Liquid Chromatography

1. Introduction and Column Packing Material

2. Retention Mechanisms in Liquid Chromatography

3. Method Development

4. Column Preparation

5. General Instrumental aspects

6. Detectors

(Chapter 4 and 5 in The essence of chromatography)

Column Preparation

1. Column is the central part of liquid chromatography.

2. Column Packing Methods

A. Dry-Packing Procedures: for rigid particles with diameter greater than 20 μm.

B. Down-Fill Slurry Packing: for rigid particles with diameter smaller than 20 μm.

C. Up-Fill Slurry Packing: for rigid particles with diameter smaller than 20 μm.

Down-Fill Slurry Packing

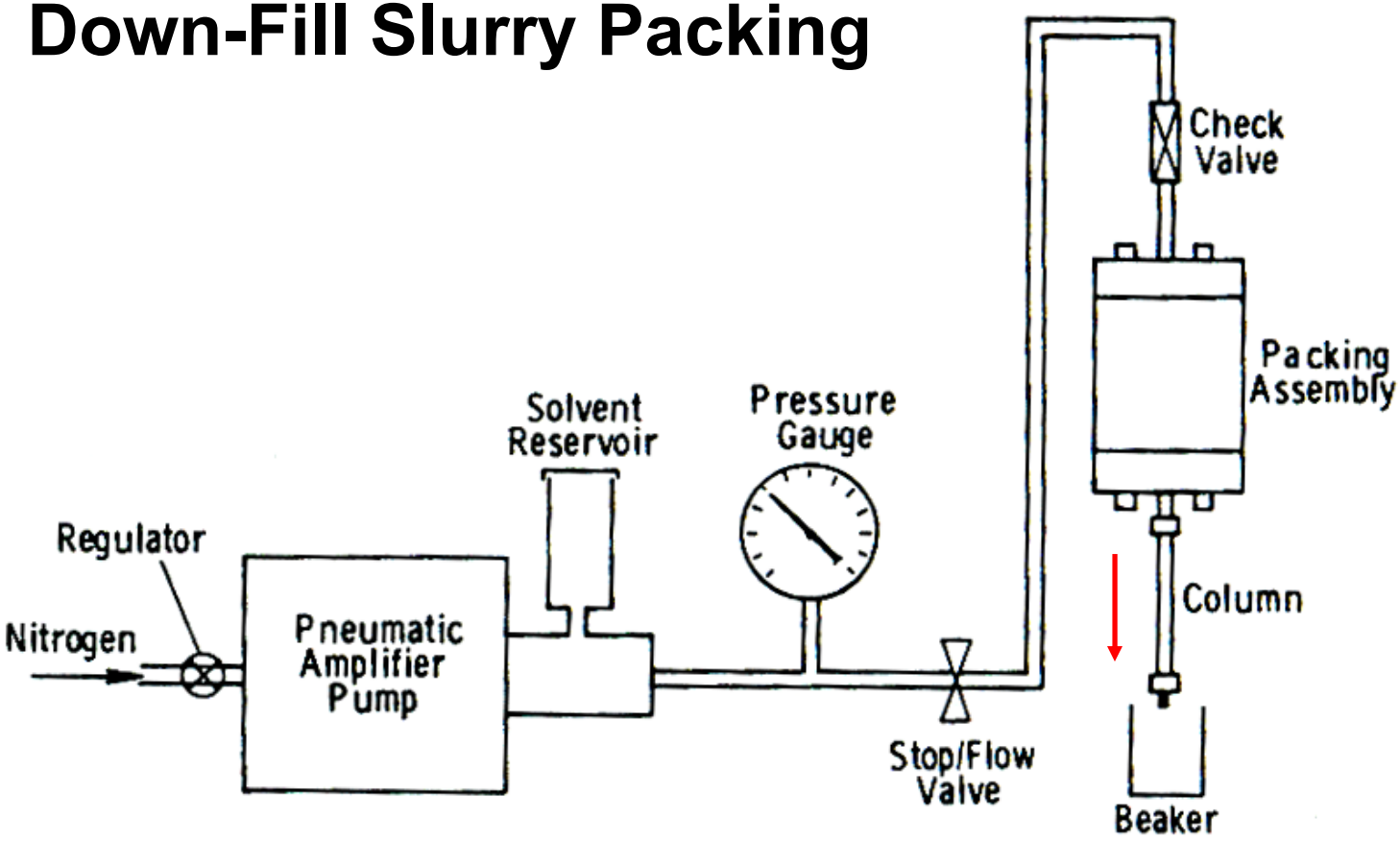


Figure 4.31. Down-fill slurry packing apparatus. (From ref. [658]. ©Elsevier)

Up-Fill Slurry Packing

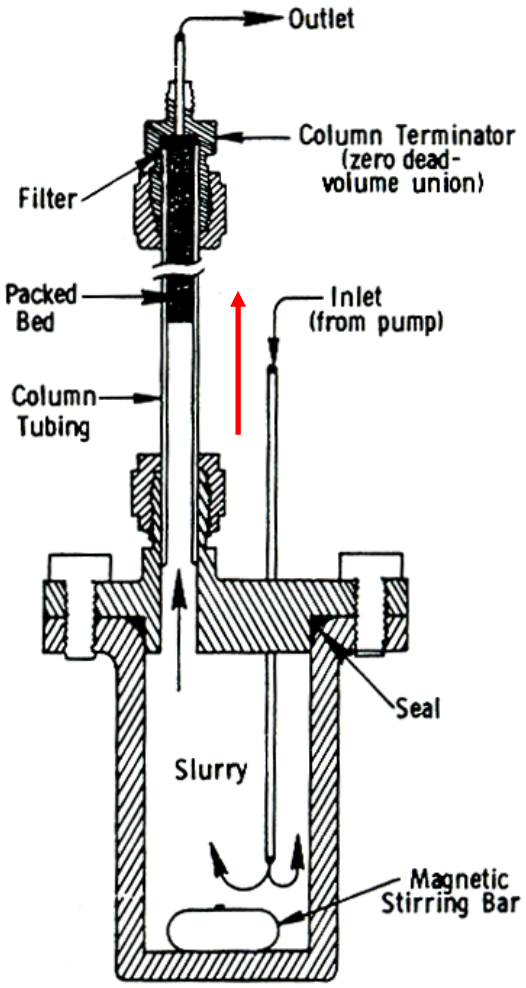


Figure 4.32. Up-fill slurry packing apparatus.

This method is used for conventional Diameter columns. It is unsuitable for packing small diameter columns.

