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Journal of Chromatography A, 730 (1996) 195–201

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic separation of some 2- and 4-hydroxy derivatives of 2′-benzoylbenzoic acid on Florisil and silica gel columns

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Abstract

A series of 2′-benzoylbenzoic acid derivatives including some pairs of positional isomers and other related compounds were chromatographed by HPLC using a normal-phase system with polar adsorbents (Florisil and silica) and 2-propanol, 1,4-dioxane and tetrahydrofuran as modifiers of the eluent. The results obtained were compared as $\log k'_{\text{Florisil}}$ vs. $\log k'_{\text{silica}}$ relationships to ascertain the differences in distribution and chemical character of surface-active centres of the investigated adsorbents and the mechanism of elution in different eluent systems

Keywords: Stationary phases, LC; Mobile phase composition; 2′-Benzoylbenzoic acid derivatives; Organic acids

1. Introduction

Florisil (magnesium silicate), a polar adsorbent, has been found to be suitable for the separation of closely related compounds. Our previous papers described the application of Florisil to the separation of especially complex natural mixtures such as the furocoumarin fraction of some plants from the Appiaceae family [1], also on a micropreparative scale [2,3], and parallel investigations of the behaviour of various model substances by TLC [4]. The interesting properties of Florisil in the separation of, e.g., positional isomers and other closely related compounds, encouraged further investigations on the separation of aromatic acids and aldehydes [5] and steroid hormones [6] by continuous TLC and also the HPLC analysis of mono-, bi- and trifunctional model solutes of different chemical character using specially prepared Florisil for HPLC [7,8]. The results obtained for Florisil were compared with those obtained for silica (or alumina) as $\log k'_{\text{Florisil}}$ vs. $\log k'_{\text{silica}}$ relationships and also by the comparison of selectivities ($\Delta\log k'$ or ΔR_M values) of the separation of, e.g., some pairs of isomers on the adsorbent surfaces.

2′-Benzoylbenzoic acids represent an interesting series of positional isomeric compounds differing from one another by one functional group; they have been investigated by Ruminski and co-workers [9,10] using reverse-phase systems, by Bieganowska and co-workers [11,12] by reversed-phase ion-pair high-performance thin-layer and column chromatography and by Ciszewska and Soczewinski [13] using liquid–liquid partition chromatographic systems.

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0021-9673/96/$15.00
SSDI 0021-9673(95)01029-7

The aim of this work was the investigation of the chromatographic behaviour of 2′-benzoylbenzoic acid derivatives, including positional isomers and other similar compounds, with various eluent system on Florisil and silica, in order to obtain better recognition of the distribution and chemical character of surface-active centres and to elucidate the mechanism of adsorption on the adsorbent surface in eluent systems with different polar modifiers.

2. Experimental

The chromatographic experiments were performed at 19 ± 1°C using a Type 302 liquid chromatograph (Techma Robot, Warsaw, Poland) equipped with a syringe pump (at a flow-rate of the 1.2 ml/min) and a UV detector (set at 254 nm). Single solutes dissolved in the eluent were injected with a 5-μl injection valve.

HPLC-grade 10-μm Florisil was prepared in the Institute of Inorganic Chemistry (Gliwice, Poland). Florisil (Fluka, Buchs, Switzerland) for TLC was milled in a Model 100 AFG counter-flow-fluid mill (Alpine, Augsburg, Germany). Particle segregation of ground Florisil was carried out in a Model 100 MZR pneumatic separator (Alpine). The column (150 × 4 mm I.D.) was packed with 10-μm Florisil [specific surface area (BET) 340 m^2/g, total porosity 46.2%] by the slurry method. It had an efficiency of about 3000 theoretical plates determined by the adsorption of phenylamine and 1-nitronaphthalene using 2% tetrahydrofuran in *n*-heptane as eluent. The void volume of the column was determined by injection of pure *n*-heptane. For further details, see Ref. [7].

The chromatographic experiments with silica were performed using a column (250 × 4 mm I.D.) packed with 10-μm Si 100 (Merck, Darmstadt, Germany) produced by Polish Reagents (Lublin, Poland).

