Liquid Chromatography

1. Introduction and Column Packing Material

2. Retention Mechanisms in Liquid Chromatography

3. Method Development

4. Column Preparation

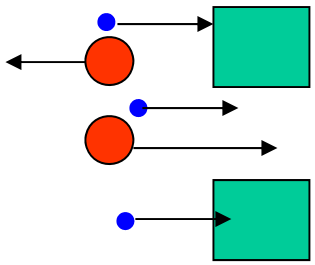
5. General Instrumental aspects

6. Detectors

(Chapter 4 and 5 in The essence of chromatography)

Size exclusive chromatography for polymer and bio-polymer

Standard entropy effect



$$K = \left(1 - \frac{2a}{d_c}\right)^2$$

Retention of a solute is dependent on Standard entropy effect.

Diameter of the pore is very important for solute selectivity.

Multiple pore sizes should be used for separate solutes with different sizes.

GPC: Gel permeation Chromatography (polymer scientists)
GFC: Gel filtration Chromatography (biochemists)

Material and trade name	Fractionation range* (molecular weight)
Dextran	
Sephadex G-10	0–700
Sephadex G-25	1000–5000
Sephadex G-50	1500–30,000
Sephadex G-75	3000–70,000
Sephadex G-100	4000–150,000
Sephadex G-150	5000–300,000
Sephadex G-200	5000–800,000
Polyacrylamide	
Bio-gel P-2	100–1800
Bio-gel P-6	1000–6000
Bio-gel P-60	3000–60,000
Bio-gel P-150	15,000–150,000
Bio-gel P-300	60,000–400,000
Agarose	
Sepharose 2B	2×10^6–25×10^6
Sepharose 4B	3×10^5–3×10^6
Sepharose 6B	10^4–20×10^6
Bio-gel A-0.5 M	10,000–500,000
Bio-gel A-15 M	40,000–15×10^6
Bio-gel A-150 M	1×10^6–150×10^6

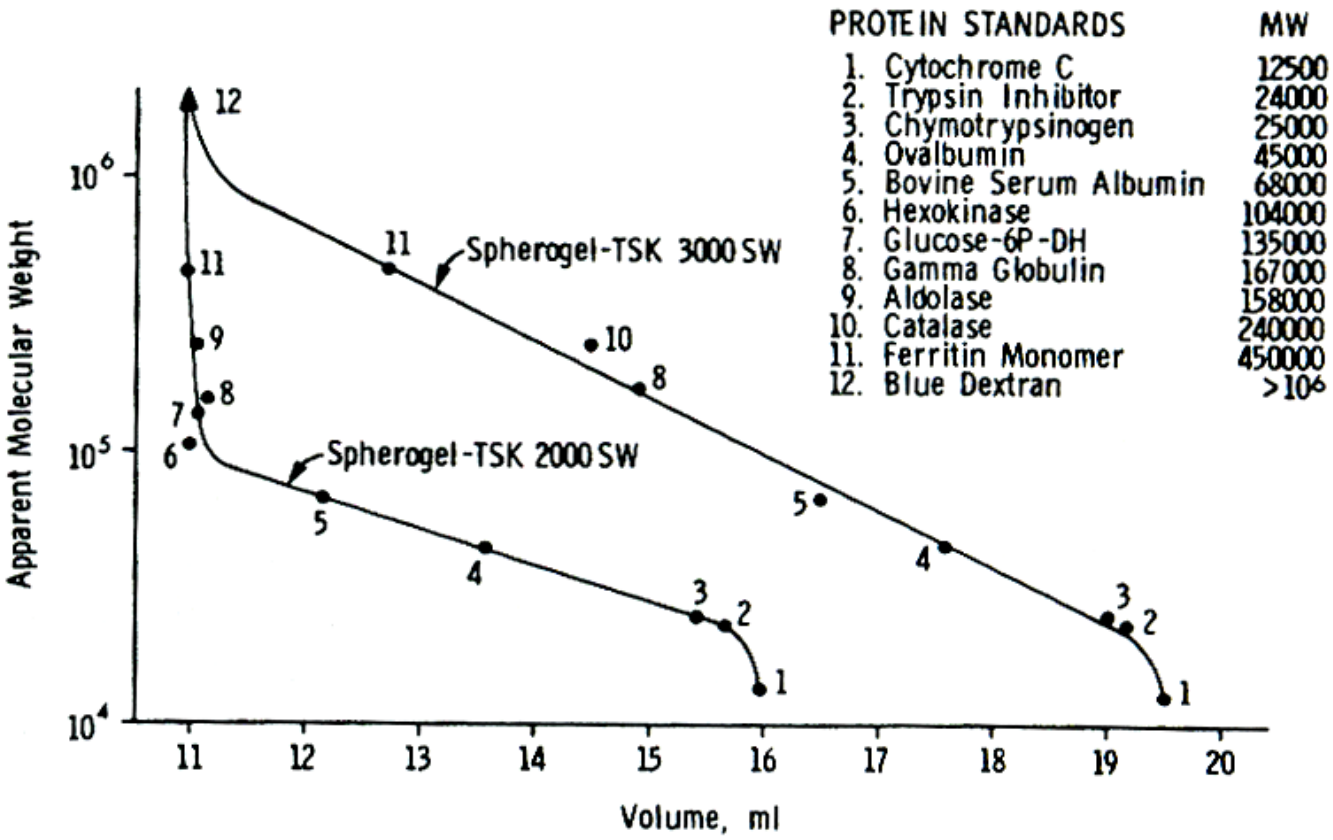
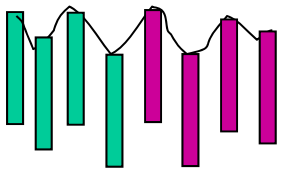


Figure 4.19. Calibration curves for the separation of proteins on Spherogel TSK-SW 2000 and TSK-SW 3000 packings. Mobile phase: phosphate buffer 0.2 M, pH = 6.8.

