#### Chromatography Definition

Chromatography is defined as physical method of separation, in which the mixture of Analytes is separated using two phases, one is stationary phase and other is mobile phase which percolates through the stationary phase. The separation occurs because of difference in affinity between Analytes and stationary phase.

### Branches of Chromatography

Mobile phase (MP)	Stationary phase(SP)	Type of Chromatograp hy	Principle	Abbrevia tion
Gas	Solid	Gas-Solid	Adsorption	GSC
Gas	Liquid	Gas-Liquid	Partition	GLC
Liquid	Solid	Liquid-Solid	Adsorption	LSC
Liquid	Liquid	Liquid- Liquid	Partition	LLC
Liquid	Porous solid	Size Exclusion	Separation on size	SEC
Liquid	Ion exchange Resin	Ion exchange	Ion exchange	IEC
Liquid	Thin layer of solid on glass	Thin layer	Adsorption	TLC
Liquid	Paper	Paper	Adsorption	Paper

#### Comparison between LC and GC Techniques

- GC
- 1. Stationary phase: Solid/liquid
- 2. Mobile phase: GAS
- 3. Mobile phase does not take part in separation
- 4. Volatile Organic/inorganic compounds only
- 5. Works at comparatively low pressure
- 6. Works on both packed as well as capillary columns
- 7. Fast and better efficiency obtained
- 8. Selective columns for applications
- Range of selective detectors available for application
- 10. Environmental friendly technique

- LC
- 1. Stationary phase: Solid/Liquid
- 2. Mobile phase: LIQUID
- 3. Mobile phase takes active part in separation
- 4. Volatile as well as non volatile compounds can be separated
- 5. Works at high pressure
- 6. Only packed columns for analysis
- 7. Slow and poor efficiency
- 8. Very few selective columns available
- 9. Few selective detectors available
- 10. Solvents eluted after separation needs to be disposed off properly hence non environmental friendly technique

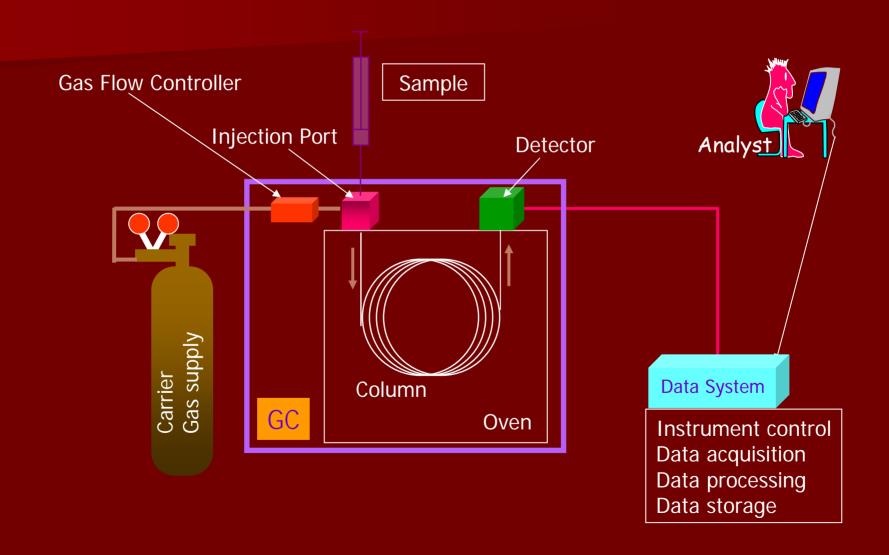
### Gas Chromatography

- A physical separation method
- It involves the distribution of component between two phases
  - stationary phase solid < (10%)</p>
    - liquid > (90%)
  - mobile phase gas

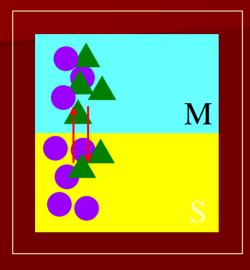
## Why use GC?

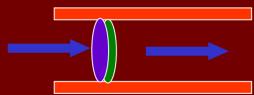
- **Short Analysis Time**
- Wide Choice of Stationary Phase
- **Wide Choice of Detectors**
- **■** Ease of Operation

#### Gas Chromatograph Main Components



### Separation of compounds





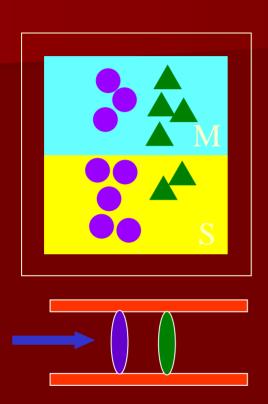
- When analytes are introduced into the column, the molecules distribute between the stationary and mobile phases
- The molecules in the mobile phase are carried down the column

M = mobile phase (carrier gas)

S = stationary phase

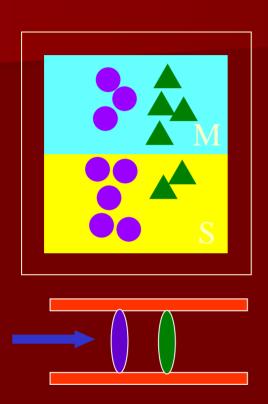
Those in the stationary phase are temporarily immobile and do not move down the column

#### Separation of compounds



• All molecules of the same compound travel through the column at nearly the same rate and appear as a band of molecules (called sample band)

### Separation of compounds

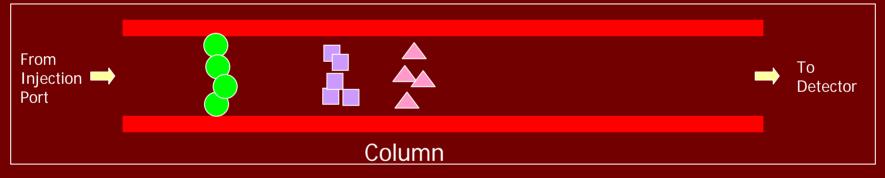


Sample band of compound which is less 'soluble' in the stationary phase moves faster, because more of the molecules spend more time in the mobile phase (carrier gas)

#### Separation in column

Retention Time
= time spent by a
compound inside
the column

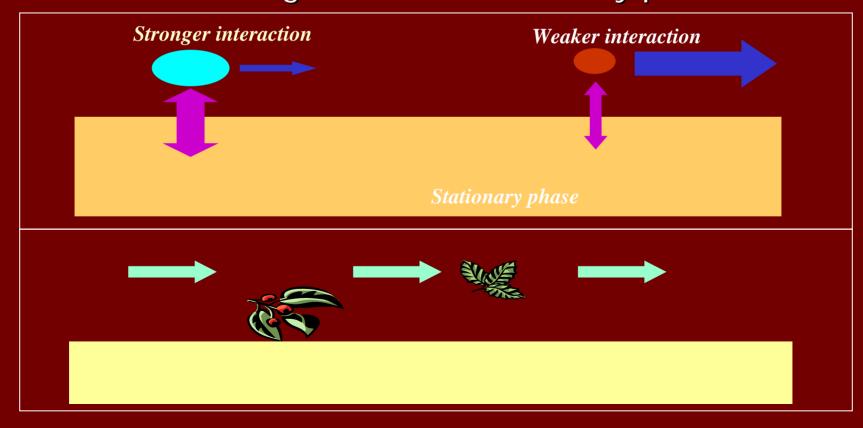






## Migration rates of compounds in column (1)

 Different migration rates of compounds can be achieved if these compounds have different interaction strengths with the stationary phase



## Migration rates of compounds in column

- Migration rate of compounds in column depend on:
  - Compound chemical structure
  - Stationary phase chemical structure
  - Column temperature

#### Retention Time (t<sub>R</sub>)

The time an analyte takes to travel through the column

A measure of the amount of time an analyte spends in the column

Sum of the time spent in the stationary phase and the mobile phase

# Retention time of an unretained compound (t<sub>M</sub>)

The time an unretained compound takes to travel through the column

 Unretained compound travels down the column at the same rate as the mobile phase (carrier gas)

