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)

Retention Mechanisms in Liquid Chromatography

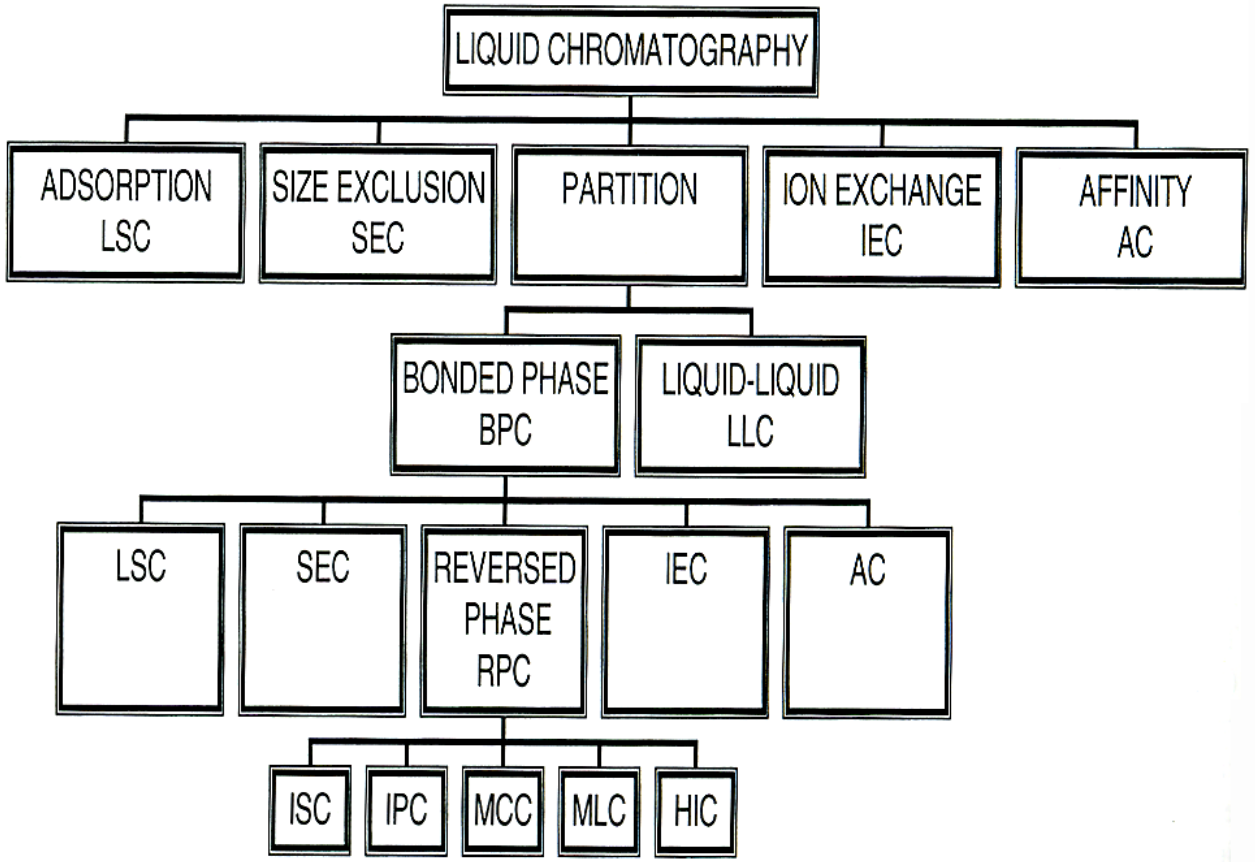


Figure 4.1. Family tree of liquid chromatographic separation modes. LSC = liquid-solid (or normal-phase) chromatography; SEC = size-exclusion chromatography; IEC = ion-exchange chromatography; AC = affinity chromatography; BPC = bonded-phase chromatography; LLC = liquid-liquid chromatography; RPC = reversed-phase chromatography; ISC = ion-suppression chromatography; IPC = ion-pair chromatography; MCC = metal-complexation chromatography; MLC = micellar-liquid chromatography; and HIC = hydrophobic-interaction chromatography.

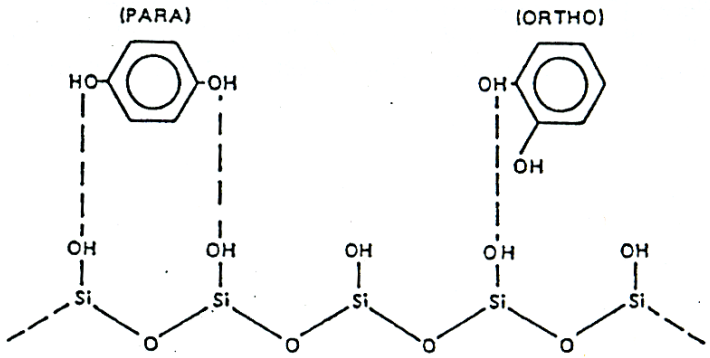
A. Adsorption Chromatography

1. A LC technique which separates solutes based on their adsorption to an un-derivatized solid particles is known as adsorption chromatography, or liquid-solid chromatography.

