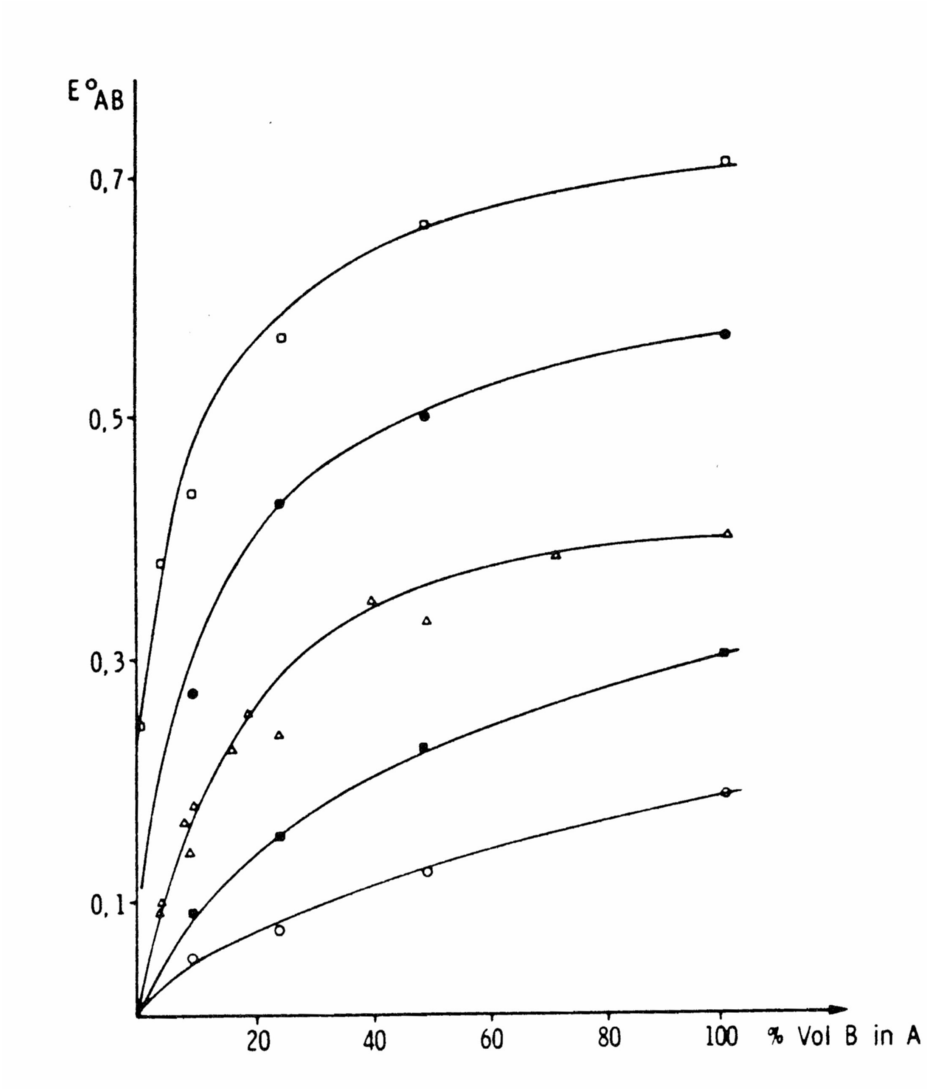
Relative Solvent Strength with Normal Phase Solvents in Adsorption Mode



Note: Solvent Strength in Normal Phase and Silica Is function of the Log of retention.

