Journal of Chromatography, 353 (1986) 109–121
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 736

FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. TRENDS IN SELECTIVITY OF 53 CHALCONES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON ALKYL- OR PHENYL-BONDED STATIONARY PHASES

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SUMMARY

The separation of 53 chalcones, giving two series of the *E-s-cis* and *Z-s-cis* isomers by reversed-phase high-performance liquid chromatography on four non-polar chemically bonded stationary phases (Zorbax ODS, Zorbax C_8, Spherisorb C_6 and μBondapak-phenyl), is presented in the form of tabulated capacity factors, and an attempt is made to elucidate the physico-chemical message coded in the numerical values obtained. Correspondence factor analysis is utilized, facilitating extraction of the relevant information from the set of experimental data. The computational results obtained ensure a better insight into the processes that occur during the chromatographic separation and allow their discussion at the molecular interactions level.

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0021-9673/86/$03.50

INTRODUCTION

Investigation of the potential ability of chromatographic techniques to produce experimental data of high physico-chemical relevance is an important trend, useful in various interdisciplinary correlations, particularly for medicinal chemistry[1], and aims to couple information derived by different experimental and computational methods[2–9]. Owing to the richness of raw chromatographic data, a detailed analysis of their information content requires appropriate data processing techniques, such as factor analysis[10] and topological analysis[11,12], as we have demonstrated in gas chromatography with series of esters[12] and hydrocarbons[10,13,14].

We were prompted to test the applicability of factor analysis to high-performance liquid chromatographic (HPLC) data in order to elucidate the potential main factors responsible for the selectivity in modelling the activity of series of drugs[1], *i.e.*, more generally for analytical applications and physico-chemical development. Such an analysis requires equal attention to be devoted to changes in molecular interactions due to variations in the chromatographic conditions and to changes due to variations in solute structures.

The chromatographic conditions can be modified in two ways. One can change the stationary and/or mobile phase, and the changes in the relative retentions of investigated compounds caused by these modifications can be treated as a source of information about the ability of compounds to undergo different kinds of intermolecular interactions, on a thermodynamic basis[15].

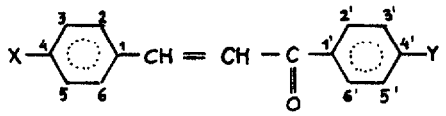
The choice of a suitable group of test compounds is particularly significant in well planned model experiments. The structures of these compounds can reveal the wide range of possibilities of the technique employed, simultaneously giving a unique characteristic of their structural and/or electron properties. Nevertheless, the key step is the correct interpretation of the experimental results. The mixed mechanism of chromatographic separations often tends to be overlooked, and under these circumstances it can be regarded as an important research task to deduce the contributions of the individual mechanisms to the process of separation. One can expect that these results will correlate well with the mechanisms of interaction in complex living systems, generally described as biological activity. Aiming to achieve a broad insight into the complex relationships in the selected chromatographic systems, we concentrated on substituted chalcones as the chromatographed test substances. Their relatively complex structure is an important factor, which helps to reveal the sensitivity of the applied systems. A wide range of polarities enabled us to utilize normal- and reversed-phase conditions. These investigations facilitate comparisons of the investigated substances and of the chromatographic systems.

In this work the chromatographic data were obtained with different reversed-phase HPLC columns but a constant methanol–water mobile phase. Information contained in these data was extracted with the help of the correspondence factor analysis (CFA) method[10,16].

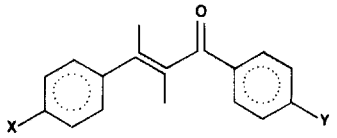
EXPERIMENTAL

Reagents

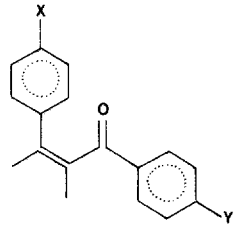
The general formula of the chalcones that were considered is:



where X and Y are listed in Table I. The chalcones are denoted as X–Y in the text. *E-s-cis*-Chalcones were synthesized[17,18] while the corresponding *Z-s-cis*-isomers (denoted here by asterisks) were formed spontaneously in dichloromethane solution. Only $N(Me)_2$–NO_2 appeared in each chromatographic system as the *E-s-cis*-isomer, showing no evident tendency to isomerize to the *Z-s-cis*-isomer (checked in about twenty different chromatographic systems). The structures of these two types of isomers are



E-s-cis



Z-s-cis

The mobile phase consisted of HPLC-grade methanol, purchased from Merck (Darmstadt, F.R.G.), mixed with Millipore-purified water.

