CHE680
Advanced Analytical Chemistry
Lecture 10

Jamie Kim
Department of Chemistry
Buffalo State College

GC vs. HPLC

- Mobil phase: Gas
- Flow rate: gas pressure by regulator
- Stationary phase: Solid with different functional groups
- FID, TCD, ECD, NPD, MS, etc

- Retention time
- Peak area
- Internal & external stds

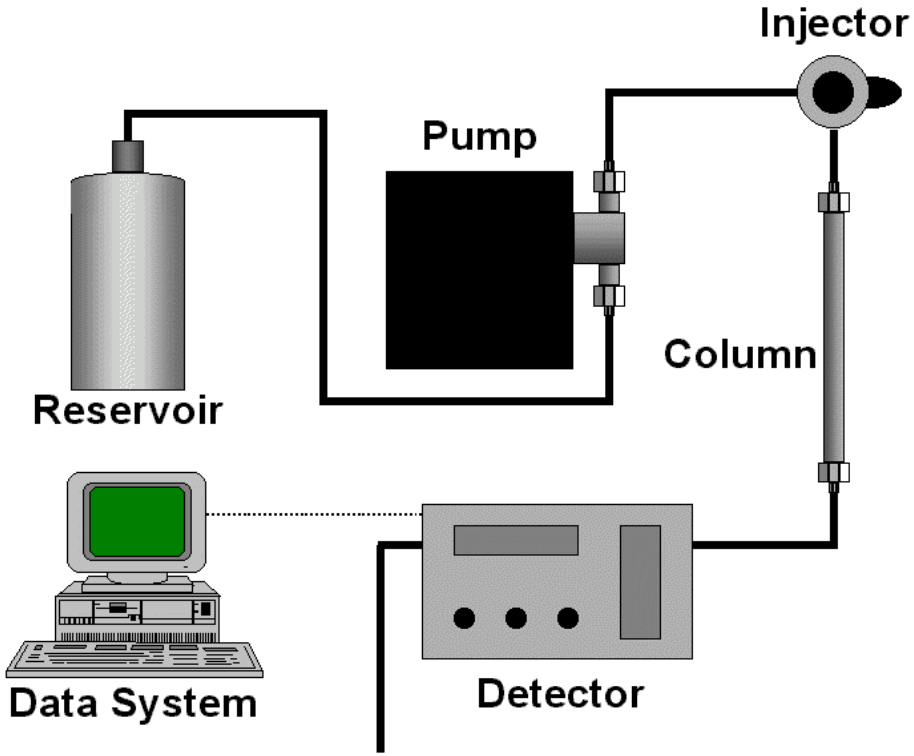
- Liquid
- Liquid pressure by pump
- Solid with different functional groups

- UV, fluorescence, electrochemical, refractive index, MS

- Retention time
- Peak area
- Internal & external stds

HP 1050 HPLC

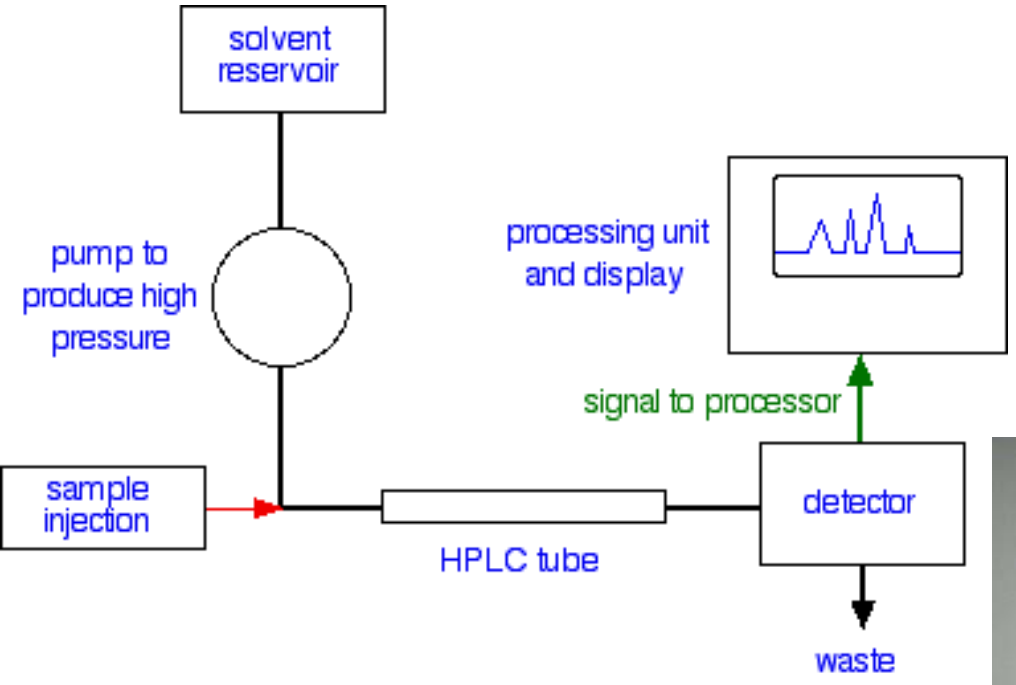
- Have a look at HP1050 HPLC and its modules (parts) located in SC459
- Data collection using a standard reference sample

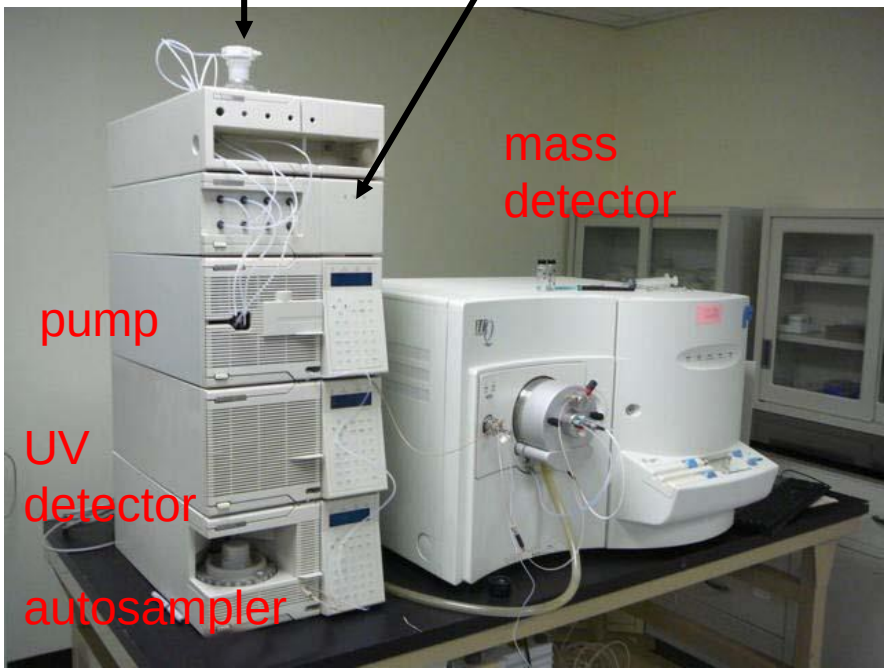


HPLC

- High performance (pressure) liquid chromatography (HPLC) makes use of a liquid (a mixture of liquids) as a mobile phase.
- The pressure of liquid can be increase up to ~ **400 bar (1 bar = 14.5 psi, 1 atm ~1013 mbar = 1.013 bar)** for fast analysis using a pump.
- Columns contain a very much smaller particle size (~5 μm) for the column packing material with different functional groups.
- Separation is the result of differences in interactions between the stationary phase and the molecules flowing past it.
- HPLC is highly automated and employs extremely sensitive detectors.

Schematics





HP 1050 and Thermo LCQ Mass detector

Normal Phase HPLC

- Although it is called "normal", it isn't the most commonly used form of HPLC.
- The column is filled with tiny silica particles (polar), and the solvent is non-polar, hexane, for example.
- A typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm.
- Polar compounds in the mixture being passed through the column will stick longer to the polar silica than non-polar compounds will.
- The non-polar ones will therefore pass more quickly through the column.