$$\text{Log}\ (k'_1 / k'_2) = \alpha A (\varepsilon_A - \varepsilon_B)$$

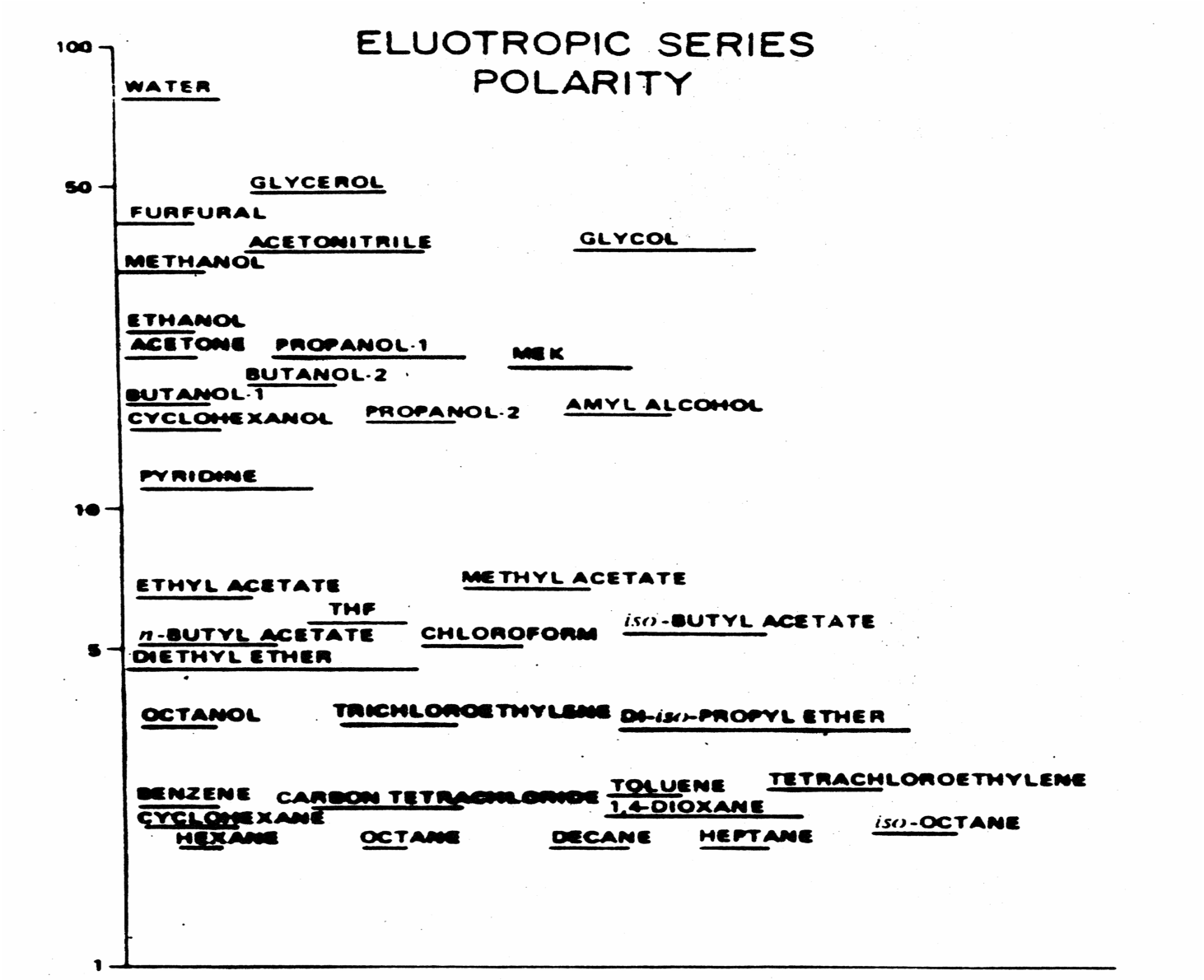
Where k'_1 and k'_2 are **capacity factors.**

α is a measure of the interaction strength of the adsorbent surface.

A is the area of the adsorbent surface.

ε_A and **ε_B are the solvent strength of mobile phase 1 and 2.**

C.F. Poole and S.K. Poole, "Chromatography Today", 1991, Elsevier Science, p.384.



From Training Class Handouts Waters Inc.

Classification of Solvents According to their Solvents Strengths

The most enduring approach to solvent classification used by LC chromatographers, especially doing small molecule normal and reverse phase separation, is L.R. Snyder's solvent strength and solvent triangle classification method.

In published papers from 1974-78, Snyder reported classifying about 75 solvents bases on their interactions with three different solutes

1. **Ethanol (assumed to have proton donating and hydrogen-bonding character)**

1. **1,4-dioxane (having various inductive or induction properties)**

2. **nitromethane (having permanent dipole interactions)**

L.R. Snyder, "Classification of the Solvent Properties of Common Liquids", *J. Chromatogr. Sci.*, 1978, 16, 223-234.

Each of their individual properties are weighted and summed according to:

$$X_{total} = X_e + X_d + X_n = 1.00$$

Where

$X_e = \log (K'_g)_e \; / \; P'$ (the solvents relative strength to interactive with ethanol and receive a proton, so a proton acceptor).

$X_d = \log (K'_g)_d \; / \; P'$ (the solvents relative strength as as a proton donor).

$X_n = \log (K'_g)_n \; / \; P'$ (the solvents relative strength for permanent dipole interactions).

Note the P' based scale had 0.0 (for hexane) and 10.2 (for water).

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

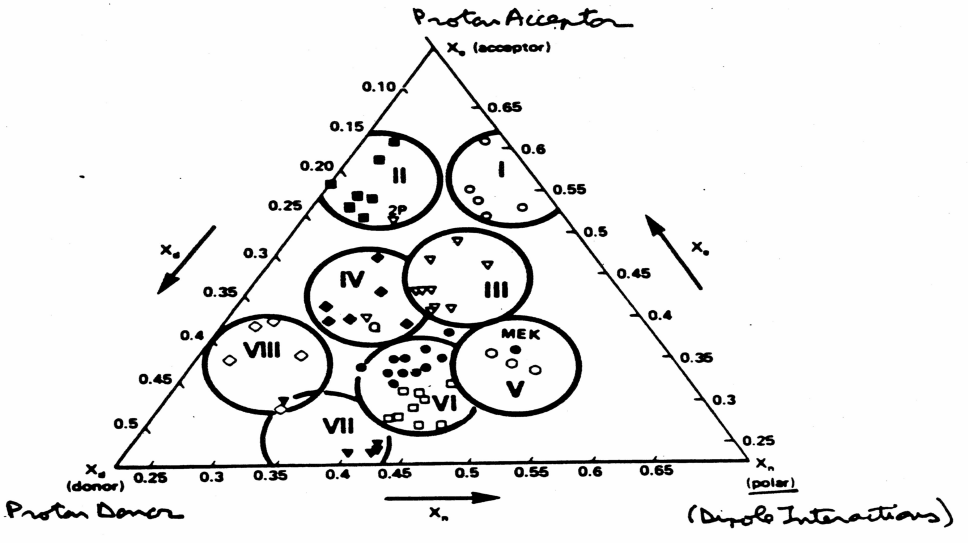
Solvent	Selectivity group	Solvent strength		Solvent selectivity		
		(P')	(S_i)	x_e	x_d	x_n
n-Butyl Ether	I	2.1		0.44	0.18	0.38
Diisopropyl Ether		2.4		0.48	0.14	0.38
Methyl *t*-Butyl Ether		2.7				
Diethyl Ether		2.8		0.53	0.13	0.34
n-Butanol	II	3.9		0.59	0.19	0.25
2-Propanol		3.9	4.2	0.55	0.19	0.27
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