Equivalent to the time a compound spends in the mobile phase

#### Retention factor (k)

- Another measure of retention
- Ratio of the amount of time a compound spends in the stationary and mobile phases
- A measure of retention by the stationary phase
- Previously called capacity factor, or partition factor

$$k = \frac{t_R - t_M}{t_M}$$

#### Distribution constant (K)

- Ratio of analyte concentration in the stationary phase and mobile phase
- K is constant for a given compound, stationary phase, and column temperature

$$K = \frac{c_M}{c_M}$$

 $c_s$  = concentration in stationary phase  $c_M$  = concentration in mobile phase

#### Separation Factor $(\alpha)$

- A measure of the time or distance between the maxima of two peaks
- $\alpha$  = 1 means the two peaks have the same retention and co-elute

$$\alpha = \frac{\mathbf{k_2}}{\mathbf{k_1}}$$

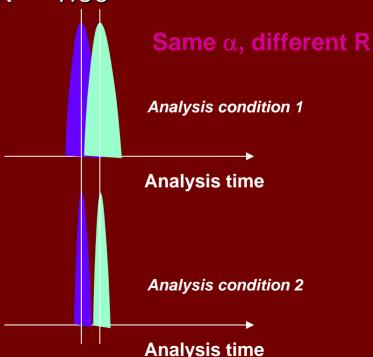
#### Resolution (R)

- A measure of overlap between two peaks; the higher the resolution, the less the overlap
- Separation (α) is only the distance between two peak maxima; resolution takes both α and the width of the peaks into account
- Baseline resolution usually occurs at R = 1.50

$$R = 1.18 \left[ \frac{t_{R2} - t_{R1}}{w_{h1} + w_{h2}} \right]$$

$$R = 2 \frac{t_{R2} - t_{R1}}{w_{b1} + w_{b2}}$$

w<sub>h</sub> = peak width at half peak heightw<sub>b</sub> = peak width at base



## Number of theoretical plates (N) or Column Efficiency

- Theoretical plates is a concept
- Theoretical plates numbers are an indirect measure of peak width for a peak at a specific retention time
- Columns with high N are considered to be more efficient than those with lower N
- A column with a high N will have a narrower peak at a given retention time
- Column efficiency is a function of:
  - Column dimensions
  - Type of carrier gas and its average linear velocity
  - Compound and its retention

$$N = 5.545 \begin{bmatrix} \underline{t}_{R} \\ w_{h} \end{bmatrix} \qquad N = 16 \begin{bmatrix} \underline{t}_{R} \\ w_{b} \end{bmatrix}$$

# Height equivalent to a theoretical plate (HETP or H)

- Another measure of column efficiency
- Small plate heights indicate higher efficiency

L = column length (mm)

N = theoretical plates number

#### GC Injection Techniques

- Types of GC injection techniques
  - Vaporization injection: sample is instantaneously vaporized upon injection (injector temperature between 140 °C to 350 °C, depending on application)
  - Cold injection: sample is injected at relatively cool injector temperature (e.g. 50 °C)

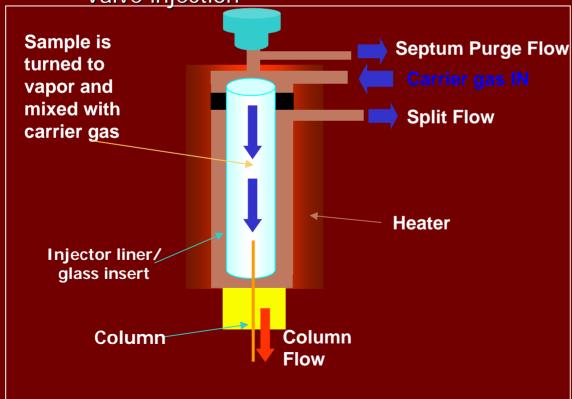
#### Injector (Injection Port)

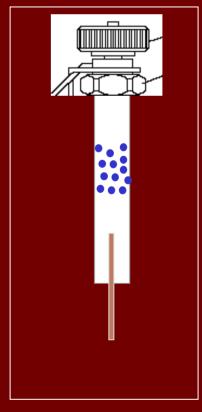
- 1. Packed Column Injector
- 2. Capillary column Injector
- Split injector
- Splitless injector
- PTV(Programming Temperature Vaporiser)
- Cool on-column injector

#### Basic GC Injector Structure

- Sample is introduced to the column through the GC injector, by using
  - Syringe injection
  - Autosampler injection

Valve injection

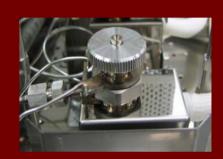




#### GC Injectors

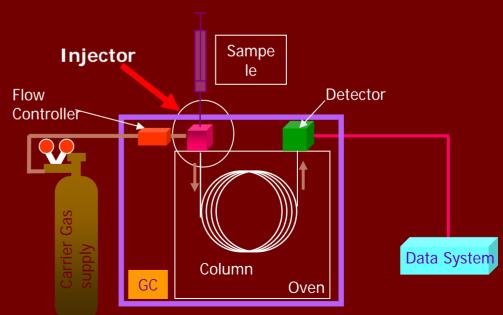
- Four types of injectors:
  - Split/splitless injector
  - Direct injector
  - Programmed Temperature Vaporization injector (not in GC-2014)
  - On-Column Injector (not in GC-2014)
  - Packed Injector (not in GC-2010)
- OCI and PTV share the same injector main body and occupies one injector position for Shimadzu GC
  - Can be used as OCI or as PTV as needed without major hardware modification
- Carrier gas supplied to the injector is controlled electronically by using electronic/digital flow controllers







Electronic Flow Controllers

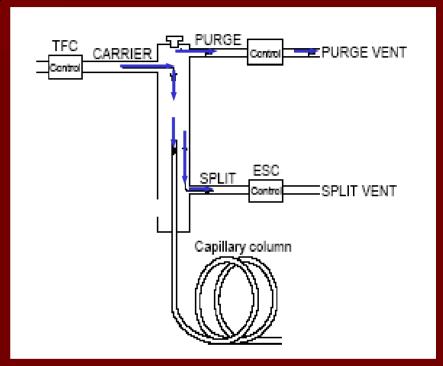


#### Split / Splitless Injection Modes

- Split injection
  - Only a portion of the sample injected is introduced to the capillary column
  - Majority of sample is vented (split) to waste
  - Typically used in major/minor component analysis
  - Typically used for higher concentration samples (higher ppm range)
  - Used in Fast GC to obtain narrow peaks
  - High pressure (pulsed) split injection
    - Pressure pulse contains sample expansion and transfers analytes to the column faster
- Splitless injection
  - Majority of sample is put onto the column
  - Relies on solvent or thermal effects for peak shape
  - Typically used in trace analysis
  - High pressure (pulsed) splitless injection
    - Pressure pulse contains sample expansion and transfers analytes to the column faster

#### Split Injection

- Sample vapor mixed with carrier gas, then flows with the carrier gas:
  - A small flow goes into the column (typically, 1-4 mL/min)
  - A much larger flow (typically 10-100 mL/min) goes out from the split vent
- SPLIT RATIO (SR) is the parameter that determines the amount of sample that goes into the capillary column
- PURGE FLOW (PF) is normally set at a low value (typically 3 to 5 mL/min)



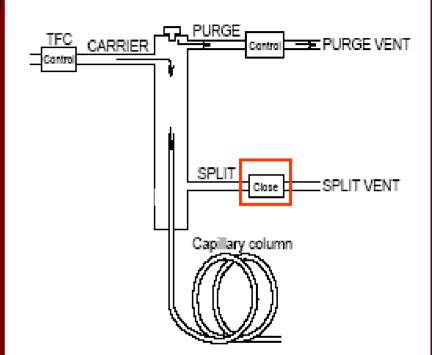
Split Ratio = 
$$\frac{\text{Split Flow (SF)}}{\text{Column Flow (CF)}}$$