3. Evaluation of column quality

Table 4.19
Test mixtures for routine quality evaluation of normal- and reversed-phase columns

Normal-phase columns	
(1)	Mixture: toluene, nitrobenzene and p-nitroaniline
	Mobile phase: isooctane-ethanol-water (84.5:15:0.5 v/v)
(2)	Mixture: naphthalene, m-dinitrobenzene and o-nitroaniline
	Mobile phase: hexane-dichloromethane-2-propanol (89.5:10:0.5 v/v)
(3)	Mixture: toluene, phenanthrene and nitrobenzene
	Mobile phase: hexane-acetonitrile (99:1 v/v)
(4)	Mixture: toluene, nitrobenzene, acetophenone, 2,6-dinitrotoluene and 1,3,5-trinitrobenzene
	Mobile phase: hexane-methanol (99.5:0.5 v/v)
Reversed-phase columns	
(1)	Mixture: resorcinol, acetophenone, naphthalene and anthracene
	Mobile phase: acetonitrile-water (55:45 v/v)
(2)	Mixture: uracil, phenol, benzaldehyde, N,N-dimethyl-3-toluamide, toluene and ethylbenzene
	Mobile phase: acetonitrile-water (65:35 v/v)
(3)	Mixture: acetone, acetophenone, anisole, benzene and toluene
	Mobile phase: acetonitrile-water (60:40 v/v)
(4)	Mixture: thiourea, phenol, 1-chloro-4-nitrobenzene, toluene, ethylbenzene, n-butylbenzene
	Mobile phase: methanol-water (80:20 v/v)
(5)	Mixture: uracil, toluene, acenaphthene, propylparaben, dipropylphthalate
	Mobile phase: methanol-water (65:35 v/v)

Major parameters: Capacity factor, plate numbers, separation factor, and asymmetry factor.

Routine Column Quality Evaluation

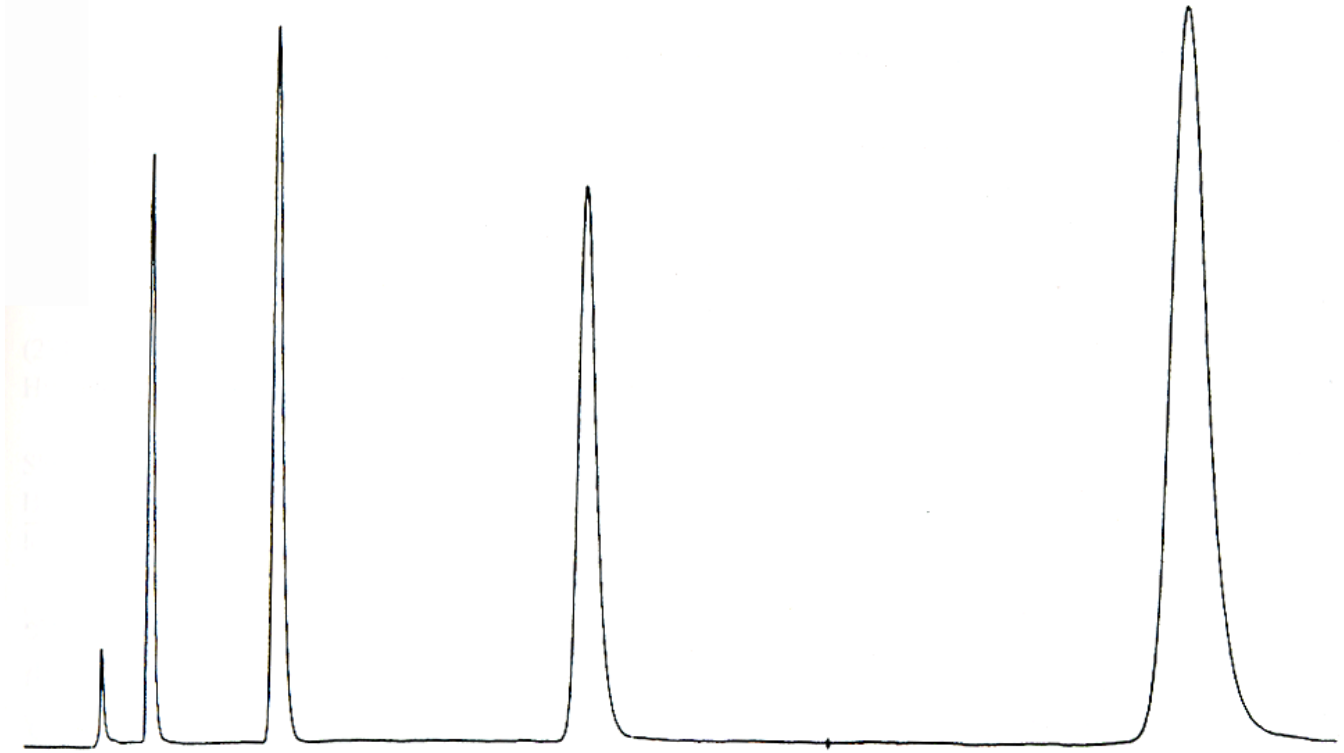
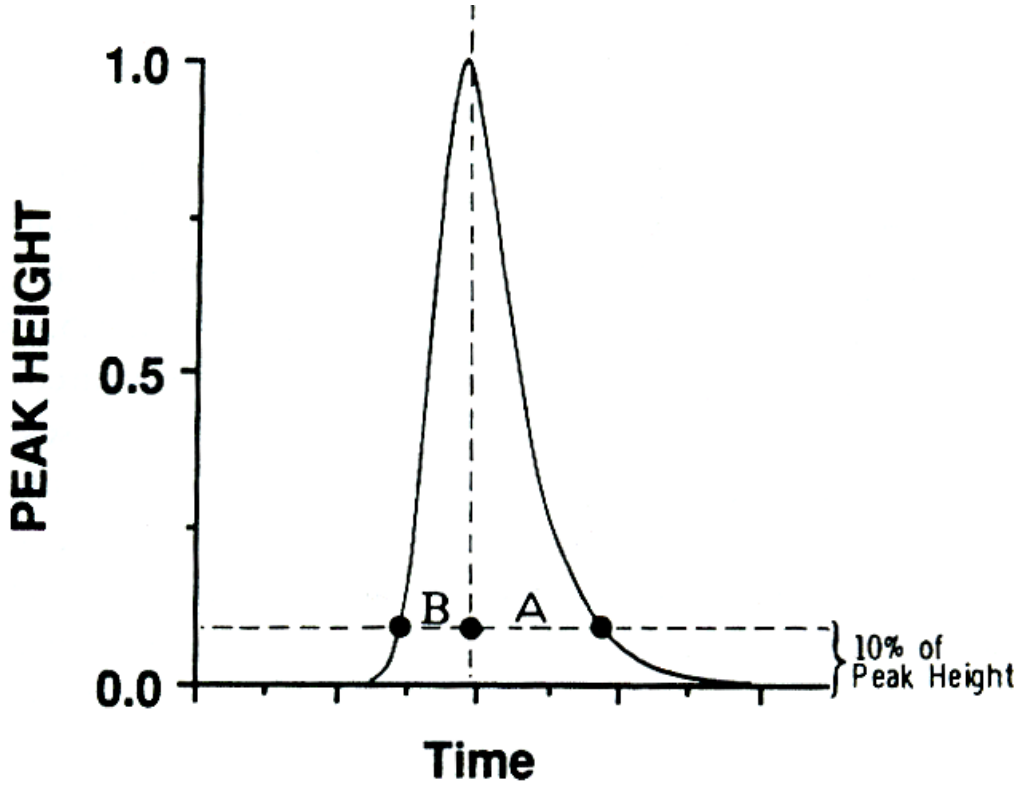