From C.K. Poole, "The Essence of Chromatography", Elsevier, 2003, p. 369.

Snyder's Classification of LC Solvents into Nine Groups

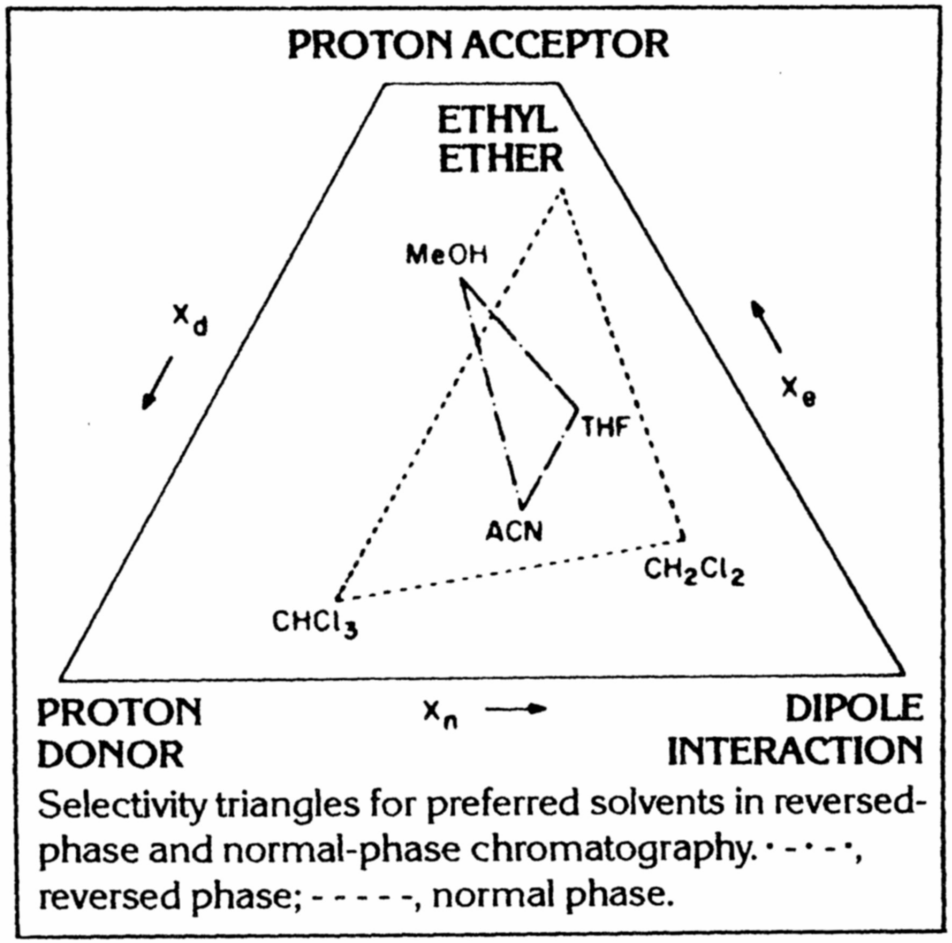
Classification of Solvents

Group	Solvents
I	Aliphatic ethers, trialkyl amines, tetramethylguanidine
II	Aliphatic alcohols
III	Pyridines, tetrahydrofuran, amides (except the more acidic formamide)
IV	Glycols, glycol-ethers, benzyl alcohol, formamide, acetic acid
V	Methylene chloride, ethylene chloride, tricresyl phosphate
VIa	Alkyl halides, ketones, esters, nitriles, sulfoxides, sulfones, aniline and dioxane
VIb	Nitro compounds, propylene carbonate, phenyl-alkyl ethers, aromatic hydrocarbons
VII	Halobenzenes, diphenyl ether
VIII	Fluoroalkanols, m-cresol, chloroform, water



From J.J. Kirkland and L.R. Snyder, American Chemical Society Short Course, "Solving Problems in Modern Liquid Chromatography, 1974.

Snyder's Solvent Triangle showing mostcommon reversed and normal phase LC solvents.