Reverse Phase (RP) HPLC

- The silica in the column is modified to be non-polar by attaching long hydrocarbon chains (C8 or C18)
- A polar solvent is used: water, methanol, acetonitrile, a mixture of them.
- There won't be as much attraction between the hydrocarbon chains attached to the silica (the stationary phase) and the polar molecules in the solution.
- Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of van der Waals dispersion forces.
- That means that now it is the polar molecules that will travel through the column more quickly.
- Reversed phase HPLC is the most commonly used form of HPLC.

Injector

HP 1050 Autosampler with a tray

Injection needle

manual injector

syringe

Solvent and Reservoir

HPLC grade solvent
No particle inside
No gas

filters

filters

HP 1050 solvent tray and thermostat

column house

Degassing

- Any gas inside HPLC will make big problems
- Dead volume, pressure change, base line change, noise, damage to pump, column, etc
- Source of gas: from solvent

- Degasser (membrane inside)
- Helium sparging

Degassing four solvents

Pump

HP 1050 pump

- Force mobile phase to the column with high pressure
- Mixing solvents

- Quaternary pump
- Binary pump

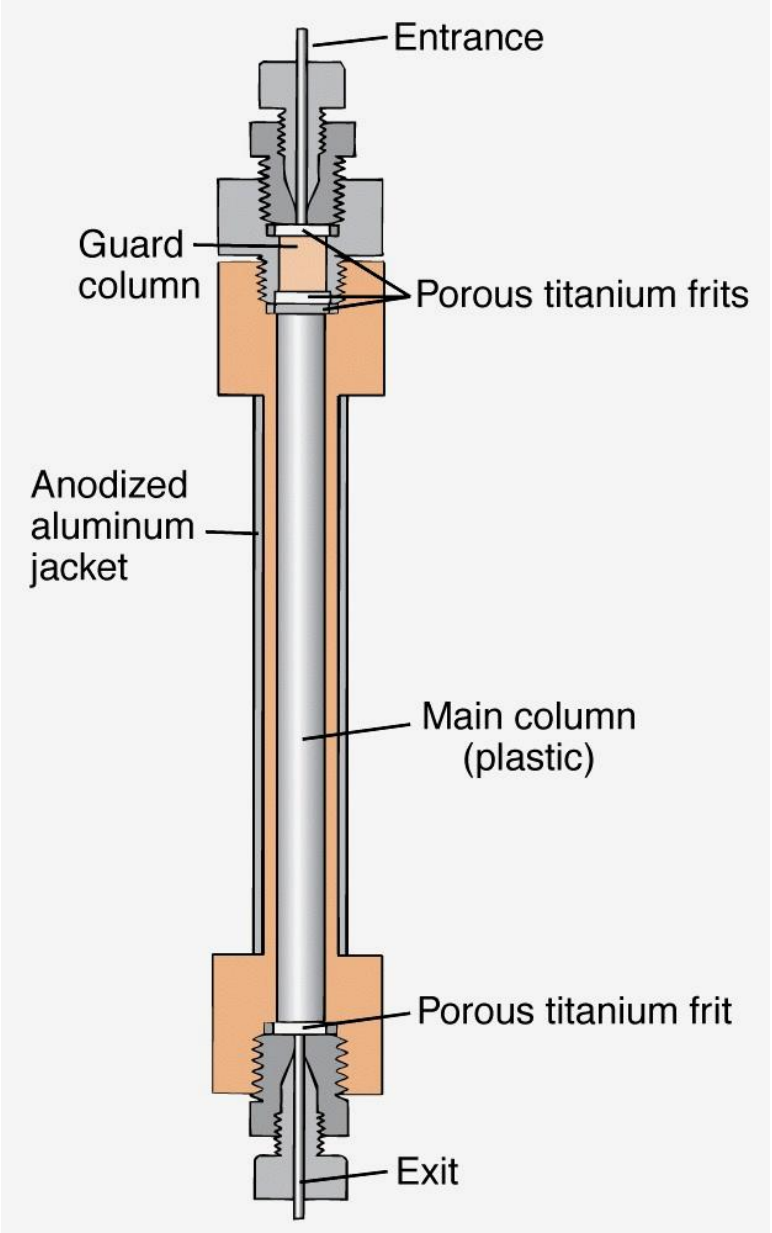
Tubing

Material	Tolerance (inches)	psi	I.D. (inches)	I.D. (mm)	O.D. (inches)	Colour
PEEK	±0.002	6100	0.005	0.13	1/16	Red
PEEK	±0.002	5800	0.007	0.18	1/16	Yellow
PEEK	±0.002	5600	0.010	0.25	1/16	Blue
PEEK	±0.002	4500	0.020	0.50	1/16	Orange
PEEK	±0.002	3500	0.030	0.75	1/16	Green
PEEK	±0.002	2400	0.040	1.00	1/16	Grey

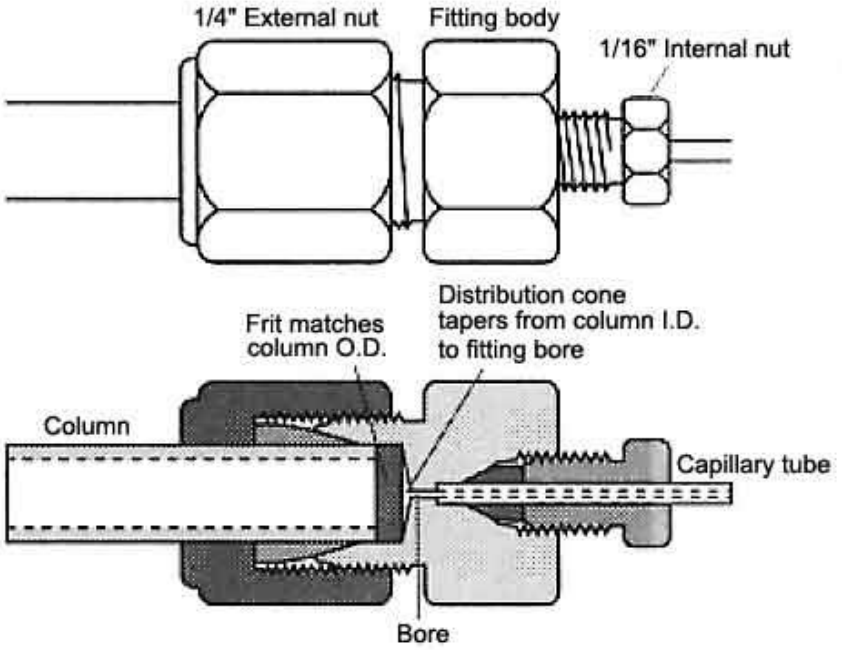
PEEK: Polyetheretherketone
PTFE: Polytetrafluoroethylene (Teflon)

Columns

Be careful about entrance and exit



Columns



Column End Fitting #994351
1/4" to 1/16" 4.6mm column I.D. with removable frit

Column Specifications

C18, 4.6 X 250 mm, 5 µm, 300 Å

bonded phase

column dimension

particle size

pore size

pH range: should be confirmed before use

Stationary Phase in HPLC

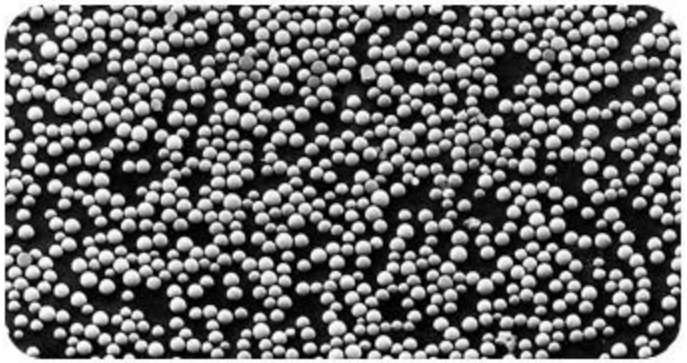
- **Chemically inert**
- **Non-soluble in any imaginable mobile phase**
- **Thermal and chemical stability**
- **Appropriate physical sorption of analyte**
- **Shape: Uniform spherical particles**

