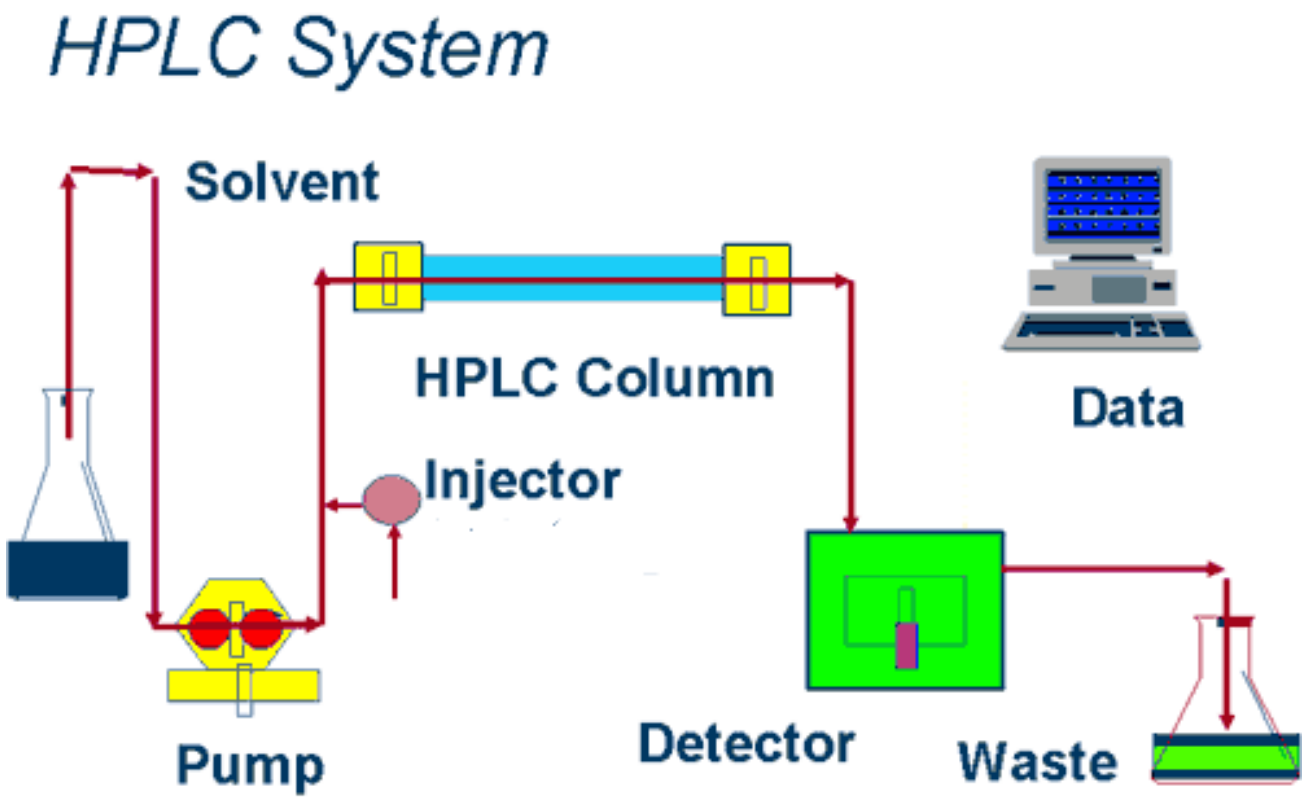
HPLC Instrumentation



Elution

Gradient elution **in liquid chromatography is analogous to temperature programming in gas chromatography.**

Increased eluent strength is required to elute more strongly retained solutes. RP-HPLC (increase organic, make more non-polar). NP-HPLC (increase solvent to make more polar)

Gradient **(gradual change) of eluent strength is used for many separations.**

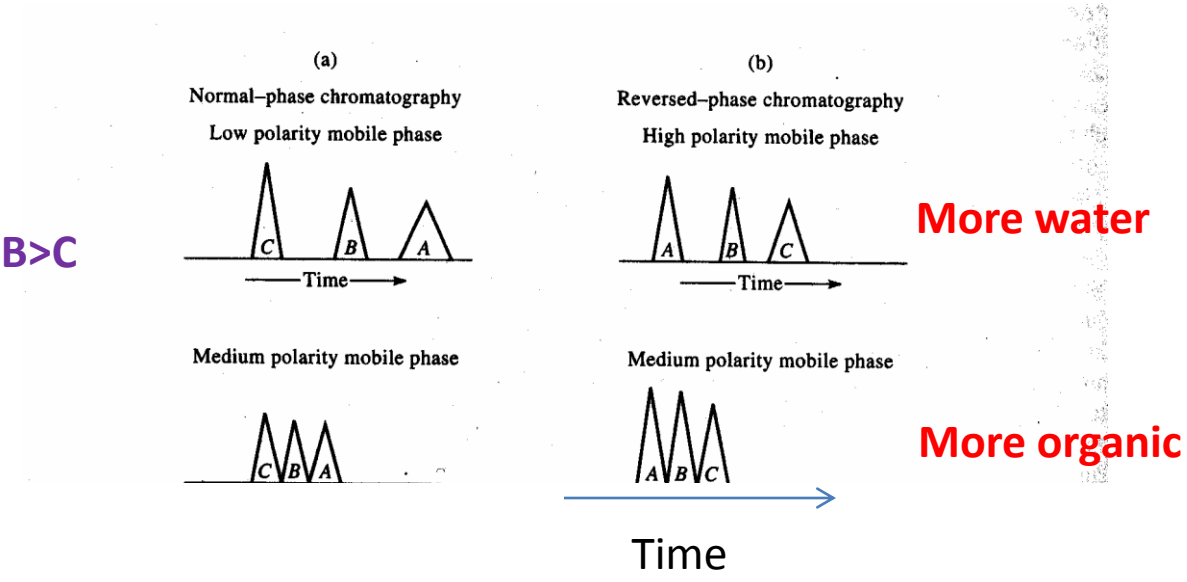
Weakly retained solutes are eluted with solvent of low eluent strength. Second solvent is mixed with the first to elute the more strongly retained solutes. Gradual increase in eluent strength.

