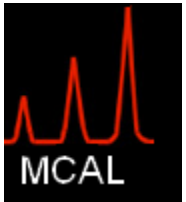
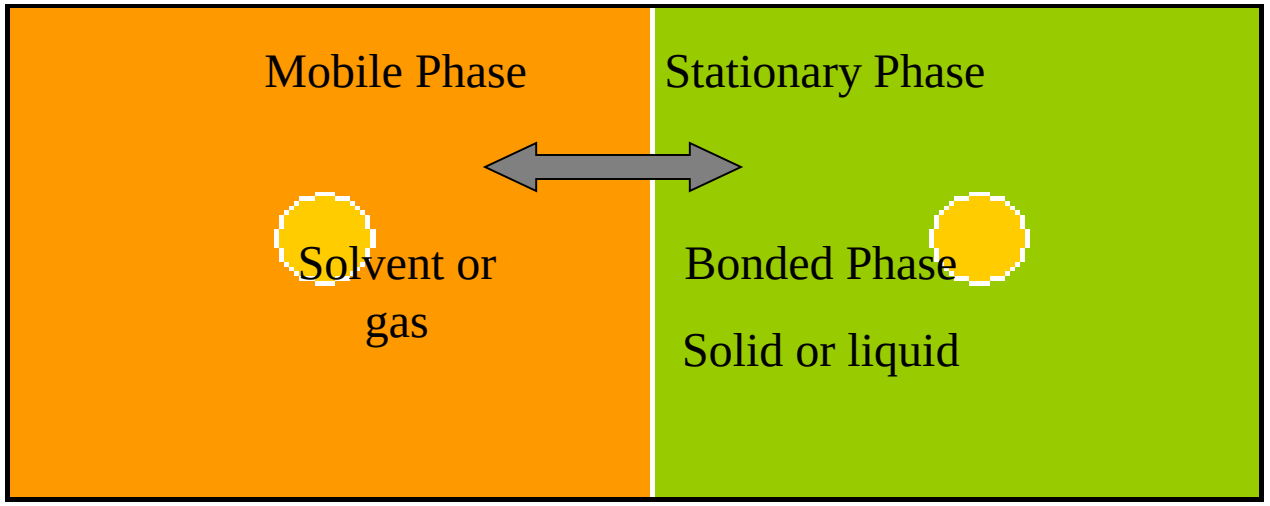
Chromatography

- Various techniques for the separation of complex mixtures that rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass.



Partitioning

- Separation is based on the analyte's relative solubility between two liquid phases or a liquid and solid



Capillary GC Column.

- Capillary columns are a thin fused-silica capillary.
- Typically 10-100 m in length and 250 µm inner diameter.
- The stationary phase is coated on the inner surface.

Separation

- The individual components are retained by the stationary phase differently.

- The components separate from each other since they are running at different speeds through the column with the eluent [gas or solvent(s)].

- At the end of the column they elute one at a time depending on their retention governed by their boiling point and polarity.

Separation

- Boiling point- lower boiling point compounds spend more time in gas phase.
- Temperature of column does not have to be above boiling point. Solids have vapor pressure.
- High vapor pressure liquids used as solvents (water 25 mm Hg whereas ether 520 mm Hg/25C).

Separation

- Polarity “like absorbs likes”.
- Polar compounds have longer retention time on Polar columns.
- Non-polar compounds have longer retention on non-polar column.
- Two types of columns in GC 3800 and 3900, a silicone based column and a PEG column (wax column).

Stationary Phases

- The most common stationary phases in gas-chromatography columns are polysiloxanes, which contain various substituent groups to change the polarity of the phase.

- The nonpolar end of the spectrum is polydimethyl siloxane, which can be made more polar by increasing the percentage of phenyl groups on the polymer.

- Polyethylene glycol (carbowax) is commonly used as the stationary phase for more polar analytes.

Separation

- Carrier gas flow- high flow rate decreases retention time.
- High gas flow may cause poor separation since components have little time to react with stationary phase.
- There is optimum gas flow for various columns. Too high a flow causes excessive back pressure.

Separation

- Column length- longer column usually improves resolution, but increases back pressure.
- Doubling length will **not** double resolution

(resolution increases according to square root of length).

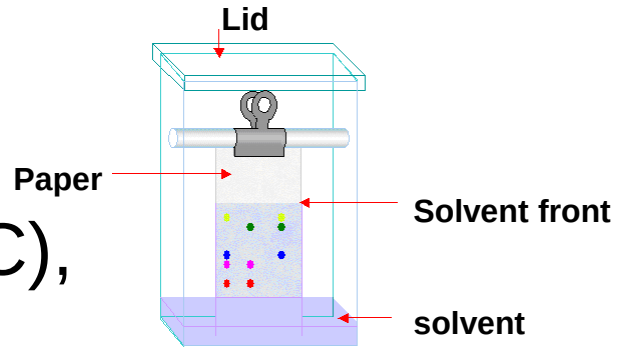
- Generally a 30 meter column gives best resolution, analysis time and head pressure

Separation

- An effective means to increase resolution is to decrease column ID.
- The efficiency of a capillary column increases (number of theoretical plates per meter) as the ID of column decrease.
- Makes sharper peaks,& decreases column bleed
- Decreasing ID decreases sample capacity.
- Ideal (most popular) ID is 0.25 mm.

Some Types of Chromatography

- **Paper** chromatography (PC),
- **Thin-layer** chromatography (TLC),



Two experiments in MCAL demonstrate:

- **Liquid** chromatography (LC, including high-performance liquid chromatography, or HPLC),

- **Gas** chromatography (GC).

GC Experiment

1) To compare the **separation of alcohols of increasing carbon number using a** general purpose **silicone column** and a **FID** detector in the Varian 3800 GC.

3) To measured sensitivity of instrument (**LOD**).

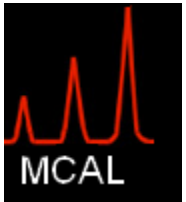
Why is LOD and LOQ important in analysis and research?

Gas Chromatography (a review)

- Chromatographic technique that can be used to separate **volatile organic** compounds.

- A gas chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, detector and a controller (integrator) and data collection and storage.