Figure 4.33. Typical routine column quality test chromatogram for a 30 cm x 4.6 mm column packed with ar octadecylsiloxane-bonded silica packing of 10 μm particle diameter. Test mixture: resorcinol (0.55 mg / ml) acetophenone (0.025 mg / ml), naphthalene (0.20 mg/ml) and anthracene (0.01 mg / ml) in acetonitrile, 10 μ injected. Isocratic separation at 23°C with acetonitrile-water (55:45) as the mobile phase with a flow rate o 1.5 ml / min.

Solute	Retention factor	Asymmetry factor	N / m	Separation factor
Resorcinol	0.2	1.00	10,725	
Acetophenone	1.4	1.08	15,900	
Naphthalene	4.3	1.20	17,875	3.30
Anthracene	9.8	1.40	18,380	2.34

Asymmetry Factor



$$\text{Asymmetry factor} = \frac{A}{B}$$

Specific column quality test for chemically bonded phases

Table 4.21
Mixtures for specific property tests of reversed-phase columns
α = separation factor, k = retention factor and A_s = asymmetry factor

Property	Measurement
(1) Walters [660]	
Hydrophobicity	α (anthracene / benzene) with acetonitrile-water (65:35)
Silanophilicity	α (N,N-diethyltoluamide / anthracene) with acetonitrile
	k (nitrobenzene) with n-heptane
(2) Kimata et al [662]	
Hydrophobicity	k for n-pentylbenzene with methanol-water (80:20)
	α (n-pentylbenzene / n-butylbenzene) with methanol-water (80:20)
Shape selectivity	α (triphenylene / o-terphenyl) with methanol-water (80:20)
Hydrogen-bonding	α (caffeine / phenol) with methanol-water (30:70)
Ion exchange (pH >7)	α (benzylamine / phenol) with methanol-0.02M phosphate buffer pH 7.6 (30:70)
Ion exchange (pH <3)	α (benzylamine / phenol) with methanol-0.02M phosphate buffer pH 2.7 (30:70)
(3) Sander and Wise [75,76,669]	
Shape selectivity	α (1,2:3,4:5,6:7,8-tetrabenzonaphthalene / benzo[a]pyrene) with acetonitrile-water (85:15)
(4) Engelhardt and Lobert [670]	
Metal impurities	$100A_s$(2,2'-bipyridyl) / A_s(4,4'-bipyridyl) with methanol-water (49:51)
(5) Cruz et al [664]	
Metal impurities	Base peak efficiency (2,7-dihydroxynaphthalene) / base peak efficiency (2,3-dihydroxynaphthalene) with acetonitrile-25 mM ammonium acetate buffer pH 7.2 (25:75). 2,3-Dihydroxynaphthalene (300 mg / l) and 2,7-dihydroxynaphthalene (150 mg / l).

Comprehensive test mixtures

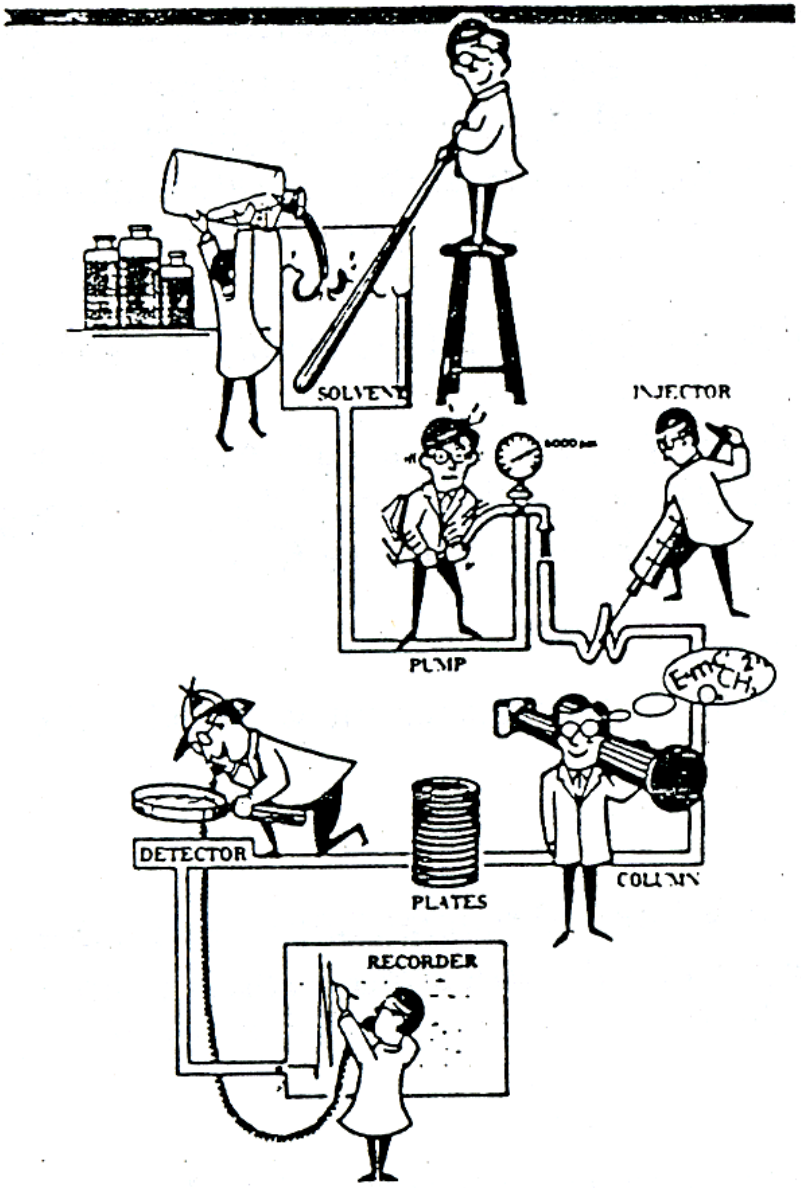
Neue and co-workers [665]

Uracil (16 mg / l), toluene (300 µl / l) or naphthalene (60 mg / l), acenaphthene (200 mg / l), propylparaben or butylparaben (20 mg / l), dipropylphthalate (300 mg / l) or dibutylphthalate (400 mg / l), propranolol (400 mg / l) and amitriptyline (100 mg / l) or doxepin (100 mg / l) with methanol-20 mM phosphate buffer pH 7 (65:35 v/v).