Linear range for separating solutes with different molecular weight.

Separation of Block Co-Polymers



Size, and functions of blocks

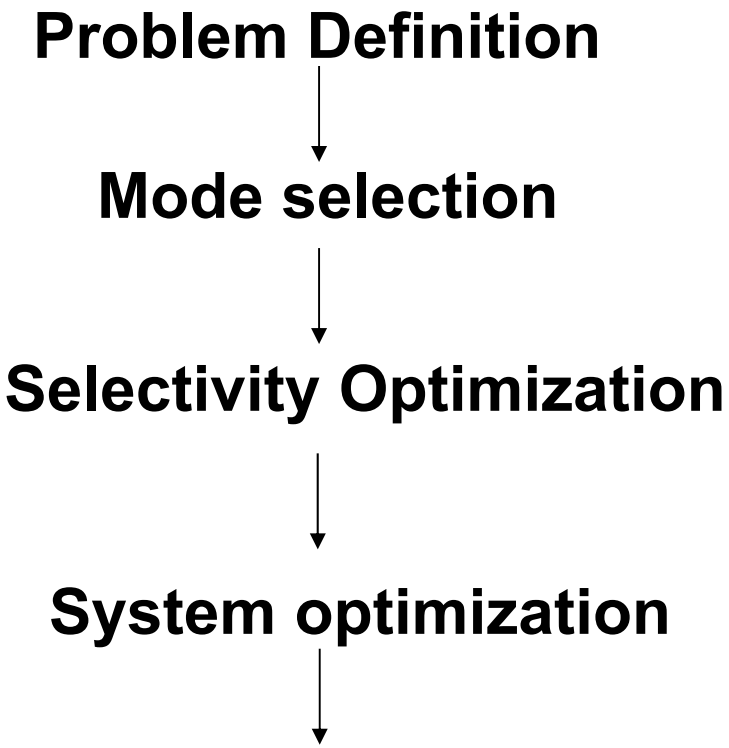
Gradient polymer elution chromatography (GPEC)

Mechanisms: (a) precipitation/re-dissolution, (b) adsorption/ de-sorption, (c) size exclusion effect.

(1) Precipitation chromatography: non-solvent/good solute elution

(2) Sudden-transition gradient polymer elution chromatography: two of non-solvents / one of good solute elution

LC Method Development



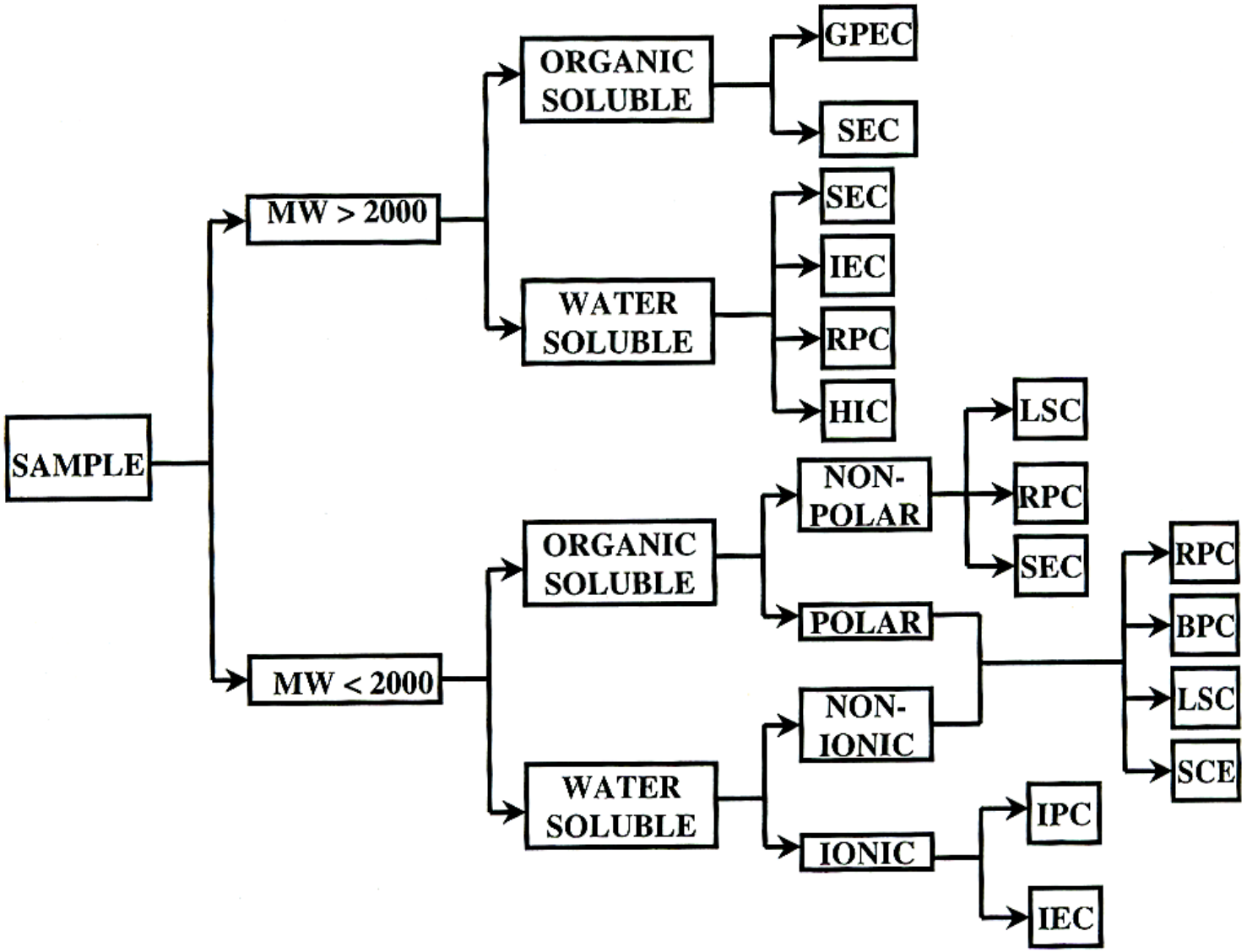
What type of LC should be used?

