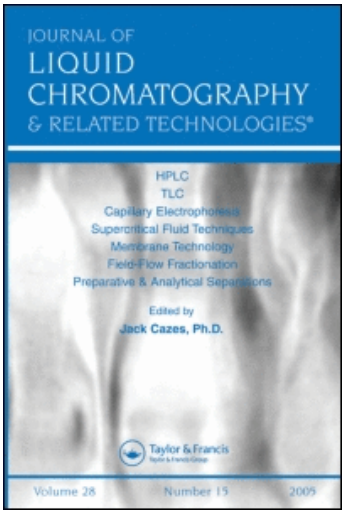

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OPTIMIZATION OF THE RESOLUTION OF THE ENANTIOMERS OF β-DIMETHYLAMINO-BUTYROPHENONE BY HPLC ON A β-CYCLODEXTRIN COLUMN

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ABSTRACT

The enantiomers of β-dimethylaminobutyrophenone have been separated by HPLC on a native β-cyclodextrin bonded column using a polar organic eluent consisting of acetonitrile/methanol/acetic acid/triethylamine. To achieve this separation, the mobile phase composition has been optimized with respect to peak resolution and analysis time using a two-levels full factorial design. The important factors controlling the separation have been identified : the resolution is mainly affected by the composition and the quantity of buffer and the retention by the amount of methanol. The polynomial equations provided by the factorial design have been modified in order to enhance their reliability in an experimental domain larger than the factorial design domain.

INTRODUCTION

Among various phenylpropylamides exhibiting analgesic activity, one of the most important is the dextropropoxyphene (α-

d-4-dimethylamino-3-methyl-1,2-diphenyl-2-butanol propionate) and its derivatives. The α-dl- and α-d-diastereoisomers possess marked analgesic activity, in contrast to the β-diastereoisomers which are substantially inactive.

The preparation of dextropropoxyphene involves 3 steps (1) : preparation of β-dimethylaminobutyrophenone by a Mannich reaction, formation of the aminocarbinol (4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol) by a Grignard reaction, followed by an esterification with propionic anhydride. The α-form was obtained by fractional crystallization of the pair of diastereoisomeric salts formed by reaction of the aminocarbinol with d-camphorsulfonic acid. In 1963, Pohland et al. (2) have shown that the (-) enantiomer of the phenone is optically stable and can be used as a raw material for the stereoselective synthesis of dextropropoxyphene. The phenone racemate was resolved by fractional crystallization of its dibenzoyltartrate salt.

In this work, we have investigated an alternative method of separation of the enantiomers of the β-dimethylaminobutyrophenone by HPLC on a chiral stationnary phase (CSP) in view of a future preparative application. The α_1-acid glycoprotein (AGP) CSP appears to have a wide applicability to molecules of pharmacological interest and is able to resolve the propoxyphene enantiomers (3). However, the AGP column has a limited stability and a low capacity. This makes preparative separations difficult or impossible. An efficient alternative method was found by Armstrong et al. (4) by using an unusual mobile phase consisting of a mixture of polar organic solvents in conjunction with the original native cyclodextrin bonded phase, to resolve racemic β-adrenergic blocking agents such as propranolol and analogous compounds. These CSP's have exceptional stability when used with these mobile phases. In addition, the separations are easily scaled to preparative proportions.

In this work, a cyclodextrin CSP with a polar organic mobile phase was used. The objectives were (i) to achieve a complete

separation of enantiomers, (ii) to understand the importance of the chromatographic conditions and (iii) to get a statistical model of the effect of the experimental variables on the separation.

First attempt of resolution using the best chromatographic conditions of Armstrong et al. (4) produced only a partial separation. An optimization procedure, varying several factors at a time was investigated. Among these, the Simplex method, that is mathematically simple and easy to implement has been widely used in analytical chemistry and sometimes in the optimization of chiral separations (see for example Ley et al., (5)). However, this method does not give informations concerning the relative importance of the experimental factors. Therefore, a two-levels full factorial design was applied to determine the magnitude of the effect of the chromatographic variables and their interactions (6). In addition, the factorial design provides a model that was modified to enhance its reliability and checked on a new set of experimental data. The advantage of the factorial design is to bring a simplified representation of a phenomenon from very few experiments. The idea was that, with some modifications of the form of the model, it could be possible to approximate more closely the behavior of the chromatography. Some of these modifications could be suggested by models existing in the chromatographic literature.

EXPERIMENTAL

Chromatographic experiments were carried out at room temperature using an HPLC pump equipped with a 20-µL injection loop and an U.V. detector. A Cyclobond-I column (250x4.6 mm i.d., Advanced Separation Technologies) packed with native β-cyclodextrin bonded to a 5 µm silica gel was used. In all cases the flow rate of the eluent was 1 ml/mn.

