



# **Phytochemical analytical techniques**

**Chromatography**

**Spectroscopy**

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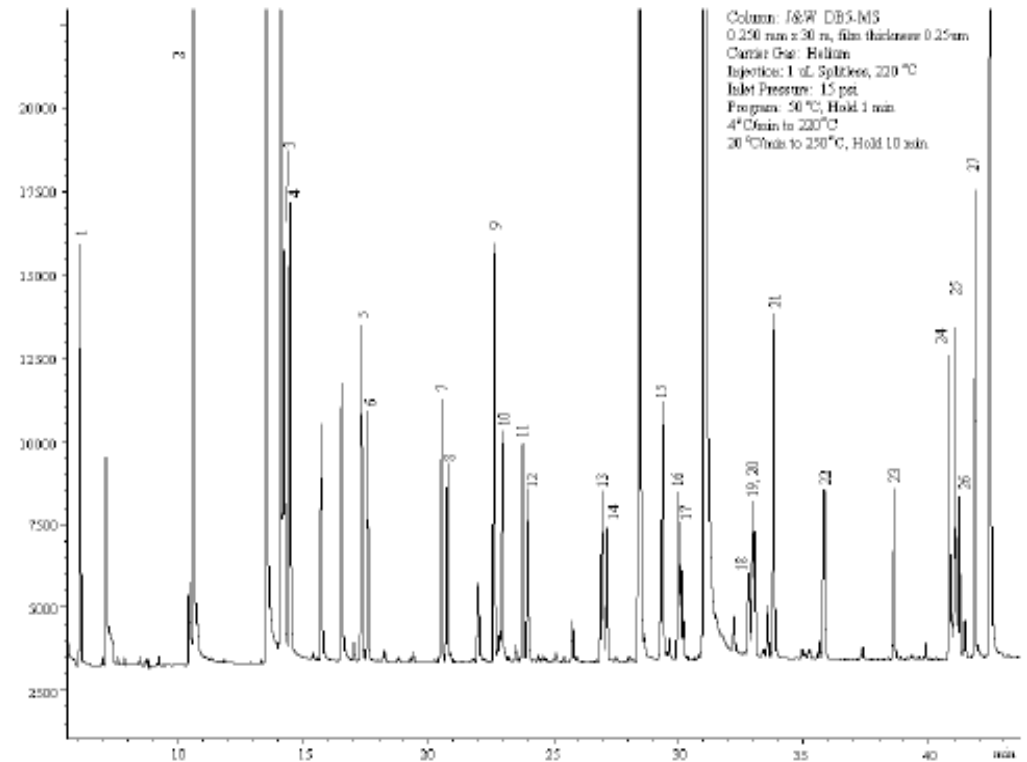
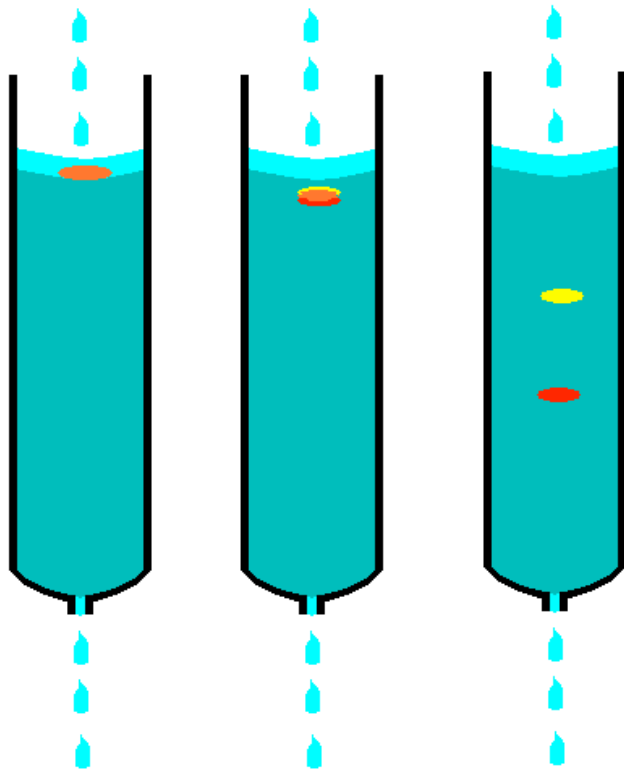
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# Chromatography

- The separation of components in a mixture that involves passing the mixture dissolved in a "mobile phase" through a *stationary phase*, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases
- Column, thin layer, liquid, gas, affinity, ion exchange, size exclusion, reverse phase, normal phase, gravity, high pressure

