(1)

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Table 27-2
Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index[a]	Viscosity, cP[b]	Boiling Point, °C	Polarity Index, P'	Eluent Strength ε^0
Fluoroalkanes[d]	1.27–1.29	0.4–2.6	50–174	<−2	−0.25
Cyclohexane	1.423	0.90	81	0.04	−0.2
n-Hexane	1.372	0.30	69	0.1	0.01
1-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

[a] At 25°C.
[b] Centipoise at 25°C. See footnote 15.
[c] On Al_2O_3. Multiplication by 0.8 gives ε^0 on SiO_2.
[d] Properties depend upon molecular weight. Range of data given.

Not valid for RP

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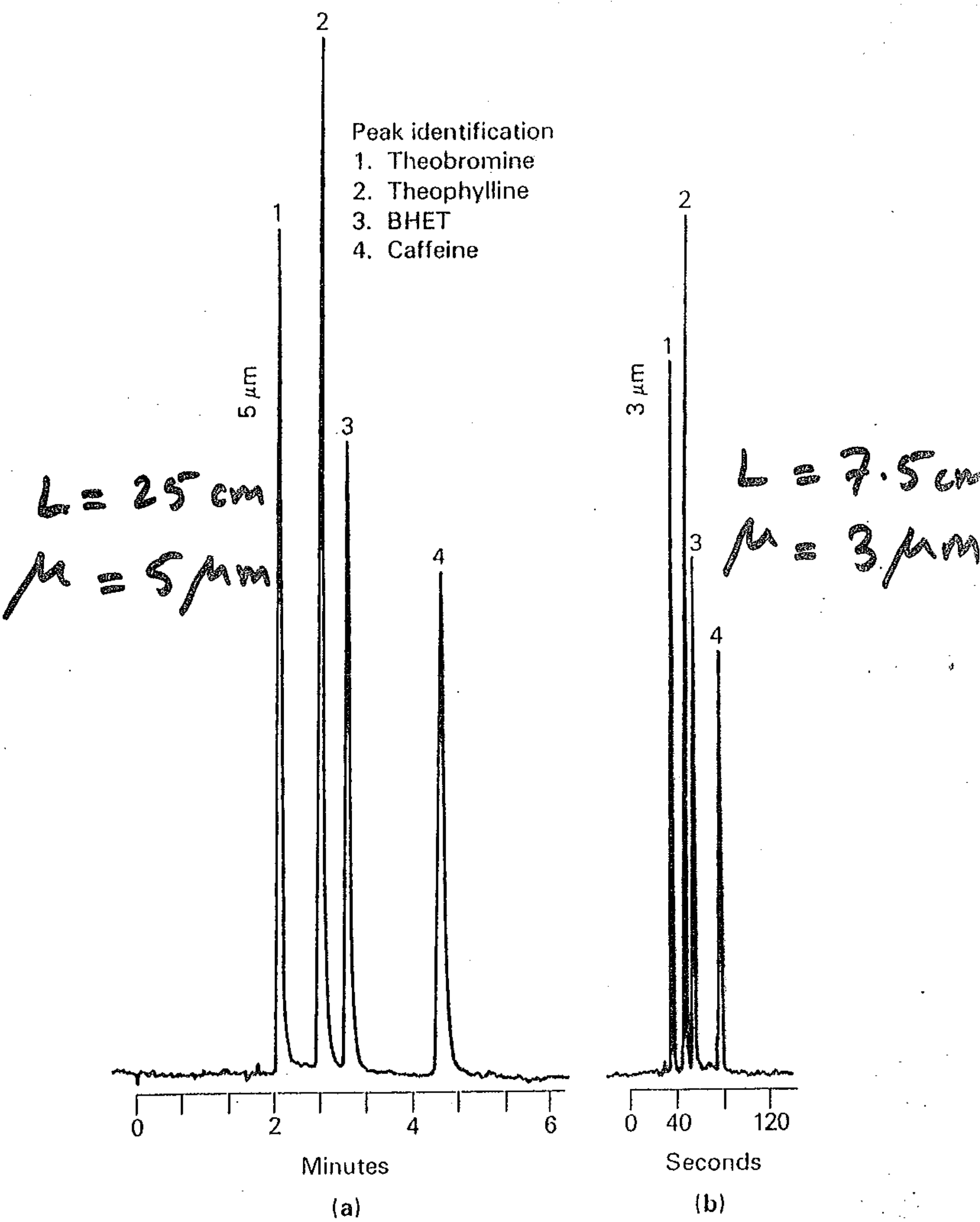