Instruments and columns

The HPLC equipment included the following components: a Bruker LC-31 pump, a Rheodyne Model 7125 injection valve, a Schoeffel Model SF 770 spectrophotometer, set at 280 nm, and Shimadzu C-R1B recorder.

The 7-μm Zorbax ODS column (250 × 4.6 mm I.D.) was obtained from DuPont (Les Ulis, France) and the 10 μm μBondapak-phenyl column (250 × 4.6 mm I.D.) from Water Assoc. (Milford, MA, U.S.A.). The 7-μm Zorbax C_8 (240 × 4.0 mm I.D.) (DuPont) and the 5-μm Spherisorb C_6 (240 × 4.0 mm I.D.) (Phase Separations, Queensferry, U.K.) columns were prepared in our laboratory by slurry packing at 6000 p.s.i. with carbon tetrachloride, followed by methanol.

Chromatographic procedures

Prior to the measurements, the columns were washed with the methanol–water (7:3) mobile phase until a constant value was obtained for the retention of chalcones. Sample solutions (2 mg per 25 ml) were prepared in dichloromethane. All data points were collected by averaging two reproducible separations. The methanol–water (7:3) mobile phase and 1 μl of 10^{-2} *M* sodium nitrate sample solution, detected at 210 nm, were used to determine the dead time, t_0, for each column. The capacity factor, k', was calculated from the retention time of the solute, t_R, according to the equation $k' = (t_R - t_0)/t_0$.

Correspondence factor analysis

The CFA method, developed by Benzécri[16] and presented elsewhere for chro-

matographic applications[10], was used to extract information from the sets of experimental data. CFA is a method of analysis of a table of positive and homogeneous data. It depends on the utilization of the elements constituting the data matrix, $K(n,p)$ (in our case an element of this matrix k_{ij} is equal to the capacity factor, k', of the ith chalcone separated in the jth chromatographic system divided by the sum of all the elements of the matrix, $|k_{ij} = k'_{ij}/\Sigma_i\Sigma_j k'_{ij}|$) to construct a new X matrix the elements of which are defined as follows:

$$x_{ij} = \frac{k_{ij} - k_{i.}\, k_{.j}}{\sqrt{k_{i.}\, k_{.j}}}$$

where:

$$k_{i.} = \sum_{j=1}^{p} k_{ij} \text{ and } k_{.j} = \sum_{i=1}^{n} k_{ij}$$

Then the variance matrix V was calculated from $V = X^{\mathrm{T}}X$ (where X^{T} is the transposed X matrix) and, to diagonalize the matrix in order to determine the set of the eigenvalues E, and the related eigenvectors B, the following equation was used:

$$B^{-1}V\,B = E$$

The ratio of the e_i eigenvalue to the sum of the eigenvalues determines the validity of the ith eigenvector and is named the "inertia percentage".

RESULTS AND DISCUSSION

Table I shows the capacity factors of 53 chalcones, separated in four reversed-phase chromatographic systems.

These systems utilized the methanol–water (7:3) as the mobile phase and Zorbax ODS, Zorbax C_8, Spherisorb C_6 and μBondapak-phenyl as the stationary phases. The application of the above mobile phase ensured good selectivity with the investigated group of compounds in all the systems examined (as an example, Fig. 1 shows the separation of some selected chalcones on Zorbax ODS). This allowed us to observe differences in retention in systems that differed only with respect to the stationary phase.

Comparison of separation mechanisms in a number of chromatographic systems for a selected group of compounds was done in terms of the separation factor, $\alpha_{a/b}$, which is the ratio of the capacity factors for two separated compounds, a and b. This separation factor is a thermodynamic measure of the difference in retention between two compounds in the systems investigated. It can be used to characterize the selectivity for the whole group of compounds in the individual chromatographic system, and to describe differences in the specific selectivity between these systems. The group of compounds chosen for the analysis additionally enables us to discuss the separation of the *E-s-cis*- and *Z-s-cis*-isomers.

The CFA method seems particularly useful for the simultaneous comparison of the different characteristics of the chromatographic systems mentioned above, mostly owing to its sensitivity to deviations from proportionally in both rows and columns of the data matrix K. Results of this approach are given in Fig. 2.

