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ISOHYDRIC SOLVENTS IN LIQUID–SOLID COLUMN CHROMATOGRAPHY

IMPORTANCE FOR THE REPRODUCIBILITY OF CHROMATOGRAPHIC SEPARATIONS AND APPLICATION TO THE EXPERIMENTAL DETERMINATION OF MOBILE PHASE POLARITY

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SUMMARY

Isohydric solvents are defined as solvents with water contents corresponding to the same activity of the adsorbent.

The influence of the water content of the mobile phase on the activity of the adsorbent and on the reproducibility of separations was studied. A practical means of determining suitable water contents of solvents and solvent mixtures is described.

The use of isohydric solvents greatly improves the reproducibility of chromatographic separations. The water contents of isohydric solvents may constitute a scale of relative polarities. In mixtures of isohydric solvents, good linear relationships were obtained between solute capacity factors (k') and the inverse of the molar fraction of water ($1/N_{H_2O}^{isoh}$).

INTRODUCTION

The control of the purity of chemicals, particularly in the pharmaceutical industry, is of increasing importance and, for this reason, traditional analytical methods are now often completed by chromatographic techniques. High-performance liquid chromatography (HPLC) in particular has numerous advantages over other chromatographic techniques such as gas (GC) and thin-layer chromatography (TLC) for the quantitative analysis of non-volatile and heat-labile molecules.

The object of this study was to apply HPLC to the routine analysis of chemicals of pharmaceutical interest and to establish means for obtaining reliable results that could be handled by a completely automatic retrieval system. In order to attain this objective, perfect reproducibility of the chromatograms was necessary. Perfect reproducibility of chromatograms is very difficult to achieve, especially when the column is to be used for different separations involving various solvents, largely

because of the variation of the activity of the adsorbent and, according to the literature, the activity of an adsorbent depends on its water content[1,2].

The activity of an adsorbent can be standardized by different means, such as heating (as in TLC) or by adding a known amount of water to a previously dehydrated adsorbent[1,3–5]. Unfortunately, such techniques are not suitable for high-speed repetitive analysis on the same column.

Many workers have attempted to use the thermodynamic equilibrium of water between the adsorbent and the mobile phase[6–12]. For example, Schlitt and Geiss[13] recommended the use of a solvent with a water content isotonic with the activity of the adsorbent. However, practical means are not clearly defined, as indicated by the following recommendations: "adding just enough water to the solvent so that solvent and adsorbent are in thermodynamic equilibrium with respect to water" and determining "by trial and error . . . the right water content, that is, the water content which leads to identical retention volumes in the first and the second injection"[14].

An usual method is to employ partially water-saturated solvents[3,7,8,11,14,15], which does not solve the problem of water-miscible solvents, which are widely used in the analysis of pharmaceuticals either as pure solvents or as polar modifiers for non-polar solvents.

As the water content of the mobile phase is a very useful parameter in adsorption chromatography, its influence on chromatographic separations in particular was investigated.

EXPERIMENTAL

Chromatographic equipment

The chromatograph used was a Varian Aerograph Model 4200 liquid chromatograph with a stop-flow injector, a 254-nm UV detector, a refractive index detector and a temperature-controlled bath.

Analytical columns of I.D. 1/8 in. (2.15 mm; length 50 cm), 1/4 in. (4 mm; length 15 cm)and 3/8 in. (7.3 mm; length 4, 5 and 10 cm) were constructed from stainless-steel tubing and terminated by stainless-steel frits of 2-μm porosity. Injections were made using Hamilton precision micro-syringes.

Adsorbents

All of the experiments were carried out on Spherosil XOA 600, a spherical porous silica from Rhone-Poulenc having a specific surface area of about 580 m^2/g, a mean pore diameter of about 90 Å and a pore volume of about 0.9 ml/g. Experimental supports with mean particle diameters of 5, 7 and 10 μm were used. Spherosil XOA 600 (5 μm) is now commercially available from Prolabo (Paris, France; Cat. No. 28 271.10).

The separation ability of Spherosil as a packing material in HPLC has been studied previously[16,17].

Reagents and samples

Methanol, isopropanol, chloroform, carbon tetrachloride, 1,2-dichloroethane, methylene chloride, cyclohexane and 2,2,4-trimethylpentane were of spectrophotometric grade and tetrahydrofuran, dioxan and acetic acid were of analytical grade, all

from Prolabo. Ethyl acetate and acetonitrile were of spectrophotometric grade (Uvasol quality), diisopropyl oxide was of analytical grade and triethylamine was of synthesis grade, all from Merck (Darmstadt, G.F.R.).

The solutes used are indicated in the figure legends. Aromatic samples were purchased from Prolabo and pharmaceutical compounds were Rhone-Poulenc products.

Adsorption isotherms

The method described by Chovin[18] was used. A known amount of activated Spherosil was shaken with a known volume of solvent (for example, 1 g and 25 ml) until equilibrium was attained. The initial and final water contents of the solvent were measured with a coulometric apparatus [19,20] (Automate Bizot et Constant pour le dosage de l'eau, Prolabo, Cat No. 06 756-02).

Water contents of isohydric solvents

To determine and to adjust the water content of an isohydric solvent, we used a column with a large volume made from a 150-ml Whitey sample cylinder (length 20 cm, I.D. 3.4 cm) (Whitey Research Tool Co., Emeryville, Calif., U.S.A.), with a stainless-steel frit of 2-μm porosity. The influent and effluent water contents were measured with the coulometric apparatus specified above.

REPRODUCIBILITY OF CHROMATOGRAPHIC SEPARATIONS

Influence of water content of mobile phase

By injecting a solute at regular intervals, we measured the time required for equilibrating an activated column with different solvents (methanol, ethyl acetate and hexane). For example, Fig. 1 shows the variation of the retention time of chlorpromazine eluted with ethyl acetate containing 0.056% of water. Obviously, the initial activity of the adsorbent is modified and is reaching another level dependent on the water content of the solvent.

A new equilibrium of the chromatographic system is obtained after a time depending on the nature of the solvent and on its miscibility with water. With non-polar solvents such as hexane and heptane, the time to attain this equilibrium is very long[12,21], which leads to non-reproducible separations with such solvents (the water contents of different commercial bottles of solvent are never identical).

The retention times and selectivity depend on the activity of the adsorbent, determined by the water content of the solvent[7,10,22], as shown in Fig. 2. Considerable variations in retention times can be obtained with little variation of the water content of the solvent, especially at the lowest water contents (which correspond to good quality commercial solvents), but the variations of retention time with respect to the activity of the adsorbent are not the same for all of the solutes and result in modifications of selectivity[4,21].

These results indicate the necessity for controlling the water content of the solvents, but another problem is to decide the amount of water that should be added to each solvent.

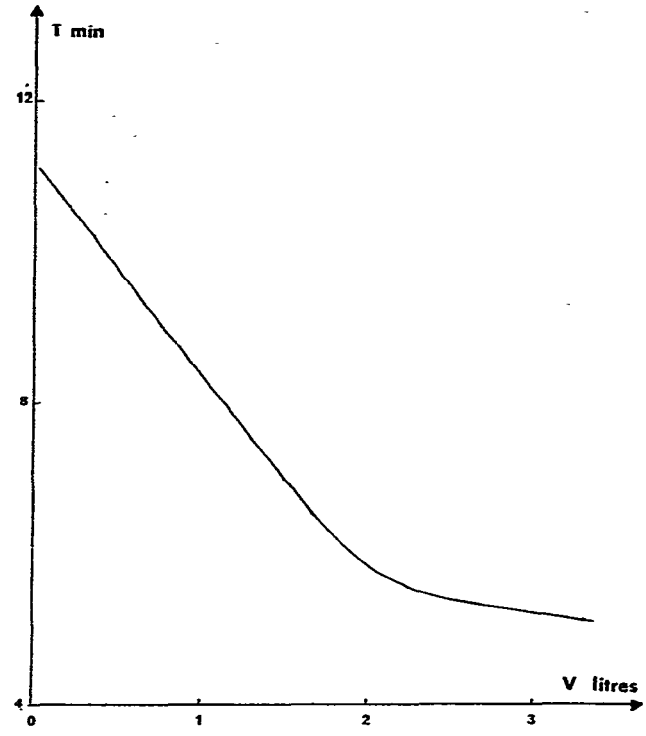

Fig. 1. Variation of retention time of chlorpromazine with volume of equilibrating solvent. Analytical column of Spherosil XOA 600, 10 μm. Initial activation by heating to 140°. Eluent: ethyl acetate containing 0.056% of water.