### Splitless Injection

- Two main steps in Splitless Injection:
  - Sampling Time
  - After Sampling Time
- SAMPLING TIME is the parameter that determines the amount of sample that goes into the capillary column
  - Sampling time is usually set to 2 min maximum
- SPLIT RATIO still needs to be set
  - Split Ratio is set to give Split Flow of about 20 to 30 mL/min
  - e.g. For column flow of 1 mL/min, set Split Ratio = 20 to 30

## Splitless Injection (During Sampling Time)

- Split flow line is closed for a period of time called Sampling Time
  - No gas flows through the split vent
  - Almost all of sample vapor goes into the column
- To obtain good peak shapes: Column temperature should be set to a low value (guideline: 10 °C below the solvent boiling point)

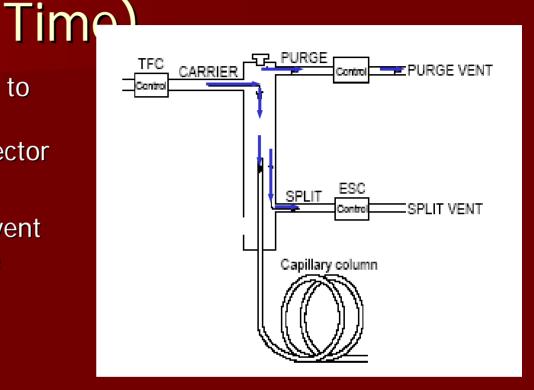


Total Flow Column Flow Purge Flow =

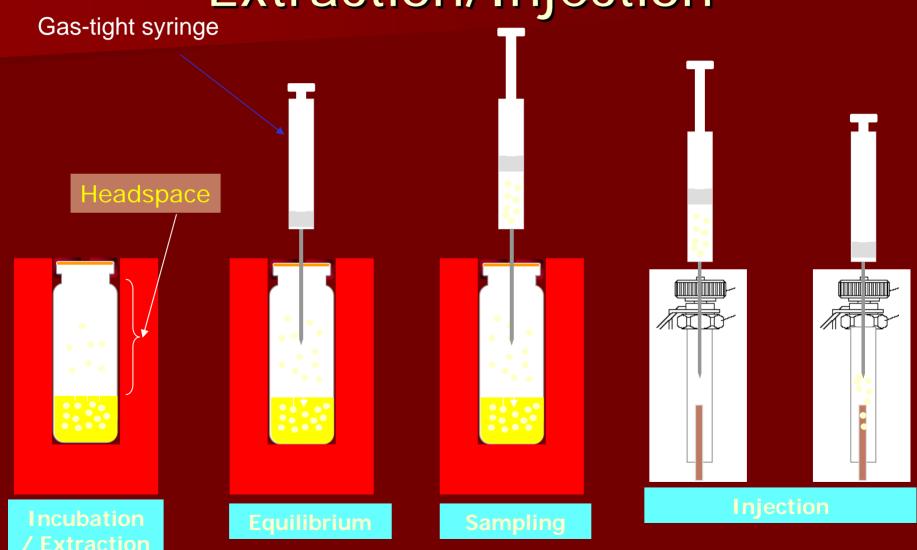
### Splitless Injection (After Sampling

 Split flow line is re-opened to purge out the remaining solvent vapor from the injector

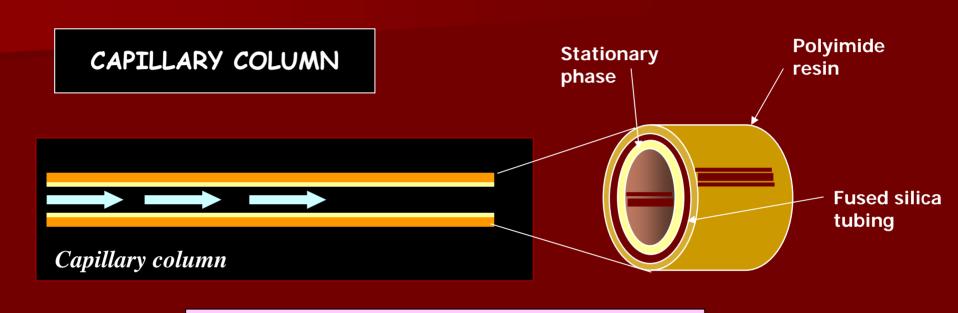
If purging is not done, solvent peak will interfere with the analysis results



Headspace (HS) Extraction/Injection



#### GC Column

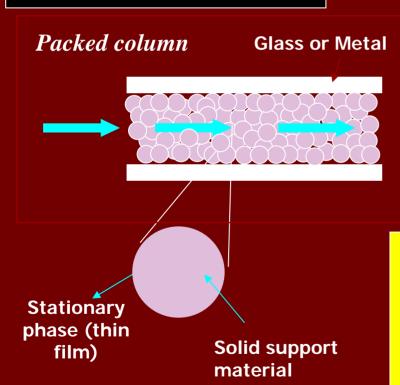


Length: 10 - ~100 m

ID (inner diameter): 0.1 mm – 0.53 mm Stationary phase film thickness: 0.1-5 um

#### GC Column

#### PACKED COLUMN



Length: 0.5-20 m ID (Inner Diameter): 2-4 mm

#### Packing Material:

- -Adsorbent (molecular sieve, activated alumina, silica gel
- -Solid support coated with thin film of stationary phase (refer to the picture)

### Packed or Capillary Column?

- General consideration : Resolution
  - Packed column
    - Low resolution
    - For small number of components
  - Capillary column
    - High resolution
    - Multi-component analysis

## Stationary Phase Selection Guide

- 100% dimethyl polysiloxane
  - Non-polar
  - Analytes examples: solvents, petroleum products, flavours, saturated hydrocarbons
- 5% diphenyl 95% dimethyl polysiloxane
  - Non-polar
  - Analytes examples: flavours, pesticides, aromatic hydrocarbons
- 35% diphenyl 65% dimethyl polysiloxane
  - Medium polarity
  - Analytes examples: nitrogen containing pesticides
- Polyethylene glycol
  - Polar
  - Analytes examples: FAMEs, fatty acids, flavours, alcohols

#### Purity and Flow rate of Carrier Gas

- 1. The higher purity of the carrier gas will prolong the column life and improve the detector senstivity.
- 2. Impurities traps or filters should be installed at the gas lines
- 3. **Hydrogen** >99.99%
  - Helium >99.995%
  - Nitrogen >99.999%
- 4. Recommended flow rate of carrier gas
  - -packed column 40 ~60 ml/min
  - -capillary column 0.5~20 ml/min

#### Columns

#### 1. Column Materials

- a) Tubing
  - i) Metal -Stainless steel Copper, Nickel, Aluminium
  - ii) Glass Pyrex
    - Fused Silica
- b) Packing Material
  - i) Adsorbent (GSC)
  - ii) Dispersant (GLC)

## Type of Columns

## 1. Packed Columns

- Length 0.5~20 m
- I. D. 2~4 mm

## 2. Capillary columns

- -Length 10~100 m
- -I.D. 0.1~0.53 mm

## Capillary Column

Most widely used column for different types of analysis

#### 2. Advantages:

- High Resolution, Short Analysis and High Detection Limit
- Inertness
- Thermally and Chemically Stable
- Low Bleeding and Ease of Regeneration

## Type of Columns

Bore, I. Dmm	Length (m)	Film Thickness um	Application
0.1	10-15	0.1	High Separtion, Fast analysis, very small column load
0.2	25-100	0.25-0.5	High separation, generally used in split type analysis
0.3	25-50	0.5-1.0	Used in splitless, on-column injection method, column load> 0.2 mm
0.5	10-12	1.0-5.0	Separtion ability equal to that of packed column. Large column load

## Which is more suitable for Sample Analysis?

Capillary system is more suitable.

#### **Because**

- The sample extracted from matrix has a lot of components.
- The concentration of these components is usually at low level.