2. Adsorption chromatography was the first type of column liquid chromatography developed (Tsweet, 1903). However, it is currently not widely used as other LC methods.

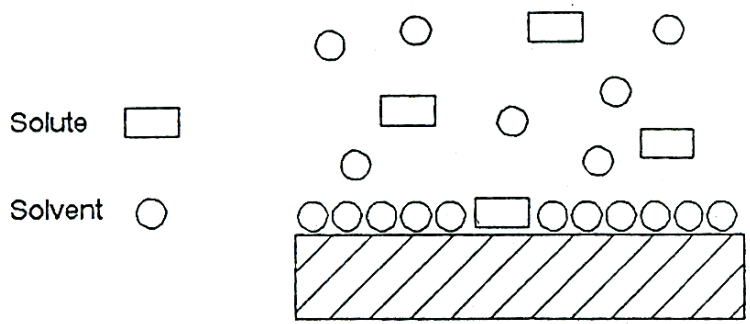
3. Like gas-solid chromatography, supports in adsorption chromatography have the potential disadvantages of having very strong retention of some solutes and may be even cause catalytic changes in solutes. However, this is not as a big problem in LC as it is in since the mobile phase composition can be varied to control solute retention and lower operating temperature of LC make catalytic reactions less likely than in GC.

4. One advantages of adsorption chromatography, as is also true for GSC, is that it is able to retain and separate some compounds that can not be separated by other methods. One such application is in the separation of geometrical isomers.



5. Mechanism

(a) Retention of solute in adsorption chromatography can be viewed as solute A displacing n moles of solvent M from a surface.



$$A_{mp} + nM_{sp} \rightleftharpoons A_{sp} + nM_{mp}$$

(b) Based on this model, the value of k for solute A can be given by

$$\log(k) = \alpha' (S^0 - A_s \varepsilon^0) + \log(V_a W_s / V_m)$$

Where: V_a = Volume of adsorbed solvent in column per gram of support

W_s = Weight of support in column

V_m = Volume of bulk mobile phase in column, or void volume

A_s = Area on surface occupied by solute A

ε^0 = adsorption energy of M per unit area of support

α'=Adsorption activity parameter (↑α' as support ↑polarity)

