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Retention prediction for β-adrenergic blocking drugs in normal-phase liquid chromatography

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Abstract

The slope–intercept and the convergence model were used for describing the normal-phase retention data for a set of β-adrenergic blocking drugs obtained on a cyanopropyl column using various proportions of hexane–2-propanol–0.1% propylamine as mobile phase. Moreover, a quantitative structure–retention relationship with log *P* as descriptor was derived. The normal-phase retention data were also found to be rather strongly correlated with the data obtained for the same β-adrenergic blocking drugs in reversed phase. This allowed to derive an equation for the prediction of normal-phase retention data from reversed-phase data and vice versa.

1. Introduction

Reversed-phase liquid chromatography (RPLC) is the most commonly used chromatographic mode. However, for some applications normal-phase liquid chromatography (NPLC) has been shown to be very useful, such as for group separations and the separation of isomeric compounds [1].

Several models have been proposed to explain the retention mechanism in normal phase. Scott and co-workers [2–5] have considered solute–mobile phase and solute–stationary phase interactions for silica gel. The Snyder–Soczewinski adsorption model is based on the displacement of solvent molecules from the column surface by solute molecules [6–11]. Another model considering hydrogen bonding and other interactions was proposed by Chang and co-workers [12,13] for an aminopropyl column. At present, the Snyder–Soczewinski model can be considered to be the most useful model for describing solute retention in NPLC. A critical review of the Scott–Kucera and the Snyder–Soczewinski model has been presented by Snyder and Poppe [14]. In general, these models introduce several physical parameters which, however, are unknown or very difficult to ascertain. This, of course, limits the practical application of these models for retention prediction purposes. For this reason, more empirical models have been developed. Jandera [15] as well as Cooper and Hurtubise [16] proposed a so-called slope–intercept relationship for predicting solute retention in normal-phase chromatography. Kowalska [17] developed an approach based on the quantification of the efficiency of intermolecular interactions among the components of the mobile phase.

For retention prediction in RPLC we recently proposed a quantitative structure–retention relationship [18] and a model based on the conver-

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gence concept [19]. Both models relate the change in retention (log k') to the volume percentage of the organic modifier in the mobile phase, on the one hand, and the structure of the compound, reflected by the calculated log P value, on the other hand. These models were successfully applied for retention prediction of a heterogeneous set of acidic, basic and neutral compounds. The parameter log P, which reflects the hydrophobic–lipophilic character of a molecule, has been extensively used for investigating structure–retention relationships, generally in RPLC [20]. However, some studies have shown that retention data obtained in normal-phase thin-layer chromatography (NP-TLC) can also be correlated with log P values [21,22].

In this work the chromatographic behaviour of β-adrenergic blocking drugs on a cyanopropyl column is investigated. These compounds belong to a class of clinically important drugs, which are generally associated with poor chromatography. Good separations for basic compounds on such a column were obtained in the presence of 0.1–0.5% (v/v) propylamine in the mobile phase [23] and for this reason this tailing suppressor will be used in this study. Another important characteristic of this group is that it covers a wide range in polarity, which is necessary to investigate the usefulness of log P for retention prediction purposes in NPLC.

The aim of this work is firstly to investigate the validity of the slope–intercept and the convergence model and afterwards to derive a quantitative structure–retention relationship (QSRR) using the descriptor log P in NPLC. Secondly, we will compare the retention data obtained for the β-adrenergic blocking drugs in NPLC with the retention data for the same drugs in RPLC [24] to investigate the feasibility to transfer retention data between both chromatographic systems.

2. Experimental

2.1. Standards and reagents

All standards were of pharmaceutical grade and donated by their manufacturers. The stock solutions (500 μg/ml) were prepared in 2-propanol and diluted to the final concentration with hexane. Both solvents were of analytical grade (Merck, Darmstadt, Germany). Propylamine (PA) was obtained from Fluka (Buchs, Switzerland).

