

Gas Chromatography

Principle, Instrumentation and Application

Dr. A. Amsavel, M.Sc., B.Ed., Ph.D.



What is Chromatography?

- Chromatography is a separation technique used for Qualitative and Quantitative Analysis.
- Essential for testing of chemicals in An Industries
- Technique developed in 1941 by Martin & Synge and were awarded Nobel Prize in 1952 (partition chromatography)
- In 1951 first GC experiment was performed by Martin & James
- In 1955, the first commercial apparatus for gas-liquid chromatography appeared on the market
- Around mixture of 200 related compounds can be tested in preset GCs



Chromatographic Separation

Chromatography is based on a **Physical equilibrium** that results when a solute is transferred between the mobile and a stationary phase.

K = Distribution coefficient *or* Partition ratio

$$K = C_s / C_M$$

- C_s - Molar conc. of the solute in the stationary phase
- C_M - Molar conc. of the solute in the mobile phase.



Gas Chromatography

Gas Chromatography is a chromatographic technique. Where Gas is used as ***Mobile phase***.

Stationary phases are two types; Solid or Liquid

- Gas-Solid Chromatography (*GSC*):

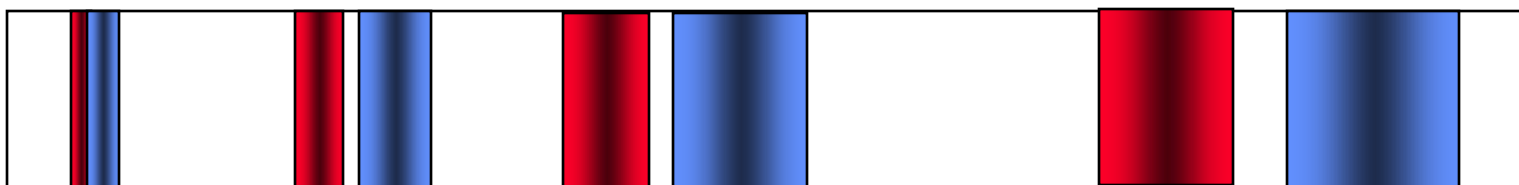
Separation is based on physical adsorption , but not widely used.

- Gas-Liquid Chromatography (*GLC*):

GLC - Separation is based partition between mobile phase (MP) and Stationary Phase (SP). Used extensively.

Separation in GC

- In a mixture, each component has a **different distribution coefficient**, and thus spends a different amount of time **absorbed on solid** or **partition on liquid** stationary phase and moving carrier gas
- The **sample** then has the **opportunity to interact** with the stationary phase as it moves past it.



Samples that **interact greatly**, which **move more slowly**.

Samples that **interact weakly**, which **move more quickly**.

Because of this difference in rates, the samples can then be

Separated into their components.





GC : Separation Principle

- Compounds that have a **greater affinity** for the stationary phase spend more time in the column and thus **elute later** and have a longer **retention time (R_t)**.
- Affinity for the stationary phase is based on intermolecular interactions and the polarity of the stationary phase.



Advantage of GC

GC is used for separating and analyzing the volatile compounds.

- Advantage
- Efficiency and analysis speed:
- Easy and fast
- Accuracy and reproducibility
- Low sample quantity ($\mu\text{g}/\text{ng}$) / low detection level
- Can test wide range of organic compounds
- High resolution and High Sensitivity (ppm)
 - Used to analyze azeotropic mixtures and samples with close boiling points, some isotopes, cis-trans isomers, adjacent or inter trans isomers, optical isomers, *etc.*



Definition

- **Carrier Gas** : Mobile phase or carrier gas.

Carrier gas in GC is to move the solutes along the column.

- **Retention time.** is defined as the time elapsed between the injection of the sample and the appearance of the maximum peak response of the eluted sample zone.
- **Relative retention (RRT):** Relative Retention time of a component is relative to that of another used as a reference, obtained under identical conditions.
- **Resolution (R_s):** The resolution is the separation of two components in a mixture
- **Make-up gas.** Career gas flow at detector (20-40 mL/m) is provided for maximum performance of detector. This additional flow (make-up gas) is adjusted at detector. Carrier gas and make up gas is mixed at the outlet of the column and enters to the detector.



Definition

- **HETP-** It is the distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in liquid phase.

Larger the number of theoretical plates/ smaller the HETP, the more efficient the column for separation.

HETP = Length of column/n ;

Where n = number of theoretical plates = $16 \times \frac{x^2}{y^2}$

- **Retention Volume:** (1) $V_R = t_R \times F$ (retained) (2) $V_M = t_M \times F$ (non-retained)
- **WCOT:** Wall-coated columns are capillary tubes coated with a thin layer of the liquid stationary phase
- **SCOT:** Support-Coated Open Tubular columns have finely divided layer of solid supported material deposited (~30 mm) on the inner wall, on to surface of the capillary on which the liquid stationary phase is coated

Definition

- ❑ **Number of theoretical plates (N):** A measure of column efficiency. For Gaussian peaks, it is calculated by: $N = 16(tR/W)^2$
where tR is the retention time of the substance, and W is the peak width at its base
- ❑ **Resolution (R_s):** The resolution is the separation of two components in a mixture, calculated between peaks (1&2); tR - Retention time & W – peak width
$$R_s = 2 \times (tR_2 - tR_1)/(W_1 + W_2)$$
- ❑ **Symmetry factor (A_s):** Also known as the “tailing factor”, of a peak is calculated by: $A_s = W_{0.05}/2f$ (Fig-1)
Where $W_{0.05}$ is the width of the peak at 5% height from base and f is the distance from the peak maximum to the leading edge of the peak.
- ❑ **The signal-to-noise (S/N) ratio:** is a useful system suitability parameter. The S/N ratio is calculated : $S/N \text{ ratio} = 2H/h$) Fig-2