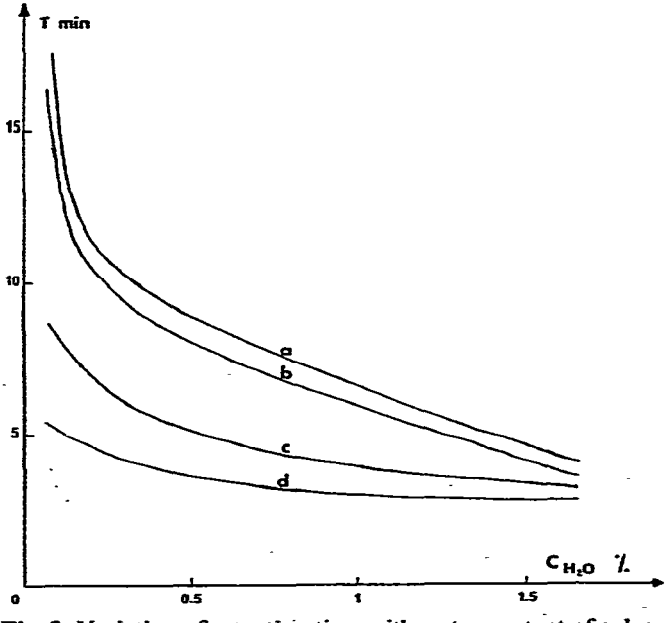

Fig. 2. Variation of retention time with water content of solvent after equilibration. Solutes. a, 5 293 R.P. (5-oxychlorpromazine); b, 5 185 R.P. [2-chloro-10-(3-methylaminopropyl)phenothiazine]; c, 5 244 R.P. (5,5-dioxychlorpromazine); d, chlorpromazine. Eluent: ethyl acetate–water. Column: 50 × 0.2 cm Spherosil XOA 600, 10 μm. Flow-rate: 3.3 ml/min.

Definition of isohydric solvents

In order to establish how much water should be added to solvents, we plotted the amount of water adsorbed by an adsorbent *versus* the water content of the solvent. Fig. 3 shows typical adsorption isotherms on silica.

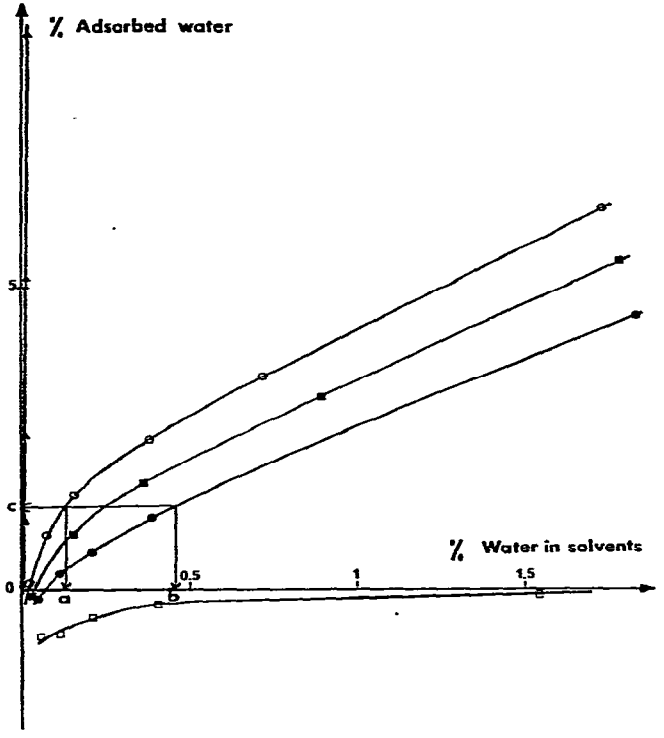

Fig. 3. Adsorption isotherms of water on silica in different solvent mixtures. ▲, 2,2,4-Trimethylpentane; ○, ethyl acetate; ■, ethyl acetate–methanol (98:2); ●, ethyl acetate–methanol (95:5); □, ethyl acetate–methanol (75:25). Adsorbent: Spherosil XOA 600, 7 μm. Initial activation by heating to 140°.

In plotting such curves, the most important problem is to obtain a reproducible initial activity of the adsorbent. The usual method, heating at *e.g.* 140°, gives imprecise results. The reproducibility is improved by heating under a stream of nitrogen but not sufficiently to allow an accurate calculation of the amount of water adsorbed.

As water is a special and ubiquitous solute (as a constituent of silica and an impurity in solvents), it is not possible to know the absolute amount of water adsorbed. The zero level is only an arbitrary value referring to a given standardization of the adsorbent (e.g., heating to 140° under a stream of nitrogen).

The curves in Fig. 3 show that the amount of water adsorbed depends on the nature of the solvent. Non-polar solvents can easily yield some water to the adsorbent, while polar solvents (such as methanol) can dehydrate it. Moreover, the adsorption isotherms in Fig. 3 show that, by adjusting two solvents of different polarities to suitable different water contents (*a* and *b*), the same amount of water will be adsorbed (*c*).

In spite of the fact that the absolute amount of water adsorbed cannot be measured, the activity of the adsorbent can be kept constant, even if unknown. In order to achieve this, *we define as isohydric different solvents which correspond to the same activation level of a given adsorbent.* In other words, isohydric solvents have water contents such that when equilibrating them one after another with an adsorbent there is no change in the activity of this adsorbent.

Experimental determination of water contents of isohydric solvents

Any adsorbent can be standardized by equilibration with a solvent of constant and known water content. This method leads to a stable and reproducible but unknown activity of the adsorbent. Hence the problem is to establish the amount of water that other solvents must contain in order to keep constant this activity of the adsorbent. With this objective, an experimental method was devised using a chromatographic column of silica.

The column is first equilibrated with a standardization solvent with a known water content, and then eluted with another solvent. If the second solvent has by chance such a water content that it is isohydric with the standardization solvent, the adsorption and desorption of water on silica will be cancelled out. In most instances, however, the second solvent is not isohydric with the standardization solvent and is either too dry or too wet. If it is too dry, some water will be desorbed from the silica and eluted from the column. Alternatively, if the water content of the solvent is too high, excess water will be adsorbed on the silica. In both instances, the adsorbent will gradually attain a different degree of activation.

However, this new equilibrium will be reached first at the top of the column and will then proceed down to the bottom. If the column has a sufficient capacity, the solvent will be eluted for some period of time in equilibrium with the standardized adsorbent of the lower part of the column and consequently, during this period, will be isohydric with the standardization solvent.

This is the method we used for determining the isohydric water content of different solvents and solvent mixtures. A silica column is first equilibrated with a standardization solvent, *e.g.*, a solvent of medium polarity such as ethyl acetate, containing a medium amount of water (0.060%). Equilibrium is reached when the percentage of water is the same at the column inlet and outlet (let a be the percentage of water in the standardization solvent). Another solvent is eluted consecutively through the column and typical curves such as those shown in Figs. 4 and 5 are obtained when the water content of the eluate is plotted against elution volume.

If the second solvent is less polar than the standardization solvent (Fig. 4), its isohydric water content (b) will be lower than that of the standardization solvent. There are three possibilities:

(1) The second solvent is isohydric with the standardization solvent: after a rapid change during the replacement of the solvents, the water content of the eluate will rapidly be stabilized at the same value (b) as that of the column inlet.

(2) The water content of the solvent (c) is lower than the isohydric value (b): this value (b) will still be obtained in the eluate for a restricted time with transfer of water from the adsorbent to the solvent. When the column capacity is exceeded, the percentage of water will decrease to reach the column inlet value (c), corresponding to a different activity of the adsorbent.

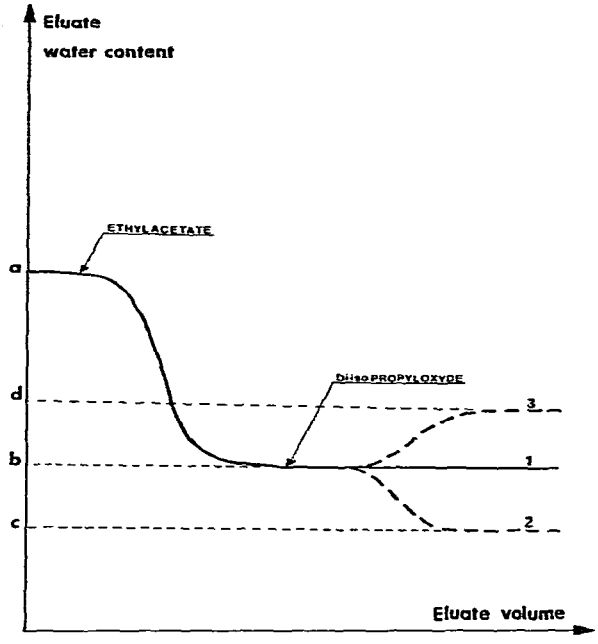

Fig. 4. Variation of water content of eluate in replacing standardization solvent with a second less polar solvent. 1 = Second solvent isohydric with the standardization solvent; 2 = too little water in the second solvent; 3 = too much water in the second solvent.

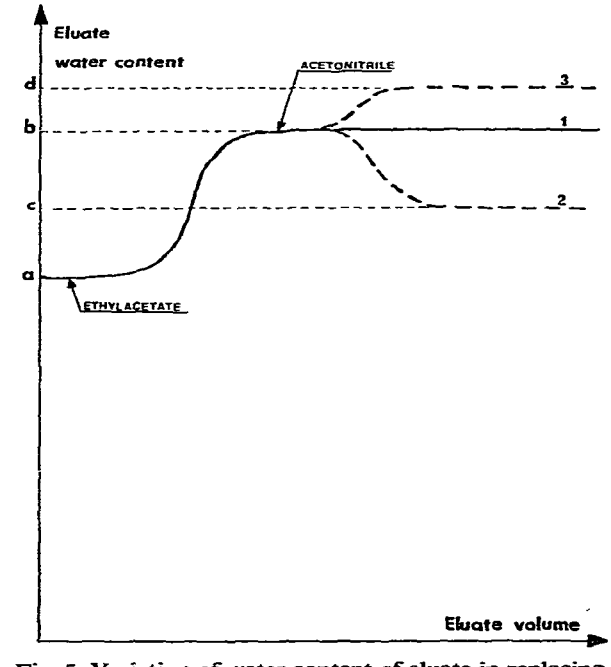

Fig. 5. Variation of water content of eluate in replacing standardization solvent with a second more polar solvent. 1 = Second solvent isohydric with the standardization solvent; 2 = too little water in the second solvent; 3 = too much water in the second solvent.