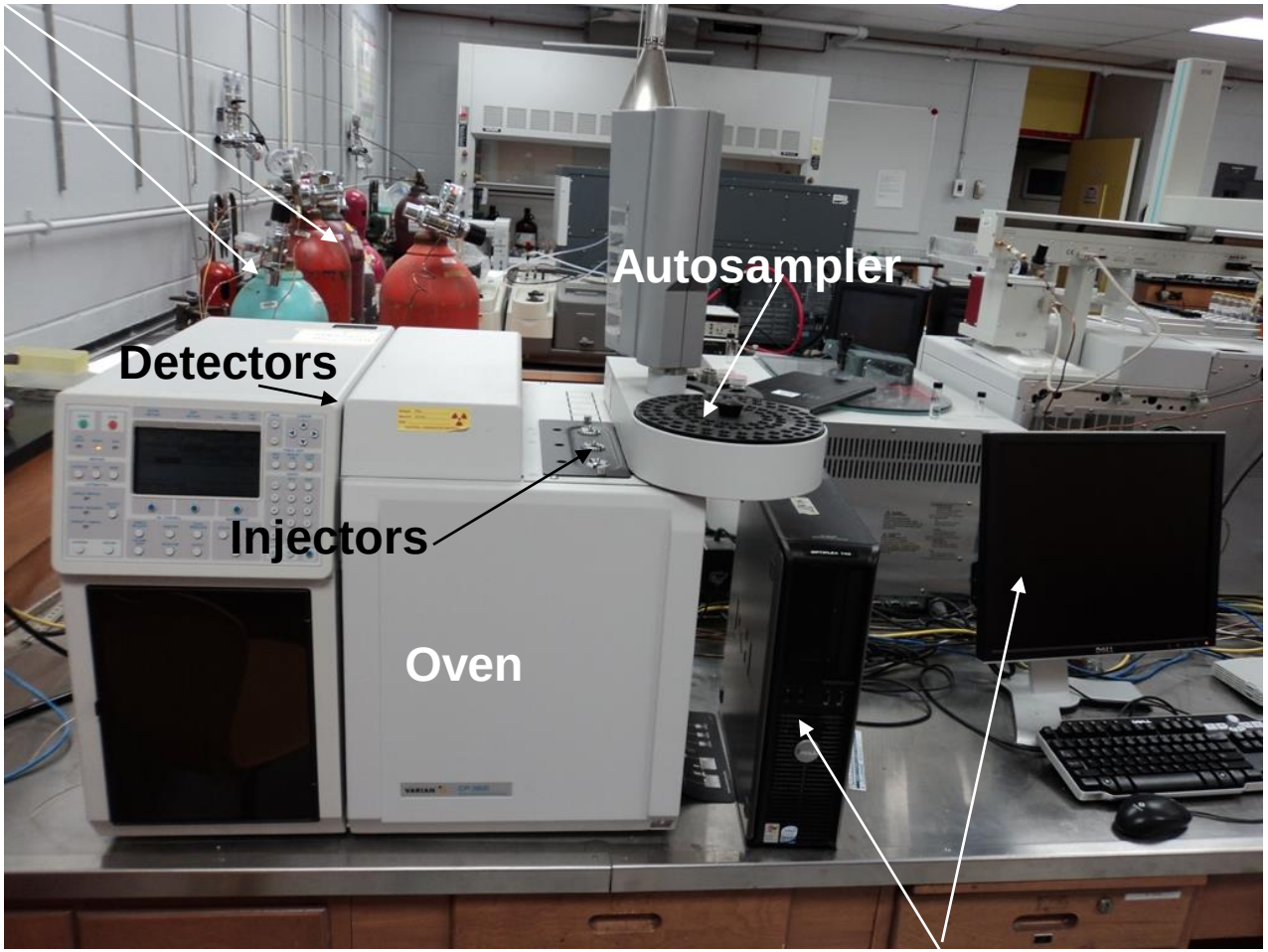
- Organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.



Bruker Improving the Quality of our Beer – New 456-GC Off-Flavor Beer Analyzers

Varian (Agilent) 3800 GC

Gas tanks He, N_2, H_2, Air



Computer control and data acquisition

Stationary Phases

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- The nonpolar end of the spectrum is polydimethyl siloxane, which can be made more polar by increasing the percentage of phenyl groups on the polymer.

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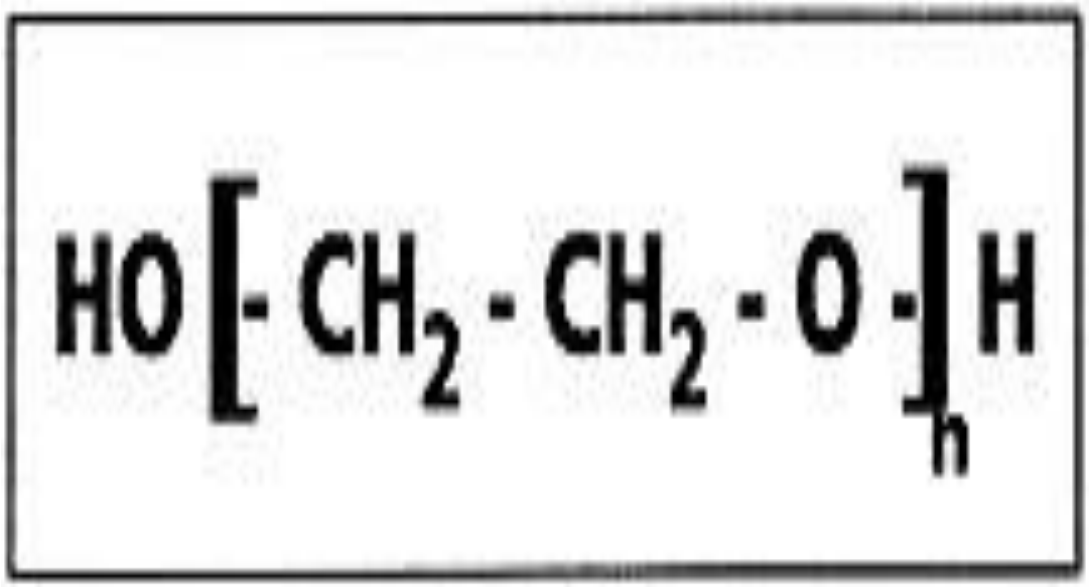
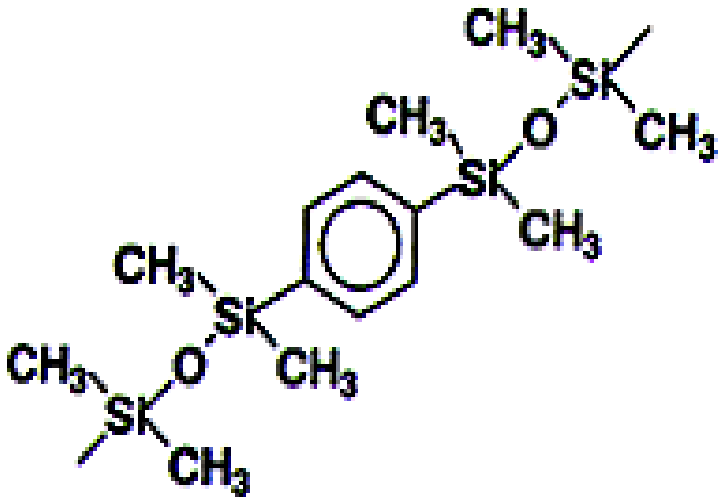
Polysiloxanes

R =	CH_3	methyl
	$CH_2CH_2CH_2CN$	cyanopropyl
	$CH_2CH_2CF_3$	trifluoropropyl
		phenyl

Polyethylene glycol



Low Bleed Phases (Arlenes)



Varian (Agilent) Factor Four Capillary GC Columns.