Stationary phase and mobile phase, Temperature

Column length, particle size, flow rate, instrument configuration, sample injection…

Accuracy, sensitivity, specificity, detection Limit, quantification limit, linearity

Mode Selection



Problem Definition

↓

Mode selection

↓

Selectivity Optimization

↓

System optimization

↓

Method Validation

Stationary phase and mobile phase,

Temperature….

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

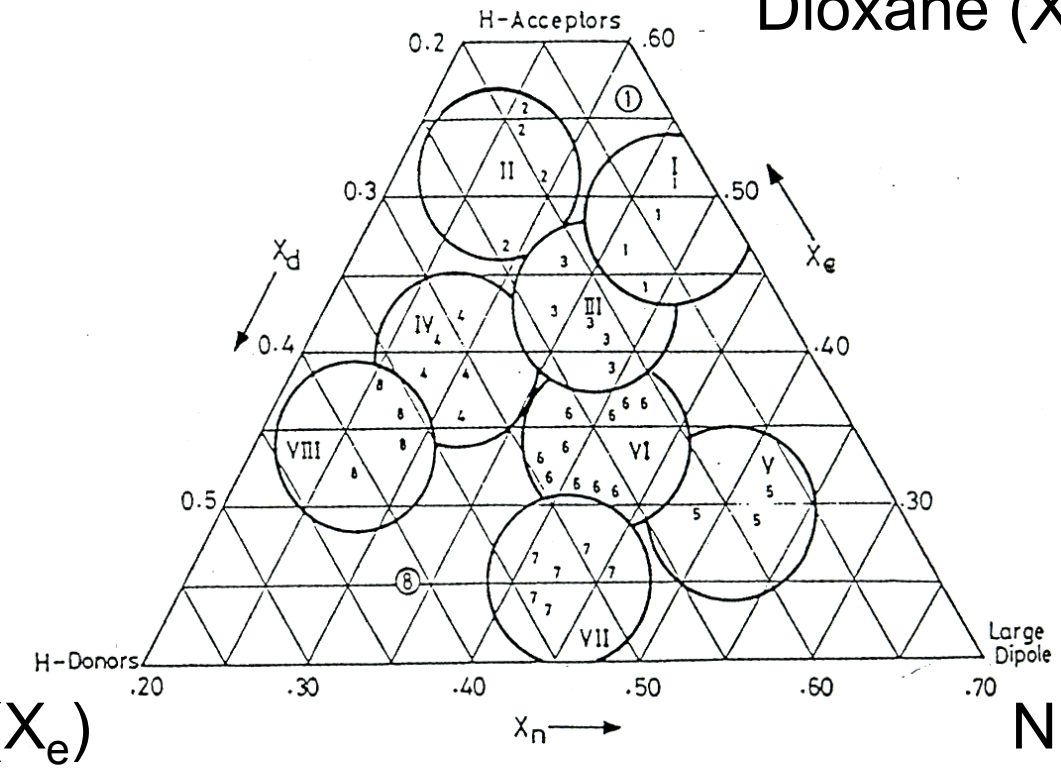
1. Solvatochromic solvent selectivity parameters

Table 4.15
Solvatochromic solvent selectivity parameters.
(Italicized solvents are only weakly attached to a group. A ? indicates that the value is unknown or uncertain)

Solvent	Solvatochromic parameters		
	π_1^*	α_1	β_1
n-Butyl Ether	0.27	0	0.46
Diisopropyl Ether	0.27	0	0.49
Diethyl Ether	0.27	0	0.47
(*Triethylamine*	*0.14*	*0*	*0.71*)
Pyridine	0.87	0	0.64
Dimethylformamide	0.88	0	0.69
Dimethyl sulfoxide	1.00	0	0.76
(*Nitrobenzene*	*1.01*	*0*	*0.39*)
Dichloromethane	0.82	0.30	0
(*Chloroform*	*0.58*	*0.44*	*0*)
Ethyl Acetate	0.55	0	0.45
Methyl Ethyl Ketone	0.67	0.06	0.48
Dioxane	0.55	0	0.37
Acetone	0.71	0.08	0.48
Tetrahydrofuran	0.58	0	0.55
(*Acetonitrile*	*0.75*	*0.19*	*0.31*)
Toluene	0.54	0	0.11
Benzene	0.59	0	0.10
(*1,1-Dichloroethane*	*0.81*	*0*	*0*)
n-Butanol	0.47	0.79	0.88
2-Propanol	0.48	0.76	0.95
1-Propanol	0.52	0.78	?
Ethanol	0.54	0.83	0.77
(*Methanol*	*0.60*	*0.93*	*0.62*)
Acetic Acid	0.64	1.12	?
Formamide	0.97	0.71	?
Water	1.09	1.17	0.18
(*2,2,2-Trifluoroethanol*	*0.73*	*1.51*	*0*)

2. Snyder solvent triangle classification method

Dioxane (X_d)



Ethanol (X_e)

Nitromethane(X_n)

