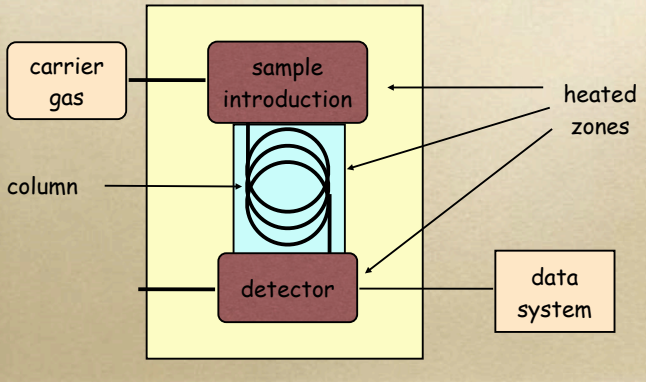
Gas chromatography

First instrumental chromatographic method developed commercially.

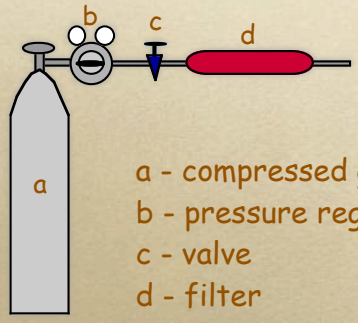
Reason - it is relatively easy to produce a stable flow and pressure for the mobile phase - carrier gas.

All that is really needed is a tank of compressed gas, pressure regulator and a valve.

Schematic of a packed column gas chromatograph



Flow control

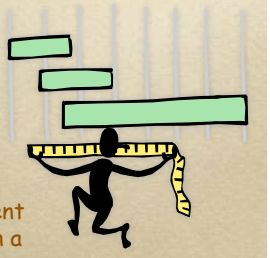


a - compressed gas cylinder
b - pressure regulator
c - valve
d - filter

Flow measurement

While flow is relatively easy to control, it still must be measured.

Bubble meters - post column or detector measurement of flow. Cheap and relatively accurate.



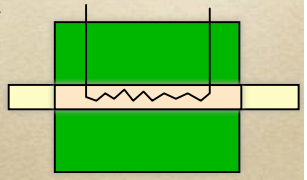
Rotameters - pre-column measurement of flow via position of ball floating in a calibrated glass tube.

Flow measurement

Electronic flow sensor

A modified thermal conductivity detector.

Permits continuous measurement of flow over a reasonably large range.



Must be calibrated for accurate flow measurement.

Response will also vary based on carrier gas used.

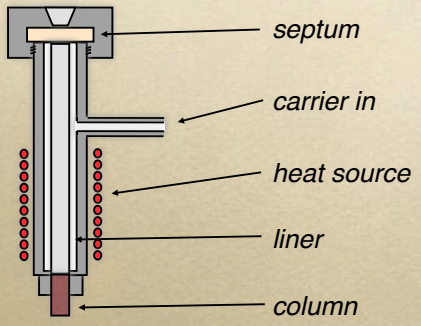
Injection methods

With packed column GC, this is a pretty simple portion of the system.

Two basic approaches

Injection ports
Sampling loops / valves

Injection port



Injection port

Purpose of port is to flash evaporate your sample and introduce it into the column.

T_{INJ} 25 - 50°C above T_{column}

Injection is through a septum.

Septum must be
- stable at the T_{inj}
- replaced regularly to maintain seal

Injection port

Liner.
Provides a known area for the flash vaporization.

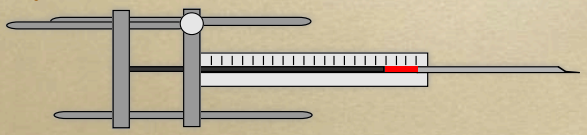
Typically made of glass although metal liners may be used. Some instruments don't have liners. Some columns will extend through the port, directly to the septum.

It can and should be replaced at regular intervals - all non-volatile materials and degradation products end up here.

Syringes

Syringes are used to introduce a known volume of a liquid or gas samples .

Adapters can be used to help control the volume injected.



Syringes

Various styles are available

- Fixed needle
- Removable needle
- Several needle lengths and angles

Sample volumes from < 1 μl an up
Body loading
Through the barrel plungers

Syringes

'Through the barrel' syringe equipped with a Chaney Adapter and removable needle.

Autoinjector

Syringe injection methods

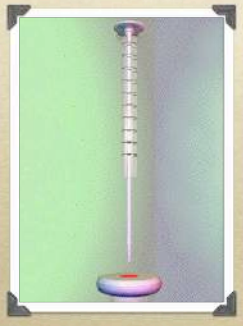
Major source of precision error is from poor injection technique.

Both automatic and manual injection methods are available.

If automatic equipment is present, use it. If not, several approaches can be tried to help reduce injection errors.

Syringe injection

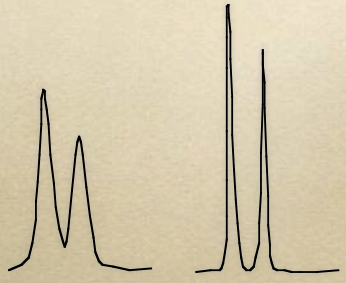
The first step is to make sure that the syringe is properly loaded -- containing no air bubbles.



Syringe injection

Samples should be injected as a plug.

Rapid and consistent injection is necessary in order to obtain acceptable precision.



Syringe injection

After injection, it is relatively easy to determine how much material remains in the needle.

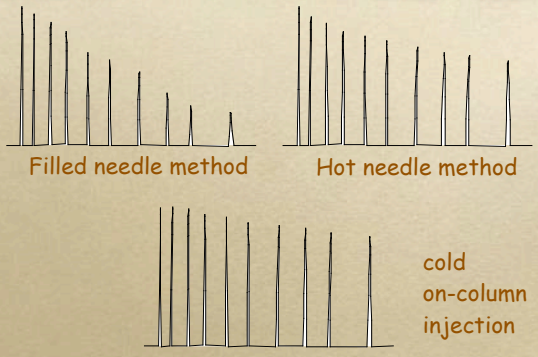
Syringe injection

When loading a syringe, you can see how much sample is in the barrel but not in the needle.