Fig-1

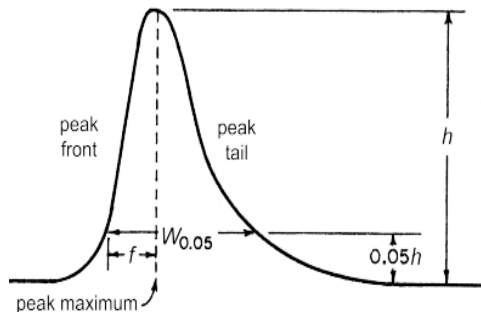
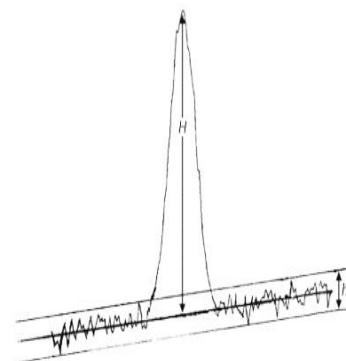


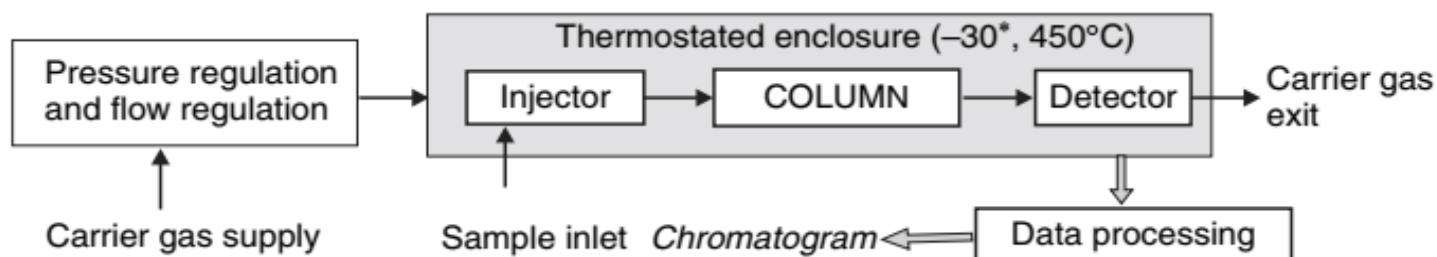
Fig-2



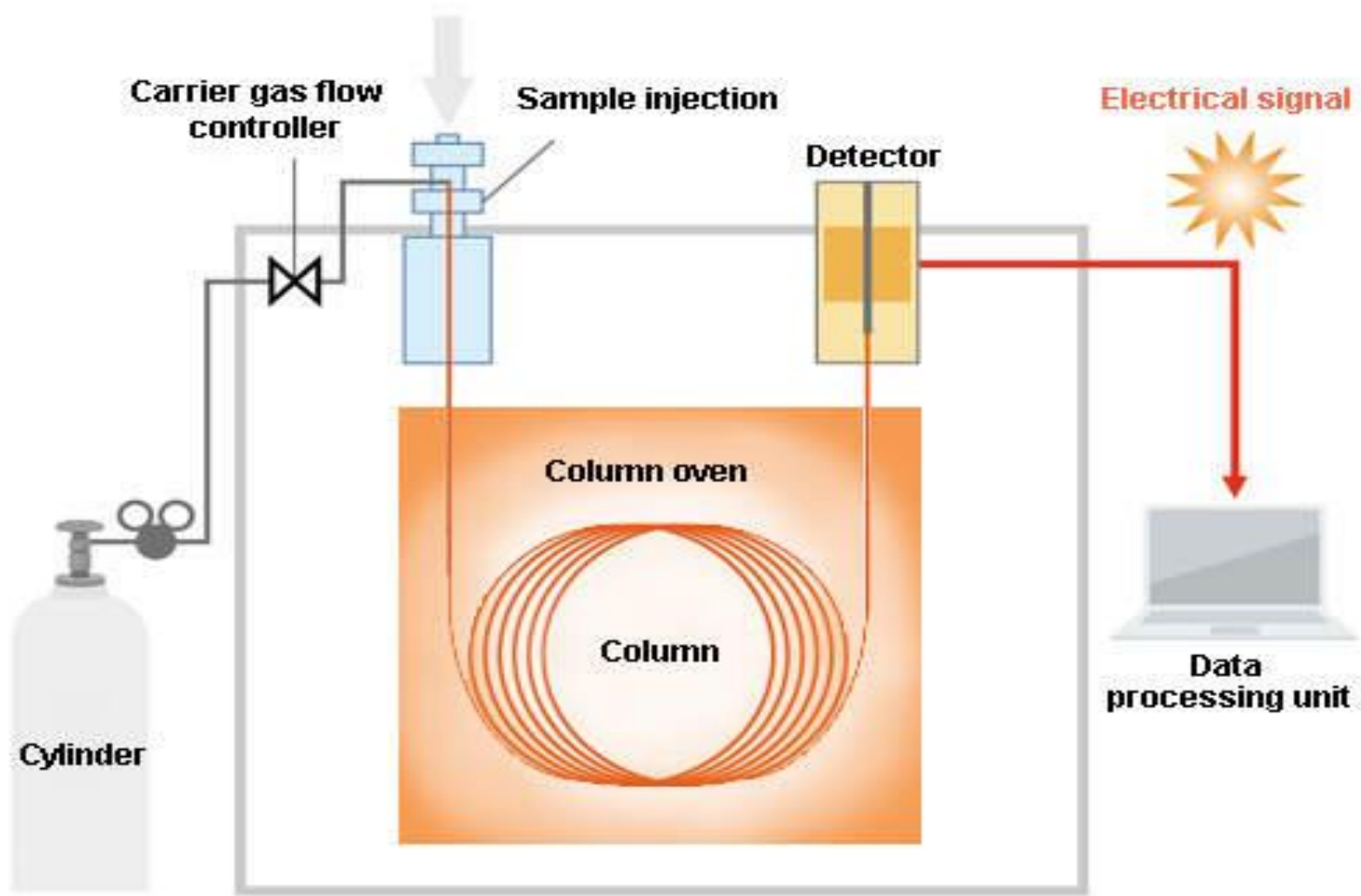
GC Instrumentation

Gas Chromatograph consists:

1. Carrier Gas source
2. Injection Port / Injector
3. Column Oven and Column
4. Detector &
5. Data processing/Software.



GC –Typical Depiction





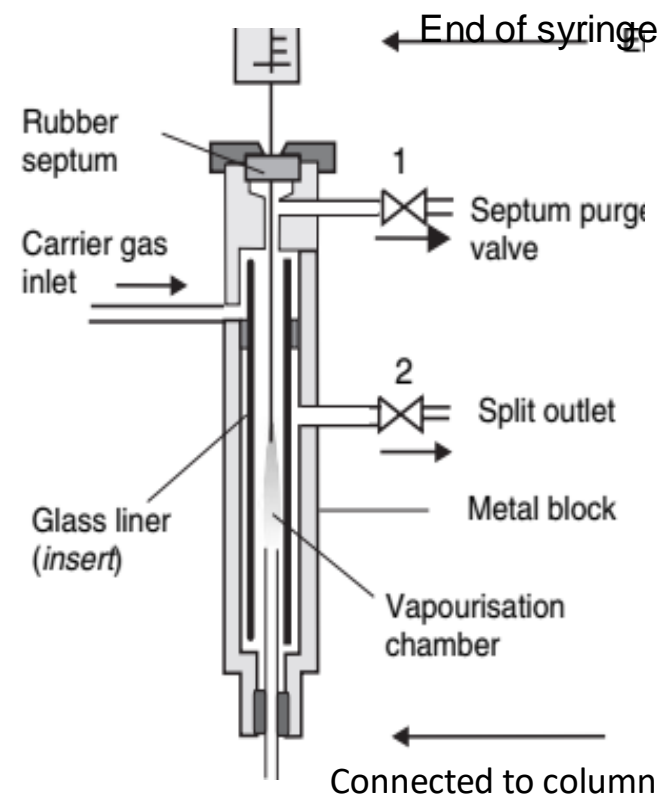
GC Instrumentation

GC Parts and It's Function:

- The injection port, column, and detector are temperature controlled and can be modified while analysis . Temperature can be optimized as desired for better separation, resolution and peak symmetry
- Carrier gas : Helium, Nitrogen, or hydrogen, depending on the column and detector in use.
- Column is brain or heart of the GC. It determines the separation of compounds
- The type of detector used depends on the nature of the compounds analyzed
- Detector output is recorded as a function of time, and the instrument response, measured as peak area or peak height are proportional to the amount present

GC: Injector

- Injector is an ***Inlet of the sample*** to the Gas chromatograph. Carrier gas continuously flows into the injector.
- Injector is maintained at elevated temperature. Higher than boiling point of samples (normally 100 – 300 °C).
- It operates in ***Split or Splitless mode***.
- *Injector vaporize the injected sample and mix* with the carrier gas and flows to the column.
- In case of split mode (*capillary columns*), injector allows proportion (as per slit ratio) of carrier gas and sample vapour to the column. Remaining portion exit from the split outlet, which is regulated by needle valve



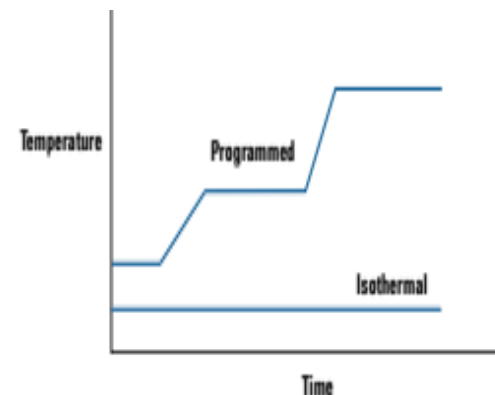


Sample Injection

- Fill sample Liquid or vapor in micro syringe.
- Overfill syringe then adjust to desired amount (0.1-1 μ L and inject.
- Do not let the sample remain in the syringe long before injecting to avoid vapor loss/ low boiling will escape.
- Hold the head of the syringe during injection. While piercing the septa , plunger may be pushed up due to pressure.
- Inject as close as possible to the column head.
- Push the plunger fairly rapidly during injection.

GC column Oven

- GC oven capacity designed to hold one or two columns and which can heat up to more than 400°C.
 - Oven can be heated or cooled a rapidly. Also designed the control temperature and set gradient with 1-100°C/min.
 - The temperature can be controlled ± 0.1 C to get reproducible separations.
 - Also can operate at low temperature with cryogenic (use of liq. N₂ or CO₂)
 - Set Isothermal or programmed temperature modes as required.
-
- **Isothermal:** Same temperature throughout the analysis.
 - **Programmed:** To get desired separation, alter or program the oven temperature (column). Set initial temperature (hold if required), increase rate of temperature change (ramp), final temperature, and hold time at the final temperature. Eg. 100°C hold 5 min ramp 10°C/min to 250°C hold for 10 min





Oven Temperature & Separation

- Oven temperature should be above the boiling point and below the degradation temperature of solute.
- Separations of compounds are temperature dependent, hence oven temperature (Column) is important.

Isothermal oven: The oven temperature is constant throughout the analysis.

- The oven is always ready for a sample analysis.
- There is no recovery time between analyses.

Programmed oven:

The oven temperature changes, usually upward, during the analysis.

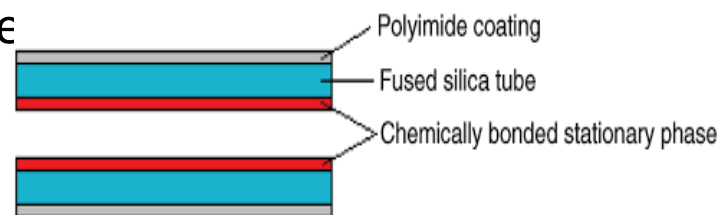
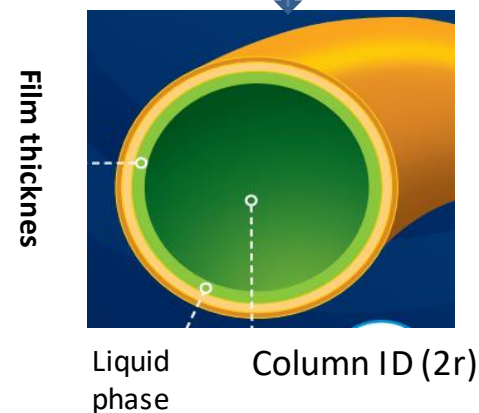
- Analysis time is reduced by proper set temperature
- Achieve good Peak separation/ resolution & peak shapes and faster analysis of complex molecules.
- *Disadvantage* is components are subjected to higher temperatures, may cause degradation of sensitive components. Excess time for cooling of oven

Capillary Column

- ❖ Fused silica used in capillary columns. It is inert and robust.
- ❖ A capillary column is an open tube coated with the stationary phase on its inside surface.
- ❖ Columns Inside Diameter (ID) range from 0.1mm to 0.53 mm
- ❖ Length is 30 meters to 100 meters
- ❖ Theoretical plates up to 300000 .
- ❖ Work with Split less (entire sample) or Split (part of sample divided before it enters the column).