2.2. High-performance liquid chromatography equipment

The chromatographic system consisted of a Varian 5000 gradient pump equipped with a Rheodyne injector (sample loop of 100 μl), a Merck–Hitachi L-4200 variable wavelength detector, operating at 0.05 A.U.F.S. and 220 nm, and a Varian CDS 401 data system. An Ultrasphere CN column (250 × 4.6 mm I.D., particle size 5 μm) was used. The mobile phase consisted of various proportions of hexane and 2-propanol, which both contained 0.1% PA. The flow rate was set at 2.0 ml/min. All experiments were performed in duplicate at 30°C. The capacity factors (k') were calculated as follows:

$$k' = (t_r - t_o)/t_o \quad (1)$$

where t_o represents the dead time of the system. The dead time was determined as the first distortion of the baseline after injection of 2-propanol, which, contrary to other methods investigated for the determination of t_o, more specifically the solvent disturbance peak, injection of pure hexane or toluene, was found to be independent of the mobile phase composition.

2.3. Computer

The regression calculations were performed using the Statistical Package for Social Sciences SPSS (Windows version 5.0.1) on an IBM PC or compatible computers.

3. Results and discussion

The retention data obtained for the β-adrenergic blocking drugs are listed in Table 1. For describing NPLC data Scott and Kucera [2] derived an equation accounting for the solute

Table 1
Chromatographic data (log k') and log P values for the β-adrenergic blocking drugs

No.	Name	log P [a]	log k' in % (v/v) 2-propanol				
			20	30	40	50	60
1	practolol	1.62	1.042	0.781	0.570	0.424	0.244
2	penbutolol	5.18	0.076	−0.034	−0.062	−0.088	−0.148
3	carazolol	3.24	0.759	0.534	0.389	0.284	0.137
4	atenolol	0.75	1.123	0.914	0.712	0.573	0.381
5	bupranolol	4.11	0.202	0.101	0.064	0.021	−0.034
6	mepindolol	2.95	n.a.[b]	0.637	0.484	0.358	0.223
7	metipranolol	3.33	0.441	0.267	0.198	0.128	0.027
8	prenalterol	1.89	0.785	0.548	0.395	0.291	0.128
9	metoprolol	2.36	0.418	0.279	0.215	0.152	0.061
10	oxprenolol	3.20	0.457	0.331	0.251	0.208	0.120
11	tertatolol	4.19	0.466	0.347	0.294	0.223	0.159
12	pindolol	2.42	n.a.	0.649	0.485	0.343	0.197
13	nadolol	1.85	0.814	0.655	0.481	0.367	0.191
14	bunitrolol	2.55	0.635	0.482	0.402	0.332	0.252
15	alprenolol	3.63	0.136	0.023	−0.014	−0.051	−0.115
16	acebutolol	2.31	n.a.	0.791	0.612	0.459	0.308
17	propranolol	3.66	0.262	0.193	0.144	0.098	0.017

Column, Ultrasphere CN; mobile phase, 2-propanol–hexane–0.1% propylamine
[a] Log P value calculated according to the Rekker fragmental method [35,36].
[b] n.a. = not available.

interactions in chromatography. One form of the equation is:

$$1/k' = A' + B' \cdot c_p \quad (2)$$

where A' and B' are constants for a particular solute and polar solvent, and c_p represents the concentration of the polar solvent in the binary mobile phase.

Considering only solute–adsorbent and solvent–adsorbent interactions, Snyder and Poppe [14] proposed another equation for NPLC with binary mobile phases:

$$\log k'_2 = \log k'_1 - (A_s/n_b) \cdot \log X_s \quad (3)$$

where k'_1 represents the capacity factor for a solute eluted with the pure strong solvent, k'_2 is the capacity factor for a solute eluted with the binary mobile phase, A_s represents the molecular area of the solute, n_b is the molecular area of the strong solvent, and X_s is the mole fraction of the strong solvent.

