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## FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

### VIII<sup>a</sup>. CHARGE-TRANSFER *VERSUS* STANDARD STATIONARY PHASES IN NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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#### SUMMARY

The separation mechanism and selectivity of the separation of a group of the 4,4'-substituted *E-s-cis* and *Z-s-cis* isomeric chalcones on charge-transfer stationary phases were compared with those on diol, CN, NO<sub>2</sub>, etc., stationary phases. Correspondence factor analysis and the hierarchical ascending classification method were used. The results obtained provide evidence of the efficiency of charge-transfer stationary phases in the separation of configurational isomers.

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#### INTRODUCTION

In recent years the effects of charge-transfer interactions have played an important role in high-performance liquid chromatographic (HPLC) separations. Chemically bonded charge-transfer phases are often applied to separate coal liquids and other aromatic mixtures. In order to broaden the range of applicability of charge-transfer stationary phase and to define their specific properties more closely, we have compared the retention mechanisms of a wide range of electron-rich

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<sup>a</sup> For Part VII, see *J. Chromatogr.*, 395 (1987) 183.

compounds on charge-transfer stationary phases and on standard chemically bonded stationary phases of the diol, CN, NO<sub>2</sub>, etc., type, which are commonly used in normal-phase HPLC.

## EXPERIMENTAL

### Samples

A series of 4- and 4'-substituted chalcones (*E-s-cis* and *Z-s-cis*),  $\text{XC}_6\text{H}_4\text{CH}=\text{CHCOC}_6\text{H}_4\text{Y}$ , were chromatographed using eleven normal-phase HPLC systems; the substituents X and Y are given in Table II. *E-s-cis* chalcones were synthesized<sup>1</sup> and the corresponding *Z-s-cis* isomers formed spontaneously in dichloromethane solution. Throughout this paper *E-s-cis* chalcones are referred to as X-Y and *Z-s-cis* chalcones as X-Y\*.

### Chromatographic systems

The mobile phase was HPLC-grade heptane-tetrahydrofuran (97:3, v/v), purchased from Merck (Darmstadt, F.R.G.). The stationary phases are listed in Table I.

### Instruments

The HPLC equipment consisted of a Bruker LC-31 pump, a Rheodyne Model 7125 injection valve, a Schoeffel Model SF 770 spectrophotometer as detector, set at 280 nm, and a Shimadzu C-R1B recorder.

### Chromatographic procedure

Prior to measurements, the columns were washed with the mobile phase until a constant value was obtained for the retention of chalcones. Sample solutions (2 mg

TABLE I  
COLUMN PACKINGS

Stationary phases 7-11 were synthesized in the Laboratoire de Chimie Organique du Silicium et de l'Etain of the Université de Bordeaux, France, and were kindly donated by Dr. G. Felix.

No.	Column packing <sup>a</sup>	Dimensions (mm × mm)	Supplier	Ref.	Abbreviation
1	Zorbax NH <sub>2</sub>	250 × 4.6	DuPont		NH <sub>2</sub>
2	LiChrospher 100 diol	250 × 4.6	Merck		diol
3	MicroPak CN	300 × 4	Varian		CN
4	Zorbax ODS	150 × 4.6	DuPont		ODS
5	Zorbax C <sub>8</sub>	90 × 4	DuPont		C <sub>8</sub>
6	RSIL NO <sub>2</sub>	250 × 4.6	Alltech		NO <sub>2</sub>
7	Spherisorb AP	90 × 4		2	AP
8	Spherisorb DNAP	90 × 4		3	DNAP
9	Spherisorb DNB	90 × 4		4	DNB
10	Spherisorb TCP	90 × 4		5	TCP
11	Spherisorb TB	90 × 4		6	TB

<sup>a</sup> AP = *n*-propyl picryl ether; DNAP = 3-(2,4-dinitroanilino)propyl; DNB = 3,5-dinitrobenzamidopropyl; TCP = tetrachlorophthalimidopropyl; TB = caffeine.

per 25 ml) were prepared in dichloromethane. All data points were collected as averages from two or three reproducible separations. The dead time,  $t_0$ , of the column was determined using benzene and cyclohexane markers. The capacity factor,  $k'$ , was calculated from the retention time of the solute,  $t_R$ , according to the equation  $k' = (t_R - t_0)/t_0$ .

#### Data processing

A set of "abstract" factors affecting the selectivity in the normal-phase HPLC systems were extracted by correspondence factor analysis (CFA)<sup>7,8</sup>, which is a method for the analysis of a table of positive data arranged as matrix elements  $k_{ij}$  in  $n$  rows ( $i = 1, 2, \dots, n$ ) and  $p$  columns ( $j = 1, 2, \dots, p$ ). CFA can be used to analyse how a studied data matrix  $K$  differs from a hypothetical matrix  $K^*$ , its elements defined by

$$k_{ij}^* = (k_{i.} \cdot k_{.j})/k$$

where

$$k_{i.} = \sum_{j=1}^p k_{ij}; \quad k_{.j} = \sum_{i=1}^n k_{ij}; \quad k = \sum_{i=1}^n \sum_{j=1}^p k_{ij}$$

The characteristic property of a hypothetical  $K^*$  matrix is that its rows are proportional to each other, and so are its columns.