(3) The water content of the solvent (*d*) is higher than the isohydric value (*b*): the percentage of water in the eluate will reach and maintain this value (*b*) for a period, during which the column will adsorb the excess of water from the solvent. Subsequently it will increase up to a higher value (*d*).

Similarly, typical curves are obtained (Fig. 5) when the second solvent is more polar than the standardization solvent ($b > a$).

In all instances the water content of the eluate is determined during a certain period by the initial activity of the adsorbent. Consequently, it is possible to know the amount of water that the solvent must contain in order to be isohydric with the standardization solvent.

Maximum accuracy is obtained when the difference between the polarities of the solvents is small. Consequently, each isohydric solvent can be used as standardization solvent to measure the isohydric water content of a solvent of similar polarity and so on .The same method can also be applied to mixed solvents, but in some instances the curves may be complicated because of de-mixing phenomena at the beginning of the elution.

Values for solvents isohydric with ethyl acetate with a water content of 0.050% are given in Table I for Spherosil XOA 600. The values in Table II refer to ethyl acetate with a water content of 0.060% as the standardization solvent and were obtained with a column of greater capacity, giving more precise results.

TABLE I

WATER CONTENTS OF ISOHYDRIC SOLVENTS

Standardization solvent: ethyl acetate containing 0.050% of water. Adsorbent: Spherosil XOA 600.

Solvent	*Water content (%, v/v)*	*Water saturation* (%, v/v)*	*Percentage of water saturation*
Ethyl acetate–methanol (80:20)	1.1	—	—
Ethyl acetate–methanol (90:10)	0.5	—	—
Ethyl acetate–methanol (95:5)	0.2	—	—
Ethyl acetate	0.050	2.97 (25°)	1.7
Methylène chloride	0.007	0.26 (25°)	2.7
Chloroform	0.006	0.11 (23°)	5.6
1,2-Dichloroethane	0.005	0.19 (20°)	2.7
Carbon tetrachloride	0.002	0.010 (24°)	20.0
2,2,4-Trimethylpentane	~0.0008	0.004 (20°)	21.0

* Taken from J. A. Riddick and E. E. Toops, Jr., *Technique of Organic Chemistry, Volume VII, Organic Solvents —Physical Properties and Methods of Purification*, Interscience, New York, 2nd ed., 1955.

Tables I and II also include the literature values for the water solubilities of solvents of low polarity and the calculated percentages of water saturation corresponding to the water content of the isohydric solvents. It is obvious that the percentages of water saturation are not identical for all isohydric solvents, but generally decrease with increasing polarity of the solvent. It follows, therefore, that it would be impossible to maintain a constant activity of the adsorbent by adjusting the water content of each solvent to the same percentage of water saturation (*e.g.*, 50% water saturated), such solvents not being isohydric.

TABLE II

WATER CONTENTS OF ISOHYDRIC SOLVENTS

Standardization solvent: ethyl acetate containing 0.060% of water. Adsorbent: Spherosil XOA 600.

Solvent	*Water content (%, v/v)*	*Water saturation* (%, v/v)*	*Percentage of water saturation*
Methanol	5.2	—	—
Dimethylformamide	4.7	—	—
Isopropanol	0.7	—	—
Dioxan	0.14	—	—
Acetonitrile	0.22	—	—
Tetrahydrofuran	0.13	—	—
Ethyl acetate	0.060	2.97 (25°)	2.0
Diisopropyl oxide	0.008	0.63 (20°)	1.3
1,2-Dichloroethane	0.007	0.19 (20°)	3.7
Cyclohexane	~0.0004	0.008 (20°)	5.1

* See footnote to Table I.

Use of isohydric solvents

When using isohydric solvents, it is possible to employ the same column with different solvents with no change in the activity of the adsorbent, and this property has many uses.

Rapid equilibration of a column. Equilibration of a column with a non-polar solvent may take several hours, as found by many workers[12,21] and also in our experiments (Figs. 1 and 6), especially if the adsorbent has been activated by heating. This is due to the low solubility of water in such solvents. However, equilibration of the column can be achieved in a few minutes by means of a polar and more water-miscible solvent that is isohydric with the non-polar solvent, with a rapid return to this non-polar solvent, as illustrated in Fig. 6 (curve A, equilibration time 100 min; curve B, total equilibration time 15 min by passing only 20 ml of polar solvent).

Change of eluting solvent. A change of solvent may be necessary if a different chromatographic analysis is to be performed on the same column or an increase in eluting strength in the same analysis is desirable. In this instance, the change of solvent may be continuous (gradient) or sudden (step-gradient). The use of isohydric solvents permits the change of eluting solvent to be effected in the minimal time, as the activity of the column is kept constant.

Figs. 7a and 7b illustrate rapid changes of analysis on the same column. We chromatographed consecutively a mixture of three phenothiazine compounds (i, k and l) with a mobile phase (A) containing 45% of 2,2,4-trimethylpentane and 5% of methanol and a mixture of seven phenothiazine compounds (a, b, c, d, e, f, g and h) with a very polar mobile phase (B) containing 5% of 2,2,4-trimethylpentane and 45% of methanol. The results are summarized in Table III.

Fig. 7a shows the change from A to B and Fig. 7b from B to A. In both instances the second analysis was effected with reproducible retention times after equilibration for 2 min. The peaks eluted during this period are attributed to the components of the mobile phases, the proportions of which are changing.

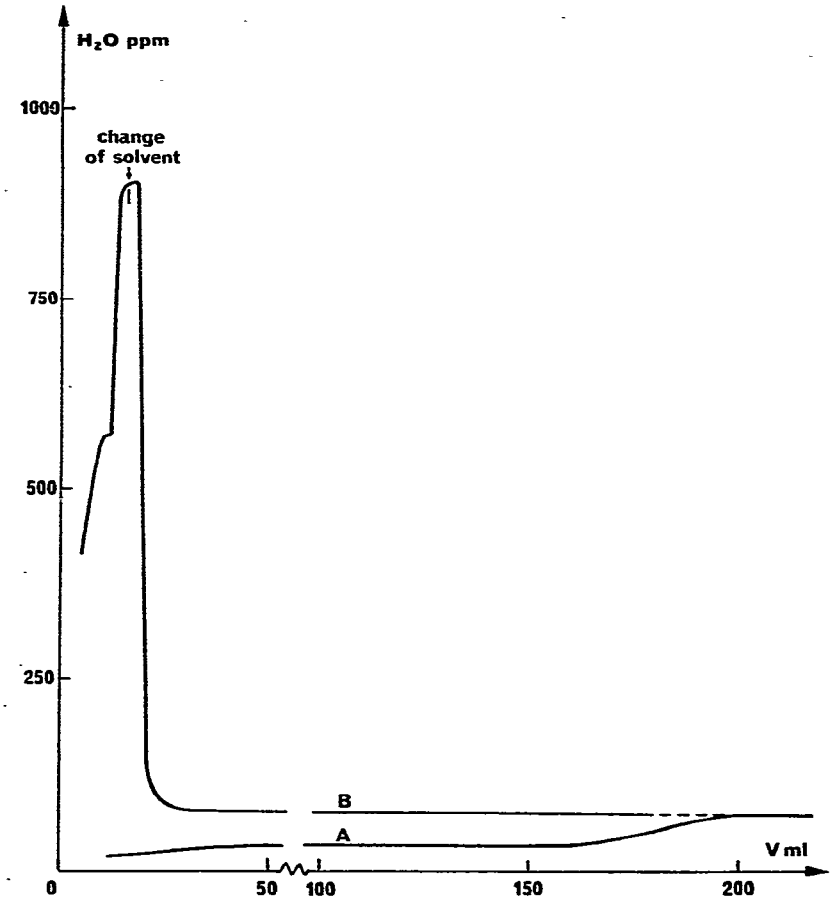

Fig. 6. Equilibration of a chromatographic column with a weak polar solvent. Variation of water content of effluent with volume of equilibrating solvent. Column: 10 × 0.73 cm Spherosil XOA 600, 5 μm. Initial activation by heating to 110° for 18 h. Flow-rate: 2 ml/min. Curve A: direct equilibration with 2,2,4-trimethylpentane–diisopropyl oxide (50:50), water content 0.0060%. Curve B: first 8-min equilibration with ethyl acetate, water content 0.0900%, isohydric to above mixture, then with the previous mixture.