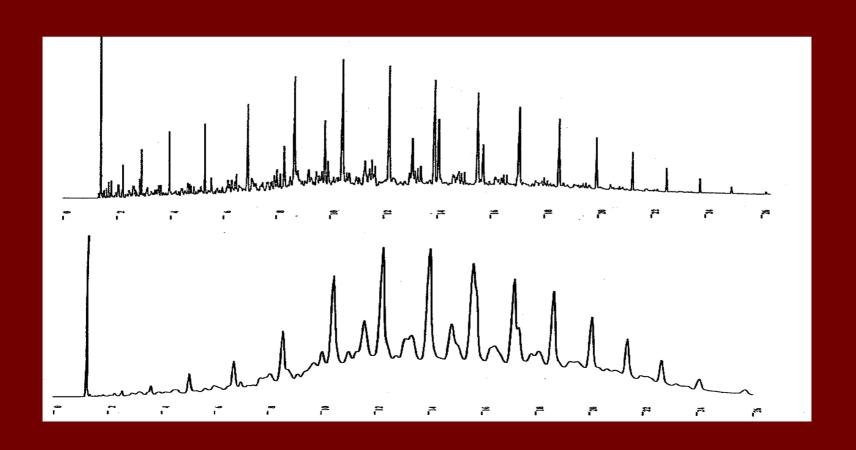
### Suitable Flow Rate

## Flow rate is important to get the good column performance.

ID (mm)	Flow Rate for He (ml/min)	Type of column
0.10	0.2 - 0.6	narrow bore
0.25	0.6 - 1.3	semi narrow bore
0.32	1.4 - 2.2	semi wide bore
0.53	4.0 - 20.0	wide bore
2.00	40.0 - 60.0	packed

## Comparison of Packed and Capillary Column

Analysis of light oil



## Make-up Gas for Capillary System

- 1.Standard detectors required a total gas flow of 40-60 ml/min for best sensitivity and peak shape
- 2. Make-up gas is added as a supplement gas flow for the capillary column (0.5-20 ml/min) to obtain a suitable flow.
- 3. The make-up gas can be the same as carrier gas or otherwise.

### Suitable Flow Rate and Sample Loading for Capillary Column

I.D.	Flow Rate for He (ml/min)	Capacity (ng per component)
0.1	0.2-0.6	10-150
0.25	0.6-1.3	40-400
0.32	1.4-2.2	60-250
0.53	4-20	80-600

### Sample Introduction

Gas

: Introduced by gas chamber or gas-tight syringe

Liquid

: Introduced by microsyringes

Solid

: Introduced by microsyringes after dissolved in a suitable solvent or using special direct injection device

### Injection Volume

#### 1. Packed Column

Gas: 0.5-50 ml

**Liquid** : 0.1-1ml

#### 2. Capillary column

Gas : 0.1-1 ml

Liquid : 0.004-2.0ul

#### Column Oven

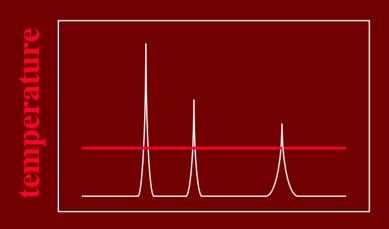
1. The column oven temperature should be high enough for the analysis to be completed in reasonable time and low enough to obtain the desired separation of sample mixture.

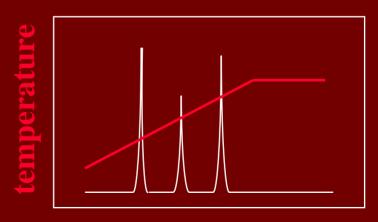
2. Isothermal Analysis

3. Temperature Programming Analysis

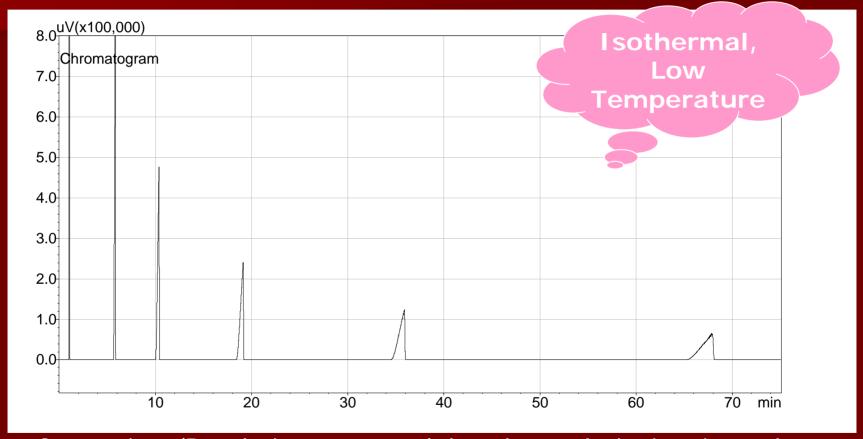
## Column Oven

# Isothermal Analysis Temperature Programming Analysis



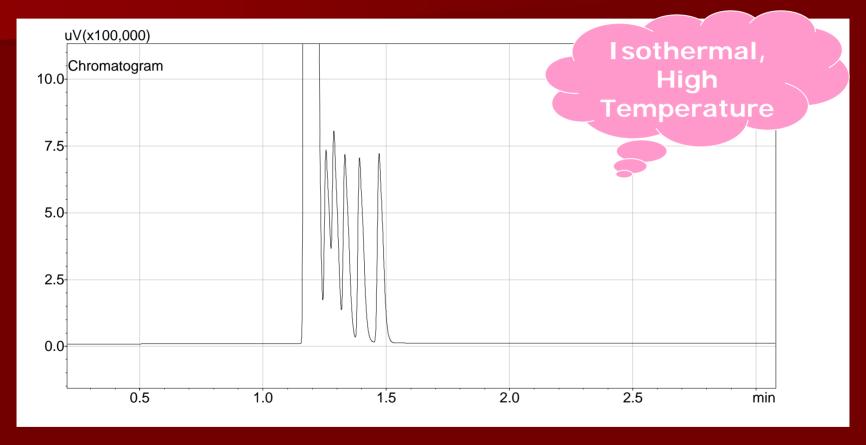


## Column Temperature Eg.1



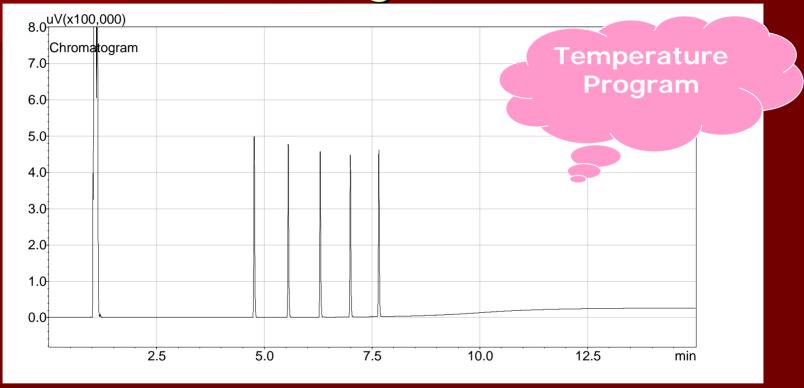
Separations/Resolutions are good, but the analysis time is too long.

## Column Temperature Eg.2



Short analysis time, but separations/resolutions are not good.

## Column Temperature Eg.3



Start from low temp and then raise the temp to a higher one in a certain rate of increase.

```
e.g.: 50^{\circ}C (2 min) \rightarrow 20°C/min \rightarrow 200°C (5 min)
80°C (5 min) \rightarrow 10°C/min \rightarrow 150°C (3 min) \rightarrow 5°C/min \rightarrow 180°C (15 min)
```

■ The lower the rate of temp increase, the better the resolution is.