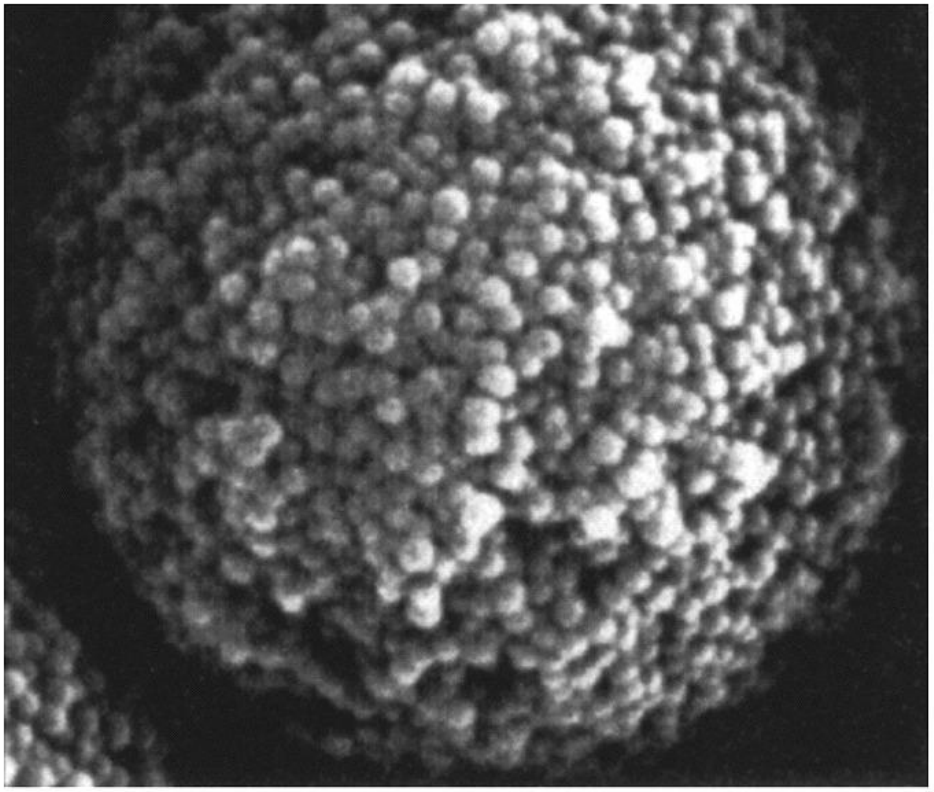
Particle Materials

PARTICLE MATERIALS

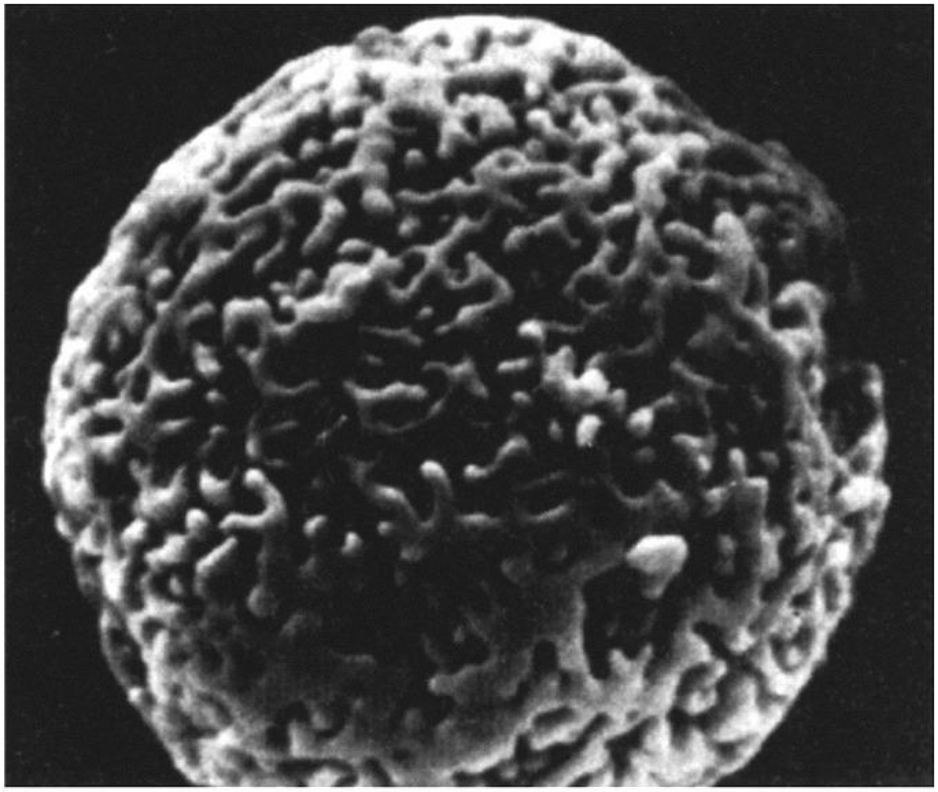
Particle materials are made of silica and polymers. Silica is by far the most popular particle material. Silica is made from sols and high purity organo silanes. Silica based particles are useful between pH 1.0 - 7.5. Silica particles resolve better than polymer based particles.

Polymer particles are useful in situations where pH extremes are required (2 - 14). Some polymer materials may be used as reversed-phase columns without a ligand attachment. The most common polymer for particle material is polydivinylbenzene.