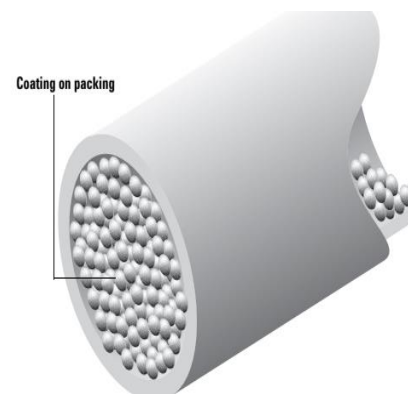


Fused silica tube wall



Packed Column

- ❖ Stationary phase is coated on a finely-divided inert material packed into a metal, glass, or plastic tube
- ❖ 1/8- or 1/4-inch outside diameter.
- ❖ Length 1-5 meters
- ❖ Stainless steel—durable, but a reactive surface , may cause component loss or peak tailing.
- ❖ Glass (deactivated surface) better peak shape, but fragile
- ❖ Teflon tubes are also used
- ❖ 4000 theoretical plates
- ❖ Low resolution and limited separation & use

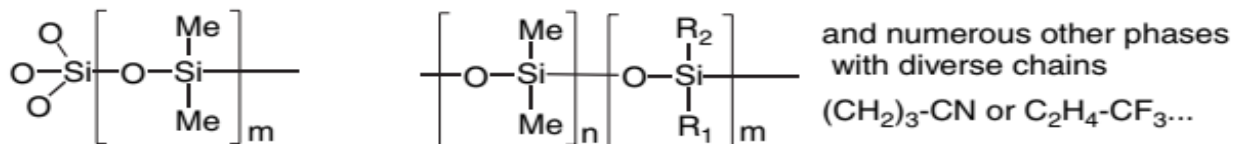


Stationary Phase

Chemical nature and functional groups:

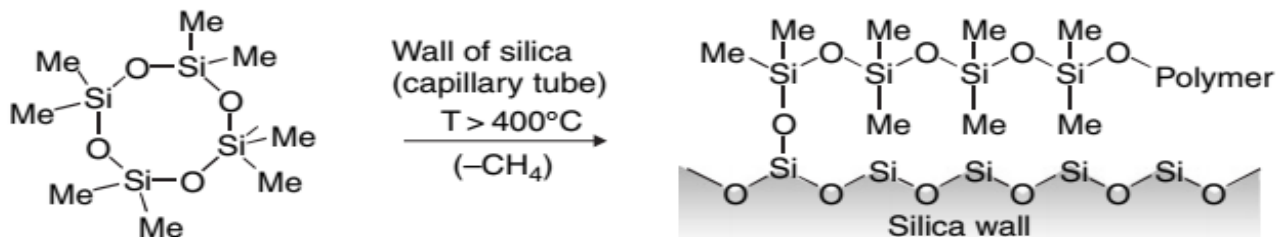
- Polydimethyl siloxane — **Non-polar to moderately polar**
- Phenyl methyl silicones (5 to 50% phenyl) — **Moderately polar to polar**
- Polyglycol and Cyanopropyl phenyl- **Very polar**

Bonded polysiloxanes (examples):

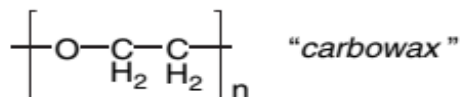


ex. R₁ and R₂ = Ph m = 95% and n = 5%

Method of formation of a bonded phase



Polyethyleneglycols





Carrier Gas flow

Type	Diameter	Carrier flow rate, mL/min		
		Hydrogen	Helium	Nitrogen
Packed	1/8-inch od	30	30	20
Packed	1/4-inch od	60	60	50
Capillary	0.05 mm id	0.2 to 0.5	0.1 to 0.3	0.02 to 0.1
Capillary	0.1 mm id	0.3 to 1.0	0.2 to 0.5	0.05 to 0.2
Capillary	0.2 mm id	0.7 to 1.7	0.5 to 1.2	0.2 to 0.5
Capillary	0.25 mm id	1.2 to 2.5	0.7 to 1.7	0.3 to 0.6
Capillary	0.32 mm id	2 to 4	1.2 to 2.5	0.4 to 1.0
Capillary	0.53 mm id	5 to 10	3 to 7	1.3 to 2.6

GCs provide electronic pneumatic control (EPC).

Capillary Column Choice

Internal diameter (ID)	Resolution	Speed	Capacity	Outcome
0.1 mm	Excellent	Excellent	Good	Good
0.25 mm	Better	Better	Better	Better
0.32 mm				
0.53 mm	Good	Better	Excellent	Excellent

Column length	Resolution	Speed
Long (60–100 m)	High	Slow
Medium (25–30 m)	Good compromise between resolution and speed	
Short (5–10 m)	Moderate	Fast