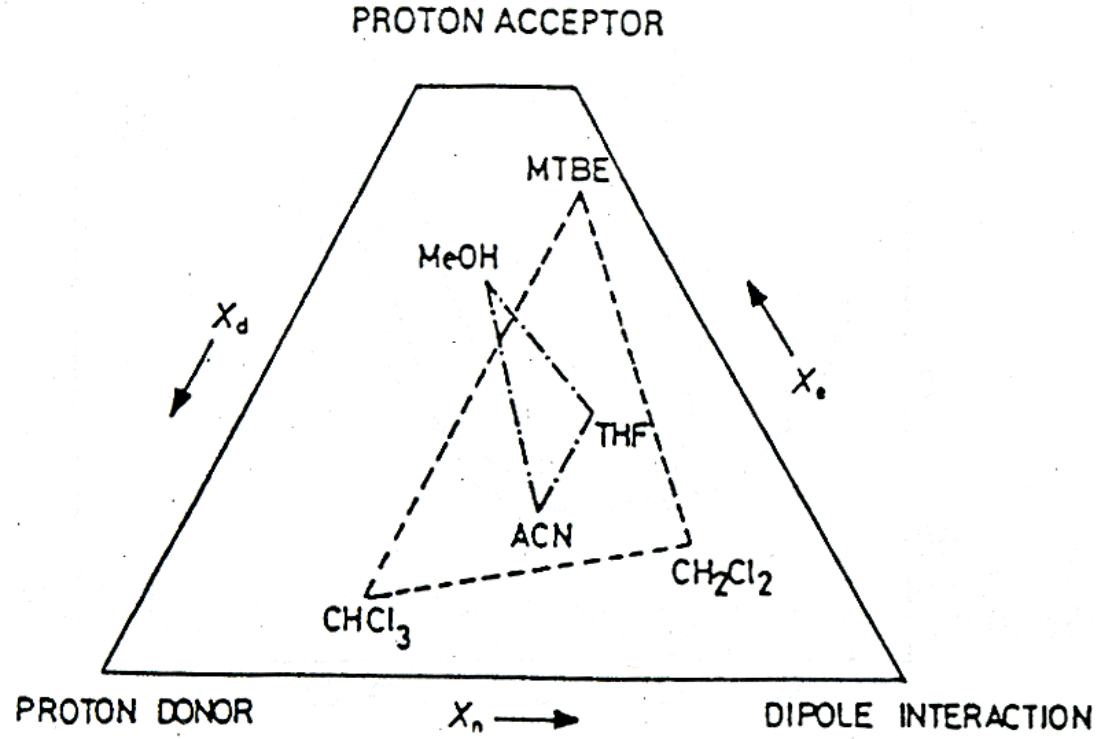
a. solvent strength: a parameter for estimating the solvent's ability To cause migration in a chromatography system.

b. solvent selectivity: the factor to distinguish the solvents that have suitable solvent strength for a separation.

Table 4.14
Solvent strength and selectivity parameters based on Snyder's selectivity triangle.
(S_i is an empirical solvent strength parameter for reversed-phase chromatography)

Solvent	Selectivity group	Solvent strength		Solvent selectivity		
		(P')	(S_i)	x_e	x_d	x_n
n-Butyl Ether	I	2.1		0.44	0.18	0.38
Diisopropyl Ether		2.4		0.48	0.14	0.38
Methyl *t*-Butyl Ether		2.7				
Diethyl Ether		2.8		0.53	0.13	0.34
n-Butanol	II	3.9		0.59	0.19	0.25
2-Propanol		3.9	4.2	0.55	0.19	0.27
1-Propanol		4.0		0.54	0.19	0.27
Ethanol		4.3	3.6	0.52	0.19	0.29
Methanol		5.1	3.0	0.48	0.22	0.31
Tetrahydrofuran	III	4.0	4.4	0.38	0.20	0.42
Pyridine		5.3		0.41	0.22	0.36
Methoxyethanol		5.5		0.38	0.24	0.38
Dimethylformamide		6.4		0.39	0.21	0.40
Acetic Acid	IV	6.0		0.39	0.31	0.30
Formamide		9.6		0.38	0.33	0.30
Dichloromethane	V	4.3		0.27	0.33	0.40
1,1-Dichloroethane		3.5		0.30	0.21	0.49
Ethyl Acetate	VI	4.4		0.34	0.23	0.43
Methyl Ethyl Ketone		4.7		0.35	0.22	0.43
Dioxane		4.8	3.5	0.36	0.24	0.40
Acetone		5.1	3.4	0.35	0.23	0.42
Acetonitrile		5.8	3.1	0.31	0.27	0.42
Toluene	VII	2.4		0.25	0.28	0.47
Benzene		2.7		0.23	0.32	0.45
Nitrobenzene		4.4		0.26	0.30	0.44
Chloroform	VIII	4.3		0.31	0.35	0.34
Dodecafluoroheptanol		8.8		0.33	0.40	0.27
Water		10.2	0	0.37	0.37	0.25

Different types of solvents can be selected by using liquid from Different solvent.



Solvent selectivity triangle for preferred solvents (– · – · – · –) for RPLC and (– – – –) for NPLC.

For NPLC (capacity factors): $k_b/k_a = 10^{(P'_A - P'_B)/2}$

For RPLC (capacity factors): $k_b/k_a = 10^{(S'_A - S'_B)/2}$

Solvent Strength for Mixtures:

For NPLC, $P_T = \Phi_1 P_1 + \Phi_2 P_2 + \ldots$

For RPLC, $S_T = \Phi_1 S_1 + \Phi_2 S_2 + \ldots$

Φ: Volume fraction of solvents 1, 2 and …

By varying the types of solvents used in mobile phase mixtures and keeping the total strength constant, the overall retention of solutes remains about the same, but changes of solvent selectivity will lead to the the change of resolution between two adjacent peaks (i.e. changing α will affect resolution)

$$\mathbf{R_s = [N^{1/2}/4][(\alpha - 1)/(\alpha)][k_2/(1 + k_2)]}$$

Solvent Strength for Mixtures:

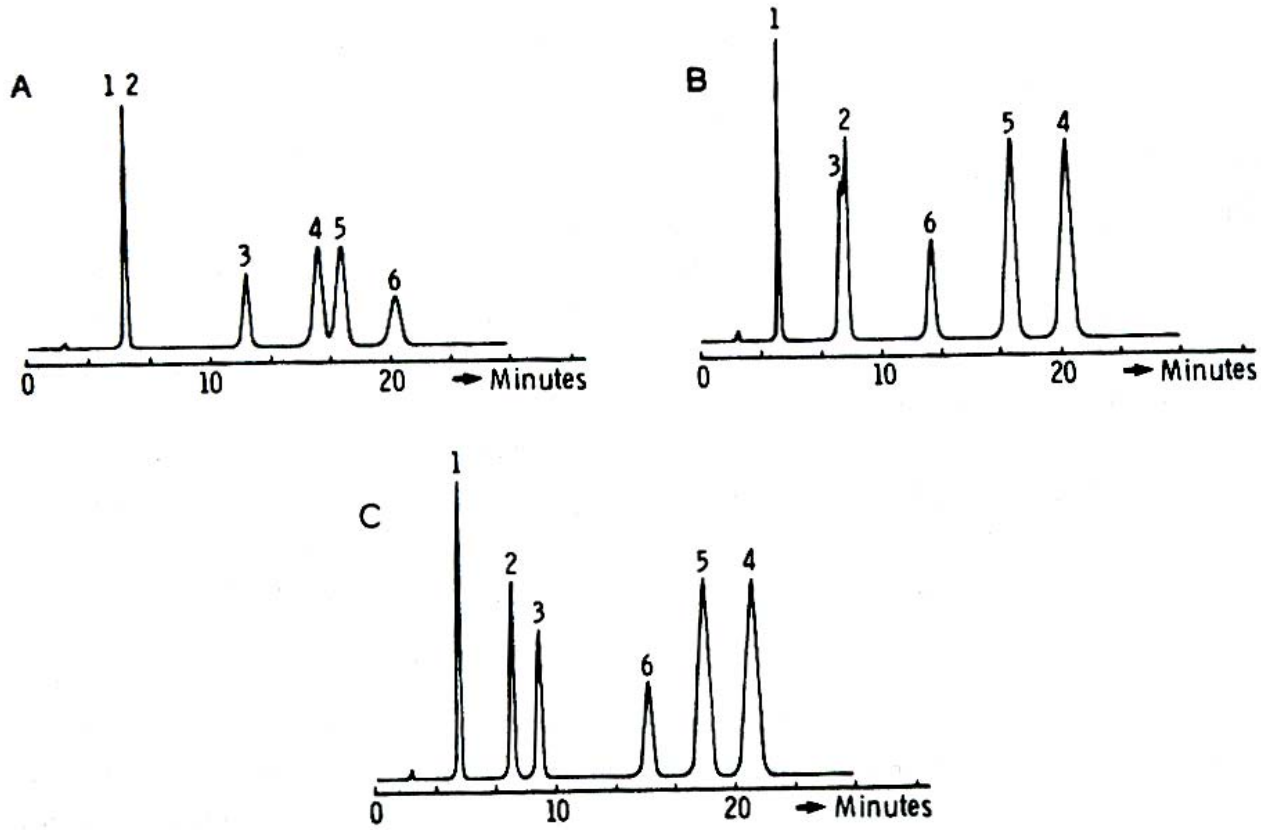


Figure 4.24. An example of the use of a ternary solvent system to control mobile phase selectivity in reversed-phase chromatography. A, methanol-water (50:50); B, tetrahydrofuran-water (32:68); and C, methanol-tetrahydrofuran-water (35:10:55). Identification: 1 = benzyl alcohol; 2 = phenol; 3 = 3-phenylpropanol; 4 = 2,4-dimethylphenol; 5 = benzene; and 6 = diethylphthalate. (From ref. [583]; ©Elsevier)