As eluents, *n*-heptane solutions of 2-propanol, tetrahydrofuran (for HPLC; Merck), 1,4-dioxane (for HPLC; Romil Chemicals, Sepshed, UK) and glacial acetic acid (Polish Reagents) were used.

3. Results and discussion

The experimental results for the solutes listed in Table 1 are illustrated graphically as plots of log k' against log c (concentration of polar solvent in the eluent) obtained for Florisil (see Fig. 1) using 2-propanol. Linearity of the plots was confirmed by the high regression parameters of the equation

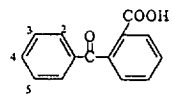
$$\log k' = a - m \log c \quad (1)$$

($r > 0.98$) (see Table 1). This indicates the agreement of the results obtained with the Snyder–Soczewinski model of adsorption [14,15].

The absolute values of the slopes of plots of log k' against log c are about 1 ($m < 1$) for alkyl (**14**, **16**), phenyl (**17**) or alkoxy (**18–20**) and phenoxy (**21**) derivatives of 2′-benzoylbenzoic acid. It indicates one-point attachment of these solutes to active centres of Florisil, but they have a carboxylic group of class AB [16] and also a C=O group of class B in the *ortho* position with respect to the first one. The alkoxy group present in the molecule does not cause any increase of the slope values. Hence the one-point attachment occurs because the adsorption energy of the COOH group is much higher than that of the other polar groups and the eluent strength suitable for it is high in relation to other groups [14]. Also, the 2-hydroxy derivatives of benzoylbenzoic acid with a second group of class AB in the molecule (**2–8**) interact only by one point with the surface-active centres. This effect is caused by blocking of the functional groups of the solute molecules by interactions of internal H-bond type, or by steric hindrance. Isomers with the OH group in the *para* position to the C=O group have higher slope values of ca. 1.5 (see Table 1), which indicates one- and two-point interactions with the adsorbent surface or a contribution of solvation effects (see compounds **9–14**).

The comparison of the adsorption properties of Florisil and silica is presented as correlations of log k' values obtained using the same eluent and two different adsorbents in Figs. 2–4. Large differences in selectivity of the investigated adsorbents are shown in Fig. 2 when 2-propanol

Table 1
Parameters of the equation $\log k' = a - m \log c$ for the system Florisil–2-propanol–*n*-heptane + 2% acetic acid for the compounds investigated



No.	Substituents				Intercept	Slope	*n*	*r*
	2	3	4	5				
1	–	–	–	–	1.21	0.82	5	0.993
2	OH	–	–	–	0.95	0.74	6	0.987
3	OH	CH_3	–	–	0.72	0.74	6	0.998
4	OH	–	CH_3	–	0.92	0.80	5	0.997
5	OH	–	–	CH_3	0.91	0.76	5	0.987
6	OH	C_2H_5	–	–	0.63	0.76	5	0.998
7	OH	–	–	Cl	0.82	0.81	5	0.991
8	OH	–	OCH_3	–	1.21	0.77	5	0.995
9	–	–	OH	–	2.59	1.56	5	0.998
10	–	CH_3	OH	–	2.39	1.44	5	0.998
11	–	$CH(CH_3)_2$	OH	–	1.09	0.71	5	0.986
12	–	OCH_3	OH	–	2.56	1.43	4	(0.998)
13	–	Cl	OH	–	2.29	1.45	5	0.999
14	OH	–	OH	–	2.19	1.47	5	0.998
15	–	–	CH_3	–	1.18	0.82	5	0.983
16	–	–	C_2H_5	–	1.08	0.84	6	0.996
17	–	–	C_6H_5	–	1.12	0.83	5	0.984
18	–	–	OCH_3	–	1.48	0.75	5	0.998
19	–	OCH_3	OCH_3	–	–	–	–	–
20	–	–	OC_2H_5	–	1.36	0.76	5	0.995
21	–	–	OC_6H_5	–	1.21	0.94	5	0.996