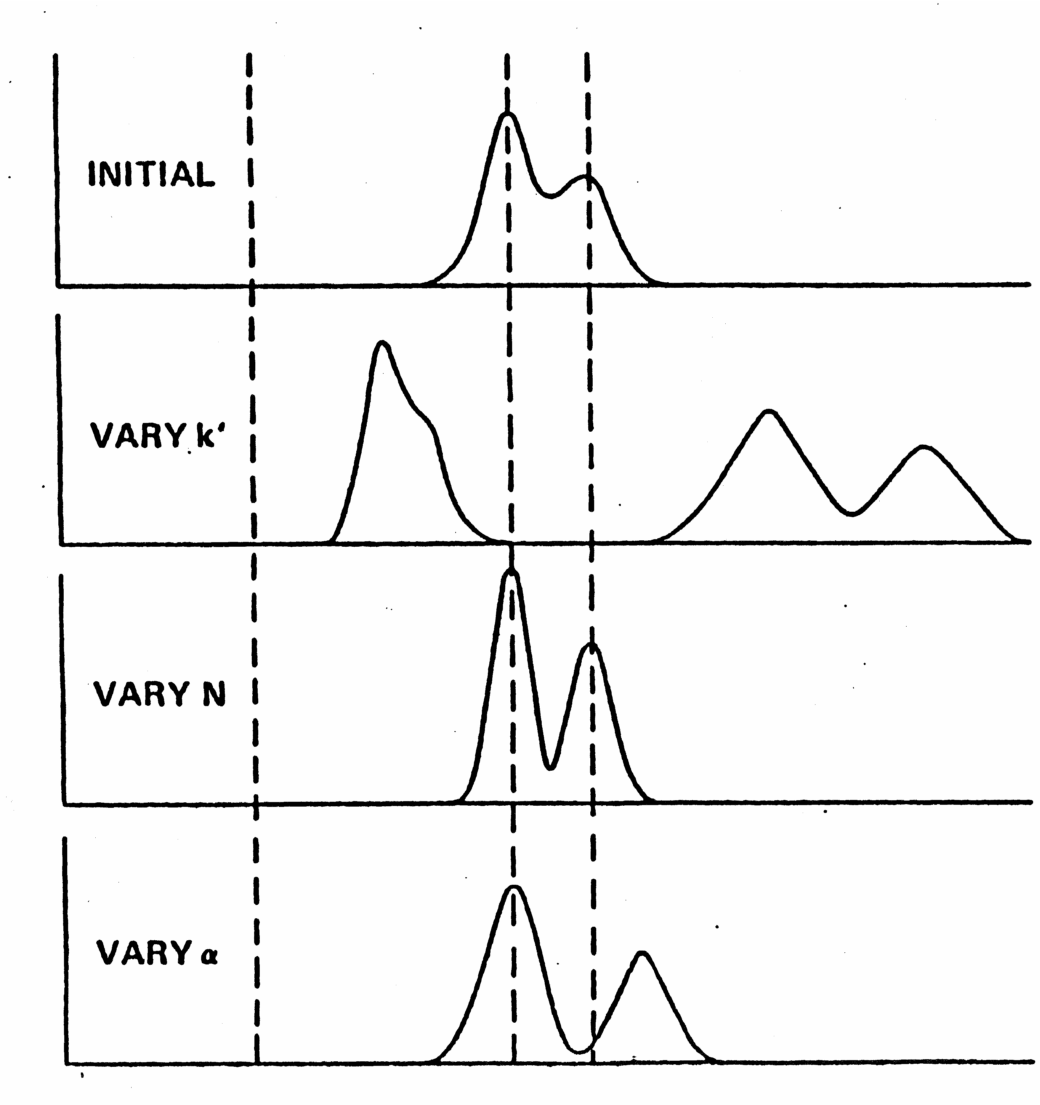


Selectivity triangles for preferred solvents in reversed-phase and normal-phase chromatography. · - · - ·, reversed phase; - - - - -, normal phase.

Fig. came from J.L. Glajch, J.J. Kirkland, K.M. Squire and J.M. Minor, Dupont's Technical Note, E-40956 March 1981, but there is an extended presentation in L.R. Snyder, J.J. Kirkland, J.L. Glajch "Practical HPLC Method Development", 1997, Wiley-Interscience Publication, pp. 233-264.

Steps in the Optimization of a Liquid Chromatographic Separation.