$S_M = 3.0$ $S_{THF} = 4.4$ $S_W = 0$

Selection of Isocratic elution method using solvent mixtures

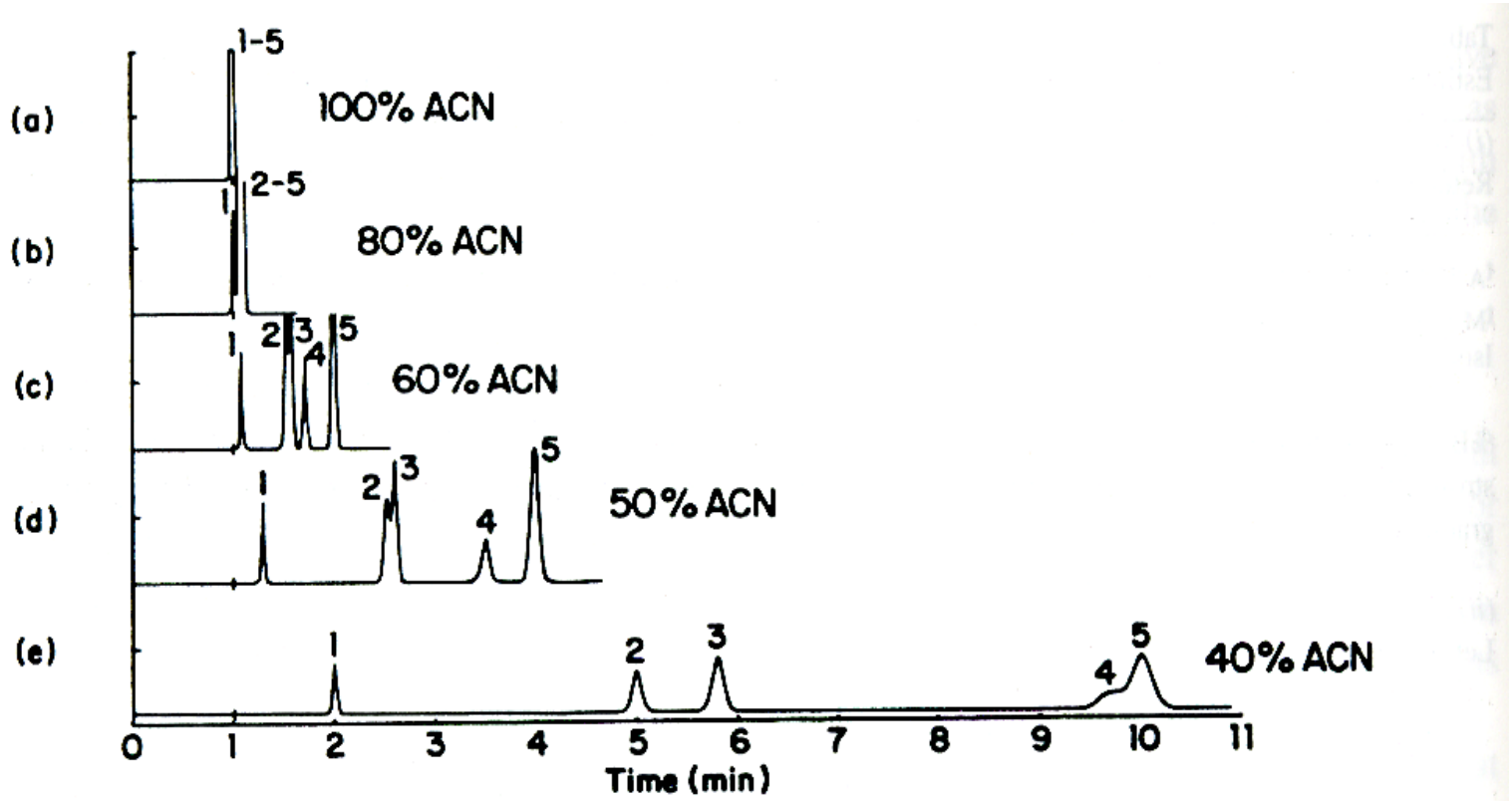


Figure 4.25. Sequential, isocratic elution using a stepwise reduction in solvent strength to identify a binary solvent of acceptable strength for elution of a five component mixture.

From strong solvents to weak solvents!

Search strategies for Optimizing Isocratic Separation (I)

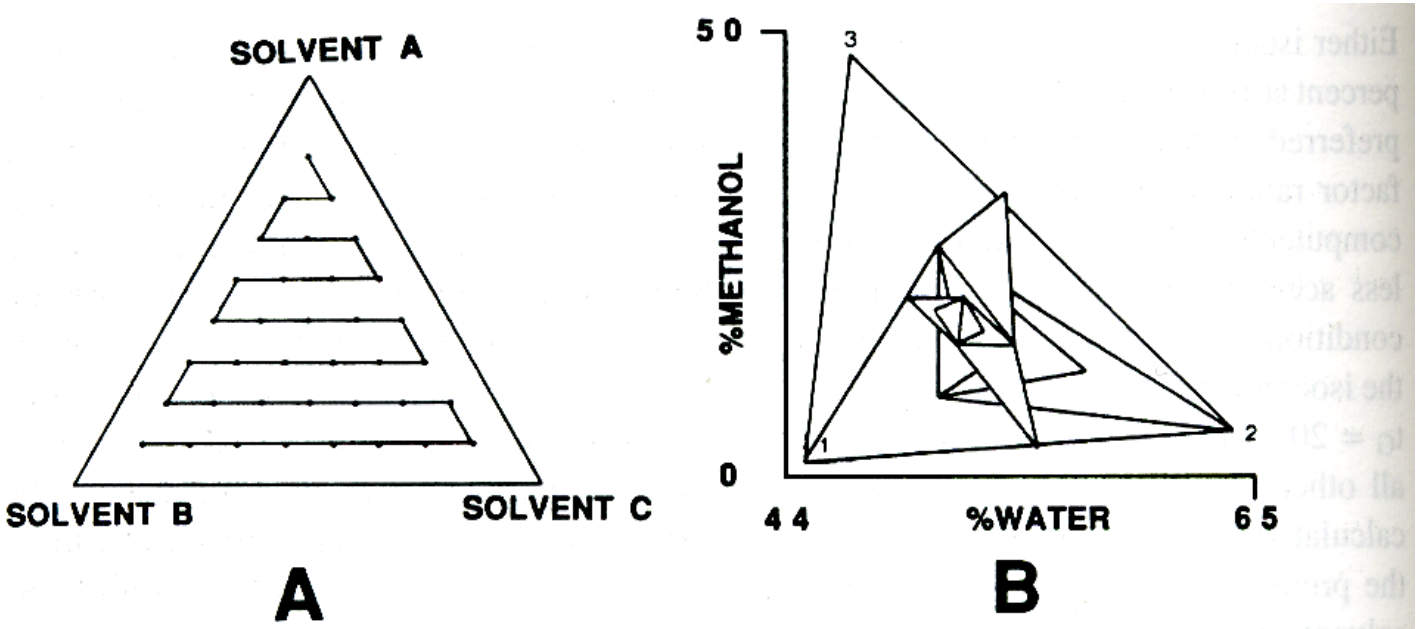


Figure 4.26. Experimental design for mobile phase composition optimization using a grid search (A) and simplex search (B).

