a stopcock attached to control the solvent flow, and may have a fritted

plate to support the adsorbent. Some columns may have a flask attached to the top of the column to act as a solvent reservoir. The variation in size allows one to select the best column for the separation. A large diameter column will handle larger amounts of compound. The resolution of the column depends both upon diameter and length of the adsorbent in the column. Resolution increases with increasing length, and decreases with increasing diameter. Thus, 25 g of adsorbent will provide a better separation in a 1 cm diameter column than in a 2 cm diameter column.

Adding the Sample: Only use the minimum amount of solvent when adding the sample to the column. Once the packing is complete, the sample can be loaded directly to the top of the column. Normally, a minimum amount of a polar solvent, 5-10 drops, is used to dissolve the mixture. The solution is then carefully added to the top of the column using a pipette without disrupting the flat top surface of the column. A thin horizontal band of sample is best for an optimal separation. After the sample is loaded, a small layer of white sand is added to the top of the column. This will help to keep the top of the column level when adding solvent eluent. Once the mixture is added and the protective layer of sand is in place, continuously add the solvent eluent while collecting small fractions at the bottom of the column. Using a pipette to add the first bit of solvent on top of the packing, sample, and sand will minimize disturbance of the column and diluting the sample. Collecting small fractions (1-3 ml) is important to the success of your column separation. Fractions that are too small can always be pooled together; however, if the collected fractions are too large, you may get more than one compound in any particular fraction. If this occurs, the only way to complete the separation is to redo the chromatography. Since column chromatography is time consuming, collecting large fractions is discouraged.

Monitoring the Column: If the mixture to be separated contains colored compounds, then monitoring the column is very simple. The colored bands will move down the column along with the solvent and as they approach the end of the column, collect the colors in individual containers. Use the color as your guide. However, most organic molecules are colorless. In this case, the reaction must be monitored by TLC. Spot each fraction on a TLC plate. Four or five fractions can be spotted on a single TLC plate. Develop the plate and use the observed spot or spots to determine which compound is in each of the collected fractions. Spotting some of the starting material or the product (if available) on the TLC plate as a standard will help in the identification.

Isolating the Separated Compounds:

Evaporation: Once you believe all the materials have been removed from the column, the colors of the materials or TLC results should indicate which fractions contain the compound(s) you are interested in isolating. Combine the like or same fractions and evaporate the solvent. The pure separated compound will be left behind. Recrystallization may be used to further purify a solid product. However, on a milligram scale, there is usually not enough material to do this.

FLASH CHROMATOGRAPHY:

Flash can help speed up the flow rate of your column: Column chromatography is often very time consuming. Allowing the solvent to elute through the column one drop at a time takes patience. One method to speed up the process is to use Flash Chromatography. This method uses a pressure of about 10 psi of air or nitrogen to force the mobile phase through the column. Because the rate of the mobile phase is increased, in general, this method gives a poorer separation. However, by using a finer grade of alumina or silica, flash chromatography can be used to increase the speed without lowering the quality of the separation. The particle size of the stationary phase is generally finer in flash column chromatography than in gravity column chromatography. Silica gel grades in the technique is mesh 230 – 400 (40 – 63 µm) [7-9] Early liquid-chromatographic columns were glass tubes with diameters of perhaps 10 to 50 mm that held 50-500cm lengths of solid particles for the stationary phase. To ensure reasonable flow rates, the particle size of the solid was kept larger than 150 to 200µm*;* even then, flow rates were at best a few tenths of a milliliter per minute. Attempts to speed up this classic procedure by application of vacuum or pressure were not effective because increases in flow rates were accompanied by increases in plate heights and accompanying decreases in column efficiency. Early in the development of liquid chromatography, it was realized that large decreases in plate heights could be expected to accompany decreases in the particle size of packings. It was not until the late 1960s, however, that the technology for producing and using packings with particle diameters as small as 5 to 10 μm was developed. This technology required sophisticated instruments that contrasted markedly with the simple devices that preceded them. The name high-performance liquid chromatography (HPLC) is often employed to distinguish these newer procedures from their predecessors, which still find considerable use for preparative purposes.