## Detector

1. Detector is to indicate the presence and measure the amount of component eluted out from the column.

2. High detector temperature should be set to prevent condensation of sample

## Type of Detectors

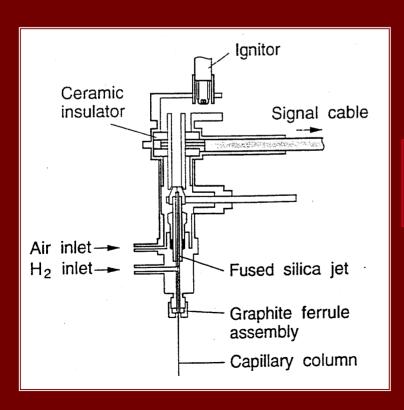
- 1. Flame Ionization Detector (FID)
- 2. Thermal Conductivity Detector (TCD)
- 3. Flame Thermionic Detector(FTD)
- 4. Flame Photometric Detector(FPD)
- 5. Electron Capture Detector (ECD)
- 6. Mass Spectrometer Detector (MSD)
- TCD, FID, MSD are general detectors.

## Type of Analysis

- FID :-Detects any compounds that can be oxdised in hydrogen/air flame
- ECD:-Selective to electronegative moieties e.g.halogens
- FTD:-Selective to organic N or P compounds
- **FPD:-Selective to P or S containing compounds**
- TCD:-Detects any component including  $N_2$  and  $O_2$  except the gas used for the carrier gas.

### FID

Most organic compounds can be detected.

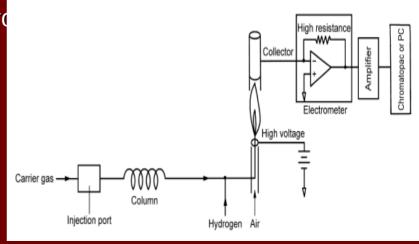


These compounds are oxidized in hydrogen / air flame

CH 
$$\xrightarrow{\text{Oxidation}}$$
 CHO<sup>+</sup> + **e**

#### Flame Ionization Detector (FID)

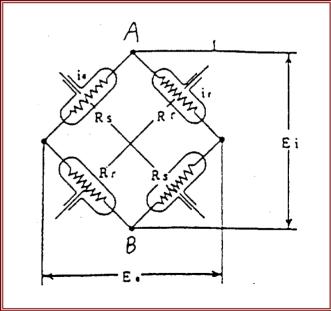
- For organic compounds analysis
- Hydrogen and air are needed to create the flame.
- Sample is brought to hydrogen flame and converted into ions. Current will be generated.
- The current is proportional to the amount of the organic compound present
- Advantages like
- High Sensitivity to organic compounds
- Little or no response to water CO2, the common carrier gas impurities hence zero signal to when no sample is present.
- Stable baseline which is not mostly afftected by fluctuations in carrier gas temp, flow rate and pressure.
- Good linearity over a wide range of sample conc. range.
- Ion quantity generated is almost pro atoms in a compound, but:
  - Presence of halogens in the compound decreases sensitivity





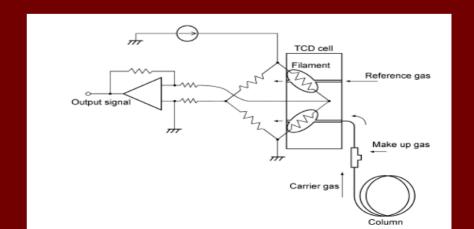
Any components including N2 and O2 except the gas used for carrier gas can be detected by TCD

Wheatstone bridge is used as principle of detection.



## Thermal Conductivity Detector (TCD)

- Detects almost all compounds except the carrier gas.
- The TCD filament is heated by applying a current
- When carrier gas + sample gas passes over the filament, the temperature of the filament increases, because the thermal conductivity of the sample compounds is less than that of the carrier gas alone.
- The changes in filament temperature affect its resistance
- The resistance change is measured and produces the signal
  - Advantages over FID
- Responds to all organic as well as inorganic compounds
- Non destructive type of detector hence can be used for trapping separated components and preparative analysis
- Low cost and versatile.



## **ECD**

Very selective and sensitive to electrophilic compounds (e.g.

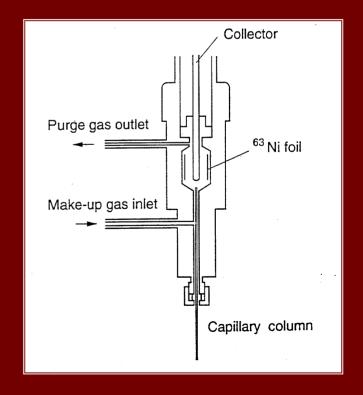
halogens)

$$N_2$$
 $N_2^+ + e^-$ 

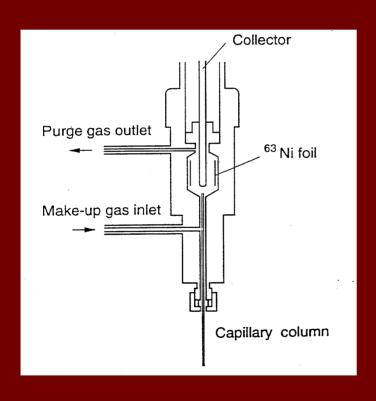
PCB + e

PCB - (neutral compound)

reverse

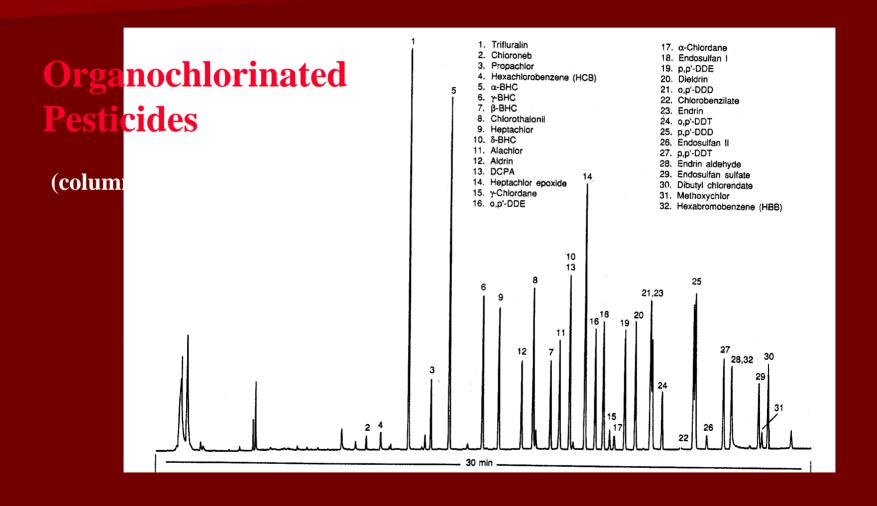


## Electron Capture Detector (ECD)



- Detects electrophilic compounds, e.g.: halogenated compounds
- Good for low concentration of these kind of compounds
- Contains <sup>63</sup>Ni radioactive
- Examples of analyses:
  - analysis of PCBs (polychlorinated biphenyls)
  - analysis of PBBs (polybrominated biphenyls)
  - analysis of PBDEs (polybrominated diphenyl ethers)
  - analysis of organochlorine pesticides (DDT, BHC, etc.)

## ECD Application



## Qualitative Analysis

#### 1. Direct Comparison of Retention Time

The simplest procedure in the identification of unknown.

A known standard is first analysed under the specific GC conditions, followed by the unknown also under the similar conditions.