As an extreme example of the usefulness of isohydric solvents, we chromatographed alternatively on the same column a mixture of six phenothiazine compounds (a, b, c, d, e and f) with a polar mobile phase (B) containing 14.5% of methanol and a mixture of aromatic compounds (g, h and i) with a non-polar mobile phase (A) (almost 100% of trimethylpentane). The chromatograms obtained are shown in Fig. 8 and the results are summarized in Table IV. In this instance, when changing from A to B, reproducible retention times were obtained after a few minutes of equilibration. On changing from B to A, this reproducibility was attained after 100 min, as shown in Fig. 9, because the polar components of mobile phase B are firmly adsorbed on the silica and are desorbed only slowly by 2,2,4-trimethylpentane.

In gradient or step-gradient elutions, the change of polarity is usually not so

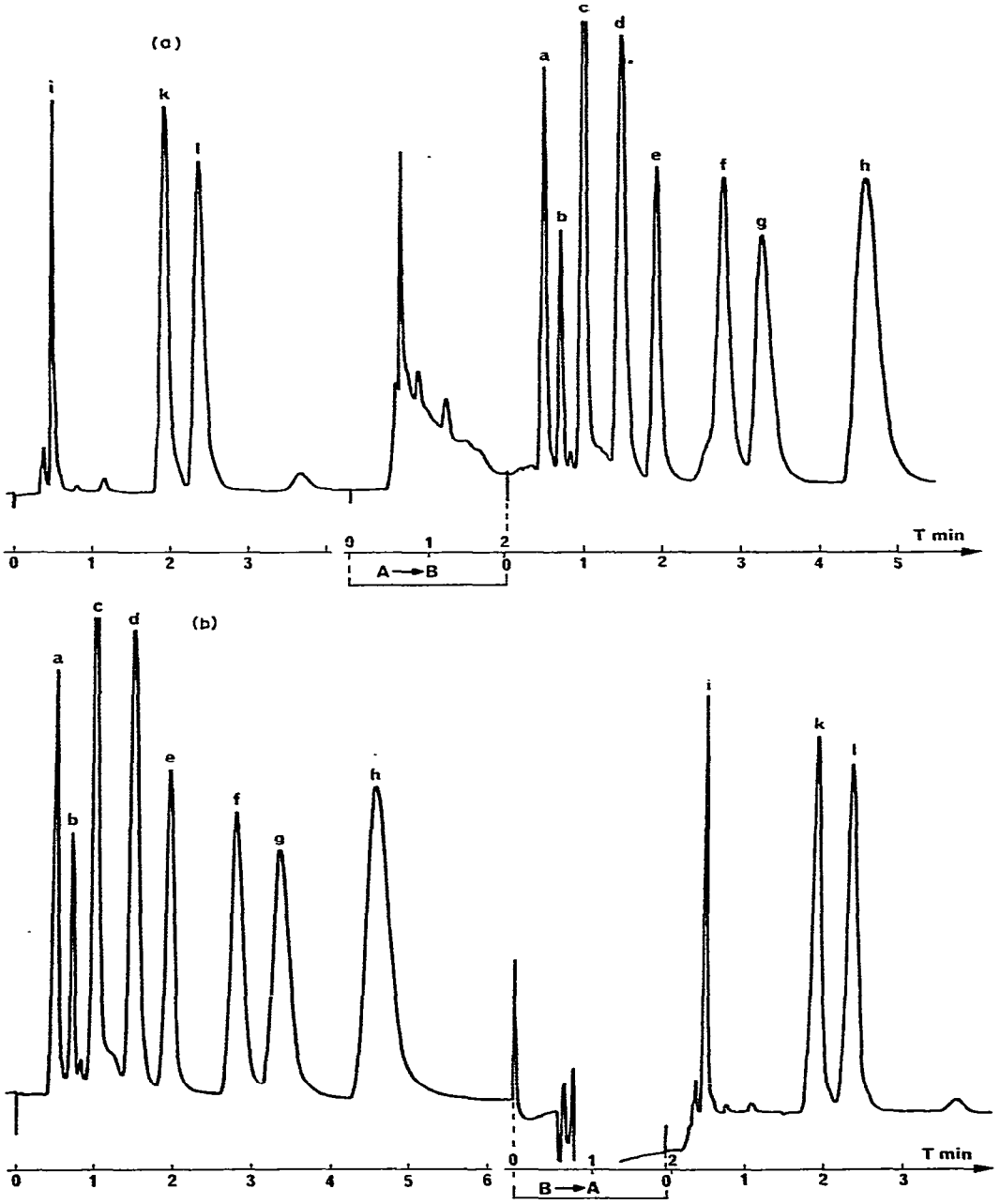

Fig. 7. Successive analyses on the same column. Column: 5 × 0.73 cm Spherosil XOA 600, 5 μm. Mobile phases: see Table III. Flow-rate: 280 ml/h. Solutes: a and i, 4 688 R.P. (3-chlorophenothiazine); b, 4 573 R.P. (chlorphenethazine); c and k, 4 560 R.P. (chlorpromazine); d, 3 276 R.P. (promazine); e, 5 244 R.P. (5,5-dioxychlorpromazine); f, 5 293 R.P. (oxychlorpromazine); g, 5 185 R.P. [2-chloro-10-(3-methylaminopropyl)phenothiazine]; h, 9 477 R.P. (N-oxychlorpromazine); i, 3 277 R.P. (promethazine). Detection: UV, 280 nm. (a) Change from analysis A to analysis B; (b) change from analysis B to analysis A.

TABLE III

SUCCESSIVE ANALYSES ON THE SAME COLUMN (FIG. 7) – NUMERICAL DATA

Mobile phase: analysis A: 2,2,4-trimethylpentane 45, diisopropyl oxide 50, methanol 5, triethylamine 0.2 water content 0.26%; analysis B: 2,2,4-trimethylpentane 5, diisopropyl oxide 50, methanol 45, triethylamin 0.2; water content 2.34%.

Equilibration time (min)		*Retention time (min)*										
		Analysis A			*Analysis B*							
A	*B*	*i*	*k*	*l*	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*
10		0.50	1.95	2.40								
15.8		0.51	1.95	2.41								
20		0.51	1.95	2.41								
	2.4				0.49	0.71	1.00	1.49	1.96	2.83	3.35	4.69
	9.2				0.50	0.72	1.01	1.51	1.98	2.85	3.41	4.69
	15.6				0.49	0.71	1.01	1.50	1.98	2.86	3.43	4.69
2.2		0.50	1.98	2.43								
7.2		0.51	1.98	2.43								
11.8		0.50	1.96	2.41								
	2.2				0.50	0.71	1.00	1.49	1.97	2.83	3.35	4.66
	9.0				0.49	0.71	1.01	1.51	1.98	2.86	3.43	4.66
	15.4				0.50	0.72	1.01	1.51	1.99	2.86	3.44	4.62
2.1		0.50	1.95	2.40								
6.4		0.50	1.96	2.41								
10.6		0.50	1.95	2.39								
	2.0				0.49	0.71	0.99	1.47	1.94	2.79	3.30	4.62
	8.7				0.50	0.71	1.00	1.48	1.95	2.81	3.36	4.60
	15.0				0.50	0.71	1.00	1.49	1.96	2.82	3.38	4.61
2.0		0.50	1.92	2.36								
6.2		0.50	1.93	2.37								
	2.0				0.48	0.70	0.98	1.45	1.92	2.77	3.27	4.58
	7.8				0.49	0.70	0.98	1.46	1.93	2.78	3.32	4.56
2.0		0.50	1.92	2.35								
6.5		0.50	1.93	2.36								
11.8		0.50	1.93	2.36								
16.2		0.50	1.93	2.37								
20.5		0.50	1.93	2.37								
Mean value, T_R:		0.50	1.95	2.39	0.49	0.71	1.00	1.49	1.96	2.82	3.37	4.64
Relative standard deviation, σ (%)		0.8	1.0	1.1	1.4	0.9	1.1	1.4	1.2	1.2	1.7	1.0

large. However, the use of isohydric solvents minimizes the recording of undesirable ghost peaks, greatly improves the reproducibility of separations and accelerates the return to the initial conditions.

Fig. 10 represents a chromatogram obtained with gradient elution using isohydric solvents and the results summarized in Table V show the reproducilibity of the retention times, which is excellent considering that these analyses were performed by different operators over a 2-month period.

Change of activity of adsorbent. In perfecting a chromatographic separation on the same column, the analyst usually takes little notice of an important factor, namely the activity of the adsorbent, because of the difficulty of defining, determining and controlling this activity. Isohydric solvents are a practical means not only of keeping

TABLE IV

SUCCESSIVE ANALYSES ON THE SAME COLUMN (FIG. 8) – NUMERICAL DATA

Mobile phase: analysis A: 2,2,4-trimethylpentane 100, triethylamine 0.01; water content 0.0010%; analysis B: 2,2,4-trimethylpentane 35.5, diisopropyl oxide 50, methanol 14.5, triethylamine 0.2; water content 0.73%.