S^0=Adsorption energy of A on support

Solvent strength ε^0 can be tuned using a two-solvent strategy

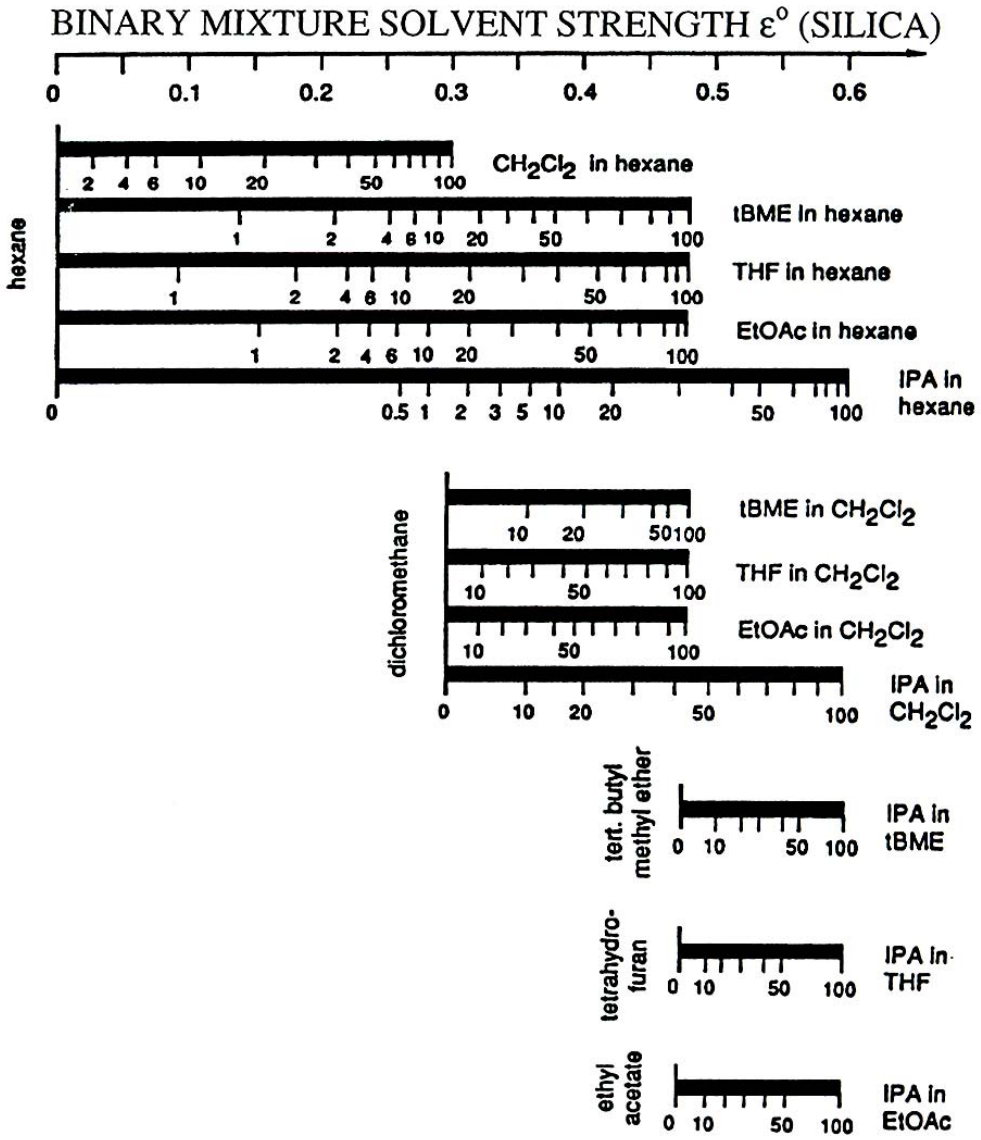


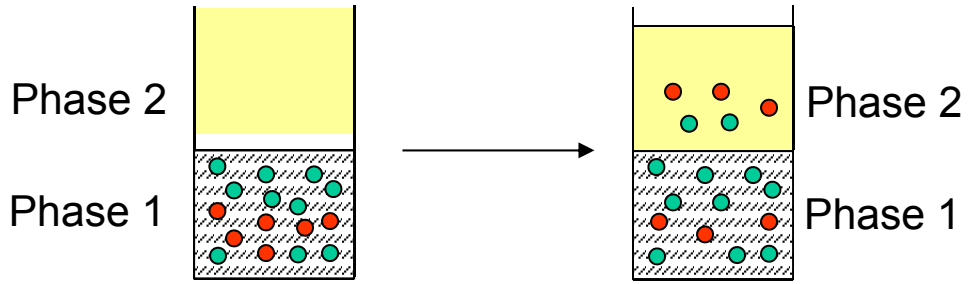
Figure 4.16. Elution strength of solvent mixtures for liquid-solid chromatography on silica gel. Solvent identity: tBME = methyl t-butyl ether; EtOAC = ethyl acetate; IPA = 2-propanol; and THF = tetrahydrofuran. (From ref. [372]. ©Elsevier).

6. Solid Supports

Adsorbent	Surface Type	Use
Silica	Slightly Acidic	General Purpose-Basic Compounds
Alumunia	Slightly Basic	General Purpose-Acidic Compounds
Charcoal	Nonpolar	Nonpolar Compounds
Florisil	Strongly Acidic	General Purpose-Basic Compounds
Polyamides	Basic	Phenols and Aromatic Nitro Compounds
Others (Clay, kiesel-guhr diatomaceous earth, celite, etc.)	Relatively Non-Polar	Polar Compounds

B. Partition Chromatography

(1) Partition chromatography, or liquid-liquid chromatography is a Chromatographic technique in which solute are separated based on their partition between a liquid mobile phase and a liquid stationary phase coated on a solid support.



(2) The support material used in partition chromatography is usually silica. Un-bonded and banded stationary phase.

(3) Mechanism:

The retention of solute in partition chromatography is given by:

$$k = K_D (V_s/V_m)$$

(4) Applications of partition Chromatography

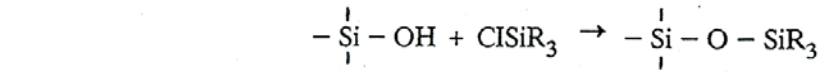
Field	Typical Mixtures
Pharmaceuticals	Antibiotics, Sedatives, Steroids, Analgesics
Biochemical	Amino acids, Proteins, Carbohydrates, Lipids
Food products	Artificial sweeteners, Antioxidants, Aflatoxins, Additives
Industrial chemicals	Condensed aromatics, Surfactants, Propellants, Dyes
Pollutants	Pesticides, Herbicides, Phenols, PCBs
Forensic chemistry	Drugs, Poisons, Blood alcohol, Narcotics
Clinical medicine	Bile acids, Drug metabolites, Urine extracts, Estrogens

