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OPTIMIZATION OF THE PREPARATIVE SEPARATION OF HYDROXYBIPHENYL ISOMERS

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SUMMARY

    The separation of hydroxybiphenyl isomers was carried out on silica gel. The choice of the separation conditions was difficult owing to the low solubility of the *para* isomer. Three kinds of the mobile phase were employed, their elution strengths being selected in such a manner that the selectivity factor varied in the range *ca.* 2.6–7.7. The rate of broadening of a band was investigated as a function of mass overloading and solubility of the sample in the mobile phase for similar k' values. The optimal column loadability was established for 99.0% purity, taking into account the column throughput and the volume of the fractions. One operation in a 300 × 50 mm I.D. preparative column yielded 1.25 and 0.63 g of pure *para* and *ortho* isomers, respectively.

INTRODUCTION

    Mixtures of *ortho, meta* and *para* isomers are well separated on silica gel. The selection of appropriate conditions for preparative separations involves choosing a gel of large specific surface area and a mobile phase that ensures maximum throughput. If the separation is aimed at obtaining small amounts of substances, optimization with respect to the particle size of the packing and the column dimensions is of less significance.
    The effect of the selectivity factor, α, and capacity factor, k', on the throughput of the process is already generally known. Among numerous studies, the most useful seem to be those of Gareil and co-workers[1-3], who gave the equation

$$C_v = C_m \exp\left(-\frac{v - V_0 - V_m}{\tau}\right) \tag{1}$$

where C_v is the concentration of the sample for an elution volume v, V_0 is the injection volume, V_m is the column hold-up volume and C_m and τ are model parameters. A limitation to the applicability of this equation to the selection of separation conditions is the poor solubility of the separated compounds in the mobile phase. A so-called linear optimization, *i.e.*, controlling the process throughput through a change

in sample volume, is recommended in this instance[3]. However, if a mixture is complex and two or more components are isolated, an increase in sample mass through an increase in its volume is rarely possible. There is a possibility of increasing the sample mass without changing its volume by injection of the sample in a solvent stronger than the mobile phase, in which the samples are readily soluble. Such a procedure can result in precipitation of the sample components of poor solubility at the top of the packing. When the precipitation and progressive dissolution occur in the mobile phase, the concentrations of the sample in both phases can be constant, depending on the solubility. A section of approximately constant concentration can also appear in the outlet profile and the descent of the peak to the baseline can be delayed with respect to the analytical peak. At the same time, the front of the peak can be shifted towards the start if the concentration in the mobile phase exceeds the linear range of the isotherm. Hydrodynamic effects[4] can accompany precipitation of the sample at the top of a column.

Injection of a sample in a solvent stronger than the mobile phase need not result in precipitation of the sample, but conditions prevailing in the initial section of a column until the stronger solvent leaves this section are difficult to define. It can be anticipated that the band will be broadened owing to acceleration of migration of its frontal part caused by the stronger solvent leaving the band. The change in k' in the band will be accompanied by the change in the degree of overloading. The degree of band spreading resulting from these phenomena will depend on the volume of solvent introduced with the sample, its elution strength and the capacity factor, k', of the substance. In some papers peak distortion as result in injection of the sample in a solvent stronger than the mobile phase has been mentioned[5,6].

EXPERIMENTAL

Adsorbents
Silica gel, $d_p = 17 \mu$m, obtained by repeated sedimentation of H 60 gel, (Macherey, Nagel & Co., F.R.G.) was used.

Solvents
n-Hexane (analytically pure) (Reachim, U.S.S.R.), dioxane, isopropanol and methylene chloride (analytically pure) (POCH, Poland) were used as solvents.

Apparatus
The apparatus employed for preparative-scale liquid chromatography was equipped with a pump of output up to 270 ml/min and a UV-254 detector with a measuring vessel of capacity 10 mm^3. The preparative chromatograph was laboratory constructed. Sampling of the substances was performed by means of a valve with a loop. Columns of dimensions 300 × 3.3 mm I. D. and 300 × 50 mm I.D. were used. Analytical columns were used with a KB 5113 chromatograph (Kabid, Poland).