TABLE I

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

Columns: 1 = Zorbax ODS, 2 = Zorbax C_8, 3 = Spherisorb C_6 and 4 = μBondapak-phenyl. Mobile phase, methanol–water (7:3); flow-rate, 1.5 ml min^{-1}; detection, UV at 280 nm. Abbreviations: tBu = *tert.*-butyl, iPr = isopropyl, Et = ethyl, Me = methyl, Ø = phenyl. The *Z-s-cis*-chalcones are denoted by asterisks.

No.	*Chalcone X–Y*	*Column*			
		1	*2*	*3*	*4*
1	H–CF_3	11.23	8.52	3.38	3.64
2	H–tBu	24.66	18.66	6.23	6.51
3	H–iPr	18.72	13.53	4.85	5.41
4	H–H	5.83	4.28	1.85	2.48
5	F–H	4.92	4.89	1.84	2.40
6	H–F	5.39	4.40	1.93	2.50
7	H–Et	12.80	9.50	3.59	4.30
8	H–Me	8.50	6.34	2.59	3.12
9	F–Me	8.83	6.85	2.53	3.14
10	F–F	5.29	4.24	1.91	2.45
11	Me–Ø	53.42	22.38	6.78	9.90
12	MeO–Me	8.76	6.64	2.59	3.53
13	Me–MeO	9.65	6.97	2.73	3.59
14	F–MeO	5.36	4.36	1.93	2.67
15	H–NO_2	5.89	3.89	1.65	2.85
16	MeO–Ø	32.46	15.87	4.84	8.28
17	F–NO_2	4.85	3.77	1.67	2.76
18	NO_2–Me	6.49	4.76	2.85	3.31
19	NO_2–H	4.02	3.25	1.48	2.62
20	MeO–MeO	5.83	4.69	1.94	3.89
21	MeO–NO_2	5.91	4.16	1.73	3.22
22	NO_2–F	4.32	3.43	1.54	2.60
23	$N(Me)_2$–NO_2	8.51	6.44	2.45	4.77
24	NO_2–MeO	4.56	3.53	1.57	2.96
25	NO_2–NO_2	4.82	3.00	1.55	2.91
26	NH_2–H	1.36	1.59	0.74	1.31
27	H–OH	1.88	2.00	0.96	1.46
1*	H–CF_3*	7.19	6.53	2.81	2.68
2*	H–tBu*	16.63	14.19	5.15	5.25
3*	H–iPr*	11.68	18.16	3.98	4.36
4*	H–H*	3.22	3.17	1.49	2.00
5*	F–H*	3.68	3.46	1.62	2.10
6*	H–F*	3.47	3.36	1.59	2.85
7*	H–Et*	7.85	7.87	2.90	3.46
8*	H–Me*	5.89	1.62	2.85	2.52
9*	F–Me*	5.70	5.89	2.22	2.74
10*	F–F*	3.93	3.64	1.7	2.14
11*	Me–Ø*	38.93	17.42	6.78	8.84
12*	MeO–Me*	5.84	5.16	2.15	3.83
13*	Me–MeO*	5.45	5.87	2.17	2.96
14*	F–MeO*	3.65	3.6	1.68	2.35
15*	H–NO_2*	3.30	3.82	1.37	2.31

(Continued on p. 114)

TABLE I *(continued)*

No.	*Chalcone X–Y*	*Column*			
		1	*2*	*3*	*4*
16*	MeO–Ø*	21.56	12.31	4.19	7.09
17*	F–NO_2*	3.68	3.26	1.48	2.34
18*	NO_2–Me*	4.27	4.83	1.80	2.88
19*	NO_2–H*	2.77	2.76	1.31	2.30
20*	MeO–MeO*	3.74	3.59	1.60	2.67
21*	MeO–NO_2*	4.36	3.47	1.50	2.77
22*	NO_2–F*	2.93	2.87	1.34	2.20
24*	NO_2–MeO*	2.89	2.96	1.36	2.56
25*	NO_2–NO_2*	2.72	2.47	1.32	2.37
26*	NH_2–H*	1.36	1.59	0.74	1.31
27*	H–NO_2*	1.15	1.46	0.75	1.15