(5) Bonded stationary phase

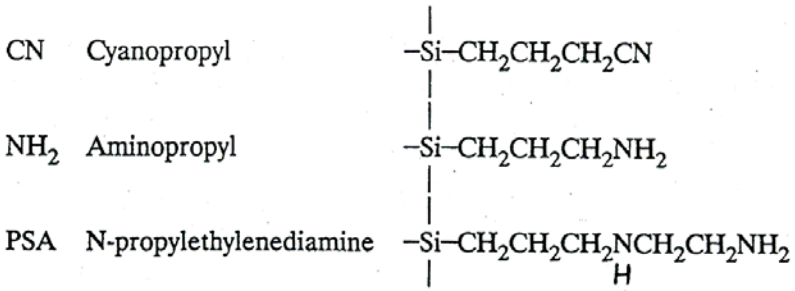
Normal-phase LC (stationary phase is more polar that mobile phase)

Reversed-phase LC (stationary phase is less polar that mobile phase)

(a) Normal phase liquid Chromatography (NPLC)



h.) Common bonded-phases used in NPLC are as follows:



i. Since NPLC has a polar stationary phase, it retains polar compounds Most strongly. However, It may be used for Separation of non-polar As well as polar compounds

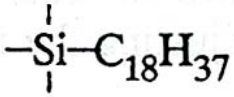
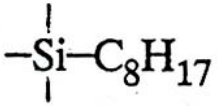
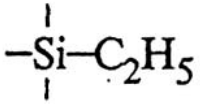
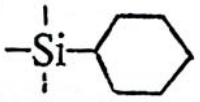
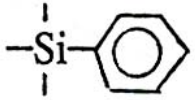
Partition chromatographic systems for separation of some important compound classes

Stationary phase	Mobile phase	Compounds separated
Normal-phase systems		
Dimethylsulphoxide, Ethylene diamine, Ethylene glycol, Nitromethane	C-5 to C-8 alkanes modified with 0–20% halomethanes/MeCN/ THF/dioxanes, etc.	Terpenoids, Steroids, etc.
β,β′-Oxypropionitrile	7% Chloroform/hexane	Insecticides
Polyethylene glycol 600	Hexane	Non-ionic detergents
Trimethylene glycol	Hexane	Pesticide metabolites
Tris(cyanoethoxy)propane	Iso-octane	Phenols
Water	*n*-Butanol	Sugars
Water/EtOH/Iso-octane (ternary, aqueous phase)	Organic phase	Steroids
		Metal complexes
CH_2Cl_2/MeOH/water (ternary, aqueous phase)	Organic phase	Corticosteroids

ii. Strong mobile phase in NPLC is polar liquids, such as water or methanol.

Weak mobile phase in NPLC is non-polar liquids, such as an organic solvent.

(b) Reversed-phased liquid chromatography (RPLC) is another type of partition chromatography.