Mixture to be separated
The main components of the post-reaction mixture were *ortho* and *para* isomers of hydroxybiphenyls.
Hydroxybiphenyls are characterized by high molar absorptivities at $\lambda = 254$

nm. An increase in the concentration of the substances in the eluent resulted in the linear dynamic range of the detector being rapidly exceeded. For this reason, the retention volume was determined for the peak front instead of at its maximum. In order to determine the concentration distribution in the band, fractions were collected and the concentration of the component was assayed chromatographically in the system silica gel/dioxane–hexane (1:9, v/v).

RESULTS AND DISCUSSION

The purpose of this work was to obtain standards of *ortho* and *para* isomers of hydroxybiphenyls from a raw mixture. A post-reaction mixture was separated on silica gel using various binary mobile phases prepared by mixing hexane and a polar solvent. It was established that the isomers were well separated using various solvent mixtures. They were also well separated from other compounds present in small amounts.

The separation of a post-reaction mixture on an analytical scale is shown on the Fig. 1. Owing to the type of detector used, three polar solvents were selected for mixing with hexane in further work: methylene chloride, dioxane and isopropanol. Values of k' and α are listed in Table I and the solubilities of the *para* isomer in the various solvents are given in Table II. It can be seen that the solubility of the *para* isomer is low, and there is therefore a problem with the preparation and injection of the sample. (The solubility of the *ortho* isomer is good; *e.g.*, in 1:99, v/v, dioxane–hexane it is 17 mg/ml). In the separation of hydroxybiphenyls, the injection of samples of large volume and low concentration would be disadvantageous in the presence of impurities with k' values slightly lower than that of the *ortho* isomer.

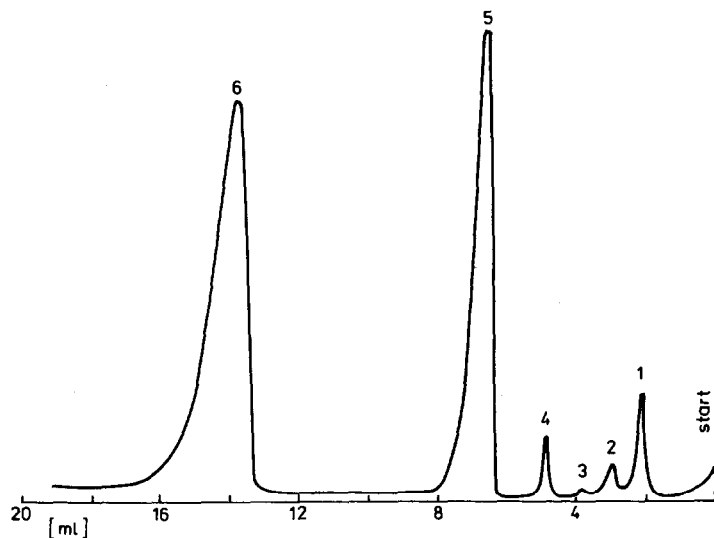Fig. 2 presents the "peaks" obtained after introduction into an analytical col-

Fig. 1. Separation of hydroxybiphenyls on an analytical scale. Column, 300 × 3.3 mm I.D.; stationary phase, silica gel (d_p = 17 μm); mobile phase, dioxane–hexane (5:95, v/v). Peaks: 1–4 = unknown substances; 5 = *ortho* isomer; 6 = *para* isomer.

TABLE I

COMPARISON OF THE EFFECTS OF DIFFERENT MOBILE PHASES ON CHROMATOGRAPH-
IC PARAMETERS

Sample, mixture of *ortho* and *para* isomers of hydroxybiphenyls; column, 300 × 3.3 mm I.D., silica gel
(d_p = 17 μm).