# Chromatography



# High Pressure (Performance) Liquid Chromatography - HPLC

- **Developed in 1970' s**
- **Uses high pressures (6000 psi) and smaller (5  $\mu$ m), pressure-stable particles**
- **Allows compounds to be detected at ppt (parts per trillion) level**
- **Allows separation of many types of polar and nonpolar compounds**



# HPLC Modalities

- **Reversed phase** – for separation of non-polar molecules (non-polar stationary phase, polar mobile phase)
- **Normal phase** – for separation of non-polar molecules (polar stationary phase, non-polar/organic mobile phase)
- **HILIC** – hydrophilic interaction liquid chromatography for separation of polar molecules (polar stationary phase, mixed polar/nonpolar mobile phase)

# HPLC Columns



Glass



PEEK®



Stainless Steel



Analytical



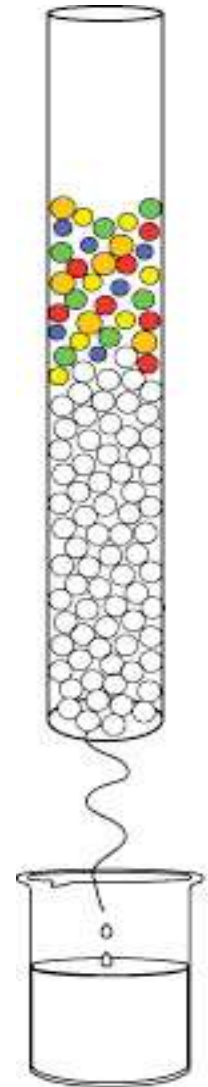
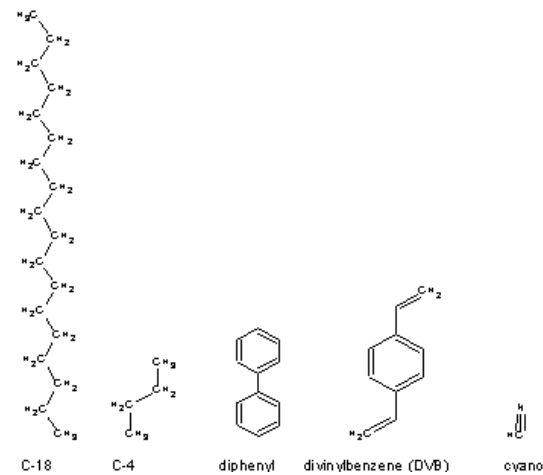
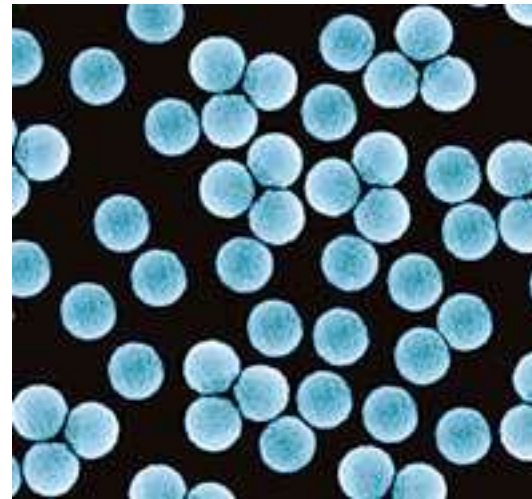
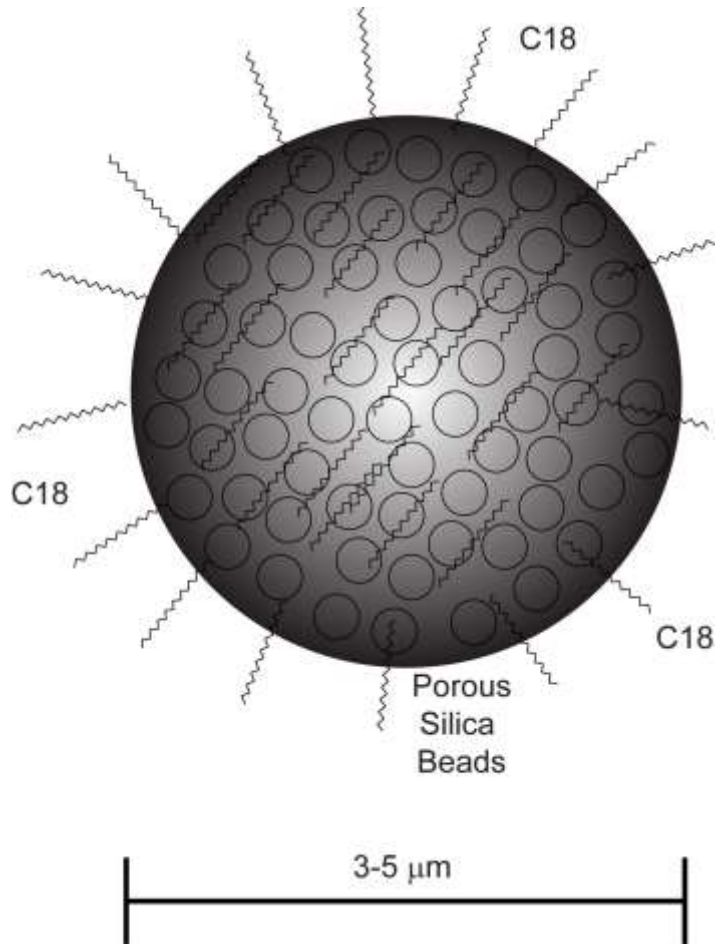
Preparative