Soczewinski and Matysik [25] and Jandera and co-workers [26] have shown that the more convenient volume percentage of the more polar organic solvent can be used. In a first step, the dependence of log k' as a function of the mobile phase composition was investigated. As can be observed in Fig. 1, these plots are quite similar to those observed in RPLC [13], which indicates

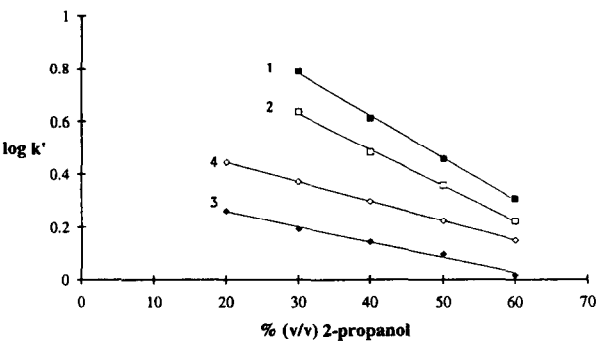

Fig. 1. Plot of log k' *vs.* the volume percentage 2-propanol in the mobile phase for the β-adrenergic blocking drugs acebutolol (1), mepindolol (2), propranolol (3) and tertatolol (4).

that the data can also be described using the model currently used in RPLC [27]:

$$\log k' = \log k'_w - S \cdot X_m \quad (4a)$$

where $\log k'_w$ represents the logarithm of the capacity factor for a solute in pure water and X_m is the volume fraction of the organic modifier in the binary mobile phase. To avoid confusion, in the NPLC context $\log k'_x$ is used instead of $\log k'_w$, as shown in Eq. 4b:

$$\log k' = \log k'_x - S \cdot X_m \quad (4b)$$

where $\log k'_x$ represents the logarithm of the capacity factor in pure hexane. The feasibility to apply Eq. 4b to our data would permit comparison of NPLC data with RPLC data for the β-adrenergic blocking drugs.

The values for the slopes (S), the intercepts ($\log k'_x$) in Eq. 4b and the corresponding correlation coefficient (r) are listed in Table 2. Since the linearity of the plots of $\log k'$ *vs.* the percentage of 2-propanol in the mobile phase was not investigated in the region below 20% of 2-propanol, the $\log k'_x$ values represent hypothetical extrapolated values. In Fig. 1 the regression lines for several β-adrenergic blocking drugs are presented. One can note that the results are in accordance with the convergence concept. Indeed, the lines for these β-adrenergic blocking drugs converge towards a single point (see below). Moreover, these plots showed quite good linearity over a rather wide range of 2-propanol compositions. As in RPLC, there seems to be a slight departure from the model. This was verified through analysis of the residuals. The pattern observed showed no indication of a violation of the linearity assumption. This will also be shown in a later part of this paper (see Eq. 11). Finally, the regression coefficients were used to calculate the standard error of estimate (*i.e.*, the standard deviation of the residuals) for the $\log k'$ prediction. This statistic demonstrates the practical value of the retention prediction. For the complete set of β-adrenergic blocking drugs a value of 0.0210 was obtained, which shows that this model provides rather accurate retention prediction. Overall, the results demonstrate that Eq. 4b can be applied for describing retention data for β-adrenergic blocking drugs in a system CN–hexane.

The occurrence of convergence indicates that the slope and intercept values for the different β-adrenergic blocking drugs are correlated, as can be seen in Fig. 2. Using linear regression

Table 2
Slopes (S), intercepts ($\log k'_x$) and correlation coefficients (r) for the relationship of $\log k'$ *versus* the volume fraction of the organic modifier in the mobile phase

No.	Slope (S)	Intercept ($\log k'_x$)	r
1	−1.9533	1.3936	−0.9941
2	−0.5015	0.1496	−0.9602
3	−1.4929	1.0178	−0.9907
4	−1.8262	1.4713	−0.9978
5	−0.5543	0.2927	−0.9818
6	−1.3679	1.0408	−0.9992
7	−0.9668	0.5990	−0.9823
8	−1.5702	1.0573	−0.9902
9	−0.8403	0.5611	−0.9878
10	−0.7975	0.5924	−0.9862
11	−0.7384	0.5933	−0.9898
12	−1.4986	1.0929	−0.9995
13	−1.5344	1.1154	−0.9983
14	−0.9859	0.7868	−0.9859
15	−0.5764	0.2265	−0.9741
16	−1.6012	1.2629	−0.9991
17	−0.5841	0.3765	−0.9954

Column, Ultrasphere CN; mobile phase, 2-propanol–hexane–0.1% propylamine. Numbering of the compounds as in Table 1