During injection, some sample can volatilize from the needle tip, causing poor precision.

This can result in sample discrimination- worst for capillary column injections.

Example of sample discrimination. C_{10} - C_{24} n-alkanes



Filled needle method

Hot needle method

cold on-column injection

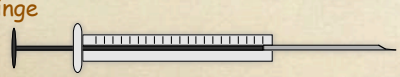
Syringe injection

Several techniques can be tried to help improve manual injection precision.

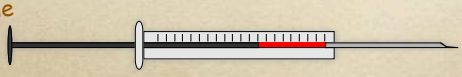
Try different, less volatile solvent
Use syringe guide
Use 'through the barrel' syringe
Measure needle volume
"Hot needle" method
Syringe loading

Needle volume correction

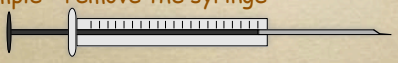
Load sample into syringe



Read the volume



Rapidly inject the sample - remove the syringe



Start run then pull back on syringe

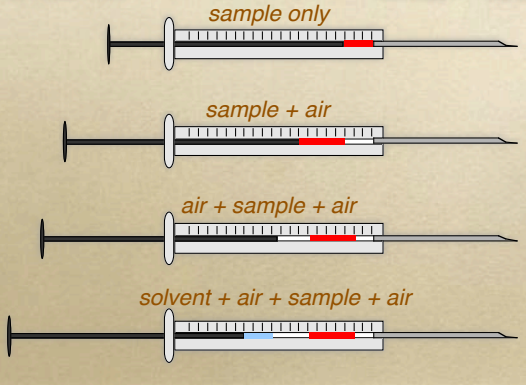
Subtract the needle volume from the total

Hot needle method

1. Draw sample into syringe barrel.
2. Next, draw 2-3 µl air into barrel.
3. Insert needle into injection port and allow to heat for a few seconds.
4. Rapidly inject sample and withdraw the needle.

This insures that all sample is injected and the 'hot needle' assists in solvent volatilization.

Syringe loading methods



Sample size

Liquids

0.1 - 10 µl is typical

Gases

0.5 - 5 ml is typical

Injection precision with a syringe is +/- 1%

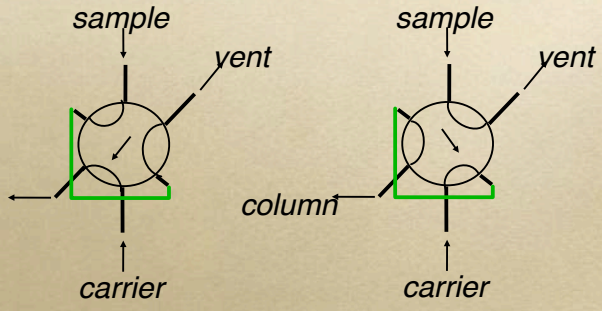
Gas sampling loops

Introducing a constant amount of a gas can be difficult with a syringe.

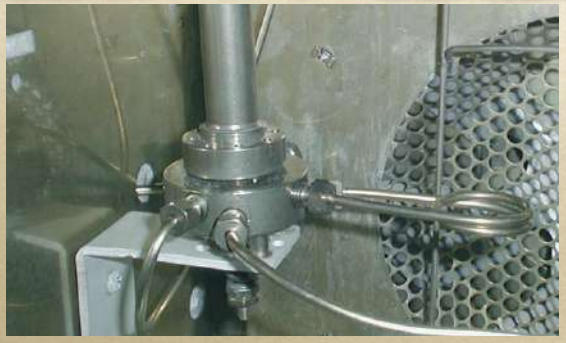
Gas sampling loops and valves offer a high precision (+/- 0.1%) means of introducing gases.

Equipment is relative inexpensive and only requires a constant temperature for easy use.

Sampling loops



6 port valve



10 port valve

The lower portion is normally covered with insulation.

It's been removed so you can see the ports.

Thermal desorption

- An alternate method of 'injecting' samples.
- Samples are trapped on an adsorbent and then thermally desorbed and injected.

Thermal desorption

Columns

- Heart of the separation process.
- Vast number of materials have been evaluated.
- It is usually best to refer to various catalogs as an up to date reference.
- Can be classified by tubing diameter and packing type.