Composition mobile phase (v/v)	Range of capacity factor, k'	Range of selectivity factor, α
Methylene chloride– hexane (15:85–45:55)	56.6–8.7	6.9–5.6
Dioxane–hexane (1:99–7:93)	26.6–4.0	4.9–2.6
Isopropanol– hexane (0.25–99.75–1:99)	13.3–2.6	7.7–3.3

umn of 4.0 mg of the *para* isomer in 0.2 ml of the methylene chloride–dioxane–hexane
(7:1:2, v/v), and in 3.0 ml of the mobile phase (*i.e.*, after diluting 0.2 ml of the sample
to 3.0 ml). The elution profiles obtained with different injection volumes are very
similar. The shift of the front of the peak indicates mass overloading and the lack of
a distinct maximum is probably the result of poor solubility.

The concentration of a substance in the mobile phase can be calculated for a
given k' value assuming that the sample at the top of the packing occupies a space
corresponding to $\sqrt{N}$ plates, *i.e.*, the limiting value above which overloading begins
to affect the shape of the outlet profile[7]. For example, in dioxane–hexane (1:99, v/v)
mobile phase, k'_{para} = 26, with a mass of substance of 4.0 mg and $\sqrt{N}$ = 32, a
concentration in the mobile phase of 2.1 mg/ml is obtained, whereas solubility is 0.89
mg/ml and the linear range of the isotherm is 0.64 mg/ml (3.8 · 10^{-3} M). These data
indicate that explanation of the concentration distribution in the band described
above is probably valid. A similar concentration distribution in the band was ob-
served in hexane–isopropanol as the mobile phase.

TABLE II

SOLUBILITY OF *para* ISOMER IN DIFFERENT MOBILE PHASES

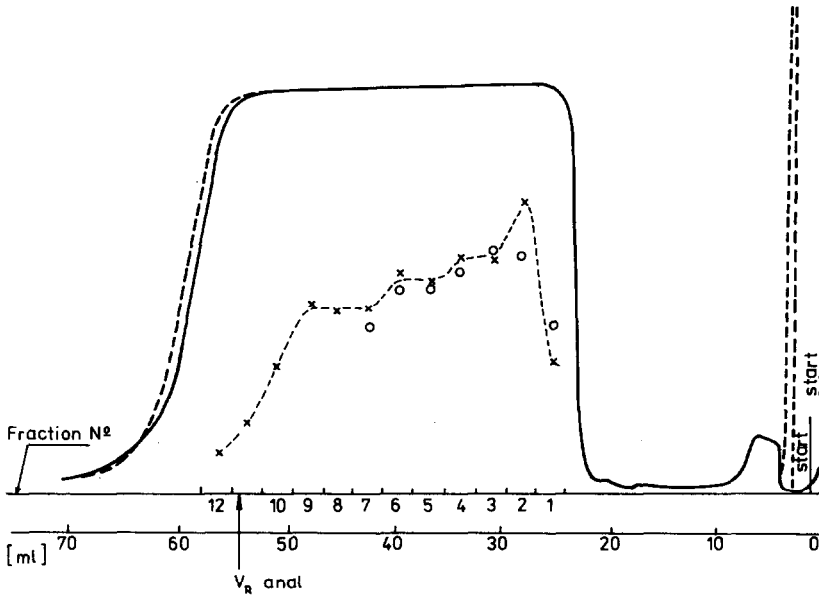| Mobile phase	Composition (v/v)	Solubility (mg/ml)	k'
Methylene chloride– hexane	15:85 25:75 40:60	1.22 2.42 5.02	56.6 9.5
Dioxane–hexane	1:99 2.5:97.5 7:93	0.89 2.02 6.15	26.6 4.0
Isopropanol– hexane	0.25:99.75 0.5:99.5 1:99	0.46 0.91 1.75	13.3 2.6

Fig. 2. Elution curves of *para* isomer. Mobile phase, dioxane–hexane (1:99, v/v). Broken line and (×), sample volume 0.2 ml; solid line and (○), sample volume 4.0 ml; sample mass, 4 mg; column as in Fig. 1.