The difference in the retention times should not be more than +/-0.1 min for proper identification

#### Quantitative Analysis

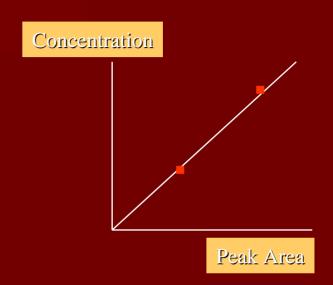
## 1. External Standard Method

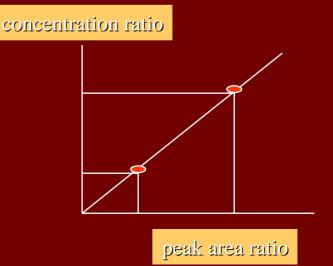
- Concentration of an unknown A  $C_A = C_1/A_1 \times A_A$  C = concentration , A = peak area

## 2. Internal Standard Method

- Concentration of an unknown X

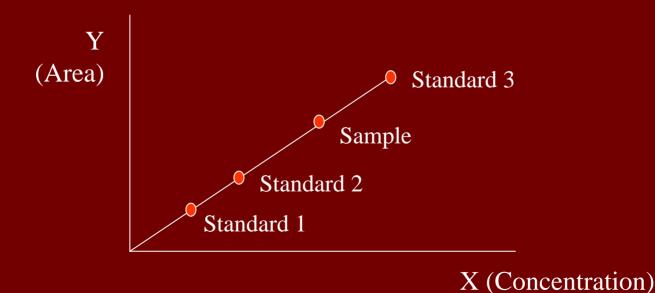
$$C_x = (C_A/C_S)/(A_A/A_S) \times A_X/A_{IS} \times W_{IS}$$
IS, S=internal standards





#### **External Standard Method**

 The peak area or height of standard sample (2known concentration) is compared to that of the sample of unknown concentration



## Introduction to GC solution Software

### **GCsolution Software**



## Components in GCsolution



#### **Analysis**

- -Click this figure to access GC Real Time Analysis.
- -Function: to perform system startup, set up system configuration, develop method/analysis conditions, perform data acquisition, perform system check, system shutdown

#### Postrun

- -Click this figure to access GC Postrun
- -Function: to see chromatograms of data acquired, process peaks, create calibration curves, and quantify unknown samples, create reports.

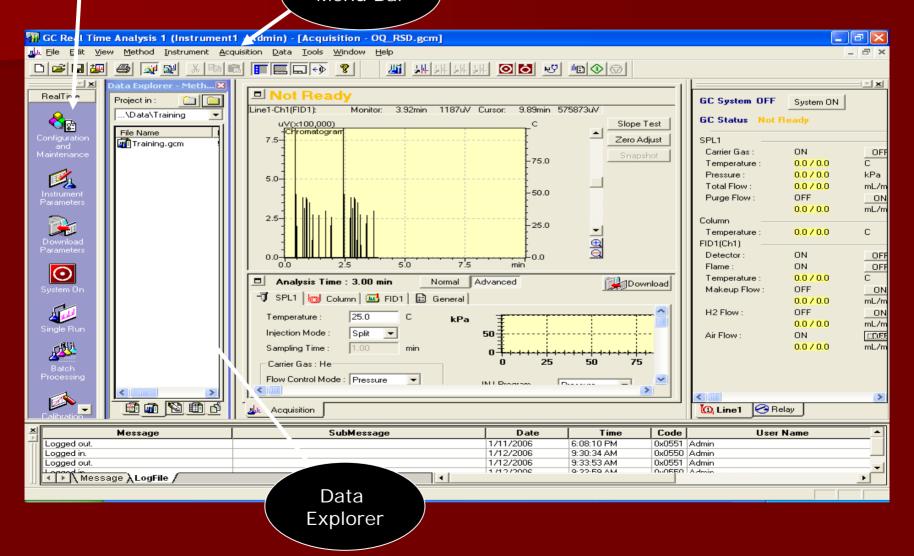
#### Offline Editor

- -Click this figure to access GC Real Time Analysis (Editor)
- -Function: to develop method, system configuration, batch table offline

## GC Real Time Analysis

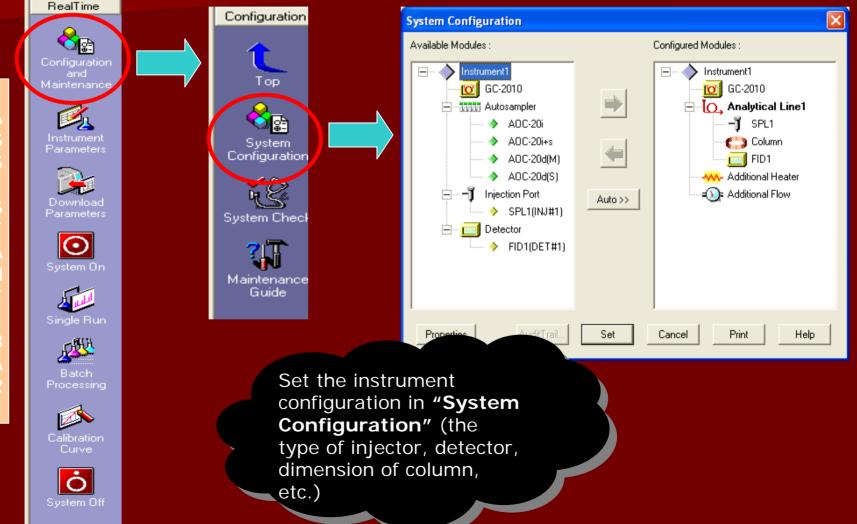
GC Real Time Analysis
Assistant Bar

Menu Bar

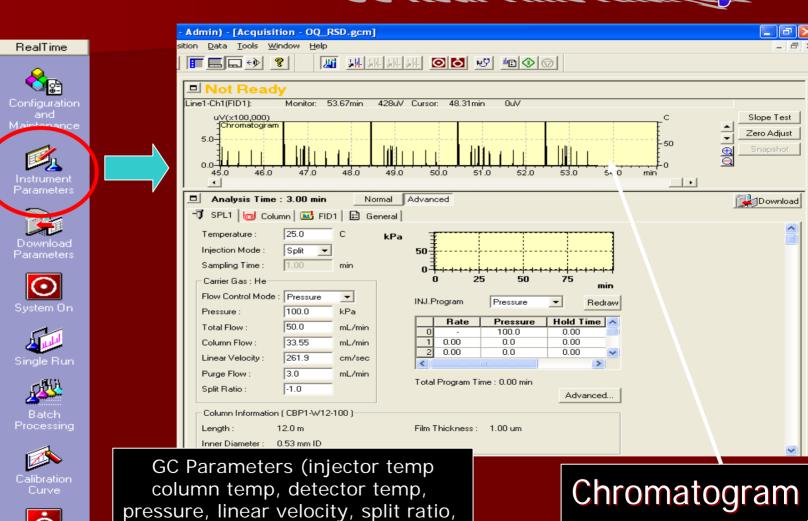


### System Configuration

#### GC Real Time Analysis



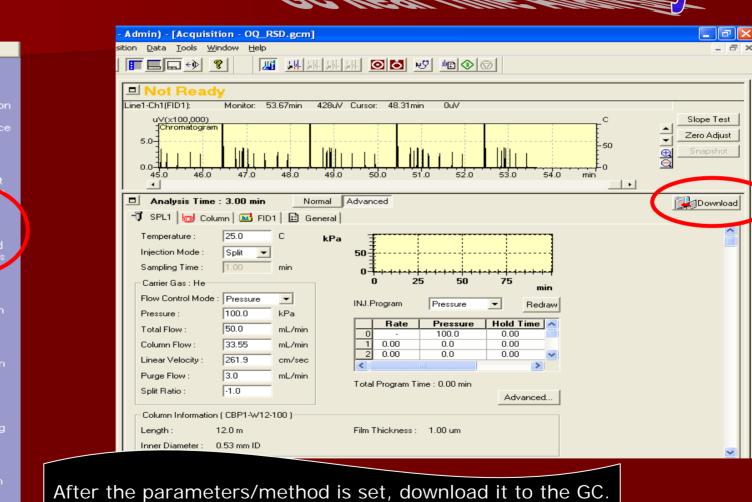
## 



column flow, etc) are set here.