FIGURE 27-6 Comparison of chromatograms with (**a**) a conventional 25.0-cm column packed with 5.0 μm particles and (**b**) a 7.5-cm column with 3.0 μm particles. (Courtesy of Beckman Instruments Inc., Fullerton, CA.)

partition chromatography as well. For lower molecular weight ionic species, ion-exchange chromatography is widely used. Small polar but nonionic species are best handled by partition methods. In addition, this procedure is frequently useful for separating members of a homologous series. Adsorption chromatography is often chosen for separating nonpolar species, structural isomers, and compound classes such as aliphatic hydrocarbons from aliphatic alcohols.

27B Column Efficiency in Liquid Chromatography

The discussion on band broadening in Section 25B–2 is generally applicable to liquid chromatography. The present section illustrates the important effect of stationary phase particle size and describes two additional sources of zone spreading that are sometimes of considerable importance in liquid chromatography.

27B-1 EFFECT OF PARTICLE SIZE OF PACKINGS

An examination of the mobile-phase mass transfer coefficient in Table 25–2 reveals that C_M in Equation 25–8 is directly related to the square of the diameter of the particles making up a packing. As a consequence, the efficiency of an HPLC column should improve dramatically as the particle size is decreased. Figure 27–2 is an experimental demonstration of this effect, where it is seen that a reduction of particle size from 45 to 6 μm results in a 10-fold or more decrease in plate height.

It is noteworthy that none of the plots in this figure exhibits the minimum that is pre-