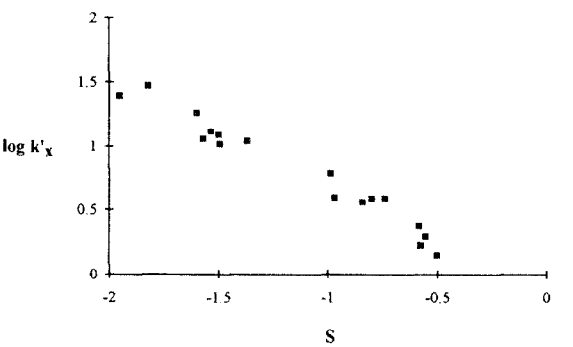

Fig. 2. Graph of slope (S) and intercept ($\log k'_x$) values for the set of β-adrenergic blocking drugs in NPLC.

analysis the following results were obtained for the straight line:

$$S = -1.1515(\pm 0.1281) \cdot \log k'_x - 0.2173(\pm 0.1149) \quad (5)$$
$$n = 17 \; s = 0.099 \; r = 0.980 \; F(\text{eq.}) = 367 \; p < 0.00005$$

The number of data points (n), the standard deviation of the residuals (s), the correlation coefficient (r), the calculated F value of the derived equation and its significance level (p) are provided. These results indicate a very good correlation between the slope and intercept values. Such significant correlations in RPLC were also observed by Schoenmakers [27], Braumann *et al.* [28], Hafkenscheid and Tomlinson [29], Jandera [30], Cooper and Hurtubise [31], and Baty and Sharp [32]. To our knowledge such correlations in NPLC have only been described by Jandera [15] and Cooper and Hurtubise [16].

Cooper and Hurtubise [16] used this so-called slope–intercept relationship for retention prediction for structurally related compounds. These authors also proposed a similar retention prediction model in RPLC [31]. The Cooper–Hurtubise NPLC model requires the calculation of the total solubility parameter values for the mobile phase [33,34], whereas in the RPLC model the volume fraction of the organic modifier is directly related to log k'. Considering the applicability of Eq. 4b to our data we investigated the usefulness of the RPLC slope–intercept model to predict the retention of β-adrenergic blocking drugs in NPLC:

$$\log k' = (1 - p \cdot X_m) \cdot \log k'_x - q \cdot X_m \quad (6)$$

where p and q represent the slope and intercept value for the plot of S *vs.* $\log k'_x$ (from Eq. 4b), respectively. This equation can be obtained by substituting Eq. 5 into Eq. 4b. Eq. 6 was used to predict the retention data for the set of β-adrenergic blocking drugs. As can be observed in Fig. 3, the predicted values (calculated by Eq. 6) correspond very well with the experimental values:

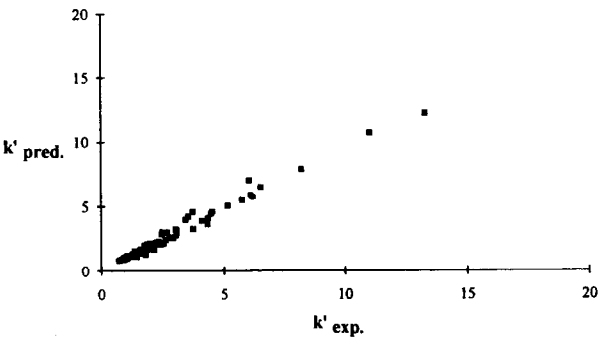

Fig. 3. Correlation of the predicted (calculated using Eq. 6) and experimental k' values for the set of β-adrenergic blocking drugs.

$$k'_{\text{pred.}} = 0.9597(\pm 0.0501) \cdot k'_{\text{exp.}} + 0.0863(\pm 0.0347) \quad (7)$$
$$n = 82 \; s = 0.293 \; r = 0.990 \; F(\text{eq.}) = 4036 \; p < 0.00005$$

The regression coefficients are accompanied by their 95% confidence intervals according to the t-test. Since 1 falls into the first and 0 into the second confidence interval, this is consistent with the equation $k'_{\text{pred}} = k'_{\text{exp}}$.

One must be aware that this retention prediction method presents a major disadvantage, namely for a new substance it requires an experimental k' value at a particular mobile phase composition for the calculation of the log k'_x value. Only then can k' values at other mobile phase compositions be predicted for an unknown compound. This, of course, limits the application of Eq. 6 for retention prediction purposes. Still, the results indicate that p and q values can be very useful for retention prediction in NPLC.