Direct Search

Interpretive method

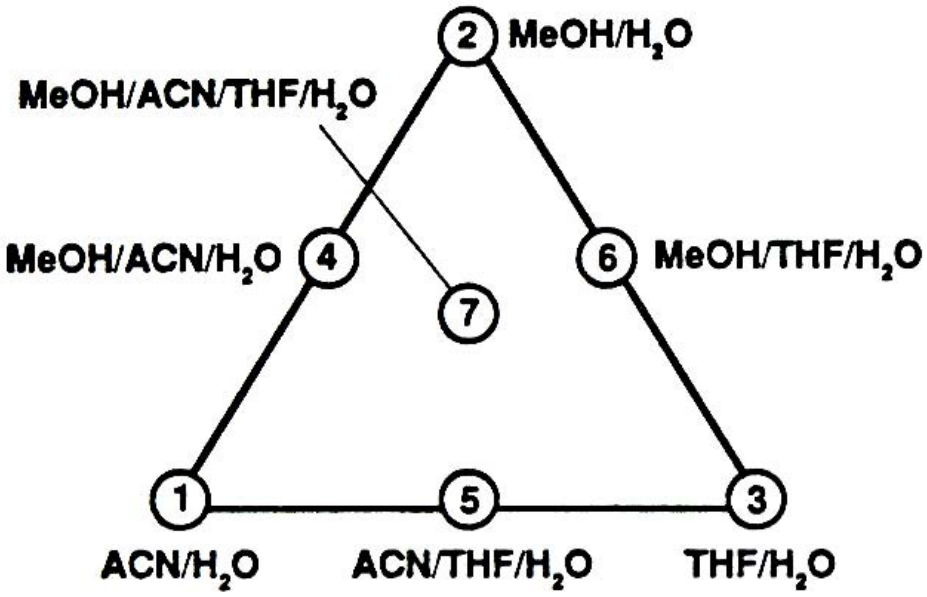


Figure 4.27. Experimental design for selectivity optimization in reversed-phase chromatography by the mixture-design technique. (From ref. [375]; ©John Wiley & Sons)

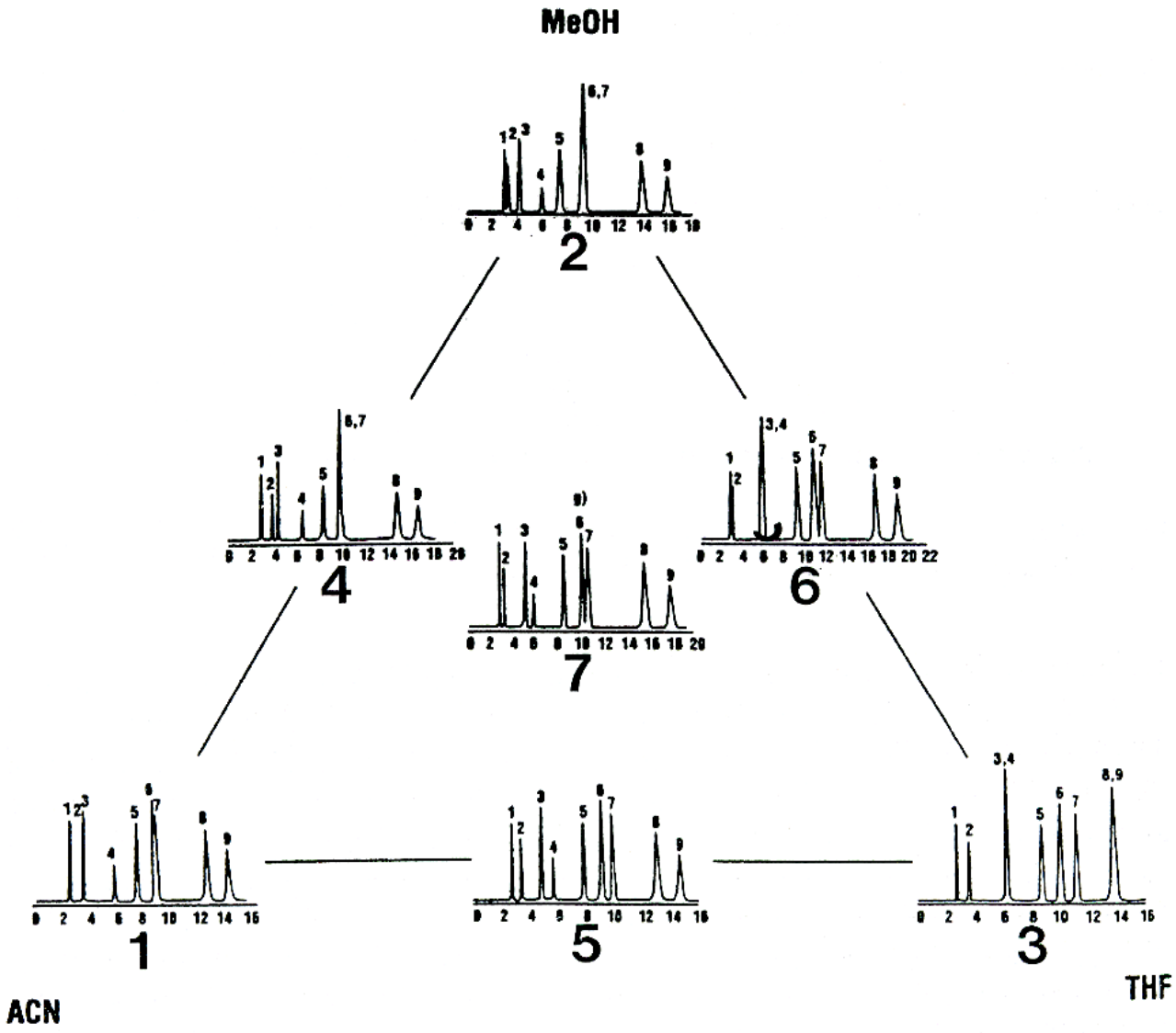


Figure 4.28. Separation of nine substituted naphthalene compounds using the mixture-design statistical technique to optimize the composition of the mobile phase (From ref. [375]; ©John Wiley & Sons).