Equilibration time (min)		*Retention time (min)*								
		Analysis A			*Analysis B*					
A	*B*	*g*	*h*	*i*	*a*	*b*	*c*	*d*	*e*	*f*
8		0.97	1.07	1.15						
16		1.01	1.15	1.30						
25		1.06	1.25	1.50						
30.5		1.07	1.27	1.55						
37.4		1.09	1.31	1.62						
45.6		1.08	1.31	1.65						
69.8		1.20	1.48	1.92						
78.4		1.20	1.49	1.96						
84.4		1.21	1.51	1.99						
90.5		1.21	1.52	2.02						
98.6		1.21	1.53	2.05						
106		1.21	1.54	2.08						
112.6		1.22	1.55	2.10						
120		1.22	1.56	2.12						
124		1.20	1.53	2.07						
130		1.21	1.55	2.10						
	7.5				0.89	1.40	1.87	2.88	3.75	5.47
	16.8				0.89	1.39	1.89	2.90	3.76	5.48
	25.5				0.90	1.40	1.89	2.91	3.78	5.50
	33.6				0.89	1.39	1.88	2.89	3.76	5.46
10.6		1.05	1.17	1.24						
25		1.14	1.33	1.57						
35		1.17	1.39	1.70						
45		1.19	1.44	1.81						
56.3		1.20	1.47	1.88						
65		1.22	1.51	1.95						
74.2		1.22	1.52	2.00						
85		1.24	1.55	2.06						
95		1.26	1.57	2.10						
104.1		1.23	1.56	2.11						

constant this activity, but also of varying it in known and reproducible ways. This can be done by changing from one series of isohydric solvents to another, with different water contents for the same solvents.

Preliminary results seem to indicate that a new series of isohydric solvents can easily be inferred from an existing series related to the same adsorbent, by simply multiplying each water content by the same factor, provided that the water content is not too close to the saturation value.

As a practical example, the chromatograms in Fig. 11 show the same chromatographic separation using as the mobile phase the same solvent mixture with different water contents. Each of these analyses corresponds to a different activity of the adsorbent and a different series of isohydric solvents.

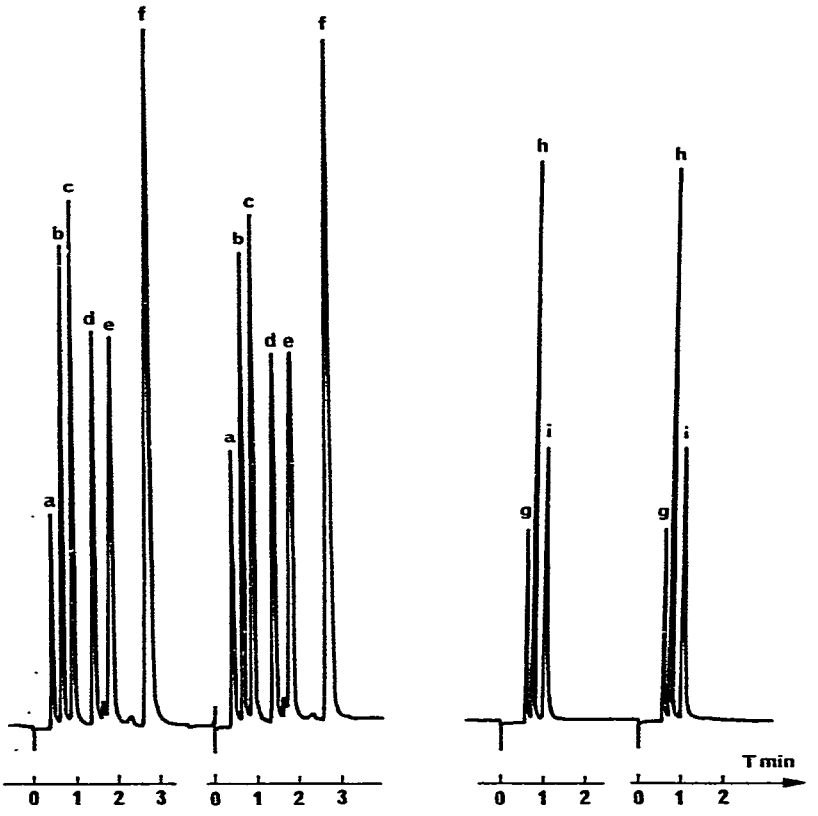

Fig. 8. Successive analyses on the same column. Column: 10 × 0.73 cm Spherosil XOA 600, 5 μm. Mobile phases: see Table IV. Flow-rate: 240 ml/h. Solutes: a, unknown; b, 7 044 R.P. (levomepromazine); c, 4 560 R.P. (chlorpromazine); d, 8 599 R.P. (dimethothiazine); e, 8 909 R.P. (propericiazine); f, 6 847 R.P. (oxomemazine); g, toluene; h, naphthalene; i, anthracene. Detection: UV, 280 nm.

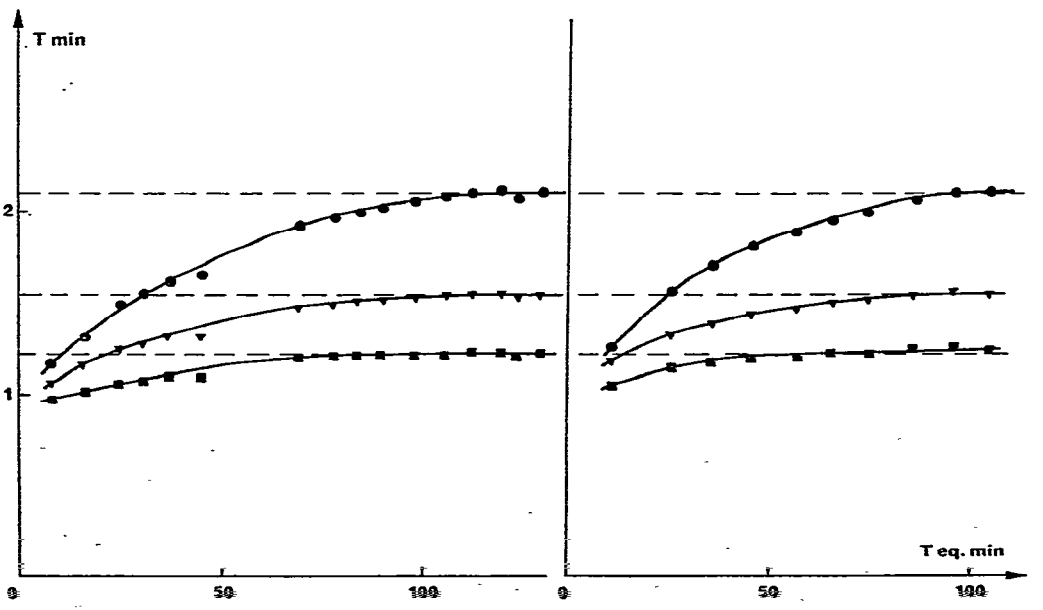

Fig. 9. Variation of retention times of toluene, naphthalene and anthracene with equilibration time, in chromatograms of Fig. 8 (numerical data in Table IV).

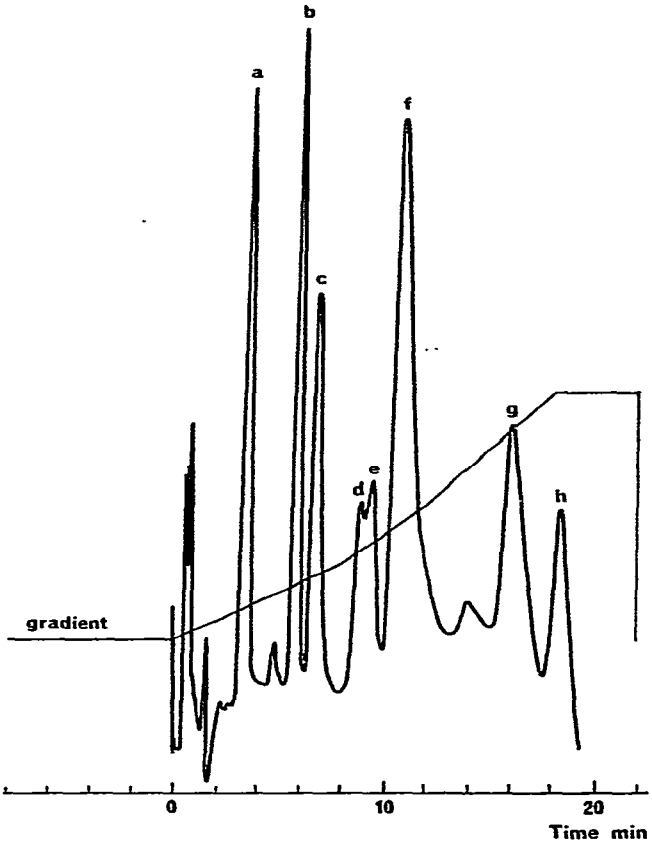

Fig. 10. Chromatographic separation with polarity gradient. Column: 4 × 0.73 cm Spherosil XOA 600, 5 μm. Non-polar solvent A: 2,2,4-trimethylpentane–diisopropyl oxide–ethyl acetate–acetonitrile–acetic acid (50:37.5:7.5:5:0.2), water content 0.037%. Polar solvent B: methanol–diisopropyl oxide–ethylacetate–acetonitrile–acetic acid (50:37.5:7.5:5:0.2), water content 5.2%. Adsorbent activation state, $\alpha_a = 2$. Mobile phases: see Table V. Flow-rate: 2 ml/min. Solutes: a, fludrocortisone 21-acetate; b, fludrocortisone; c, 9α-fluoroprednisolone; d, 16α-hydroxyfludrocortisone; e, fludrocortisone 21-acetamide; f, triamcinolone; g, 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-D-homoandrost-4-ene-3,17-dione; h, 9α-fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-D-homoandrost-1,4-diene-3,17-dione. Detection: UV, 254 nm.