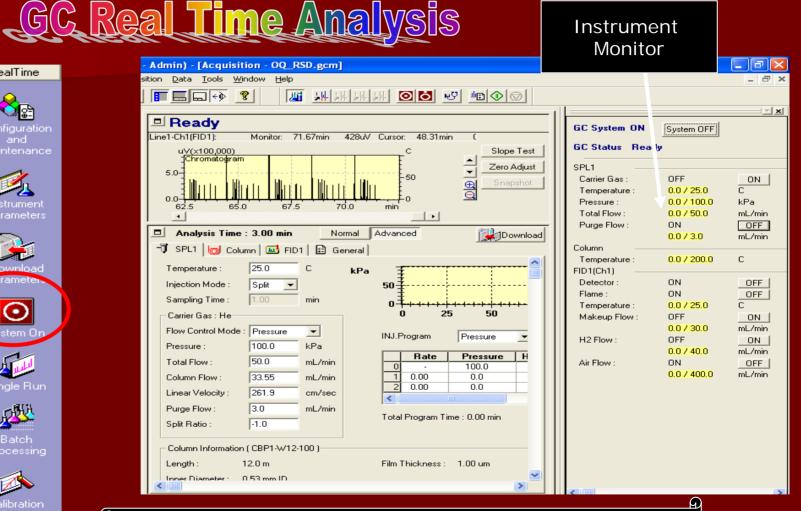
RealTime

## Instrument Carameter Malysis



After the parameters/method is set, download it to the GC. Save the parameters/method in Method File. RealTime

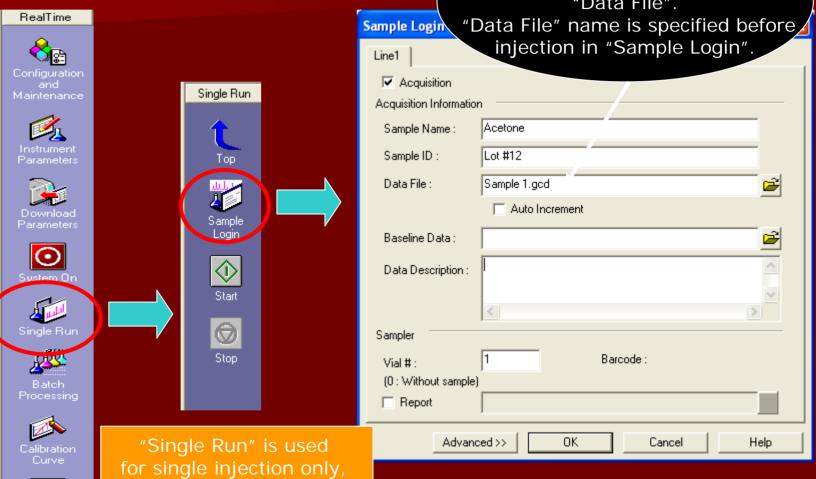
#### Instrument Parameters



Click "System On" if the GC System is still Off. Instrument Monitor displays the GC Parameters set values and actual values.

# Single Run GC Real Time Analysis

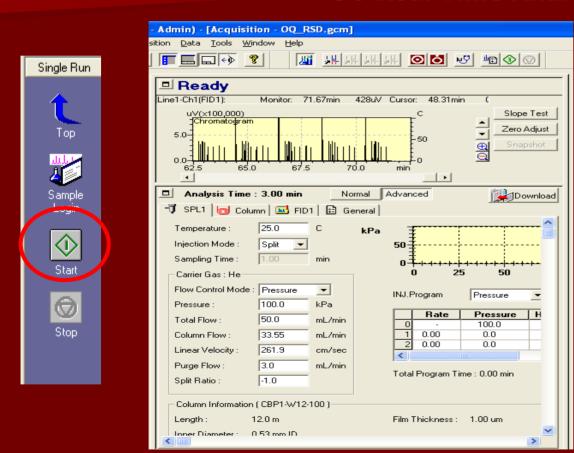
other data will be saved in "Data File".



either by manual injection or by an auto-injector.

### Single Run

GC Real Time Analysis



For **Manual Injection**, click the "Start" Button. Wait until the GC Status displays "Ready/Standby". Inject the sample to the injector, and press "Start" on GC.

For Automatic Injection using Auto-Injector, click the "Start" Button.
The auto injector will automatically inject the sample.

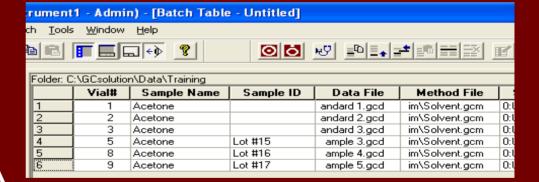
### **Batch Processing**

GC Real Time Analysis



Batch Processing is used, if it is desired to analyze more than one time in a row.

Normally, this is used with an auto-injector.

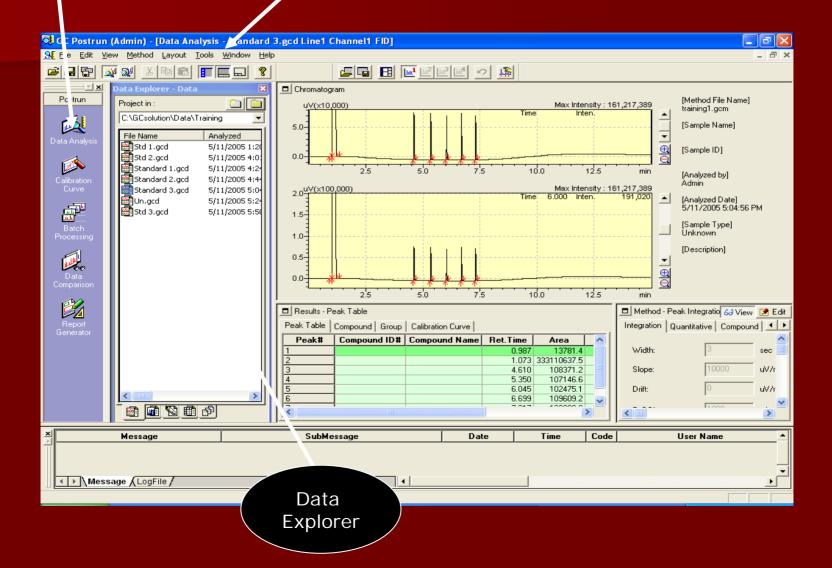


After the batch table above is created, save it in the batch file.

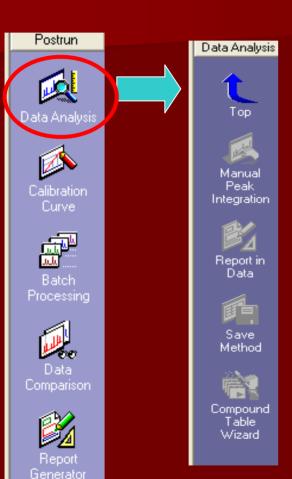
Afterwards, click the "Start" button to begin the analyses.