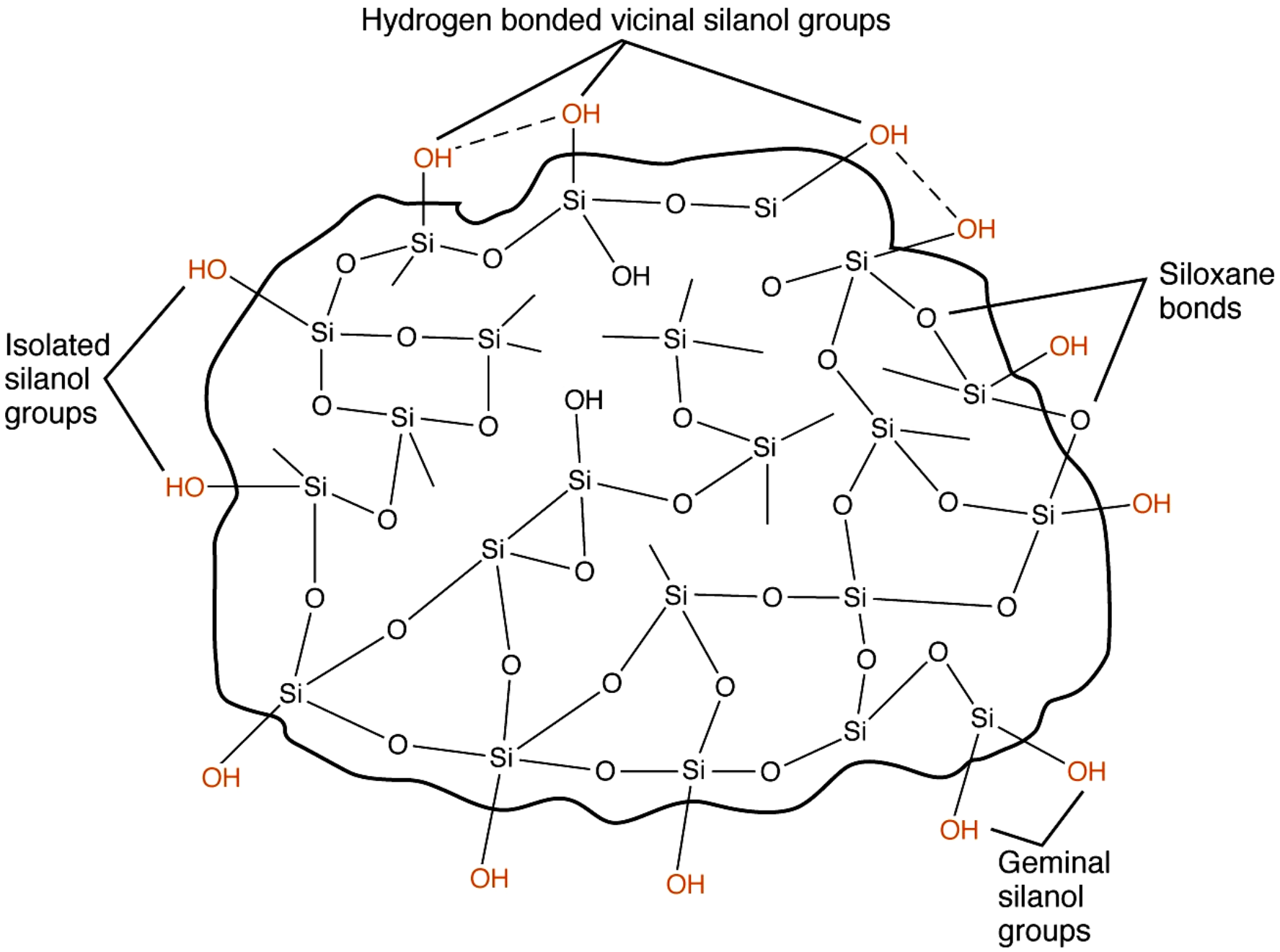




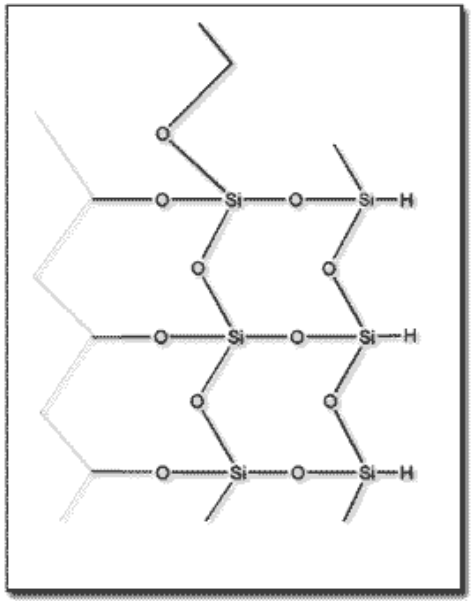
(a)



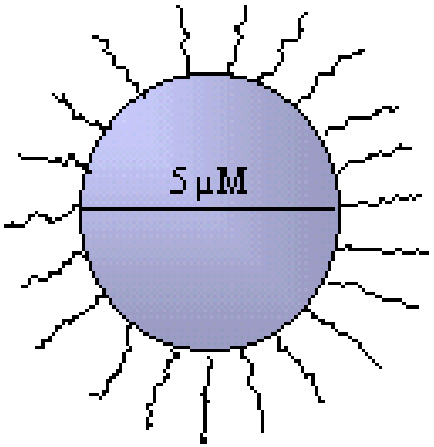
(b)


Hydrogen bonded vicinal silanol groups
Isolated silanol groups
Siloxane bonds
Geminal silanol groups
OH
HO
Si
O

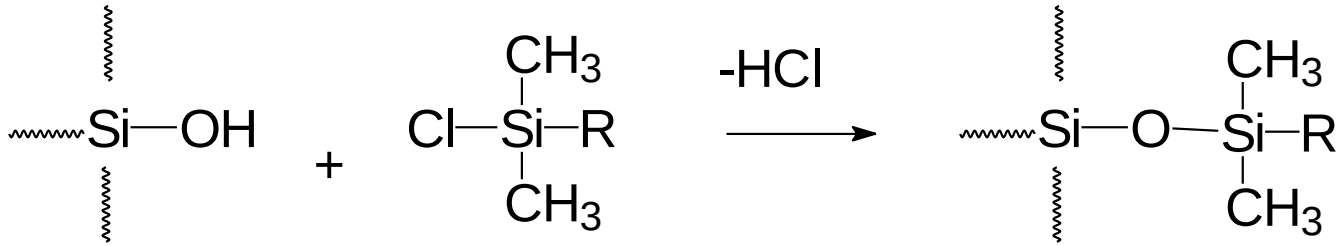
Chemical Surface Modification

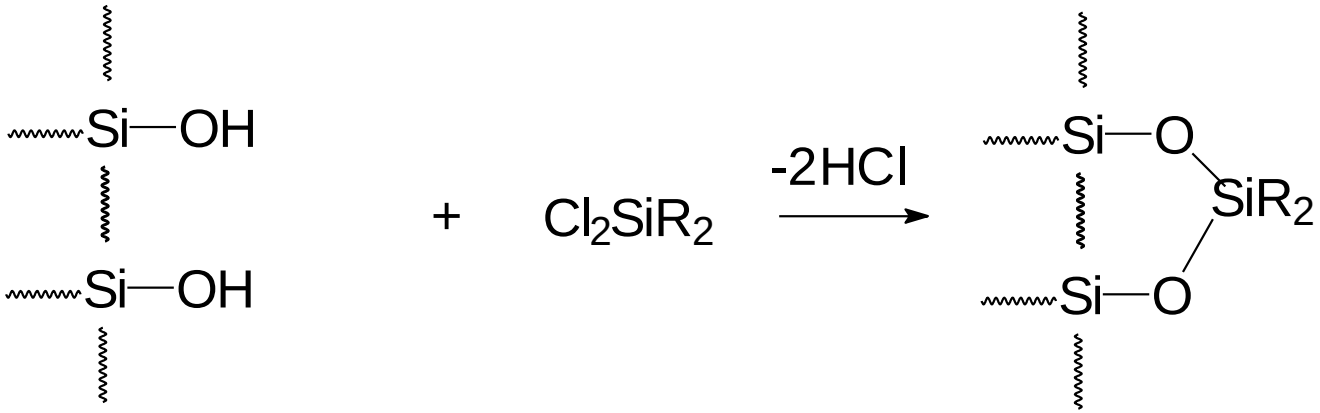


	BEH Particle				
	C_{18}	C_8	Shield RP18	Phenyl	HILIC
Chemistry					
Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl	—
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²	—
Carbon Load*	18%	13%	17%	15%	—
Endcap Style	Proprietary	Proprietary	TMS	Proprietary	—
pH Range	1-12	1-12	2-11	1-12	1-8

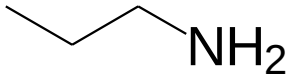


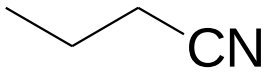
To give you an idea of scale a 5 µM silica particle is 30,000 Å across. You could fit 166, 300 Å pores across the equator of this particle. As you can see the C18 chains are not to scale.