Discussion

The isohydric water contents in Tables I and II are strictly valid only for the adsorbent used (Spherosil XOA 600, 580 m^2/g). However, if there is only a slight difference in the specific surface area, the isohydric water contents will be approximately transposable. The water content of a solvent mixture can be calculated from the water contents of the individual solvents and we found that mixing pure isohydric solvents results approximately in an isohydric blend. From the values in Tables I and II, it is therefore possible to know the amount of water that a complex mobile phase must contain in order to be isohydric.

TABLE V

REPRODUCIBILITY OF GRADIENT SEPARATION

See Fig. 10.

Multinear gradient

Step	*Duration (min)*	*Initial B (%)*	*Variation of B (%/min)*	*Final B (%)*
Equilibration	8	8	0	8
1	8	8	+0.5	12
2	4	12	+0.8	15.2
3	6	15.2	+1.0	21.2
4	4	21.2	0	21.2

*Reproducibility of retention time**

Parameter	*Solute*							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
Number of measurements	20	20	20	12	5	20	20	6
Mean value of retention time (min)	3.40	5.71	6.71	8.72	9.38	10.35	16.01	18.33
Standard deviation	0.08	0.12	0.15	0.19	0.22	0.34	0.35	0.19
Relative standard deviation (%)	2.4	2.1	2.2	2.2	2.4	3.3	2.2	1.0

* Analysis performed by two different analysts over 2 months.

MOBILE PHASE POLARITY

New relative polarity scale

Numerous classifications of solvents with respect to polarity have been proposed as a function of various parameters such as dielectric constant, dipole moment, elution strength, solubility coefficient and polarity index. The classification of isohydric solvents according to their water contents leads to the same order as most of the other proposed polarity scales, as is obvious from Tables I and II. In Fig. 12, for example, is plotted the relationship between Snyder's elution strength (ε^0) and the logarithm of the water content in a series of isohydric solvents for which the numerical data are given in Table VI.

Consequently, we tried to use the water content of isohydric solvents as a relative polarity scale. The existence of a relationship between polarity and water content of the mobile phase has been proposed before[22,23]. Such a relative polarity scale may have some advantages compared with other proposals. Firstly, each polarity value can be measured directly in a simple and rapid way; secondly, the measurement can be performed even with a complex mobile phase; thirdly, the polarity values refer to a constant activity of the adsorbent, as recommended by Snyder[24]; and finally, the polarity scale established in this way is completely valid for the adsorbent used by the operator.

In order to check the validity of this new polarity scale, we considered existing relationships between the capacity factors (k') and water contents of isohydric solvents.

Pure solvents. With pure solvents, no simple and precise relationship between

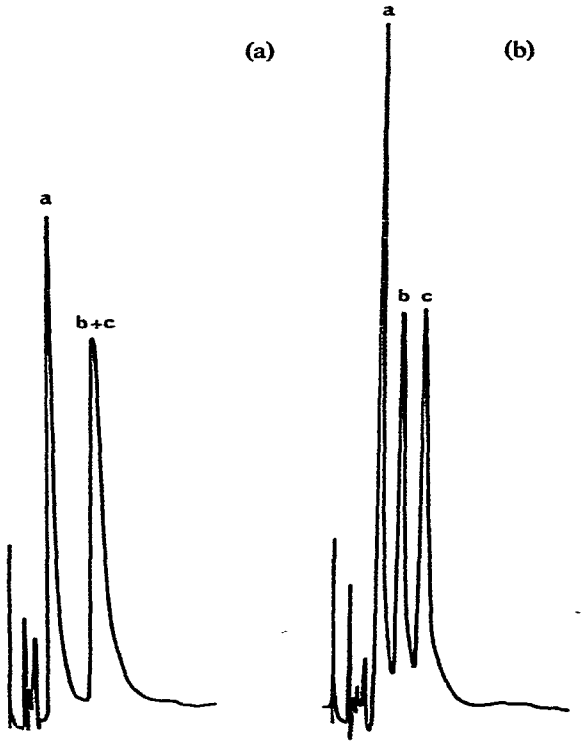

Fig. 11. Chromatographic separation in two different activation states. Column: 5 × 0.73 cm Spherosil XOA 600, 5 μm. Non-polar solvent A: 2,2,4-trimethylpentane–diisopropyl oxide–triethylamine (50:50:0.2). Polar solvent B: methanol–diisopropyl oxide–triethylamine (50:50:0.2). Mobile phase: A–B (80:20). Flow-rate: 3 ml/min. Solutes: a, benzocaine; b, tetracaine; c, procaine). (a) Water contents of solvents: A, 0.004%; B, 1.95% (adsorbent activation state, $\alpha_a = 0.75$). (b) Water contents of solvents: A, 0.007%; B, 3.5% ($\alpha_a = 1.35$).

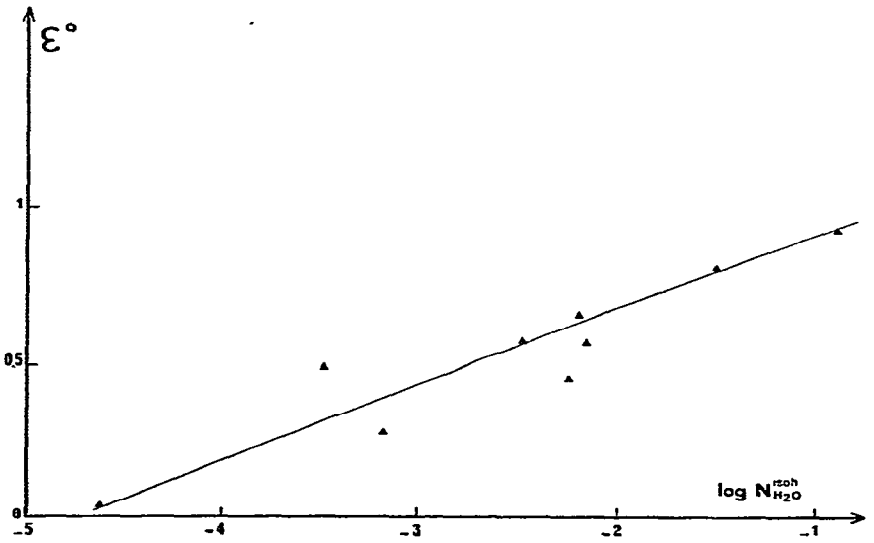

Fig. 12. Correlation between the elution strength parameter of Snyder (ε°) and log $N^{isoh}_{H_2O}$.

TABLE VI

POLARITY SCALE FOR ISOHYDRIC SOLVENTS

Standardization solvent: ethyl acetate containing 0.060% of water. Adsorbent: Spherosil XOA 600; activation state, $\alpha_a = 1$.

Solvent	ε°	*Water content (%)*	$N_{H_2O}^{isoh}$	$1/N_{H_2O}^{isoh}$
Dimethylformamide		4.7	$1.7 \cdot 10^{-1}$	5.7
Methanol	0.95	5.2	$1.1 \cdot 10^{-1}$	9.1
Isopropanol	0.82	0.7	$2.9 \cdot 10^{-2}$	34
Dioxan	0.56	0.14	$6.6 \cdot 10^{-3}$	152
Acetonitrile	0.65	0.22	$6.4 \cdot 10^{-3}$	156
Tetrahydrofuran	0.45	0.13	$5.9 \cdot 10^{-3}$	170
Ethyl acetate	0.58	0.060	$3.3 \cdot 10^{-3}$	310
Diisopropyl oxide	0.28	0.0080	$6.3 \cdot 10^{-4}$	$1.6 \cdot 10^{3}$
1,2-Dichloroethane	0.49	0.0070	$3.1 \cdot 10^{-4}$	$3.2 \cdot 10^{3}$
Cyclohexane	0.04	~0.0004	$2.4 \cdot 10^{-5}$	$42 \cdot 10^{3}$
2,2,4-Trimethylpentane	0.01	~0.0002	$1.8 \cdot 10^{-5}$	$54 \cdot 10^{3}$

k' and water content was found. On plotting the logarithm of the capacity factor (k') *versus* the logarithm of the molar fraction of water in isohydric solvents ($N_{H_2O}^{isoh}$), no linear curves but broken lines were obtained if the polarity is modified discontinuously on passing from one pure solvent to another. Similar results were obtained with other proposed expressions of polarity, which can be attributed to solute–solvent interactions, described by Snyder as "solvent secondary effects"[25].

Mixtures of solvents. In order to maintain continuous variations of the "solvent secondary effects", we prepared two mixtures of isohydric solvents leading to a complex solvent (A) with a weak elution strength and a very polar solvent (B); their compositions are given in Fig. 13.

We then plotted the capacity factors for various solutes *versus* the molar fraction of water in various mixtures of A and B used as mobile phases and obtained the hyperbolic curves shown in Fig. 13. The hyperbolic character of the curves $k' = f(N_{H_2O}^{isoh})$ is corroborated by plotting k' *versus* $1/N_{H_2O}^{isoh}$ (Fig. 14). Fairly good linear relationships are obtained, allowing a good prediction of the capacity factors with the proportions of polar and non-polar solvents used; the polarity is then expressed by $1/N_{H_2O}^{isoh}$.