Polarity of Stationary Phase

100% dimethyl polysiloxane

5% phenyl - arylene - 95% dimethyl polysiloxane

5% diphenyl/95% dimethyl polysiloxane

20% diphenyl/80% dimethyl polysiloxane

6% cyanopropylphenyl/94% dimethyl polysiloxane

35% diphenyl/65% dimethyl polysiloxane

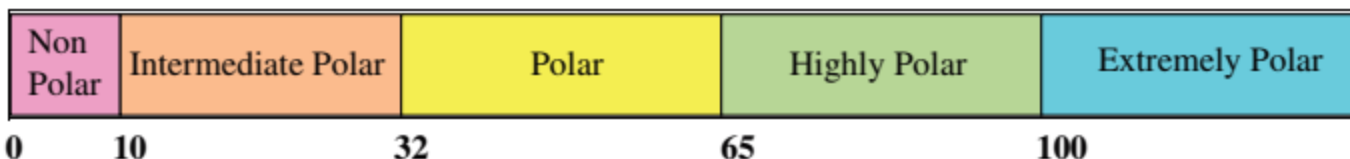
Trifluoropropylmethyl polysiloxane

50% diphenyl/50% dimethyl polysiloxane

14% cyanopropylphenyl/86% dimethyl polysiloxane

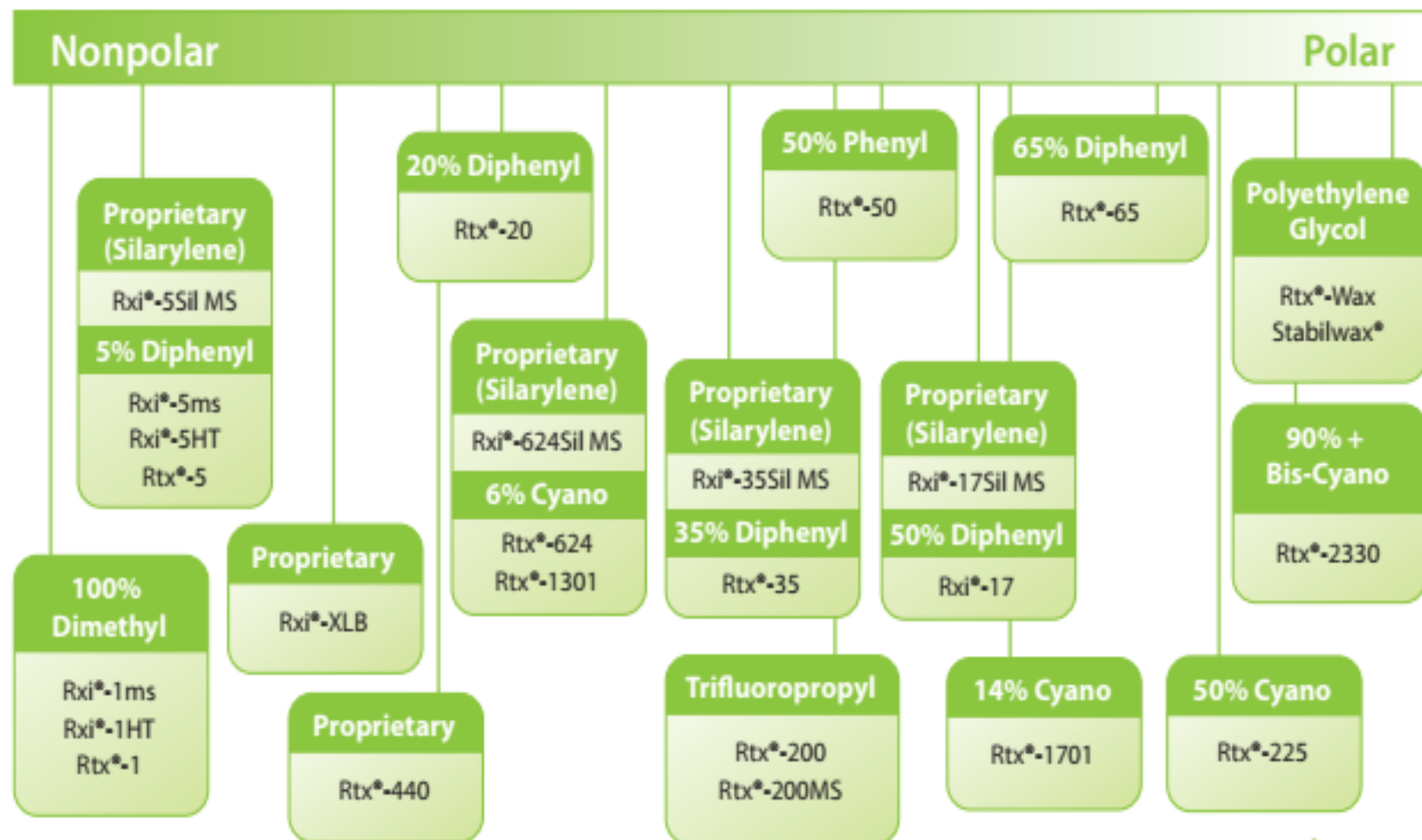
50% cyanopropylphenyl/50%Phenylmethyl polysiloxane

Polyethylene Glycol



Polarity based on ionic liquid. Number is calculated using McRaynold constant

Commercial Columns



In general, maximum operating temperature decreases as polarity increases.

Note that silarylene columns typically differ in selectivity and have higher temperature limits than their conventional counterparts.



Column Selection Criteria

Column Selectivity is based on Chemical nature of stationary phase.

Low selectivity: Elute together. High selectivity: Peaks separate well.

GC Column Selection Parameters

- Dimensions : Column length , ID, film thickness
- Conditions : temperature , flow rate
- Composition –Stationary phase composition, carrier gas
- Phase selection : Principle that "like dissolves/separates like."
- A non-polar column for analyses of non-polar compounds.
- A polar columns for the separation of polar compounds.

Note: Bonded phases are preferred. Immobilized and/or chemically bonded (cross linked) within the column.



Retention Mechanism of Column

- Non-polar columns are primarily dispersive. Based on Van-der Waals forces. Intermolecular attraction between Stationary phase and compound. Larger size/ compounds with higher boiling points have longer retention. Phases with phenyl functional groups can also undergo a moderate amount of π - π interactions. Elution order is based on boiling points.
- Intermediate polar and polar columns are strongly dispersive. Moderate amounts of hydrogen bonding and basic interactions. Phases with phenyl functional groups can also undergo π - π dipole-dipole, and dipole-induced dipole interactions. Phases with Cyanopropyl groups undergo strong dipole-dipole and moderate basic interactions.
- Highly polar columns are strongly dispersive, very strongly dipole-dipole, and very strongly dipole-induced dipole. Moderately basic interactions are also possible.



Column Efficiency

Internal Diameter (mm)	Efficiency: Plates/Meter (N/m)	Efficiency: Total Plates (N)	Capacity Each Analyte (ng)
0.53	1,300	39,000	1000-2000
0.32	2,300	69,000	400-500
0.25	2,925	87,750	50-100
0.20	3,650	109,500	<50
0.18	4,050	121,500	<50
0.10	7,300	219,000	<10

Note: Theoretical values for 30 meter columns, calculated @ a $k = 6.00$ and 85% coating efficiency



Influence of Column ID

- The efficiency ie plates (N) or plates per meter (N/m) increases as the ID of the column decreases.
- Efficiency (number of theoretical plates) and sample capacity (amount enters into column) determines the separation.
 - Less sample -Sharp peak, but low detection level
 - Higher sample may lead to overload/broad peak
- Optimize either efficiency or sample capacity to get narrow and well-resolved peaks.



