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Separation of barbiturates with micellar liquid chromatography and optimization by a second order mathematical design

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Abstract

A series of active experiments for the micellar chromatographic separation of seven barbiturates was carried out. The influence of the principal factors, namely the micelle concentration, the amount of organic modifier and the mobile phase pH, on the retention behaviour was quantified. The established response hypersurfaces allow to predict the areas of the multifactorial space where an improved separation of those peak pairs which are difficult to resolve can be expected. The correctness of this approach was proven experimentally. © 1998 Elsevier Science B.V.

Keywords: Micellar liquid chromatography; Experimental design; Optimization; Second order design; Barbiturates

1. Introduction

Micellar liquid chromatography (MLC) represents a rather uncommon mode of reversed-phase high-performance liquid chromatography (RP-HPLC) although micellar mobile phases show several advantages compared to the conventional hydro-organic eluents such as low cost, low toxicity, the possibility of simultaneous separation of ionic and nonionic compounds, direct injection of serum or plasma samples, low volatility, uncommon separation selectivity (e.g. a reversal of the elution order with a change in micelle concentration) or the enhancement of separation selectivity by an increase of the organic modifier content [1–4]. Modeling and prediction of chromatographic retention, which should lead to an improved separation, is a complicated process in MLC because the chromatography is influenced by a large number of factors, e.g. the type and concentration of the surfactant, the type and volume fraction of organic modifier, or the mobile phase pH. The influence of different factors on the chromatographic separation can be studied by passive (traditional) and so-called active experiments. A passive experiment is monofactorial, i.e. only one factor is varied at fixed values of all the others. Under such conditions, the study of interaction of factors is very complicated or practically impossible. Therefore active experiments, carried out via mathematical design, need to be performed which reveal the interaction of factors. The realization of active experiments for the establishment of a mathematical model of chromatographic separation has its particular features by using RP-HPLC complicated by the existence of secondary chemical equilibria in the mobile phase, viz. ion-pair chromatography (IPC)

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[5,6] and MLC. The simultaneous optimization of different factors in MLC by using iterative regression optimization strategies has been demonstrated in several excellent papers [7–10]. This optimization approach is based on linear relationships between influencing factors and retention; when deviations from linearity are remarkable, higher order models are necessary [8].

Chromatographic behaviour in RP-HPLC with micellar and submicellar mobile phases was successfully studied by using barbiturates as model compounds [11,12]. In our work a group of seven barbiturates was selected as model mixture, see Fig. 1. It contains compounds of different polarity, e.g. polar barbital and nonpolar benzonal. The selection of these barbiturates is also stimulated by their wide medical application as drugs with antiepileptic, narcotic and sedative actions. Some of them are often used simultaneously, e.g. phenobarbital and hexamydin or phenobarbital and benzonal, some are metabolized in the organism and transformed into each other, e.g. benzonal, halonal and hexamydin are metabolized to phenobarbital.

