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Hydrophobicity parametrization of progesterone and corticosteroid derivatives by high performance liquid chromatographic technique. I: Linear case

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Abstract

Partition chromatographic parameters, determined by the theoretical concepts underlying chromatographic techniques, were explored as an alternative means of measuring lipophilicity of drugs. The influence of the nature and polarity of mobile phases on the resolution of a chromatographic systems was analyzed in a group of progesterone and corticosteroid derivatives which possess different functionalities attached at steroid skeleton. Optimal chromatographic conditions were established and extraneous variables were eliminated by appropiate statistical tests. Quantitative relationships with standard lipophilia index were obtained and the usefulness and predictive potential of equations proposed are evaluated.

Keywords: Progesterone derivatives; Chromatographic retention time; Lipophilic index parametrization; Anova study; Regression analysis

1. Introduction

Numerous workers have realized that there is a close parallel between the retention of drugs on reversed phase high-presure liquid chromatographic (HPLC-RP) columns and the octanol-water partition coefficients, and they have tried to link this correlation to biological activity (Ponec et al., 1986; Walter and Kurz, 1988; Kadir et al., 1990; Pozzo et al., 1991).

The passage of active molecules through biological membranes and the maintaining of effective concentrations of these molecules in the site of action is greatly influenced by their lipophilia (Tojo et al., 1987; Nogrady, 1988). This parameter has traditionally been described by the partition coefficient, and the standard method to determine this is that known as the 'shake-flask'

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method (Gago et al., 1987; Leo, 1987; Minick et al., 1987; James, 1988). However, this procedure has been found to present many practical difficulties affecting its reliability, and therefore its practical use is limited, compared to others that are more rapidly implemented.

Chromatographic methods of hydrophobicity parametrization are getting more and more popular due to their reliability and feasibility and make it possible to predict biological efficacy of drugs in quantitative structure-activity relationships studies (QSAR) (Caron and Shroot, 1984; Garst and Wilson, 1984, Hafkenrscheid and Tomlinson, 1989; Gami and Kaliszan, 1992, Kaliszan, 1992).

Liquewise, this technique allow determination of the different lipophilia produced by each chemical group attached in a particular molecule (Sánchez et al., 1992).

Retention in RP-HPLC system is mainly due to hydrophobic interations, originating from a net repulsion between stationary phase and the water of mobile phase as well as the unpolar moiety of the solute molecule (Munson, 1984).

There are currently available column-filling materials that, in conjunction with a suitable choice of mobile phases (modified in polarity, pH or dielectric constant), provide systems wich allow it to act as a net distribution system, according to the theoretical concepts underlying chromatographic techniques (Lindsay, 1987). However, in the choice of an HPLC system for measuring lipophilicity, potential sources of error must be controlled.

To study the resolution of a chromatographic system, an HPLC technique was applied that incorporates optimal principles of reported methods, i.e. the use of different organic solvents in the mobile phase so that resolution capacity may be ajusted, and the use of a heavily sylated reverse phase column (stationary phase) to reduce undesired eluent interations with silated sites. Different parameters of chromatographic retention were calculated in a series of progesterone and other hydroxyl derivatives which are used as drug models.

In these drugs, the hydrophilicity was progressively increased by adding substituents at different positions on the steroidal skeleton, capable of specific intermolecular interactions and undergoing ionization in aqueous solutions (Ponec et al., 1986; Tojo et al., 1987).

In this paper, all the chromatographic conditions are carefully established: variables of interest were compared and extraneous variables are eliminated. Emphasis is placed on the estimation of elution times to predict chromatographic selectivity factors. The influence of the polarity of the mobile phase on the resolution chromatographic systems was also analyzed. After all, quantitative relationships, between the chromatographic capacity factors and the conventional partition coefficient, were obtained and the usefulness of equations are evaluated.

2. Materials and methods

Progesterone; progesterone 17 hydroxy; progesterone 11 hydroxy; deoxycorticosterone; hydrocortisone acetate; triamcinolone acetonide; corticosterone; hydrocortisone and prednisolone were purchased from Sigma Chemical Company (St. Louis, MO, USA).

All chemicals used for preparation of mobile phases were of reagent grade H.P.L.C. and analytical reagent grade; and were purchased from Merck (Darmstadt, Germany).