C18	Octadecyl	
C8	Octyl	
C2	Ethyl	
CH	Cyclohexyl	
PH	Phenyl	

i. Effect of hydrocarbon chain length

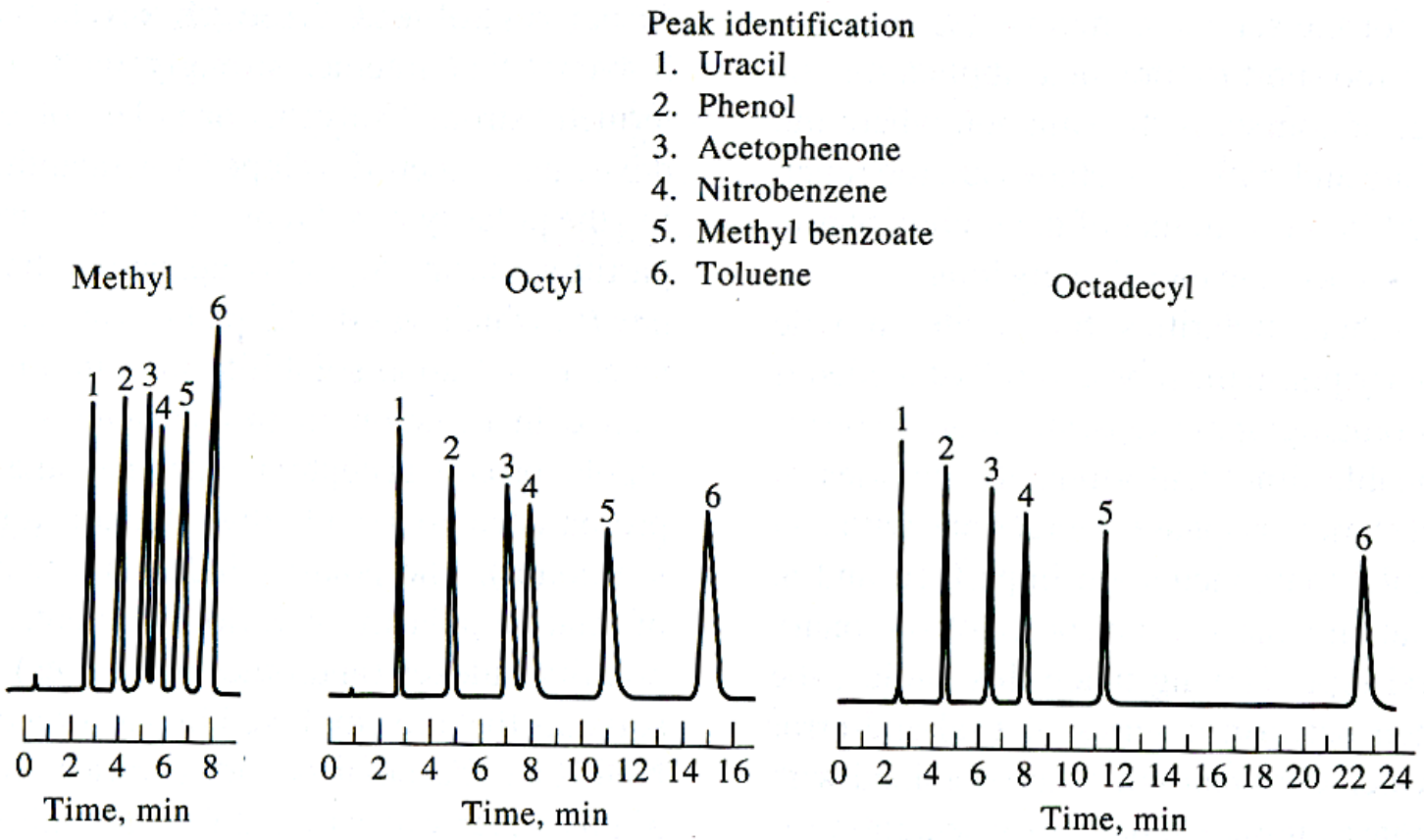


Figure 28-15 Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.

ii. Strong mobile phase in RPLC is non-polar liquid
weak mobile phase in RPLC is polar liquid

iii. Differences in retention between NPLC and RPLC

Solutes

phenol

nitrobenzene

Toluene

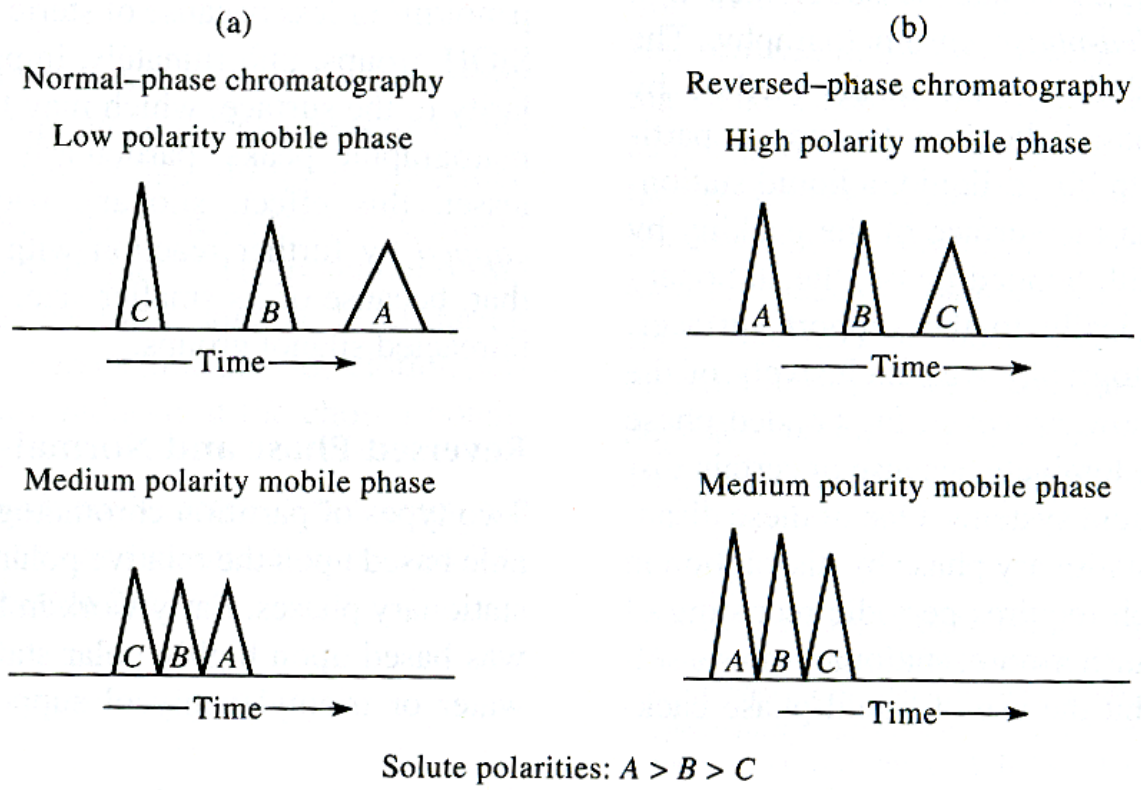


Figure 28-14 The relationship between polarity and elution times for normal-phase and reversed-phase chromatography.