Engelhardt and co-workers [671,672]

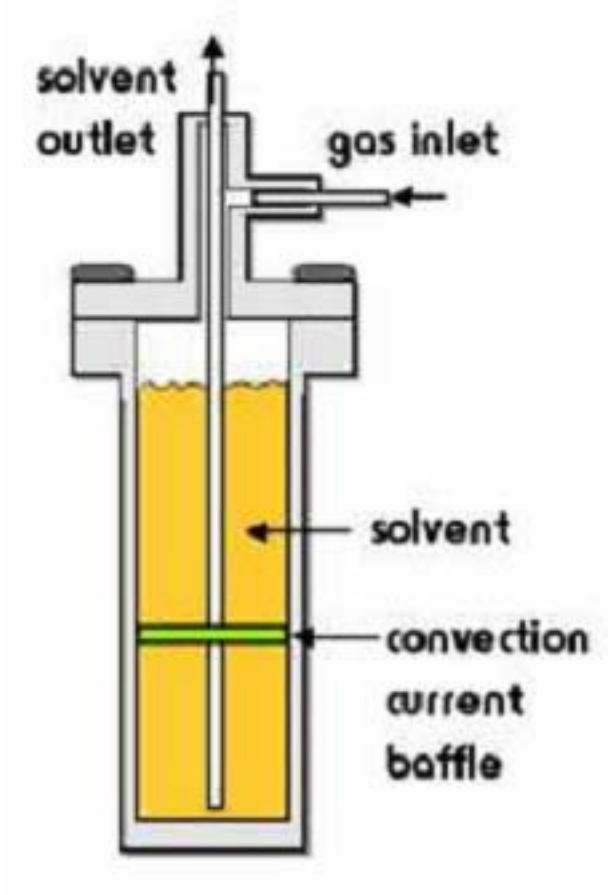
Thiourea (12 mg / l), toluene (870 mg / l), ethylbenzene (867 mg / l), ethylbenzoate (523 mg / l), aniline (81.7 mg / l), o-touidine (79.8 mg / l), p-toluidine (20 mg / l), N,N-dimethylaniline (38.2 mg / l), phenol (120 mg / l) with methanol-water (55:45)

General Instrumental aspects



1. Pumps
2. Injectors
3. **Detectors**

Direct pressure pump



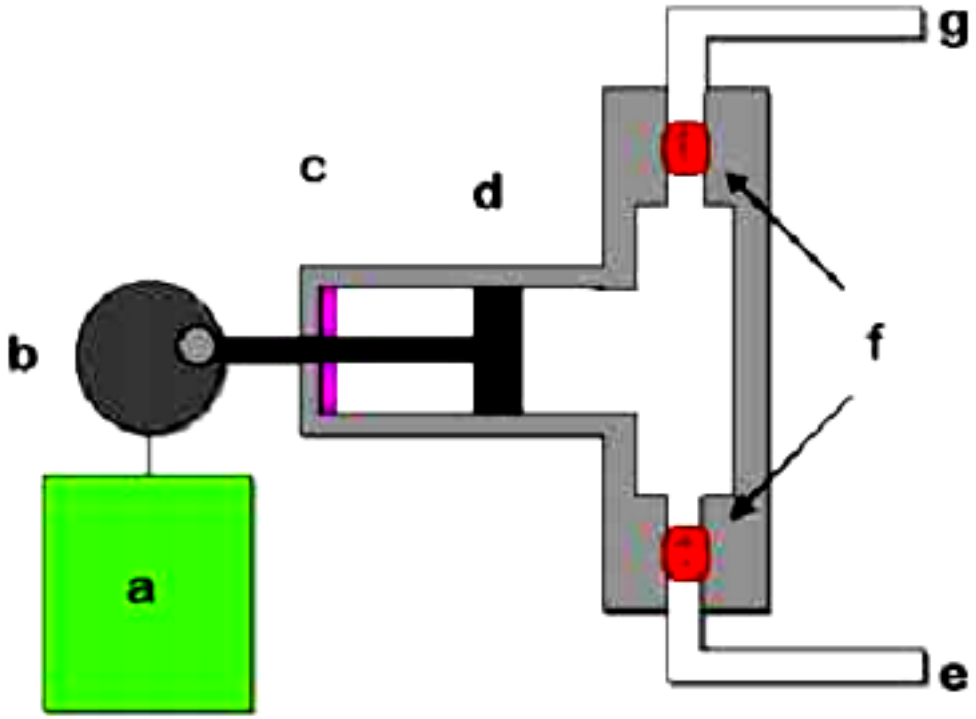
Gas pressure is applied from an external gas tank using a high Pressure regulator.

No pressure pulses are produced

The solvent reservoir is limited

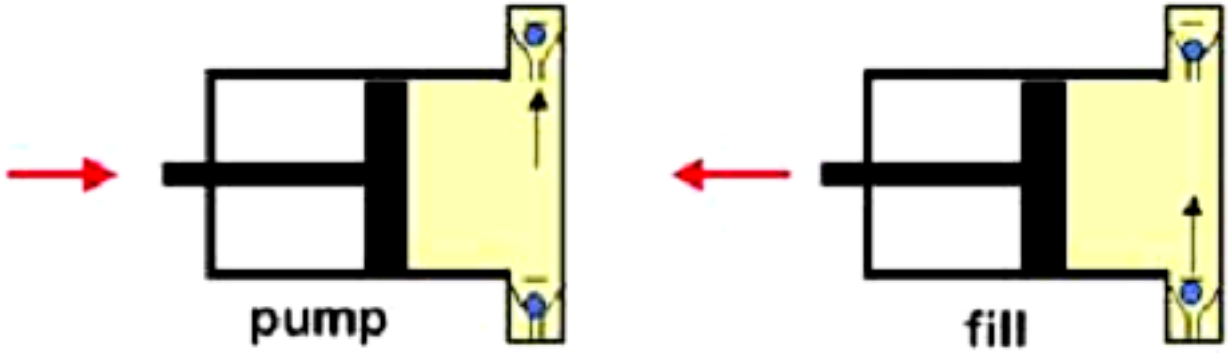
A major problem is introduction Of gas into the solvent.