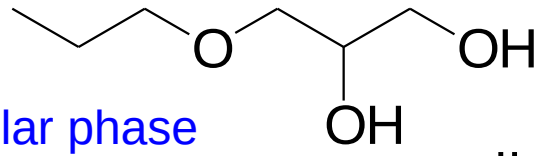


amino





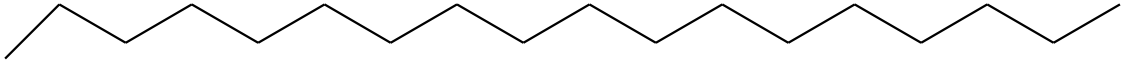
cyano



Polar phase

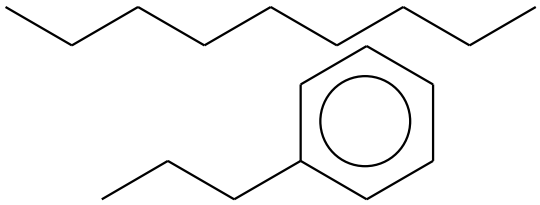
diol

octadecyl



Nonpolar phase

octyl



phenyl

Detectors

UV Detectors: most common

- Single wavelength (VWD)
- Multiple Wavelength (MWD)
- Diode Array Detector (DAD)

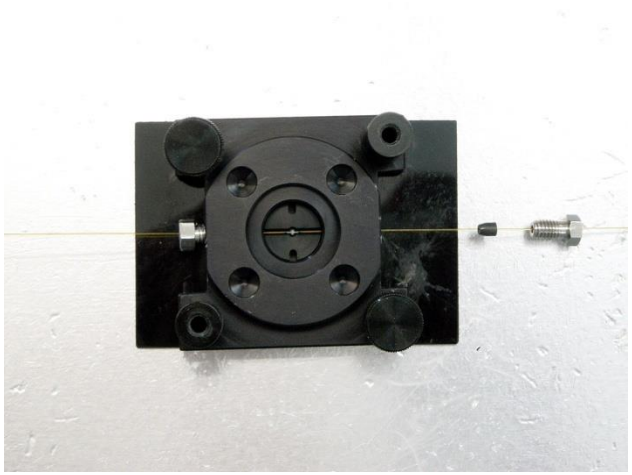
Refractive Index Detectors (RID)
Fluorescence Detectors (FD)
Electrochemical Detector (ECD)
Mass Detector (MS)

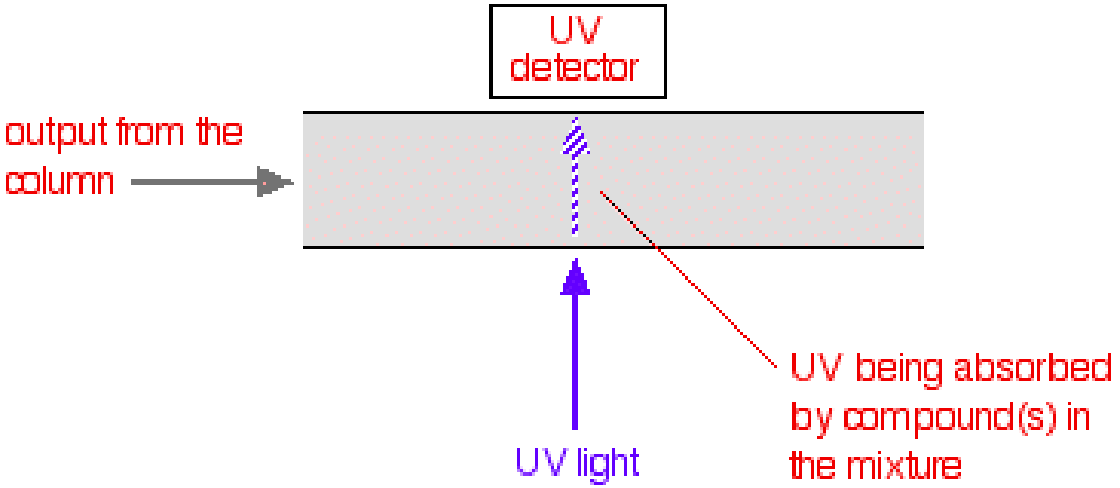
HP 1050 VWD

Detector Comparison

Detector	Sensitivity (nm)	selectivity	Ease of Use
VWD (UV)	500	Low	Easy
DAD (UV)	700	Low	Easy
Refractive Index	10000	Low	Easy
Mass Selective	10000	Low	Easy
Mass Spectral	10	Moderate	Skilled
Fluorescence	1	Very High	Easy
Electro-chemical	1	Very High	Skilled

UV Detectors

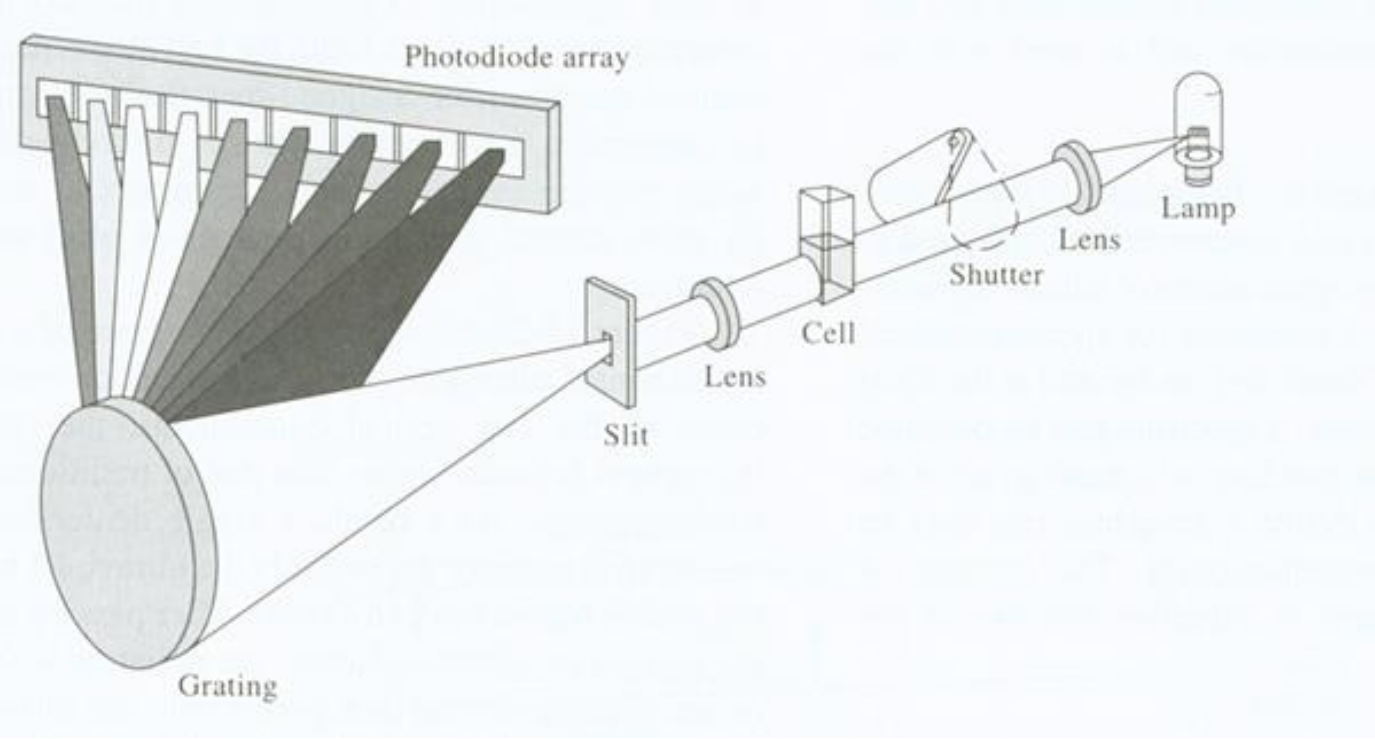




- Many organic compounds absorb UV light of various wavelengths.
- The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.
- You might wonder why the solvents used don't absorb UV light. They do! But different compounds absorb most strongly in different parts of the UV spectrum. Methanol, for example, absorbs at wavelengths below 210 nm, and water below 205 nm.
- If you were using a methanol-water mixture as the solvent, you would therefore have to use a wavelength greater than 210 nm to avoid false readings from the solvent.

Diode Array Detectors

Diode array: array of photosensitive semiconductors after the light goes through the sample.