- Capillary columns are a thin fused-silica capillary.
- Typically 10-100 m in length and 250 µm inner diameter.
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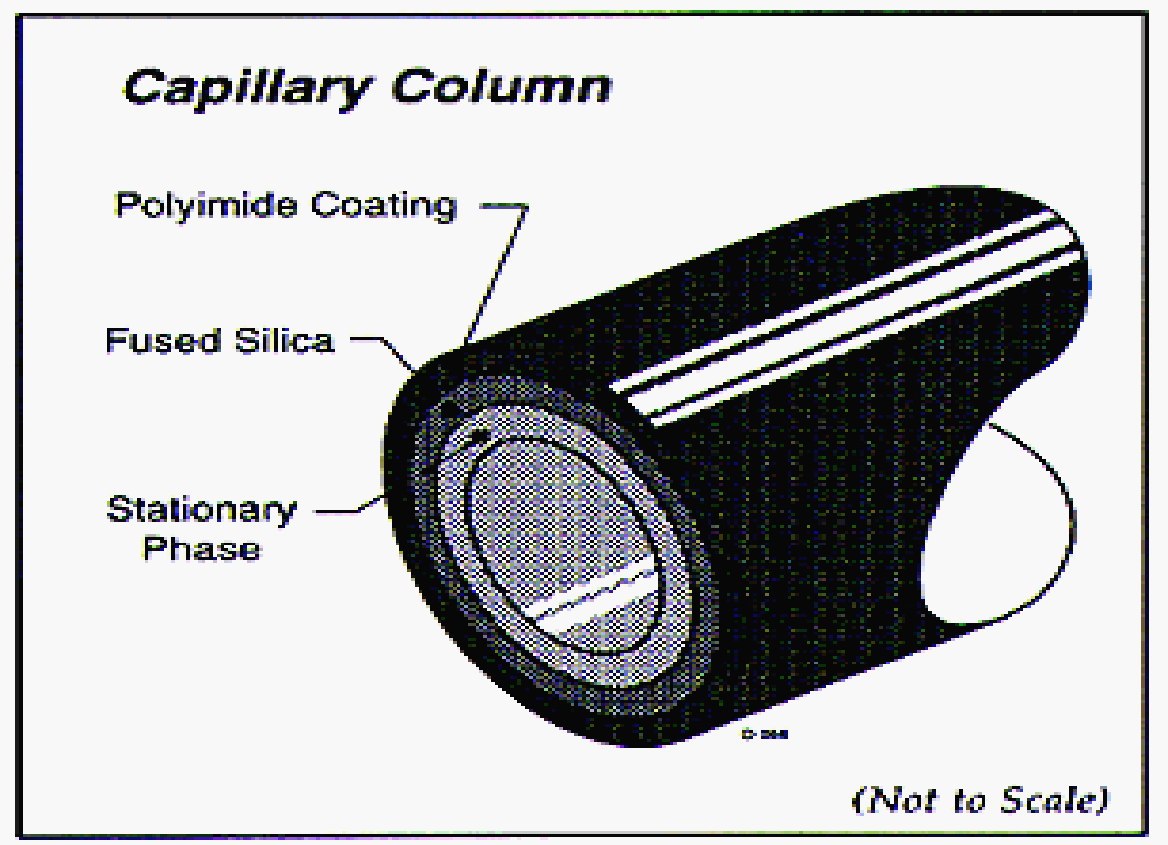
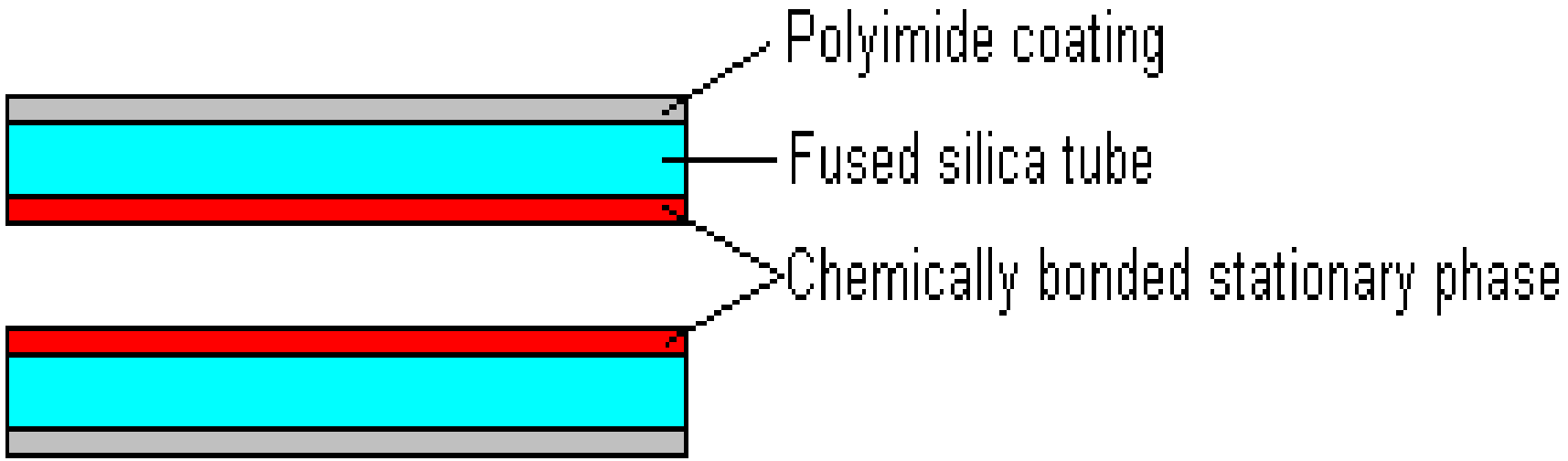
Capillary Columns