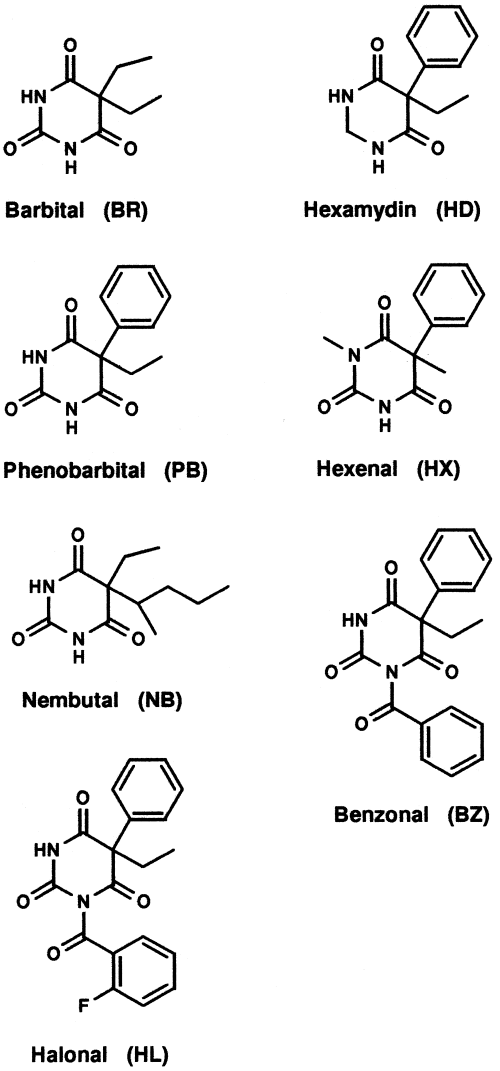

Fig. 1. Structures of the barbiturates used in this study.

Our previous work dealt with the use of a first order mathematical design for the description of the separation of barbiturates by MLC [13]. An empirical regression equation was deduced which describes the influence of micelle concentration, amount of organic modifier and mobile phase pH (as principal factors) on the selectivity and resolution of the chromatographic separation. A strong interaction between principal factors was revealed. This indicates that the use of higher order mathematical equations is essential for the precise description of the quantitative relationships. Such equations are found via second order design methods as presented in this paper.

2. Experimental

2.1. Conditions

Column: 6.2 cm×2 mm I.D.; stationary phase: Separon-C_{18}, 5 μm (Lachema, Brno, Czechia); flow-rate: 50 μl/min; temperature: ambient; instrument: Milichrom 4 (Nauchpribor, Oryol, Russia); detection: UV 220 nm.

Sodium dodecyl sulfate (SDS) was purchased from AO Ekros (St. Petersburg, Russia). Barbital (BR), hexamydin (HD), phenobarbital (PB), nembutal (NB) and hexenal (HX) were obtained from a plant of medical preparations (Kiev, Ukraine). Benzonal (BZ) and Halonal (HL) were kindly supplied by the Laboratory of Organic Synthesis of the Tomsk Polytechnic Institute (Tomsk, Russia). *n*-Pentanol and *n*-heptanol were obtained from a synthetic products plant (Novocherkask, Russia).

The column dead volume was determined by the

injection of water and was defined as the mobile phase volume between injection and the first deviation of the baseline. The solutes were dissolved in methanol. Micellar mobile phases were prepared by dissolving the appropriate amount of SDS (see Table 1) in 0.05 *M* sodium dihydrogenphosphate solution. The desired pH was adjusted with phosphoric acid or sodium hydroxide and finally the required amount of organic modifiers, pentanol–heptanol (3:1, v/v) was added.

The separation behaviour of the barbiturates was investigated with twenty eluents of different composition within the range of the second order mathematical design. For the improvement of separations three pairs of solutes were then chosen: HL and BZ as a very demanding separation problem and, as easier problems, HD and PB as well as HX and NB.

The optimization parameters Y_1–Y_3 represent the following values:

$Y_1 = R_s$ (BZ, HL), i.e. the resolution, R_s, of BZ and HL;

$Y_2 = \alpha$ (HD, PB), i.e. the separation factor, α, (selectivity) of HD and PB;

$Y_3 = \alpha$ (HX, NB), i.e. the separation factor of HX and NB.

The choice of R_s as the optimization parameter for the pair BZ and HL was indicated by the hydrophobic nature of these compounds. In comparison with other barbiturates, BZ and HL are eluted late and as rather broad peaks. Therefore it was necessary to take their peak widths into account. For the other pairs, HD and PB and HX and NB, narrow, symmetrical peaks were obtained, therefore peak width is of minor importance and the selectivity α was chosen as optimization parameter; high selectivity is a prerequisite for good resolution.

As principal factors *Z* (or operational initial values) the following parameters were chosen:

Z_1 = micelle concentration in the mobile phase (m*M*);

Z_2 = the amount of organic modifier in the mobile phase (ml/l);

Z_3 = the mobile phase pH.