A way to extend the applicability of Eq. 6 is to consider not only the relationship of log k' *vs.* the volume fraction of the organic modifier in the mobile phase, but also to incorporate a parameter which characterizes or reflects the structure of the compound. Recently, we proposed a model based on theoretical considerations for retention prediction in RPLC [19]:

$$\log k'_i = \frac{(\Gamma \cdot \sum f + C' - \log k'_{cv})}{(0\%\text{MPS} - x_{cv}\%\text{MPS})} \cdot \%\text{MPS} + \Gamma \cdot \sum f + C' \quad (8)$$

where Σf represents the log P value of the solute calculated according to the Rekker hydrophobic fragmental system [35,36], x_{cv}%MPS and log k'_{cv} are the values for the convergence point (see below), %MPS represents the volume percentage of the organic modifier in the mobile phase, and Γ and C' are the slope and intercept values for the relationship of Σf *vs.* log k'_w.

Eq. 8, which relates the change in retention (log k') to the volume percentage of the organic modifier in the mobile phase, on the one hand, and the structure of the compound, reflected by the calculated log P value, on the other hand, was applied to predict the retention of the set of β-adrenergic blocking drugs in NPLC. The calculated log P values for the different drugs are listed in Table 1.

In a first step the convergence point was determined as described previously [19]. The lines for the β-adrenergic blocking drugs were found to converge towards the point with x-coordinate 83 (in % 2-propanol) and y-coordinate −0.145. The latter value corresponds to the mean log k' value at that percentage of 2-propanol for all the β-adrenergic blocking drugs. The x-coordinate and the y-coordinate represent x_{cv}%MPS and log k'_{cv}, respectively. The occurrence of convergence can also be observed in the data published by Soczewinski and Kuczmierczyk [37].

To obtain the values for the constants Γ and C' one has to consider the relationship between the calculated log P values and the extrapolated log k'_x values (Fig. 4). The following equation was derived by linear regression:

$$\log k'_x = -0.3249(\pm 0.1064) \cdot \log P + 1.7429(\pm 0.3284) \quad (9)$$
$$n = 17 \; s = 0.219 \; r = 0.859 \; F(\text{eq.}) = 42$$
$$p < 0.00005$$

The statistics for Eq. 9 indicate a significant correlation between both parameters. Numerous publications described the usefulness of log k'_w as a means for the estimation of the hydrophobicity–lipophilicity of a drug in RPLC [19]. Contrary to RPLC, in NPLC retention as reflected by the log k'_x value decreases as the compound becomes more lipophilic.

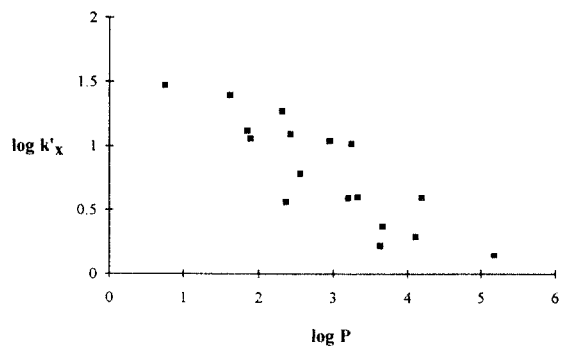

Fig. 4. Relationship between the estimated log P value and the log k'_x values for the set of β-adrenergic blocking drugs in NPLC.

The calculated log P values, the values for the convergence point and the constants Γ and C' were then used to predict the retention of the β-adrenergic blocking drugs in NPLC. From Fig. 5 and from the statistics one can conclude that the predicted values (calculated by Eq. 8) correspond rather well with the experimental values:

$$k'_{\text{pred.}} = 0.8526(\pm 0.0635) \cdot k'_{\text{exp.}} + 0.3435(\pm 0.2164) \quad (10)$$
$$n = 82 \; s = 0.618 \; r = 0.948 \; F(\text{eq.}) = 715$$
$$p < 0.00005$$