Types of columns

Conventional

1/8-1/4" OD, stainless steel or glass tube
6 - 20 feet in length

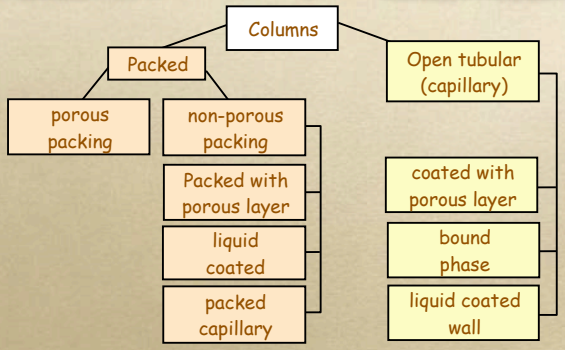
Preparative

> 1/4"
>10 feet in length

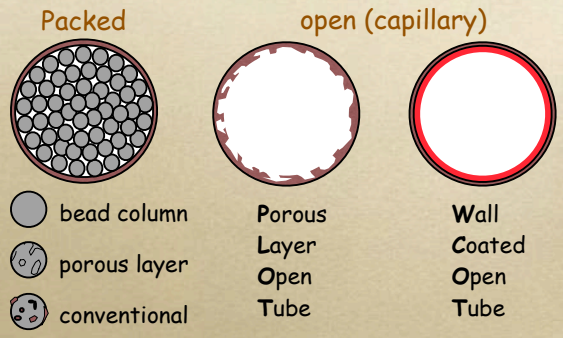
Capillary

0.1 - 0.5 mm ID
10 - 100 meters in length

Types of columns

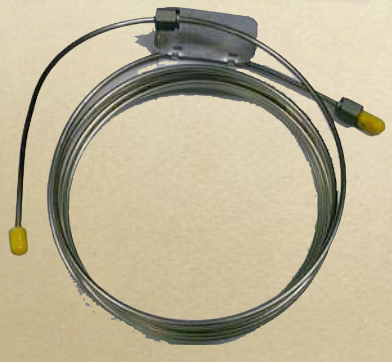


Types of columns



1/4" packed column

1/8" packed column

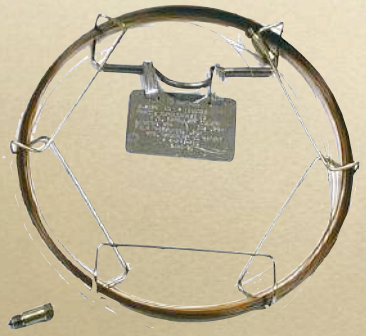


Stainless steel capillary column

This is a real antique.

All of these were internally coated.

Fused silica capillary column



Packed column selection

We'll deal with capillary columns in the next unit. For now, let's go over how to select an appropriate column.

Unless you're developing new packing materials or methods, the best starting point is to consult a chromatographic catalog.

They provide a wealth of information regarding cost, temperature limits, sample applications . . . .

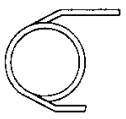
Examples of stationary phases

Phase [USP Code] (Solvent)	Temp. (°C) Min/Max
OV-351, 10g (C) (suggested substitute: SP-1000)	50/270
OV-1701, vinyl, 3g	0/250
β,β-Oxydipropionitrile, 50g (M)	0/75
Phenyldiethanolamine succinate **[G12]**, 25g (C)	0/230
Polyethylene glycol adipate (EGA) **[G23]**, 25g (A)	
Polyethyleneimine, 50g (A)	0/175
Polyphenyl ether (5 rings) OS-124, 25g (A)	0/200
Polyphenyl ether (6 rings) OS-138, 25g (A)	0/225
Polypropylene glycol, 50g (M)	0/150
Polypropyleneimine, 10g (C)	0/200
PPE-20 (poly-M-phenoxylene) (C)	125/375

Examples of packings

Packing Description	Use	Min./Max. Temp. (°C)
Activated Alumina		
Alumina F-1, 60/80 50/100	Light Hydrocarbons	300 max.
GP 10% Apiezon L/2% KOH on 80/100 Chromosorb W AW	Amphetamines	225 max.
10% Apiezon L/2% KOH on 80/100 Chromosorb W AW	Amines	50/225
GP 35% BC-120 on 100/120 Chromosorb P AW-DMDCS	Benzene	0/125
35% BC-150 on 100/120 Chromosorb P AW-DMDCS	Benzene	0/240
Carbopack B		
Carbopack B, 60/80	Light Hydrocarbons	>300
Carbopack B HT, 60/80, (hydrogen treated)		225
40/60 Carbopack B HT® 100 Monopak®	Sulfur Gases	150 max.
4% CARBOWAX 20M/0.8% KOH/80/90 Carbopack B Monopak®	Amines	220 max.
5% CARBOWAX 20M/GP 80/90 Carbopack B	Blood Alcohols	225 max.
4% CARBOWAX 20M /80/120 Carbopack B-DA▼	C2-C5 Acids	200 max.
5.0% CARBOWAX 20M/80/120 Carbopack B AW Monopak®	Alcoholic Beverages	225 max.
6.6% CARBOWAX 20M/80/120 Carbopack B AW	Alcoholic Beverages	225 max.
5% Fluorad on 60/80 Carbopack B (see Packed Column section)	Freons	150 max.
2.5% Ucarite NW/80/90 Carbopack B	Alcohols, Esters, Ketones, General Halogenated Organics	200 max.
1% SP-1000/60/80 Carbopack B		225 max.

Examples of empty columns

Chromatograph	Sketch (not to scale)
HP 5880, 5890 Configuration B• TCD only	

	X Y S	Length	OD	ID (mm)
X	11.02" 280mm	6'/1.83m	¼"	2
Y	7.09" 180mm	10'/3.05m	¼"	2
S	9"	6'/1.83m Other◆	¼"	4

Packed column examples

Supports, Coated (Cat. No. of packing)

10% Carbowax 20M on 80/100 Chromosorb W AW, 6 ft. (1-1994)
10% Carbowax 20M on 80/100 SUPELCOPORT, 6 ft. (1-1810)

3% OV-17 on 80/100 SUPELCOPORT, 6 ft. (1-1953)

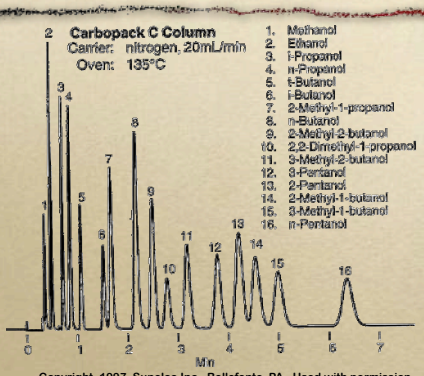
10% SP-1000 on 80/100 SUPELCOPORT, 10 ft. (1-1872)
10% SP-1000 on 80/100 SUPELCOPORT, 20 ft. (1-1872)