Advantage: speed, sensitivity,

Disadvantage: resolution is 1 nm, vs 0.1 nm for normal UV

Table 25-2 Eluotropic series and ultraviolet cutoff wavelengths of solvents for adsorption chromatography on silica

Solvent	Eluent strength (ϵ°)	Ultraviolet cutoff (nm)
Pentane	0.00	190
Hexane	0.01	195
Heptane	0.01	200
Trichlorotrifluoroethane	0.02	231
Toluene	0.22	284
Chloroform	0.26	245
Dichloromethane	0.30	233
Diethyl ether	0.43	215
Ethyl acetate	0.48	256
Methyl *t*-butyl ether	0.48	210
Dioxane	0.51	215
Acetonitrile	0.52	190
Acetone	0.53	330
Tetrahydrofuran	0.53	212
2-Propanol	0.60	205
Methanol	0.70	205

The ultraviolet cutoff for water is 190 nm.

SOURCES: L. R. Snyder, in *High-Performance Liquid Chromatography* (C. Horváth, ed.), Vol. 3 (New York: Academic Press, 1983); *Burdick & Jackson Solvent Guide*, 3rd ed. (Muskegon, MI: Burdick & Jackson Laboratories, 1990).

Coupling HPLC to a Mass Spectrometer

- When the detector is showing a peak, some of what is passing through the detector at that time can be diverted to a mass spectrometer.
- There it will give a fragmentation pattern which can be compared against a computer database of known patterns.
- That means that the identity of a huge range of compounds can be found without having to know their retention times.

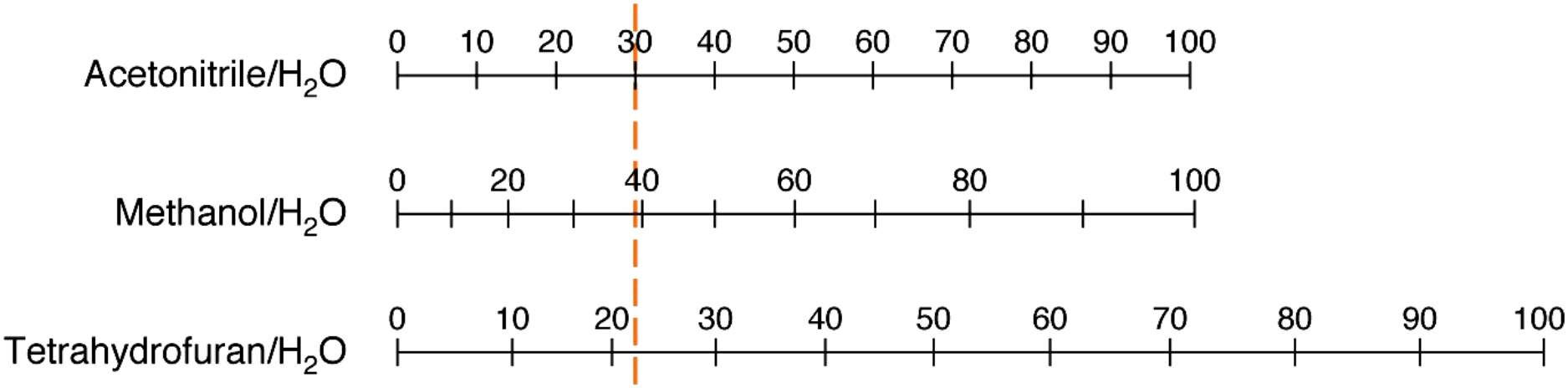
Table 25-4 Starting conditions for reversed-phase chromatography[1]

Stationary phase:	C_{18} or C_8 on 5-μm-diameter spherical silica particles. Less acidic Type B silica (Figure 25-7) is preferred. For operation above 50°C, sterically protected silica (Figure 25-8) is preferred.
Column:	0.46 × 15 cm column for 5-μm particles[a] 0.46 × 7.5 cm column for 3.5-μm particles (shorter run, same resolution)
Flow rate:	2.0 mL/min
Mobile phase:	CH_3CN/H_2O for neutral analytes CH_3CN/aqueous buffer[b] for ionic analytes 5 vol % CH_3CN in H_2O to 100% CH_3CN for gradient elution
Temperature:	35°–40°C if temperature control is available
Sample size:	25–50 μL containing ~25–50 μg of each analyte

a. A 0.30 × 15 cm column reduces solvent consumption to $(0.30/0.46)^2 = 43\%$ of the volume required for 0.46-cm diameter, reducing the flow to (0.43)(2.0 mL/min) = 0.86 mL/min.

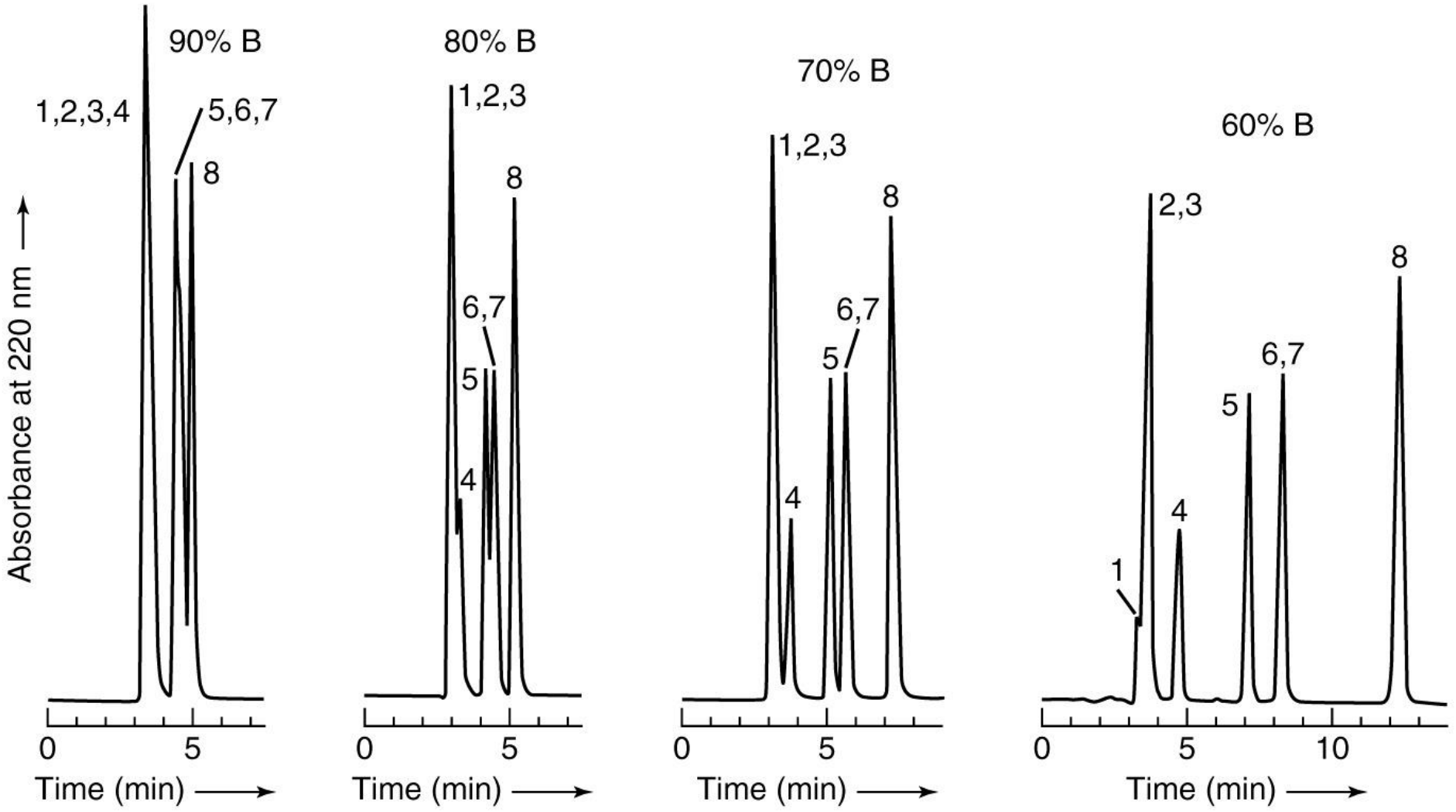
b. Buffer is 25–50 mM phosphate/pH 2–3 made by treating H_3PO_4 with KOH. K^+ is more soluble than Na^+ in organic solvents and leads to less tailing. Add 0.2 g sodium azide per liter as a preservative if the buffer will not be used quickly.