FACTORIAL DESIGN RESULTS

According to Armstrong et al. (4), the separation of β-blockers can be obtained with acetonitrile as main solvent and requires small amounts of methanol (1-10% by volume) and very small amounts of glacial acetic acid and triethylamine (0.2-1.2% by volume).

After some vain attempts with other additives (water, isopropanol), three experimental variables were selected : the amount of acetic acid/triethylamine buffer, X_1, the acetic acid/triethylamine ratio, X_2 and the amount of methanol, X_3. For each natural variable, the two levels associated to the +1 and -1 levels of the corresponding coded variables are indicated in Table 1. The quality of the separation was estimated through the resolution Rs, measured on the chromatogram as:

$$Rs = 2\,(t_2 - t_1)/\,(t_{w1} + t_{w2}),$$

and the capacity factor of the first eluted peak,

$$k'_1 = t_1/t_0 - 1.$$

t_i being the retention time and t_{wi} the peak width at half-height.

The chromatographic conditions of the eight (2^3) experiments are reported in Table 2, together with the separation results. In the formalism of full fractional design, the results of the separation can be approximated by a linear combination of the coded variables :

$$Y = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_3 + a_{12} X_1 X_2 + a_{13} X_1 X_3 + a_{23} X_2 X_3 + a_{123} X_1 X_2 X_3 \qquad (1)$$

It should be noted that the capacity factor was correlated in a logarithm form, for reasons discussed in the "Modelization..." section. The magnitude of the coefficients corresponding to main factors and two-factors interactions, tabulated in Table 2, indicates the relative importance of each variable. It can be seen that the resolution mainly increases with the acid/amine ratio, X_2 and slighly with the buffer percentage, X_1. The interaction term $b_{23} = -0.029$ means that the influence of the percentage of

TABLE 1
Values of the Experimental Factors in the Factorial Design.

	Factors	level -1	level +1
X_1	percentage of buffer	0.02	0.2
X_2	acid/amine ratio	6	30
X_3	percentage of methanol	1	10

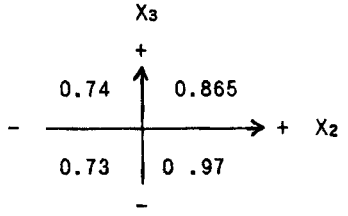
TABLE 2
Chromatographic Conditions and Responses (Resolution Rs and Capacity Factor k'_1 of the First Eluted Enantiomer) in the Factorial Design.

Run	x_1	x_2	x_3	Rs	k'_1
1	+1	+1	+1	0.93	3.19
2	-1	+1	+1	0.80	3.95
3	+1	-1	+1	0.74	1.95
4	-1	-1	+1	0.74	2.60
5	+1	+1	-1	1.04	9.25
6	-1	+1	-1	0.90	10.38
7	+1	-1	-1	0.78	4.50
8	-1	-1	-1	0.68	3.71

methanol depends on the level of X_2. This is clearly shown on the interaction diagram (scheme 1) obtained in the following way : the right top number (0.865) corresponds to the mean value of Rs for the two experiments performed with both high acid/amine ratio (X_2 = +1) and high percentage of methanol (X_3 = +1).

The amount of methanol has a negative effect on the resolution only at high acid/amine ratio. The same result is observed for the interaction X_1- X_3.

On the contrary, the capacity factor mainly depends on the amount of methanol, and whatever the level of the others factors, an increase of methanol will reduce the capacity factor.



Scheme 1

SUPPLEMENTARY EXPERIMENTS

In the factorial design, the best resolution (1.04) has been obtained at low methanol amount, and high acetic acid amount in the eluent (high acid/amine ratio and high percentage of buffer). But the separation is not complete and further experiments have been performed in the direction indicated by the factorial design, first to improve this result and second to investigate the effect of the individual parameters in order to test the models. These experiments are referred in the following as the "new set of experiments". It consists of 43 experiments, in which the percentage of buffer was varied between 0.02 and 0.4 %, the acetic acid/triethylamine ratio r between 5 and 100 and the percentage of methanol between 0 and 20 %.

A maximum resolution of 1.17, corresponding to a baseline separation was obtained with 2 % methanol, 0.15 % buffer, and r = 50. In these conditions, the capacity factor is quite high (12.0).

Figures 1 and 2 show that when only the percentage of methanol, or of buffer, is varied the resolution reaches a maximum value, around 3 % of methanol, or 0.15 % of buffer. In both cases, the retention factor decreases with an increasing percentage of additives. When the acid/amine ratio r is increased, both k' and Rs increase and reach a plateau at r = 50 (Fig. 3).