Eq. 8 has been shown to correspond with

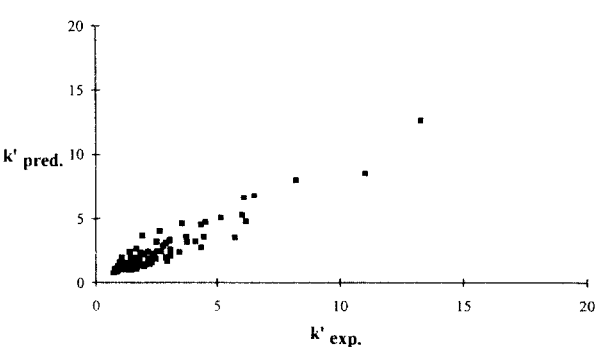

Fig. 5. Correlation of the predicted (calculated using Eq. 8) and experimental k' values for the set of β-adrenergic blocking drugs.

another model developed by multiple linear regression [34]. This frequently used statistical method for the analysis of chromatographic data, and more specifically for QSRR studies, has also been applied in this study for the interpretation of the NPLC retention data. Multiple linear regression was carried out by considering the dependent variable log k' and the independent variables log P, %MPS, the interaction term and the quadratic terms. The following equation was derived with the stepwise method (values of p-to-enter and p-to-remove 0.05 and 0.10, respectively):

$$\log k' = -0.0208(\pm 5.55.10^{-3}) \cdot \%\text{MPS} - 0.3150(\pm 0.0756) \cdot \log P + 0.0035 (\pm 1.777.10^{-3}) \cdot \%\text{MPS} \cdot \log P + 1.6791(\pm 0.2371) \quad (11)$$

$$n = 82 \; s = 0.123 \; \text{Mult.}R = 0.896 \; \text{Adj.}R^2 = 0.796 \; F(\text{eq.}) = 106 \; p < 0.00005$$

The terms %MPS, log P and the interaction term were found significant at $p < 0.00005$. The quadratic terms were found insignificant. The latter results justify the application of Eq. 4b in this study, *i.e.* the assumption of linearity of log k' *vs.* the volume fraction of the organic modifier.

From Eq. 11 one can note that the log k' values decrease as a function of the volume percentage of the organic modifier in the mobile phase and also in function of the hydrophobicity–lipophilicity of the compound.

Some authors [38–40] have claimed that cyanosilica behaves much like a deactivated silica when less polar mobile phases, such as dichloromethane are used. This means that the cyanopropyl groups show little participation as adsorption sites. However, it has been shown that as the polarity of the solvent increases, for instance with alcohols as mobile phase component, the effect of the residual silanol group is masked, leaving the cyanopropyl groups to function as the principal adsorption sites. Since log k' values obtained on a CN stationary phase are correlated with log P it can be concluded that the β-adrenergic blocking drugs interact with the propyl chains of the stationary phase. It is important to mention that the Ultrasphere CN column contains residual silanol groups. The β-adrenergic blocking drugs have been shown to interact strongly with the silanol groups resulting in a mixed retention mechanism [24]. However, Weiser *et al.* [40] suggested that 2-propanol deactivates the silanol groups. Moreover, in this study the attachment of solute to the strong adsorption sites (the residual silanol groups) is eliminated by the addition of 0.1% (v/v) propylamine to the mobile phase [23]. Overall, one can conclude that the cyanopropyl groups constitute the adsorption sites in these packings. One must be aware that as a result of the strong adsorption of 2-propanol onto the surface silanol groups and the addition of an alkylamine to the mobile phase, these chromatographic conditions are not favorable for isomeric separations, since one then needs localization effects (*i.e.* interaction with residual silanol groups) to play an important role.