The principle of CFA is to associate with every row of the  $K$  matrix a point in  $p$ -dimensional space so that the distance between two rows  $i$  and  $i'$  clearly shows the deviation from proportionality between them. Simultaneously, a second cluster of  $j$  points associated with every column of the matrix  $K$  is built in  $n$ -dimensional space, so that the distance between two columns  $j$  and  $j'$  shows their deviation from proportionality.

To establish the relative locations of the  $(n + p)$  points (as the rows and columns play a symmetrical role in CFA), a small-dimensional subspace is constructed, in which the cluster of projected points is most extended. The factors of CFA that define this subspace are the eigenvectors of a correspondence matrix obtained as the covariance matrix of the original data divided by the square root of the row and column sums.

The results of the analysis can be demonstrated as a projection of the  $(n + p)$  points on the planes defined by the successive main axes of cluster inertia (the CFA factors), e.g., on the plane defined by the two main axes (axes 1 and 2), then on the plane defined by axes 2 and 3, etc.

In order to determine the number of factors (i.e., the dimension of the constructed subspace) we use principal component analysis (PCA)<sup>9</sup>. The results of PCA indicated that when comparing the retentions of chalcones using the eleven chromatographic systems as many as seven factors ought to be taken into consideration. The error of the data reproduction is then 5.05%. It is known<sup>8</sup> that the number of significant CFA factors is equal to the number of significant PCA factors minus 1, which means that in the CFA six factors need to be taken into consideration.

To establish the similarity of the chromatographic behaviours of solutes and/or chromatographic systems, the hierarchical ascending classification (HAC)<sup>10</sup> was used,

the basis of which can be presented in the following way. Let  $n$  denote the number of objects,  $N$  the number of observations on these objects and  $x_{ij}$  (where  $i=1,2,\dots,n$  and  $j=1,2,\dots,N$ ) the numerical value related to the  $j$ th observation performed on the  $i$ th object. The arranged series of numerals  $\{x_{i1}, x_{i2}, \dots, x_{iN}\}$  can be regarded the observation vector of the  $i$ th object, and the individual numerals  $x_{ij}$  are components of this vector. These components determine the position of the  $i$ th object in the  $N$ -dimensional observation space. Hence, from the geometrical standpoint the considered objects can be viewed as a set of  $n$  points in the  $N$ -dimensional observation space, in which the inter-object distances can furnish a criterion of their similarity. These distances can be defined in a number of different ways<sup>10</sup>; in this paper inter-object distance is expressed by the Euclidean distance measure.

The first step in the HAC procedure depends on calculating the distances between all pairs of object, and from the  $n$  objects one chooses those two which are the closest to one another (*i.e.*, in this case, in which the inter-object distance is the smallest). These two objects are fused to form a group, and then replaced by the coordinates of their centroid. In the next step, one calculates distances between the pairs of  $(n - 1)$  objects, and again the closest two are joined. The procedure is repeated until all the objects are eventually agglomerated. The subsequent agglomerations are presented in the form of a classification tree (*i.e.*, dendrogram), and each agglomeration level corresponds to a numerical value representing the distance of the objects. Because we consider those objects for which the distance is zero to be identical (similarity = 100%), and the objects for which the relatively greatest distance is observed to be dissimilar (similarity = 0%), the relative scale of object similarity varies from 100 to 0%.

## RESULTS AND DISCUSSION

### *Polarity of phases*

The experimental results obtained for the chromatographic systems with stationary phases 1–5 (see Table I) have been published<sup>11</sup>. The capacity factors,  $k'$ , for chalcones chromatographed using the remaining six chromatographic systems are presented in Table II.

With respect to the mean capacity factors ( $\bar{k}'_j = \sum_i^{38} k'_{ij}/38$ ; see Table III), the investigated chromatographic systems can be arranged in the order DNAP > DNB > NH<sub>2</sub> > TCP > CN > NO<sub>2</sub> > TB > AP > diol > ODS > C<sub>8</sub>. Hence the polarities of the charge-transfer stationary phases cover a fairly wide range (the mean capacity factor for DNAP is almost five times higher than that for AP; Table III).

### *Retention mechanism*

Examination of the  $\log k'_{ij}$  vs.  $\log k'_{i'j'}$  relationship (where  $j$  and  $j'$  denote the  $j$ th and  $j'$ th stationary phases, respectively) can help in comparing the retention mechanisms of the studied group of chalcones in the individual chromatographic systems. Let us assume the following classification rules according to Melander *et al.*<sup>12</sup>: with a correlation coefficient  $r \geq 0.95$  for the  $\log k'_{ij}$  vs.  $\log k'_{i'j'}$  relationship, the retention mechanisms can be regarded as homo- or homeoenergetic; and with  $r < 0.95$ , the retention mechanisms on the  $j$ th and  $j'$ th stationary phases can be regarded as heteroenergetic.

