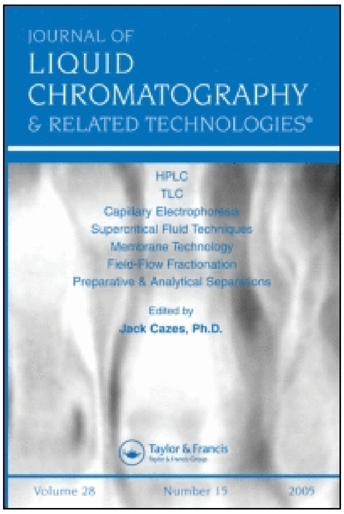

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THE EFFECT OF BINARY SOLVENT COMPOSITION AND POLARITY ON SEPARATIONS IN REVERSED PHASE THIN LAYER AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[1,2]

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ABSTRACT

The effect of the solvent's composition and polarity on separation in reversed phase thin layer and high performance liquid chromatography is discussed. These results show that retention times cannot be predicted merely from the polarity of the binary mobile phase. Although organic modifiers with the same physico-chemical properties and from the same solvent group were used, the retention times obtained using binary mobile phases having the same polarity, were different. It was also observed that normal chain carbon alcohols gave retention times different from those with a branched chain (n-propanol vs. iso-propanol), and the longer the alcohol chain the higher the R_f value. The results also show that not only the organic modifier used is important but the solute mixture used.

INTRODUCTION

It is not easy to select the best solvent system for adsorption, partition or ion exchange chromatography. Important considerations are the nature of the material being separated (polar, non polar or ionic), and the solid

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phase (silica gel, alumina, cellulose, ion exchange, or reverse phase C_2, C_8 or C_{18}). The appropriate solvent can only be selected when these two factors have been decided. When binary solvent mixtures are used, the analyst must consider the solubility of the solute in the solvent, the effect of solvent demixing, solvent strength (polarity) and hydrogen bonding. In ion exchange, the buffer used, pH and ionic strength are important. Selection of a binary solvent mixture and prediction of the elution times for the components of a mixture may not be that simple. In reverse, and normal phase liquid chromatography elution times of the solute are a function of the properties of the stationary and mobile phase.

Synder (1) gave the following equation for calculating the polarity of a binary solvent mixture used in reversed phase liquid chromatography:

$$P' = \phi_1 P_1 + \phi_2 P_2 \qquad (I)$$

P' is the polarity of the mixture, ϕ_1 and ϕ_2 are the volume fractions of the two solvents and P_1 and P_2 are the polarity of the pure solvents. This relation does not apply to normal phases in which the calculations are more complex (1).

The relation between retention time (R_t) and capacity factor (K') is described by the following equation:

$$K' = (R_t - R_{to})/R_{to} \qquad (II)$$

Where R_{to} is equal to the retention time of unretained solute.

Our objectives were to determine (a) the effect of the binary mobile phase polarity and composition on separation; and (b) how the solvent affects the bonded alkyl chain and, thus, the separation in reversed phase thin layer (TLC) and high performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials: Solvents were glass distilled (Burdick and Jackson). Chemicals were analytical grade (Aldrich Chemical Co.) and used without further purification. Reversed phase (RP-18) and silica gel TLC plates were purchased from Whatman, Inc. Standard TLC tanks and equipment were used. Plates were spotted with 5 μl disposable micropipettes. The mobile phase was a binary alcohol/water, and alcohol/alcohol mixture. The alcohols were methanol (MeOH), ethanol (EtOH), n-propanol (n-PrOH), iso -propanol (i-PrOH), tert-butanol (t-BuOH) and iso-pentanol (i-POH). Results with other binary solvent mixtures were also examined.

Apparatus: A modular HPLC system consisting of Laboratory Data Control (LDC) Constametric I and II pumps attached to an LDC Gradient Master, a Chromatronix dual-channel uv absorbance detector, a Rheodyne injector, and a strip-chart recorder operated at 0.2 in/min. was used.

The RP-18 reversed phase column (Merck) and μ-porasil column (Waters Associates) were 250 mm X 4.6 mm prepacked with 10 μm particle size materials. 10 μl samples were injected. Experiments were run at room temperature using a mobile phase flow rate of 1.2 ml/min. Retention times, peak widths (W) and resolution (R_s) were determined with a Packard 1865 A/D converter connected to the UV detector output of the liquid chromatograph. The output from the data system was recorded on a 9866A thermal line printer (Hewlett-Packard).