Such complex mixtures, although very interesting for certain types of chromatography, are not always necessary. If simply 2,2,4-trimethylpentane is used as the non-polar solvent A and diisopropyl oxide as the polar solvent B, good linear relationships are also obtained, as shown in Fig. 17.

Relationship with activity of adsorbent

Similar results were obtained with different activities of the adsorbent. This activity is modified by using different series of isohydric solvents (by modification of the water content of the standardization solvent), as explained above.

We arbitrarily expressed the state of activation of the chromatographic system by the parameter α_a. This parameter, valid for a given adsorbent only, is defined as unity for the water content of the isohydric solvents in Table V (ethyl acetate contain-

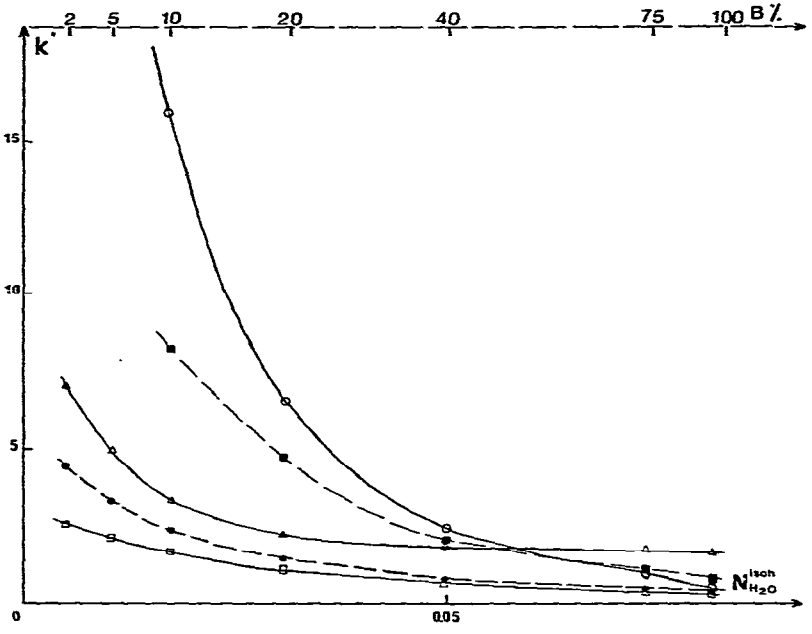

Fig. 13. Relationship between solute capacity factors (k') and water molar fractions ($N_{H_2O}^{isoh}$) of isohydric solvent mixtures. Column: 15 × 0.4 cm Spherosil XOA 600, 7 μm. Non-polar solvent A: 2,2,4-trimethylpentane–diisopropyl oxide–ethyl acetate–acetonitrile–triethylamine (50:37.5:7.5:5:0.2), water content 0.019%. Polar solvent B: methanol–diisopropyl oxide–ethyl acetate–acetonitrile–triethylamine (50:37.5:7.5:5:0.2), water content 2.6%. Flow-rate: 1 ml/min. Solutes: □, phenol; ● barbital; △, chlorpromazine; ■, *p*-aminophenol; ○, spiramycin II. The percentage of solvent B in the mobile phase is indicated on the axis at the top of the figure.

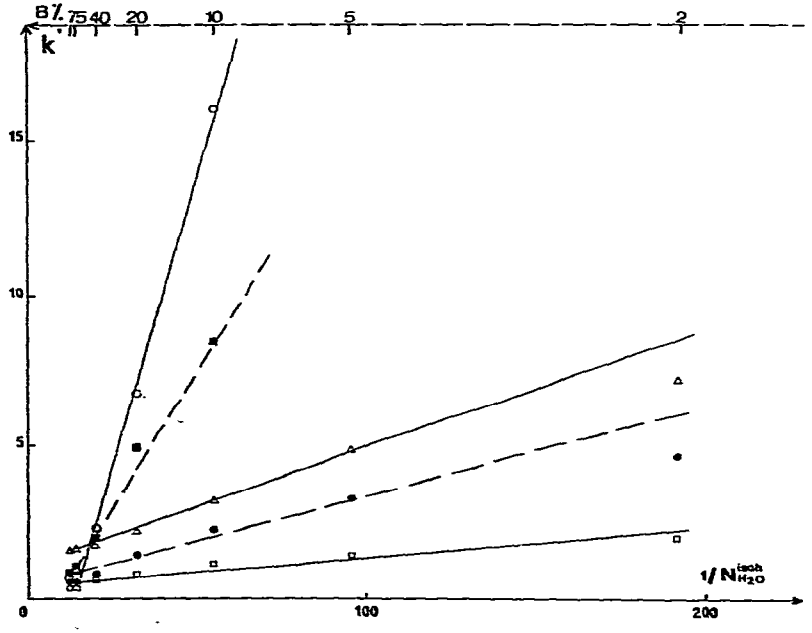

Fig. 14. Relationship between solute capacity factors (k') and the inverse of the water molar fractions ($1/N_{H_2O}^{isoh}$) of isohydric solvent mixtures. Conditions and solutes as in Fig. 13.

ing 0.060% of water as the standardization solvent). When the water content of the standardization solvent is doubled, we can say that the corresponding isohydric solvents will give an activation state $\alpha_a = 2$. Figs. 15, 16 and 17 show linear relationships between k' and $1/N_{H_2O}^{isoh}$ for $\alpha_a = 1$, 0.385 and 1.8, respectively.

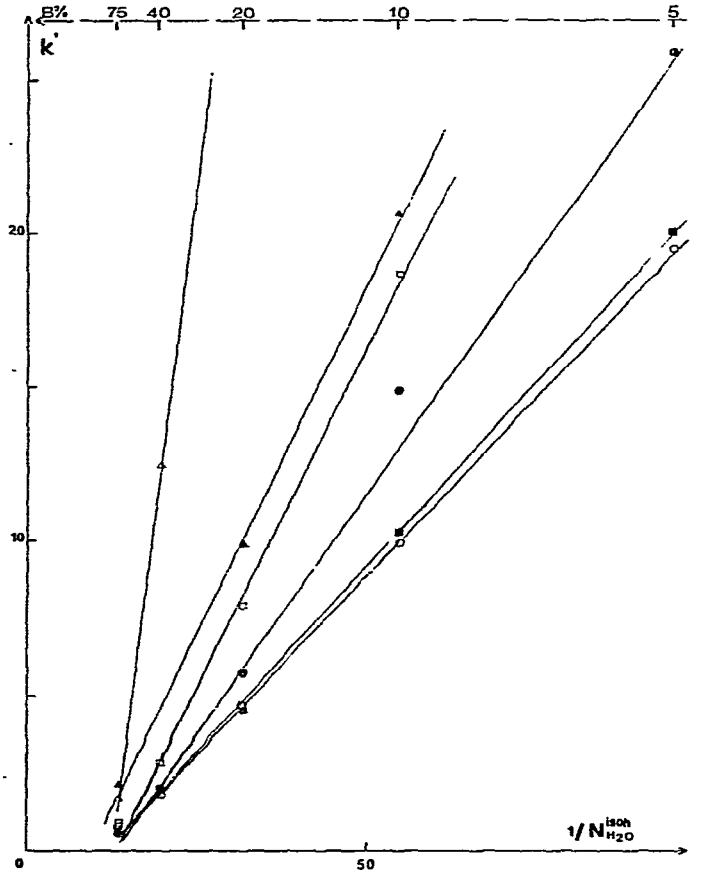

Fig. 15. Relationship between solute capacity factors (k') and the inverse of the water molar fractions ($1/N_{H_2O}^{isoh}$) of isohydric solvent mixtures. Chromatographic conditions as in Fig. 13; adsorbent activation state, $\alpha_a = 1$. Solutes: ○, spiramycin III; ■, paracetamol; ●, triamcinolone; □, spiramycin I; ▲, 27 267 R.P. {5-[(4-methyl-1-piperazinyl)carbonyloxy]-6-(5-chloro-2-pyridyl)-7-oxo-5,6-dihydropyrrolo-[3,4-*b*]-pyrazine}; △, ketoprofen.

These linear relationships are verified when the polarities of the mobile phase and of the solutes are varied in very broad ranges (Figs. 15–17). The slopes of the curves depend on the polarity of the solute and the solute–solvent interactions. For the same polarity interval, the slopes can be different when using different isohydric solvent mixtures of same polarity (expressed by $1/N_{H_2O}^{isoh}$). The slopes depend on the activity of the adsorbent and generally increase when α_a is decreased (the slopes are increased, not k').