Internal Diameter (i.d.)  
1mm – 50mm

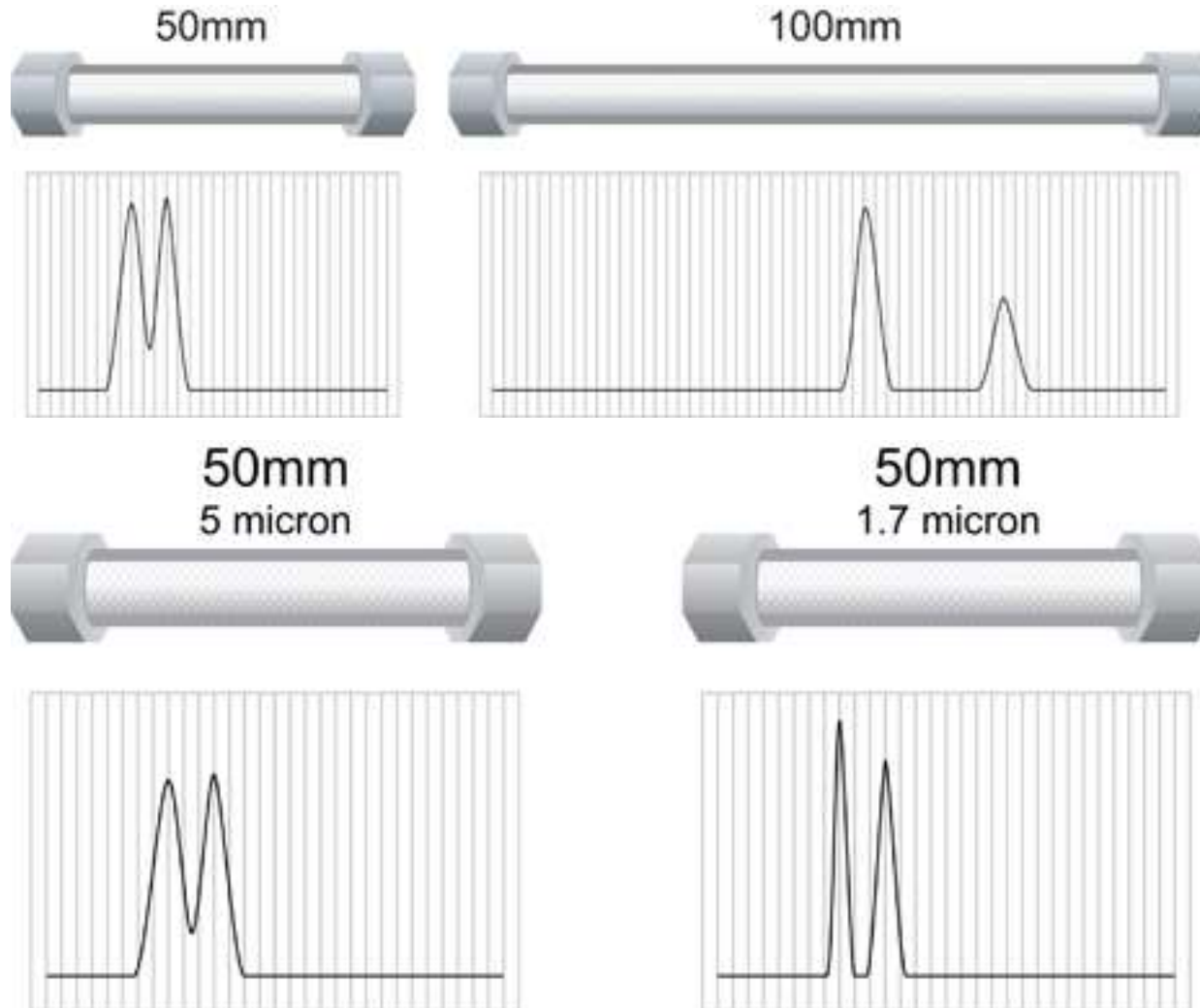


Length  
20mm – 500mm

# Reverse Phase Column