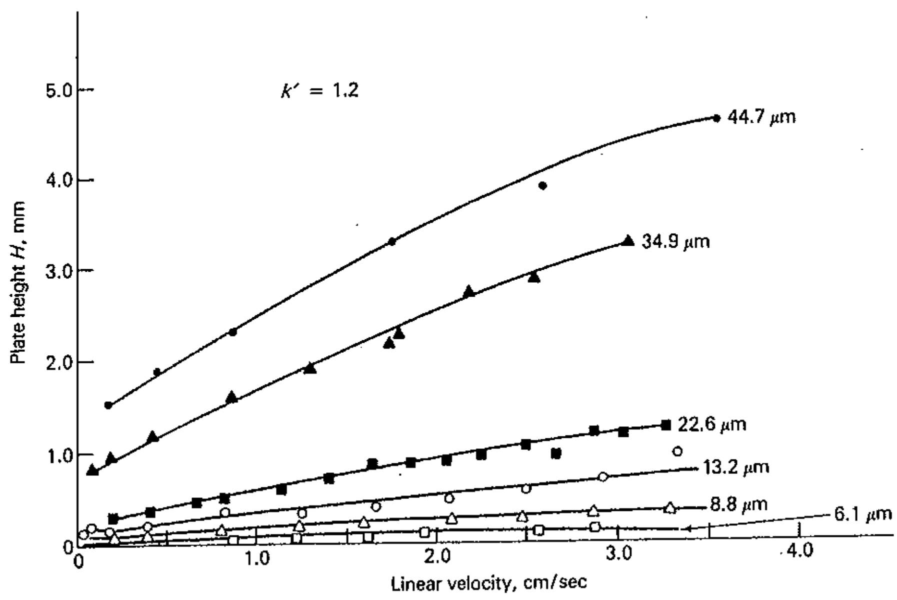
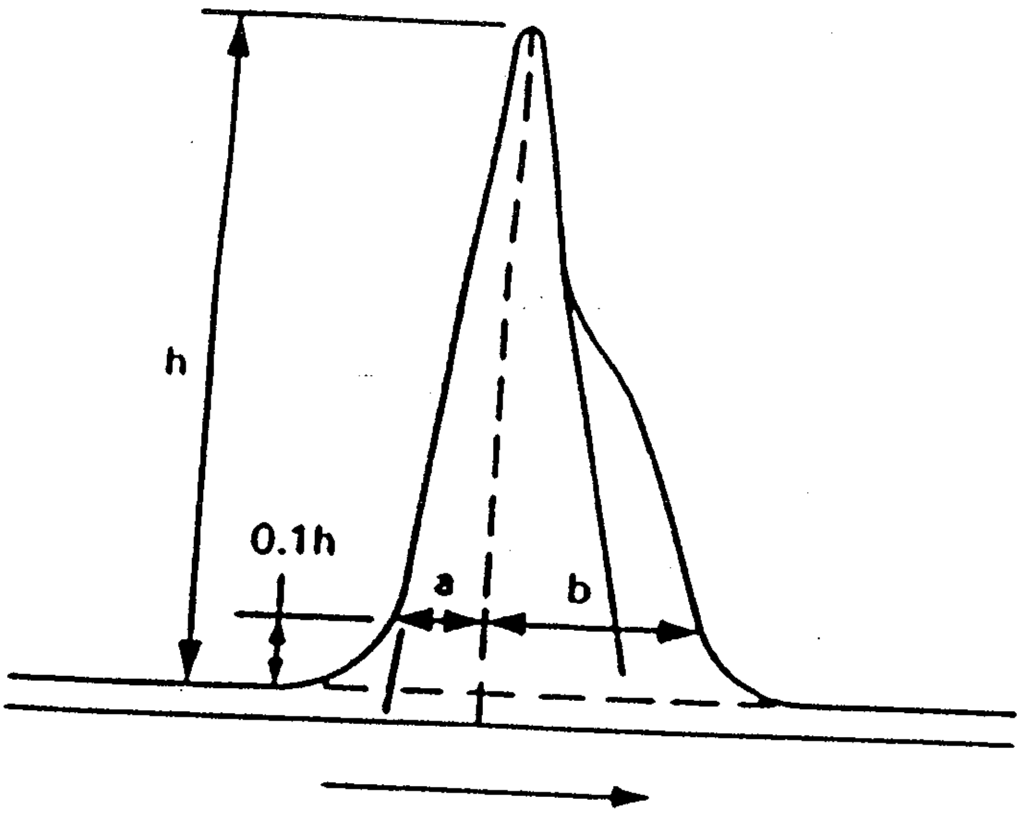


FIGURE 27-2 Effect of particle size of packing and flow rate upon plate height H in liquid chromatography. Column dimensions: 30 cm × 2.4 mm. Solute: N,N-diethyl-n-aminoazobenzene. Mobile phase: mixture of hexane, methylene chloride, isopropyl alcohol. (From: R. E. Majors, *J. Chromatogr. Sci.*, **1973**, *11*, 92. With permission.)

Peak Symmetry



$$\% S = \frac{a}{b} \times 100$$

% S = Percent Symmetry

mixing solvents for isocratic elution

(5)

Table 27-3
Classes of Mobile Phases

I.	Aliphatic ethers and alkyl amines.
II.	Aliphatic alcohols.
III.	Tetrahydrofuran, pyridine derivatives, dimethylsulfoxide, amides (except formamide).
IV.	Formamide, acetic acid, benzyl alcohol, glycols.
V.	Methylene chloride, 1, 2-dichloroethane.
VI.	Halogenated alkanes, esters, ketones, dioxanes, nitriles, aniline.
VII.	Benzene and benzene derivatives, aliphatic nitro compounds.
VIII.	Chloroform, *m*-cresol, water, fluoroalkanols.