TABLE II  
CAPACITY FACTORS,  $k'$ , FOR 38 *E-s-cis* AND *Z-s-cis* CHALCONES SEPARATED ON DIFFERENT COLUMNS

No.	Chalcone <i>X-Y</i>	Stationary phase					
		<i>NO</i> <sub>2</sub>	<i>AP</i>	<i>DNAP</i>	<i>DNB</i>	<i>TCP</i>	<i>TB</i>
1	H-CF <sub>3</sub>	1.74	0.92	2.84	2.40	2.52	1.78
2	H- <i>t</i> -C <sub>4</sub> H <sub>9</sub>	2.69	1.33	7.01	5.77	3.25	1.85
3	H- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	2.77	1.37	7.07	5.79	3.70	1.91
4	H-H	2.92	1.61	6.14	5.38	5.12	2.41
5	F-H	1.93	1.66	6.18	5.10	5.17	2.61
6	H-F	2.61	1.35	4.70	3.99	4.32	2.30
7	H-C <sub>2</sub> H <sub>5</sub>	3.06	1.55	7.77	6.42	4.97	2.14
8	H-CH <sub>3</sub>	3.42	1.79	8.56	7.07	6.32	2.57
9	F-CH <sub>3</sub>	3.89	1.81	8.22	6.51	6.14	2.70
10	F-F	3.13	1.50	4.99	3.97	4.41	2.57
11	CH <sub>3</sub> O-CH <sub>3</sub>	10.42	4.90	29.57	21.78	21.02	6.05
12	CH <sub>3</sub> -CH <sub>3</sub> O	5.82	4.70	31.88	21.89	20.33	6.12
13	F-CH <sub>3</sub> O	5.49	4.67	22.57	15.57	17.91	6.45
14	H-NO <sub>2</sub>	6.05	2.77	12.01	9.38	10.10	6.83
15	NO <sub>2</sub> -CH <sub>3</sub>	10.35	4.43	23.96	17.06	16.80	8.97
16	NO <sub>2</sub> -H	9.78	4.33	18.91	13.81	14.59	2.21
17	CH <sub>3</sub> O-CH <sub>3</sub> O	12.23	10.84	82.46	49.44	47.92	13.69
18	NO <sub>2</sub> -F	12.44	4.29	19.34	12.74	13.59	9.88
19	CH <sub>3</sub> O-C <sub>6</sub> H <sub>5</sub>	14.67	6.72	43.26	29.38	36.92	10.63
20	CH <sub>3</sub> -C <sub>6</sub> H <sub>5</sub>	5.40	2.82	17.00	12.36	13.19	4.61
21	H-CF <sub>3</sub> *	1.39	0.67	1.63	1.63	1.25	1.16
22	H- <i>t</i> -C <sub>4</sub> H <sub>9</sub>	1.89	0.89	3.65	4.18	1.51	1.31
23	H- <i>i</i> -C <sub>3</sub> H <sub>7</sub> *	1.96	0.94	3.69	4.22	1.72	1.37
24	H-H*	2.02	1.09	3.12	3.60	2.45	1.67
25	F-H*	1.59	1.02	2.71	2.81	2.32	1.70
26	H-F*	1.93	0.96	2.48	2.75	2.10	1.48
27	H-C <sub>2</sub> H <sub>5</sub> *	2.11	1.02	3.99	4.55	2.30	1.51
28	H-CH <sub>3</sub> *	2.32	1.22	4.20	4.90	2.97	1.83
29	F-CH <sub>3</sub> *	2.12	1.09	3.37	3.60	2.65	1.76
30	F-F*	2.03	1.02	2.37	2.36	2.08	1.67
31	CH <sub>3</sub> O-CH <sub>3</sub> *	4.70	2.48	9.09	11.61	8.40	3.54
32	CH <sub>3</sub> -CH <sub>3</sub> O*	3.39	2.90	10.94	12.77	8.40	4.01
33	F-CH <sub>3</sub> O*	4.72	2.63	8.05	8.36	6.74	4.01
34	H-NO <sub>2</sub> *	4.61	1.91	6.22	5.64	5.20	3.99
35	NO <sub>2</sub> -CH <sub>3</sub> *	7.53	3.50	15.18	13.10	9.01	7.28
36	NO <sub>2</sub> -H*	7.39	3.65	13.23	11.20	8.43	1.56
37	CH <sub>3</sub> O-CH <sub>3</sub> O*	10.78	5.82	23.19	27.41	19.84	7.99
38	NO <sub>2</sub> -F*	8.78	3.84	13.67	10.65	8.30	7.88

The results in Table IV indicate that the separation mechanism on most pairs of stationary phases is heteroenergetic;  $r > 0.95$  only for 15 of the 55 pairs of systems.

A detailed analysis of the chromatographic data and the specific properties of the individual stationary phases was performed with the help of CFA. The data matrix was constructed of the capacity factors for 38 chalcones developed with the eleven chromatographic systems. Fig. 1 gives an example of chalcones and chromatographic systems projected on the plane defined by the two main CFA axes.