Automated systems will include components normally found on more expensive high performance liquid chromatography (HPLC) systems such as a gradient pump, sample injection ports, a UV detector and a fraction collector to collect the eluent. Typically these automated systems can separate samples from a few milligrams up to an industrial many kilogram scale and offer a much cheaper and quicker solution to doing multiple injections on prep-HPLC systems.

HPLC:

HPLC is one of the most useful tools available for quantitative analysis. Reversed phase chromatography refers to the use of a polar mobile phase with a non polar stationary phase in contrast to normal phase is employed with a non polar mobile phase. [10,11]

HPLC as compared with the classical technique is characterized by:

- Diameter (2-5 mm), reusable stainless steel columns;
- Packing with very small (3, 5 and 10 mm) particles and the continual development of new substances to be used as stationary phases;
- High inlet pressures and controlled flow of the mobile phase;
- Sample introduction without the need for large samples;
- Continuous flow detectors capable of handling small flow rates and detecting very small amounts;

An elution with a single solvent of constant composition is termed isocratic.

In gradient elution, two (and sometimes more) solvent systems that differ significantly in polarity are employed. The ratio of the two solvents is varied in a Pre-programmed way, sometimes continuously and sometimes in a series of steps. Gradient elution frequently improves separation efficiency. Modern high performance liquid chromatographic instruments are often equipped with proportionating valves that introduce liquids from two or more reservoirs at rates that vary continuously.[12]

Advances in column technology, high pressure pumping systems and sensitive detectors has transformed liquid column chromatography into a high speed, high efficiency method of separation. This advanced technology is based upon the use of small bore (2.5 mm–internal diameter) columns and small particle size (3-50 µm) that allow fast equilibrium between stationary and mobile phases. This small particle column technology requires high pressure pumping system capable of delivering the mobile phase at high pressure, as much as 300 atmospheres, to achieve flow rates of several ml per minute. Since it is often necessary to use small amounts of analyte (usually less than 20 µg) with the column packing, sensitive detectors are needed.

The column is one of the most important components of the HPLC chromatograph because the separation of the sample components is achieved when those components pass through the column. Normally, columns are filled with silica gel because its particle shape, surface properties, and pore structure help to get a good separation. Silica is wetted by nearly every potential mobile phase, is inert to most compounds and has a high surface activity which can be modified easily with water and other agents. Silica can be used to separate a wide variety of chemical compounds, and its chromatographic behavior is generally predictable and reproducible.

Pumping Systems: The requirements for liquid-chromatographic pumps are severe and include:

(1) Generation of pressures of up to 6000 psi (lb/in^2);

(2) Pulse-free output;

(3) Flow rates ranging from 0.1 to 10 ml/min;

(4) Flow reproducibilities of 0.5 % relative or better; and

(5) Resistance to collision by a variety of solvents.

It should be noted that the high pressures generated by liquid-chromatographic

pumps do not constitute an explosion hazard because liquids are not very compressible.

Thus, rupture of a component results only in solvent leakage. To be sure, such leakage may constitute a fire hazard

Sample Injection Systems: The most widely used method of sample introduction in liquid chromatography is based on sampling loops. These devices are often an integral part of modem liquid chromatography equipment and have interchangeable loops that provide a choice of sample sizes ranging from 5 to 500 µl. The reproducibility of injections with a typical sampling loop is a few tenths of a percent relative.

Describing the 5 major HPLC components and their functions:

Pump:

- The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in milliliters per min (ml/min).
- Normal flow rates in HPLC are in the 1-to 2-ml/min range.
- Typical pumps can reach pressures in the range of 6000-9000 psi

(400 to 600 bar).

- During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient).

Injector:

- The injector serves to introduce the liquid sample into the flow stream of the mobile phase.
- Typical sample volumes are 5-to 20-microliters (μL).
- The injector must also be able to withstand the high pressures of the liquid system.
- An auto sampler is the automatic version for when the user has many samples to analyze or when manual injection is not practical.

Column:

- Considered the "heart of the chromatograph" the column's stationary phase separates the sample components of interest using various physical and chemical parameters.
- The small particles inside the column are what cause the high backpressure at normal flow rates.
- The pump must push hard to move the mobile phase through the column and this resistance causes a high pressure within the chromatograph.