Effects of Column ID.

Column Length (m)	Inlet Pressure (psi)	Peak 1 Retention (min)	Peak 1/2 Resolution (R)	Efficiency: Total Plates (N)
15	5.9	8.33	0.8	43,875
30	12.0	16.68	1.2	87,750
60	24.9	33.37	1.7	175,500

Note: Theoretical values for 0.25 mm ID columns with 85% coating efficiency, 145 °C isothermal analyses, helium at 21 cm/sec, k (peak 1) = 6.00



Attributes of Column ID

ID-0.10mm - 0.20mm	ID -0.25mm -0.32mm	ID 0.53mm
<u>Characteristics</u> <ul style="list-style-type: none">• Highest efficiency• Shorter analysis time• Lower sample loading capacity	<u>Characteristics</u> <ul style="list-style-type: none">• High efficiency• Good performance for analysis time and sample loading capacity	<u>Characteristics</u> <ul style="list-style-type: none">• Good efficiency• Longer analysis time• Higher sample loading capacity• May require higher flow rates than MS detectors can tolerate
<u>Application</u> <ul style="list-style-type: none">• Highly complex samples• Fast GC• GC-MS• Split injection	<u>Application</u> <ul style="list-style-type: none">• Complex samples• Wide concentration range• Split, splitless, direct, headspace	<u>Application</u> <ul style="list-style-type: none">• Packed column replacement• Purity analysis• Split, splitless, direct, headspace

As inner diameter increases, efficiency decreases, sample loading capacity increases, optimal flow rate increases, and analysis time increases.



Influence of Column Length

Column length

- Longer columns provide greater resolution, but increase back pressure
- To increasing resolution is to reduce column ID
- Column resolution is proportional to the square root of column length.

Factor to consider :

- Shorter columns are recommended when great resolution is not required, such as for screening purposes
- Longer Columns Increase Cost and Analysis Time



Impact of Film Thickness

Optimal film thickness should be depending on the application.

Most 0.25 mm ID columns have a 0.25 or 0.50 μm film thickness.

Decreasing Film Thickness (0.1 μm to 0.5 μm)

- Peaks are sharper in lower film thickness.
- High resolution and increase signal-to-noise.
- Reduced column bleed, can use of max. operating temperature.
- Elute with shorter retention times and at lower temperatures.
- Useful: Medium and high molecular weight compounds

Increasing Film Thickness (1 μm to 10 μm)

- Reduce the analyte-tubing interaction
- Increased sample capacity and Increased analyte retention. (good- purity testing)
- The drawback is increased peak widths (less resolution)
- Increased column bleed, and a reduced maximum operating.
- Useful: Volatile, low molecular weight compounds



Attributes of Film Thickness

0.1 μm to 0.5 μm

Characteristics

- Shorter retention times
- Lower bleed
- Higher maximum temperatures
- Lower sample loading capacity
- High resolution for high molecular weight compounds

Applications

Medium and high molecular weight compounds

1 μm to 10 μm

Characteristics

- Longer retention times
- Higher bleed
- Lower maximum temperatures
- Higher sample loading capacity
- High resolution for volatiles and low molecular weight compounds

Applications

- Volatile, low molecular weight compounds
- High concentration samples (e.g., purity testing)



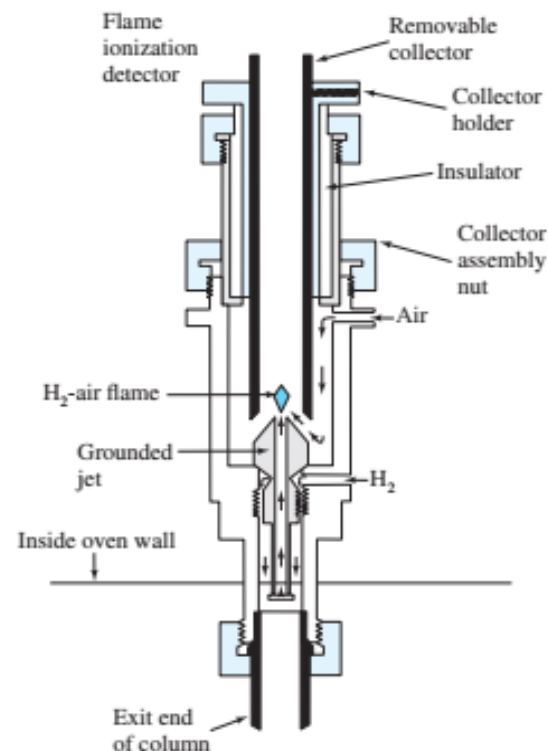
Detectors

- Detectors sense the separated components and provide a signal. Selection of detectors based on the compounds
- Detectors are destructive or non-destructive of the analytes.
- All the detectors are either concentration-dependent or mass dependant. Can connect multiple detectors for good response
- The detector set with correct temperature to prevent decomposition.

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	1 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen and phosphorous compounds	0.1 pg/s (P) 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s 2 pg S/s 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR (FTIR)	Organic compounds	0.2 to 40 ng

Flame Ionization Detector (FID)

- The carrier gas from the column mixes with hydrogen and is burned (flame) in air
- Carbon-containing material enters from column to the flame generated the ions / charged particles
- FID consists two electrodes, one is the jet where the flame burns, another electrode is wire / grid kept tip of the flame as collector.
- When charged particles enters between the electrode (pd of 100 to 300 V), collector collects the ions and current raises as per concentration.
- After amplification of the weak current, it creates the chromatogram.
- FID can be used only for the compound, which can create ions in a flame.