Polarity of Mobile Phase



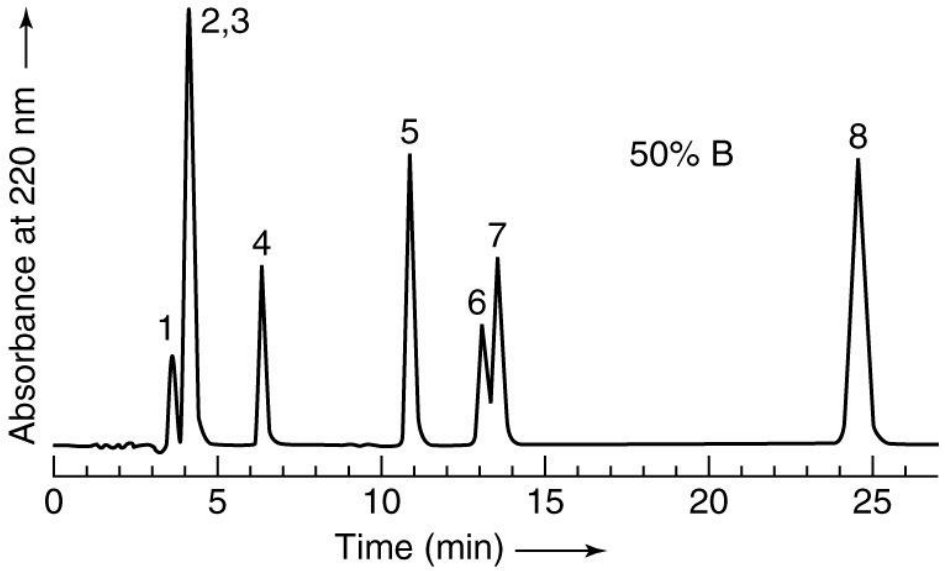
Percentage of solvents having the same eluent strength