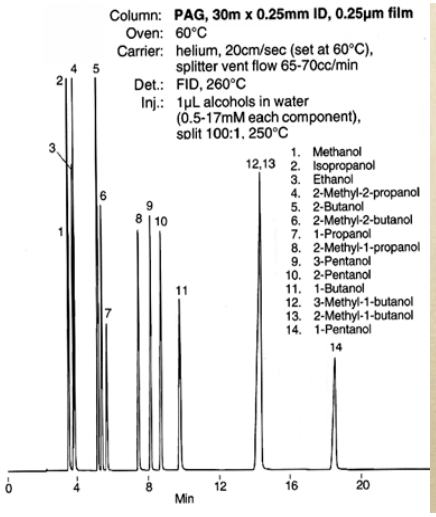
5% SP-1200/1.75% Bentone 34 on 100/120 SUPELCOPORT, 6 ft. (1-2134)

5% SP-1200/1.75% Bentone 34 on 100/120 SUPELCOPORT, 6 ft. (1-1734) *For EPA Method 602*

3% SP-2100 on 100/120 SUPELCOPORT, 6 ft. (1-1738)
10% SP-2100 on 80/100 SUPELCOPORT, 6 ft. (1-2140)
10% SP-2100 on 80/100 SUPELCOPORT, 10 ft. (1-2140)
10% SP-2100 on 100/120 SUPELCOPORT, 6 ft. (1-1989)
10% SP-2100 on 100/120 SUPELCOPORT, 10 ft. (1-1989)
20% SP-2100/0.1% Carbowax 1500 on 100/120 SUPELCOPORT, 10 ft. (1-1821)
10% SP-2330 on 100/120 Chromosorb W AW, 6 ft. (1-1851)

Example application





Example application

When all else fails!

Most chromatographic supply companies offer toll free technical support.

Before calling, be ready to answer with

- Instrument and model.
- Injection and detection method.
- If capillary or packed columns are desired.
- Type of sample, solvent and concentrations of each component expected.

Kovats retention index

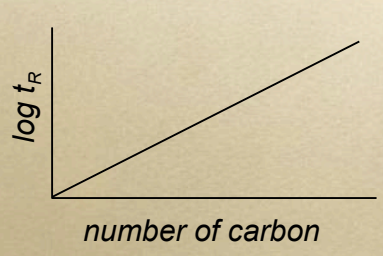
Each chromatographic setup will vary to some degree.

Retention times for a known set of species can be hard to reproduce from one lab to another or even one instrument to another.

Retention indexing helps to standardize your results.

Kovats retention index

Method is based on results of homologous series where log $t_R \alpha$ n .



Kovats retention index

The index value for any material can be found from:

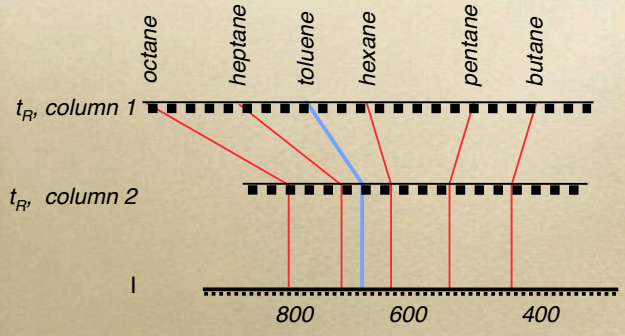
$$I = 100\frac{\log V^{O}_{R(unk)} - \log V^{O}_{R(n-C_n)}}{\log V^{O}_{R(n-C_{n+1})} - \log V^{O}_{R(n-C_n)}} + 100n$$

V^{o}_{R} - net retention volume, can use t_R

$V^{o}_{R}(n\text{-}C_n) < V^{o}_{R}(unknown) < V^{o}_{R}(n\text{-}C_{n+1})$

n-paraffins are used as reference standards and must bracket the unknown.

Kovats retention index



Kovats retention index

All that is really being done is to normalize each component compared to n-paraffins.

It assumes that you are dealing with either identical or at least very similar columns or packings.

Packing that have large differences can result in peaks eluting in different orders - the method would then be useless

McReynolds constants

Method to evaluate a wide range of phases.

- Based on measuring performance for a set of representative substances.
- Often provided by suppliers
- Can tell if two phases should give comparable performance or if a phase is better for specific functional groups.

McReynolds constants

Group	Substance	Symbol
aromatic, olefinic	benzene	X′
alcohols, phenols, acids	1-butanol	Y′
ketones, ethers, esters, aldehydes	methyl-n-propyl ketone	Z′
nitro, nitriles	nitropropane	U′
bases, aromatic hetrocyclics	pyridine	S′

McReynolds constants

Squalane is used as the reference material and all other packings are normalized to it.

Packing	T_{MAX}	X′	Y′	Z′	U′	S′
squalane	150	0	0	0	0	0
SE-30	350	15	53	44	64	41
OV-7	350	69	113	111	171	128
Carbowax 20M	250	322	536	368	572	59

Temperature programming

The column sits in an oven.

If the temperature is held constant during the entire analysis it is isothermal.

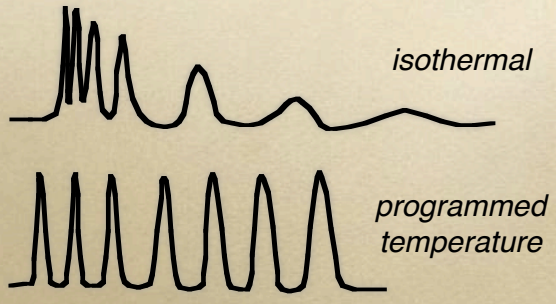
If you vary the temperature during the analysis, you typically use a temperature program.

Why bother?

Temperature programming

- With homologues, the retention time increases exponentially with the number of carbon.
- As t_R increases, width increases and the height decreases, making detection impossible after a few peaks have eluted.
- Since solubility of a gas in a liquid decreases as temperature goes up, we can reduce the retention of a material by increasing T_{column}.