The factor space obtained is six-dimensional, the percentage contributions of the

TABLE III  
MEAN CAPACITY FACTORS,  $\bar{K}$ , AND MEAN SELECTIVITY PARAMETERS,  $\bar{\alpha}_{X-Y/X-Y^*}$ , FOR THE INVESTIGATED CHROMATOGRAPHIC SYSTEMS

No.	System	$\bar{K}$	$\bar{\alpha}_{X-Y/X-Y^*}$
1	NH <sub>2</sub>	9.76	1.10
2	diol	2.50	1.40
3	CN	7.28	1.26
4	ODS	1.34	1.21
5	C <sub>8</sub>	0.76	1.02
6	NO <sub>2</sub>	5.05	1.45
7	AP	2.68	1.52
8	DNAP	13.03	2.18
9	DNB	10.29	1.56
10	TCP	9.31	2.15
11	TB	4.05	1.50

individual factors to the total cluster inertia being 70.66, 8.90, 6.93, 5.89, 3.24 and 2.78%. The mutual distance of the chromatographic systems in the six-dimensional factor space is  $\chi^2$ , defined by

$$\chi^2(j,j') = k \sum_{i=1}^n (k'_{ij}/k_{.j} - k'_{i'j}/k_{.j'})/k_i.$$

where

$$k_i = \sum_{j=1}^p k'_{ij}$$

$$k_{.j} = \sum_{i=1}^n k'_{ij}$$

$$k = \sum_{i=1}^n \sum_{j=1}^p k'_{ij}$$

TABLE IV  
NUMERICAL VALUES OF THE CORRELATION COEFFICIENT,  $r$ , OF THE LOG  $k'_{ij}$  vs. LOG  $k'_{i'j'}$ , RELATIONSHIPS FOR 55 PAIRS OF THE CHROMATOGRAPHIC SYSTEMS

	NH <sub>2</sub>	diol	CN	ODS	C <sub>8</sub>	NO <sub>2</sub>	AP	DNAP	DNB	TCP	TB
NH <sub>2</sub>	1.000										
diol	0.975	1.000									
CN	0.990	0.993	1.000								
ODS	0.938	0.919	0.925	1.000							
C <sub>8</sub>	0.945	0.900	0.920	0.960	1.000						
NO <sub>2</sub>	0.931	0.956	0.952	0.867	0.853	1.000					
AP	0.933	0.963	0.952	0.918	0.910	0.952	1.000				
DNAP	0.849	0.906	0.884	0.882	0.823	0.923	0.973	1.000			
DNB	0.856	0.882	0.877	0.831	0.851	0.911	0.968	0.982	1.000		
TCP	0.857	0.920	0.893	0.862	0.822	0.922	0.978	0.975	0.957	1.000	
TB	0.857	0.886	0.868	0.834	0.793	0.867	0.892	0.869	0.853	0.890	1.000

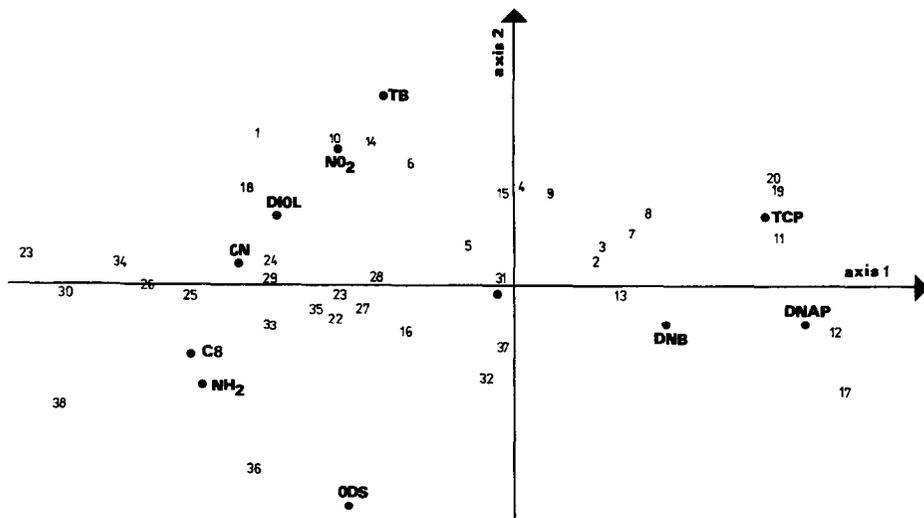


Fig. 1. Projection of chalcones (compounds 1–38; see Table II) and the chromatographic systems on the plane defined by the two main CFA axes.

$p$  and  $n$  are the number of stationary phases and compounds, respectively, and  $k'_{ij}$  is the capacity factor of the  $i$ th solute in the  $j$ th chromatographic system.

The  $\chi^2(j, j')$  distance can be used as a criterion of similarity of the separation mechanisms on the  $j$ th and  $j'$ th stationary phases<sup>13</sup>, because  $\chi^2(j, j')$  describes the deviation from proportionality between the capacity factors measured on the column pairs ( $j$  and  $j'$ ); for the chromatographic systems showing a homoenergetic separation mechanism,  $\chi^2(j, j') = 0$ . Analogously, the  $\chi^2(i, i')$  distance can serve as a measure of similarity of the chromatographic behaviours of the  $i$ th and  $i'$ th compound, and  $\chi^2(i, j)$  permits a rapid evaluation of the specificity of the  $j$ th chromatographic system toward the  $i$ th compound.

Assuming the  $\chi^2$  distance to be a measure of similarity or differentiation of the investigated objects, we classified chalcones and the chromatographic systems by means of the HAC. The results obtained presented in form of a classification tree are shown in Fig. 2.