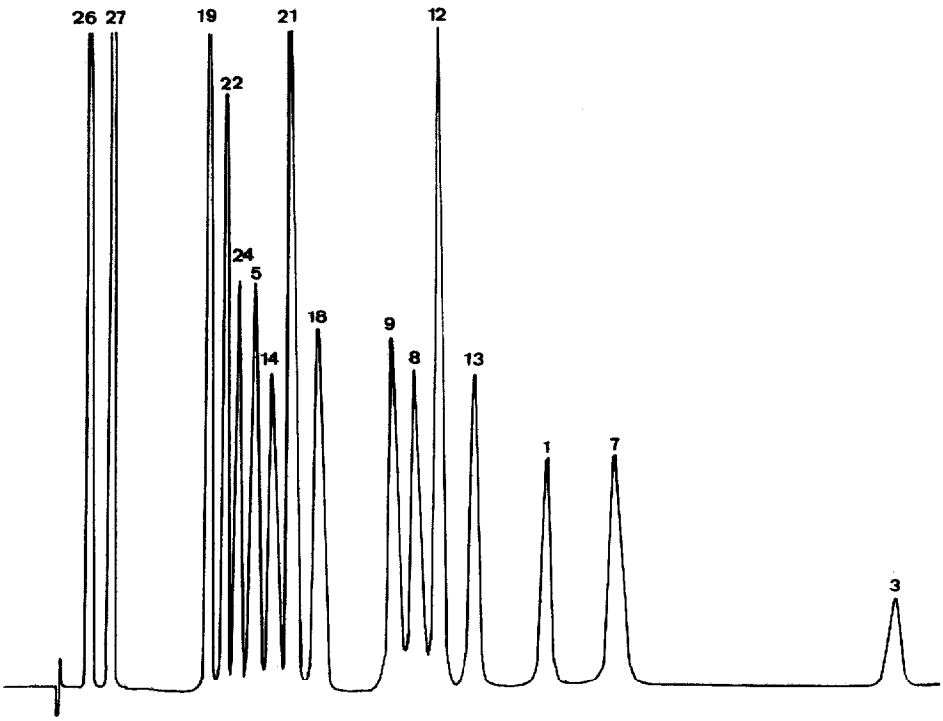


Fig. 1. Separation of some selected *E-s-cis*-chalcones on a Zorbax ODS column (250 × 4.6 mm I.D.). Mobile phase, methanol–water (7:3); flow-rate, 1.5 ml min^{-1}; detection, UV at 280 nm. Peak numbers correspond to Table I.

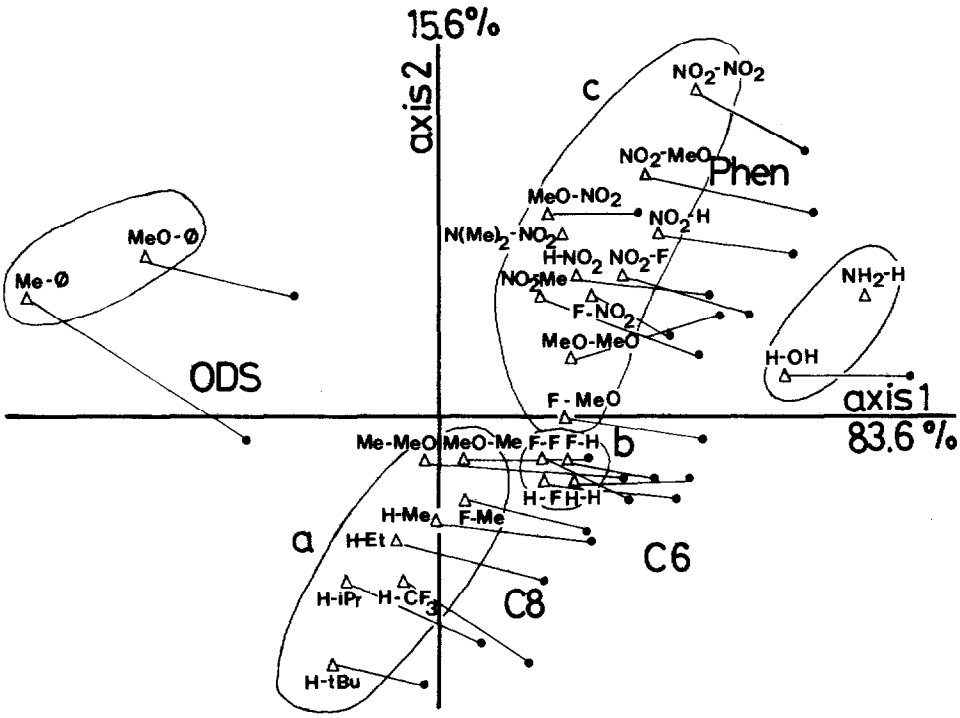


Fig. 2. Simultaneous projection of 53 chalcones (△, *E-s-cis*; ●, *Z-s-cis*) and four chromatographic systems on to the plane determined by the two main inertia axes extracted from CFA.