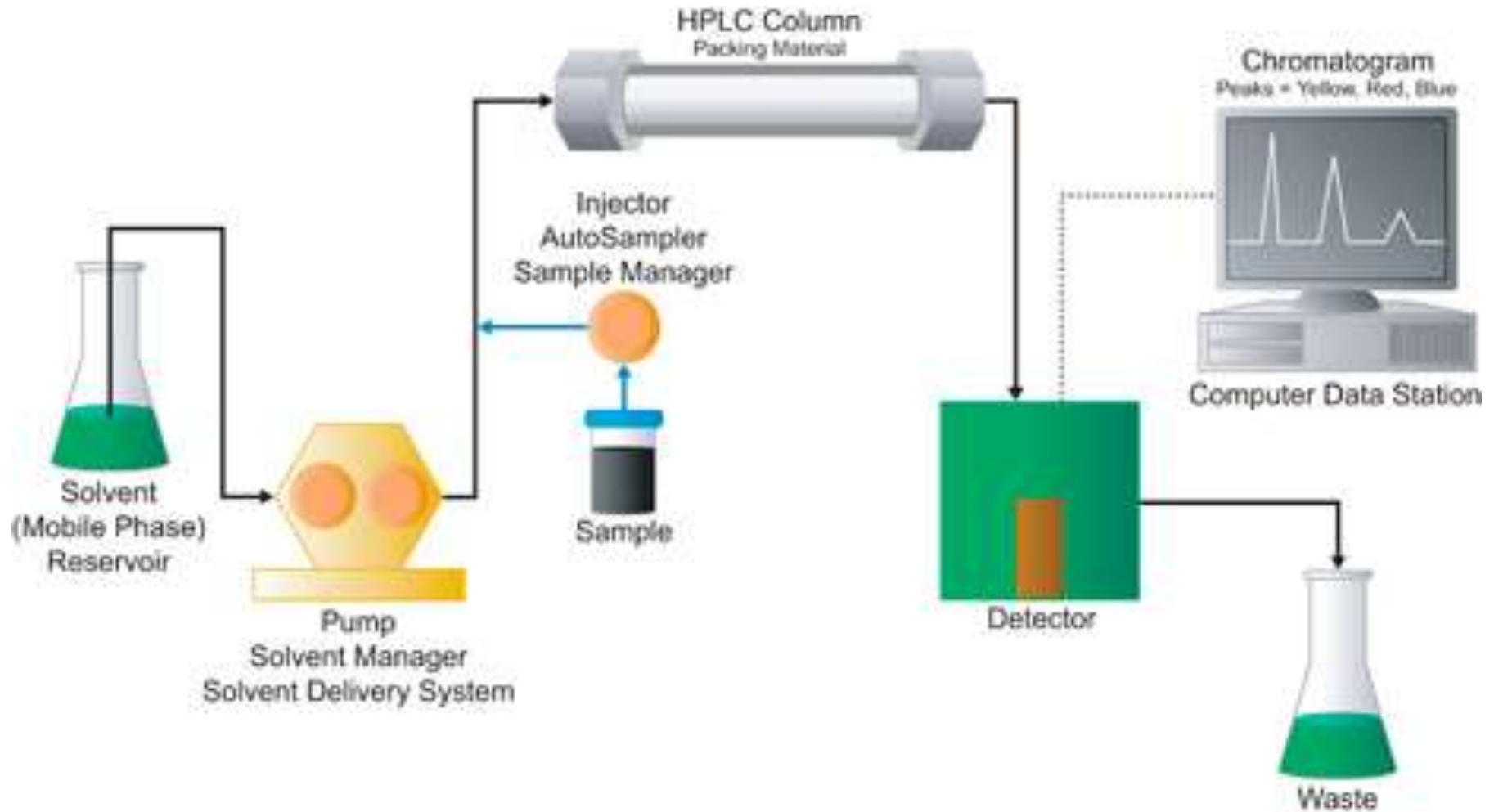


# HPLC Separation Efficiency





# HPLC Schematic



# Gradient HPLC Schematic