The data in Fig. 2 indicate that only at an aggregation level of 21% do the investigated chromatographic systems form three distinct sub-groups: (A) NH<sub>2</sub>, diol, CN, NO<sub>2</sub>, ODS and C<sub>8</sub>; (B) TB; and (C) DNB, AP, TCP and DNAP. The stationary phase TB seems slightly closer to those in the sub-group A than to those in sub-group C. Among the phases in sub-group A the greatest similarity is observed for diol and CN. In sub-group C the pairs TCP–DNAP and DNB–AP as the most similar.

At an aggregation level of 50% we can distinguish the following sub-groups of the investigated chalcones: (a) H–H\*, F–CH<sub>3</sub>\*, F–CH<sub>3</sub>O\*, NO<sub>2</sub>–H\*, NO<sub>2</sub>–CH<sub>3</sub>\*, H–F, F–F, H–NO<sub>2</sub>, H–CF<sub>3</sub> and NO<sub>2</sub>–F; (b) NO<sub>2</sub>–F\*, H–CF<sub>3</sub>\*, F–F\* and H–NO<sub>2</sub>\*; (c) NO<sub>2</sub>–H; (d) NO<sub>2</sub>–H\*; (e) H–*t*-C<sub>4</sub>H<sub>9</sub>, H–*i*-C<sub>3</sub>H<sub>7</sub>, H–C<sub>2</sub>H<sub>5</sub> and H–CH<sub>3</sub>; (f) H–*t*-C<sub>4</sub>H<sub>9</sub>, H–*i*-C<sub>3</sub>H<sub>7</sub>, H–C<sub>2</sub>H<sub>5</sub>, H–CH<sub>3</sub>, H–H, F–CH<sub>3</sub>, NO<sub>2</sub>–CH<sub>3</sub>, F–H, F–CH<sub>3</sub>O, CH<sub>3</sub>O–CH<sub>3</sub>\*, CH<sub>3</sub>O–CH<sub>3</sub>O\* and CH<sub>3</sub>–CH<sub>3</sub>O\*; and (g) CH<sub>3</sub>O–C<sub>6</sub>H<sub>5</sub>, CH<sub>3</sub>–C<sub>6</sub>H<sub>5</sub>,

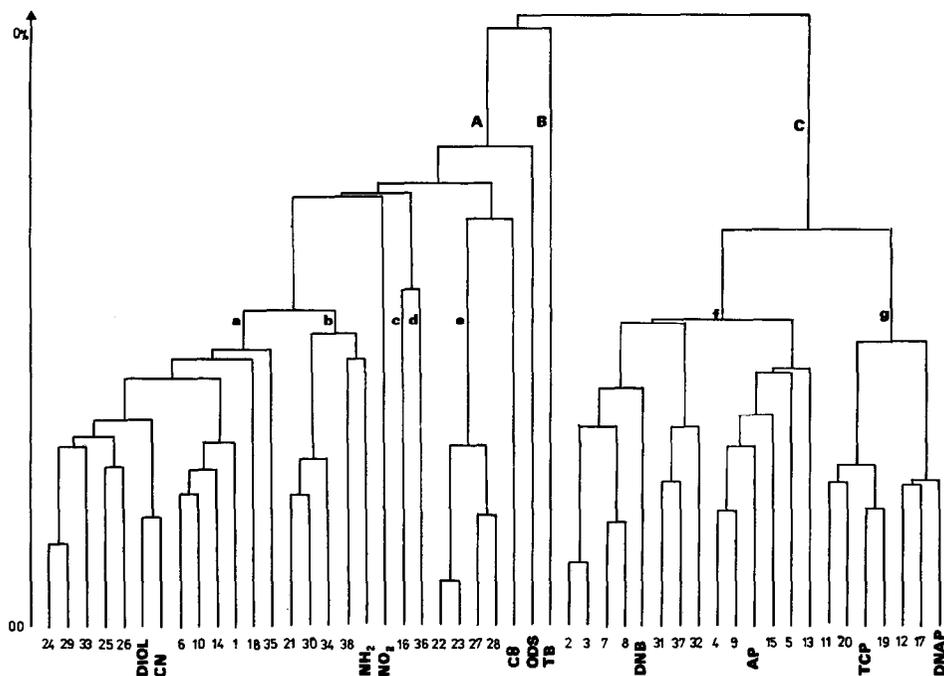


Fig. 2. Classification tree from the HCA for 38 chalcones and 11 normal-phase HPLC systems. The classification criterion is the euclidian distance in the six-dimensional factor space built by means of CFA.

$\text{CH}_3\text{O}-\text{CH}_3$ ,  $\text{CH}_3-\text{CH}_3\text{O}$  and  $\text{CH}_3\text{O}-\text{CH}_3\text{O}$ . These sub-groups of solutes are based on their specific behaviour toward the individual stationary phases. Thus the solutes in sub-group a show a relatively higher retention on stationary phase  $\text{C}_8$ , those in sub-group b on  $\text{NH}_2$ , those in sub-group c on diol and CN, etc. The specific properties of the charge-transfer stationary phases in sub-group C depend on their relatively more effective interactions (higher  $k'$  values) with chalcones in sub-group f and g.