The two main axes selected by CFA have contributions of 83.6 and 15.6% to the total inertia of the cluster. They define the plane in Fig. 2 in which projection of the 53 compounds and four columns offers an accurate representation of their behaviour in the initial cluster. The Zorbax ODS (51.0%) and the μBondapak-phenyl (32.1%) columns, and Me–Ø (34.9%) and MeO–Ø (11.4%) have the greatest contribution in isolating the first axis, while the μBondapak-phenyl (48.4%) and the Zorbax C_8 (37.0%) columns, and H–tBu* (12.4%), H–tBu (12.2%), MeO–Ø (7.9%) and Me–Ø (6.5%) predominate in isolating the second axis. (After elimination of these compounds, Me–Ø, MeO–Ø, Me–Ø* and MeO–Ø*, which contribute most to the total cluster inertia, we obtain another projection from the CFA analysis, in which the arrangement of compounds and of the chromatographic systems changes in relation to the newly extracted axes, but it remains mutually constant in comparison with the previous projection.)

Let us first consider the arrangement of the *E-s-cis*-chalcones in the projection given in Fig. 2. One can easily see that the phenyl-substituted chalcones are most distinctly separated from the remaining compounds. The above-mentioned chalcones give relatively high values of the separation factor, $\alpha_{X-Y/H-H}$, on Zorbax ODS (see Table II). It is probably caused by their strong hydrophobicity, combined with their distinct donor–acceptor properties. Apart from the 4'-phenylchalcones, other clusters are observed: in cluster a, compounds with so-called hydrophobic substituents are

grouped, for which the separation factor, $\alpha_{X-Y/H-H}$, in each chromatographic system exceeds 1, and shows the following dependence on the individual stationary phases:

$$\alpha_{X-Y/H-H}\,(\text{ODS}) > \alpha_{X-Y/H-H}\,(C_8) > \alpha_{X-Y/H-H}\,(C_6) > \alpha_{X-Y/H-H}\,(\text{phenyl})$$

For the small cluster b, incorporating fluorine in their structure, the sequence given above undergoes certain perturbations, although the numerical values of $\alpha_{X-Y/H-H}$ do not differ significantly. Cluster c includes the polar-substituted chalcones for which the separation factor is greater for the μBondapak-phenyl than for the alkyl stationary phases. For a few compounds in this group we observe that $\alpha_{X-Y/H-H}$ (phenyl) > 1 and $\alpha_{X-Y/H-H}$ (alkyl stationary phases) < 1. This means that the given pair of substituents (*i.e.*, NO_2–MeO, NO_2–NO_2, F–NO_2, NO_2–H, NO_2–F) in the latter cluster induces an increase in retention on the phenyl phase in comparison with the unsubstituted chalcone.

The above-mentioned regularity gives evidence of the significant role played by specific interactions in the mechanism of chalcone separations on the μBondapak-phenyl phase. Also, NH_2–H and H–OH (Nos. 25 and 26 in Table II), which give $\alpha_{X-Y/H-H} < 1$ in all the applied chromatographic systems but the greatest value for the phenyl stationary phase, are separated from the other *E-s-cis*-chalcones.

Division of the investigated compounds into the clusters enumerated above and the respective differences in the specific selectivities can be elucidated by applying the relevant physico-chemical interpretation to both of the discussed axes. All the previously mentioned regularities allow the conclusion that the first axis reflects the changes in hydrophobicity of compounds and columns, while the second describes the changes in retention caused by their different abilities to interact specifically. Good correlations between the chalcone coordinates on the first axis and the hydrophobic Hansch (π) or Rekker (f) substituent parameters[19,20] serve to confirm this hypothesis. These relationships are given in the form of the following equations:

$$X_{E\text{-s-cis-chalcone}} = 0.101 - 0.193\pi_X - 0.142\pi_Y \quad (r = 0.981;\ 27 \text{ compounds})$$
$$X_{E\text{-s-cis-chalcone}} = 0.178 - 0.209f_X - 0.148f_Y \quad (r = 0.953;\ 27 \text{ compounds})$$

As these equations indicate, the contribution of a given substituent to the coordinate x on axis 1 depends on the substitution site. This phenomenon can be explained by the different influence of the substituents in positions 4 and 4′ on the electron density in the chalcone core; the charge density at the carbonyl oxygen increases on introduction of electron-releasing substituents and decreases in the presence of electron-withdrawing groups, the stronger effect being exerted by the substituent attached to the acetophenone part of the chalcone[21].