Partition Chromatography

✓ **Normal-phase chromatography – uses a polar stationary phase and a less polar solvent.** ***Eluent strength is increased by adding a more polar solvent. No use of water!!***

✓ **Reversed-phase chromatography – uses a non-polar stationary phase.** ***Eluent strength is increased by adding a less polar solvent. Water is often constituent of mobile phase.***

Polarity: A>B>C



HPLC Detectors

TABLE 28-1 Performance of HPLC Detectors

HPLC Detector	Commercially Available	Mass LOD* (typical)	Linear Range† (decades)
Absorbance	Yes	10 pg	3–4
Fluorescence	Yes	10 fg	5
Electrochemical	Yes	100 pg	4–5
Refractive index	Yes	1 ng	3
Conductivity	Yes	100 pg–1 ng	5
Mass spectrometry	Yes	<1 pg	5
FTIR	Yes	1 µg	3
Light scattering	Yes	1 µg	5
Optical activity	No	1 ng	4
Element selective	No	1 ng	4–5
Photoionization	No	<1 pg	4

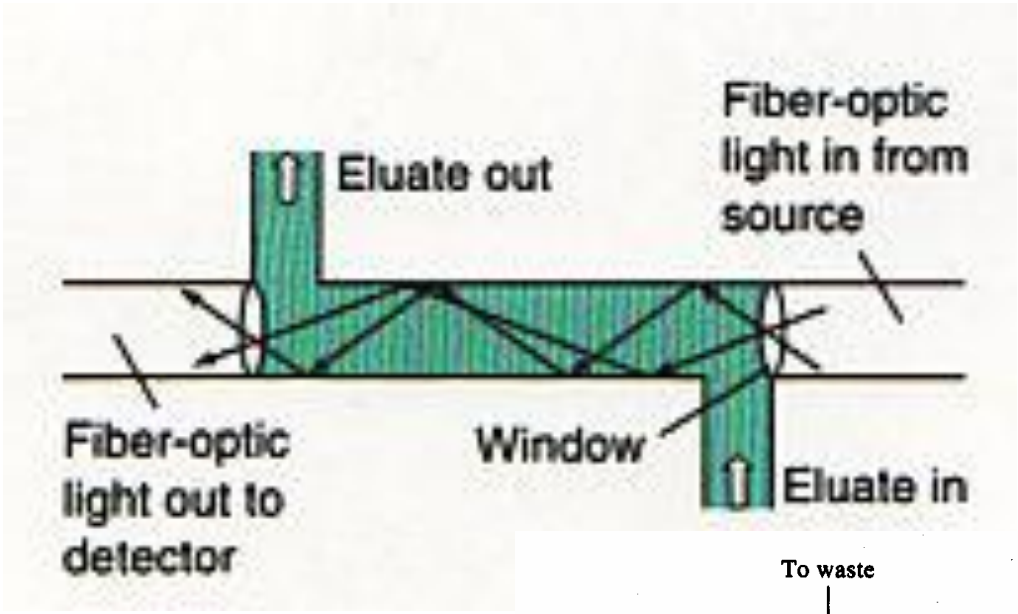
Sources: From manufacturer's literature; *Handbook of Instrumental Techniques for Analytical Chemistry*, F. Settle, ed., Upper Saddle River, NJ: Prentice-Hall, 1997; E. S. Yeung and R. E. Synovec, *Anal. Chem.*, **1986**, *58*, 1237A.

*Mass LODs (limits of detection) depend on compound, instrument, and HPLC conditions, but those given are typical values with commercial systems when available.

†Typical values from the preceding sources.

Mass LOD (g) = concentration (mol/L) x inj. vol. (L) x FW (g/mol)

Detectors (UV/Vis or Fluorescence)



Detector volume small like injection volume (10-20 µL)

$$A = \varepsilon b C$$

$$F \alpha \varepsilon b C P_o \Phi$$

What is the path length in these cells?