Reciprocating Pump



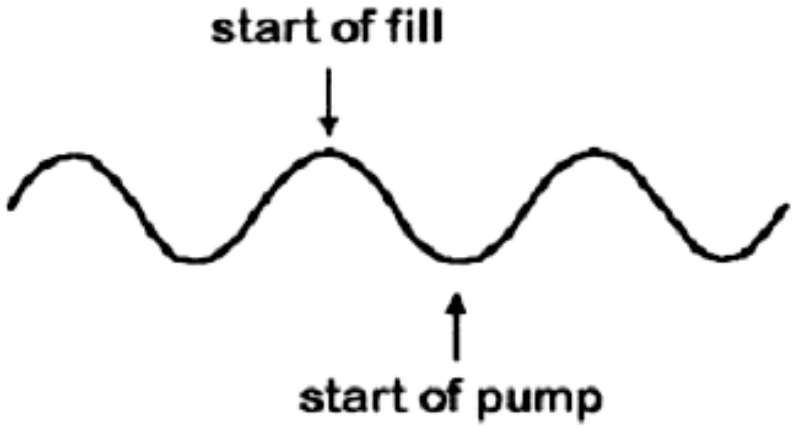
a. Motor
b. Gear
c. Seal
d. Piston
e. Solvent in
f. Check valves
g. Solvent out

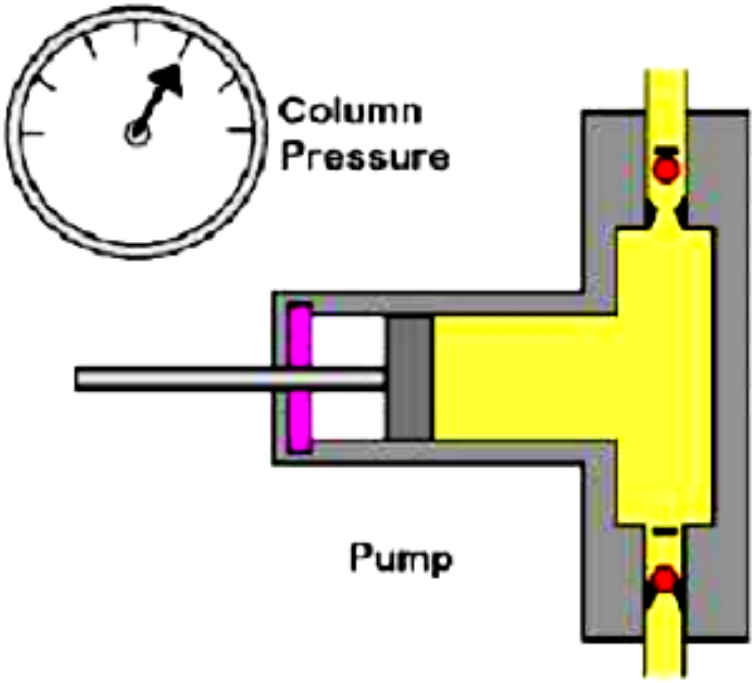
Reciprocating Pump



One of the most common type of system
Unlimited reservoir system
A major problem is that it produces variable pressure

Pressure Variances

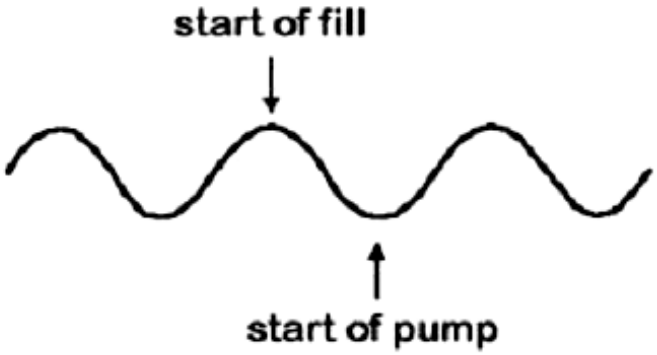




Since the pump must spend at least a portion of its time filling, there is a pressure drop during the phase.

This effect must be minimized because it would greatly affect your sensitivity and detection limit.

Reciprocating Pump

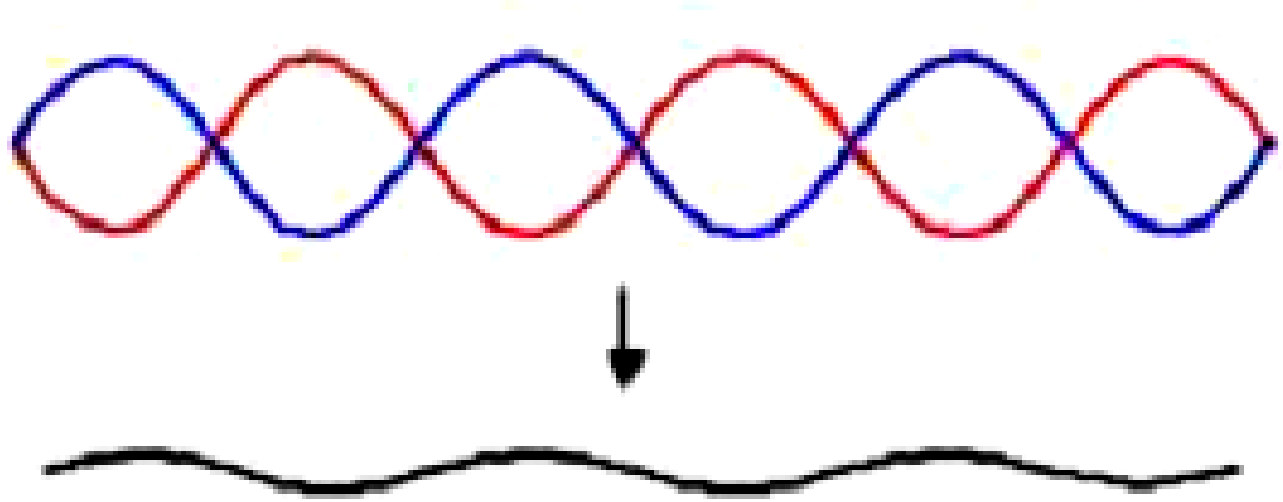


One approach is to have a more rapid fill cycle

This does not eliminate the problem by reduce it

Reciprocating Pump

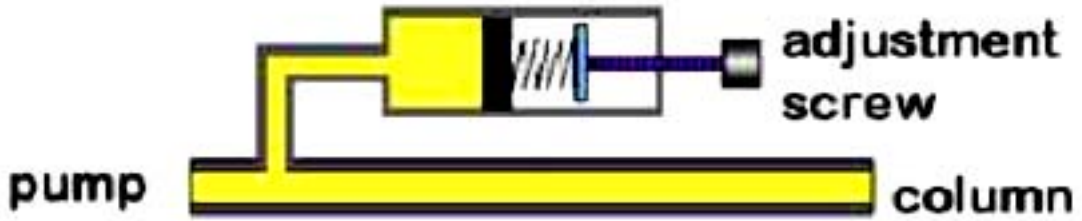
One could also use two or more pumps working in tandem