Example



Temperature programming

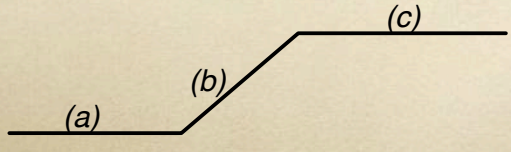
Factors to consider:

- Variations in solubility of solutes
- Changes in volatility of solutes
- Stability of solutes
- Flowrate changes
- Stability of stationary phase

Must stay within T_{min}/T_{max} of column.

Other factors are found experimentally.

A temperature program



a - initial temperature and time
b - ramp (°C/min)
c - final hold time and temperature

Most GCs will allow for a more complex program.

Temperature programming

General steps to create a program assuming that the separation is possible.

1. Determine initial temperature and time based on best possible separation of first few peaks.
2. Repeat 1 for the last few peaks to find the best final temperature and time.
3. Experiment with various ramps to account for the rest of the components.

Detectors

We need a way to measure our eluents as they evolve from the column.

Virtually every method of directly or indirectly observing eluents as been looked at.

We'll cover some of the more common types.

Detectors

Each can be roughly classified based on

Destructive vs. nondestructive

General vs. some discrimination
vs. very discriminating

Let's start by reviewing some general concepts such as detection limit and sensitivity.

Properties of a good detector

High sensitivity - possible selectivity

Rapidly respond to concentration changes

Large linear range

Stable with respect to noise and drift

Low sensitivity to variations in flow, pressure and temperature

Produces an easily handled signal

Detector Response Characteristics

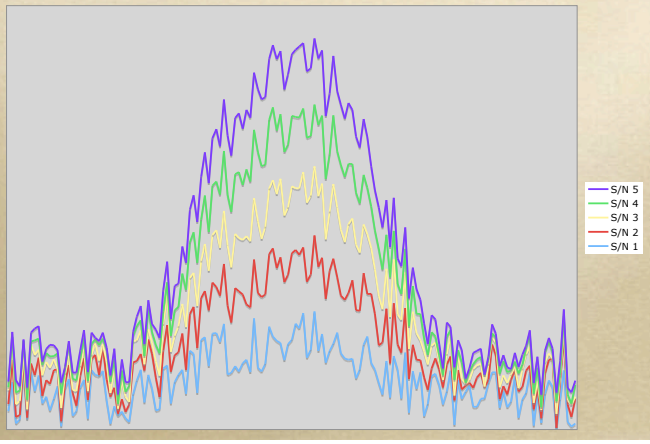
Sensitivity
Response per amount of sample. Slope of response/amount curve.

Minimum detectable level (MDL)
The amount of sample in which the peak height is 2 or 3 times the noise height.

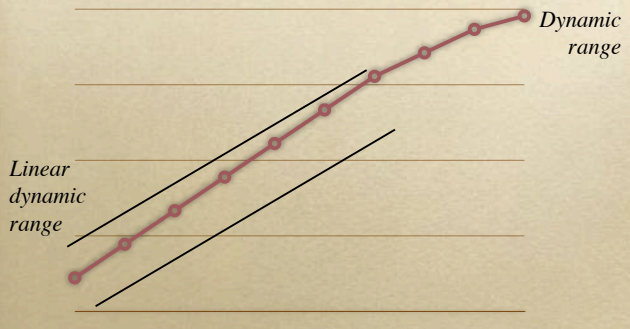
Dynamic Range.
Range where detector gives increasing response with increasing amount (amount/ml carrier gas.)

Linear Dynamic Range.
Range when detector gives linear response (amount/ml carrier gas.)

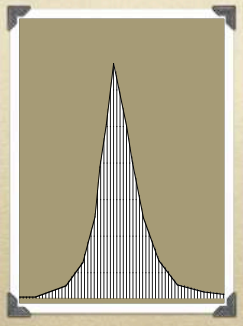
Signal to noise example



Dynamic Range



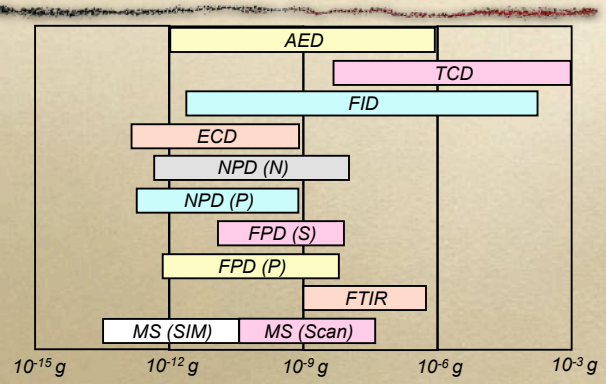
Concentration Linear range.



Linear range of detector will tell you maximum at any given measurement.

Linear range for analysis will work work with the sum of all measurements -- range is much larger than detector range.

GC detectors sensitivities and ranges



Thermal conductivity detector

- General purpose
- Nondestructive
- Limit of detection ~ 400 pg/ml carrier
- Linear range ~ 10^6

Mode of detection

Change in resistance of a wire based on variations in the thermal conductivity of the gas evolving from a column.

Representative thermal conductivity values, 100°C

Species	Thermal conductivity 10^5 cal/cm sec °C
hydrogen	49.93
helium	39.85
nitrogen	7.18
ethane	7.67
water	5.51
benzene	4.14
acetone	3.96
chloroform	2.33

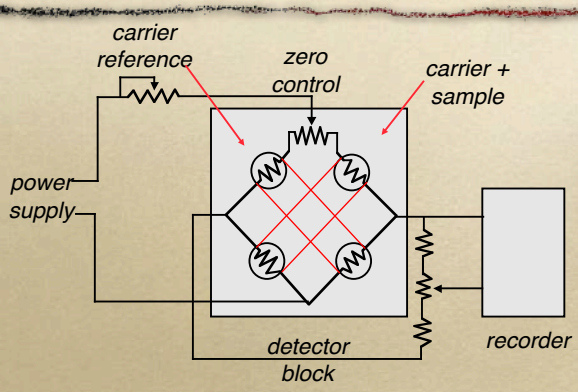
Thermal conductivity detector

While hydrogen has the largest TC value, helium is commonly used - less reactive.