RESULTS AND DISCUSSION

Table 1 shows the separation of benzo(e)pyrene (BeP) and anthracene spotted on reversed phase C_{18} (TLC) plates and developed in binary alcohol/water mixtures having the same polarity (5.60), calculated according to eq. (I). Note that solvent demixing occurred when n-BuOH/H_2O, and i-PenOH/H_2O were used and, as a result, high R_f values were obtained and the results had to be discarded. However, when mixtures of MeOH/H_2O, EtOH/H_2O and n-PrOH/H_2O were used the R_f values for BeP and anthracene were different. Also, note that the solvent mixtures n-PrOH/H_2O and i-PrOH/H_2O gave different elution times, with i-PrOH/H_2O giving lower R_f values for both BeP and anthracene. When the R_f values were compared using i-PrOH/H_2O and t-BuOH/H_2O, the longer carbon chain alcohols gave higher R_f values, but the separation factor α remained the same.

Since solvent demixing occurred with i-PrOH/H_2O and n-BuOH/H_2O pairs, it was decided to (a) use a lower polarity value (4.3) and to substitute methanol

TABLE I

SEPARATION OF BENZO[E]PYRENE AND ANTHRACENE ON RP-18 TLC PLATES USING A BINARY ALCOHOL/WATER MIXTURES HAVING THE SAME POLARITY (5.60)

SOLVENT	RATIO (v/v)	R_fX100 BeP	ANTHRACENE	α
$MeOH/H_2O$	90.2/9.8	18	33	1.83
$EtOH/H_2O$	78/22	23	38	1.65
$n\text{-}PrOH/H_2O$	74.2/25.8	35	51	1.46
$i\text{-}PrOH/H_2O$	73/27	24	38	1.58
$t\text{-}BuOH/H_2O$	75.4/24.6	28	44	1.57
$i\text{-}POH/H_2O$	70.8/29.2	68*	77*	-
$n\text{-}BuOH/H_2O$	73/27	63*	71*	-

*Solvent Demixing

$\alpha = R_f$ anthracene/R_f BeP

or acetone (AC=0) for water (Table II). The table shows that although the nine binary solvent mixtures have the same polarity, different R_f and α values were obtained for both BeP, and anthracene in each solvent mixture. R_f X 100 values ranged from 49-80 for BeP, and from 61-85 for anthracene. Note that, for example, $n\text{-}BuOH/H_2O$, n-BuOH/MeOH, and n-BuOH/AC=0 all gave different R_f values for both BeP and anthracene. This is because water, methanol and acetone each belong to a different solvent group (1). Differences in retention times of BeP and anthracene were observed when the plate was spotted and developed in the following pairs of binary solvent mixtures, n-BuOH/MeOH and t-BuOH/MeOH; and n-PrOH/MeOH and i-PrOH/MeOH, with the normal chain solvent giving the higher R_f values.

In another experiment, the volume of alcohol was kept constant at 90 ml (Table III) and 95 ml (Table IV), while the volume of water was calculated according to the following eq:

$$V_1 P_1 + V_{H_2O} \times P_{H_2O} = V_2 P_2 + V_{xH_2O} \times P_{H_2O} \qquad \text{(III)}$$

TABLE II

EFFECT OF BINARY SOLVENT COMPOSITION ON R_f AND α VALUES IN REVERSED PHASE TLC (P' = 4.3)

SOLVENT	RATIO (v/v)	R_fX100 BeP	R_fX100 ANTHRACENE	α
EtOH	neat	63	73	1.16
n-PrOH/H_2O	95.2/4.8	60	70	1.17
n-PrOH/MeOH	72.7/27.3	71	81	1.14
n-BuOH/H_2O	93.6/6.4	60	76	1.27
n-BuOH/MeOH	66.7/33.3	72	78	1.08
i-PrOH/MeOH	66.7/33.3	56	68	1.21
t-BuOH/MeOH	80/20	49	61	1.24
n-BuOH/AC=O	66.7/33.3	80	85	1.06
n-PrOH/AC=O	72.7/27.3	75	81	1.08