A second order mathematical design allows the variation of the principal factors within wider ranges compared to a first order design as it was used in our previous paper [13] (which explains the choice of principal factors and the conditions which limit their variation). The levels of all the principal factors *Z* as well as the respective intervals of variation (h) are listed in Table 1. With these data the relationship between the factors *Z* and their respective dimensionless coded variables *x* are as follows [14]:

$$x_1 = \frac{Z_1 - 100}{30} \quad (1a)$$

$$x_2 = \frac{Z_2 - 50}{15} \quad (1b)$$

$$x_3 = \frac{Z_3 - 5}{1.5} \quad (1c)$$

3. Results and discussion

3.1. Calculation of the response hypersurfaces

A second order design can be performed by using different methods, e.g. a three level experiment (3^n type), an orthogonal design of the second order, or a rotatable design of second order [15]. The realization of experiments of the 3^n type requires to carry out a great number of trials; e.g. in the case of three-

Table 1
Conditions of multifactorial experiments

Factor	Level			Interval (h)
	Z_{min}	Z_0	Z_{max}	
Z_1: micelle concentration (m*M*)	70	100	130	30
Z_2: organic modifier content (ml/l)	35	50	65	15
Z_3: pH	3.5	5.0	6.5	1.5

factorial experiments the number of essential trials makes $3^3=27$. The number of trials with the orthogonal design is comparatively reduced but the obtained precision of the response hypersurface in the factorial space is different in the various directions. This circumstance creates certain difficulties for the experimenter because the direction of the shift in the factorial space is unknown in advance. These disadvantages for the improvement of response are not present in the rotatable design of second order. It does not require to carry out such a great number of trials as in the 3^n type design. Additionally, the response hypersurface is determined practically with the same precision in the different directions of the factorial space. Therefore, the rotatable design [15] was selected for the determination of the regression equation of second order.

The matrix of the rotatable design for the three-factorial experiment of second order is shown in Table 2 together with the results, i.e. the numerical values of the optimization parameters Y_1–Y_3. x_0 reflects the so-called dummy variable which accepts only the value of $+1$ and is used for the calculation of the coefficient b_0. For the calculation of the coefficients b_{12}, b_{13} and b_{23}, reflecting the interaction of factors, the scalar products of x_1x_2, x_1x_3 and x_2x_3 on the respective vector-columns Y_1 to Y_3 were used. In order to calculate the square effects b_{11}, b_{22} and b_{33}, the scalar products of x_1^2, x_2^2 and x_3^2 on the Y_1 to Y_3 vector-columns were utilized analogously. A total of 20 experiments were performed at regularly arranged points within the factorial space as well as in the centre (experiments 15–20).

Y_1, the resolution of BZ and HL, is highest in experiment 3 with $x_1=x_3=-1$ and $x_2=+1$ (micelle concentration 70 m*M*, organic modifier concentration 65 ml/l, pH 3.5). A separation at these conditions is shown in Fig. 2 where $Y_1=R_s=0.8$. For the two pairs HD and PB, HX and NB the separation factors are highest in experiment 7 with $x_1=-1$, $x_2=x_3=+1$ (micelle concentration 70 m*M*, organic modifier concentration = 65 ml/l, pH 6.5), see Fig. 3; the separation factors are $Y_2=\alpha$(HD, PB)=1.42 and $Y_3=\alpha$(HX, NB)=1.37.

These results were processed according to known rules [15] and gave the empirical second order

Table 2
Matrix of design and experimental results

Trial number	Matrix of design				Results		
	x_0	x_1	x_2	x_3	Y_1	Y_2	Y_3
1	+1	−1	−1	−1	0.57	1.05	1.11
2	+1	+1	−1	−1	0.33	1.16	1.10
3	+1	−1	+1	−1	0.80	1.25	1.17
4	+1	+1	+1	−1	0.21	1.36	1.15
5	+1	−1	−1	+1	0.44	1.05	1.00
6	+1	+1	−1	+1	0.74	1.20	1.12
7	+1	−1	+1	+1	0.33	1.42	1.37
8	+1	+1	+1	+1	0.02	1.15	1.12
9	+1	−1.682	0	0	0.36	1.23	1.03
10	+1	+1.682	0	0	0.20	1.09	1.02
11	+1	0	−1.682	0	0.32	1.14	1.08
12	+1	0	+1.682	0	0.56	1.09	1.10
13	+1	0	0	−1.682	0.48	1.05	1.02
14	+1	0	0	+1.682	0.47	1.16	1.02
15	+1	0	0	0	0.52	1.15	1.08
16	+1	0	0	0	0.62	1.13	1.06
17	+1	0	0	0	0.55	1.15	1.08
18	+1	0	0	0	0.62	1.21	1.10
19	+1	0	0	0	0.70	1.15	1.06
20	+1	0	0	0	0.65	1.12	1.06