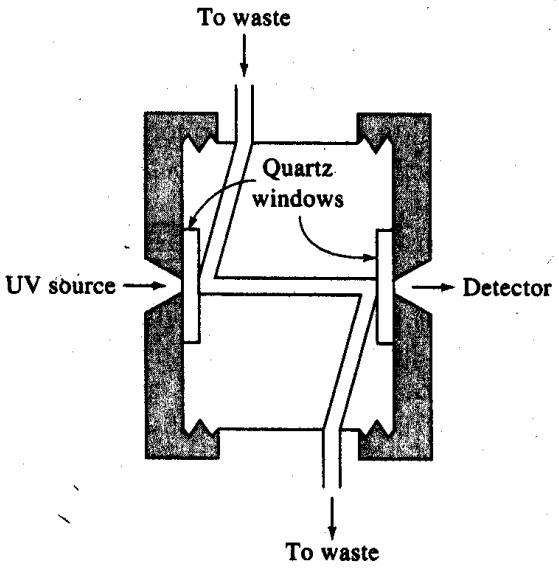


Figure 28-9 Ultraviolet detector cell for HPLC.

Fluorescence detection is 90° relative to excitation light

Detectors (Mass Spectrometry)

Qualitative and Quantitative Analysis

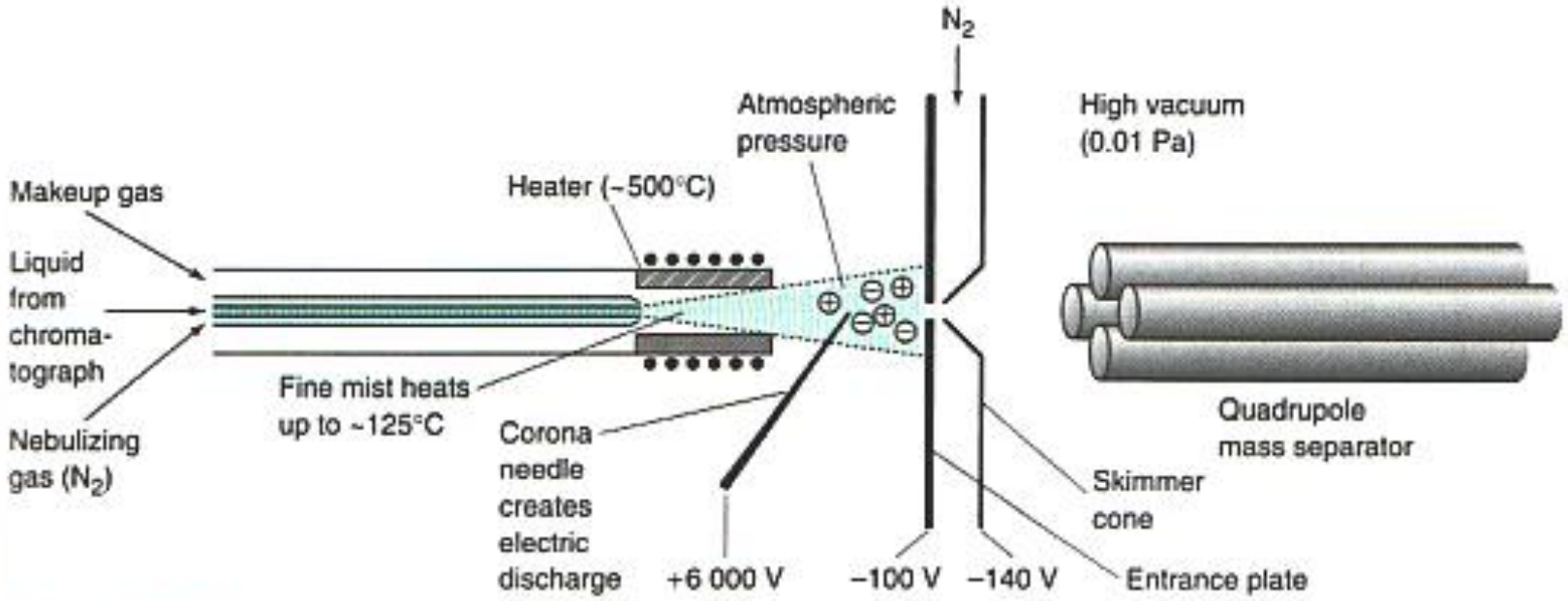
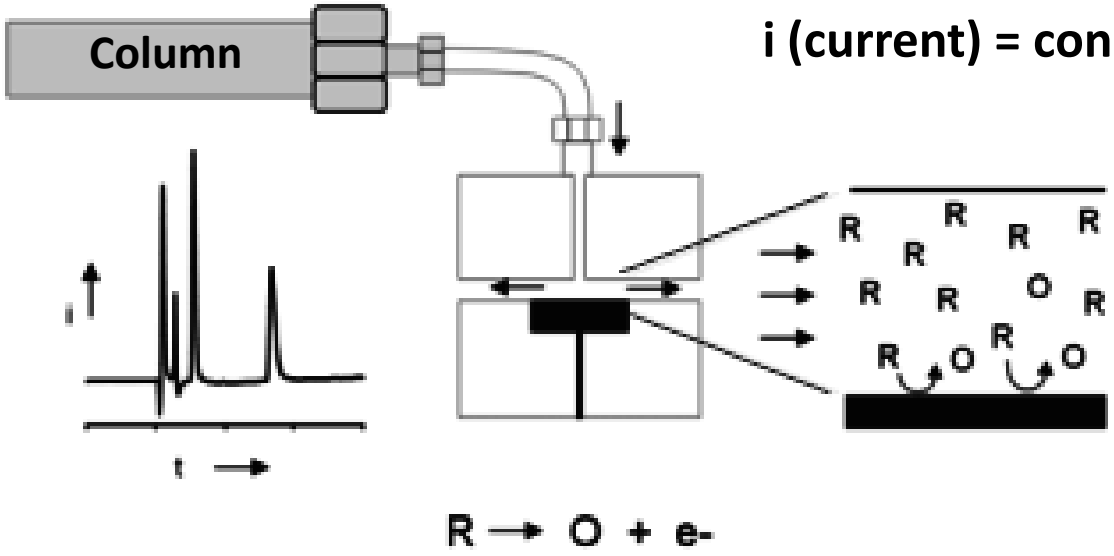