TABLE III

EFFECT OF BINARY MOBILE PHASE COMPOSITION ON R_f AND α VALUES OF BENZO[E]PYRENE AND ANTHRACENE USING CONSTANT ALCOHOL BUT VARIABLE WATER VOLUME

SOLVENT	RATIO (v/v)	P'	R_fX100 BeP	R_fX100 ANTHRACENE	α
MeOH/H_2O	90/10	5.6	18	33	1.83
EtOH/H_2O	90/17	5.2	29	44	1.52
n-PrOH/H_2O	90/19.7	5.1	38	51	1.34
i-PrOH/H_2O	90/20.6	5.1	29	44	1.52
n-BuOH/H_2O	90/20.6	5.1	53	63	1.19
t-BuOH/H_2O	90/18.8	5.2	36	52	1.44
i-POH/H_2O	90/22.4	5.0	65	73	1.12

Where V_1 and V_2 are volumes of pure alcohols 1 and 2, P_1 and P_2 are the polarity of pure alcohols 1 and 2, V_{H_2O} is the known volume of water added and $V_{x(H_2O)}$ is calculated volume of water. V_1 and V_2 were kept constant at 90 ml. V_{H_2O} was 10 ml. The experiment was performed to see the effect the water in the mobile phase had on (a) separation and (b) the C_{18} alkyl chain. The binary solvent polarity values of the mixture were not constant and varied by ± 0.1 units, except for MeOH/H_2O (Tables III and IV). The tables indicate that, although the amount of alcohol is constant, the R_f values of both BeP and anthracene, increased with increasing amount of water for both the normal, and the branched chain alcohols, with the latter giving lower R_f values. A few conclusions can be drawn from the data in Tables III and IV. (a) The R_f X 100 values ranged from 33-73 for anthracene, and from 18-65 for BeP in 90ml alcohol/water (Table III) while they ranged from 47-79 for anthracene and from 27-72 for BeP in 95ml alcohol/water (Table IV); (b) better separation factors were found with the branched chain alcohols; and (c) the same R_f values were obtained for both compounds in EtOH/ H_2O, and i-PrOH/ H_2O, although there was more water in the i-PrOH/H_2O binary mixture, which may indicate that the solutes are more soluble in i-PrOH than in methanol.

The results (Tables I-IV) indicate that R_f values were higher as the amount of water in the mobile phase was increased, which may indicate a lack of interaction between the solute and the solid phase, and in turn collapse of the C_{18} alkyl chain in the solvent as the volume of water increased. It also could indicate a solute-solvent interaction. Tables III and IV show that, although

TABLE IV

EFFECT OF BINARY MOBILE PHASE COMPOSITION ON R_f AND α VALUES OF BENZO[E]PYRENE AND ANTHRACENE USING CONSTANT ALCOHOL BUT VARIABLE WATER VOLUME

			R_fX100		
SOLVENT	RATIO (v/v)	P'	BeP	ANTHRACENE	α
MeOH/H_2O	95/5	5.4	27	47	1.74
EtOH/H_2O	95/12.5	5.0	30	46	1.53
n-PrOH/H_2O	95/15.2	4.9	43	57	1.33
i-PrOH/H_2O	95/16.2	4.8	33	47	1.42
n-BuOH/H_2O	95/16.2	4.8	72	79	1.10
t-BuOH/H_2O	95/14.3	4.9	46	63	1.37
i-POH/H_2O	95/18.0	4.7	70	78	1.11

the same volume of water was used in the i-PrOH and n-BuOH mixtures, different R_f and α values were obtained, which suggests that the alcohol used plays an important role in the separation process.

In view of these results another experiment was undertaken in which three different groups of compounds with different chemical properties were spotted on C_{18} reversed phase TLC plates and developed under the following conditions (a) pure alcohols: (b) constant alcohol/water ratio (90/10); (c) constant water but variable alcohol ratio; (d) constant alcohol but variable water ratio; and (e) constant polarity (5.1) using methanol/alcohol and water/alcohol. The three group of compounds selected for this study were: (1) anthraquinones [anthraquinone (A), methyl- (M) and ethylanthraquinone (E)]; (2) naphthalene (N) and biphenyl (B); and (3) dimethyl- (MP) and diethyl- (EP) phthalates.