$$Rs = (k' / (1 + k')) / ((n)^{1/2} / 4) / ((\alpha - 1) / \alpha)$$



Vary % composition of mobile phase

Optimize flow rate

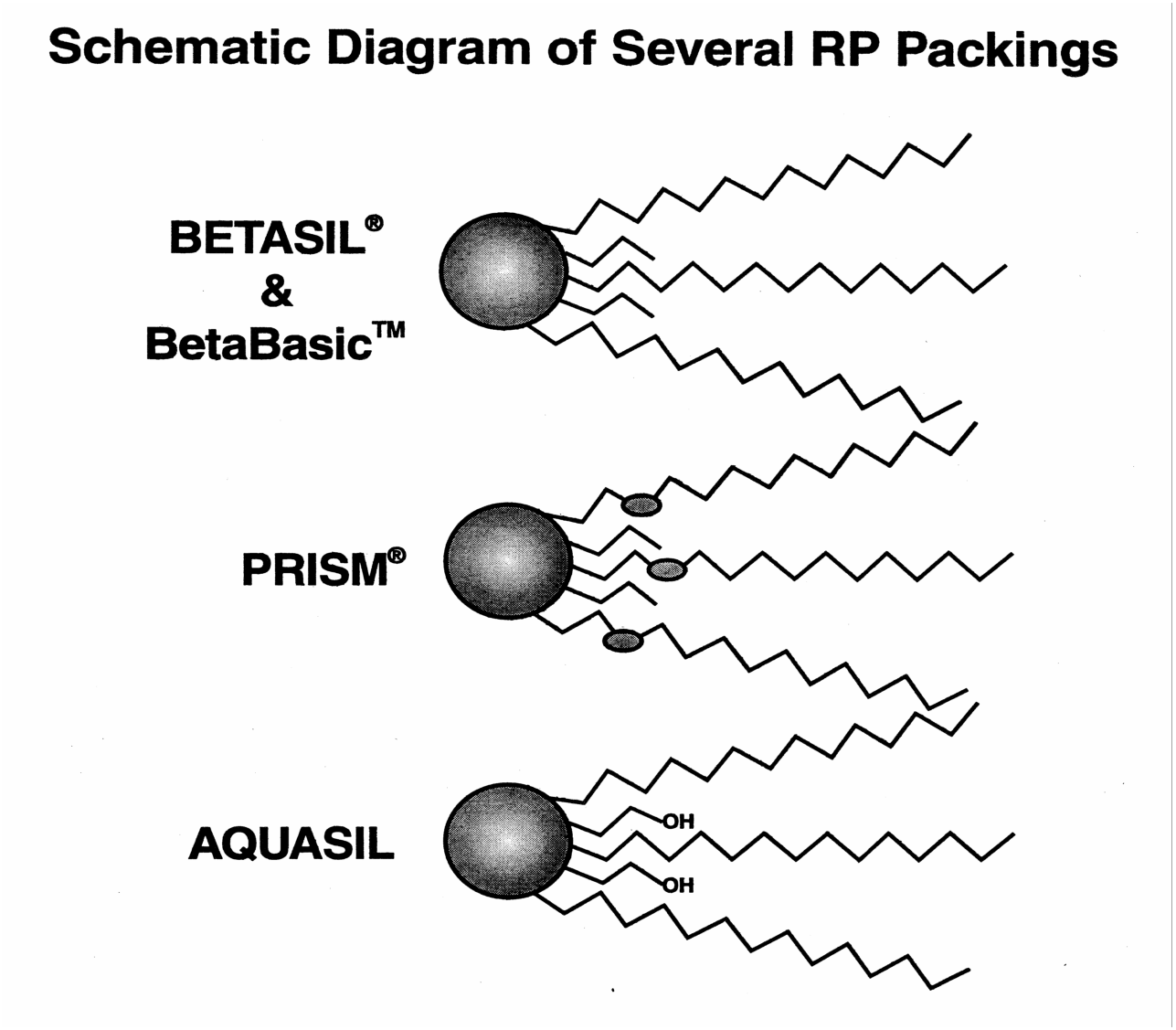
Add more column

Change solvent class

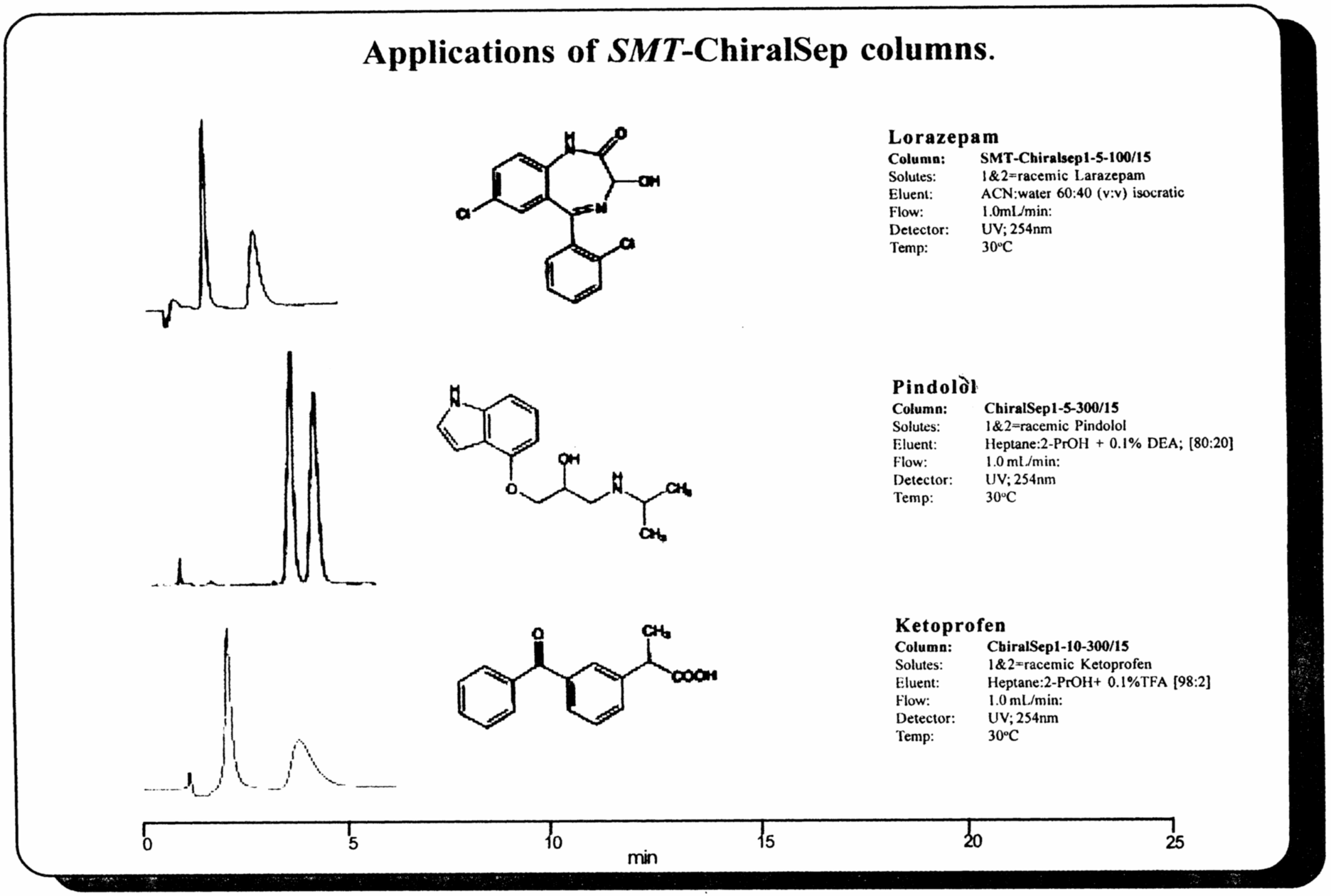
Change type of bonded stationary phase

From D.A. Skoog, "Principles of Instrumental Analysis", 3 rd. ed., 1985, p. 743.

Schematic of certain of the newer embedded reverse-phase C-18 phases.



From a Keystone Scientific (Thermo/Electron) Sales Brochure, 1997, Frame # 96113.



From a Sales Brochure on the new "Self-Assembled Monolayers" by Separation Methods Technologies, Newark, Delaware, 2005, p.47.

Use of the new Monolith (Continuous Bed) Silica Column.

It consists of a single, porous silica rod, of macropores, that allows for low pressure drops, hence high flow rates with acceptable column efficiencies.

Open structure and wide pores allow for the separation of biological molecules from oligonucleotides and proteins to plasmids and whole virus. The smaller disks allows for micrograms microgram separations, while larger disks can be used to separate 5 mg. upto 500 grams in capacity.

At flow rates of several column volumes per minute without loss of resolution or capacity.

The Protein A column can be used to quantify IgG in less than 1 minute. An 8 ml DEAE CIM column can be used to quickly purify preparative amounts of supercoiled plasmid DNA. Also available are phases to separate: QA, DEAE, SO3, CM, Epoxy, Protein A, Protein G, RPC, EDA, C2, C4 and custom available. Works with any standard medium or HPLC systems. From an on-line circular of BiaSeparations, Ljubjana, Slovenia http://www.biaseparations.

Hydrophobic Interaction Chromatography (HIC)

- Start with an aqueous buffer of high ionic strength to salt the protein onto the low- pressure, wide-pore, soft silica surface.
- Next follow with an aqueous gradient starting with high to increasing low salt concentration to selectively displaced the protein.
- Since solvent stength is controlled by varying the ionic strength, the activity of the protein maybe maintained! often an ammonium sulfate gradient from 3.0 to 0.0 Molar is used).

Showing the two positions of a 6-port valve-and-loop injector.