It can be noted that Armstrong et al. (4) observed the same behavior in the resolution of propranolol. They interpret the

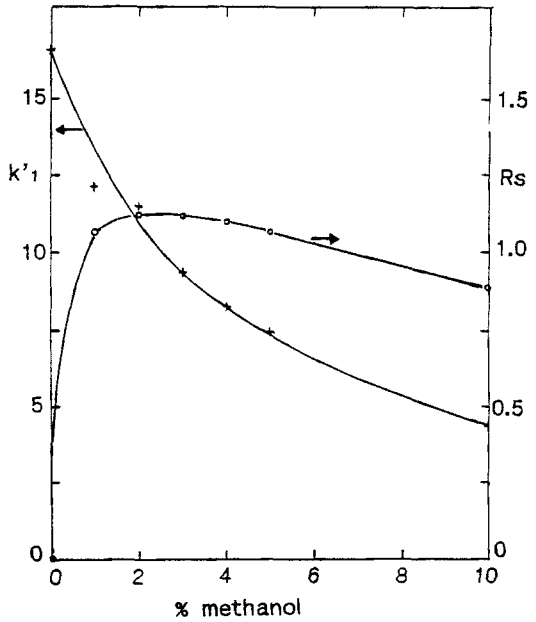


FIGURE 1. Influence of the amount of methanol in the mobile phase on the capacity factor k'_1 (+) and the resolution Rs (o). Conditions: Column, Cyclobond-I (250x4.6 mm, 5 µm); mobile phase : 0.2 % (by volume) of the acetic acid/triethylamine buffer at an acid/amine ratio, 50 in acetonitrile; flowrate, 1 mL/mn; U.V. detection at 254 nm.

retention results by a better affinity for the stationary phase of the protonated amine relatively to the free enantiomer, leading to a slower elution. Actually, because of the high acid/amine ratio, there is an excess in acid that will be able to protonate the amine group of the enantiomer. But, as on the one hand, the acid excess is produced by an increase in either the buffer amount or the r ratio, and on the other hand, the buffer amount decreases the retention while the r ratio increases it, an additional mechanism, involving direct competition between buffer and enantiomers for the inclusion in the β-cyclodextrin cavity, can be

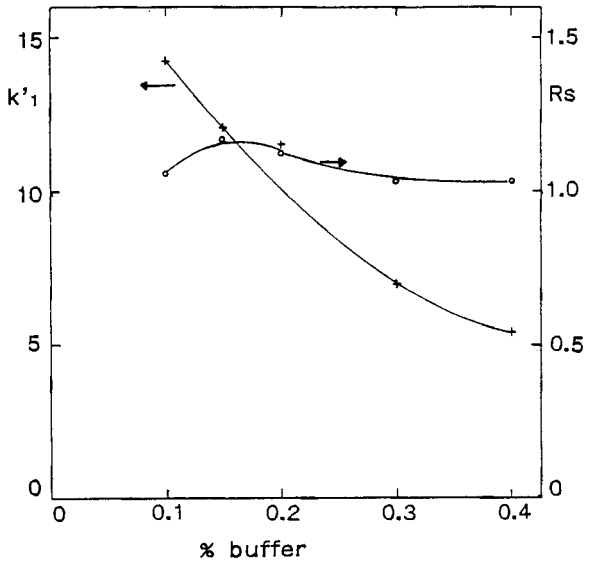


FIGURE 2. Influence of the amount of acetic acid/triethylamine buffer in the mobile phase on the capacity factor k'_1 (+) and the resolution Rs (o). Conditions : Column, Cyclobond-I (250x4.6 mm, 5 µm); mobile phase : 2 % (by volume) of methanol in acetonitrile, acid/amine ratio, 50; flowrate, 1 mL/mn; U.V. detection at 254 nm.

invoked. Inclusion of methanol in the cavity is also probably the cause of the observed reduction in retention time.

MODELIZATION OF RETENTION AND RESOLUTION

The polynomial model provided by the factorial design (Eq. 1) is expressed in terms of coded variables (level +1 or -1). To check the reliability of the model over the new set of experimental data, a transformation of natural variables to coded variables is needed. The simplest one is a linear transformation :