Detector:

- The detector can see (detect) the individual molecules that come out (elute) from the column.
- A detector serves to measure the amount of those molecules so that the chemist can quantitatively analyze the sample components.
- The detector provides an output to a recorder or computer that results in the liquid chromatogram (i.e., the graph of the detector response).

Computer:

- Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis).

The identification (ID) of individual compounds in the sample:

- The most common parameter for compound ID is its retention time(the time it takes for that specific compound to elute from the column after injection);
- Depending on the detector used, compound ID is also based on the chemical structure, molecular weight or some other molecular parameter.

The measurement of the amount of a compound in a sample

There are two main ways to interpret a chromatogram (i.e. perform quantification):

- Determination of the peak height of a chromatographic peak as measured from the baseline;
- Determination of the peak area.

GAS-LIQUID CHROMATOGRAPHY:

Gas chromatography (GC), is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture. [13,14]

In gas chromatography, the mobile phase (or "moving phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (an homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different time, known as the retention time of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas mobile phase, whereas in column chromatography the stationary phase is a solid and the mobile phase is a liquid. (Hence the full name of the procedure is "Gas–liquid chromatography", referring to the mobile and stationary phases, respectively.) Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas. Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. microscale).

The following devices are common types of GC detectors:

- Thermal Conductivity Detector (TCD)
- Flame Ionization Detector (FID)
- Nitrogen-phosphorus Detector
- Electron Capture Detector (ECD)
- Mass Spectrometers
- Thermionic Detectors (TID)
- Atomic Emission Detectors (AED)

The choice of detector will depend on the analyte and how the GC method is being used (i.e., analytical or preparative scale)

In GC the carrier gas is simply stated as just a carrier to transport the vaporized solute molecules through the column during the partitioning process. Carrier gases are compressible gases that expand with increasing temperature. This results in a change in the gas viscosity. The selection and linear velocity of the carrier gas will affect resolution and retention times. Carrier gases should be inert to the stationary phase and free of detectable contaminants. Helium is the most common mobile phase in gas-liquid chromatography.

Sample Injector: (syringe / septum)**Column:** 1/8" or 1/4" or 6-50" tubing packed with small uniform size, inert support coated with thin film of nonvolatile liquid.

Carrier Gas Viscosity (µP): Temperature programming changes the viscosity of a carrier gas resulting in a decrease in linear velocity/flow over the programmed range when run in a constant pressure mode. Hydrogen is the best choice for capillary GC due to diffusivity and a broad working range as long as safety concerns and proper controls are in place.[15]

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY:

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about ninefold decrease in analysis time as compared to the conventional HPLC system using sub-2µm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3-5µm particle size analytical columns without compromise on overall separation. Reducing these separation times without reducing the quality of the separation would mean that important analytical information could be generated more quickly. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. As it is very well known from Van Deemter equations, the efficiency of chromatographic process is proportional to particle size decrease. According to this model describing band broadening, it describes relationship between height equivalent of theoretical plate (HETP) and linear velocity, one of the terms (path dependent term), is dependent on a diameter of particle packed into the analytical column. Smaller particle diameter can significantly reduce HETP which results in higher efficiency and the flatter profile of Van Deemter curve. Consequently, the mobile phase flow-rate increase does not have negative influence to the efficiency as it could be observed at 10 or 5 µm particle. The negative aspect of small particle packed columns used in HPLC is, however, high back-pressure generating.[16,17]

Detectors:

Tunable UV (TUV) Detector is a tunable, dual wavelength ultraviolet/visible (UV/Vis) detector that offers optimal linearity, resolution and sensitivity for UPLC®/UV separations.

Photodiode Array (PDA) Detector allows your laboratory to detect and quantify lower concentrations of sample analytes and compare spectra across wavelengths and broad concentration ranges.

Evaporative Light Scattering (ELS) Detector is designed specifically for optimal UPLC®/ELS performance in a small footprint.

When your analytes have poor to no UV/Vis response, or don't ionize well by mass spectrometry, the ACQUITY UPLC ELS Detector with the ACQUITY UPLC System lets you analyze more molecules (including sugars, triglycerides, phospholipids, antibiotics, and natural products) in a single analytical run.

TQD- Tandem Quadrupole MS Detector for UPLC/MS/MS and HPLC/MS/MS applications.

SQD-Combines the resolution, sensitivity, and speed of UltraPerformance technology with Single Quadrupole MS Detection.