2.1. Instruments

The determinations were carried out using a chromatographic system (Waters Associates Inc., Milford, MA, USA) equipped with a M-45 solvent delivery system and a M-994 Diode Array detector system. Injections were made by a Rheodyne inyector (Berkeley, CA, USA); the loop injector load is 20 microliters. The chromatographic column used was a reversed-phase microbondapack C-18 (Waters Division of Millipore Co.).

2.2. Chromatographic method

Different combinations of methanol:water (55–80:45–20) were used as the mobile phase for generating well-defined liquid chromatographic peaks for each of the progesterone derivatives.

Table 1
Experimental parameter (logK) for HPLC-RP analysis

Progesterone derivatives	Log k″ value at methanol percentage of:			
	80	70	60	0:Extrap
Progesterone (A)	0.0910	0.4120	0.4630	1.6360
17-Hydroxy-progesterone (B)	0.1550	0.1340	0.1880	0.274
11-Hydroxy-progesterone (C)	−0.3590	0.1330	−0.0390	1.031
Deoxycorticosterone (D)	−0.3540	0.0750	0.1280	1.6360
Hydrocortisone acetate (E)	−0.3820	−0.1250	−0.0760	0.8760
Triamcinolone acetónide (F)	−0.4230	−0.1640	−0.1140	0.8470
Corticosterone (G)	−0.3680	−0.1180	−0.0720	0.8500
Hydrocortisone (H)	−0.5380	−0.3010	−0.2510	0.6412
Prednisolone (I)	−0.6610	−0.4320	−0.3580	0.4618

All mobile phases were aspirated through a filter Millex Special H0EM 407HO (Millipore, Co.,) and degassed in an ultrasonic bath before use. At ambient conditions, a flow rate were maintained at 0,7 ml/min.

The retention times of the test compounds selected for this study were measured at different concentrations of each progesterone derivative. The capacity factors logK″ y Rq were calculated in the usual way by using the ratio: $K'' = (Tr - To/To)$y $Rq = \log(Tr - To/Tr)$, where Tr is the retention time of the compound to be analized and To is the column dead time (Lindsay, 1987).

The column dead volumes were determined by measure of the peak of methanol with pure methanol as the eluent.

Concentration of drugs were determined computing its calibration curve, constructed from a series of standard solution (Shah et al., 1992).

2.3. Statistical method

The most powerful statistical tool to verify the internal validity of an analytical procedure, as criterion of accuracy, is the analysis of variance (ANOVA). One and two-way variance analysis was realized on the resolution chromatographic data, applying the test of least significant difference (test LSD) and other test of the ANOVA study (Polgar and Thomas, 1988; Bolton, 1990). This statistical procedure allows comparison of the effects of each experimental variable and eliminates the extraneous variables which might affect the outcome for chromatographic resolution.

To establish quantitative relationships between the parameters studied, and know the predictive performance of their association model, lineal simple regression and stepwise multiple lineal regression analysis was applied. The variance study associated with each relation model was realized and its suitability for inclusion in the experimental design was determined (Bolton, 1990). For fitting the experimental data, ANOVA, simple and multiple linear regression subroutines of the statistical software Statgraphic (Statistical Graphics System, 1992) were applied.

3. Results and discussion

The results of experimental parameters, capacity factors chromatographic (logK and Rq), together with partition coefficient (expressed in logarithmic form) are presented in Tables 1 and 2. Each set of data is the mean value of nine experiments corresponding at three mobile phases: *Phase 1*: methanol — water 80:20; *Phase 2*: methanol — water 70:30; and *Phase 3*: methanol — water 60:40, respectively.

3.1. Results of variance analysis

The ANOVA procedure results, applying the least significant difference test (LSD) and other

Table 2
Experimental parameter (Rq) for HPLC-RP analysis

Progesterone derivative	Rq value at methanol percentage of:			
	80	70	60	logP
Progesterone	−0.258	−0.142	−0.128	3.87
17-Hydroxy-progesterone	−0.385	−0.239	−0.217	3.06
Deoxycorticos-terone	−0.513	−0.265	−0.241	2.88
Hydrocortisone acetate	−0.533	−0.368	−0.341	2.44
Triamcinolone acetónide	−0.563	−0.391	−0.362	2.37
Corticosterone	−0.523	−0.364	−0.338	2.17
Hydrocortisone	−0.648	−0.477	−0.444	1.93
Prednisolone	−0.747	−0.537	−0.537	1.46

test availables in the ANOVA study, are presented in Table 3 and Figs. 1–3 (means graphs and interaction graphs). The comparison in the one way ANOVA test of both chromatographic parameters showed a less variable capacity factor (logK): differences non-significant statistically in the multiple range test were observed and less significant results are obtained in the statistic 'F ratio'.