Note that, since we are studying the interaction of the solvent-solute-bonded chain, we are ignoring the optimization of the separation factor(α). Table V lists R_f values for the three groups of compounds in pure alcohols. It can be seen that R_f values did not increase with increasing chain length of the alcohol (except methanol), which indicates that solute-solid phase interaction, due to the solubility of the C_{18} alkyl chain in the alcohols studied, is not

TABLE V

SEPARATION OF ANTHRAQUINONE, METHYL- AND ETHYL ANTHRAQUINONE, NAPTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPTHALATES IN PURE ALCOHOLS USING RP-18 TLC PLATES

	$R_f X100$		
SOLVENT	ANTHRAQUINONES	N+B	PHTHALATES
MeOH	53, 48, 48	58	69, 66
EtOH	72	78	88
PrOH	75	80	88
i-PrOH	71	76	83
n-BuOH	70	75	78
i-BuOH	73	79	85
t-BuOH	76	80	88

Note: When one number is reported it means that no separation was achieved in that solvent.

affected by the alcohol used. When constant ratios of alcohol/water v/v were used, the R_f values obtained for each group of compounds in the binary solvent mixture were a function of the solute. The R_f values for naphthalene and biphenyl and the anthraquinones increased with the increase in normal alcohol chain length. The phthalates gave comparable R_f values in MeOH/H_2O, EtOH/H_2O, n-PrOH/H_2O and n-BuOH/H_2O. When the branched chain alcohol/water mixtures were used (Table VI), the R_f values for each group of compounds remained approximately constant (±0.03 Units).

When the results in pure alcohol (Table V) were compared with those in (90/10) alcohol/water (Table VI), differences in R_f values were observed. It was also observed that MeOH/H_2O (90/10) resolved the components of all the three groups of compounds. The addition of 10% water to MeOH and EtOH (Table VI) resulted in lower R_f values for the anthraquinones than when developed in the pure alcohol. No differences in R_f values were observed when water was added to n-PrOH, i-PrOH, i-BuOH or t-BuOH. However, higher R_f values were obtained when water was added to n-BuOH. Almost the same effect was observed for naphthalene, biphenyl and the phthalates (Table VI).

TABLE VI

SEPARATION OF ANTHRAQUINONE, METYL ANTHRAQUINONE, ETHYL ANTHRAQUINONE, NAPTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES IN CONSTANT ALCOHOL/WATER RATIO (90/10) USING RP-18 TLC PLATES

	R_fX100		
MOBILE PHASE	ANTHRAQUINONES	N+B	PHTHALATES
MeOH-H_2O	47, 40, 36	53, 47	73, 66
EtOH-H_2O	45, 50, 50	52	70, 67
PrOH-H_2O	75	75	89
i-PrOH-H_2O	70	71	87
n-BuOH-H_2O	80	81	92
i-BuOH-H_2O	76	76	79
t-BuOH-H_2O	76	80	88
i-POH-H_2O	76	76	90

Table VII gives the R_f values of the three groups of compounds developed in alcohol/water. The volume of water was kept constant (10 ml), while that of methanol (90 ml) was replaced by a volume of an alcohol having the same polarity as that of methanol (0.90 X 5.1 = 4.59). In this experiment we wanted to see the effect of keeping the polarity of the alcohol and the volume of the water constant. Note that the polarity of each binary solvent mixture is not constant according to equation (I). The results show (Table VII) that resolution of the mixture in each group is achieved only when $MeOH/H_2O$ and $EtOH/H_2O$ were used, but no separation was achieved using an alcohol with a chain longer than C_2, although the three groups of compounds tested have different chemical properties. This indicates that solvent-solute or solute-solid phase (C_{18}) interaction was eliminated and no separation was achieved using C-3 or higher chain alcohols. Table VII also shows that, although the R_f values increased from MeOH to n-PrOH, they were very similar to those obtained with the other alcohols (n-ProH to t-BuOH).