FSOT Column

Cross section of a Fused Silica Open Tubular Column

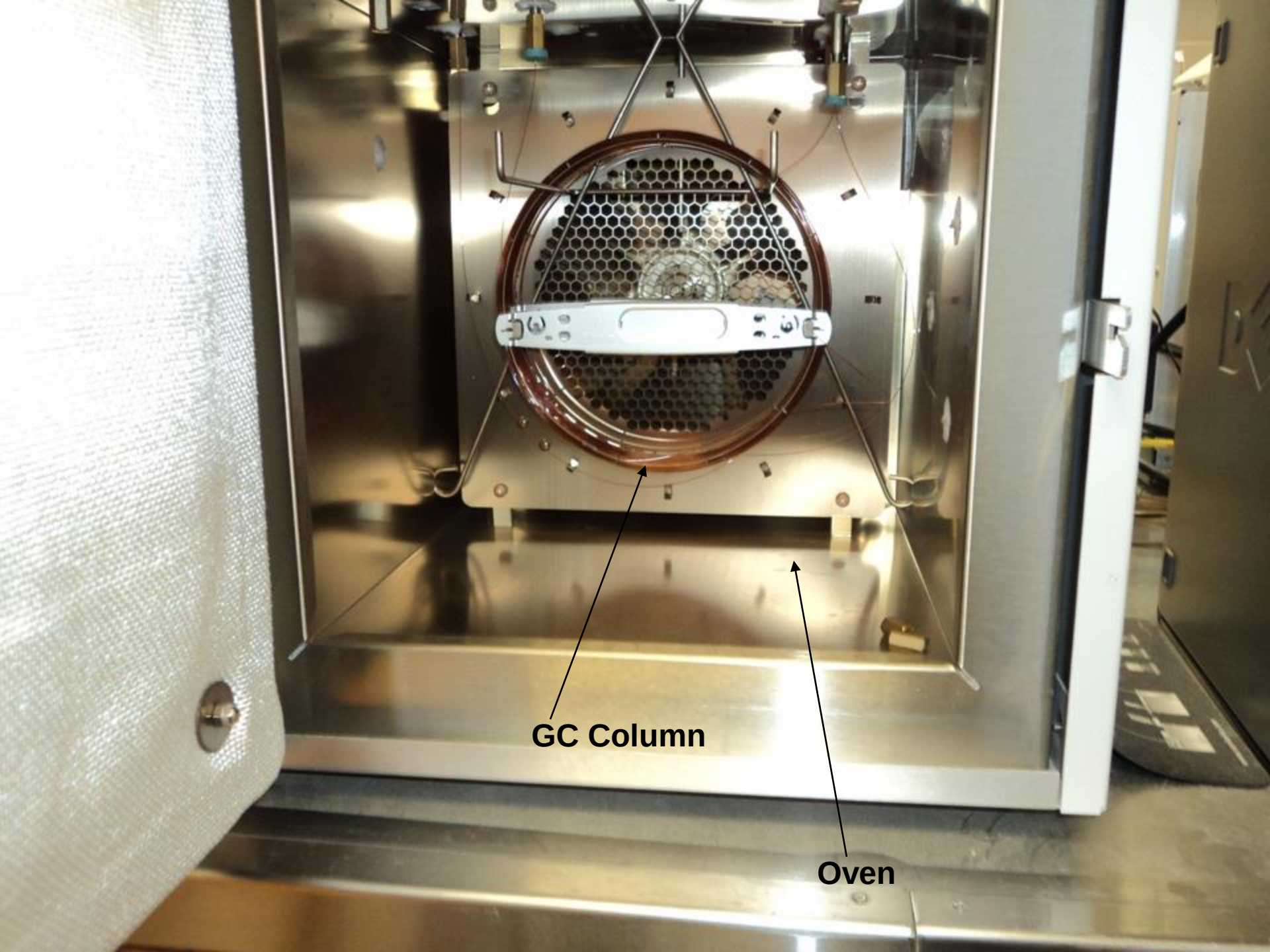


Capillary Columns

- Capillary columns provide much higher separation efficiency than packed columns but are more easily overloaded by too much sample.

- You need to dilute your sample to get good symmetrical (Gaussian) peaks

- Solvent peak dominates chromatogram due to normalization of peaks.


GC Column
Oven

MCAL GC Columns

GC 3800- one columns FID detector:

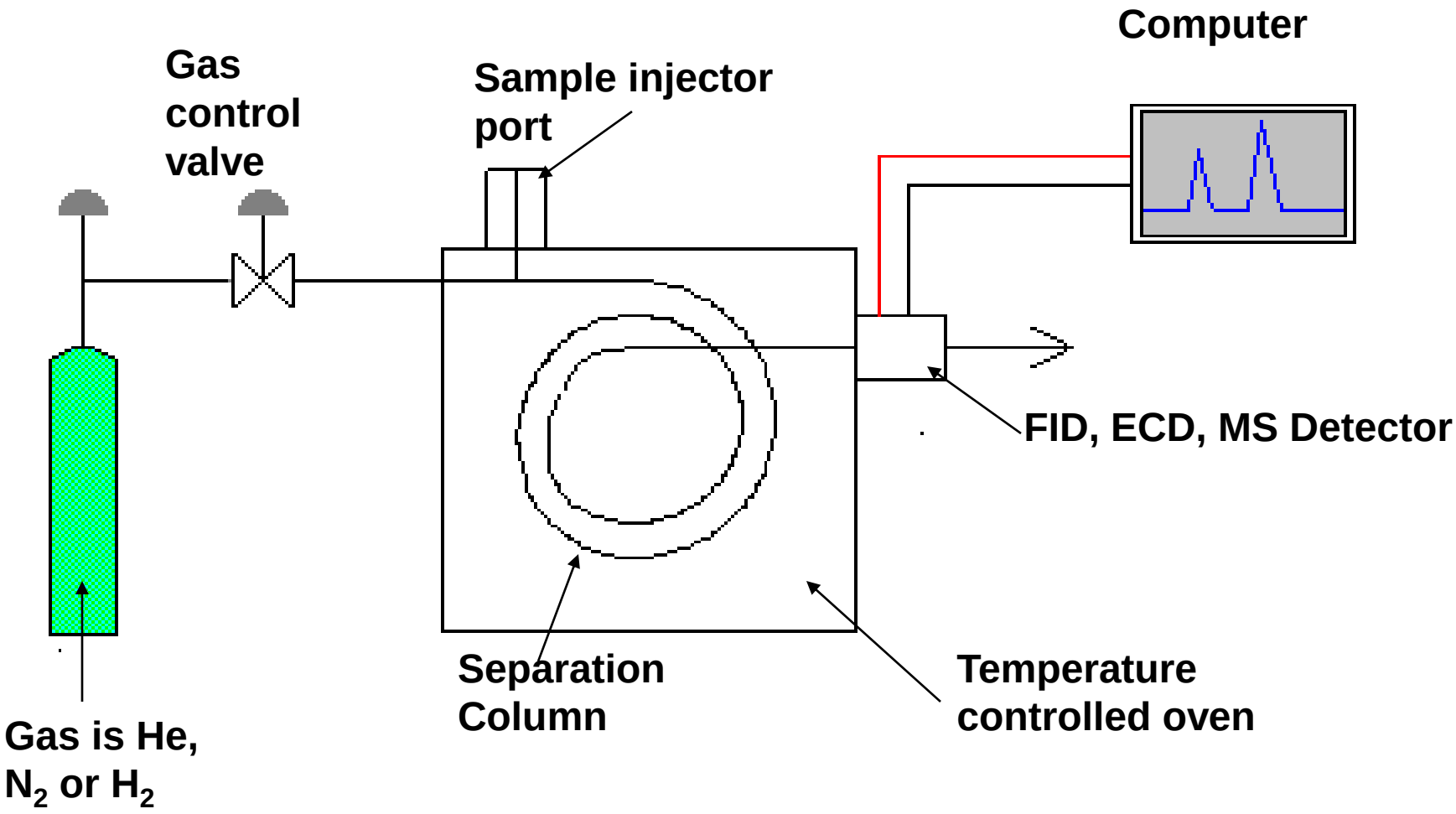
1) ***CP-sil 8 50 m, 0.2mm, .33um 5% phenyl 95% dimethyl polysiloxane***

