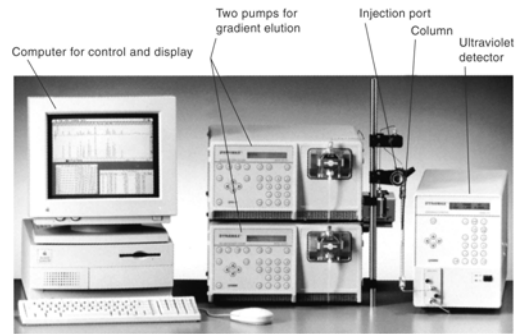


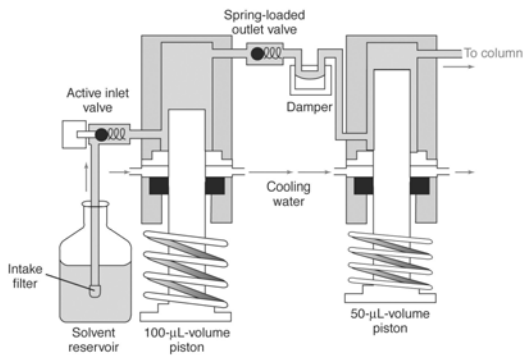
Introduction to HPLC

Special Topics
Dr. Bryan

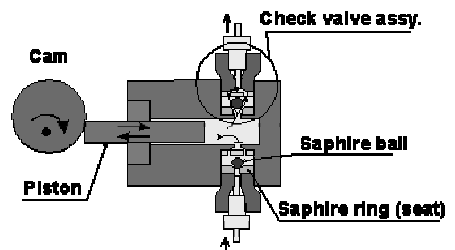
The Instrumentation



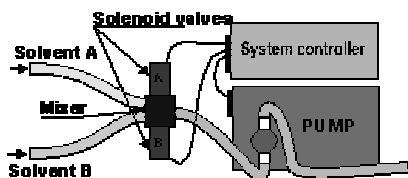
Pump Design



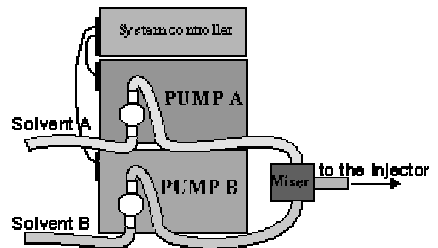
Pump Head



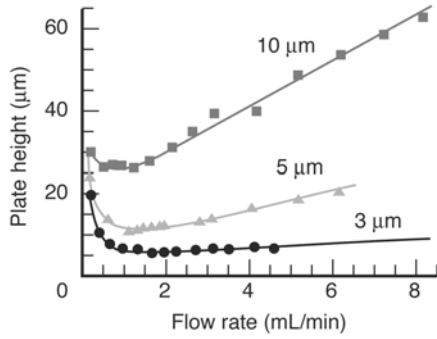
Gradient- Low Pressure Mixing



Gradient- High Pressure Mixing



HETP vs Particle Size



Performance vs Particle Size

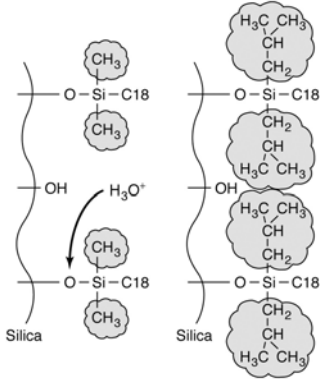
Table 25-1 Performance as a function of particle diameter

Particle size d_p (μm)	Retention time (min)	Plate number (N)	Required pressure (bar)
5.0	30	25 000	19
3.0	18	42 000	87
1.5	9	83 000	700
1.0	6	125 000	2 300

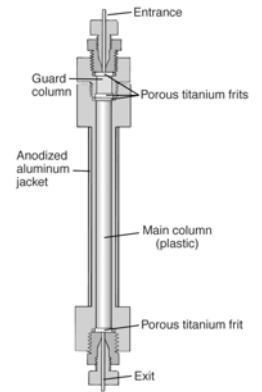
Theoretical performance of 33- μm -diameter \times 25-cm-long capillary for minimum plate height for solute with capacity factor $k' = 2$ and diffusion coefficient = $6.7 \times 10^{-10} \text{ m}^2/\text{s}$ in water-acetonitrile eluent.

SOURCE: J. E. MacNair, K. D. Patel, and J. W. Jorgenson, "Ultra-high-Pressure Reversed-Phase Capillary Liquid Chromatography with 1.0- μm Particles," *Anal. Chem.* **1999**, *71*, 700.