Arrangement of the investigated chromatographic systems along the second axis seems to be consistent with their arrangement according to the number of the residual silanol groups[22]. An attempt to correlate chalcone coordinates on this axis with parameters such as Hammett constants (σ_p^{+-}, σ_p^0, σ_p) for the substituents X and Y[23], carbonyl stretching frequencies, $\gamma_{C=O}$[24,25], dipole moments[25,26], proton-acceptor powers[27–30], polarizabilities[31] or the electron densities on the individual chalcone atoms[21,32] did not give satisfactory results. This seems to suggest that the different types of specific interactions between chalcones and the stationary phases are simul-

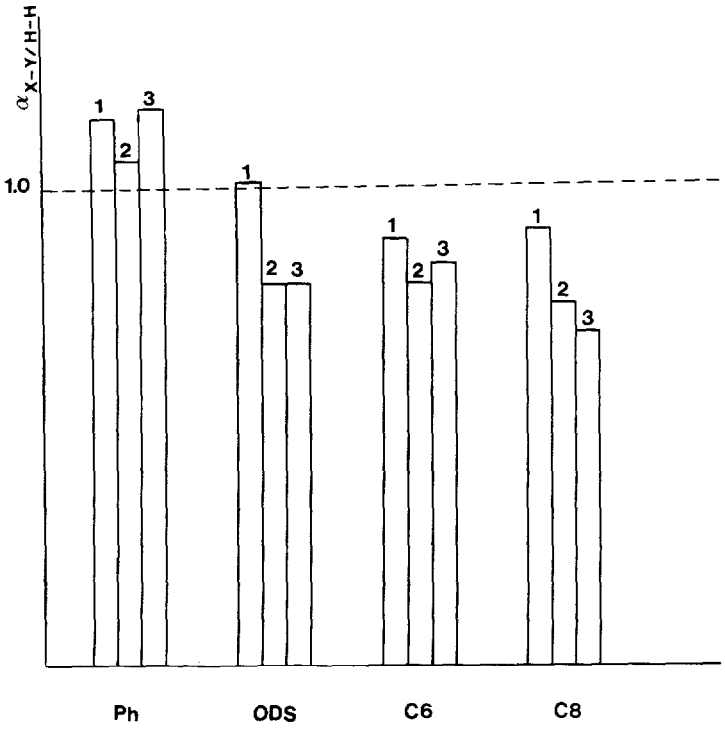


Fig. 3. Numerical values of the separation parameter $\alpha_{X-Y/H-H}$ for H–NO_2 (1), NO_2–H (2) and NO_2–NO_2 (3) on μBondapak-phenyl, Zorbax ODS, Spherisorb C_6 and Zorbax C_8 columns. Mobile phase, methanol–water (7:3); flow-rate, 1.5 ml min^{-1}; detection, UV at 280 nm.

taneously responsible for the separations discussed. From the side of the chalcone this involves carbonyl groups, phenyl rings and the various substituents, which can effectively participate in such interactions, whereas from the side of the stationary phases residual silanol groups and π-electrons from the phenyl rings of the phenyl stationary phase may be involved.

For H–NO_2, NO_2–H and NO_2–NO_2 the respective separation factors, $\alpha_{X-Y/H-H}$, obtained on all the stationary phases investigated are compared in Fig. 3.

This example demonstrates well the lack of regularity of the chromatographic effects induced by the different types of intermolecular interactions involved. On the phenyl stationary phase, the separation factors for all the compounds of interest exceed 1, which confirms our previous conclusions regarding the importance of intermolecular interactions in the process of separation. With the alkyl-bonded stationary phases the numerical values of $\alpha_{X-Y/H-H}$ surpass 1 only for H–NO_2, separated on Zorbax ODS, whereas in all the remaining instances the NO_2 substituent lowers the retention of the respective chalcones (*i.e.*, $\alpha_{X-Y/H-H} < 1$). There is one characteristic phenomenon, *viz.*, $\alpha_{H-NO_2/H-H}$ is always larger than $\alpha_{NO_2-H/H-H}$. This phenomenon is caused by a change in both the hydrophobicity of the two compounds involved and the ability of the respective functional groups to interact intermolecularly, induced by the shift of the NO_2 substituent from position 4 to 4′. The corresponding $\alpha_{X-Y/H-H}$ values are influenced by each of these two effects, which are

TABLE II

NUMERICAL VALUES OF THE SEPARATION FACTORS (*a*) $\alpha_{X-Y/H-H}$ AND (*b*) $\alpha_{X-Y*/H-H*}$ FOR *E-s-cis*- AND *Z-s-cis*-CHALCONES CHROMATOGRAPHED ON DIFFERENT COLUMNS

Columns and conditions as in Table I.