Pulse Dampers

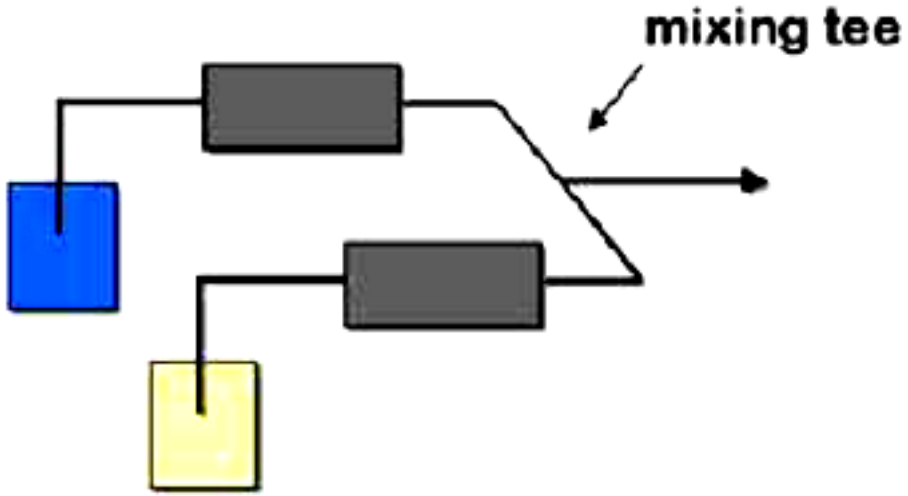
The Popular Approach to minimize flow pulse associated with Reciprocating pumps

Mechanism: Absorb the peak and valley of the pressure pulse



Gradient Controller

Dual pumping systems



A valve system can be sued on each pump that can Provide a different solvents.

Valve Injectors

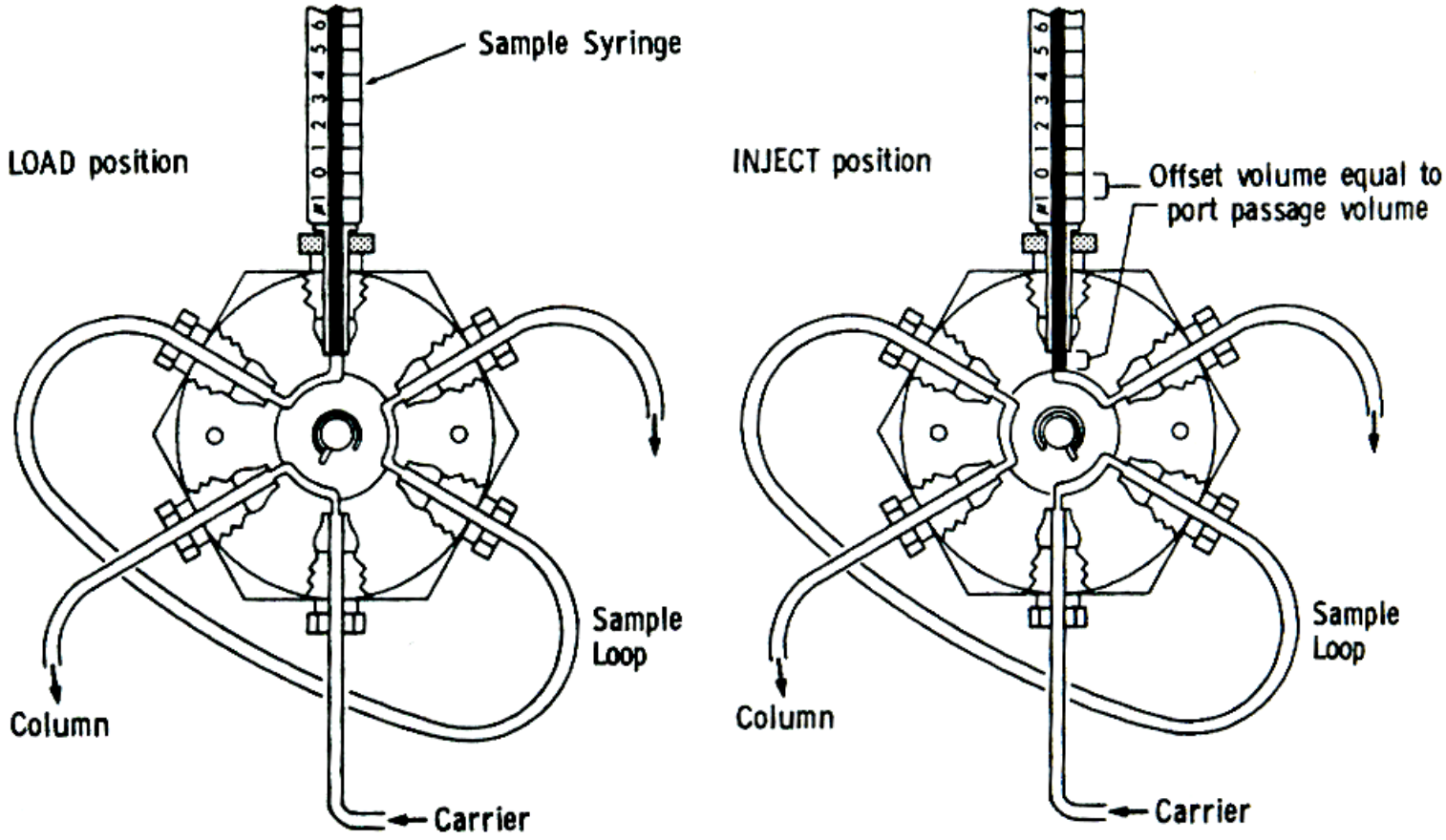
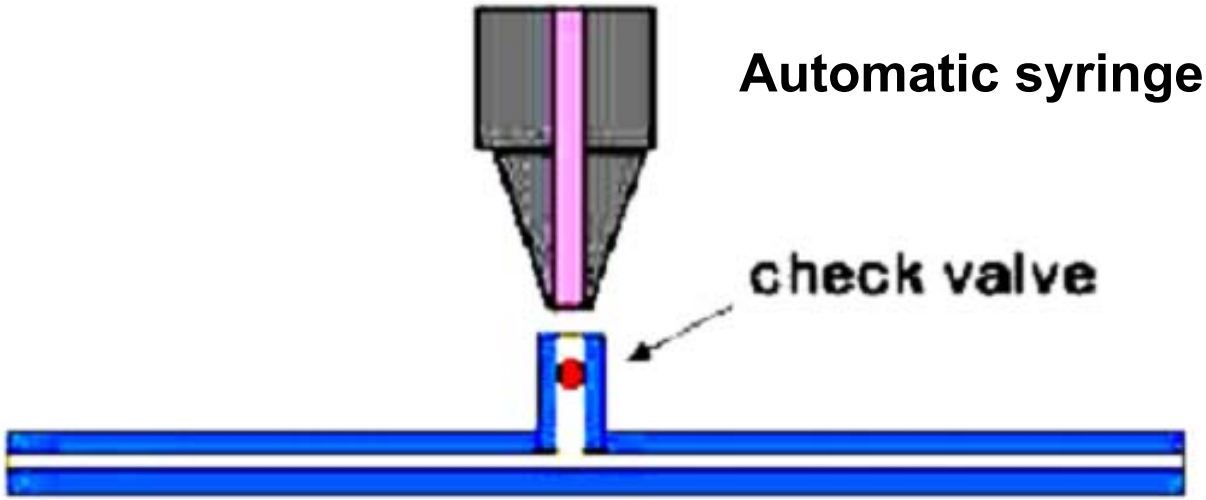


Figure 5.5. Sample injection valve showing the valve configuration in the load and inject position. (Reproduced with permission from Valco Instruments, Inc.).