Table VIII lists the R_f values obtained when the volume of alcohol was kept constant (90 ml) but the volume of water was changed to compensate for the polarity of the alcohols according to equation (III). The results indicate that better resolutions were obtained for the anthraquinones and phthalates but not

TABLE VII

SEPARATION OF ANTHRAQUINONE, METHYL- AND ETHYL ANTHRAQUINONE, NAPHTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES USING CONSTANT ALCOHOL AND CONSTANT WATER POLARITY MOBILE PHASES ON RP-18 TLC PLATES

		R_fX100		
SOLVENT	RATIO (v/v)	ANTHRAQUINONES	N+B	PHTHALATES
$MeOH/H_2O$	(90/10)	47, 40, 35	53, 57	75, 68
$EtOH/H_2O$	(107/10)	67, 64, 61	66	85, 81
$n\text{-}PrOH/H_2O$	(115/10)	73	74	88
$n\text{-}BuOH/H_2O$	(118/10)	73	74	83
$i\text{-}PrOH/H_2O$	(118/10)	71	71	86
$t\text{-}BuOH/H_2O$	(112/10)	75	74	87

TABLE VIII

SEPARATION OF ANTHRAQUINONE, METHYL-, AND ETHYL ANTHRAQUINONE, NAPHTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES USING CONSTANT ALCOHOL BUT VARIABLE WATER VOLUME ON RP-18 TLC PLATES

SOLVENT	RATIO (v/v)	R_fX100 ANTHRAQUINONES	N+B	PHTHALATES
MeOH/H_2O	(90/10)	47, 40, 35	53, 47	75, 78
EtOH/H_2O	(90/18)	63, 57, 53	56	85, 79
n-PrOH/H_2O	(90/20)	65, 61, 61	59	77, 77
n-BuOH/H_2O	(90/21)*	-	-	-
i-PrOH/H_2O	(90/21)	64, 60, 58	58	81, 78
t-BuOH/H_2O	(90/18.8)	76, 76, 76	73	92, 92

*Solvent demixing

the naphthalene or the biphenyl, which means that that the results are affected by the solute used (3).

Table IX shows the effect of replacing water with methanol to adjust the polarity of the binary solvent mixture to 5.1. The results indicate an increase in R_f values with increase in the alcohol chain length. However, the resolution of the solutes within a group was poor. This indicates that solvent-solute interactions are the predominant factor, since separation is a function of the solute distribution coefficient. Table X gives the R_f values in a binary alcohol/ water (P' = 5.1) solvent mixture. Except for naphthalene-biphenyl mixture, the results indicate better resolution with alcohol/ water than with alcohol/methanol solvent mixtures (Table IX), although the R_f values obtained for each compound are comparable, (Tables IX and X). This is because the addition of water to the mobile phase decreases the solubility of the solutes (4).

Table XI gives the retention times and K' values for the three groups of compounds using a C_{18} column and binary alcohol/water mixtures having the same P' value of 6.12 calculated according to Equation (I). Note that different K' values were obtained for each compound in each mobile phase. These results agree with those obtained using C_{18} reverse phase TLC plates.

TABLE IX

SEPARATION OF ANTHRAQUINONE, METHYL- AND ETHYL ANTHRAQUINONE, NAPTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES IN ALCOHOL/METHANOL BINARY SOLVENT MIXTURES HAVING A CONSTANT POLARITY OF 5.1 ON RP-18 TLC PLATES

SOLVENT	RATIO (v/v)	R_fX100 ANTHRAQUINONES	N+B	PHTHALATES
MeOH	100%	58, 52, 52	63	78, 73
EtOH/MeOH	86.4/13.6	61	68	75
PrOH/MeOH	82.3/17.7	66	72	78
i-PrOH/MeOH	81 / 19	67	71	80
BuOH/MeOH	81 / 19	79	83	89
t-BuOH/MeOH	83.6/16.4	68	73	83

TABLE X

SEPARATION OF ANTHRAQUINONE, METHYL- AND ETHYL ANTHRAQUINONE, NAPHTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES IN ALCOHOL/WATER BINARY SOLVENT MIXTURES HAVING A CONSTANT POLARITY OF 5.1 ON RP-18 TLC PLATES

SOLVENT	RATIO (v/v)	R_fX100 ANTHRAQUINONES	N+B	PHTHALATES
MeOH	100%	56, 51, 50	60	72, 69
EtOH/H_2O	86.4/13.6	56, 52, 49	52	73, 69
n-PrOH/H_2O	82.3/17.7	74, 70, 68	68	88, 84
BuOH/H_2O*	81.0/19.0	-	-	-
i-PrOH/H_2O	81.0/19.0	69, 66, 63	63	87, 83
t-BuOH/H_2O	83.6/16.4	65, 65, 65	64	82, 82