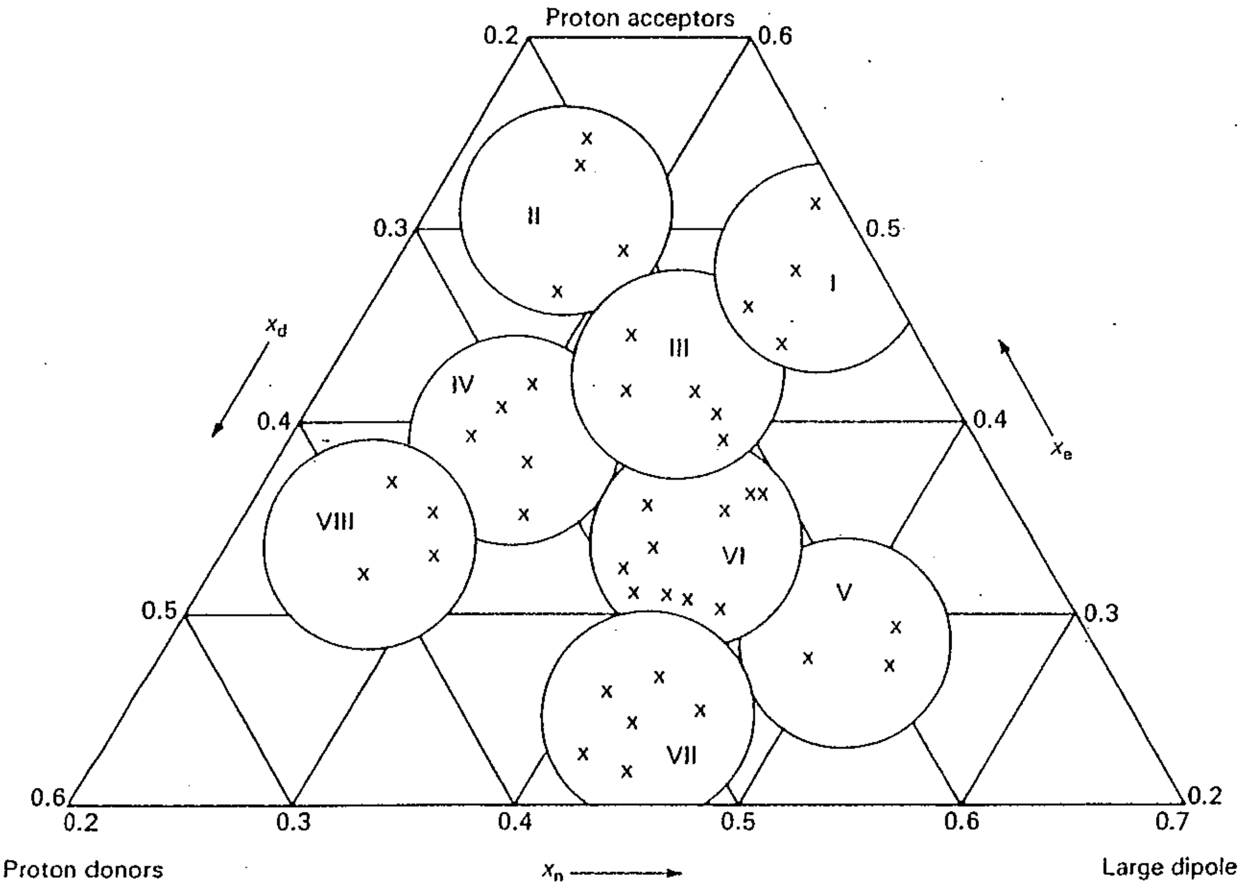


FIGURE 27-13 Mobile-phase classification triangle. Each x represents the position of one mobile phase. (From: L. R. Snyder, *J. Chromatogr. Sci.*, **1978**, *16*, 227. With permission.)