(6) Retention mechanisms: (solvation parameter model/ Kamlet-Taft parameters)

$$\log k = c + mV_x + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H$$ (Liquid chromatography)

Table 4.6
System constant ratios for several stationary phases with methanol-water (50:50) as the mobile phase

Stationary phase	System constant ratios				
	m	*r*/*m*	*s*/*m*	*a*/*m*	*b*/*m*
(i) Dimethylsiloxane-bonded phases					
Methyl	1.25	0	-0.10	-0.21	-0.79
Cyclohexyl	1.85	0	-0.15	-0.12	-0.76
Octyl	2.29	0.03	-0.26	-0.09	-0.79
Decyl	1.65	0	-0.08	-0.22	-0.76
$(CH_2)_3OC_3F_7$	1.47	-0.09	0	-0.29	-0.92
$(CH_2)_2C_6F_{13}$	1.64	-0.17	0	-0.30	-0.85
Phenyl	1.13	0	0	-0.38	-0.78
Pentafluorophenyl	1.56	0	0	-0.22	-0.80
(ii) Octadecylsiloxane-bonded phases					
Hypersil ODS	2.46	0.07	-0.27	-0.08	-0.75
Zorbax ODS	2.68	0.14	-0.31	-0.11	-0.81
Spherisorb ODS-2	2.14	0.17	-0.32	-0.22	-0.86
Capcell Pak C_{18}	2.23	0.08	-0.21	-0.34	-0.91
J.T. Baker ODS	2.03	0.08	-0.20	-0.17	-0.74
Nucleosil C_{18}	1.78	0.11	-0.29	-0.25	-0.91
Nucleosil C_{18} (HD)	2.37	0.08	-0.16	-0.08	-0.85
Partisil ODS	2.28	0.20	-0.47	-0.21	-0.91
(iii) Other phases					
Porous graphitic carbon (Hypercarb)	3.21	0.30	0.08	-0.07	-0.52
Porous polymer (PLRP-S 100)	2.77	0.16	0	-0.40	-1.01
Horizontally polymerized C_{18}/C_3	2.59	0.17	-0.45	-0.21	-0.91
J. T. Baker Butyl (WP)	1.65	0	-0.15	-0.16	-0.81
J. T. Baker $(CH_2)_3CN$	0.84	0.25	0	-0.24	-1.05
J. T. Baker $(CH_2)_3OCH_2CH(OH)CH_2(OH)$	0.80	0.26	0	-0.20	-1.18

Mobile phases effects on system constants (I)