No.	*Chalcone X–Y*	*1*		*2*		*3*		*4*	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	H–CF_3	2.23	2.23	1.99	2.06	1.83	1.88	1.47	1.44
2	H–tBu	4.90	5.16	4.36	4.48	3.37	3.46	2.62	2.62
3	H–iPr	3.72	3.63	3.16	3.20	2.62	2.67	2.18	2.18
4	H–H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	F–H	0.98	1.14	0.96	1.09	0.99	1.09	0.97	1.05
6	H–F	1.07	1.08	1.03	1.06	1.04	1.07	1.01	1.02
7	H–Et	2.54	2.44	2.22	2.23	1.94	1.95	1.73	1.73
8	H–Me	1.69	1.58	1.48	1.46	1.40	1.38	1.26	1.26
9	F–Me	1.60	1.77	1.41	1.60	1.37	1.49	1.27	1.37
10	F–F	1.05	1.22	0.99	1.15	1.03	1.14	0.99	1.07
11	Me–Ø	10.62	9.60	5.23	5.50	3.66	4.55	3.99	4.02
12	MeO–Me	1.74	1.81	1.55	1.63	1.40	1.44	1.42	1.52
13	Me–MeO	1.92	1.69	1.63	1.60	1.48	1.46	1.45	1.48
14	F–MeO	1.06	1.13	1.02	1.14	1.04	1.13	1.05	1.18
15	H–NO_2	1.01	1.02	0.91	0.95	0.89	0.92	1.15	1.16
16	MeO–Ø	6.45	6.70	3.52	3.88	2.62	2.81	3.34	3.54
17	F–NO_2	0.96	1.14	0.88	1.03	0.90	0.99	1.11	1.17
18	NO_2–Me	1.29	1.33	1.71	1.27	1.11	1.21	1.33	1.44
19	NO_2–H	0.80	0.86	0.76	0.87	0.80	0.88	1.06	1.15
20	MeO–MeO	1.16	1.16	1.10	1.13	1.05	1.07	1.24	1.34
21	MeO–NO_2	1.17	1.35	0.97	1.09	0.94	1.01	1.30	1.38
22	NO_2–F	0.86	0.91	0.80	0.90	0.83	0.90	1.05	1.10
23	NO_2–MeO	0.91	0.90	0.82	0.93	0.85	0.91	1.19	1.28
24	NO_2–NO_2	0.80	0.84	0.70	0.78	0.84	0.88	1.17	1.18
25	NH_2–H	0.27	0.42	0.37	0.50	0.40	0.50	0.53	0.65
26	H–OH	0.37	0.36	0.47	0.46	0.52	0.50	0.59	0.58

superimposed on one another. Although one could expect that substitution of NO_2–H with the second NO_2 group would result in some evident trend in the changes in the separation factors on the investigated stationary phases, in fact we observe an increase in retention on μBondapak-phenyl and Spherisorb C_6, but a decrease on Zorbax C_8 and no evident influence on Zorbax ODS.

Analogous observations and conclusions can be drawn for the *Z-s-cis*-chalcones (labelled as X–Y*). These isomers show lower capacity factors than the respective *E-s-cis* species (see Table I), although the separation factors for both series, on any given stationary phase, are very similar. Correlation of the capacity factors for the *E-s-cis* and *Z-s-cis* series can be evaluated as follows: $r = 0.996$ (ODS), 0.998 (C_8), 0.998 (phenyl) and 0.989 (C_6). Table II gives the $\alpha_{X-Y/H-H}$ and $\alpha_{X-X*/H-H*}$ values, which reflect the influence of isomerism on contributions of the X and Y substituents to retention.

From the data in Table II, it is evident that in most instances $\alpha_{X-Y*/H-H*} > \alpha_{X-Y/H-H}$. This means that substitution of the polar group, able to lower the retention

TABLE III

NUMERICAL VALUES OF THE SEPARATION FACTOR $\alpha_{X-Y/X-Y*}$ FOR CHALCONES CHROMATOGRAPHED ON DIFFERENT COLUMNS

Columns and conditions as in Table I.