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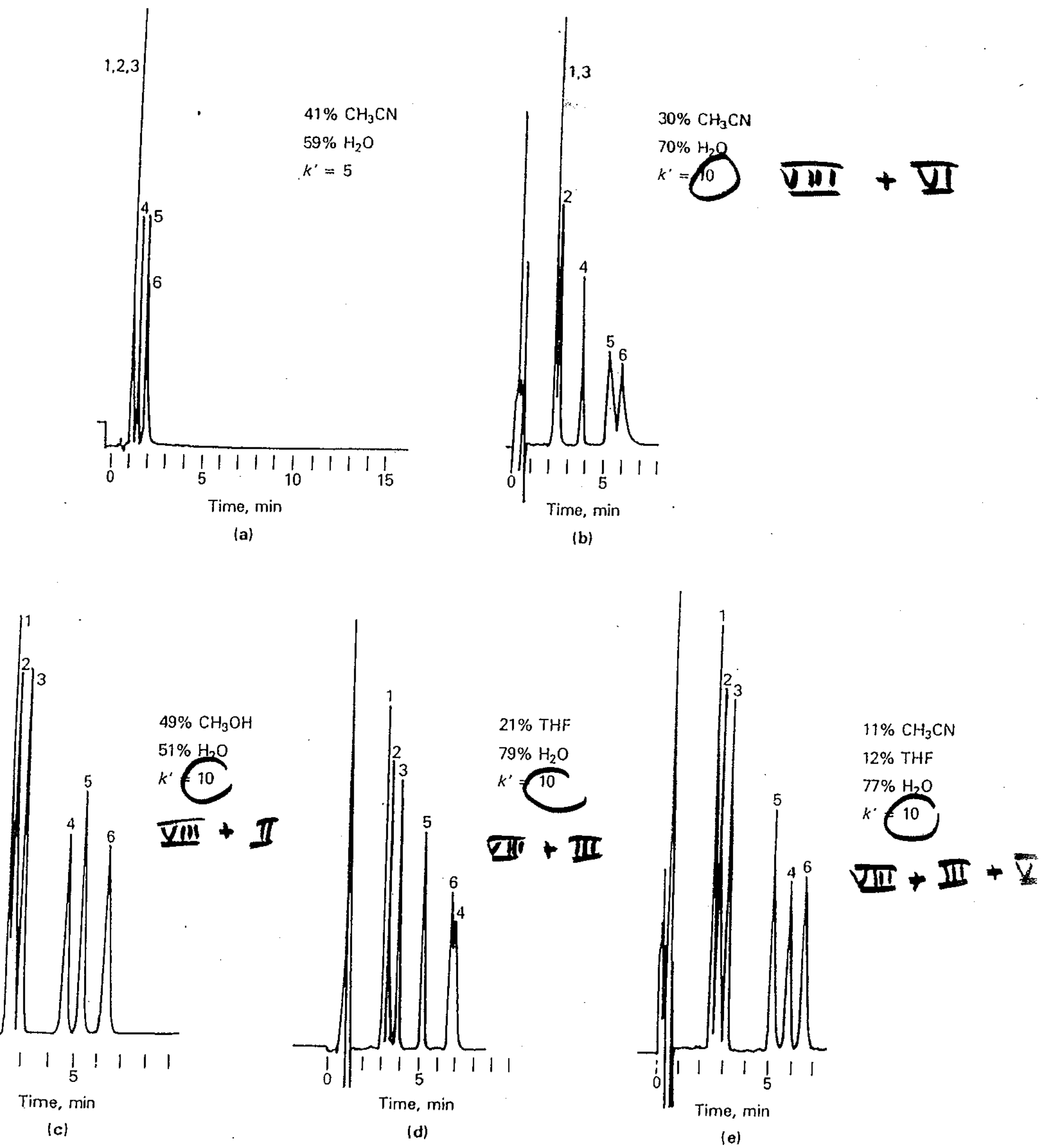
– Soft ware available to select the best mixture

(16) (6) constant $k' = 10$



27-14. Systematic approach to the separation of six steroids. The use of water to adjust k' is shown and (**b**). The effect of varying α at constant k' are shown in (**b**), (**c**), (**d**), and (**e**). Column: 0.4 × 150 acked with 5 μm C_8 bonded, reversed-phase particles. Temperature: 50°C. Flow rate: 3.0 cm^3/min. tor: UV-254 nm. THF = tetrahydrofuran. CH_3CN = acetonitrile. Compounds: 1) prednisone; tisone; 3) hydrocortisone; 4) dexamethasone; 5) corticosterone; 6) cortoexolone. (Courtesy of nt Instrument Systems, Wilmington, DE.)