Since the retention data for the β-adrenergic blocking drugs in NPLC were found to be correlated with the descriptor log P, we compared the retention data in NPLC with data obtained in RPLC [24]. In a first step, the slope and intercept values for the log k' *vs.* X_m relationships in RPLC were calculated (Table 3). Since these plots manifest curvature these values were determined within a limited methanol range. For all compounds the log k' for 40 and 50% methanol were used, except for penbutolol. For this compound only log k' values for 50 and 60% methanol were available. One must be aware that, firstly, the log k'_w obtained in this manner represent hypothetical extrapolated intercepts, and, secondly, that these values depend on the concentrations of methanol used for the linear regression [20]. At both levels acceptable k' values (*i.e.*, k' values situated between 0.5 and 15) were obtained for most of the β-adrenergic blocking drugs investigated. As can be observed in Fig. 6, the slope and intercept values for the different β-adrenergic blocking drugs in RPLC are strongly correlated. Using linear re-

Table 3
Slopes (S), intercepts (log k'_w) and correlation coefficients (r) for the relationship of log k' *versus* the volume fraction of the organic modifier in the mobile phase [24]

No.	Slope (S)	Intercept (log k'_w)
1	−2.0400	0.4670
2	−3.8300	3.1910
3	−4.6800	2.4390
4	−1.6900	0.2450
5	−4.1400	2.7980
6	−2.9700	1.2470
7	−5.1500	2.9690
8	−1.4300	0.1540
9	−3.3400	1.6840
10	−4.1900	2.3540
11	−4.8000	2.9980
12	−1.9400	0.6300
13	−2.3900	0.8970
14	−3.4400	1.6280
15	−4.5700	2.8880
16	−3.4300	1.6330
17	−4.3600	2.7250

Column, Nova-Pak RP18; mobile phase, methanol–phosphate buffer (pH 4.0, $\mu = 0.1$). Numbering of the drugs as in Table 1.

gression analysis the following results, including statistics, were obtained for the straight line:

$$S = -1.0605(\pm 0.1883) \cdot \log k'_w - 1.5041(\pm 0.3936) \quad (12)$$
$$n = 17 \; s = 0.374 \; r = 0.952 \; F(\text{eq.}) = 144$$
$$p < 0.00005$$

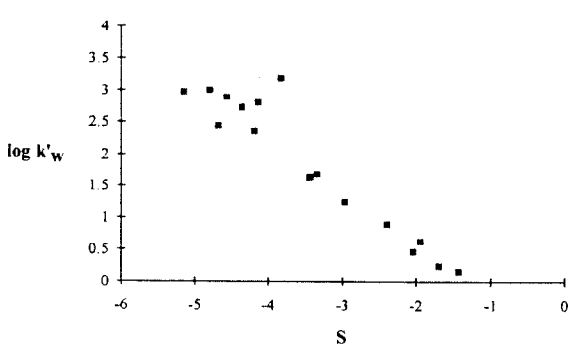

Fig. 6. Graph of slope (S) and intercept (log k'_w) values for the set of β-adrenergic blocking drugs in RPLC.

These results indicate a very good correlation between slope and intercept values.

For the relationship between the calculated log P values and the extrapolated log k'_w values (Fig. 7) the following results were obtained:

$$\log k'_w = 0.8708(\pm 0.2309) \cdot \log P - 0.7018(\pm 0.7125) \quad (13)$$
$$n = 17 \; s = 0.475 \; r = 0.901 \; F(\text{eq.}) = 65$$
$$p < 0.00005$$

The extrapolated log k'_w values are strongly correlated to the log P values. These chromatographically obtained values can hence be used to provide an estimation of the lipophilicity of a drug.

The intercept values obtained in NPLC and RPLC were then compared. As can be observed in Fig. 8 and from the statistics for Eq. 14, the intercept values in both modes are inversely correlated:

$$\log k'_w = -2.1675(\pm 0.7458) \cdot \log k'_x + 3.5582(\pm 0.6689) \quad (14)$$
$$n = 17 \; s = 0.580 \; r = 0.848 \; F(\text{eq.}) = 38$$
$$p < 0.00005$$

Some β-adrenergic blocking drugs show a particular behaviour in NPLC, for instance the compounds metoprolol (no. 9) and acebutolol (no. 16). In RPLC these solutes do not show special properties, but are retained as one would expect from their hydrophobic properties. Con-

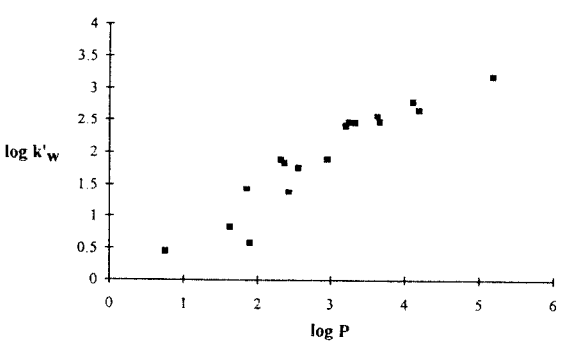

Fig. 7. Relationship between the estimated log P value and the log k'_w values for the β-adrenergic blocking drugs in RPLC.