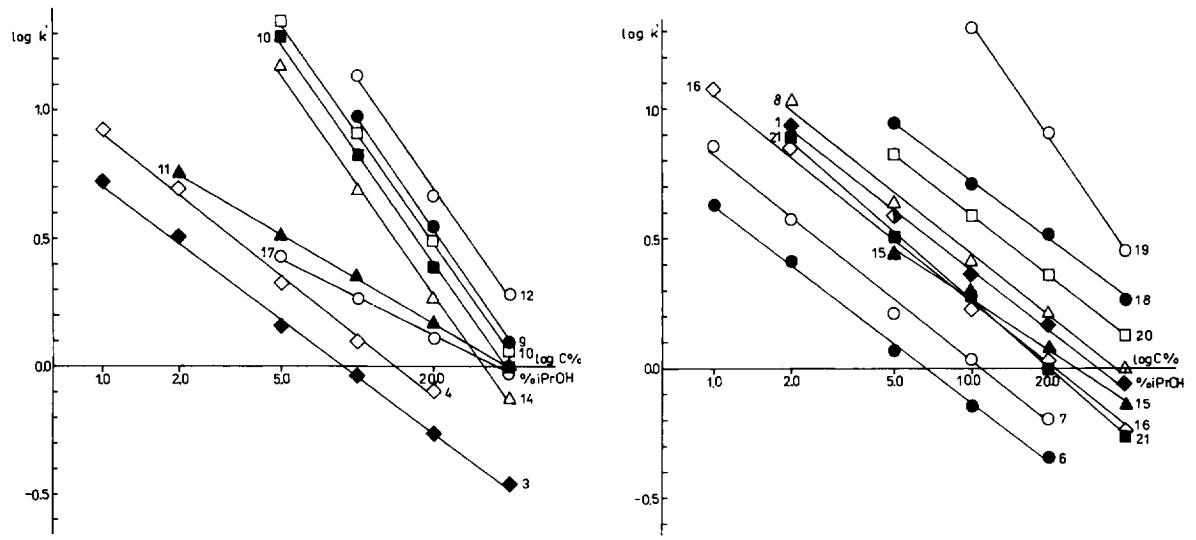


Fig. 1. Plots of log k' versus log c (concentration of modifier in the mobile phase, %, v/v) for the solutes investigated (see Table 1). System: Florisil with 2-propanol–*n*-heptane + 2% acetic acid.

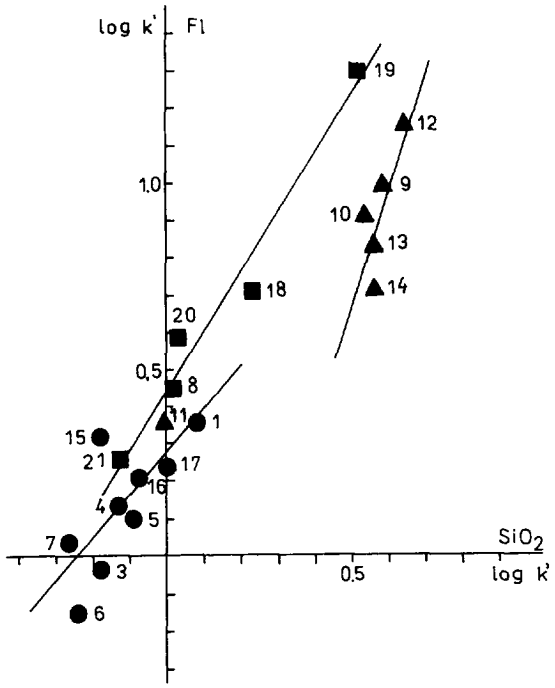


Fig. 2. Correlation between log k' values of the compounds investigated (see Table 1) on Florisil and silica. Mobile phase: 10% 2-propanol–*n*-heptane + 2% acetic acid.