Higher retention volumes (relative to the analytical peak) of the descending part of the peak with increasing mass of the samples, without a change in the sample volume, were observed for the system containing isopropanol. The results shown in
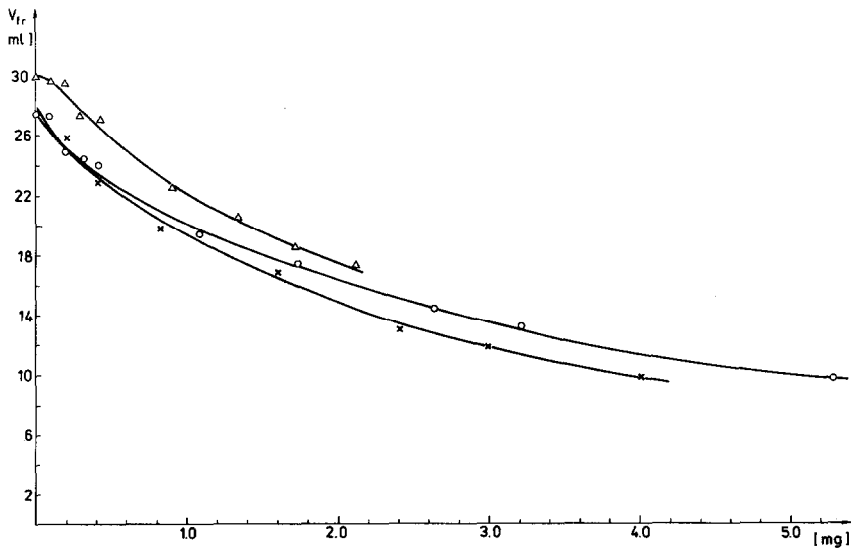

Fig. 3. Shift of the peak front *vs.* sample mass of *para* isomer. Mobile phases: △, isopropanol–hexane (0.25:99.75, v/v); ○, dioxane–hexane (2.5:97.5, v/v); ×, methylene chloride–hexane (36:64, v/v). Column as in Fig. 1.
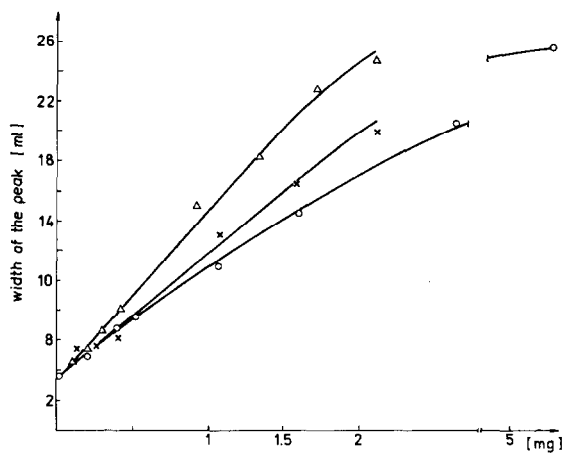
Fig. 4. Width of the peak front *vs.* sample mass of *para* isomer. Conditions and points as in Fig. 3; column as in Fig. 1.
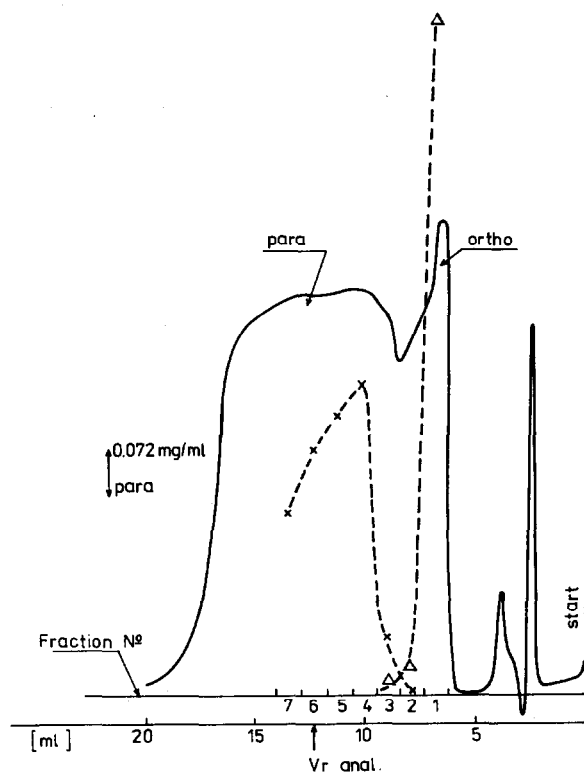

Fig. 5. Elution curves of *ortho* and *para* isomers. Mobile phase, hexane–dioxane (93:7, v/v); sample mass, 2.4 mg; sample volume, 0.2 ml; column as in Fig. 1.