*Solvent demixing

When the binary solvent mixture was composed of solvents from different solvent groups (1), the retention times were different on both silica gel and C_{18} reversed phase silica gel plates (Table XII), although they have the same P' value. It is interesting to note that the retention times of naphthalene on silica gel plates were approximately the same no matter what binary mobile phase

TABLE XI

SEPARATION OF ANTHRAQUINONE, METHYL- AND ETHYL ANTHRAQUINONE, NAPHTHALENE, BIPHENYL, DIMETHYL- AND DIETHYLPHTHALATES USING C_{18} HPLC COLUMN AND BINARY ALCOHOL/WATER MOBILE PHASES HAVING THE SAME POLARITY (6.12)

SOLVENT	RATIO (v/v)	R_{t_0}(min.)	R_t/k'	A	M	E	N	B	MP	EP
MeOH/H_2O	80/20	2.55	R_t	6.5	7.7	9.0	5.5	6.5	3.5	4.5
			K'	1.55	2.02	2.53	1.16	1.55	0.37	0.61
EtOH/H_2O	69.4/30.6	2.55	R_t	5.1	6.6	6.6	5.8	6.6	3.2	3.7
			K'	1.00	1.59	1.59	1.28	1.59	0.26	0.45
n-PrOH/H_2O	65.8/34.2	2.95	R_t	4.5	5.1	5.1	5.4	5.4	3.8	3.8
			K'	0.53	0.73	0.73	0.83	0.83	0.29	0.29
i-PrOH/H_2O	64.8/35.2	3.20	R_t	5.5	5.5	6.5	6.5	6.9	3.9	4.3
			K'	0.72	0.72	1.03	1.03	1.16	0.22	0.34

TABLE XII

SEPARATION OF A MIXTURE OF ANTHRAQUINONE, NAPHTHALENE AND DIMETHYL PHTHALATE ON REVERSED-PHASE AND SILICA GEL PLATES USING DIFFERENT SOLVENT MIXTURES HAVING THE SAME POLARITY*

			R_fX100		
SOLVENT	RATIO (v/v)	PLATE	ANTHRAQUINONE	NAPHTHALENE	Me-PHTHALATE
MeOH/H_2O	45/55	RP-18	0	0	13
CH_3CN/H_2O	52/48	RP-18	35	23	43
THF/H_2O	37/63	RP-18	8	5	21
Ethyl ether/ Hexane	52/48	Silica gel	92	99	79
$CHCl_3$/Hexane	35/65	Silica gel	50	99	25
CH_2Cl_2/Hexane	47/53	Silica gel	39	94	18

*From Reference 2.

was used. This was not the case when a polycyclic aromatic hydrocarbon mixture was separated on a μ-Porasil column using 2% ethyl acetate/hexane and 2.15% chloroform/hexane (Fig. 1).

Figures 2 and 3 show the effect of the organic modifier on the separation. Although the polarity of the mobile phases are the same, replacing acetonitrile by methanol gave different retention times, α and K' values, under the same experimental conditions.