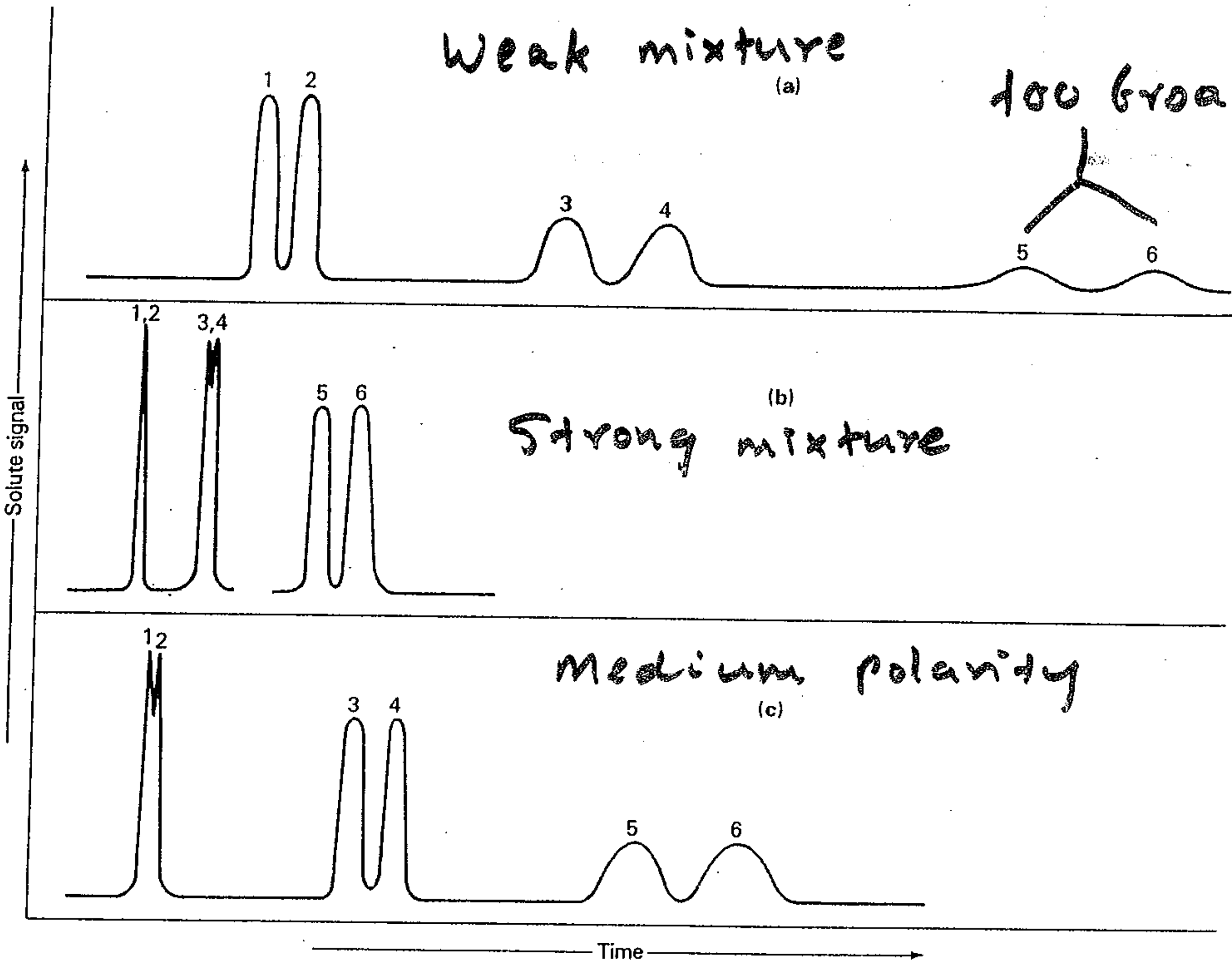


FIGURE 25-11 Illustration of the general elution problem in chromatography.