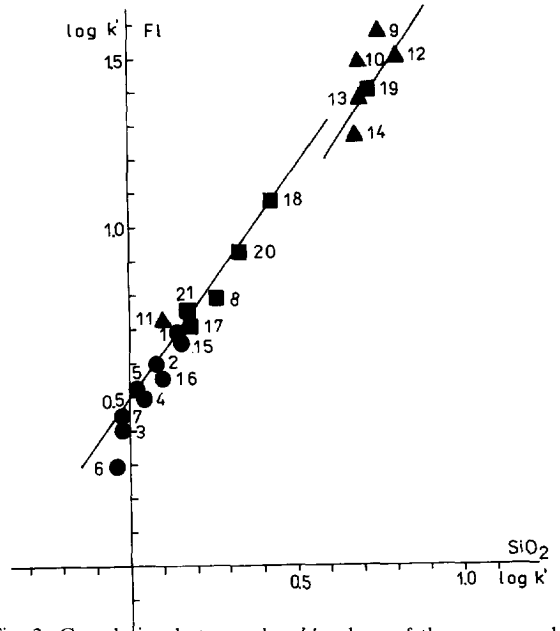


Fig. 3. Correlation between log k' values of the compounds investigated (see Table 1) on Florisil and silica. Mobile phase: 15% tetrahydrofuran–*n*-heptane + 2% acetic acid.

was used as the modifier. As was ascertained earlier [8], the adsorption of solute molecules in systems with 2-propanol is a result of displacement and solvation effects. This means that solutes interact in this case directly with active centres of the adsorbents and the differences in retention parameters show the differences in the distribution and character of the active centres of the two adsorbents. It is seen in Fig. 2 that the points of the log k'_{Florisil} vs. log k'_{silica} relationships form three groups near three separate correlation lines. *p*-Hydroxy derivatives of benzoylbenzoic acid (triangles) form the first group and the linear equation of the plot is

$$\log k'_{\text{Florisil}} = -0.850 + 3.05 \log k'_{\text{silica}};$$
$$r = 0.802 \quad (n = 5)$$

The large slope value of the line indicates a greater selectivity of separation of these compounds on the Florisil surface. The relatively low regression coefficient indicates individual differences in the adsorption mechanism of the molecules.

The second line (squares) relates to the alkoxy derivatives of benzoylbenzoic acids. The line can be expressed by the following equation:

$$\log k'_{\text{Florisil}} = 0.43 + 1.59 \log k'_{\text{silica}};$$
$$r = 0.974 \quad (n = 5)$$

The selectivity of the separation of this group of compounds is higher for Florisil. The log k' values on Florisil are in the region about one log k' unit and on silica in the region of 0.6 unit.

The remaining compounds form the third group (circles) with the equation

$$\log k'_{\text{Florisil}} = 0.26 + 1.06 \log k'_{\text{silica}};$$
$$r = 0.708 \quad (n = 10)$$

The low value of the regression coefficient indicates the individual changes in the separation selectivity on the adsorbent surfaces.

Fig. 3 shows the log k'_{Florisil} vs. log k'_{silica}

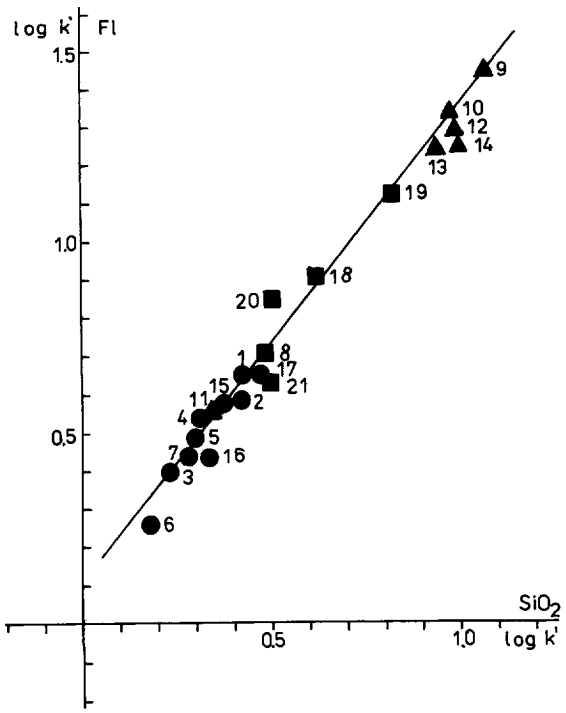


Fig. 4. Correlation between log k' values of the compounds investigated (see Table 1) on Florisil and silica. Mobile phase: 20% dioxane–*n*-heptane + 2% acetic acid.