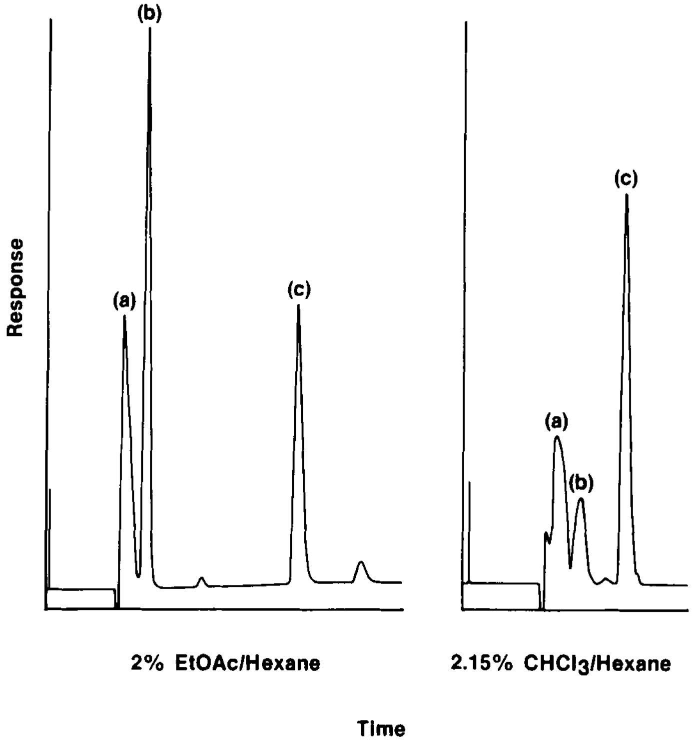

Figure 1. Comparative HPLC separation of benzo[a]pyrene (a), naphthacene (b) and anthracene (c) using a μ-porasil column and two different mobile phases having the same polarity; 2% ethyl acetate/hexane (left) and 2.15% chloroform/hexane (right).

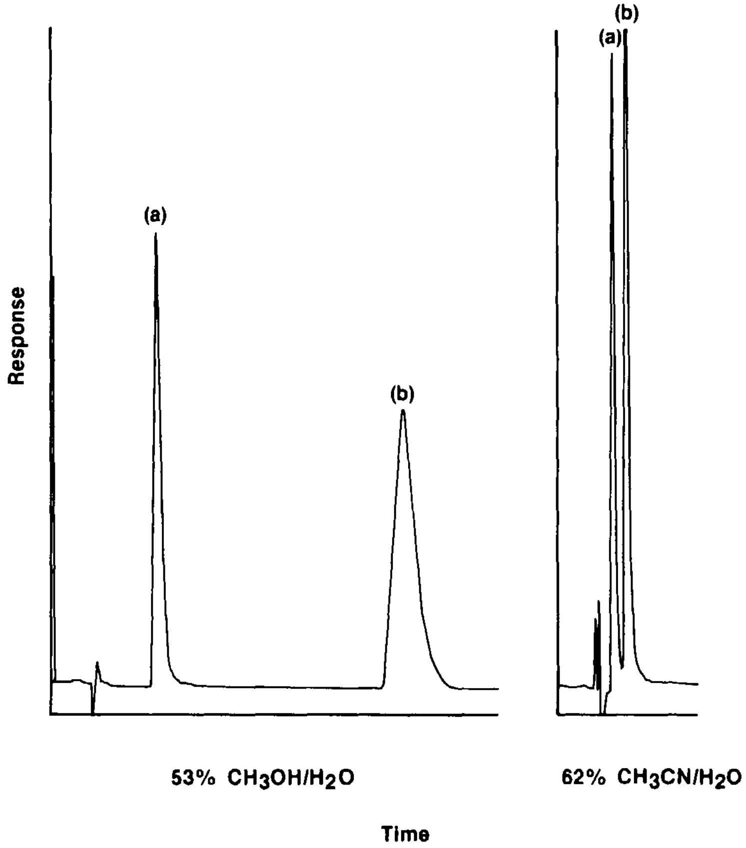

Figure 2. Comparative HPLC separation of dimethylphthalate (a) and diethylphthalate using a reversed phase C_{18} column in two different mobile phases having the same polarity; 53% methanol/water and 62% acetonitrile/water at a flow rate of 1.2 ml/min.

If the 10 ml of water in acetonitrile/water (90/10) is replaced by 20 ml of methanol, both having the same polarity, the effect on R_t, α and K' values are quite evident (Fig 4). This is due to solvent-solute and solute-solid phase interactions.