Solutes with very different polarities ⟶ cannot use Isocratic elution ⟶ Use gradient elution

(8)

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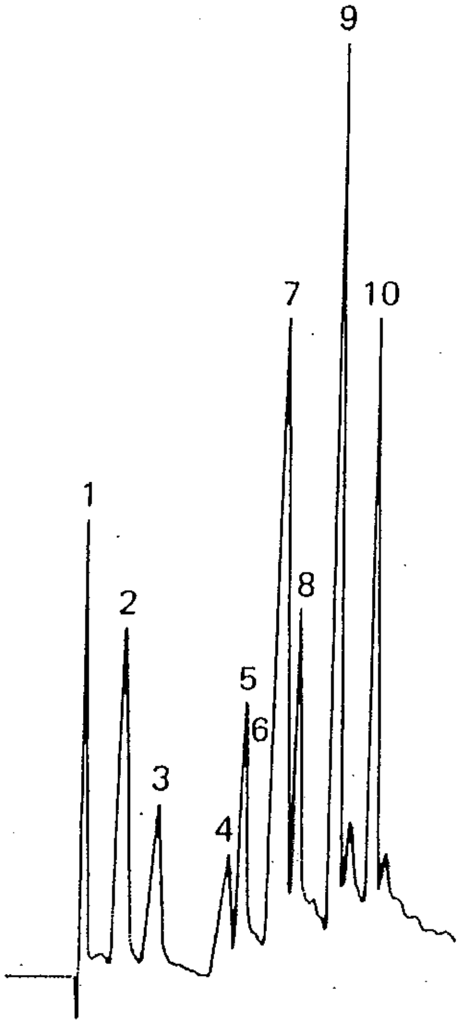


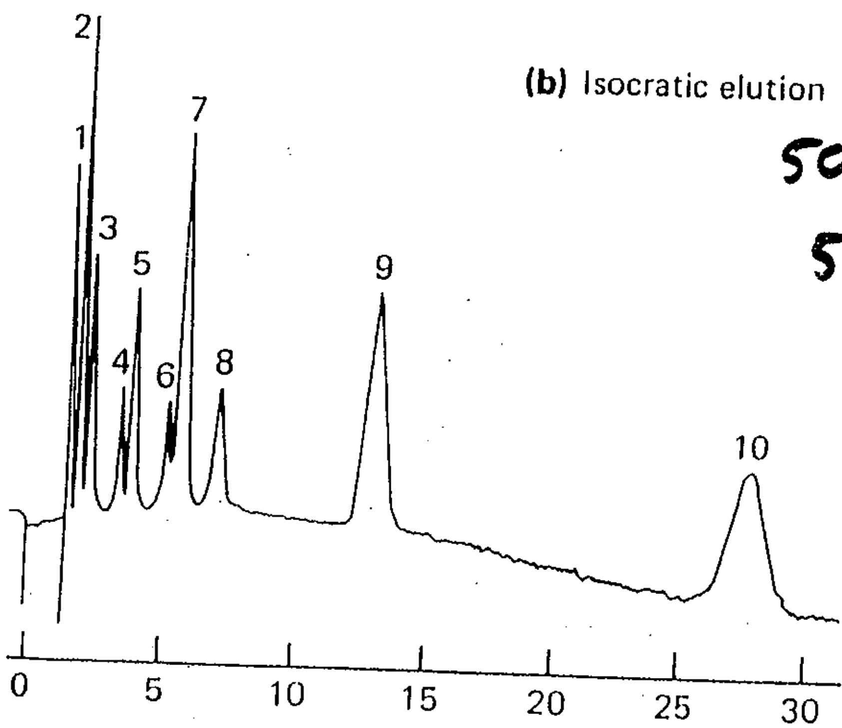
(a) Gradient elution

40% meoH 60% H2O —15 min→ 85% meo 5% H2C (?)

Peak identity

1. Benzene
2. Monochlorobenzene
3. Orthodichlorobenzene
4. 1,2,3-trichlorobenzene
5. 1,3,5-trichlorobenzene
6. 1,2,4-trichlorobenzene
7. 1,2,3,4-tetrachlorobenzene
8. 1,2,4,5-tetrachlorobenzene
9. Pentachlorobenzene
10. Hexachlorobenzene

High resolution with lower run time



(b) Isocratic elution

50% meoH
50% H2O