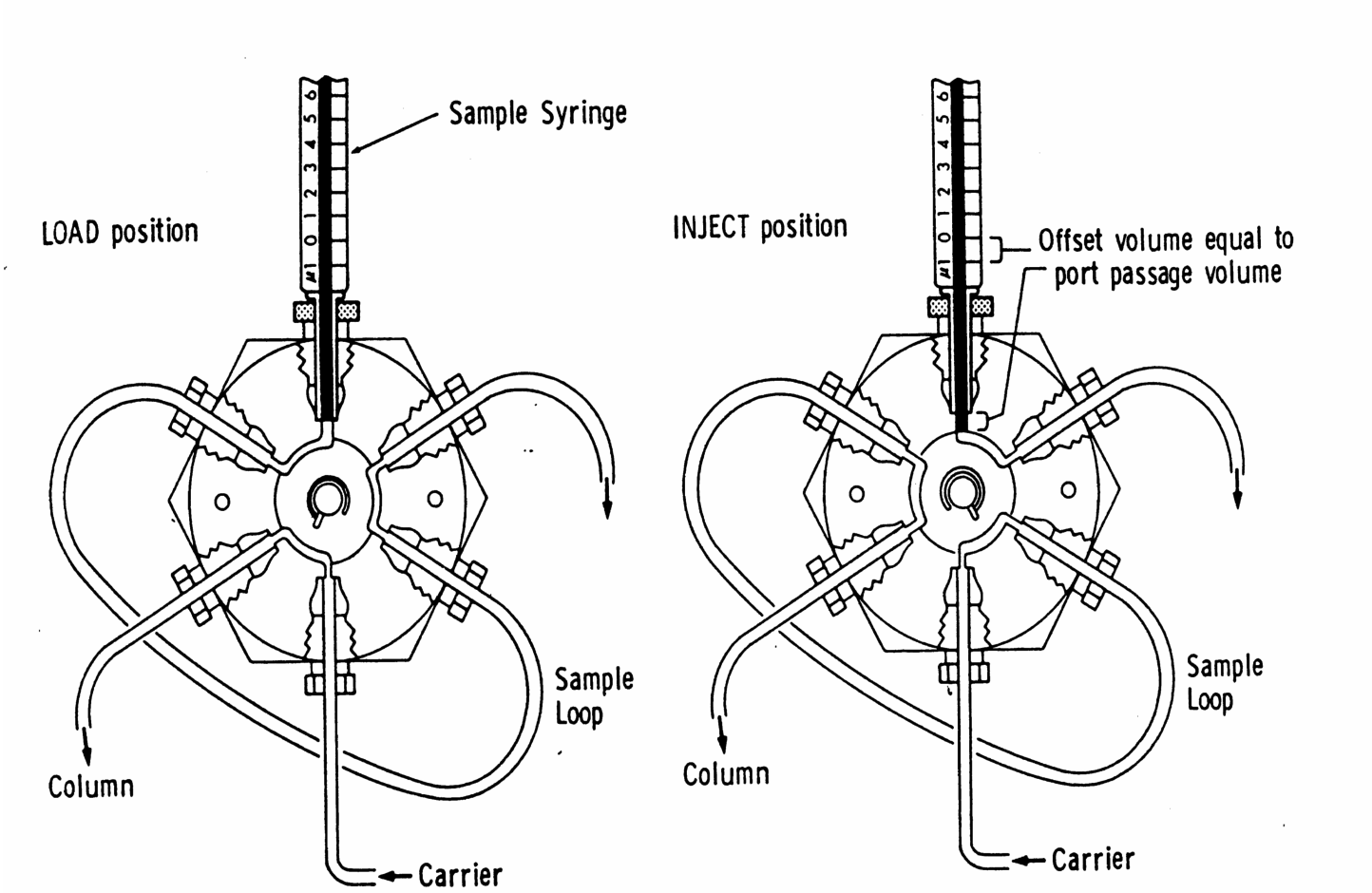
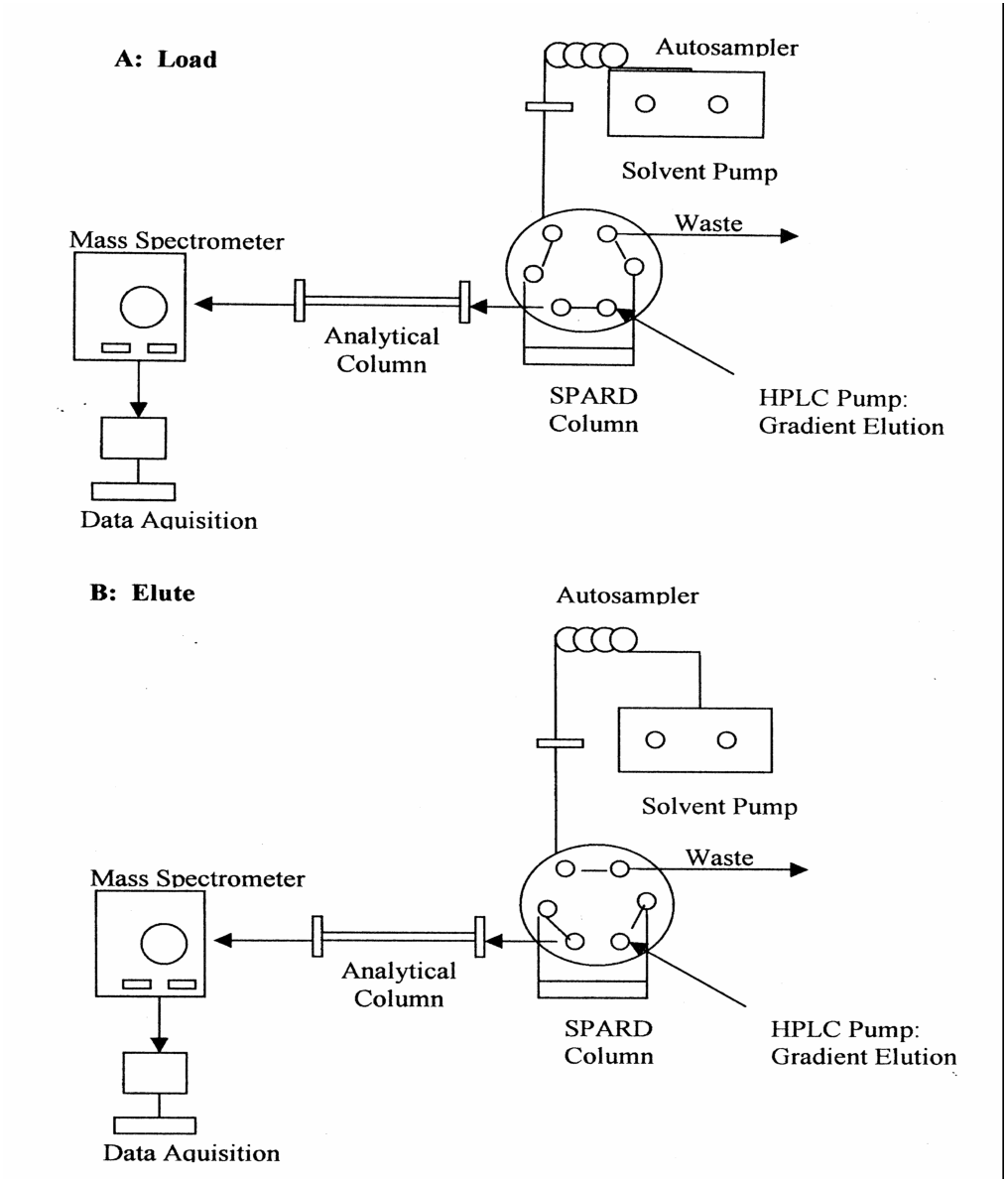