The two way ANOVA test results (high F ratio: 17,786 for the factor parameter in Table 3 and differents means values, Fig. 1) support the above differences observed between the chromatographic capacity factors logK and Rq.

According to Hafkenscheid and Tomlinson, 1984, Tayar et al., 1985a and Tayar et al., 1985b, both parameters, independently, (Fig. 3 showed no interactions) can be used for the determination of lipophilic character in glucocorticosteroids, although it is the capacity factor logK which best defines the behaviour of the mobile phase in the chromatographic resolution of a lipophilic series.

The ANOVA test was also applied to differentiate the influence of the methanol content of mobile phase on resolution power of chromatographic system. The results (Table 3) showed the high methanol content (80% v/v) mobile phase numbered 1 as more significant. With these mobile phase, lower capacity factors are obtained and some deviations from linearity were observed mainly in the progesterone derivatives of greatest lipophilia: 17-hydroxy and 11-hydroxy derivatives (Tables 1 and 2; Fig. 4). Our results confirm the finding of Leo, 1987: if the alcohol content of the chromatographic mobile phase is high (80% or greater), then its polarity properties are altered. In these circumstances, the chromatographic system no longer functions as a conventional distribution system.

The mobile phases of lower methanol percentage, numbered 2 and 3, reveal not statistically significant differences for both parameters in all statistical figures and provides less variable lipophilic data for all progesterone derivatives studied in this paper (Tables 1 and 2).

The lack of statistically significant differences between the mobile phases numbered 2 and 3 was confirmed applying multifactor ANOVA test to each. The results (Table 3 and Fig. 2: means plot) confirm the above findings and lead us to accept the null hypothesis of equality between the two phases (alpha = 0,05)

To verify the total behaviour of the chromatographic system, other mobile phases of lower methanol content should be considered, however neat aqueous eluents (lower of 30% of methanol content), due to very high cohesive density of water, are weakest in RP-HPLC and would make the chromatographic system impractical, because of the strong asymetric retention behaviour and high cohesive density of water (Otto and Wegscheider, 1983; Leo, 1987).

Fits to the complete model cannot be investigated experimentally. Hence, as proposed by Tayar et al., 1985a,b and Tayar et al., 1985b, the values for the lipophilia parameters can be ob-

Table 3
Analysis of variance (one way and multifactor ANOVA) between the chromatographic mobile phases and parameters

Factor	ANOVA test results			
Mobile Phase	F. ratio (logK)	Sign. (logK)	F. ratio (Rq)	Sign. (Rq)
1,2,3,4	25.745	0.0000*	—	—
1,2,3	2.664	0.0931	4.673	0.0210
1,3	3.184	0.0977	5.619	0.0327
1,2	4.5160	0.0510*	7.756	0.0146
2,3	0.0139	0.7192	0.151	0.7017
Mobile Phase	0.604	0.4520	—	—
Parameter	17.786	0.0002*	—	—
Interaction	0.0000	0.9871	—	—

*Denotes a statistically significant difference.

tained by linear extrapolation to an aqueous content of 100% in mobile phase (mobile phase extrapolated numbered 4 in Table 3). Differences were observed in both logK and Rq parameters in the extrapolated phase and high deviations from linearity were also observed, mainly in progesterone derivatives of high lipophilia: 17-hydroxy and 11-hydroxy derivatives.

It was thought appropiate to attribute the non-linearity primarily to a conformational changes of solute which in turn are affected by the methanol content of the mobile phases (Otto and Wegscheider, 1983; Tayar et al., 1985a; Tayar et al., 1985b).

The aforementioned authors have proposed that, for hydro-alcoholic mobile phases, quadratic extrapolations should be performed. They also consider a series of theoretical considerations which are beyond the scope of this paper, but work along this line of research is already in progress in our laboratory.

3.2. Results of regression analysis

The effect of organic modifiers in the mobile phase on retention behaviour of corticoids in HPLC systems was reported linear (Caron and Shroot, 1984; Hafkenscheid and Tomlinson, 1984; Leo, 1987; Ponec et al., 1986; Beezer et al., 1987).