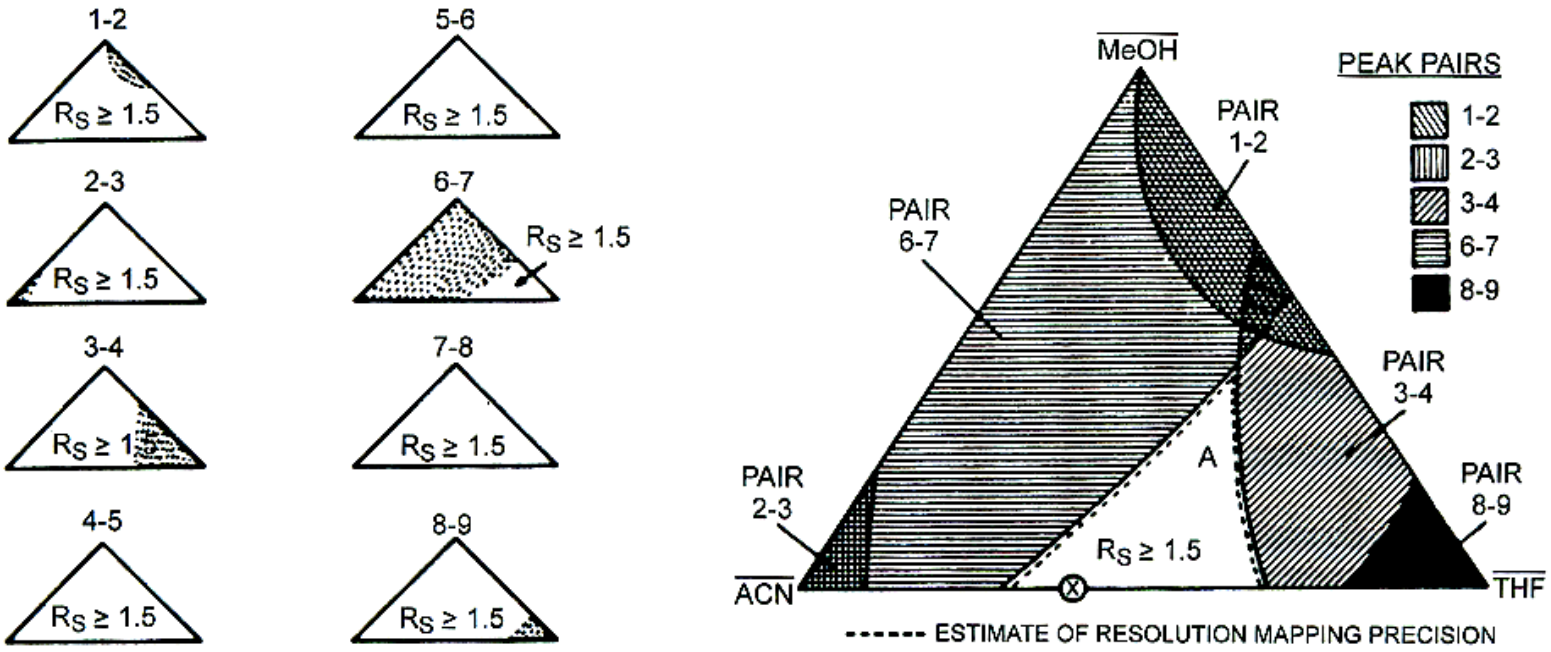
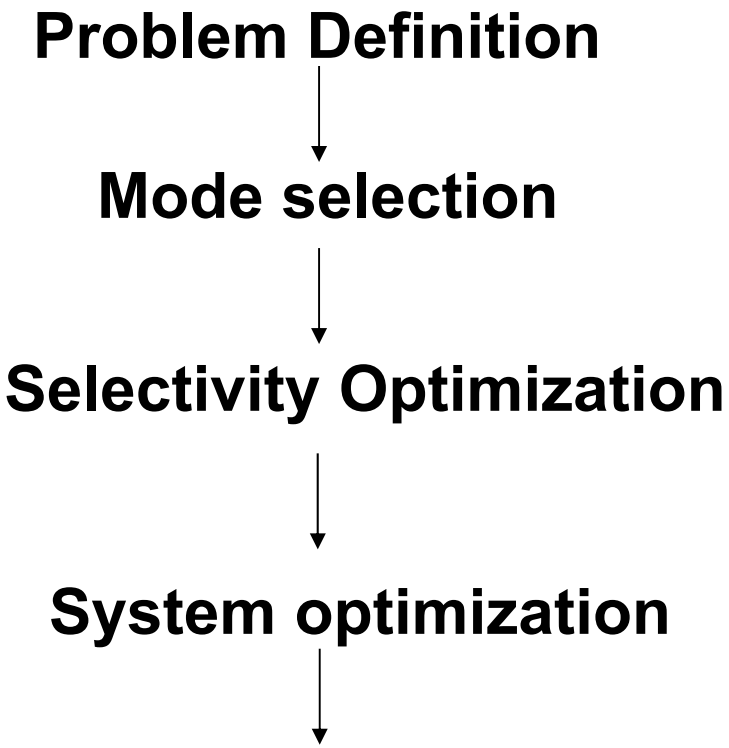


Figure 4.29. Peak-pair resolution maps and an overlapping resolution map for the separation of nine substituted naphthalene compounds by reversed-phase chromatography illustrated in Figure 4.28. (From ref. [603]; ©Elsevier)

LC Method Development



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Accuracy, sensitivity, specificity, detection Limit, quantification limit, linearity

Liquid Chromatography

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(Chapter 4 and 5 in The essence of chromatography)

1. Recently, a 40-year health statistical report has shown that excess vitamin E could cause a bad long-term consequence to human health. A scientist wishes to analyze vitamin E in various food samples by a normal-phase chromatography.

(a) A standard sample of vitamin E is injected onto a 4.1 mm ID x 10 cm normal-phase LC column using a mobile phase of 2% methanol: 98% ethyl ether. At a flow-rate of 2.0 ml/min, vitamin E elutes at 19.8 min. The void time under these conditions is 1.20 min. What mixture of methanol and ethyl ether must be used in order to achieve an elution time of 9.5 min for vitamin E on this system? ($P'_{methanol}$ = 5.1, and $P'_{ethyl\ ether}$ = 2.8)

(b) When the elusion time of vitamin E is 9.5 min, another peak from the sample appears at 9.3 min that interferes with the vitamin E's detection. Suggest a new mobile phase mixture that could be used to help improve this separation.

(c) In the case where the new mobile phase in (b) does not help the separation, suggest one other specific factor that could be changed to improve the resolution of vitamin E from the interfering compound.