Figure 22-26 Atmospheric pressure chemical ionization interface between liquid chromatography column and mass spectrometer. Aerosol is produced by the nebulizing gas flow and the heater. Electric discharge from the corona needle creates gaseous ions from analyte.
[Adapted from E. C. Huang, T. Wachs, J. J. Conboy, and J. D. Henion, *Anal. Chem.* **1990**, *62*, 713A.]

Need an interface that can take a continuous liquid eluent from LC and convert analyte to ions in the gas for MS. Electrospray ionization is also an LC interface with MS.

Detectors (Electrochemical)

Electrochemical detection (ECD) for HPLC is an extremely selective and sensitive detection technique that is applied in a number of analyses such as the neurotransmitters dopamine, serotonin and noradrenalin. In combination with the proper electronics, ECD has a linear dynamic range of more then 6 orders of magnitude. This means that concentrations can be measured as low as 50 pmole/L and as high as 100 µmole/L or more.



i (current) = const x [Analyte]

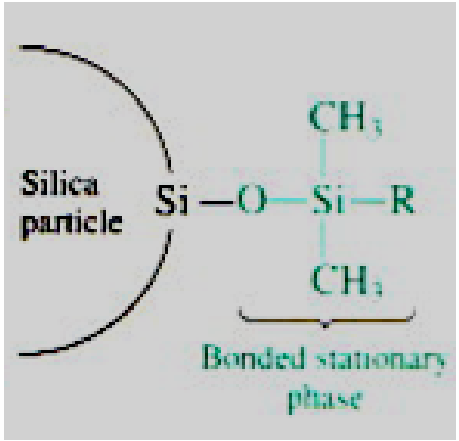
HPLC with amperometric detection.

Bonded Stationary Phases (Stable and Controlled Chemistry)

Table 22-3 Some common bonded phases for liquid chromatography

Bonded polar phases		Bonded nonpolar phases	
$R = (CH_2)_3NH_2$	Amino	$R = (CH_2)_{17}CH_3$	Octadecyl
$R = (CH_2)_3C{\equiv}N$	Cyano	$R = (CH_2)_7CH_3$	Octyl
$R = (CH_2)_3OCH_2CH(OH)CH_2OH$	Diol	$R = (CH_2)_3C_6H_5$	Phenyl
$R = (\text{spacer})CH_2\overset{+}{N}(CH_3)_2(CH_2)_3SO_3^-$	ZIC-HILIC®	$R = (CH_2)_3C_6F_5$	Pentafluorophenyl

Normal-phase LC **Reversed-phase LC**

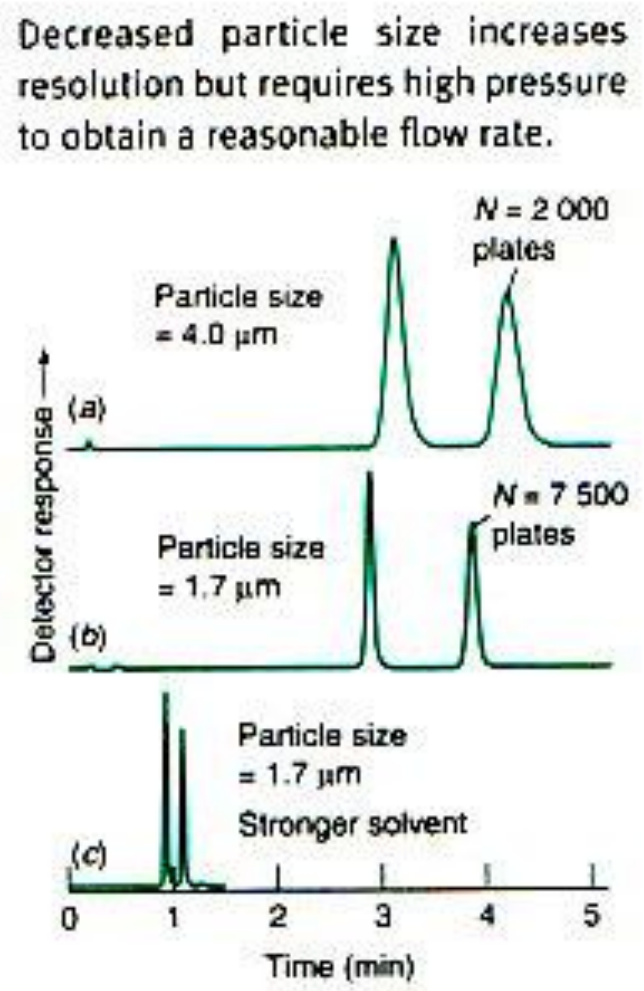


Organochlorosilane coupling chemistry!!

R group can be whatever one wants.