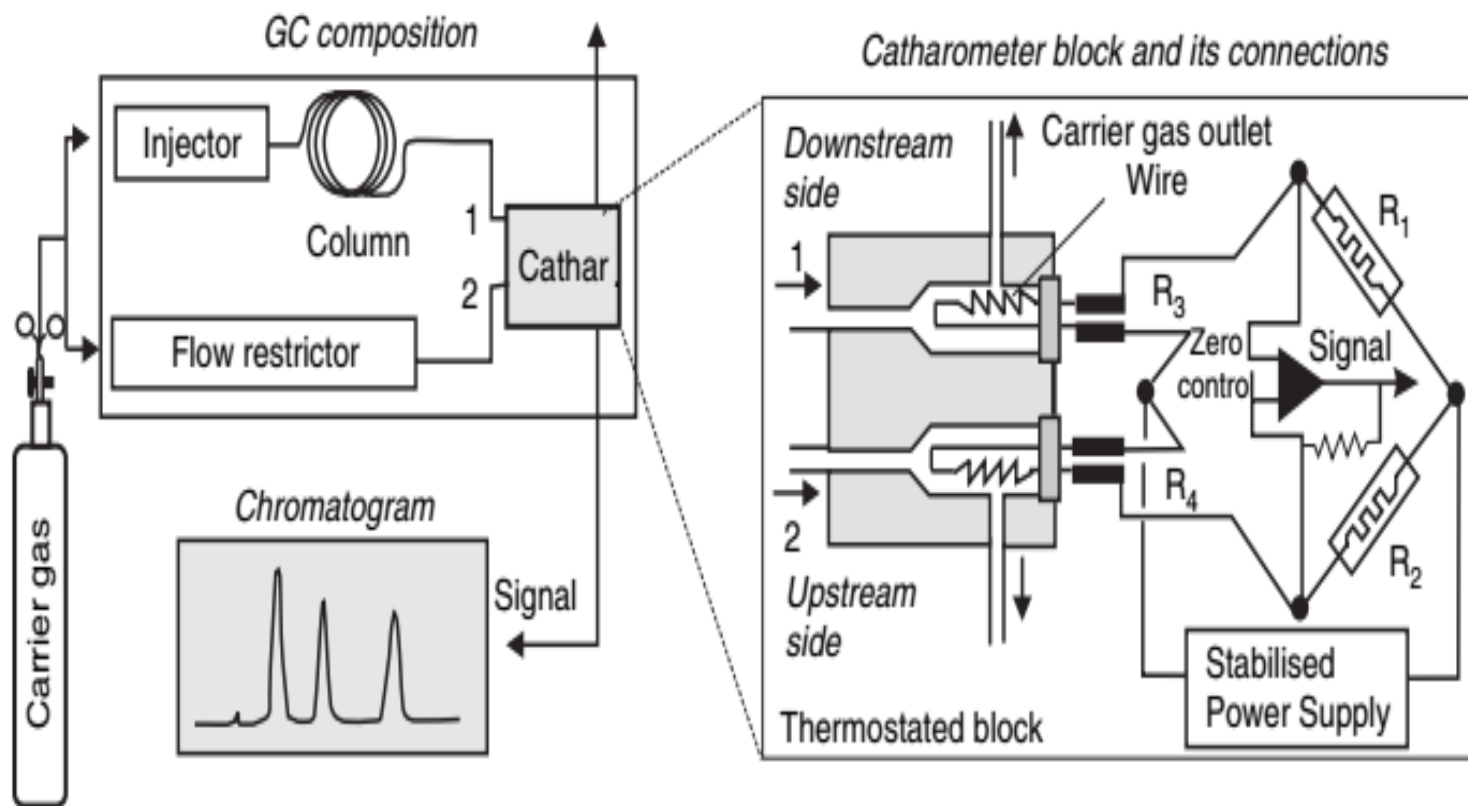




Thermal Conductivity Detector (TCD)

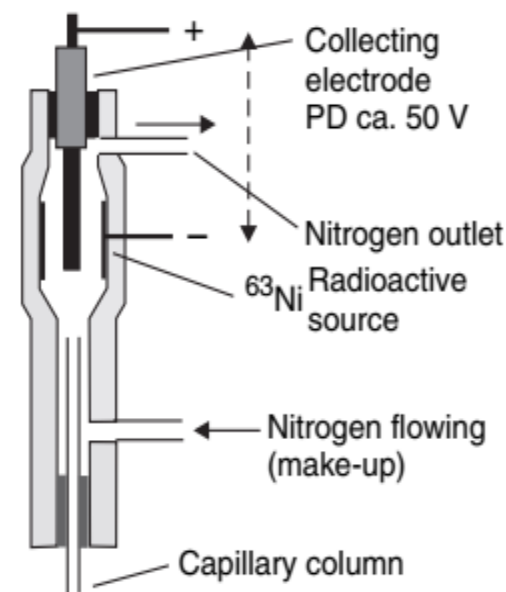
- TCD is non-destructive detector, working based on the thermal conductivity of gas mixtures as a function of their composition
- The detector consists two identical thermistors (filaments) and maintained at a constant temperature (for ref and sample from column)
- Both thermistors are located within the path of the carrier gas. One is flushed by the carrier gas evolving the column, while the other is from by a part of the carrier gas entering the injector (Figure in next page)
- In the steady state a temperature equilibrium is established between thermal conductivity of the carrier gas & electrical current in the filament.
- When a solute elutes from the column, there is a change in the mobile phase composition, which changes its thermal conductivity.
- The thermal equilibrium being disrupted and change in the resistance, which is proportional to the concentration of the compound.
- Hydrogen & helium are suitable carrier gases for TCD since high thermal conductivity. Then all other gases (compound)

Thermal Conductivity Detector (TCD)



Electron Capture Detector (ECD)

- The electron capture detector has found wide use in environmental samples due to its very high sensitivity to halogen-containing components (eg. Herbicides & pesticides).
- A radioactive isotope, usually ^{63}Ni , in the detector cell emits beta particles. These collide with carrier gas to create showers of low-energy free electrons. Two electrodes and a polarizing voltage collect the electrons as a current.
- Some molecules can capture low-energy electrons to form negative ions.
- When such a molecule enters the cell, some of the electrons are captured and the collected current decreases. This change converts into signal/ peak





GC Application

- Qualitative Analysis – by comparing the retention time or volume of the sample to the standard against the sample.
- Retention time or relative retention time can be used for identification for eluted compound. Retention times are characteristic of the compounds they represent (but are not unique). Mass Detector gives the mass value to identify precisely.
- Other detector – FTIR, NMR, Mass detector can be combined
- Quantitative Analysis- Peak area / Peak height of elution peak is proportional to the quantity / concentration. Peak response is based of the detector used.
- Method used for Quantitative Analysis shall be validated
- Type of estimation- Area normalization, Internal standard, Calibration and standard, Standard addition and etc