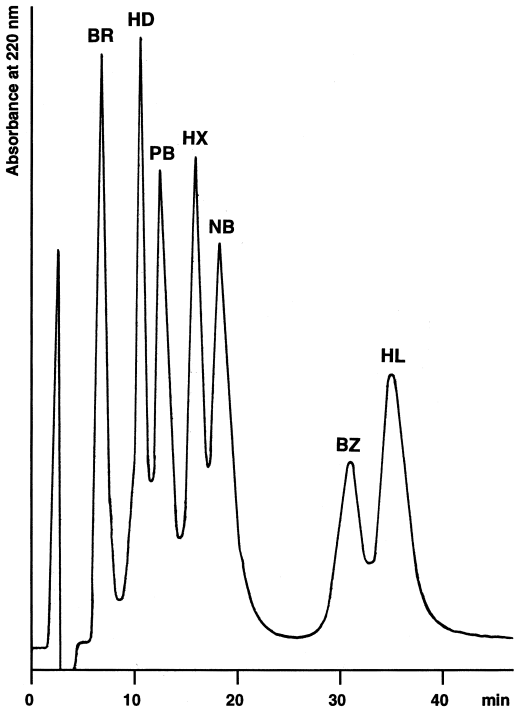

Fig. 2. Separation of barbiturates under the conditions of trial 3: $Z_1 = 70$ mM, $Z_2 = 65$ ml/l, $Z_3 =$ pH 3.5.

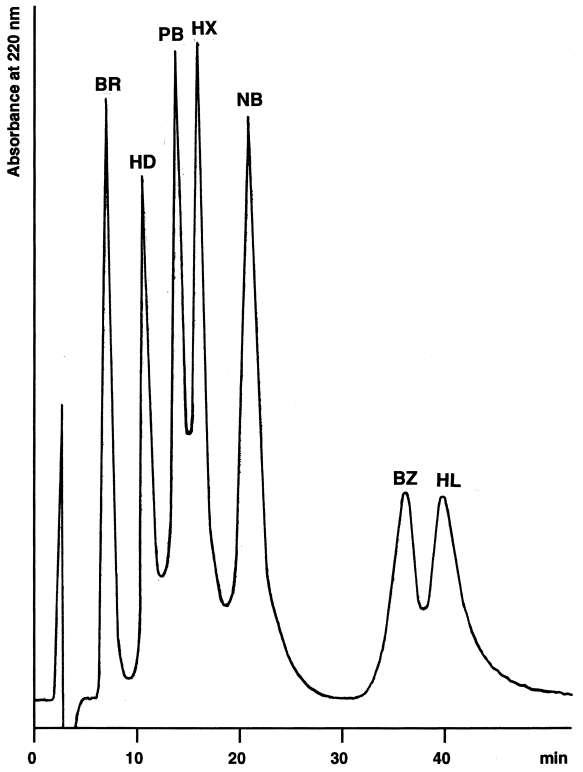

Fig. 3. Separation of barbiturates under the conditions of trial 7: $Z_1 = 70$ mM, $Z_2 = 65$ ml/l, $Z_3 =$ pH 6.5.