#### Separation of substitutional isomers

Let us consider the relative retentions of the pairs of *E-s-cis* substitutional isomers  $\text{NO}_2-\text{H}$ ,  $\text{H}-\text{NO}_2$ ;  $\text{F}-\text{H}$ ,  $\text{H}-\text{F}$ ; and  $\text{CH}_3-\text{CH}_3\text{O}$ ,  $\text{CH}_3\text{O}-\text{CH}_3$  (see Fig. 3a). It can be stated that the relatively best separation is observed for the isomer pair  $\text{NO}_2-\text{H}$  and  $\text{H}-\text{NO}_2$ . With stationary phases 1–10  $\alpha_{\text{NO}_2-\text{H}/\text{H}-\text{NO}_2}$  changes from 1.42 to 1.59. On stationary phase TB the elution order for this pair of isomers reverses, and consequently  $\alpha_{\text{NO}_2-\text{H}/\text{H}-\text{NO}_2} = 0.32$ . The selectivity of the separation of the isomer pair  $\text{F}-\text{H}$  and  $\text{H}-\text{F}$  is lower than in the previous instance, although it is satisfactory on each stationary phase ( $\alpha_{\text{F}-\text{H}/\text{H}-\text{F}}$  varies in the range 1.13–1.31, and the elution order reverses only on the  $\text{NO}_2$  phase, while the respective  $\alpha_{\text{F}-\text{H}/\text{H}-\text{F}} = 0.74$ ). In contrast to the aforementioned examples, separation of the substitutional isomers  $\text{CH}_3-\text{CH}_3\text{O}$  and  $\text{CH}_3\text{O}-\text{CH}_3$  is much poorer ( $\alpha_{\text{CH}_3-\text{CH}_3\text{O}/\text{CH}_3\text{O}-\text{CH}_3}$  range from 0.96 to 1.08). A satisfactory separation of these isomers is achieved exclusively on the  $\text{NO}_2$  phase ( $\alpha = 0.56$ ).

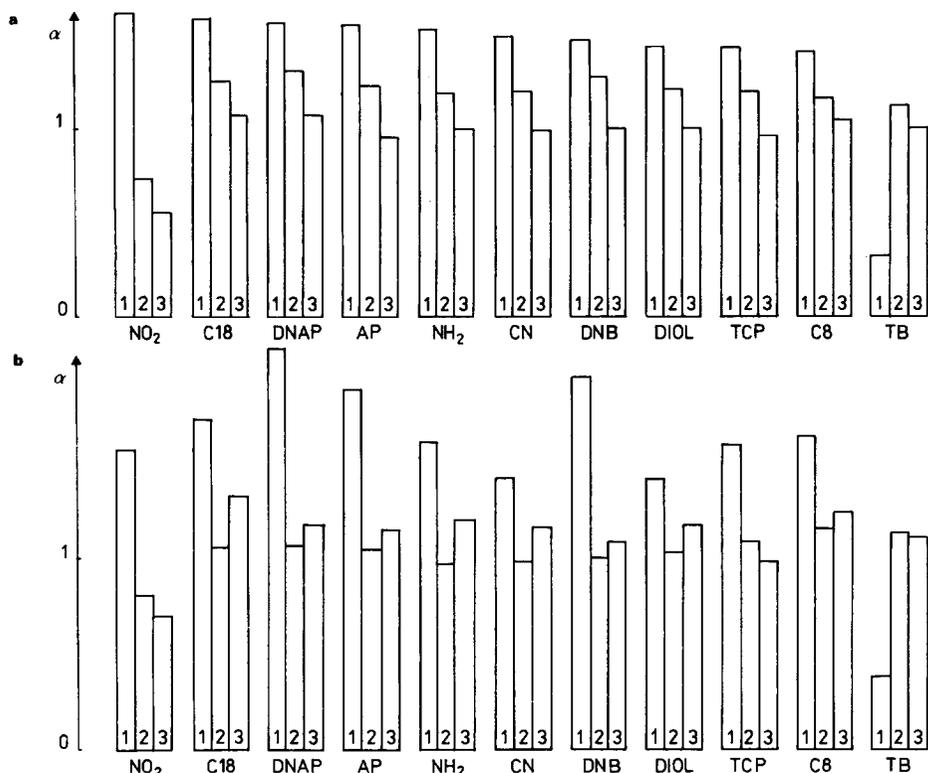


Fig. 3. Comparison of the selectivity parameter,  $\alpha$ , for the pairs of substitutional isomers (1)  $\text{NO}_2\text{-H}$ ,  $\text{H-NO}_2$ , (2)  $\text{F-H}$ ,  $\text{H-F}$  and (3)  $\text{CH}_3\text{-CH}_3\text{O}$ ,  $\text{CH}_3\text{O-CH}_3$ , with configurations (a) *E-s-cis* and (b) *Z-s-cis*.