Automatic Injectors



This method allow for adjustment of sample size.
The motor driven syringe can provide sufficient pressure
To inject sample past the check valve.

Trace Enrichment Sampling

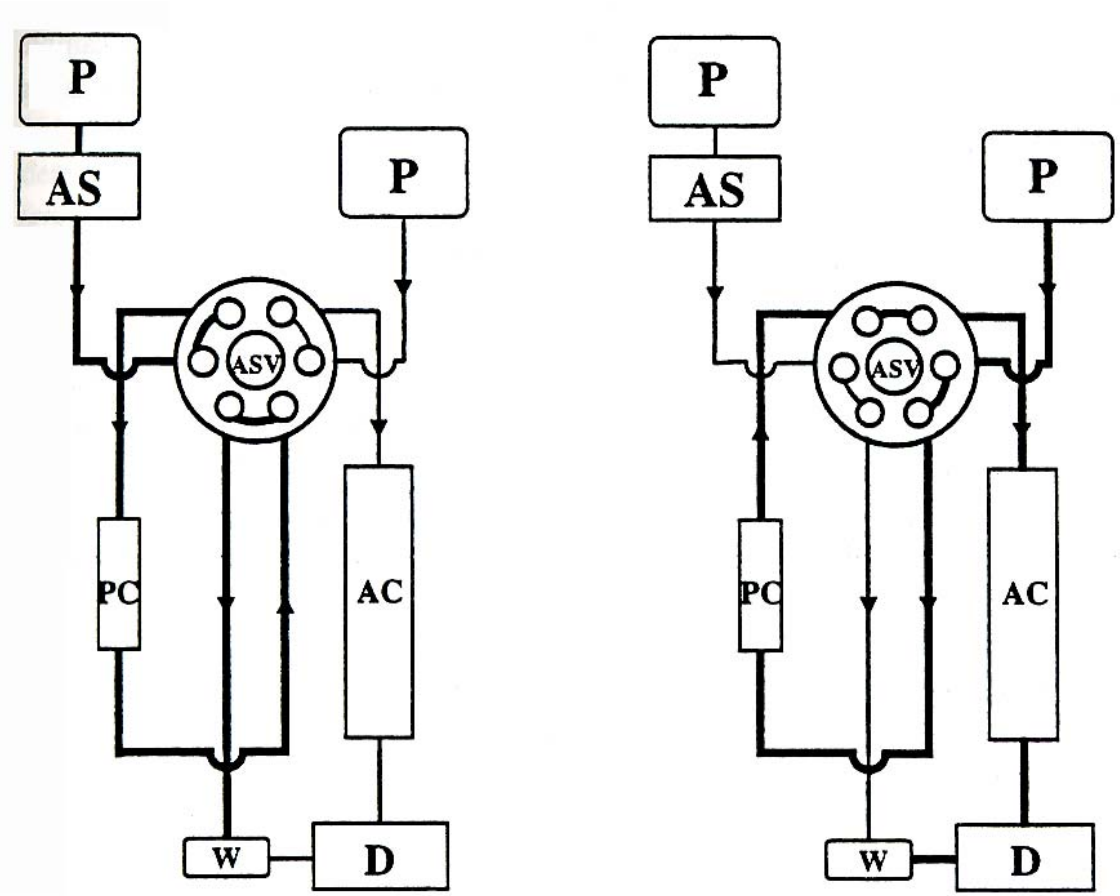


Figure 5.6. Arrangement for automated coupled-column switching in liquid chromatography. Position (I) is for sample application and fractionation and position (II) for transfer of extracted analytes and subsequent separation. P = pump, AS = autosampler, ASV = automated switching valve, PC = precolumn, AC = analytical column, D = detector and W = waste. (From ref. [41]; ©Elsevier).

Multi-Dimensional LC

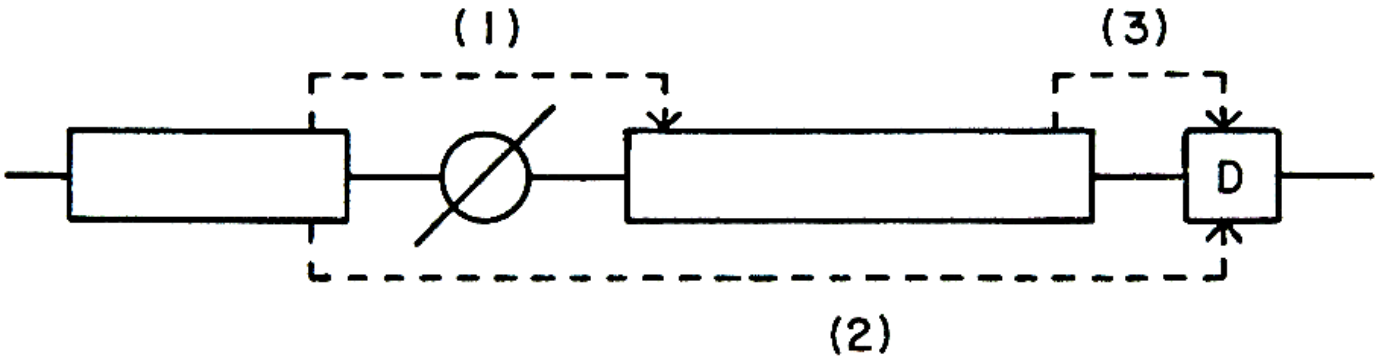


Figure 5.8. Schematic diagram of a two-column system for the separation of a sample containing components with a wide range of retention factors.