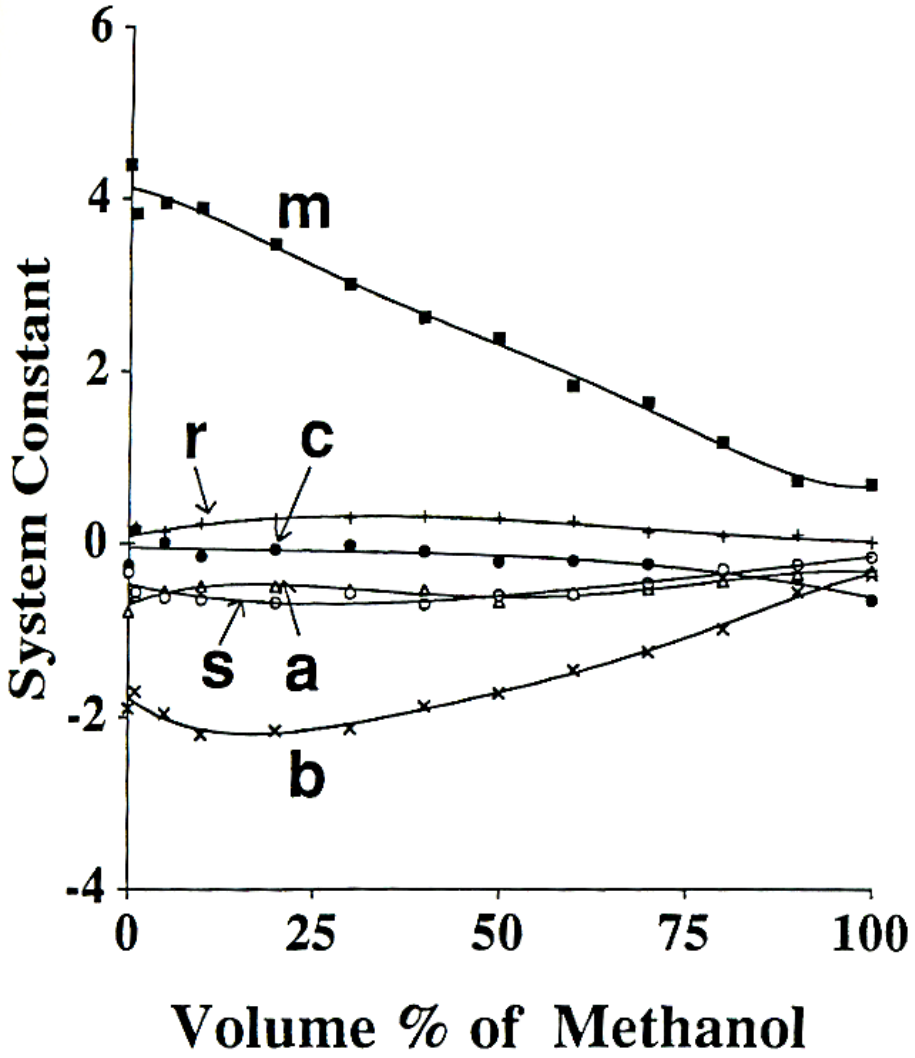


Figure 4.13. System map for an octadecylsiloxane-bonded silica sorbent with methanol-water mixtures as mobile phase.

$$\log k = c + mV_x + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H$$

Mobile phases effects on system constants (II)

Table 4.5
Influence of solvent type on the system constants of the solvation parameter model for a cyanopropylsiloxane-bonded silica sorbent in reversed-phase chromatography
($s = 0$ in all cases)

Solvent	Volume fraction (% v/v)	System constant m	r	a	b
Methanol	50	0.84	0.21	-0.20	-0.88
	40	1.09	0.24	-0.22	-1.15
	30	1.45	0.32	-0.24	-1.36
2-Propanol	50	0	0.15	-0.27	-0.10
	40	0.29	0.16	-0.27	-0.41
	30	0.84	0.20	-0.29	-1.05
Acetonitrile	50	0.40	0.05	-0.18	-0.54
	40	0.64	0.09	-0.21	-0.80
	30	0.98	0.15	-0.24	-1.06
Tetrahydrofuran	50	0.47	0	-0.11	-0.67
	40	0.70	0	-0.06	-0.93
	30	1.18	0	0	-1.45