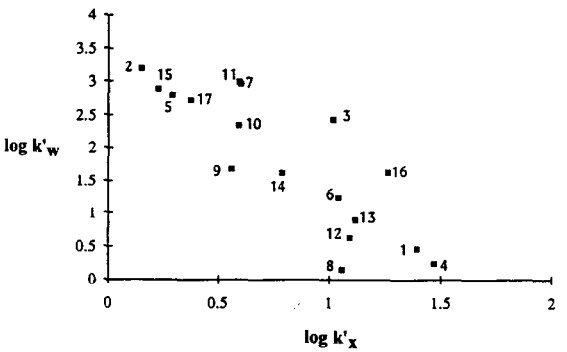

Fig. 8. Correlation between the intercept values obtained in NPLC (log k'_x) and RPLC (log k'_w) for the set of β-adrenergic blocking drugs.

sequently, other specific interactions occur in NPLC. Metoprolol (no. 9) possesses an aliphatic–O–fragment, whereas acebutolol (no. 16) contains two other functional groups, namely–CONH and–$COCH_3$ (Table 4). The latter functions can lead to strong polar interactions with the cyanopropyl groups of the stationary phase (1).

Since the retention data in both chromatographic systems were found to be correlated, an equation was derived by multiple regression to transfer the retention data for β-adrenergic blocking drugs obtained in RPLC [24] to NPLC:

$$\log k'_{NP} = -0.2922(\pm 0.0570) \cdot \log k'_{RP} - 0.0224(\pm 2.517.10^{-3}) \cdot \%MPS + 1.4051(\pm 0.1212) \quad (15)$$

$$n = 65 \; s = 0.114 \; \text{Mult.}R = 0.917 \; \text{Adj.}R^2 = 0.836 \; F(\text{eq.}) = 164 \; p < 0.00005$$

All terms were found significant at $p < 0.00005$. Since the retention data were compared at the same level, *i.e.* the same percentage of the organic modifier in the mobile phase, Eq. 15 can also be used to transfer NP retention data to RP data. This equation can therefore be very useful for HPLC method development.

The analogy between the RP system (with aqueous eluents) and the NP system (with non-aqueous eluents) is somewhat unexpected. This phenomenon could be related to the weakly polar properties of a CN type of column. It would therefore be most interesting to investigate the applicability of this approach on more polar adsorbents, such as pure silica.

Table 4
Overview of the functional groups (corresponding with Rekker fragments) present in the different β-adrenergic blocking drugs

No.	–NHCO (ar.)	–NH– (ar.)	$-CONH_2$ (al.)	–Cl (ar.)	–COO (ar.)	–OH (ar.)	–O– (al.)	$-CH{=}CH_2$ (al.)	–O– (ar.)	–S– (al.)	–OH (al.)	–CN (ar.)	–CONH (ar.)	$-COCH_3$ (ar.)
1	*													
2														
3		*												
4			*											
5				*										
6		*												
7					*									
8						*								
9							*							
10								*	*					
11										*				
12		*												
13											**			
14												*		
15								*						
16													*	*
17														

Numbering of the compounds as in Table 1.

4. Conclusions

The experimental data presented in this work indicate that the slope–intercept and convergence model can be used for describing the retention data of the β-adrenergic blocking drugs for this chromatographic system, *i.e.* an Ultrasphere CN column and a mobile phase composed of hexane–2-propanol–0.1% (v/v) propylamine.

The retention mechanism of the β-adrenergic blocking drugs in NPLC is governed by two types of interactions, namely hydrophobic–lipophilic interactions, on the one hand, and polar interactions, on the other one.

One can also observe that a CN–hexane system is less useful for the analysis of less polar compounds, in which these possess weak retention. However, the chromatographic conditions used in this study are particularly suitable for the more polar β-adrenergic blocking drugs, which show insufficient retention in RPLC.

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