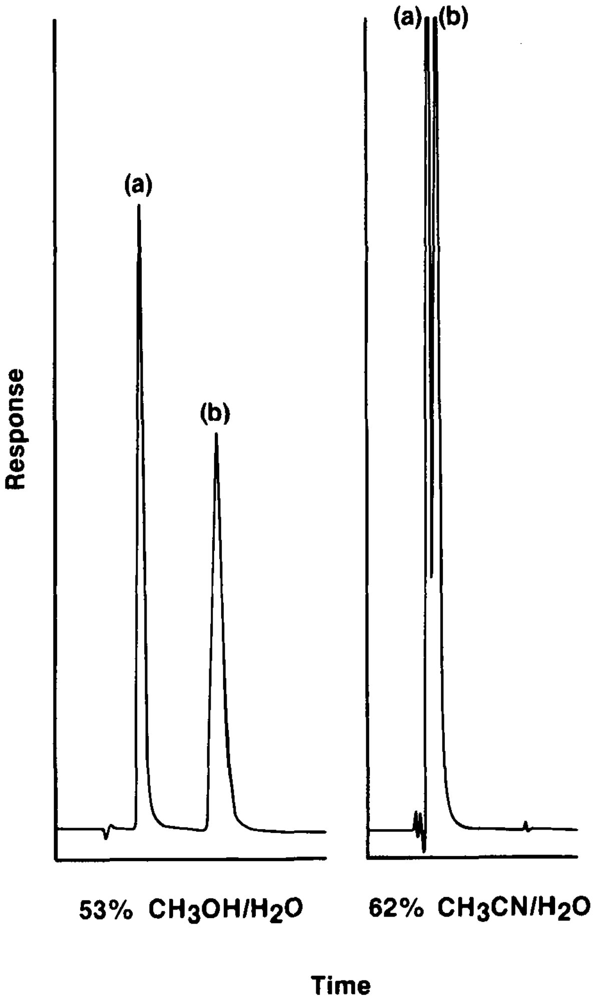

Figure 3. Comparative HPLC separation of benzene (a) and naphthalene (b) using a reversed phase C_{18} column and two different mobile phases having the same polarity; 53% methanol/water (left) and 62% acetonitrile/water (right), at a flow rate of 1.2 ml/min.

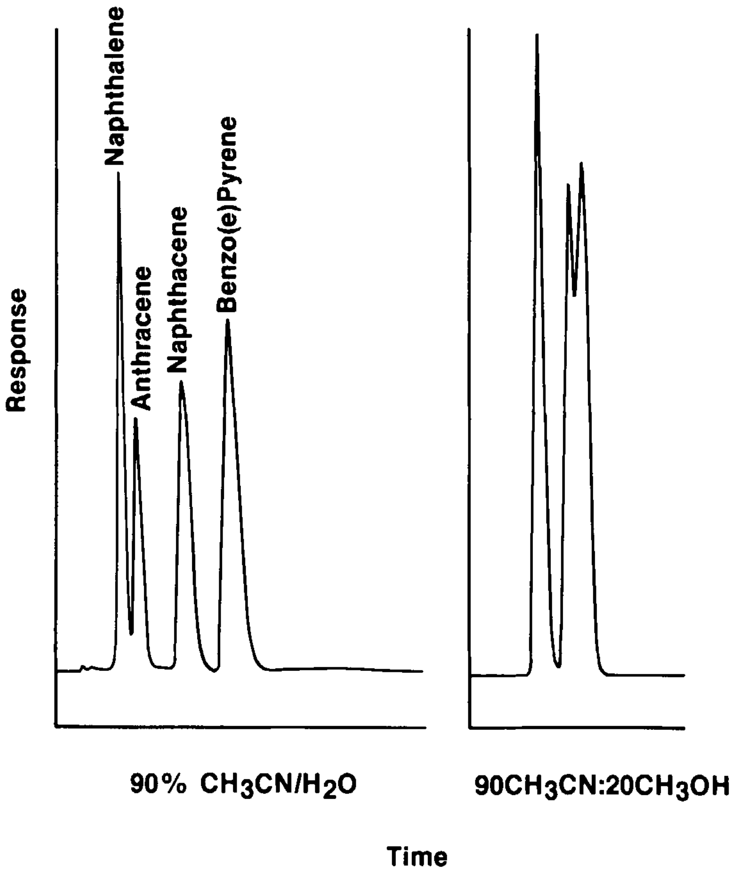

Figure 4. Comparative HPLC separation of naphthalene, anthracene, naphthacene and benzo[e]pyrene using reversed phase C_{18} column, and two different mobile phases acetonitrile/water (90/10) and acetonitrile/methanol (90/20) at a flow rate of 1.2 ml/min.

The data presented here clearly indicates that the polarity of the mobile phase is not as important as other parameters in liquid chromatography, such as solvent-solute, and solvent-solid phase interactions.

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