Particle Size Effect of Stationary Phase

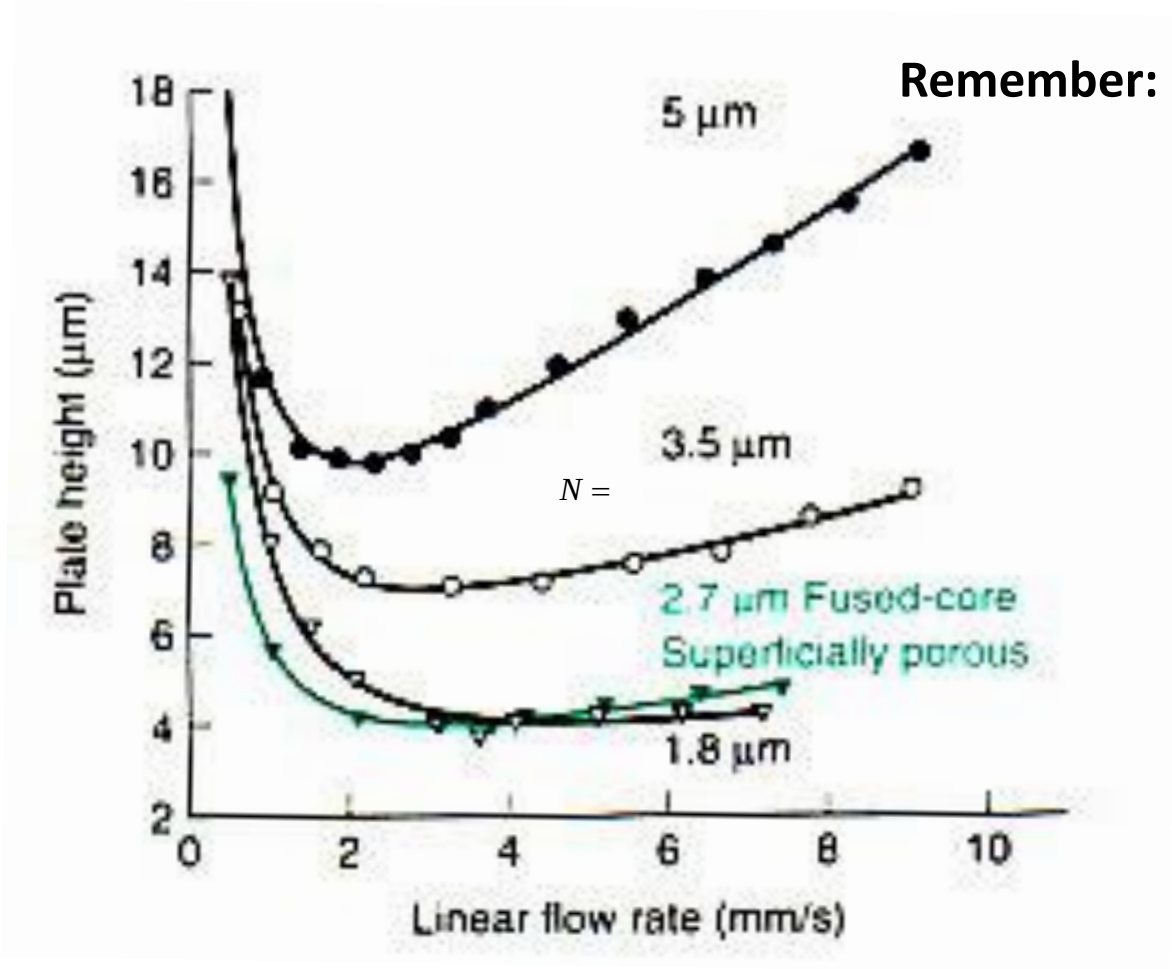
Smaller particles, more efficient packing, lower A term.



Stronger solvent, greater affinity for mobile phase (better solubility)

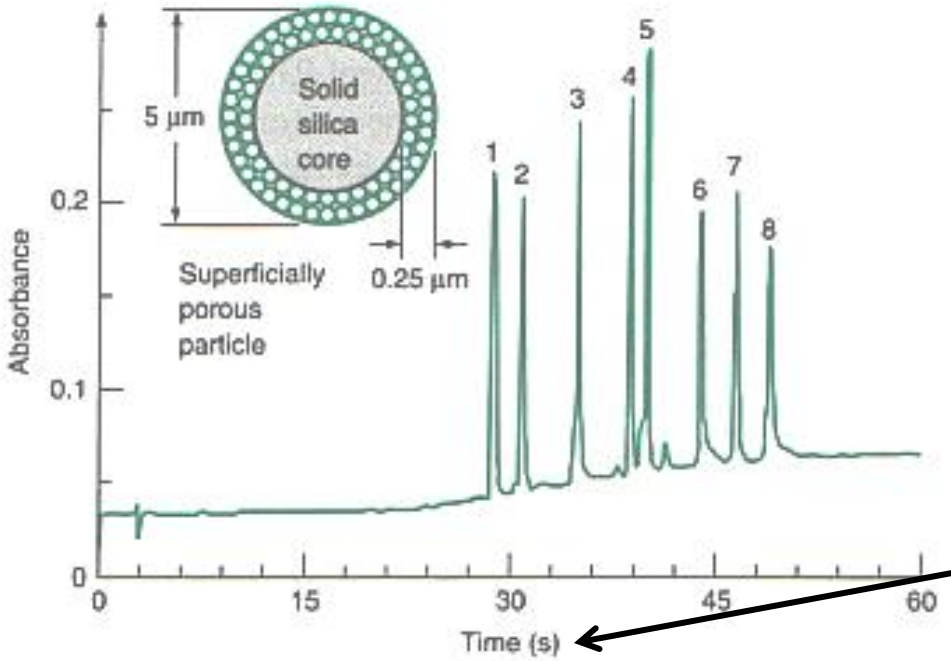
Effect of Particle Size of Stationary Phase

Remember: $N = \frac{L}{H}$



Smaller particle size, more efficient packing and improved plate numbers (efficiency of separation).