Type of Samples for GC Analysis

- Elemental analysis : % composition of elements C, H, N, sulfur in organic compounds...
- All volatile organic compounds like Solvents , liquid reagents...
- Silyl derivatives: Some of the organic compounds can be derivatized using Trimethylchlorosilane to reduce the boiling point and test. Eg. Glucose, fructose, sorbitol etc.
- Residual solvent from the solid material using Headspace samples with GC
- Compound can be converted to the volatile coordination complex and tested by GC



Selection of Column

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs; steroids; PCBs
Poly(phenylmethyldimethyl) siloxane (10% phenyl)	OV-3, SE-52	350	Fatty acid methyl esters; alkaloids; drugs; halogenated compounds
Poly(phenylmethyl) siloxane (50% phenyl)	OV-17	250	Drugs; steroids; pesticides; glycols
Poly(trifluoropropyldimethyl) siloxane	OV-210	200	Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids; alcohols; ethers; essential oils; glycols
Poly(dicyanoallyldimethyl) siloxane	OV-275	240	Polyunsaturated fatty acids; rosin acids; free acids; alcohols



**The following Section is Applicable for
Pharmaceutical Industries.**



Analytical Method

- Method should demonstrate specificity, linearity, accuracy, robust, rugged and sensitive, .
- Ensure that Analytical method used is validated to meet the above characteristics.
- If test method is as per monograph, ensure that analytical method is verified for its suitability Eg USP General Chapter <1226>
- Any change in the test condition shall be within the allowable limit of Pharmacopeia (next page)

Adjustment Allowed for GC condition

Property	USP General Chapter 621	Ph.Eur. Gen. Chapter 2.2.46
Column length	$\pm 70\%$	$\pm 70\%$
Film thickness	-50% to 100%.	- 50 % to + 100% (cap. columns).
Column ID	$\pm 50\%$	$\pm 50\%$
Flow rate	$\pm 50\%$ or more, provided the linear flow velocity remains the same. Velocity adjustment is between +50% and -25%	$\pm 50\%$
Oven temperature	$\pm 10\text{ }^{\circ}\text{C}$ or Program- up to $\pm 20\%$ is permitted. (when the temperature must be changed from one value to another)	$\pm 10\text{ }^{\circ}\text{C}$
Injection Volume	The injection volume and split volume may be adjusted if detection and repeatability are satisfactory.	may be adjusted, provided detection and repeatability are satisfactory

Starting GC Analysis

- Ensure required Gas and pressure before starting GC
- Test method is downloaded & verified
- Create Sequence as per test method
 - Eg Blank, system suitability (SS), reference solution, test, and bracketing standard.
- **Data file shall be continuous (do not repeat the file no/name)**
- Load the sample and run the system suitability and once SS passes run the test
- After completion of the sequence, process the chromatograms
- Print the method, sequence and data
- Perform audit trail before batch release
- Store the data and ensure the back up of data



Integration of Peaks

- Do not integrate any peak by manually.
- Integrate all the peaks or else as per procedure
- Always use same processing method for processing of blank, standard & sample chromatograms in case of Assay & related substances, etc.
- Verify the processing parameters like
 - Threshold,
 - Width,
 - System suitability,
 - Peak names etc.
- Save the processing method
- Re-integration:
 - Do not re-integrate the chromatograms without documenting.
 - Document reason for reintegration.





Common problem in GC analysis

- System failures may occur during analysis due to
 - System over pressure
 - Leak
 - Communication error
 - Failure of system suitability
 - Peak splitting/ negative peak
 - Spurious peak
 - Bracketing standard failure



Handling of Deviation/Failure

How to handle the failure ?

- SOP shall be available and shall address the handling of Lab deviation/ incidents.
- SOP shall define clearly the deviation/incident, reporting investigation, CAPA and documentation
- Record all the deviation/incident happened in chromatographic analysis
- Process all the injections including the invalid injection and report and store the data along with Raw data.
- Do not omit any injection
- Investigate the deviation/incident and find the root cause for the failure.
- Rectify the problem, take appropriate CAPA and document
- Repeat complete sample set of injections in case of sample injection failure



How to handle the problem if any

- In case of interruption, due to power failure, computer interruption, time gap due to sample preparation or due to injection for ~4 hours and if system is in continuous state of equilibrium ;
- Inject bracketing standard and proceed otherwise restart
- Deviation in RT for sample or std, is $> 15\%$ of specified RT
- Inhibit the peak upto void volume
- Use the same integration parameter for entire set
- Reprocess shall be at the same time for entire sequence

A vertical collage of science-related images. At the top is a 3D molecular model with blue and orange spheres. Below it is a laboratory setup with glass flasks and tubes. In the middle is a globe with a grid overlay. At the bottom is a scientist in a lab coat holding a flask, with a large blue 'E' or 'B' shape in the background. The text 'Ringaskiddy' is at the very bottom.

A vertical collage of science-related images. At the top is a 3D molecular model with blue and orange spheres. Below it is a laboratory setup with glass flasks and tubes. In the middle is a globe with a grid overlay. At the bottom is a scientist in a lab coat holding a flask, with a large blue 'E' or 'B' shape in the background. The text 'Ringaskiddy' is at the very bottom.

-
- A vertical collage of science-related images. At the top is a 3D molecular model with blue and orange spheres. Below it is a laboratory setup with glass flasks and tubes. In the middle is a globe with a grid overlay. At the bottom is a scientist in a lab coat holding a flask, with a large blue 'E' or 'B' shape in the background. The text 'Ringaskiddy' is at the very bottom.





Reference

1. **Chemical Analysis: Modern Instrumentation Methods and Techniques**
Francis and Annick Rouessac and Steve Brooks
2007- John Wiley & Sons Ltd,.
2. **Fundamentals of Gas Chromatography** – Agilent manual
3. **Guide to GC Column Selection and Optimizing Separations** – Restek
4. **Analytical Separation Science**, First Edition.
Basic Overview on Gas Chromatography Columns
Md. Musfiqur Rahman, A.M. Abd El-Aty, Jeong-Heui Choi, Ho-Chul Shin, Sung Chul Shin, and Jae-Han Shim
5. **Vogel's – Quantitative Chemical Analysis- 6th edition**
6. **Principles of Instrumental Analysis- 6th Edition- D.A. Skoog et al**