FLR-Provides sensitive, selective detection for UPLC-based fluorescence applications.

QDa Detector is a mass detector built around the needs of analytical scientists for chromatographic analysis. Robust, reliable and requiring no sample adjustments, it integrates with your current LC, ACQUITY UPLC, UPC2 and purification systems.

RI Detector - Low-dispersion detection for the isocratic analysis of analytes that lack a UV chromophore. Delivers high sensitivity, baseline stability and repeatable integration of narrow UPLC peaks.

ULTRA PERFORMANCE CONVERGENCE CHROMATOGRAPHY (UPC^2)

New tool for tackling tough-to-analyze compounds including hydrophobic and chiral compounds, lipids, thermally-labile samples and polymers. Works on the principle of Supercritical Fluid

Chromatography (SFC). Supercritical fluid chromatography is one of the most important column chromatography methods after gas chromatography (GC) and high-performance liquid chromatography (HPLC). SFC combines the advantages of GC and HPLC. Also, supercritical fluid extraction is an advanced analytical technique. SFE does not have the problems experienced with liquids.[18] A supercritical fluid is the phase of a material at critical temperature and critical pressure of the material. Supercritical fluids combine useful properties of gas and liquid phases.UPC^2 provides a technique for analysis of non-volatile and semi-volatile extractables, as well as polar and non-polar compounds.[19]

UPC^2 is the equipment most similar to UPLC. It is the most advanced type of chromatographic technique present in the market. UPC^2 runs in Normal Phase chromatography.

- Avoid the usage of water for UPC^2
- Rinse the mobile phase (MP) bottle thoroughly with MP/methanol before filling it with MP.
- Carbon dioxide is the mobile phase in UPC^2. (Polarity of CO2 ≈ n-Heptane).
- Range of column temperature 40-70°C.
- Range of ABPR pressure 1750-2200psi.

Types of columns used:

- BEH 2-EP(Ethyl Pyridine)
- BEH(Ethylene Bridge Hybrid)
- CSH Fluoro-Phenyl(Charged Surface Hybrid)
- HSS C18 SB(High Strength Silica-Stable Bond) for glycerides

Composition of Mobile phases:

- Range of CO2 composition 60-100%.
- Range of binary solvent composition 0-40%.

(0020set the composition of binary solvent based on the reference manual of the column)

Injection Volume: 1-5 µl

Detectors:

Photo diode Array (PDA) Detector allows your laboratory to detect and quantify lower concentrations of sample analytes and compare spectra across wavelengths and broad concentration ranges.

QDa Detector is a mass detector built around the needs of analytical scientists for chromatographic analysis. Robust, reliable and requiring no sample adjustments, it integrates with your current LC, ACQUITY UPLC, UPC2 and purification systems.

Table 1: Various detectors used in HPLC

Type of Detectors	**Sub Type of Detectors**		
Refractive Index Detector	**Christiansen effect Detector**	**Interfero meter Detector**	**Thermal Lens Detector**
Ultraviolet Detector	**Variable wavelength UVabsorption Detector**	**Fixed wavelength UV Detector**	**Multiple dispersive UV Detector**
Fluorescent Detector	**Single wavelength excitation Fluorescence Detector**	**Multi wavelength Fluorescence Detector**	**Laser Induced Fluorescence Detector**
Transport Detector	**Moving wire Detector**	**Chain Detector**	**Disc Detector**
Electrochemical Detector	**Dynamic Detector/ Multi Electrode array Detector**	**Equilibrium Detector**	
Electric conductivity Detector			
Liquid light Scattering Detector	**Low angle Laser light scattering Detector**	**Multi angle Laser scattering Detector**	
Advanced Detectors			
Aerosol based Detectors	**Evaporative light scattering Detector**	**Charged aerosol Detector**	**Nano aerosol Detector**
Chiral detector	**Polarimetric detector**	**Circular dichroism Detector**	
Pulsed Amperometric detector			