The selectivity of separation of the analogous *Z-s-cis* substitutional isomers is considerably influenced by the stationary phase (see Fig. 3b).  $\alpha_{\text{NO}_2\text{-H}^*/\text{H-NO}_2^*}$  varies in the range 1.43–2.13 for stationary phases 1–10 ( $\alpha_{\text{NO}_2\text{-H}^*/\text{H-NO}_2^*} = 0.39$  for phase 11). The reverse situation occurs with the isomers  $\text{F-H}^*$  and  $\text{H-F}^*$ .  $\alpha_{\text{F-H}^*/\text{H-F}^*}$  is lower than  $\alpha_{\text{F-H}/\text{H-F}}$ , and the only exception in this instance is furnished by phase 11. A relatively improved selectivity of separation is observed with isomers  $\text{CH}_3\text{-CH}_3\text{O}^*$  and  $\text{CH}_3\text{O-CH}_3^*$ . Only with phase 10 is  $\alpha_{\text{CH}_3\text{-CH}_3\text{O}^*/\text{CH}_3\text{O-CH}_3^*} = 1$ , whereas for phases 1–8 and 11  $\alpha_{\text{CH}_3\text{-CH}_3\text{O}^*/\text{CH}_3\text{O-CH}_3^*}$  varies in the range 1.10–1.36. For the  $\text{NO}_2$  phase (9) the retention order of the discussed isomers reverses, and consequently  $\alpha_{\text{CH}_3\text{-CH}_3\text{O}^*/\text{CH}_3\text{O-CH}_3^*} = 0.72$ .

#### Separation of configurational isomers (*E-s-cis* and *Z-s-cis*)

The selectivity of separation of the isomeric *E-s-cis* and *Z-s-cis* chalcones varies over a very wide range on shifting from one chromatographic system to another. The mean selectivity parameters  $\bar{\alpha}_{\text{X-Y}/\text{X-Y}^*}$  determined for eighteen pairs of isomers are given in Table III and indicate that the charge-transfer stationary phases separate the *E-s-cis* and *Z-s-cis* isomers far better than the standard phases. Particularly high efficiency of separation is observed with DNAP and TCP, for which the mean selectivity parameters  $\bar{\alpha}_{\text{X-Y}/\text{X-Y}^*}$  are 2.18 and 2.15, respectively. Fig. 4 shows an

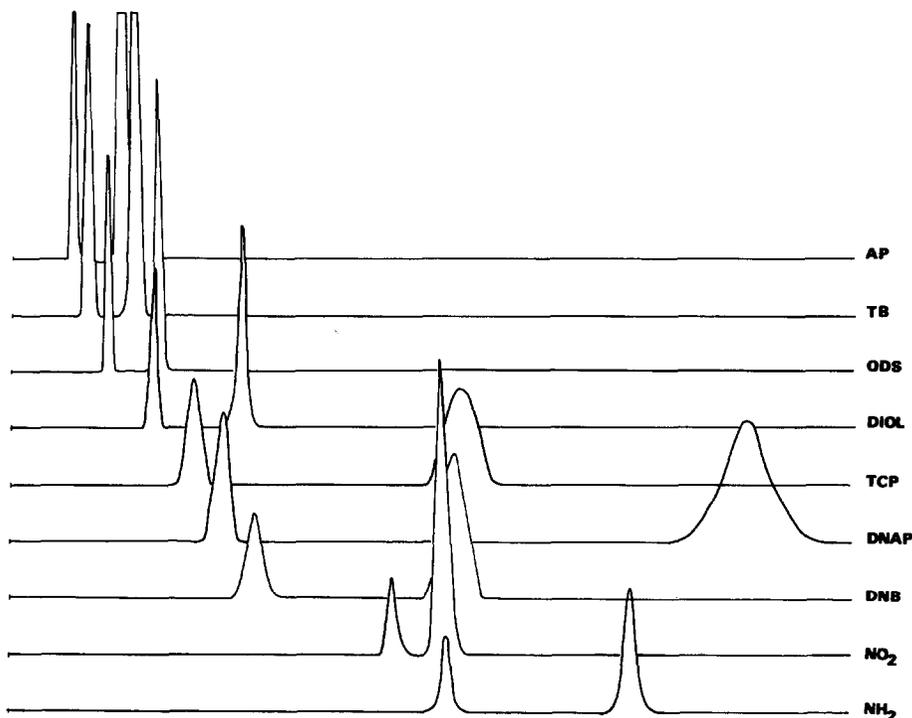


Fig. 4. Separation of the isomers  $\text{CH}_3\text{O}-\text{CH}_3\text{O}$  (*E-s-cis*) and  $\text{CH}_3\text{O}-\text{CH}_3\text{O}^*$  (*Z-s-cis*) on the selected stationary phases. Mobile phase: heptane-THF (97:3, v/v). Detection: UV, 280 nm.

example of the separation of the isomeric  $\text{CH}_3\text{O}-\text{CH}_3\text{O}$  and  $\text{CH}_3\text{O}-\text{CH}_3\text{O}^*$  chalcones on the selected stationary phases.

The selectivity of separation of the *E-s-cis* and *Z-s-cis* isomers depends, however, on the chemical nature of the substituents present in the chalcone molecule. The degree of separation similarity for the *E-s-cis* and *Z-s-cis* isomeric pairs on the eleven stationary phases can be established from the results of HAC, given in Fig. 5. This classification indicates that the isomeric monosubstituted chalcones ( $\text{H}-\text{Y}$  and  $\text{H}-\text{Y}^*$ ) appear in sub-groups A and C. The isomers with an  $\text{NO}_2$  substituent in position 4 are all in sub-group B. Sub-groups D, E and F contain the isomers with a  $\text{CH}_3\text{O}$  substituent in position 4 and/or 4' and an F substituent in position 4.