GC 3900 –one column FID detector:

1) ***Supelco wax-10 15m x 0.32 mm, 0.5 u***

   Polyetyylene glycol

Simple GC


Computer
Gas control valve
Sample injector port
FID, ECD, MS Detector
Separation Column
Temperature controlled oven
Gas is He, N_2 or H_2

Sequence of GC Injection

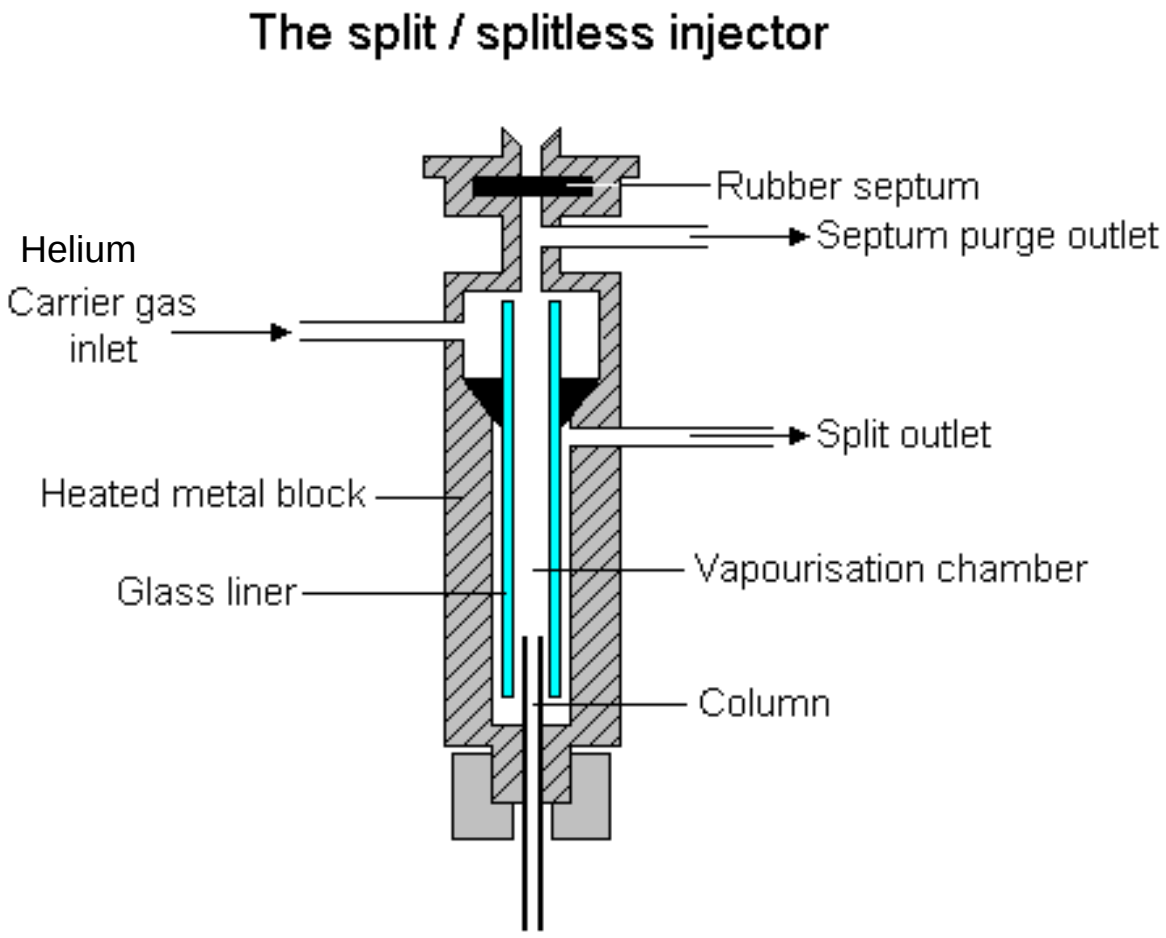
1. A small amount of liquid (microliters) is injected through a silicon rubber septum into the heated (>200^{o}C) GC injector that is lined with an inert glass tube.

2. The sample is immediately vaporized.

3. A pressurized, inert, carrier gas-which is continually flowing from a gas regulator through the injector and into the GC column-sweeps the gaseous sample, solvent, and analyte, onto the column.