Nevertheless, deviations from linearity have been explained in terms of silanophilic interactions, conformational changes of solute and changes in secondary equilibria (Tayar et al., 1991; Sánchez et al., 1992). In practice, however, the underlying mech-

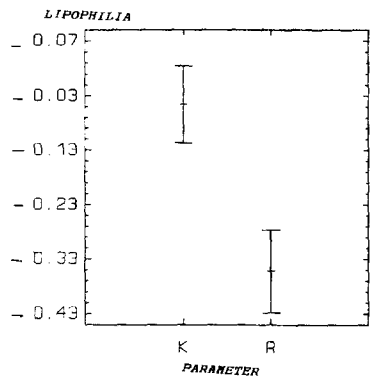

Fig. 1. Means plot of results of lipophilic character of corticoids (± standard deviations) for both chromatographic parameters.

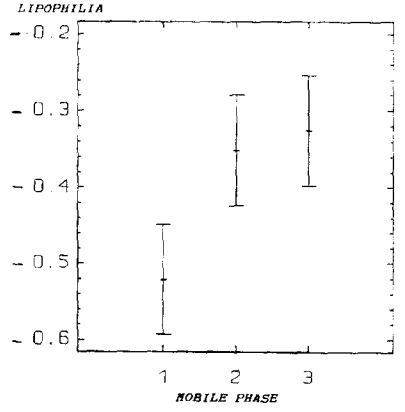

Fig. 2. Means plot of results of lipophilic character of corticoids (± standard deviations) for the different methanol content.

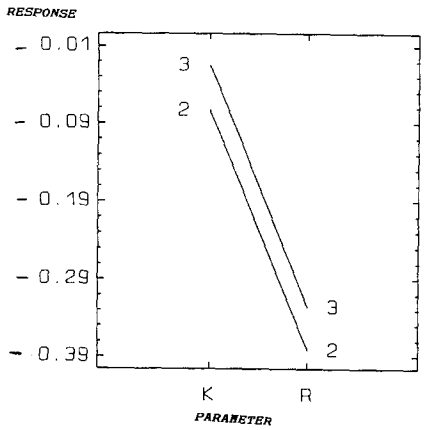

Fig. 3. Interaction plot for the mobile phase composition and the chromatographic parameter.

anism of the system are frequently complicated and it is necessary to establish an empirical approach.

To predict this behaviour by mathematical model, the quantitative relationship between partition coefficients and chromatographic retention parameters (logK and Rq) were determined by linear simple regression and sequential multiple regression.

The results of the equations parameters and statistical figures for the linear regression analysis are summarized in Tables 4 and 5 and some representative correlations are displayed in Figs. 4–6. The usefulness of the equation is evaluated statistically by the determination index (the R-squared value) and correlation coefficients (r) for the values experimentally observed, and predicted

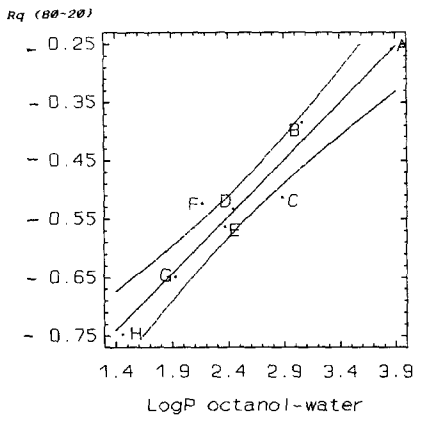

Fig. 4. Linear regression of chromatographic parameter Rq on partition coefficient (logP) for the mobile phase 80–20.

Table 4
Linear regression parameters for the relationship between logP octanol-water and chromatographic lipophilia parameter (a)

Parameter	Statistical figures		Probability level	
	Intercept	Slope	Intercept	Slope
logK 82	−1.7516	0.34298	0.0007	0.00310
Rq 82	−1.0310	0.19495	0.0000	0.00008
logK 73	−0.9574	0.35385	0.0000	0.00000
Rq 73	−0.8026	0.17868	0.0000	0.00003
logK 64	−0.8975	0.35155	0.0000	0.00000
Rq 64	−0.7571	0.17091	0.0000	0.00003

values for the regression equation (Bolton, 1990). Responses are summarized in Table 5 together with the results of the variance analysis of the regression.