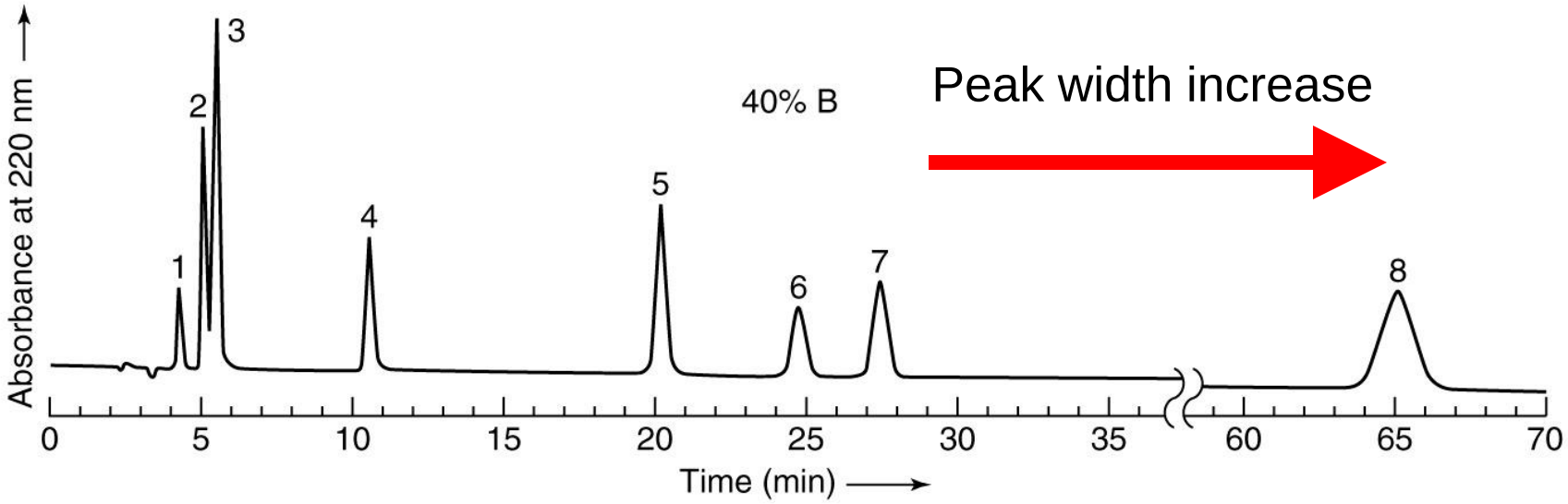
Polarity of Mobile Phase vs Resolution



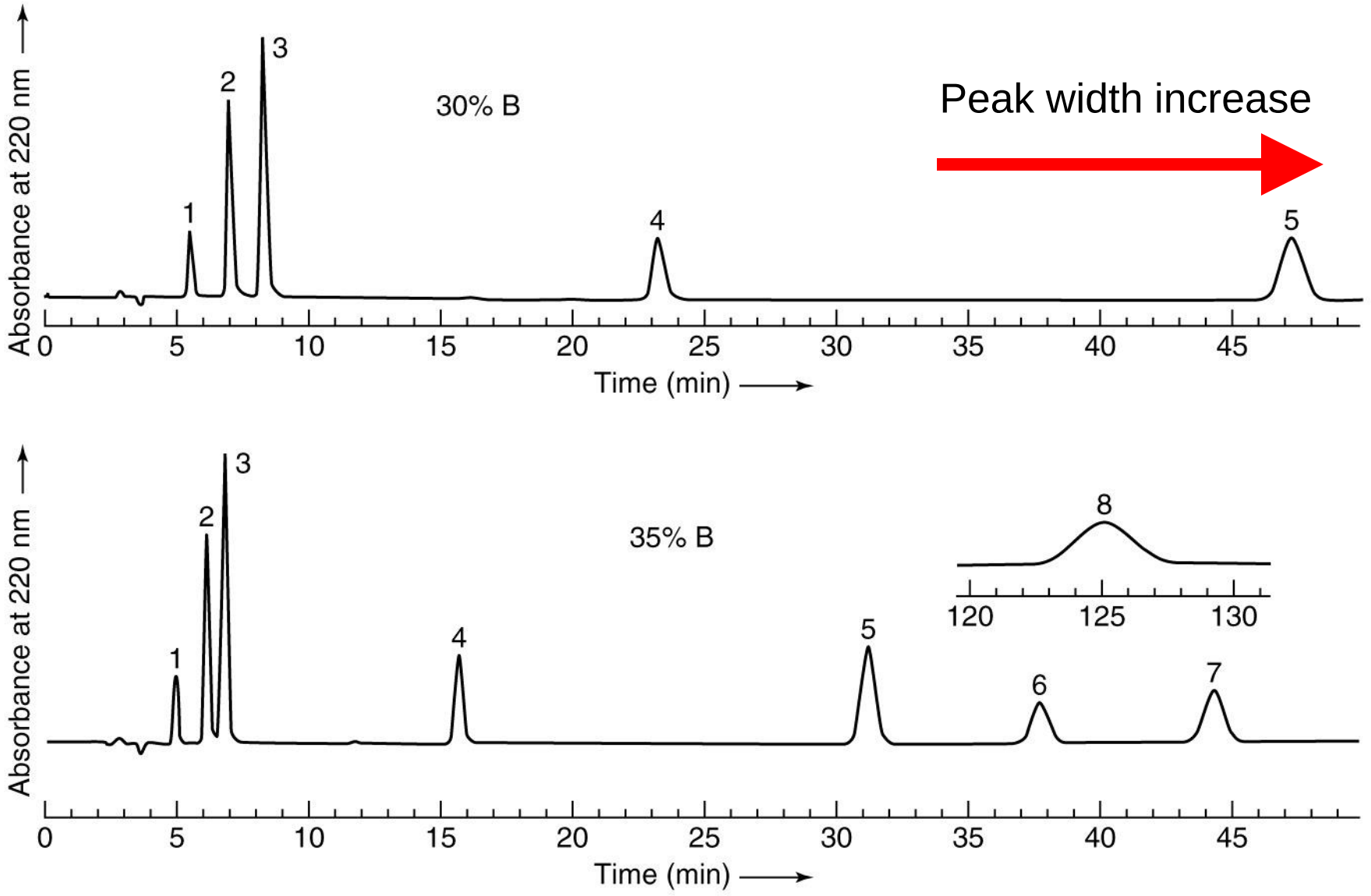
B - acetonitrile **CH_3CN**

Polarity of Mobile Phase vs Retention Time

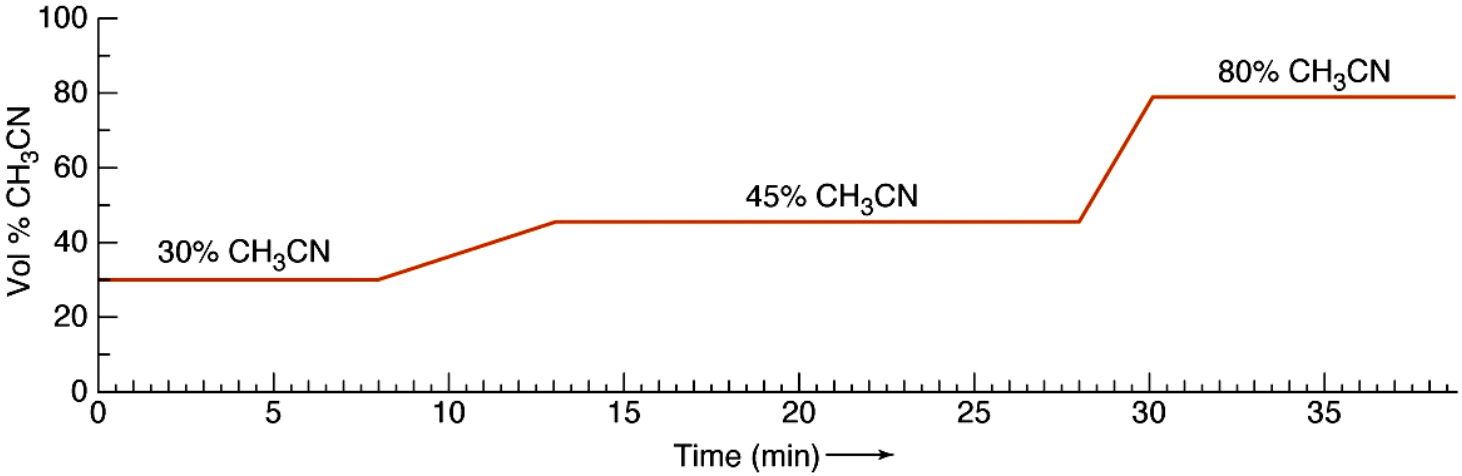


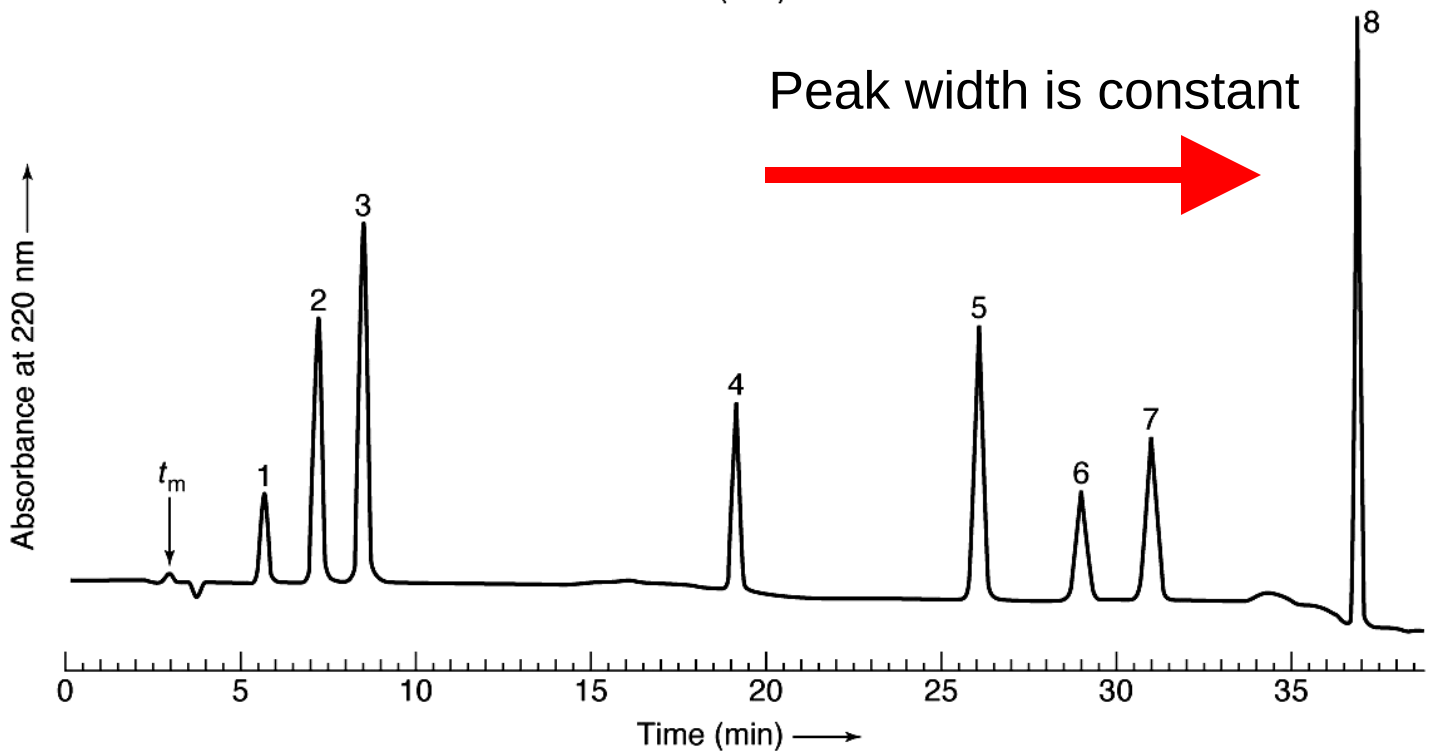


Polarity of Mobile Phase vs Retention Time



Gradient Mobile Phase





First Run of the Day

One observation is that if you start up a reverse phase analysis from a dead stop with a column that has perhaps been sitting in high aqueous conditions for up to 10 hours the analysis will give irreproducible results.

Conventional wisdom has it,

- you want to first flush the column with the highest % organic of your method for at least 3 column volumes and then bring it back to the equilibrating condition.
- Check the pressure stabilized
- This practice may have the advantage of getting you to standard equilibration conditions faster and it will also clean your column.
- A better alternative is to make the first run a blank run (or "preparation run") and then the next run can be your real analysis.
- We prefer the second option because it should get you to the standard starting conditions more accurately.

Last Run of the Day

- We store our columns in 50/50 methanol/water without any acid.
- If you are using a salt, unlikely in LC/MS, wash your entire system, solvent bottles, HPLC, solvent lines, and column, into a non-salt containing solvent.
- Salt may precipitate out and plug your HPLC or column or may cause corrosion. Usually we flush with pure water first then leave the system in 50/50 methanol: water.
- Some salts may precipitate out in high organic so an initial water wash is advised.
- The 50/50 methanol:water solution helps to stop bacterial growth which can muck up your system.
- Take care of your HPLC, it's the right thing to do!
- If you spend 30 min now, you will save 3 hr tomorrow.