Figs. 3 and 4 confirm this fact. Despite the higher rate of shift of the front of the peak in the mobile phase containing methylene chloride, its total width is still smaller than in 0.25% isopropanol, in which the peak tail was shifted by 30% with respect to the analytical peak. A shift of the back of the peak is also observed in some mobile phases containing dioxane, although to a smaller extent.

In systems containing methylene chloride or dioxane, peaks with a shape similar to a rectangular triangle were observed at higher concentrations of these solvents (Figs. 5 and 6). This can be explained by the fact that an increase in the content of the polar component in the mobile phase is accompanied by increases both in the concentration of the substance in the mobile phase (k' decreases) and in solubility. For the three polar solvents used the solubility increases most rapidly in the mobile phase containing methylene chloride. The solubility no longer influences the peak shape. When the retention of the compounds is moderate ($k'_{para} \approx 5$), for the same k' values the positions of the band fronts of both isomers are very similar. In the presence of the *para* isomer, with an increase in its amount the retention volume of the *ortho* isomer (and all other impurities except for the peak eluted with the solvent front) decreases owing to its displacement from the silica gel bed by the considerably more strongly sorbed *para* isomer. An example of the change in the retention of the *ortho* isomer is shown in Table III. The change increases with increasing retention of both isomers. In addition to the decrease in retention volume, peak narrowing approximately proportional to the decrease in retention volume is also observed.
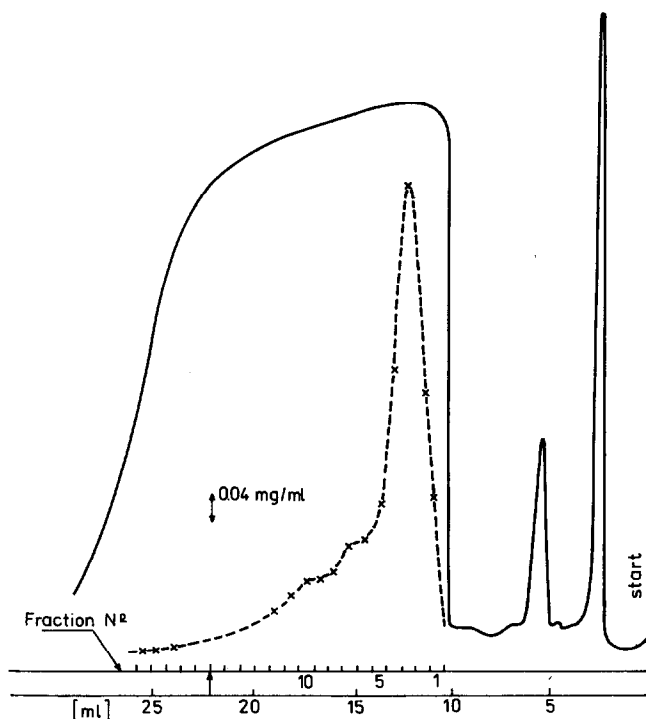

Fig. 6. Elution curve of *para* isomer. Mobile phase, methylene chloride–hexane (3:7, v/v); sample volume, 0.3 ml; sample mass, 3.0 mg; column as in Fig. 1.