Table 2: Various detectors used in Gas Chromatography

Type	Applicable Samples	Typical Detection Limit
Flame Ionization	Hydrocarbons	1 pg/s
Thermal Conductivity	Universal detector	500 pg/ml
Electron Capture	Halogenated compounds	5fg/s
Mass Spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen & phosphorous compounds	0.1 pg/s (P), 1pg/s (N)
Electrolytic Conductivity	Compounds containing halogens, sulfur, nitrogen	0.5 pg Cl/s, 2 pg S/s, 4pg N/s
Photo ionization	Compounds ionized by UV radiation	2 pg C/s
Fourier Transform IR (FT-	Organic compounds	0.2 to 40 ng

Table 3: Advantages & disadvantages of different Carrier Gases used in GC

Carrier Gas	Advantages	Disadvantages
Nitrogen	Cheap, Readily available	Long run times
Helium	Good compromise, Safe	Expensive
Hydrogen	Shorter run times, Cheap	Explosive

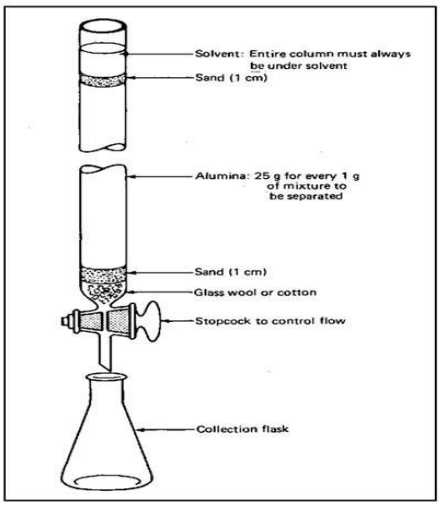

Figure 1: Schematic diagram of Column Chromatography

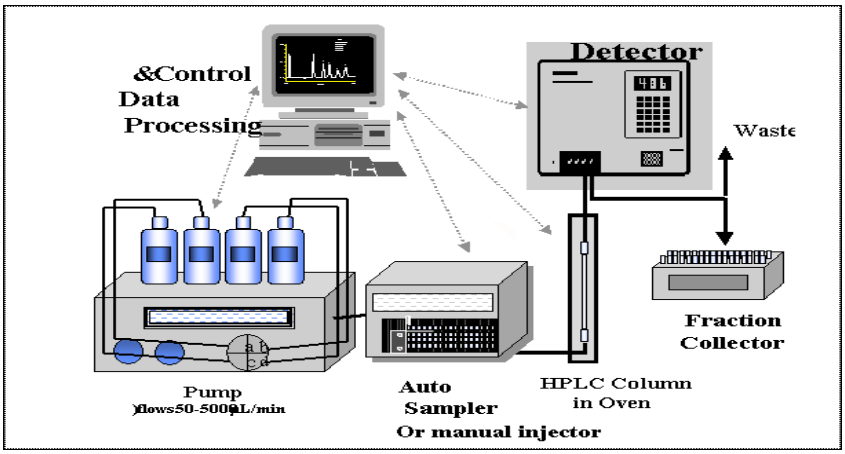

Fig 2: Schematic representation of HPLC

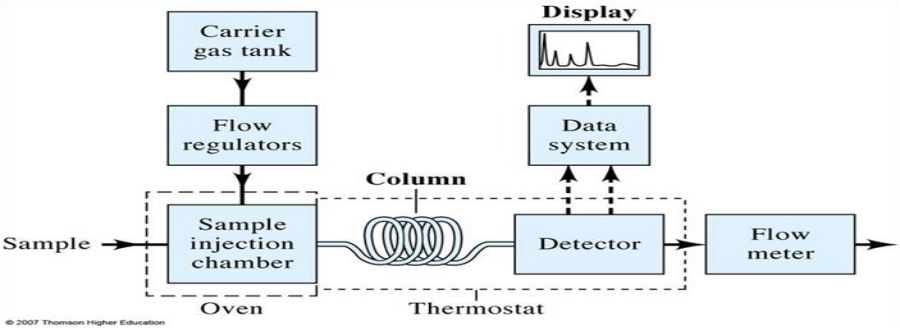

Figure 3: Schematic Representation of Gas Chromatography

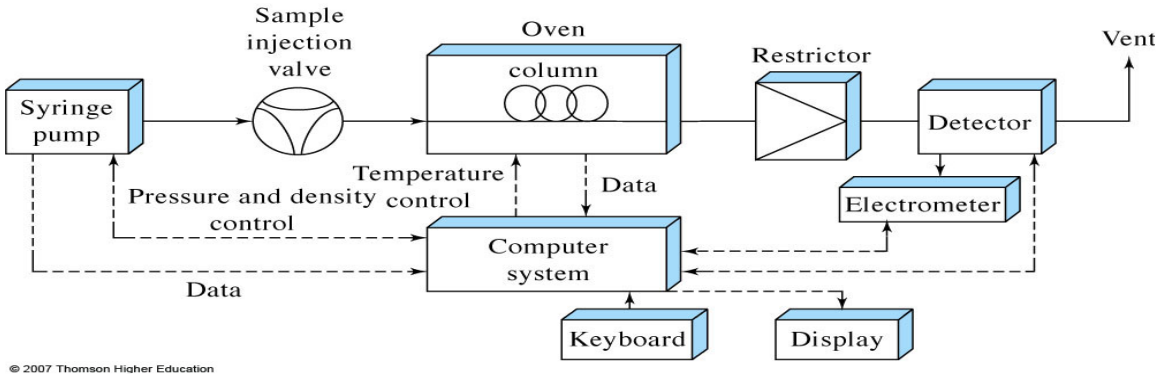

Figure 4: Schematic representation of UPCC

REFERENCES

1. Kaushal C, Srivastava B. A process of method development: A chromatographic approach. J Chem Pharm Res 2010; 2(2): 519-545.
2. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Mott MG. Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical Technology, 2002; p. 48-56.
3. Christie WW, Gill S, Nordbäck J, Itabashi Y, Sanda S, Slabas AR. New procedures for rapid screening of leaf lipid components from Arabidopsis. Phytochemical Anal 1998;9:53-57.
4. Wiklund AE, Dag B, Brita S. Toxicity evaluation by using intact sediments and sediment extracts. Marine Pollution Bulletin 2005; 50(6): 660-667.
5. Kwok YC, Hsieh DPH, Wong PK. Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Marine Pollution Bulletin. 2005; 51(8-12): 1085-1091.