The analogous classification of the stationary phases allows their similarity to be described with respect to the separation selectivity for the eighteen pairs of the *E-s-cis* and *Z-s-cis* isomeric chalcones (see Fig. 6). The classification tree obtained indicates that the stationary phases can be divided into three sub-groups: (A) AP, DNB, TB, diol, CN and  $\text{NO}_2$ ; (B)  $\text{NH}_2$ , ODS and  $\text{C}_8$ ; and (C) DNAP and TCP. It should be emphasized that the stationary phases DNAP and TCP differ to the greatest extent from the others.

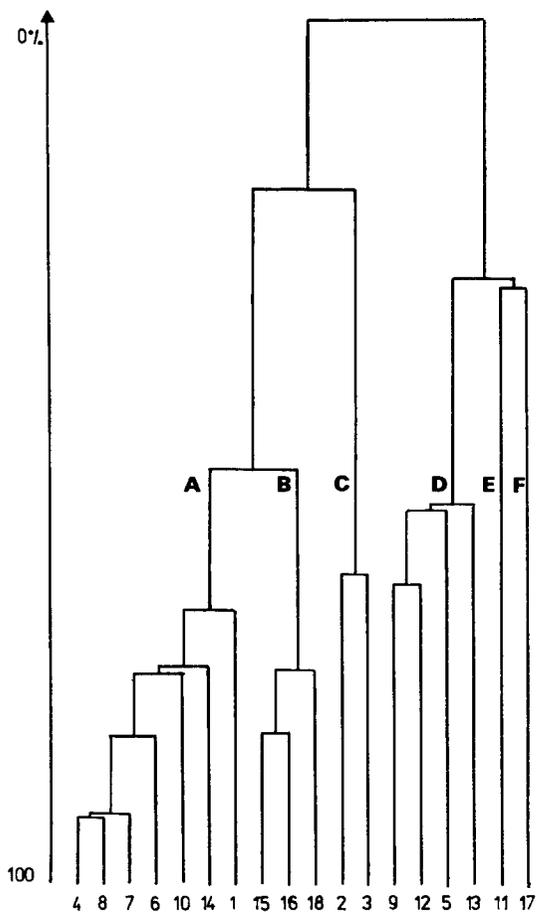


Fig. 5. Classification tree from the HAC for the selectivity parameters  $\alpha_{X-Y/X-Y^*}$  for 18 pairs of *E-s-cis* (X-Y) and *Z-s-cis* (X-Y\*) isomeric chalcones. The numbering of the parameters  $\alpha_{X-Y/X-Y^*}$  conforms with the numbering of chalcones X-Y in Table II.

CONCLUSIONS

The polarity of the investigated charge-transfer stationary phases changes to a relatively great extent in the order DNAP > DNB > TCP > TB > AP. The retention mechanism on the charge-transfer stationary phases compared with that on standard phases (diol, CN, NH<sub>2</sub>, etc.) is heteroenergetic (see Table IV) (the only exception is phase AP). The separation of the substitutional isomers on the charge-transfer phases does not differ substantially from that on the standard phases (see Fig. 3). The selectivity of separation of the *E-s-cis* and *Z-s-cis* chalcone isomers on the charge-transfer stationary phases is far better than that on the standard phases. Special attention in this respect should be paid to the phases DNAP and TCP (see Table III and Fig. 4).

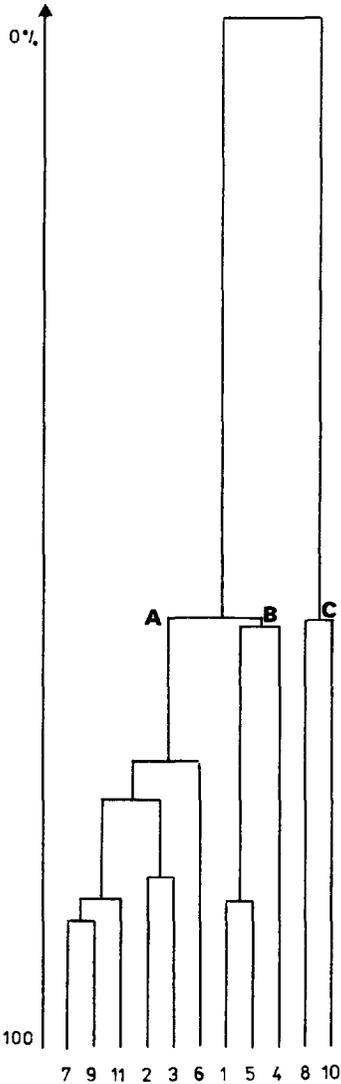


Fig. 6. Classification tree from the HAC for eleven normal-phase HPLC systems. The classification criterion is the euclidian distance in the eighteen-dimensional space of the parameters  $\alpha_{X-Y/X-Y^*}$ . The numbering of the systems is given in Table I.

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