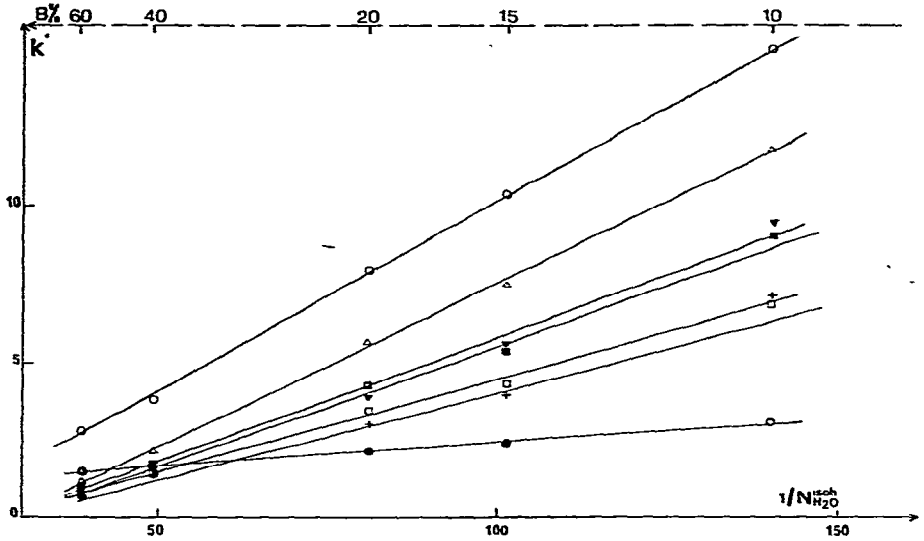

Fig. 16. Relationship between solute capacity factors (k') and the inverse of the water molar fractions ($1/N^{Isoh}_{H_2O}$) of isohydric solvent mixtures. Column: 15 × 0.4 cm Spherosil XOA 600, 7 μm. Non-polar solvent A: 2,2,4-trimethylpentane–diisopropyl oxide–ethyl acetate–acetonitrile–triethylamine (50:37.5:7.5:5:0.2), water content 0.0072%. Polar solvent B: methanol–diisopropyl oxide–ethyl acetate–acetonitrile–triethylamine (50:37.5:7.5:5:0.2), water content 1%. Adsorbent activation state, α_a = 0.385. Flow-rate: 1 ml/min. Solutes: ●, chlorpromazine; +, paracetamol; □, spiramycin III; ▲, triamcinolone; ■, spiramycin II; △, spiramycin I; ○, 27 267 R.P. (see legend of Fig. 9).

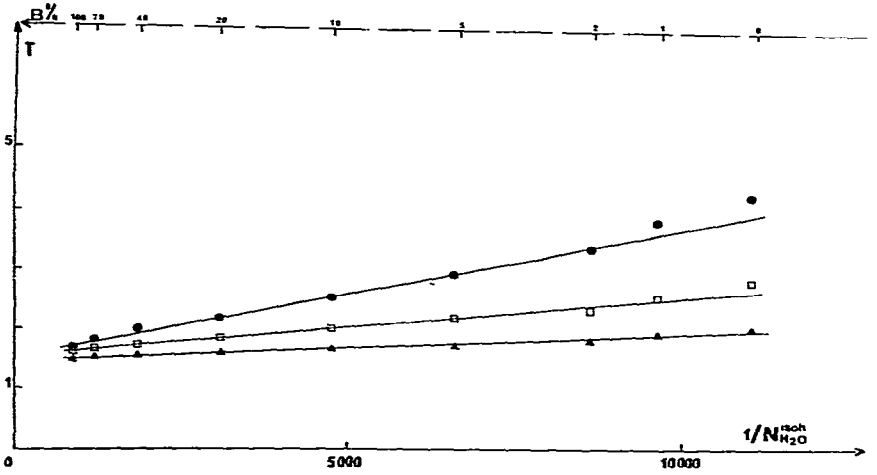

Fig. 17. Relationship between solute retention times (T) and the inverse of the water molar fractions ($1/N^{Isoh}_{H_2O}$) of isohydric solvent mixtures. Column: 4 × 0.73 cm Spherosil XOA 600, 5 μm. Non-polar solvent A: 2,2,4-trimethylpentane–triethylamine (99.99:0.01), water content 0.0010%. Polar solvent B: diisopropyl oxide–triethylamine (99.99:0.01), water content 0.0144%. Adsorbent activation state, α_a = 1.8. Flow-rate: 1 ml/min. Solutes: ▲, benzene; □, naphthalene; ●, anthracene.

Discussion

In plotting $k' = f(1/N_{H_2O}^{isoh})$ curves, some deviations from linearity may occur under certain circumstances: when k' is very low (below 1.5), when the correct water content is difficult to control (in the very low polarity range) and when the solvent is almost saturated with water. In such instances, it might be better to modify the chromatographic conditions (*e.g.*, activity of the adsorbent) if one wishes to make use of these relationships or to employ another chromatographic process (partition, reversed phase, etc.).

The use of the molar fraction of the water content of a solvent in equilibrium with an adsorbent may be compared with other proposals in the literature: some workers use the proportion of the most polar solvent in a mixture to predict the capacity factors of the solutes[26–34]. Jandera and Churáček[29] simplified Snyder's equation for a mixture and showed that the following equation can be derived:

$$k' = k'_0 \cdot c^n$$

where k'_0 is the capacity factor of the solute in the most polar solvent, n is a constant depending on the nature of the solvent and of the solute and c is the molar fraction of the polar solvent in the mobile phase.

It should be pointed out that the water content of an isohydric solvent mixture is proportional to the percentage and the water content of each solvent and, in particular, depends on the most polar solvent.

In Jandera and Churáček's relationship, we can replace c with $N_{H_2O}^{isoh}$ and derive the following equation:

$$\log k' = \log k'_0 - n \log N_{H_2O}^{isoh}$$

showing a great similarity with our experimental results. Our results show that for isohydric solvents, $n = 1$.

CONCLUSION

In all instances, the use of isohydric solvents maintaining a constant activity of the adsorbent permits very reproducible separations to be obtained, even if the column has to be eluted with different solvents (gradient, step-gradient or change of chromatographic analysis). Moreover, the use of isohydric solvents may considerably reduce the length of the equilibration step after any change of solvent in the column. The use of different series of isohydric solvents enables the activity of the adsorbent in any chromatographic study to be varied.

Finally, the linear relationships between the capacity factor and the inverse of the molar fraction of water, $1/N_{H_2O}^{isoh}$, in isohydric solvent mixtures permits one to use these values as a relative polarity scale and greatly facilitates the definition of suitable chromatographic systems for complex analytical problems.

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REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 128.
2 H. Halpaap, *J. Chromatogr.*, 78 (1973) 63.
3 R. E. Majors, *Basic Liquid Chromatography*, Varian Aerograph, Palo Alto, 1971, Ch. 5, p. 26.
4 G. Cavina, G. Moretti and A. Cantafora, *J. Chromatogr.*, 80 (1973) 89.
5 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974, p. 251.
6 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 145.
7 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974, pp. 265 and 467.
8 *Operational Information for Micropak Columns*, Varian Aerograph, Palo Alto, 1972.
9 J. J. de Stefano and H. C. Beachell, *J. Chromatogr. Sci.*, 10 (1972) 654.
10 L. R. Snyder, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, p. 128.
11 L. R. Snyder and D. L. Saunders, *J. Chromatogr. Sci.*, 7 (1969) 195.
12 C. Gonnet and J. L. Rocca, *J. Chromatogr.*, 109 (1975) 297.
13 H. Schlitt and F. Geiss, *J. Chromatogr.*, 67 (1972) 261.
14 L. R. Snyder, in J. J. Kirkland (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, p. 223.
15 R. E. Majors, *Anal. Chem.*, 44 (1972) 1722.
16 J. Vermont, M. Deleuil, A. J. de Vries and C. L. Guillemin, *Anal. Chem.*, 47 (1975) 1329.
17 M. Caude, Le Xuan Phan, B. Terlain and J.-P. Thomas, *J. Chromatogr. Sci.*, 13 (1975) 390.
18 P. Chovin, in E. Lederer (Editor), *Monographies de Chimie Organique, Tome II, Chromatographie en Chimie Organique et Biologique, Vol. I, Généralités —Applications en Chimie Organique*, Masson, Paris, 1959, p. 23.
19 J. Bizot, *Bull. Soc. Chim. Fr.*, (1967) 151.
20 J. Bizot, *Communication at the "Journées d'Information sur les Méthodes d'Analyse Physico-chimiques", Commission Interministérielle des Appareils Electriques et Electroniques de Mesure (C.I.A.M.E.), Paris, June 18–20, 1975.*
21 H. Engelhardt, J. Asshauer, U. Neue and N. Weigand, *Anal. Chem.*, 46 (1974) 336.
22 L. V. Berry and H. Engelhardt, *J. Chromatogr.*, 95 (1974) 27.
23 C. Hesse and W. Hoeverman, *Chromatographia*, 6 (1973) 345.
24 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 189.
25 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, p. 216.
26 E. Soczewinski, *Anal. Chem.*, 41 (1969) 179.
27 E. Soczewinski and W. Golkiewics, *Chromatographia*, 4 (1971) 501.
28 E. Soczewinski and W. Golkiewics, *Chromatographia*, 6 (1973) 269.
29 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
30 J. Ościk and G. Chojnacka, *J. Chromatogr.*, 93 (1974) 167.
31 J. K. Różyło, *J. Chromatogr.*, 93 (1974) 177.
32 B. Uchytil, *J. Chromatogr.*, 93 (1974) 447.
33 E. Soczewiński and G. Matysik, *J. Chromatogr.*, 111 (1975) 7.
34 M. Ciszewska and E. Soczewiński, *J. Chromatogr.*, 111 (1975) 21.