regression equations for $\overline{Y_1}$ to $\overline{Y_3}$ which represent the empirical evaluations of the conditional mathematical expectation of optimization parameters Y_1 to Y_3:

$$\overline{Y_1} = 0.5796 - 0.0650x_1 + 0.0528x_2 - 0.0230x_3 - 0.725x_1x_2 + 0.0300x_1x_3 + 0.0325x_2x_3 - 0.1074x_1^2 - 0.0826x_2^2 + 0.0093x_3^2 \quad (2)$$

$$\overline{Y_2} = 1.1479 - 0.0099x_1 + 0.0465x_2 + 0.0135x_3 - 0.0525x_1x_2 - 0.0425x_1x_3 - 0.0100x_2x_3 + 0.0200x_1^2 + 0.0041x_2^2 + 0.0006x_3^2 \quad (3)$$

$$\overline{Y_3} = 1.0690 - 0.0129x_1 + 0.0376x_2 + 0.0059x_3 - 0.0475x_1x_2 - 0.0125x_1x_3 + 0.0325x_2x_3 + 0.0041x_1^2 + 0.0270x_2^2 + 0.0023x_3^2 \quad (4)$$

These equations reflect quantitatively the relationship between principal factors and optimization parameters. In order to establish the significance of deviations from zero of the coefficients listed in Eqs. (2)–(4), i.e. the significance of regression coefficients, the standard deviations of these coefficients were calculated [15] and Student's criterion applied. For this purpose the results of trials 15–20 carried out in the centre of the plan were utilized and on this basis the scatters of reproducibility S^2_{rep} were determined:

For $\overline{Y_1}$, $S^2_{rep} = 7.47 \cdot 10^{-3}$

For $\overline{Y_2}$, $S^2_{rep} = 9.77 \cdot 10^{-4}$

For $\overline{Y_3}$, $S^2_{rep} = 2.67 \cdot 10^{-4}$

It should be noted that S^2_{rep} for $\overline{Y_1}$ is somewhat elevated which comes from random errors of observation (Table 2). The confidence probability was taken equal to 90%.

The application of Student's criterion to Eq. (2) showed that, at the 10% significance level, the coefficients $b_3 = 0.023$, $b_{13} = 0.03$, $b_{23} = 0.0325$ and $b_{33} = 0.0093$ are negligible. This means that the influence of the third factor (pH) on both the chromatographic resolution and on its interaction with the other factors is negligible. In contrast to this, the direct influence of the first and second factors (micelle concentration and amount of modifier) on the resolution, which is reflected by coefficient $b_{12} = 0.725$, is very important as well as their interaction.

The analogous statistical analysis of Eq. (3) indicates that, at the 10% significance level, the coefficients b_1, b_3, b_{23}, b_{33}, b_{33} may be negligible. This points to the very sparing influence of the third factor (pH) on chromatographic selectivity. However, its interaction with the first factor (micelle concentration) is important, and the interaction of the first with the second factor (amount of modifier) is also significant.

Concerning Eq. (4), the coefficients b_3, b_{11} and b_{33} may be neglected at the 10% significance level. In this case, the direct influence of the third factor on selectivity is weak but its interaction with the first and second factors is highly important as well as the interaction of these two factors.

The conformity of regression equations and experimental data was checked with Fisher's criterion at low levels of significance. It was found that Eqs. (2) and (3) are adequate at the 1% significance level to the experiment and that Eq. (4) is adequate at the 0.1% significance level.

3.2. Investigation of the response hypersurface

In the first step the extremal points S [15] of the response hypersurfaces of the factorial space were determined. For this purpose the derivatives of Eqs. (2)–(4) to x_1–x_3 were set equal to zero:

$$\frac{\partial \overline{Y}_i}{\partial x_j} = 0 \quad i = 1,2,3;\ j = 1,2,3 \tag{5}$$

The set of Eq. (5) represents the system of linear equations which allow to determine the coordinates of the S points of each pair of compounds of interest: $x_{1,S}$, $x_{2,S}$, $x_{3,S}$. By introducing these values in Eqs. (2)–(4) the optimization parameters at the extremal points could be calculated; they are listed in Table 3.