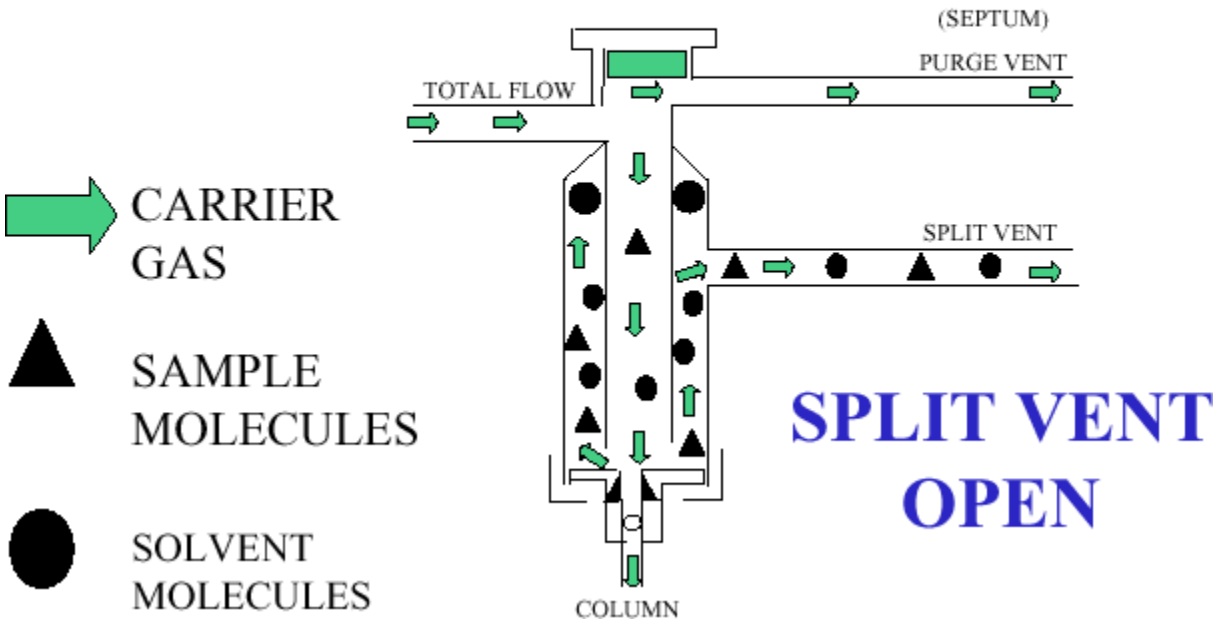
4. Septum purge: a small ancillary flow of carrier gas bathes the underside of the injector's septum so that hot vaporized sample gases can't interact and possibly stick to the septum. This improves peak shape and reproducibility.