diagram for tetrahydrofuran as modifier. As was found earlier [8], tetrahydrofuran molecules cover and deactivate the adsorbent surface. The spread of points is less pronounced and only two groups near two correlation lines are shown. The isomers with the OH group in position 4 are focused near the line

$$\log k'_{\text{Florisil}} = 0.358 + 1.5 \log k'_{\text{silica}};$$
$$r = 0.644 \quad (n = 5)$$

The selectivity of separation on Florisil is slightly better (slope = 1.5) and the low regression coefficient indicates differences in the character of the active centres of the investigated adsorbents. The other compounds form the second line:

$$\log k'_{\text{Florisil}} = 0.49 + 1.35 \log k'_{\text{silica}};$$
$$r = 0.93 \quad (n = 16)$$

The relatively high regression coefficient indicates the similarities of the character of both adsorbent surfaces covered with tetrahydrofuran molecules.

The comparison of log k' values obtained on two adsorbents using dioxane as modifier confirmed earlier conclusions that dioxane modifies both silica and Florisil surfaces [7,8,17] by the ether oxygen, so that its adsorbed molecules can interact with the second ether oxygen with the chromatographed solutes. The points of the retention parameters of the investigated compounds form a single correlation line:

$$\log k'_{\text{Florisil}} = 0.111 + 1.2 \log k'_{\text{silica}};$$
$$r = 0.990 \quad (n = 21)$$

with a very high correlation coefficient. This means that co-adsorption of solutes on the monolayer of dioxane occurs and therefore the differences in adsorption properties decrease.

Table 2 presents $\Delta\log k'$ values for the investigated compounds in relation to 2′-benzoylbenzoic acid (**1**) in all the systems investigated. It shows a comparison of the selectivities of the systems investigated. In most cases the $\Delta\log k'$ values for Florisil are higher than the corresponding values obtained for silica in three eluent systems using 2-propanol (system I), dioxane (system II) and tetrahydrofuran (system III). In only five out of 120 cases are the $\Delta\log k'$ values higher on silica in comparable eluent systems. This manifests the better adsorptive properties of Florisil especially when 2-propanol is used as modifier. This is also confirmed by the $\Delta\log k'$ values of the isomers investigated presented in Table 3.

The detailed analysis of the results permits one to ascertain the influence of the structural effects on the retention of the solutes. For example, the presence of a polar OH group in the *ortho* position to the C=O group in the molecule of benzoylbenzoic acid causes a decrease in retention (see solute **2**) whereas the same group in the *para* position causes strong adsorption of the molecule (see solute **9**). The reason for such different chromatographic behavior is the internal H-bond interactions in the *ortho*-substituted molecules (see also Table 3). Comparison of the adsorptive properties of other isomers having a

Table 2
$\Delta\log k' = \log k'_n - \log k'_1$ values for the compounds investigated on Florisil and silica with the systems (I) 10% 2-propanol–*n*-heptane + 2% acetic acid, (II) 20% dioxane–*n*-heptane + 2% acetic acid and (III) 15% tetrahydrofuran–*n*-heptane + 2% acetic acid