The regression equations were then transformed into their canonical forms. This was done by first shifting the coordinate origins to the S points (thereby the linear terms of the equations were excluded) and second by turning the coordinate axes around the S points in such a way that the members describing the interaction of factors were excluded from the regression equation. This gave the canonical equations:

$$\hat{Y}_1 = 0.60 - 0.0569X_1^2 - 0.1365X_2^2 + 0.0128X_3^2 \tag{6}$$

Table 3
Extremal points of the response hypersurfaces

Characteristic parameter of the hypersurface	Peak pair		
	BZ and HL	HD and PB	HX and NB
$X_{1,S}$	−0.4157	0.5330	0.1297
$X_{2,S}$	0.6534	−0.7724	0.0077
$X_{3,S}$	0.7685	1.2225	−0.9802
$\hat{Y}_S$	0.60	1.13	1.06
m_{11}	0.5949	−0.8084	0.4844
m_{12}	−0.8014	0.4834	−0.0883
m_{13}	0.0619	0.3358	0.8704
m_{21}	−0.7996	−0.0739	−0.7307
m_{22}	−0.5822	−0.6495	−0.5879
m_{23}	0.1474	0.7568	0.3470
m_{31}	0.0819	0.5839	−0.4810
m_{32}	0.1370	0.5869	0.8041
m_{33}	0.9872	0.5608	0.3493

$$\hat{Y}_2 = 1.13 + 0.0445X_1^2 + 0.0069X_2^2 - 0.0268X_3^2 \quad (7)$$

$$\hat{Y}_3 = 1.06 - 0.0028X_1^2 - 0.0121X_2^2 + 0.0483X_3^2 \quad (8)$$

The terms X_1–X_3 represent new canonical variables which are related linearly with the dimensionless coded variables x_1–x_3:

$$X_1 = m_{11}(x_1 - x_{1,S}) + m_{12}(x_2 - x_{2,S}) + m_{13}(x_3 - x_{3,S}) \quad (9)$$

$$X_2 = m_{21}(x_1 - x_{1,S}) + m_{22}(x_2 - x_{2,S}) + m_{23}(x_3 - x_{3,S}) \quad (10)$$

$$X_3 = m_{31}(x_1 - x_{1,S}) + m_{32}(x_2 - x_{2,S}) + m_{33}(x_3 - x_{3,S}) \quad (11)$$

The data of the coefficients m_{11}–m_{33} are also listed in Table 3.

The coefficients of the canonical Eqs. (6)–(8) have different signs. This indicates that the response hypersurfaces in the multifactorial space are hyperbolic paraboloids. Therefore, the extremal points *S* represent saddle points; the response hypersurfaces are stretched strongly along those axes which possess the lowest absolute value of coefficient: in Eq. (6) this is towards X_3 (with coefficient 0.0128), in Eq. (7) towards X_2 (0.0069), and in Eq. (8) towards X_1 (0.0028).

Eq. (6) states that a move from the saddle point *S* along the canonical axis X_3 is necessary in order to increase the optimization term Y_1. This means that the additional trials must be carried out at conditions $X_1 = X_2 = 0$, $X_3 \neq 0$. X_3 may increase or decrease, i.e. $X_3 > 0$ or $X_3 < 0$, since the power of X_3 in Eq. (6) is two. According to Eqs. (9)–(11), a rise of X_3 in the positive direction leads to high pH (pH > 10) which cannot be realized because of stationary phase decomposition. Therefore the additional experiment was carried out towards a decrease of X_3 ($X_3 < 0$) which corresponds to a lower pH. An Y_1 (resolution) value of 1.0 is obtained with $X_3 = -2.47$ which means that the values of principal factors are $Z_1 =$ 81.5 m*M*, $Z_2 = 55$ ml/l and $Z_3 =$ pH 2.5. The resulting chromatogram is shown in Fig. 4. Indeed the resulting optimization parameter $Y_1 = R_s$ (BZ, HL) was found to be ~1.0. This is a significant improvement compared to experiment 3 (Table 2 and Fig. 2) where R_s had been 0.8.