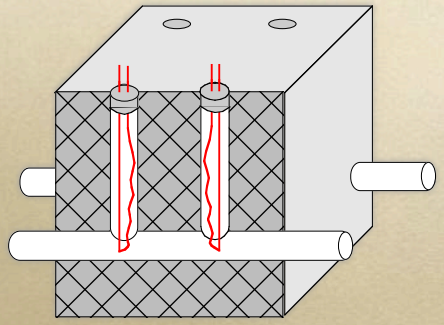
Hydrogen will give a negative peak when helium is the carrier gas.

Peak response is a function the the TC value for a species so you must standardize for each eluent of interest.

Thermal conductivity detector



Thermal conductivity detector



Thermal conductivity detector

Dual channel detectors require both an analytical column and a blank column.

- accounts for response changes due to
  - variations in temperature
  - column bleed

Single channel TCD systems are available that correct for temperature variations.

Flame ionization detector

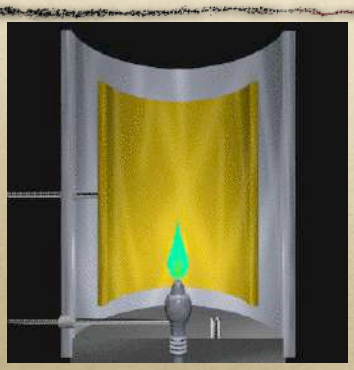
- Specific - sample must be combustible
- Destructive
- Limit of detection ~ 5 pg carbon / second
- Linear range ~ 10^7

Mode of detection

Production of ions in a flame result in a current that can be measured.

A make-up gas may be required to maintain an optimum flow - capillary columns

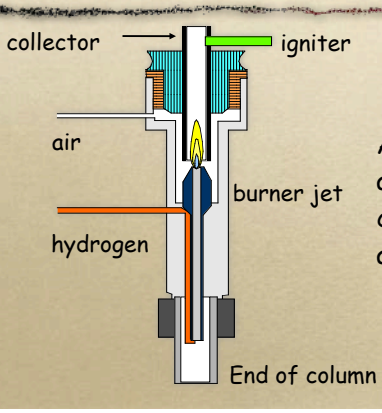
Flame ionization detector



Sample components enter at the base of the detector. They mix with hydrogen and enter the flame.

Ions are produced that can be measured.

Flame ionization detector



A make-up gas may also be present if capillary columns are to be used

Flame ionization detector

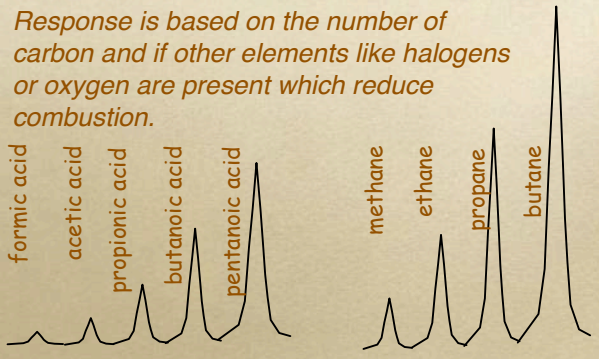
Compounds with little or no FID response

noble gases	NH_3	CS_2
NO_x	CO	O_2
H_2O	CO_2	N_2

perhalogenated compounds
formic acid, formaldehyde

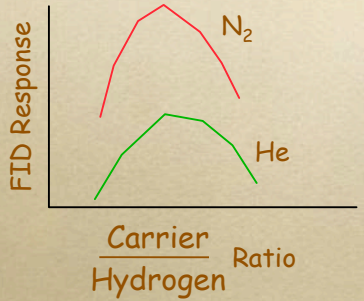
FID response

Response is based on the number of carbon and if other elements like halogens or oxygen are present which reduce combustion.



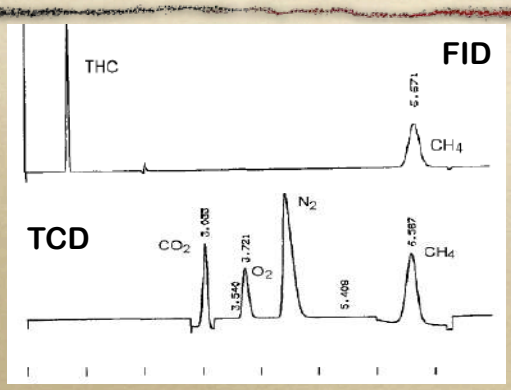
Effect of carrier gas type

The carrier gas used will affect the type of response you obtain.



View of FID and TCD

FID and TCD



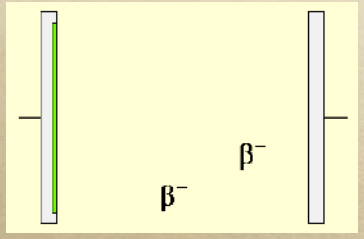
Electron capture

- Specific - sample must contain a gas phase electrophore
- Non-destructive
- Limit of detection ~ 0.1 pg Cl / second
- Linear range ~ 10^4

Mode of detection

Absorption of β particles by species containing halogens, nitriles, nitrates, conjugated double bonds, organometallics.

Electron capture

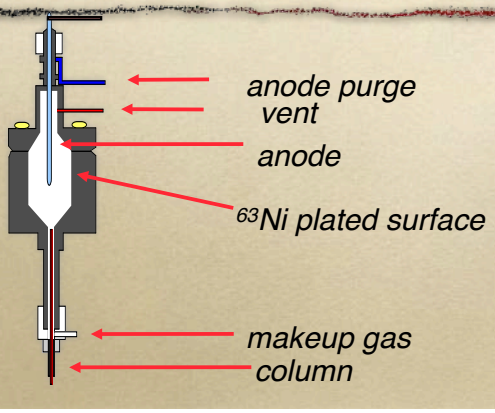


β^- are emitted by an ^{63}Ni source.