Dimension -- column

2-D Liquid Chromatography

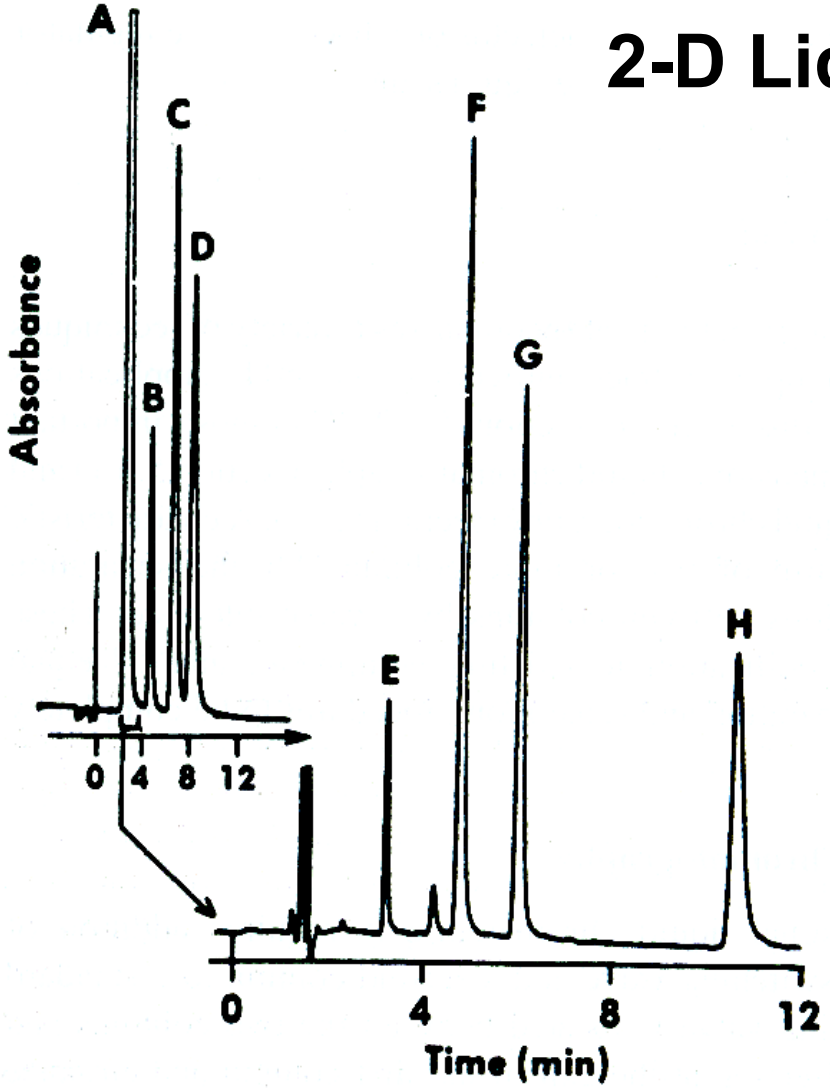
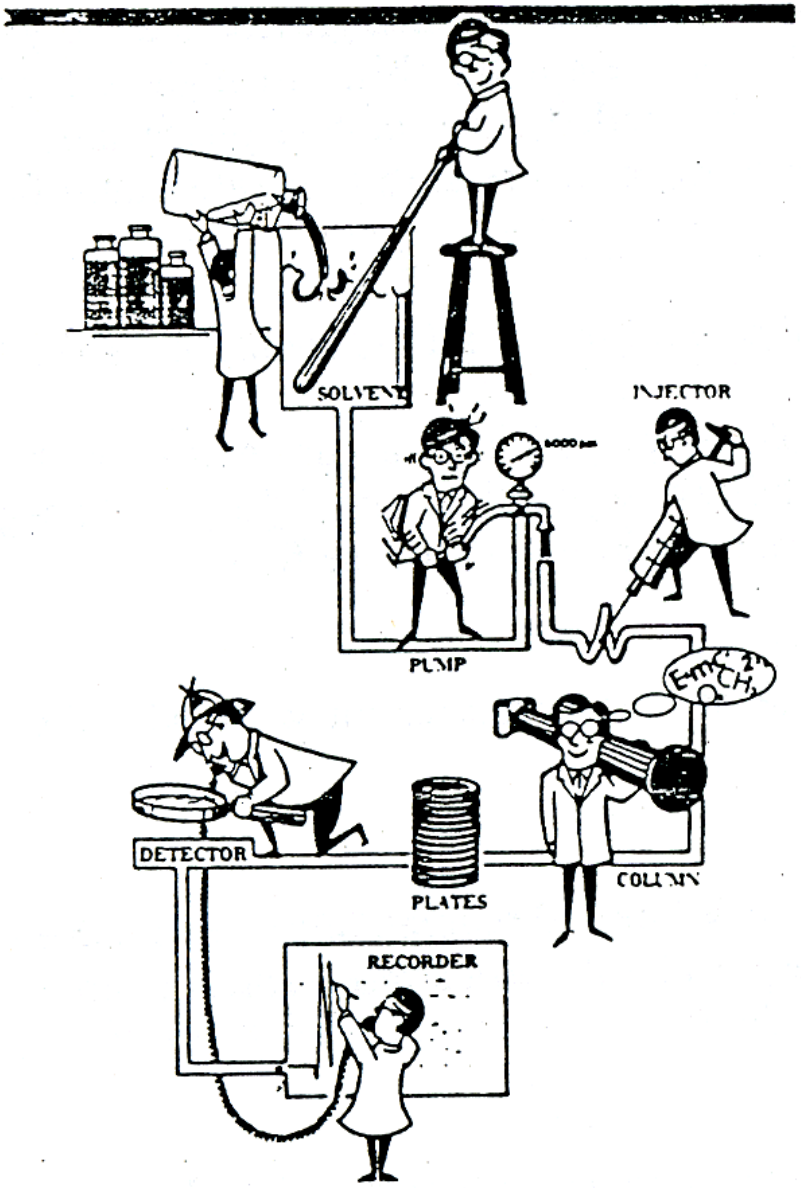


Figure 5.7. Separation of deoxyribonucleosides and their 5'- monophosphate esters by multidimensional liquid chromatography on a strong cation-exchange column (column one) and a reversed-phase column. The unseparated nucleosides, peak A, on the ion-exchange column were switched to the reversed-phase column for separation. Peak identification: A = nucleosides, B = d-CMP, C = d-AMP, D = d-GMP, E = d-CYD, F = d-URD, G = THD, and H = d-ADO. (From ref. [84]; ©Preston Publications, Inc.).

General Instrumental aspects



1. Pumps
2. Injectors
3. **Detectors**

1. Solute 1 and 2 are eluted from a reversed-phase column with retention time of 18.1 min and 23.5 min at a flow-rate of 1 mL/min, using a 10% 2-propanol: 90%water mixture as the mobile phase. The void time of the column at this flow-rate is 0.9 min. The strength of 2-propanol and water are 3.9 and 0, respectively.

a. **What % 2-propanol must be used in order to elute 1 with a retention time of 10.0 min? What will be the retention time of solute 2 under this condition.**
b. **What mixture of water and THF (S' = 4.4) will allow elution of 1 with a retention time of 10.0 mins?**