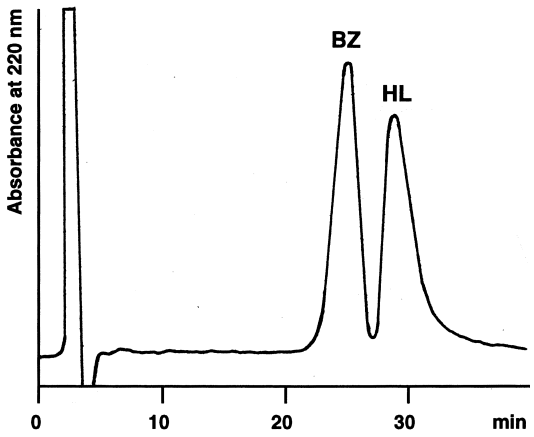

Fig. 4. Separation of BZ and HL under the conditions of an additional trial predicted by the mathematical design: $Z_1 = 81.5$ m*M*, $Z_2 = 55$ ml/l, $Z_3 =$ pH 2.5.

A mathematical analysis of Eq. (6) shows that for a further increase of the optimization parameter Y_1 it is necessary to move from the saddle point towards the direction which is related with a further decrease of mobile phase pH ($X_3 < -2.47$). Unfortunately, a lower pH than ~2.5 is not acceptable with silica-based stationary phases.

In Eq. (7) the largest positive coefficient is the one of variable X_1 (+0.0445). For the optimization of Y_2 it is therefore necessary to shift within the factorial space from the saddle point along axis X_1, i.e. to carry out an experiment with $X_3 = 0$, $X_2 \approx 0$ and $X_1 \neq 0$. But a decrease of X_1 is accompanied by a significant increase of Z_1 (e.g., when $X_1 = -2.84$, $Z_1 = 185$ m*M*) which results in a rise of mobile phase viscosity. In order to avoid this, a shift towards an increase of X_1 was performed. When $X_1 = +2.84$, the corresponding values of principal factors are: $Z_1 = 47$ m*M*, $Z_2 = 54$ ml/l, $Z_3 =$ pH 8.3; the resulting separation is shown in Fig. 5. Y_2, the separation factor α(HD/PB), increased to 1.63 (in experiment 7, α(HD/PB) had been 1.42, see Table 2).

Concerning Eq. (8) it is clear that a shift along axis X_3 is necessary to get an improved separation since the respective coefficient is the only one which is positive (+0.0483). The experiment with $X_1 = X_2 = 0$ and $X_3 \neq 0$ ($X_3 = +2.64$) was performed at $Z_1 = 60.6$ m*M*, $Z_2 = 55$ ml/l, $Z_3 =$ pH 4.6. The ob-

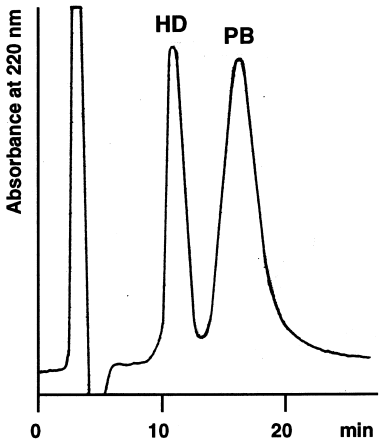

Fig. 5. Separation of HD and PB under the conditions of a second additional trial: $Z_1 = 47.0$ mM, $Z_2 = 54$ ml/l, $Z_3 =$ pH 8.3.

tained separation is shown in Fig. 6 and gave an improved separation factor α(HX/NB) = 1.49 (in experiment 7, α(HX/NB) had been 1.37, see Table 2).

These results demonstrate that the chosen mathematical design of second order can be successfully used to improve the separation of peak pairs which are difficult to resolve. It should be mentioned that the separation of other components of the mixture can be somewhat deteriorated; e.g. on Fig. 7, BZ and HL are completely separated but the separation of HD and PB is worse than on Fig. 2. The general optimization function for all components was not determined. (Such investigations are under way with the separation of vitamins by ion-pair chromatography.) The separation of BZ and HL has not been described in the literature so far either by ion-pair chromatography or by traditional RP-HPLC. Their separation by using micellar mobile phases underlines the usefulness of MLC for specific separations.