Reversed-Phase HPLC



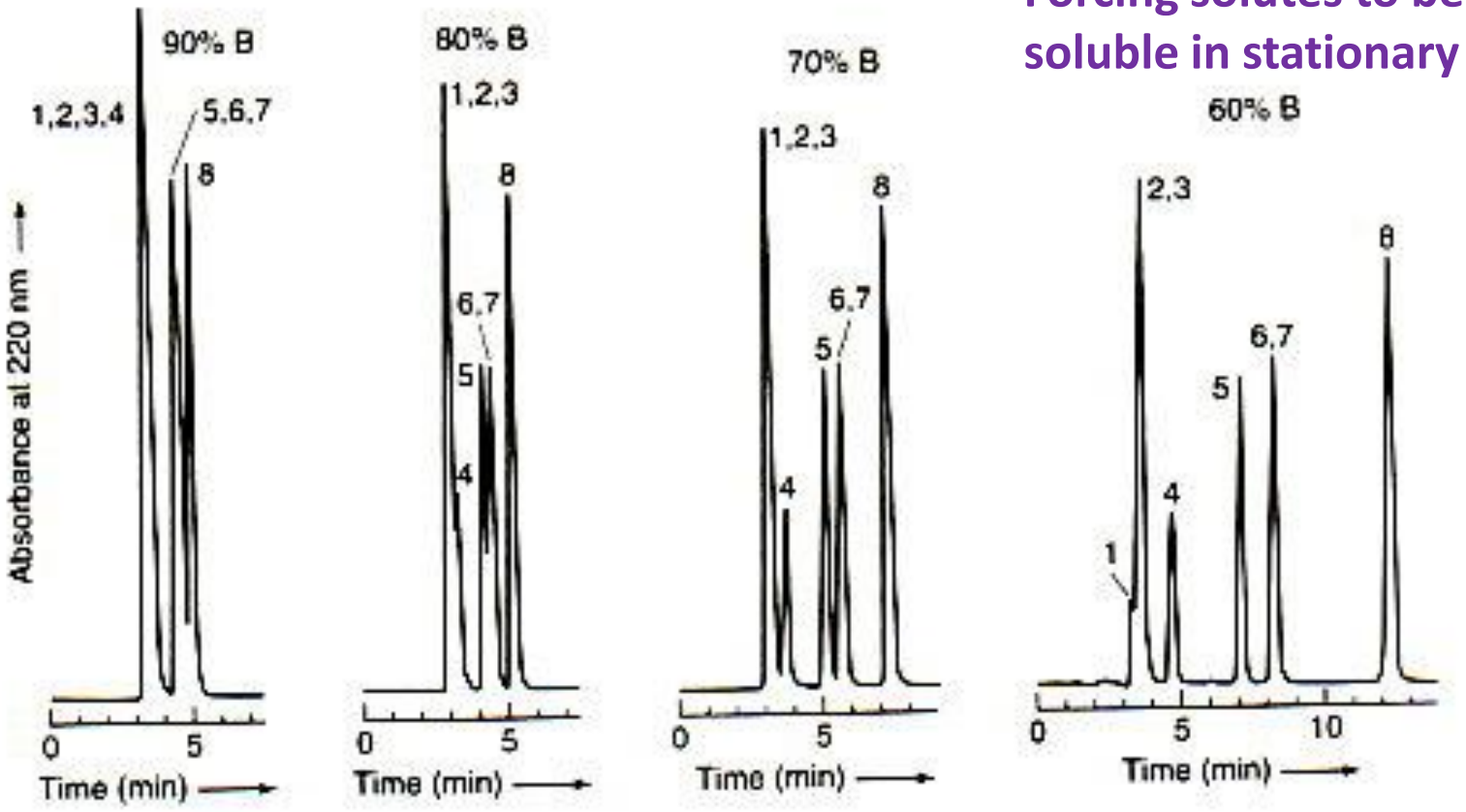
Notice the time!

Figure 22-19 Rapid separation of eight proteins on superficially porous C_{18}-silica in 75 × 2.1 mm column containing Poroshell 300SB-C18 eluted at 3 mL/min at 70°C at 26 MPa (260 bar) with ultraviolet detection at 215 nm. [From R. E. Majors, *LCGC Column Technology Supplement*, June 2004, p. 8K. Courtesy Agilent Technologies.]