GC Postrun Assistant Bar Menu Bar

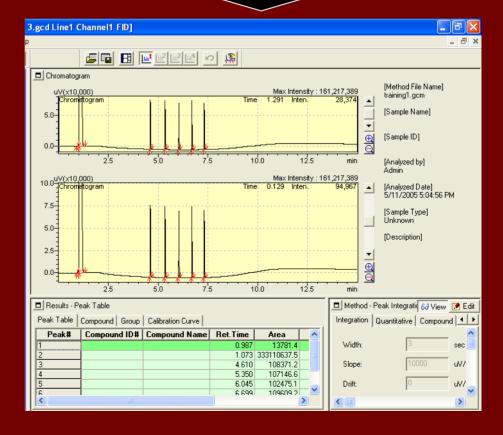




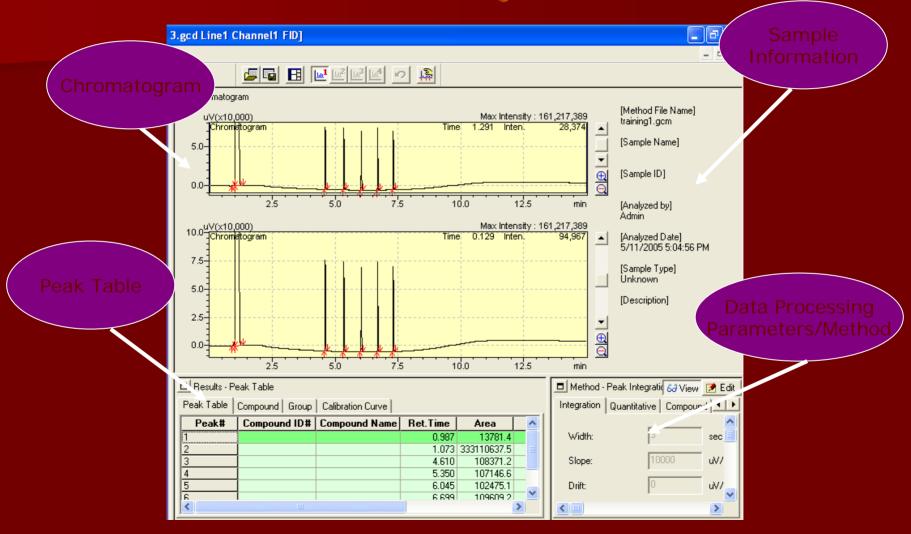
## Data Ang Postrum



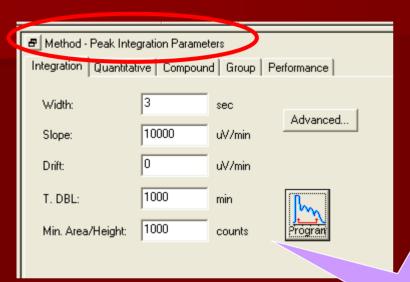
Open "Data File" in Data Analysis. Chromatogram, Sample Info, Peak Table, and Method for data processing will be displayed.



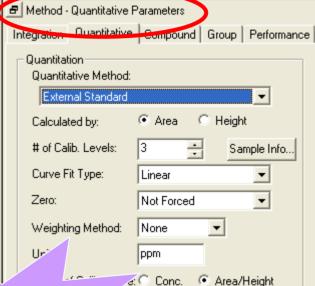
# Data Arff Postrum



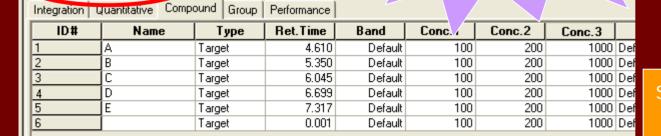
#### Data Processing Paradate Data Paradate Data Processing Paradate Data Paradate



■ Method - Compound Table



Data Processing
Parameters/Method



Save the "Method File" again, after changing these Parameters.



Compound

Table

Wizard

Type

Target

Target

Target

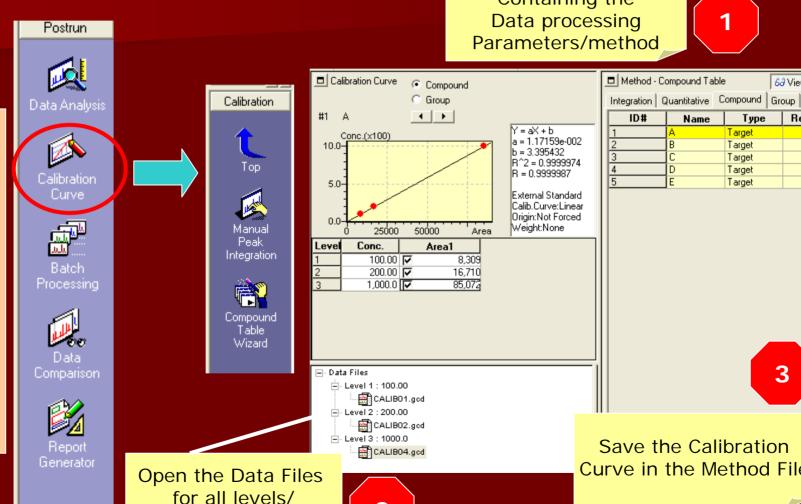
Target

Target

68 View

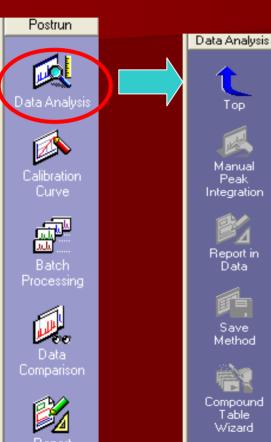
Rel

3



concentrations

Save the Calibration Curve in the Method File



Generator

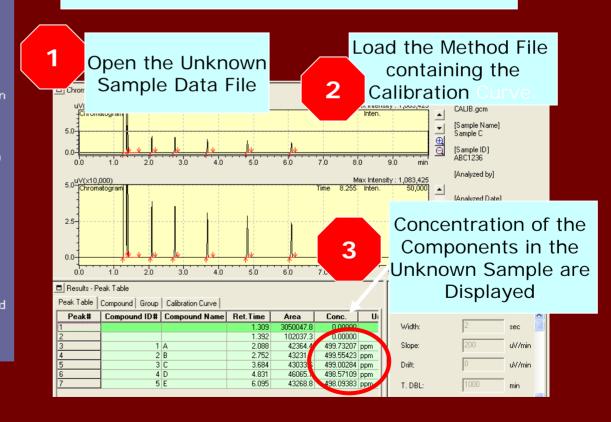
Тор

Peak

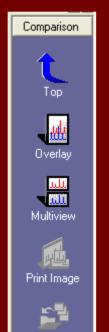
Data

Table

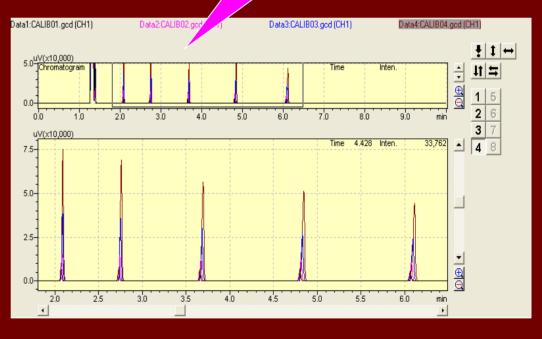
After creating & saving the Calibration Curve, the software can calculate the concentration of the unknown sample



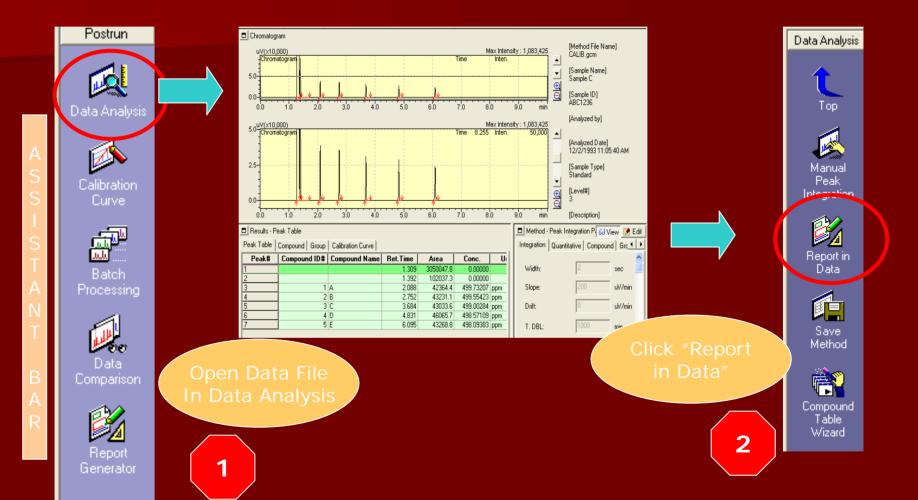




Close All Active Data Data Comparison
Can compare
Up to 8 Data Files



# Reporting Construit



Reporting in Data

