

Chromatography

The Basics of GC and HPLC

A Simple GC by GOW MAC

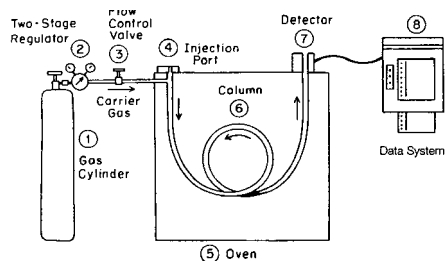
- Mobile Phase – helium gas
- Columns – two w/different polarities
- Detector – thermal conductivity



Method of Separation

- Separation based on equilibrium between vapor phase and liquid phase of analyte
- Analyte moves along column while in the vapor phase
- Limitation- Analyte should have boiling points of 300° C or less for reasonable retention times.

Block Diagram of GC



GC Columns (top view)



GC Column Packing

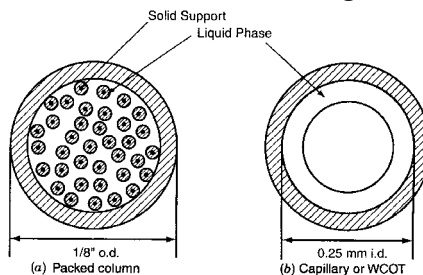


Fig. 19. Schematic representation of (a) packed column and (b) open tubular column.

Parameters that can be varied in GC

- Column temperature
- Column polarity
(nonpolar – polydimethyl siloxane, polar – polyethylene glycol “carbowax”)
- Flow rate of carrier gas (He, H₂, N₂)
- Temperature programming

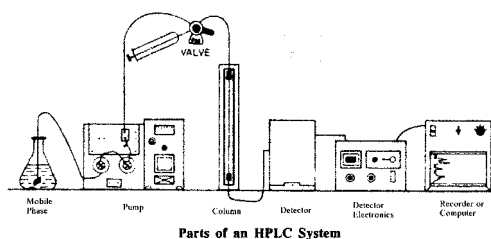
Common Detectors

- TCD – Thermal Conductivity
- FID – Flame Ionization
- Also in conjunction with IR and MS

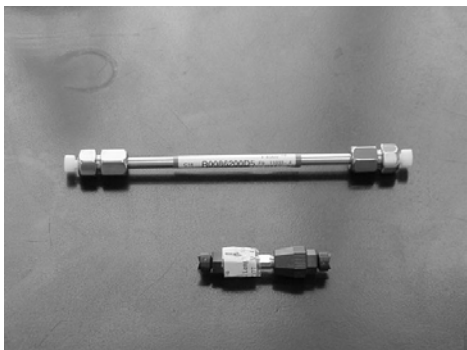
Isocratic HPLC



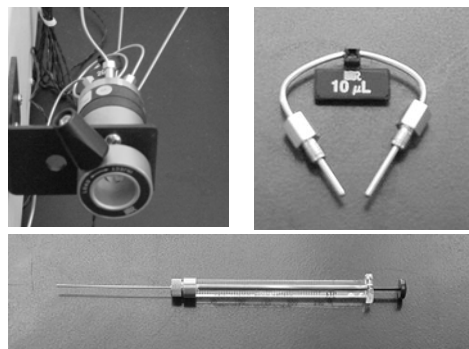
Diagram of Isocratic HPLC



HPLC Columns



HPLC injection



HPLC Detection



Method Development

- Use very pure solvents- Filter and Degas
- Adjust strength (polarity) of mobile phase
- Adjust pH of mobile phase
- Consider ion-pairing agents
- Try columns from different companies
- The power of the separation is in the mobile phase!!!!

Two Types of HPLC Methods

- Normal Phase – polar stationary phase (column) with a nonpolar mobile phase (solvent)
- Reverse Phase– nonpolar stationary phase (column) with a polar mobile phase (solvent)