Reversed-Phase HPLC

Effect of organic solvent content (mixed with water)

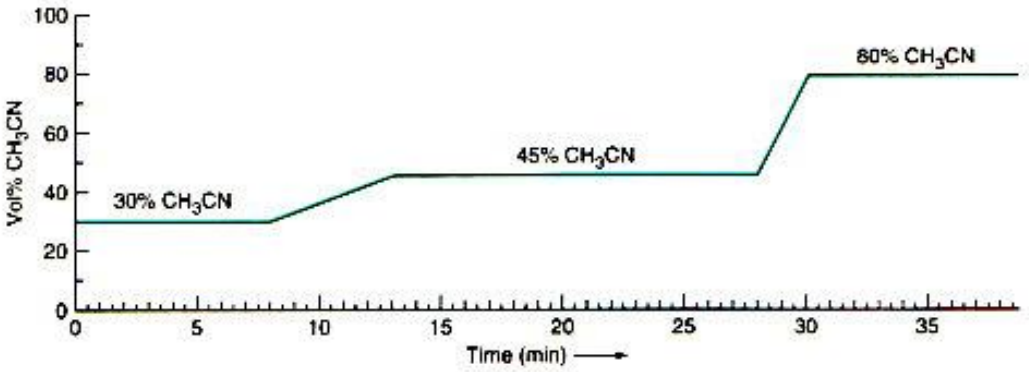
Forcing solutes to be more soluble in stationary phase!!



Decreasing organic solvent content ⟶

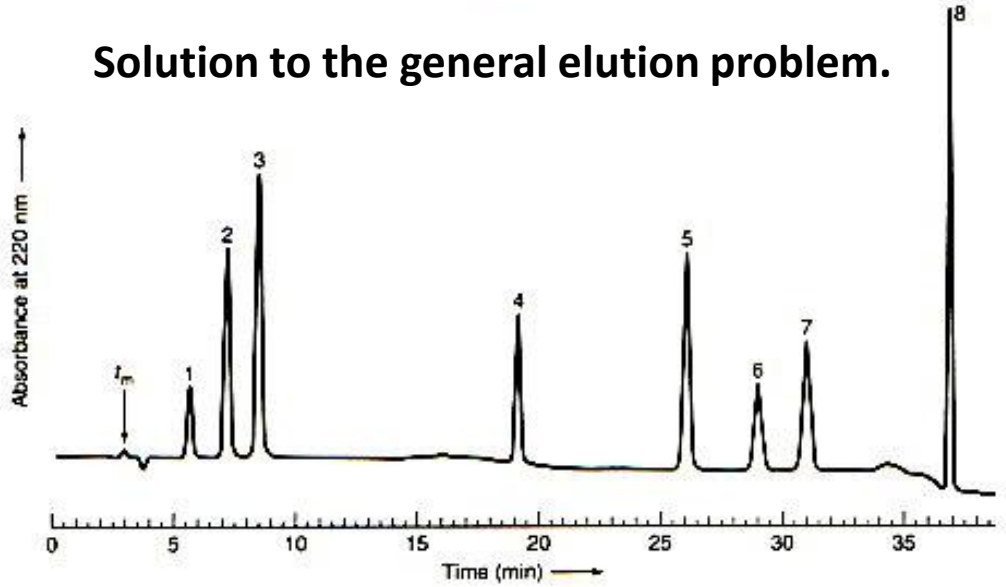
Gradient Elution in RP-HPLC

Solvent plays a key role in separation!!!!



Make the solutes more soluble in mobile phase than in stationary phase!!!

Solution to the general elution problem.



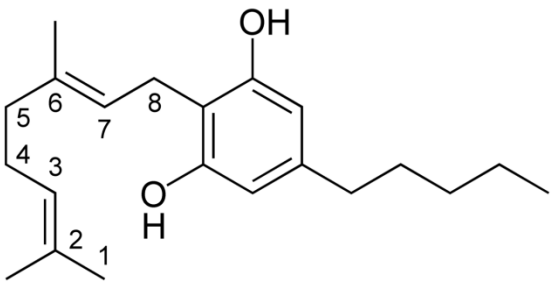
Nonpolar stationary phase. To elute solutes, must increase the eluent strength by adding organic solvent (mixed with water).

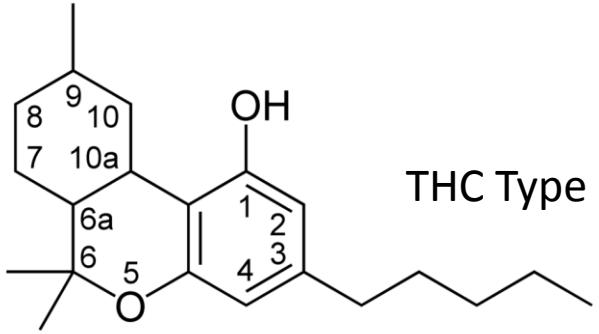
Quantitative Analysis of Naturally Occurring Cannabinoids

The analysis of natural cannabinoids is necessary not only because of potential medical uses for these compounds, but also in the regulation and quality control testing of products containing these compounds. To ensure the authenticity, quality, and amount of each cannabinoid contained in the product, an LC–MS method was developed.

This method showed certain medicinal oils or tinctures available over the internet contained naturally occurring cannabinoids.

CBG Type





THC Type

What structural features make them separable by RP-HPLC? Could UV/Vis detection be used? Why?