TABLE III

RETENTION VOLUME OF *ortho* ISOMER ACCOMPANIED BY *para* ISOMER

Amount of para isomer in sample (mg)	Retention volume of ortho isomer								
	D–H*				C–H**		P–H***		
	1	2	3	4	1	2	1	2	3
0.0	13.4	10.0	6.2	5.5	8.5	5.6	6.0	4.8	3.7
2.0	12.6	9.0	6.1	5.2	7.7	5.3	4.7	3.6	3.3
4.0	11.2	8.7	–	–	7.1	4.8	–	–	–
5.3	–	8.4	5.8	5.1	–	–	–	–	–

* D–H = dioxane–hexane: (1) 1:99; (2) 2.5:97.5; (3) 5:95; (4) 7:93.
** C–H = methylene chloride–hexane: (1) 30:70; (2) 45:55.
*** P–H = isopropanol–hexane: (1) 0.25:99.75; (2) 0.5:99.5; (3) 1:99.

Fig. 7 illustrates the dependence of the position of the peak front and the peak width for the *ortho* isomer on the mass of the *para* isomer in the sample in one of the mobile phases.

In order to facilitate extrapolation of the results, the dependence of the elution volume of the peak front was plotted against the logarithm of substance concentration in the sample (Fig. 8). Linear plots were obtained in the concentration range from 0.8 mg/ml ($4.7 \cdot 10^{-3}$ M) to 20 mg/ml (0.12 M) for all the investigated systems. Evidently, the slope and intercept of the straight lines no longer corresponded to eqn. 1 owing both to replacement of the retention volume of the peak maximum by the elution volume of the peak front and to the processes described earlier.

Table IV lists the throughputs of the *para* isomer determined experimentally in non-linear chromatography and those calculated for linear optimization[3]. The throughput was calculated according to $M = m_s/m_a V_t$, where m_s is the mass of the substance, m_a is the mass of the adsorbent and V_t is the total elution volume. As the
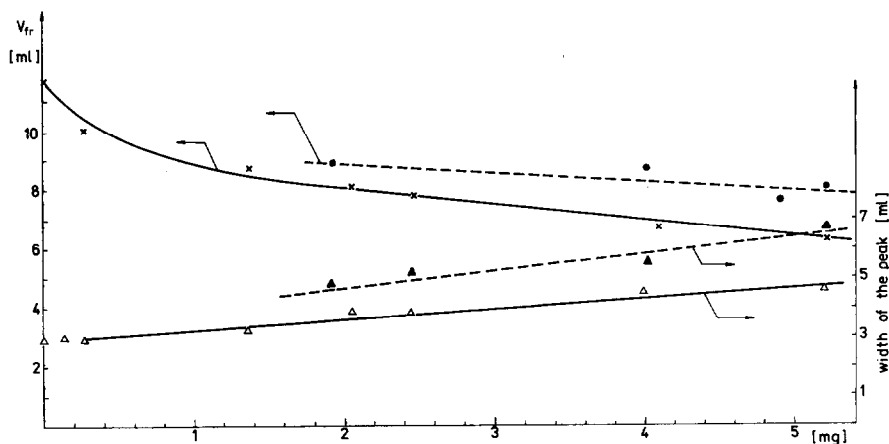

Fig. 7. Shift of the peak front and the peak width *vs.* mass of *ortho* isomer: ×, △, accompanied by *para* isomer; the masses of *ortho* and *para* isomers are equal, ●, ▲, without *para* isomer. Mobile phase, methylene chloride–hexane (25:75, v/v); column as in Fig. 1.
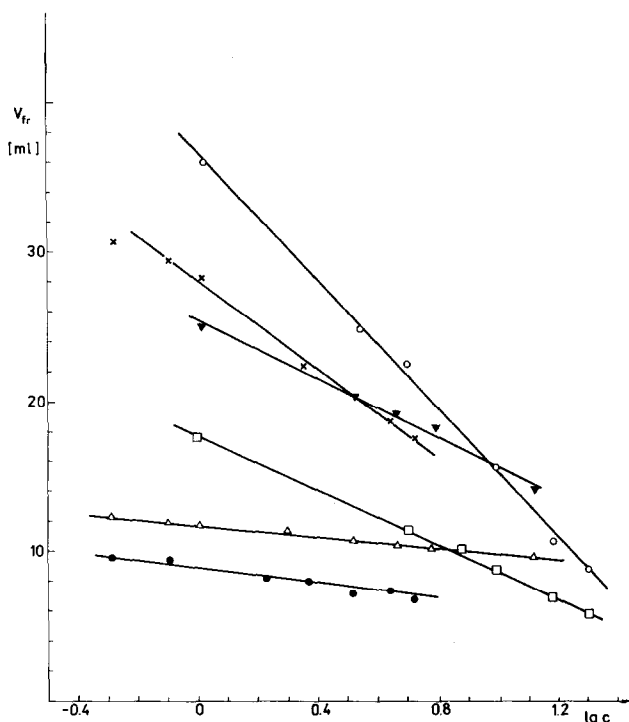
Fig. 8. Peak front elution volume *vs.* logarithm of sample concentration for different mobile phases: ●, dioxane–hexane (1:99, v/v); △, dioxane–hexane (5:99, v/v); □, methylene chloride–hexane (40:60, v/v); ▼, dioxane–hexane (2.5:97.5, v/v); ×, isopropanol–hexane (0.25:99.75, v/v); ○, methylene chloride–hexane (30:70, v/v). Column as in Fig. 1.