Electrophores will absorb β^-, reducing the current.

This is the basis for the response

Electron capture



Electron capture detector

Provides excellent trace analysis of
- halogenated compounds
- nitro group compounds
- eluents with conjugated double bonds

Most common use is environmental analysis of organochlorine pesticides

Major problem - detector is radioactive. Requires regular area testing and must be licensed.

Electron capture detector

Relative responses

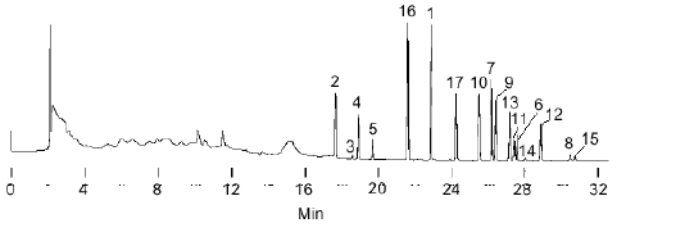
10^0	hydrocabons
10^1	esters, ethers
10^2	alcohols, ketones, monochlorides, amines
10^3	monobromides, dichlorides
10^4	anhydrides, trichlorides
10^5 - 10^6	poly halogenated, mono and diiodo

ECD example
Chlorinated pesticides in apples

Stationary Phases: PTE-5, SPB-608, SPB-1701
Column Dimensions: 30m x 0.25mm ID, 0.25µm phase film
Catalog Nos.: **24135-U** (PTE-5), **24103-U** (SPB-608), **24113** (SPB-1701)
Oven: 100°C to 280°C at 6°C/min
Carrier: helium, 40cm/sec.
Det.: ECD, 250°C
Sample: 1µL of 0.1µg/mL extract, split/splitless 45 sec, 250°C

1. Aldrin
2. α-BHC
3. β-BHC
4. γ BHC
5. δ-BHC
6. 4,4'-DDD
7. 4,4'-DDE
8. 4,4'-DDT
9. Dieldrin
10. Endosulfan I
11. Endosulfan II
12. Endosulfan sulfate
13. Endrin
14. Endrin aldehyde
15. Endrin ketone
16. Heptachlor
17. Heptachlor epoxide
18. Methoxychlor (not observed with P

PTE-5 Column

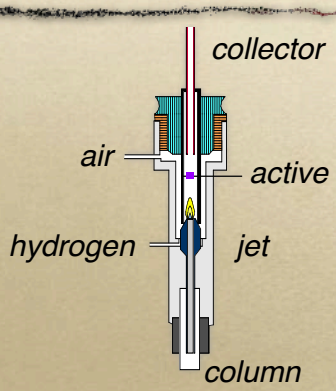


Nitrogen-phosphorous detector

- Specific - sample must contain nitrogen or phosphorous - based on operational mode.
- Destructive
- Limit of detection ~ 0.4 pg N / second
  ~ 0.2 pg P / second
- Linear range ~ 10^4

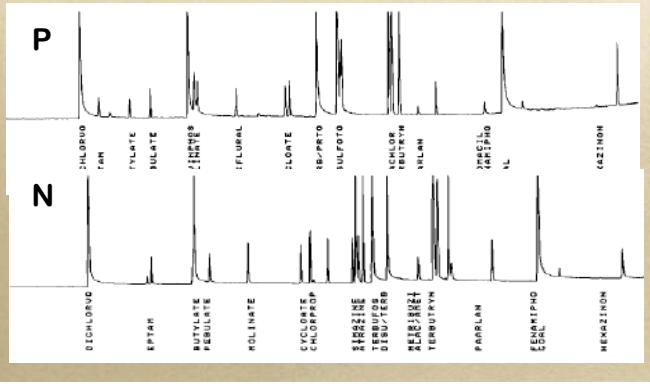
Mode of operation. Essentially a modified FID. Active element acts to block undesired species

NPD



Active element is a K or Rb salt.

Comparison of N and P output from NPD detector

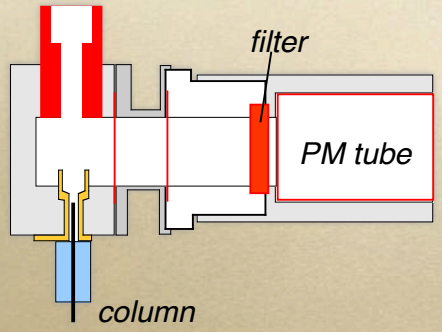


Flame Photometric Detector

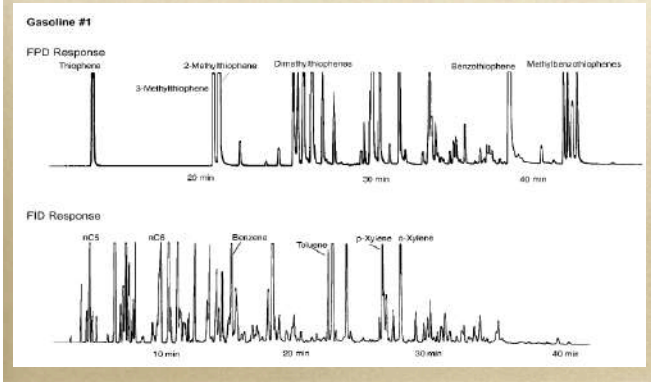
- Specific. Phosphorous or Sulfur
- Destructive
- Limit of detection ~ 20 pg S / second
  ~ 0.9 pg P / second
- Linear range ~ 10^4 P and ~ 10^3 S

Mode of operation. Directly measure light produced during combustion of sulfur or phosphorous containing species.