RP-HPLC Natural Cannabinoids

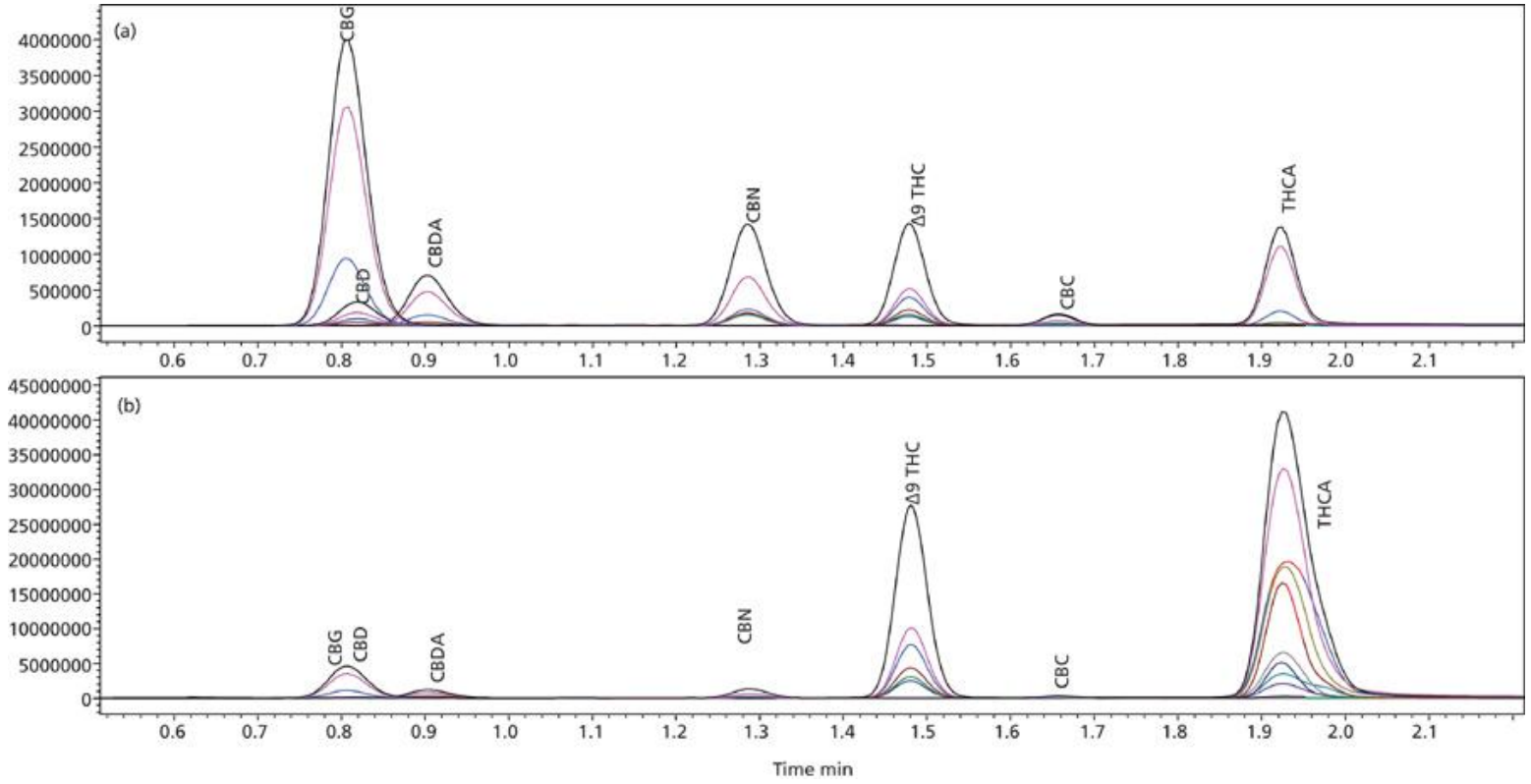


Figure 1: (a) Chromatogram of seven cannabinoids at 100 ng/mL in solvent. (b) Chromatogram of a commercially available tincture containing seven cannabinoids. Standard additions shown.

Detection Figures of Merit for Different Cannabinoids

Table I: Quantitative results for each cannabinoid at the limit of quantitation and the concentration of the commercial tincture.

Quantitative Results at LLOQ (n=6)						
Compound	LOD (ng/mL)	%RSD	%Accuracy	S/N	Weighting	Commercial Tincture
CBN	1	4.516099	99.998± 4.2%	58.96	$1/C^2$	0.016% ± 0.001%
THCA	1	7.023558	99.998 ± 9.1%	21.14	1/C	0.452% ± 0.018%
CBDA	1	6.671582	100.001 ± 5.7%	70.42	$1/C^2$	0.019% ± 0.001%
Δ9 THC	1	6.414479	99.997 ± 6.3%	85.89	$1/C^2$	0.370% ± 0.021%
CBG	1	3.666911	100.000 ± 3.7%	2397.6	$1/C^2$	0.018% ± 0.0004%
CBD	1	7.770838	100.123 ± 6.8%	107.4	$1/C^2$	0.006% ± 0.001%
CBC	2.5	8.193242	100.006± 5.7%	70.64	1/C	0.029% ± 0.006%

This work demonstrates a rapid method for the detection of naturally occurring cannabinoids by using LC-MS. All seven cannabinoids were detected at levels as low as 1 ng/mL (1 ppb or 1 pg on column) with a S/N of at least 20:1. This method is useful for quantitating cannabinoids in raw or commercial products.