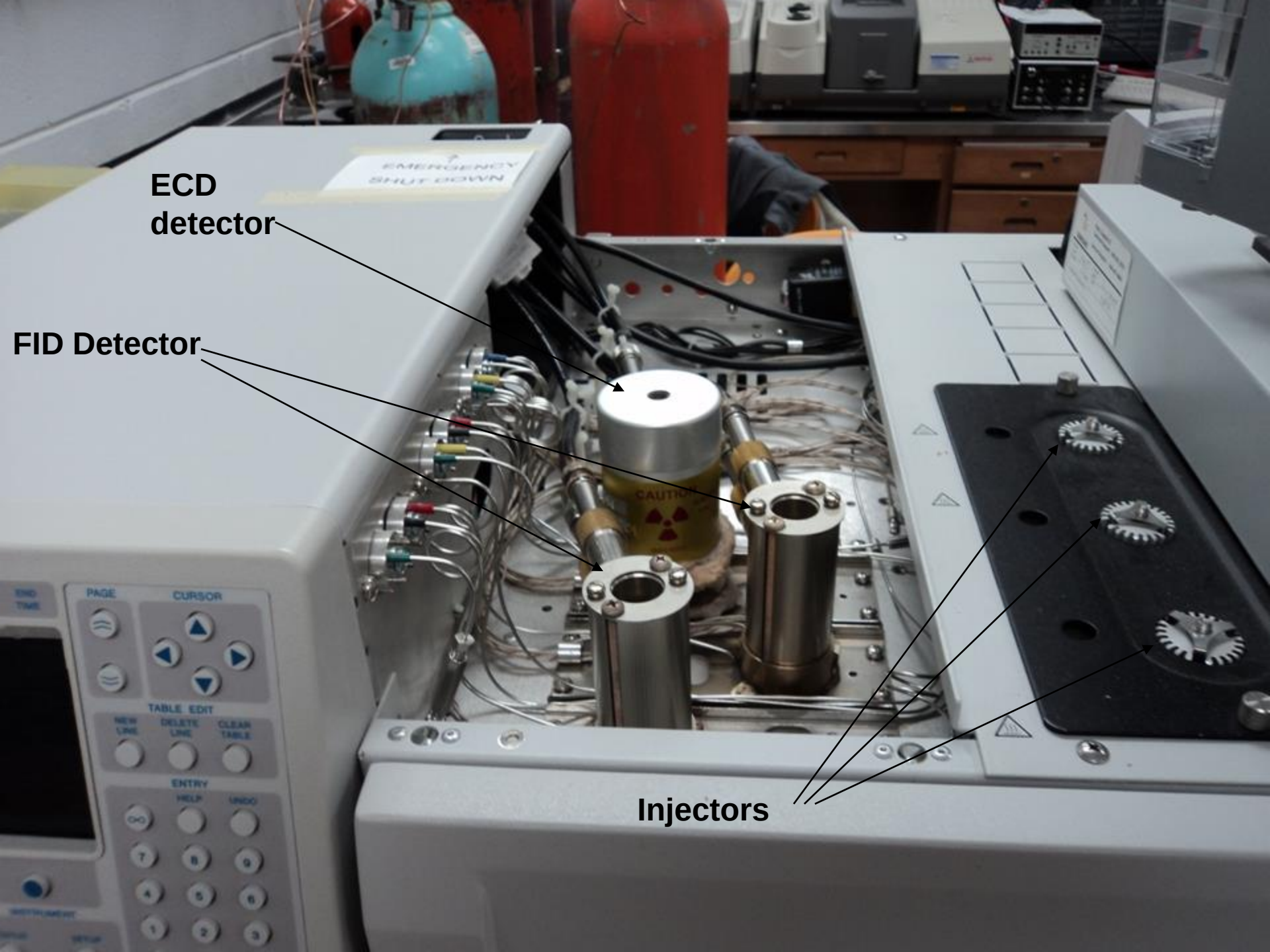
Split / Splitless Injector



Split / Splitless Injector

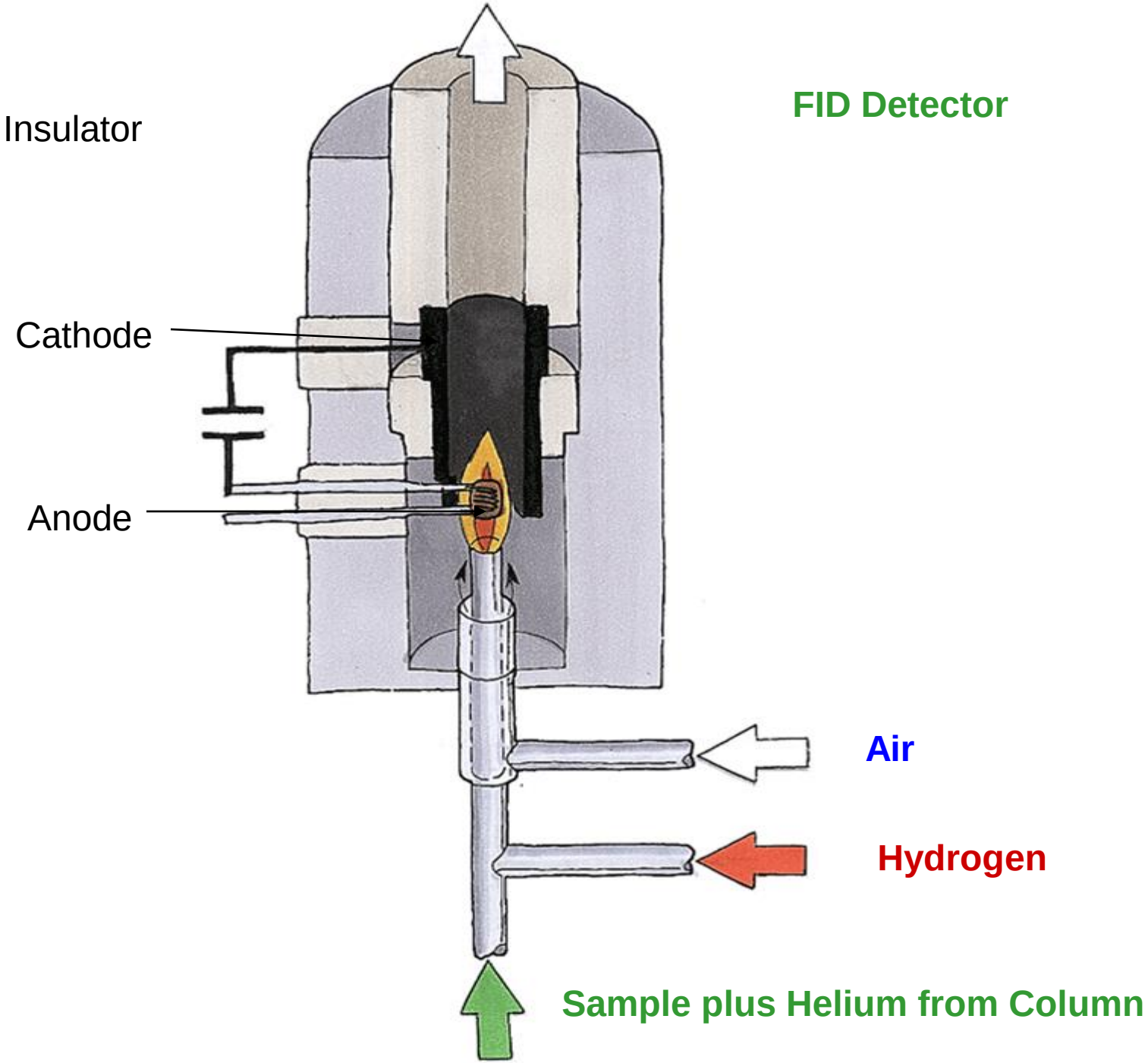
Split Injection



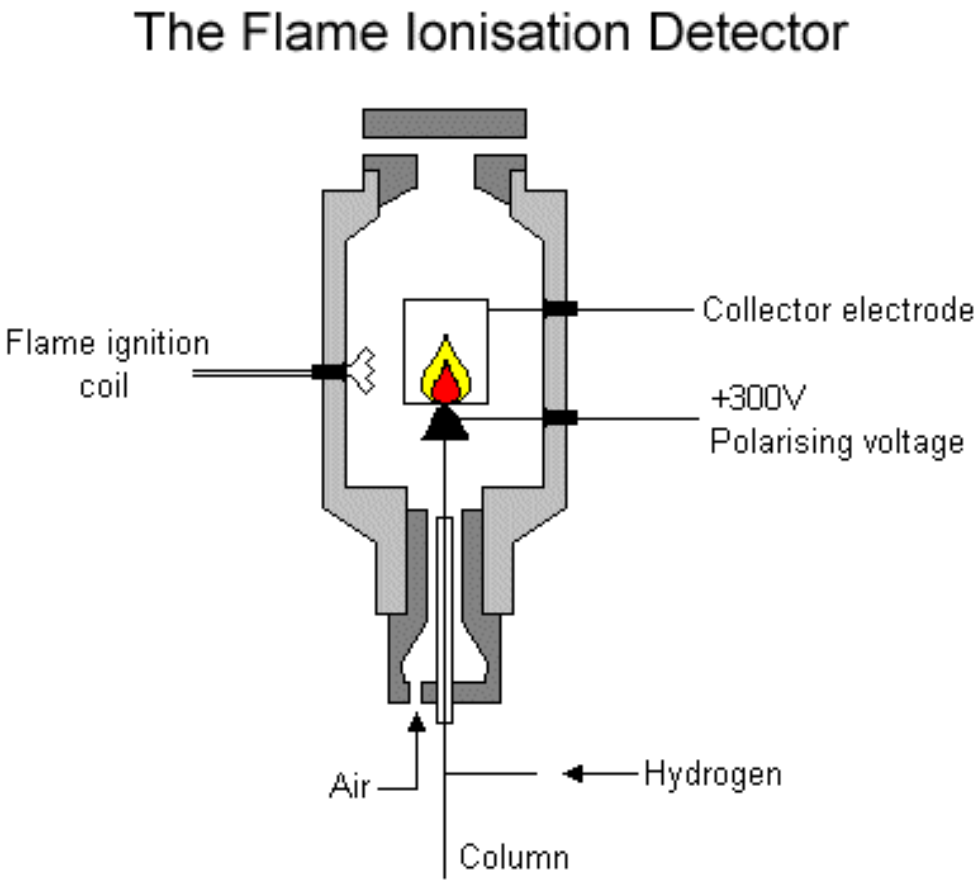

ECD
detector
FID Detector
Injectors
EMERGENCY
SHUT DOWN
CAUTION
PAGE
CURSOR
TABLE EDIT
NEW LINE
DELETE LINE
CLEAR TABLE
ENTRY
HELP
UNDO

FID Detector

- Organic compounds burning in a hydrogen-oxygen flame produce ions and electrons. These charged particles created in the combustion process create a current between the detector's electrodes.
- One electrode is the metallic jet itself, the other is above the jet. The detector housing is heated so that gases produced by the combustion (mainly water) do not condense in the detector before leaving the detector chimney.