6. Haginaka J et al. Alkaline degradation and determination by high performance by high performance liquid chromatography. Chem Pharm Bull 1984; 32: 2752-2758.
7. Liu Y, Lee ML. Ultra high pressure liquid chromatography using elevated temperature. Journal of Chromatography. 2006;1104(1-2):198–202.
8. Patel RM et al. Stability Indicating HPLC Method Development- A Review. Int Res J Pharmacy 2011; 2(5): 79-87.
9. http://www.scribd.com/doc/9508765/Physical-Properties-of-Drug.
10. Kupiec T. Quality-control analytical methods: High-performance liquid chromatography. IJPC 2004; 8(3): 223-227.
11. Arayne, MS, Sultana NF, Siddiqui A. Pak J Pharm Sci 2006; 19(4): 326-329.
12. Ngwa G, Forced Degradation Studies. Forced Degradation as an Integral part of HPLC Stability Indicating Method Development Drug Delivery Technology. 2010; 10(5).
13. Reynolds DW, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Mott MG. Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical Technology, 2002; 48-56.
14. Gentili A, Caretti F, Ascenzo GD. Simultaneous determination of water-soluble vitamins in selected food matrices by liquid chromatography/electrospray ionization and mass spectrometry, Rapid Communications in Mass Spectrometry. 2008. p. 2029–2043.
15. Swartz ME. Ultra performance liquid chromatography (UPLC): An Introduction, Separation Science Re-Defined , LCGC Supplement. 2005. p. 11.
16. An Encyclopedia of Chemical, Drugs and Biologicals, 13th ed., Merck Research Laboratories. Division of Merck & Co Inc. Whitehouse Station, NJ. The Merck index. 2001. p. 245.
17. Jeffery GH and Basselt J. Vogel's Text Book of Quantitative chemical analysis. 6th ed. Pearson education pvt. Ltd. Singapore; 2003. P. 21-23.
18. Michael E. Swartz. Ultra Performance Liquid Chromatography (UPLC): An Introduction and Review. Journal of Liquid Chromatography & Related Technologies. 2005; 28: 1253–1263.
19. Lucie Novakova, Ludmila Matysova, Petr Solich. Advantages of application of UPLC in pharmaceutical analysis. Talanta: 2006. P. 908-918.