$$X_{coded} = -1 + 2\,(X_{natural} - X_{-1})/(X_{+1} - X_{-1})$$

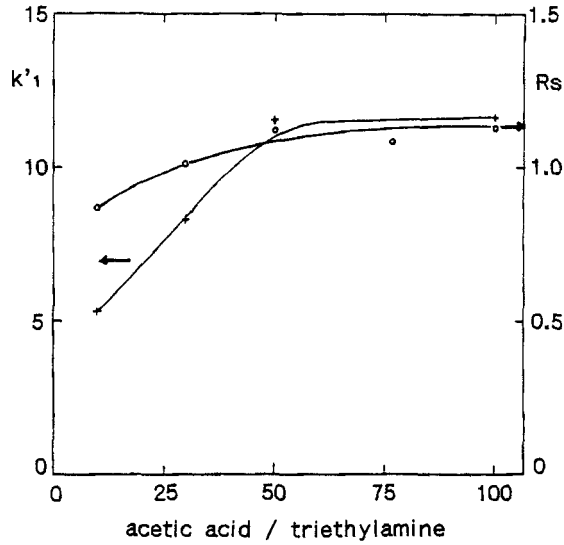


FIGURE 3. Influence of the acetic acid/triethylamine ratio in the buffer on the capacity factor k'_1 (+) and the resolution Rs (o). Conditions : Column, Cyclobond-I (250x4.6 mm, 5 µm); mobile phase : 2 % (by volume) of methanol and 0.2 % (by volume) of the buffer in acetonitrile; flowrate, 1 mL/mn; U.V. detection at 254 nm.

x_{+1} and x_{-1} being the values of the natural variables taken in the factorial design (Table 1).

Such models, with coefficients given in Table 3, represent the experimental data with a mean error of 15 % on Rs and 23 % on Log k'_1. This is a bad representation since the dispersion of the experimental points, expressed in standard deviation, is 16 % on Rs and 27% on Log k'_1. It must be noted that the capacity factor was correlated in a logarithm form, after the physicochemical model of Snyder et al. (7) giving the mobile phase effect on retention in non-chiral chromatography, in form of Log k'_1 in function of the additives molar fractions. In this work, it has been verified that the Log k'_1 model gives more accurate results than the k'_1 model.

TABLE 3
Coefficients of the Effects and Interactions of the Variables Considered in the Factorial Design, Calculated for the Resolution and for the Capacity Factor of the First Eluted Peak.

Factors		Rs	Log k'_1
average	b_0	0.826	1.442
X_1, % buffer	b_1	0.046	-0.053
X_2, acid/amine ratio	b_2	0.091	0.332
X_3, % methanol	b_3	-0.024	-0.403
interaction X_1-X_2	b_{12}	0.021	-0.029
interaction X_1-X_3	b_{13}	-0.014	-0.072
interaction X_2-X_3	b_{23}	-0.029	-0.105

The main discrepancy between model and experiments comes from the linear representation of the response when one parameter at-a-time is varied, whereas figures 2-4 show a very different behavior. Since the model is established using only two values of each variable, a certain curvature can be introduced by a non-linear transformation of natural variables to coded variables, and that without changing the coefficients of the models. In this way, the mean errors have been reduced by a factor 2-3, by modifying the acid/amine ratio r in Log(r-1) : a mean error of 5 % on the resolution, and of 12 % on the capacity factor are obtained. It is noteworthy that this transformation has a physical meaning : Log(r-1) is closely connected to the pH of the buffer solution, i.e. to the concentration of proton.

It may be added that other models have been built using a more theoretical approach, particularly by decomposing the resolution in selectivity, column efficiency and retention terms, but the final mean error has the same magnitude as in the statistical models presented here.

REFERENCES

(1) Pohland, A. and Sullivan, H.R., Preparation of α-d- and α-l-4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionyloxybutane, J. Am. Chem. Soc., 77, 3400, 1955.

(2) Pohland, A., Peter, L.R. and Sullivan, H.R., Analgesics. Stereoselective Syntheses of α-(+)- and α-(-)-4-Dimethylamino-1,2-diphenyl-3-methyl-2-propionyloxybutane, J. Org. Chem., 28, 2483, 1963.

(3) Schill, G., Wainer, I.W. and Barkan, S.A., Chiral Separation of Cationic Drugs on an α_1-Acid Glycoprotein Bonded Stationary Phase, J. Liq. Chromatogr., 9, 641, 1986.

(4) Armstrong, D.W., Chen, S., Chang, C. and Chang, S., A New Approach for the Direct Resolution od Racemic beta-Adrenergic Blocking agent by HPLC, J. Liq. Chromatogr., 15, 545, 1992.

(5) Ley, G.W., Fell, A.F. and Kayes, B., Recent Advances in Chiral Separations, Plenum Press, New York, 1991, p. 97.

(6) Box, G.E.P. and Draper, N.R., Empirical Model-Building and Response Surfaces, Wiley, New York, 1987, p. 105.

(7) Snyder, L.R., Glajch, J.L. and Kirkland, J.J., Practical HPLC Method Development, Wiley, New York, 1988, p.202.

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