Compound	Florisil			Silica		
	I	II	III	I	II	III
1	0	0	0	0	0	0
2	−0.23	−0.07	−0.10	−0.20	0.00	−0.06
3	−0.40	−0.26	−0.29	−0.26	−0.19	−0.16
4	−0.26	−0.12	−0.20	−0.20	−0.11	−0.09
5	−0.26	−0.17	−0.17	−0.18	−0.12	−0.12
6	−0.50	−0.40	−0.40	−0.31	−0.44	−0.18
7	−0.32	−0.22	−0.25	−0.35	−0.14	−0.16
8	0.05	0.05	0.10	−0.04	0.06	0.12
9	0.62	0.80	0.88	0.50	0.65	0.61
10	0.56	0.68	0.79	0.46	0.56	0.55
11	0.00	−0.10	0.03	−0.08	−0.08	−0.04
12	0.79	0.63	0.80	0.57	0.57	0.66
13	0.47	0.58	0.68	0.49	0.52	0.55
14	0.34	0.59	0.57	0.47	0.58	0.54
15	−0.04	−0.08	−0.03	−0.26	−0.04	0.01
16	−0.15	−0.21	−0.14	−0.16	−0.09	−0.04
17	−0.12	−0.01	−0.01	−0.08	0.04	0.04
18	0.35	0.25	0.38	0.16	0.20	0.28
19	1.10	0.46	0.71	0.43	0.40	0.58
20	0.23	0.19	0.23	−0.05	0.08	0.19
21	−0.10	−0.02	0.06	−0.13	0.07	0.04

polar OH group in *ortho* and *para* positions leads to the same conclusions (see $\Delta\log k'$ values of compounds **12** and **8**, **13** and **7**, and **10** and **3**). It is interesting that even 2,4-dihydroxybenzoylbenzoic acid has a lower retention than the 4-hydroxy derivative (compare solutes **14** and **9**).

The presence of an alkyl group or chlorine atom in the molecule always causes a decrease in retention (compare compounds **3–6** with compound **2**, **7** with **2**, **10** with **9** and **13** with **9**) when the alkyl group shields polar OH or C=O groups or causes steric hindrance. The effect is especial-

Table 3
$\Delta\log k'$ values of some pairs of isomers of 2′-benzoylbenzoic acid derivatives on Florisil and silica with the systems (I) 10% 2-propanol–*n*-heptane + 2% acetic acid, (II) 20% dioxane–*n*-heptane + 2% acetic acid and (III) 15% tetrahydrofuran–*n*-heptane + 2% acetic acid

Isomer Nos.	Florisil			Silica		
	I	II	III	I	II	III
9–2	0.85	0.86	0.98	0.70	0.65	0.67
12–8	0.73	0.58	0.70	0.61	0.51	0.54
13–7	0.79	0.80	0.93	0.84	0.66	0.71
10–3	0.96	0.94	1.02	0.72	0.75	0.71
3–5	−0.14	−0.09	−0.12	−0.08	−0.07	−0.04
3–4	−0.14	−0.14	−0.09	−0.05	−0.08	−0.07

ly strong when the alkyl group is larger, such as the isopropyl group in solute **11**, shielding the OH group in the *para* position and decreasing the adsorption of the molecule, comparable with the adsorption of 2′-benzoylbenzoic acid (solute **1**). The addition of an alkyl or phenyl group to the basic molecule (see compounds **15–17**) causes a weakened retention of these solutes; the addition of alkoxy groups to the molecule causes a relatively stronger retention of the compounds (see solutes **18–20**), contrary to the phenoxy group (see solute **21**).

4. Conclusions

Log k' vs. log c (concentration of modifier) relationships obtained for Florisil for 2′-benzoylbenzoic acids are linear in accordance with the Snyder–Soczewinski model of adsorption. Most of the investigated 2-hydroxy derivatives interact only by one point and 4-hydroxy derivatives of 2′-benzoylbenzoic acid interact by one or two points with the active centres of the Florisil surface.

The selectivities of the separations of the compounds investigated are better for Florisil than for silica, especially when 2-propanol is used as the modifier and displacement of adsorbed molecules by the chromatographed solutes occurs. Tetrahydrofuran shields the active centres of the adsorbents and the differences in their adsorptive properties are less pronounced.

Adsorbed dioxane modifies Florisil and silica surface via the ether oxygen so that its molecules interact with the chromatographed solutes via the second oxygen, and in this system the properties of silica and Florisil are very similar.

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