Figure 5.6 Microvolume injection valve showing the valve configuration in the load and inject position. (Reproduced with permission from Valco Instruments, Inc.)

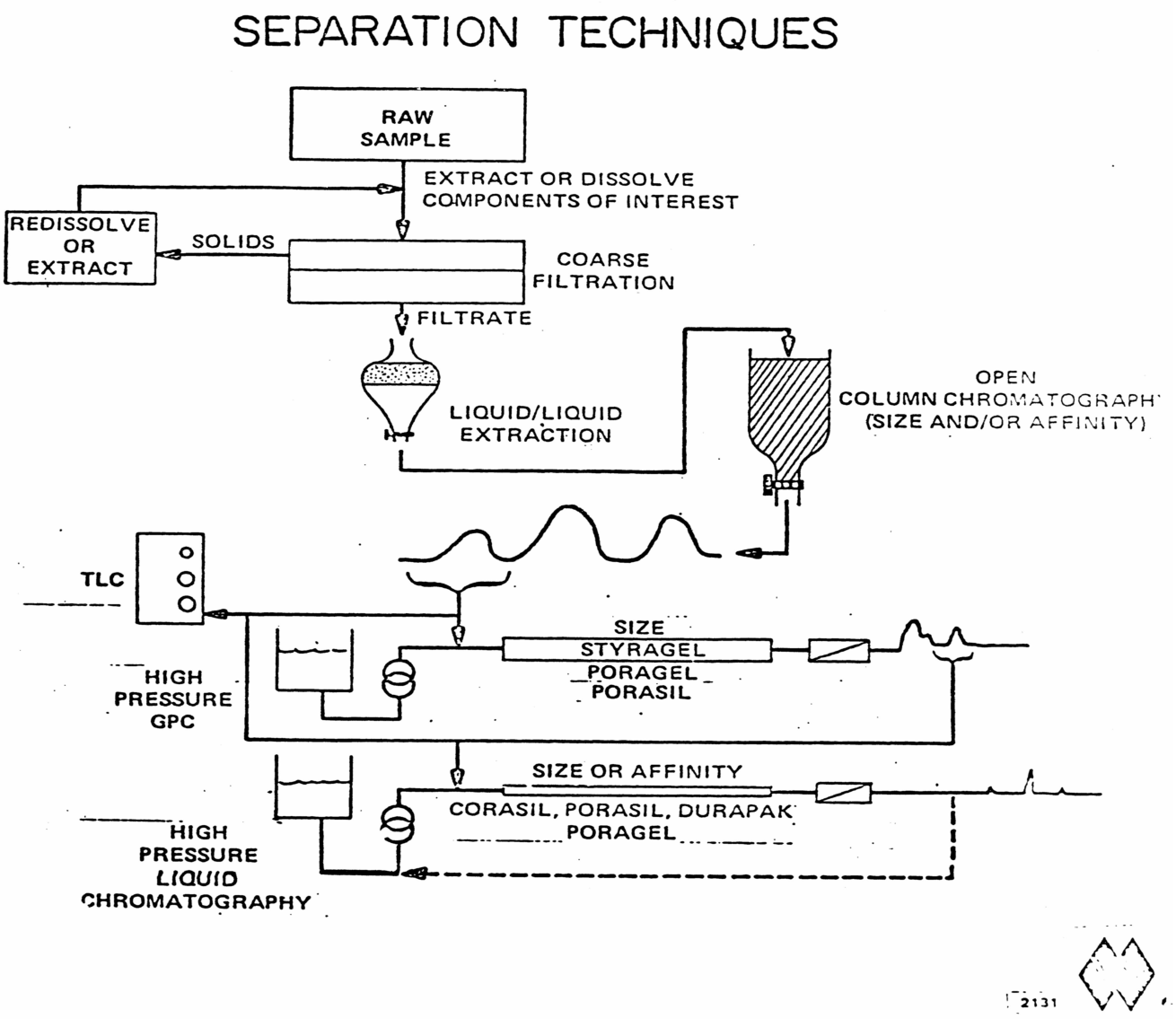
From C.F. Poole and S.K. Poole, "Chromatography Today", 1991, Elsevier Science, p. 565.

Showing how to concentrate on a pre-column, back-flush incipient to waste and to inject onto the analytical column.



From the Ph.D. Thesis of Dustin Yavrosky, UCONN, 2003

Many Times Preliminary Sample Preparation is key to Successful HPLC!



From Training Class Handouts Waters Inc.