Insulator
FID Detector
Cathode
Anode
Air
Hydrogen
Sample plus Helium from Column

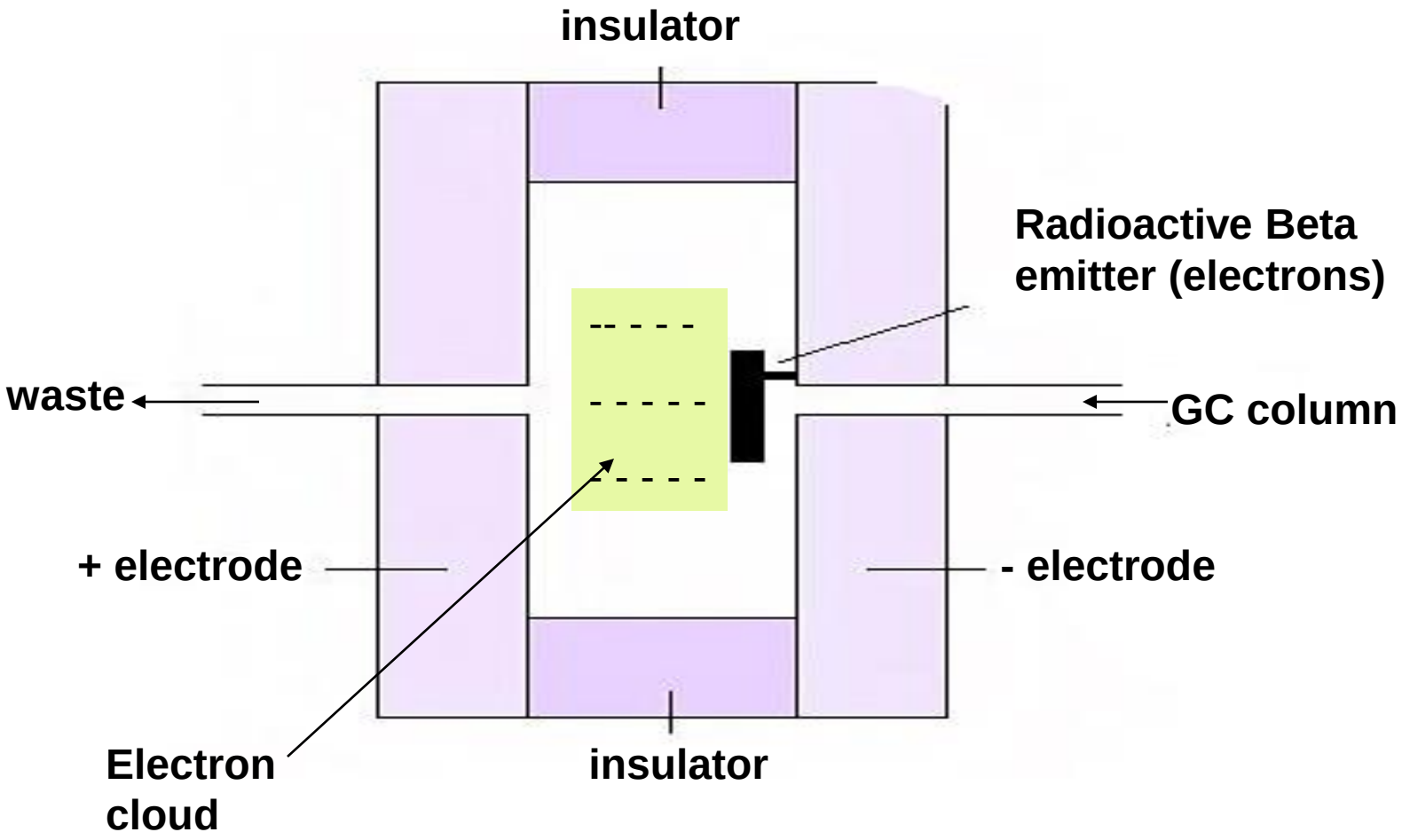
FID Detector



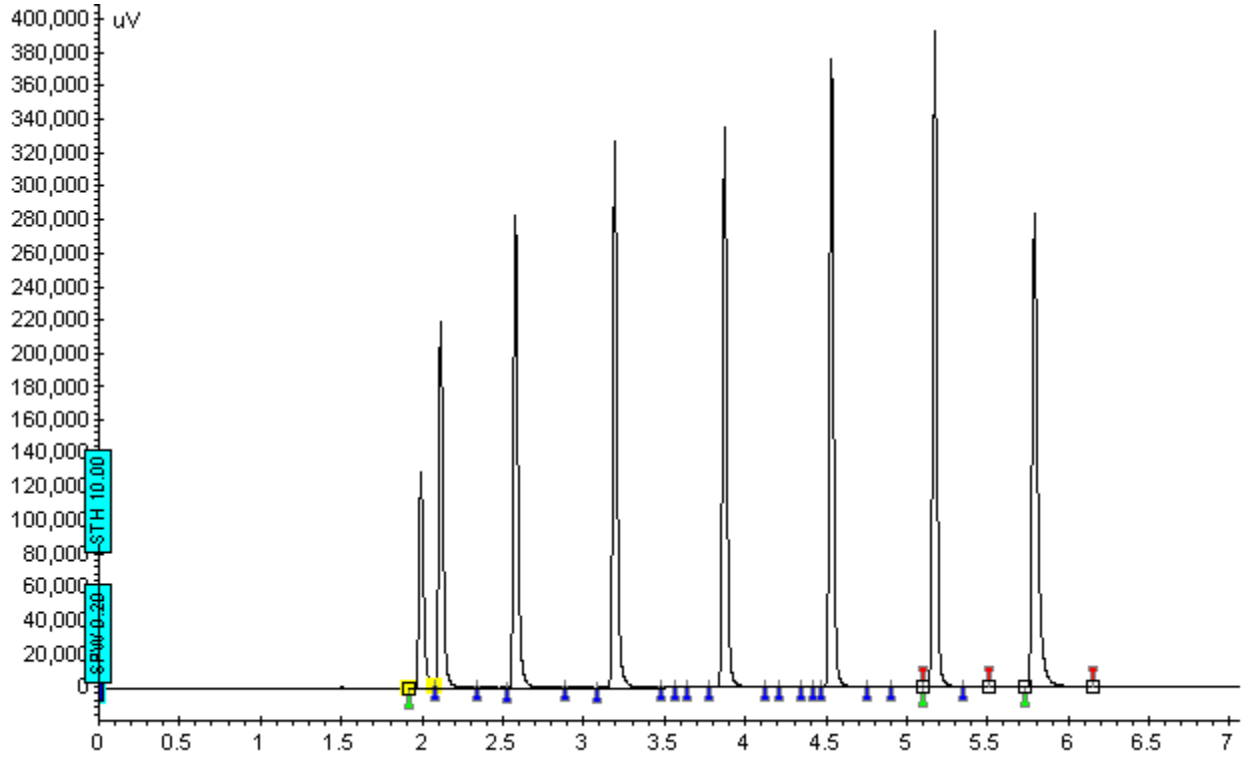
The ECD or Electron Capture Detector

- **The ECD or electron capture detector measures electron capturing compounds (usually halogenated) by creating an electrical field in which molecules exiting a GC column can be detected by the drop in current in the field**

ECD Detector Electrode



Seperation of Alcohols C1 to C8 on carbowax column



GC Exp: Fid Detector