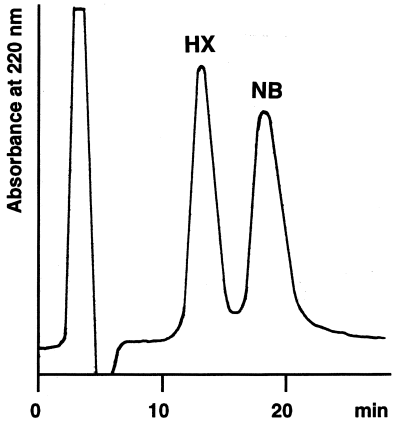

Fig. 6. Separation of HX and NB under the conditions of a third additional trial: $Z_1 = 60.6$ mM, $Z_2 = 55$ ml/l, $Z_3 =$ pH 4.6.

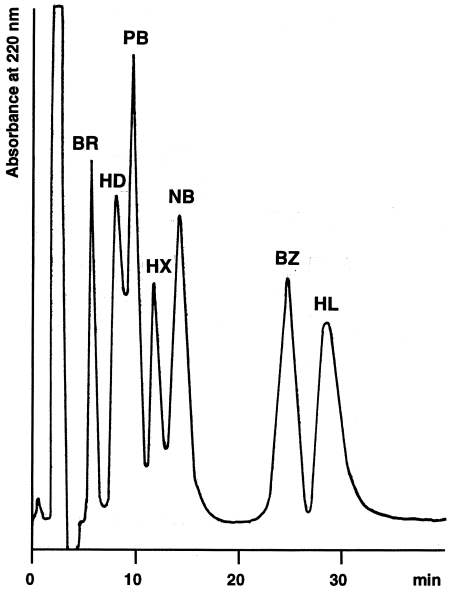

Fig. 7. Chromatogram of the mixture of all seven barbiturates under the conditions of Fig. 4.

4. Conclusions

The rotatable second order design allows description of the significant interactions between three principal factors which govern the micellar liquid chromatographic separation of barbiturates, viz. the micellar concentration, the amount of organic modifier and the mobile phase pH. With this mathematical model the respective hypersurfaces can be created and explored. The approach allows prediction of the experimental conditions for the successful separation of pairs of compounds which are difficult to resolve.

References

[1] D.W. Armstrong, Sep. Purif. Methods 14 (1985) 213.
[2] M.G. Khaledi, Biochromatogr. 3 (1988) 20.

[3] M.L. Marina, M.A. Garcia, J. Liq. Chromatogr. 17 (1994) 957.
[4] M.G. Khaledi, J.K. Strasters, A.H. Rodgers, E.D. Breyer, Anal. Chem. 62 (1990) 130.
[5] E. Tomlinson, T.M. Jefferies, C.M. Riley, J. Chromatogr. 159 (1978) 315.
[6] B.A. Bidlingmeyer, J. Chromatogr. Sci. 18 (1980) 525.
[7] J.K. Strasters, E.D. Breyer, A.H. Rodgers, M.G. Khaledi, J. Chromatogr. 511 (1990) 17.
[8] J. K Strasters, S.T. Kim, M.G. Khaledi, J. Chromatogr. 586 (1991) 221.
[9] A.H. Rodgers, J.K. Strasters, M.G. Khaledi, J. Chromatogr. 636 (1993) 203.
[10] A.S. Kord, M.G. Khaledi, J. Chromatogr. 631 (1993) 125.
[11] J. Fischer, P. Jandera, J. Chromatogr. A 728 (1996) 279.
[12] J. Fischer, P. Jandera, J. Chromatogr. B 681 (1996) 3.
[13] M.D. Rukhadze, G.S. Bezarashvili, M.V. Sebiskveradze, V.R. Meyer, Anal. Chim. Acta 356 (1997) 19.
[14] G.S. Bezarashvili, Experimentis Dagegmva (Design of Experiments), Tbilisi, State University, Tbilisi, 1989, p. 108 (in Georgian).
[15] S.l. Akhnazarova, V.V. Kafarov, Metody Optimizatsii Experimenta v Khimicheskoi Tekhnologii (Optimization Methods of Experiments in Chemical Technology), Vysshaya Shkola, Moscow, 1985, (in Russian).