No.	*Chalcone X–Y*	$\alpha_{X-Y/X-Y*}$			
		1	*2*	*3*	*4*
1	H–CF_3	1.56	1.30	1.20	1.26
2	H–tBu	1.48	1.32	1.21	1.24
3	H–iPr	1.60	1.33	1.22	1.24
4	H–H	1.56	1.35	1.24	1.24
5	F–H	1.34	1.18	1.14	1.14
6	H–F	1.55	1.31	1.21	1.22
7	H–Et	1.63	1.34	1.24	1.24
8	H–Me	1.67	1.37	1.26	1.24
9	F–Me	1.41	1.19	1.14	1.14
10	F–F	1.35	1.16	1.12	1.14
11	Me–F	1.73	1.28	1.00	1.23
12	MeO–Me	1.50	1.29	1.20	1.16
13	Me–MeO	1.77	1.37	1.26	1.21
14	F–MeO	1.47	1.21	1.15	1.14
15	H–NO_2	1.54	1.29	1.20	1.23
16	MeO–Ø	1.50	1.22	1.16	1.17
17	F–NO_2	1.32	1.16	1.13	1.18
18	NO_2–Me	1.52	1.18	1.14	1.15
19	NO_2–H	1.45	1.18	1.13	1.14
20	MeO–MeO	1.56	1.31	1.21	1.16
21	MeO–NO_2	1.36	1.20	1.15	1.16
22	NO_2–F	1.47	1.20	1.15	1.18
23	NO_2–MeO	1.58	1.19	1.15	1.16
24	NO_2–NO_2	1.48	1.21	1.17	1.23
25	NH_2–H	1.00	1.00	1.00	1.00
26	H–OH	1.63	1.37	1.28	1.27

of a given compound in comparison with H–H (*e.g.*, NO_2), results in a greater decrease in retention for the *E-s-cis*-isomers than for the corresponding *Z-s-cis*-isomers. Nevertheless, the extent of this decrease depends on the chromatographic system applied, which seems to be the reason for certain slight differences in the relative arrangement of chalcones from the *E-s-cis* and *Z-s-cis* series on CFA projection (Fig. 2). Correlation of the coordinates x on axis 1 for the *Z-s-cis* series with the hydrophobic constants of the substituents X and Y according to Hansch and Rekker results in the following dependences:

$$X_{\text{Z-s-cis-chalcone}} = 0.200 - 0.133\pi_X - 0.134\pi_Y \ (r = 0.964;\ 26 \text{ compounds})$$
$$X_{\text{Z-s-cis-chalcone}} = 0.263 - 0.154f_X - 0.140f_Y \ (r = 0.948;\ 26 \text{ compounds})$$

These relationships provide evidence that for the *Z-s-cis* series, the contributions of the 4- and 4′-substituents to the x coordinate are less differentiated than with the *E-s-cis*-isomers. This fact illustrates well the decrease in electron coupling in the *Z-s-cis* molecules, due to a lack of coplanarity with the constituent phenyl rings.

The usefulness of the investigated chromatographic systems for the separation of isomers can be judged on the basis of numerical values of the the separation factor $\alpha_{X-Y/X-Y*}$, which are collected in Table III. The results show that the separation of isomers is effective in each system, although the best results are obtained when Zorbax ODS is used as the stationary phase.

CONCLUSION

CFA allows the determination and quantification of the factors governing the selectivity of separation for the chosen group of chalcone compounds in reversed-phase HPLC. Two main factors are involved; the first is related to the hydrophobicity of the solutes and stationary phases, and the second may be attributed to their ability to interact specifically. The equations describing the relationship between the chalcone coordinate on axis 1 and the hydrophobicity of substituents X and Y involve the use of Hansch or Rekker parameters. This helps to determine the relative sensitivity of chalcones to the changes in the hydrophobic properties of stationary phases. These equations also reflect the influence of the positional isomerism and stereoisomerism. The substituent contribution to the above-mentioned sensitivity depends more on the substituent position for the *E-s-cis-* than for the *Z-s-cis*-chalcones. Nevertheless, in both instances substituents in position 4 have a greater influence than substituents in position 4'. The best selectivity of *E-s-cis-* and *Z-s-cis*-chalcone isomers is obtained on the ODS stationary phase. Specific interactions play a particular role in the separation of chalcones on the phenyl stationary phase, chiefly with nitro derivatives. This may be caused by residual silanol groups and by phenyl participation.

Such data derived from reversed-phase HPLC, describing changes in intermolecular interactions and resulting from modification of the solute structures, are of special interest from the analytical and physio-chemical points of view. To test potential specific interactions, complementary studies in normal-phase HPLC will be presented in a subsequent paper[33].

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