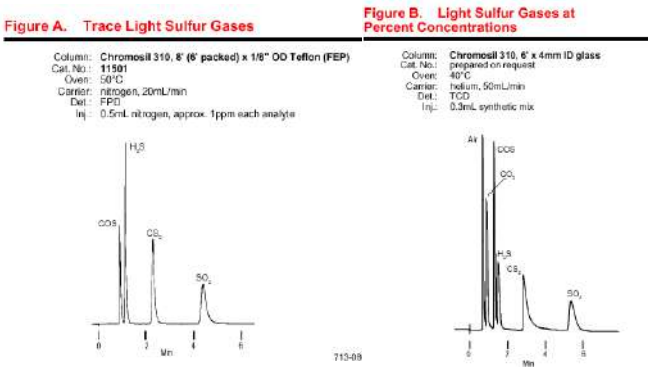
Flame photometric detector



FPD vs. FID - P mode



FPD vs. FID - S mode

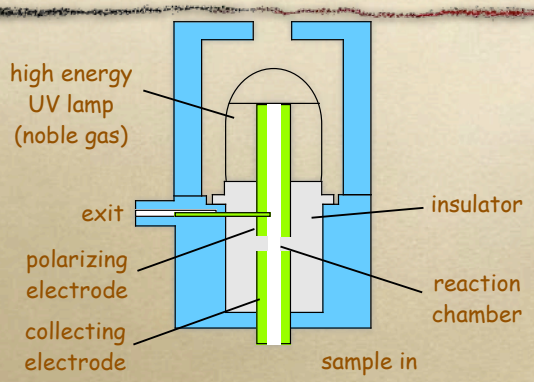


Photoionization detector

- Specific. Compounds ionized by UV (olefinic, aromatics, hetrocyclics)
- Nondestructive.
- Limit of detection ~ 2 pg carbon / second
- Linear range ~ 10^7

Mode of operation. UV light is used to directly ionize sample components. The resulting current is measured.

Photoionization detector



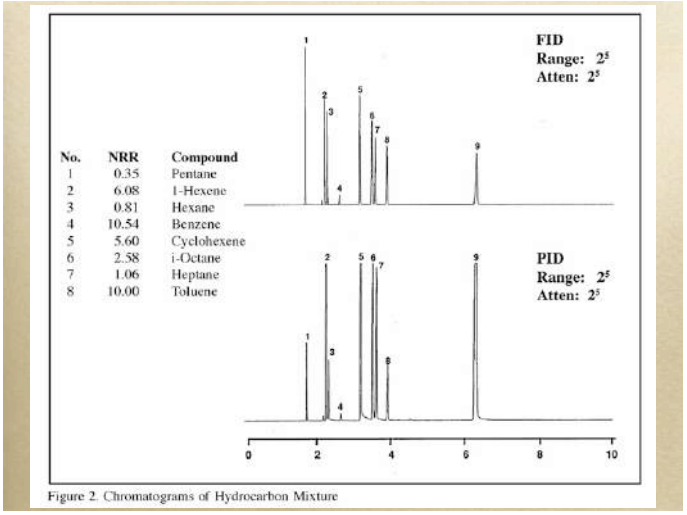


Figure 2. Chromatograms of Hydrocarbon Mixture

Electrolytic Conductivity Detector

ELCD - Hall detector

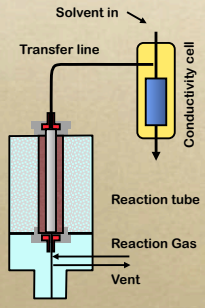
Selectivity: Halogens, sulfur or nitrogen containing species. Only one at a time. Destructive.

Sensitivity: 5-10 pg (X)
10-20 pg (S)
10-20 pg (N)

Linear range: 10^5 -10^6 (X)
10^4 -10^5 (N)
10^3 -10^4 (S)

Reaction Temp: 800-1000°C (halogens); 850-925°C (N); 750-825°C (S)

Electrolytic Conductivity Detector



Compounds are mixed with a reaction gas and passed through a high temperature reaction tube.

Specific reaction products are created which mix with a solvent and pass through an electrolytic conductivity cell.

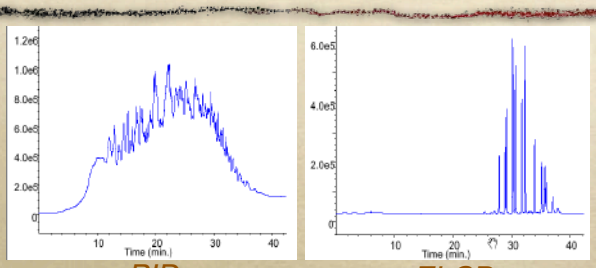
Change in electrolytic conductivity of is measured and a signal is generated.

Reaction tube temperature and solvent determine what compounds are detected.

Electrolytic Conductivity Detector

- Haloacid example.
- Catalytically reduction relies on high temperature hydrogen gas.
- The mixture is passed through a heated nickel reaction tube.
- The reduced compounds flow into the electrolytic conductivity cell via a Teflon® transfer line, where they dissolve in a solvent (typically n-propanol).
- As the n-propanol conductivity changes, the compounds are detected.

PID - ELCD comparison



PID ELCD

110 PCB standard - contaminated with Diesel Fuel - on soil

Shows eliminate of organic contaminate interference to make halogenated species easier to detect.

Halogen Specific Detector (XSD)

- Specific: Halogenated compounds.
- Selectiviy: 10^4 Cl to hydrocarbon
- Destructive.
- Limit of detection: <1 pg Cl / second
- Linear range > 10^4

Mode of operation. Compounds are oxidatively pyrolyzed at 900-1100 °C - forming free halogen atoms. Atoms are then collected on cathode, forming electrons and halogen ions (thermionic emission).

Hyphenated methods

We'll cover these approaches in another unit.

Overall - this approach amounts to attaching a GC to a second instrument that will produce qualitative data.

GC - MS
GC - FTIR
GC - AES