$$\log k = c + mV_x + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H$$

Solvent-programmed LC

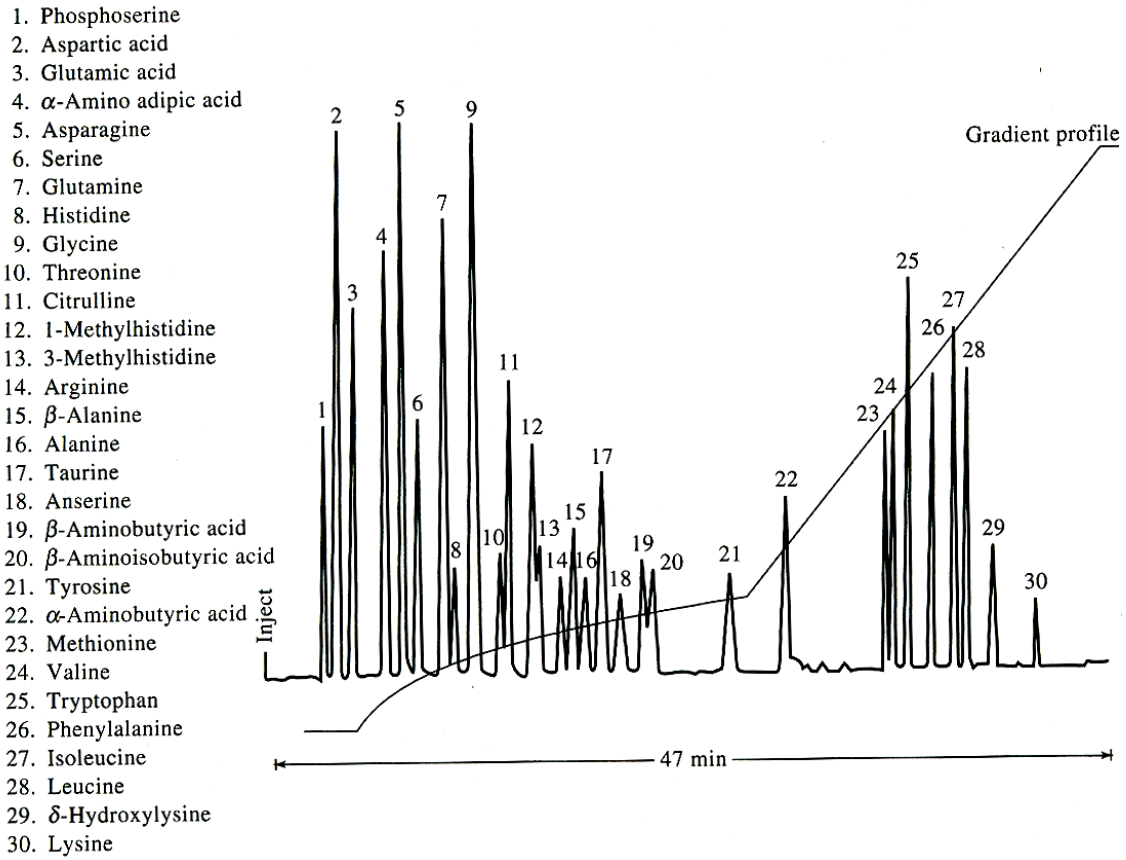
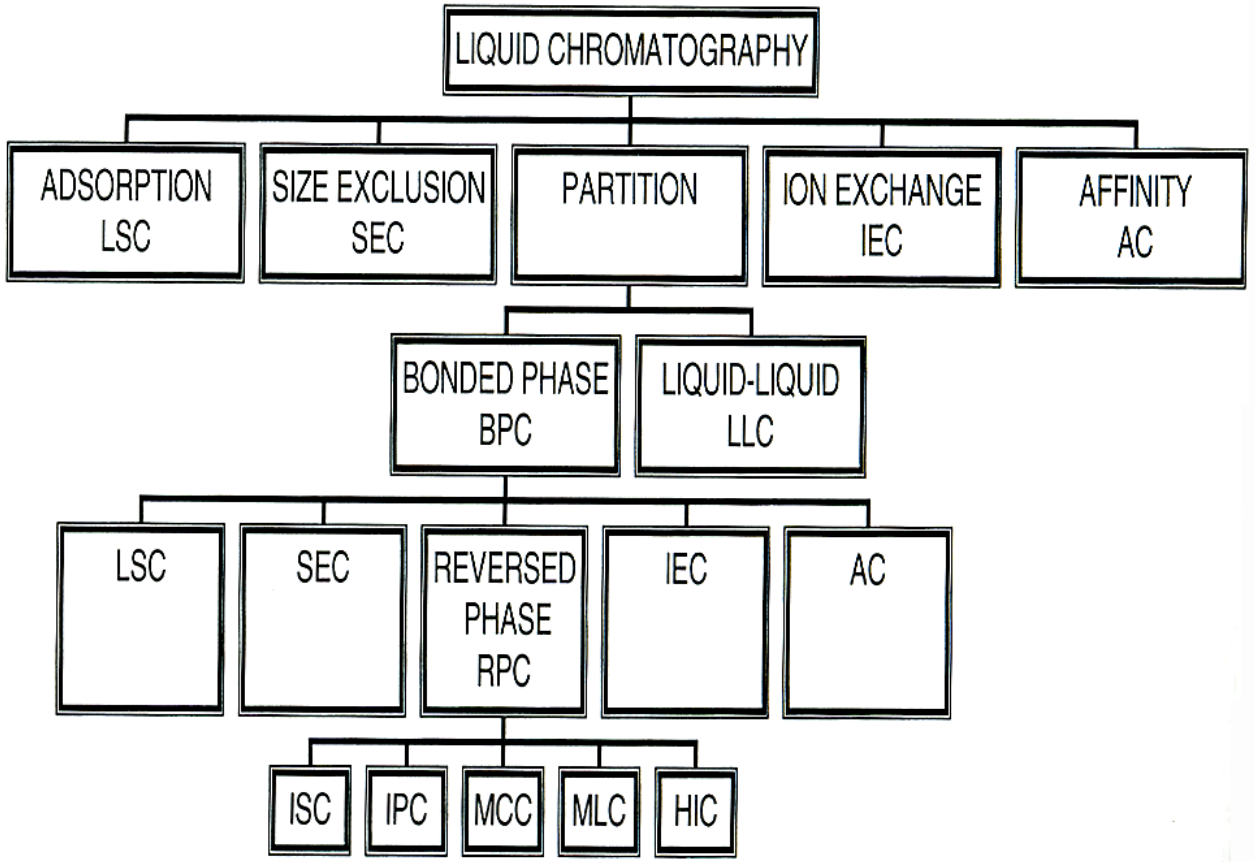


Figure 28-18 Chromatogram of orthophthalaldehyde derivatives of 30 amino acids of physiological importance. Column: 5 μm C_{18}, reversed-phase. Solvent A: 0.05 M Na_2HPO_4, pH 7.4, 96:2:2 $CH_3OH/THF/H_2O$. Fluorescence detector: excitation 334 nm; emission 425 nm. *(Reprinted with permission from R. Pfiefer et al.,* Amer. Lab., ***1983,*** *15(3), 86. Copyright 1983 by International Scientific Communications, Inc.)*

Retention Mechanisms in Liquid Chromatography



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