overload limit, a mass of the *para* isomer was selected for which the front of its peak intersected at the baseline the tangent to the descending part of the peak of the *ortho* isomer, determined for the analytial sample.

It follows from the experimental data that in chromatography of hydroxybiphenyls the throughput of the process depends to a greater extent on the value of k' than on the selectivity factor α.

In all the investigated systems a higher yield of the *para* isomer was obtained in linear chromatography. Unfortunately, in order to isolate simultaneously both isomers the sample volume, *e.g.*, in the dioxane–hexane (5:95, v/v) system, cannot exceed 1.0 ml and the output of linear chromatography decreases to 0.05 mg/ml · g. The sample can be separated in two steps, *i.e.*, isolation of the *para* isomer is followed by concentration of the sample and isolation of the *ortho* isomer. The total yield of the process is then 0.24 mg/ml · g (the time required for sample pre-concentration was not taken into account).

Tests of the described solvent compositions resulted in selection of dioxane–hexane (5:95, v/v) as the optimum mobile phase. The sample injected into an analytical column can contain at most 6.0 and 3.0 mg of *para* and *ortho* isomers, respectively, whereas the respective values for the sample injected into a preparative

TABLE IV

COMPARISON OF THROUGHPUTS OF *para* ISOMER OBTAINED WITH DIFFERENT MOBILE PHASES

M_1 is the throughput in linear chromatography, M_2 is the throughput in non-linear chromatography.

Mobile phase	Composition (v/v)	k'_{para}	α	M_1 (mg/ml · g)	M_2 (mg/ml · g)
Dioxane–hexane	1:99	26.6	4.9	0.13	0.05
	2.5:97.5	12.5	3.4	0.26	0.11
	5:95	5.4	2.9	0.30	0.25
	7:93	4.0	2.6	0.27	0.13
Methylene chloride–hexane	30:70	18.5	6.6	0.24	0.08
	40:60	9.5	5.5	0.32	0.12
Isopropanol–hexane	0.25:99.75	16.2	7.7	0.26	0.06
	0.5:99.5	6.0	5.0	0.35	0.14
	1:99	2.6	3.3	0.29	0.12

column are 1.3 and 0.7 g, respectively. One operation in the preparative column yielded 1.25 and 0.63 g of pure *para* and *ortho* isomers, respectively.

CONCLUSIONS

The preparation of a solution of a sample mixture in a solvent stronger than the mobile phase and injection of small sample volumes is possible and overcomes problems associated with low solubility of the sample components. If the separation of *ortho* and *para* isomers was satisfactory ($\alpha > 2$), decreasing the elution strength of the mobile phase, leading to an increase in k' of the *para* isomer to *ca.* 10, resulted in a substantial decrease of the throughput.

The best throughput was obtained in dioxane–hexane (5:95, v/v) at $k'_{para} = 5.4$ and $\alpha = 2.9$. As a displacement effect occurs, selection of the separation conditions should be carried out using a mixture and not the individual components.

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