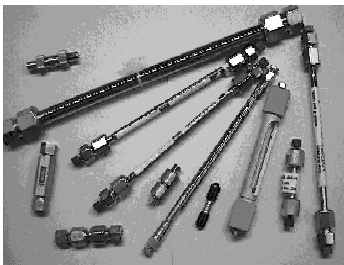
Variations in C18 packing



HPLC Column Cross Section

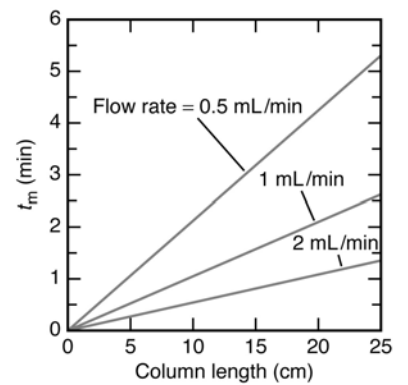


Columns Column Selection

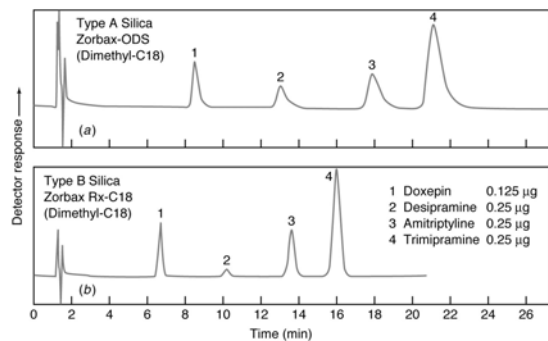


ColSel.1

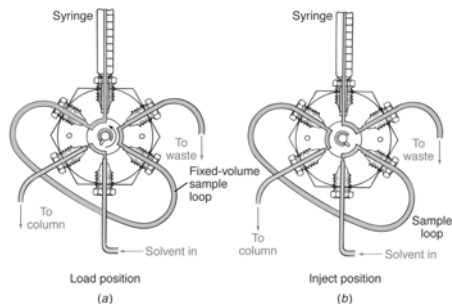
Flow Rate and Column Length



Comparison of Packing



Manual Injector (Rheodyne)



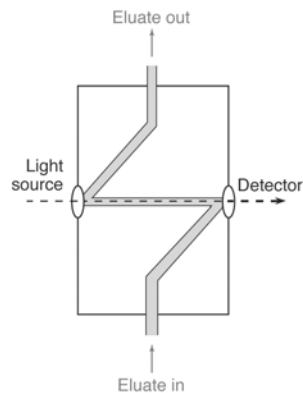
HPLC Detectors - Table

Table 25-3 Comparison of commercial HPLC detectors

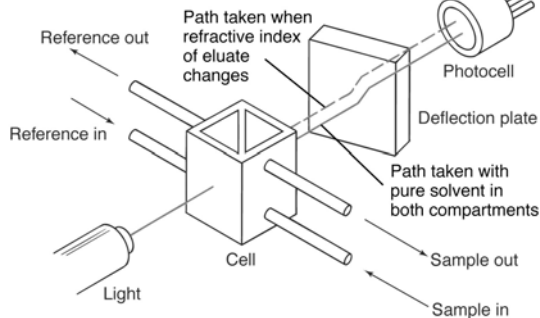
Detector	Approximate limit of detection ^a (ng)	Useful with gradient?
Ultraviolet	0.1-1	Yes
Refractive index	100-1 000	No
Evaporative light-scattering	0.1-1	Yes
Electrochemical	0.01-1	No
Fluorescence	0.001-0.01	Yes
Nitrogen ($N \xrightarrow{\text{combustion}} NO \xrightarrow{O_3} NO_2 \rightarrow hv$)	0.3	Yes
Conductivity	0.5-1	No
Mass spectrometry	0.1-1	Yes
Fourier transform infrared	1 000	Yes

a. Most detection limits from E. W. Yeung and R. E. Synovec, "Detectors for Liquid Chromatography," *Anal. Chem.* **1986**, *58*, 1237A.

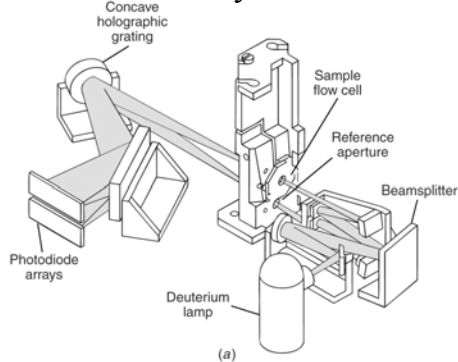
UV Flow Cell



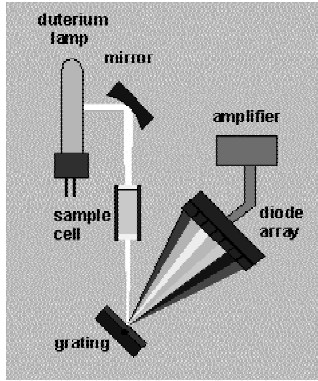
Refractive Index Detector



Diode Array Detector



Diode Array Detector II



Eluent Strength

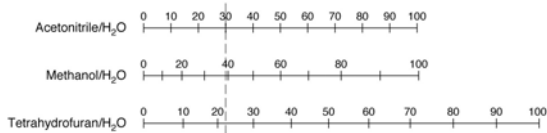
Table 25-2 Eluotropic series and ultraviolet cutoff wavelengths of solvents for adsorption chromatography on silica

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 <i>i</i> -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

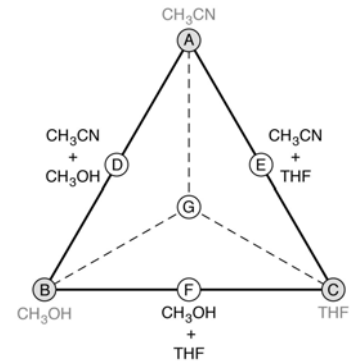
The ultraviolet cutoff for water is 190 nm.

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

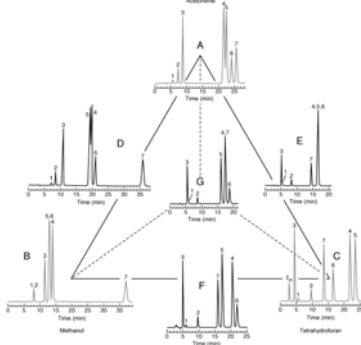
Comparison of ACN, Methanol and THF as Mobile Phases



Mobile Phase Pyramid



Mobile Phase Pyramid and Chromatograms



Altering Percent Organic in MP