It is apparent (Tables 4 and 5, Figs. 4–6) that the relationship between the capacity factors chromatographic (logK and Rq) and the partition coefficient octanol-water (logP sf in tables and figures) is linear as good regression coefficients are obtained.

The good agreement between the fitted model and the experimental data can be deduced from the residuals. The residuals, which include random effects (errors in determination of K″ and Rq) as well as inadequacies in the model, are computed as the square root of the mean square deviations between logK and Rq values measured and estimates (means squared residuals in Table 5) (Polgar and Thomas, 1988).

It was concluded that hydrophobicity parameters determined in individual HPLC systems are not highly intercorrelated and, hence, can reflect different structural features of solutes.

Linear dependences could be observed in all cases, but it was noted that the correlation is more 'sensitive' to the nature of the mobile phase (greater or lesser alcohol content) (Table 5, Fig. 4).

Thus, we consider the variables of high predictive value for the quantification of the lipophilia in corticoids to be either of the two chromatographic parameters (logK and Rq) and the mobile phase containing 70% methanol or less.

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Table 5
Linear regression parameters for the relationship between logP octanol-water and chromatographic lipophilia parameter (b)

Parameter	Statistical		Figures		
	Correlation coefficient	R squared	F. ratio	Probability level	Mean square residuals
logK 82	0.88905	79.04	22.627	0.0031	0.02010
Rq 82	0.96790	93.70	89.183	0.0000	0.00164
logK 73	0.99060	98.14	317.24	0.0000	0.01526
Rq 73	0.97800	95.65	132.01	0.0000	0.00090
logK 64	0.99170	98.35	358.30	0.0000	0.00130
Rq 64	0.97650	95.37	123.64	0.0000	0.00090

The distribution systems (logk parameter and mobile phase 2 and 3), as may be seen in Table 5, produces highest values for the determination coefficients and correlation coefficients which are practically equal to unity ($r = 0.99070$ and 0.99170, respectively). Furthermore, analysis of the variance associated with the regression model studied almost exactly explains the variability of the model itself (high F ratio for the mobile phase 2 and 3 in Table 5).

The graphic results reinforce the above conclusions, the graphs that corresponds to the mobile phases 2 and 3 are composed of points that virtually coincide with the theoretical line (Figs. 5 and 6, respectively).

It is intriguing that the parallelism observed between these findings and the above results of ANOVA test: an increase in methanol content of the hydroorganic mobile phase in RP-HPLC also results in worse correlations of capacity factors logK.

It its true that increasing concentrations of an modifier organic such as methanol or acetonitrile result in a systemic decrease of the surface tension of water wich is accompanied by lower capacity factors, although dependence is strongly nonlineal (Gago et al., 1987). To research this topics, work along this method of HPLC-RP is already in progress in our laboratory.

4. Conclusions

(1) It is possible to design chromatographic systems in which the retention of a certain solute depends solely on its relative hidrophobicity (being neglected electrostatics interactions with the solvent and stationary phase). These systems are highly practical and make it possible to distinguish substances with minimal structural differences.

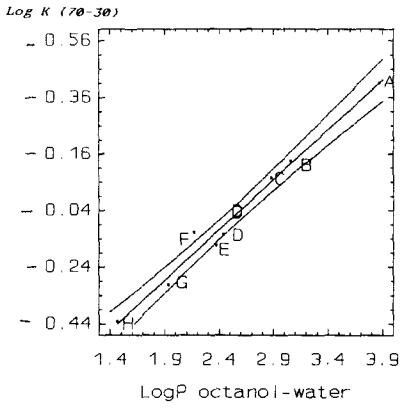

Fig. 5. Linear regression of chromatographic parameter logK on partition coefficient (logP) for the mobile phase 70–30.

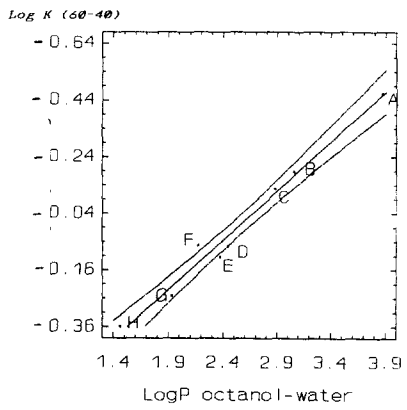

Fig. 6. Linear regression of chromatographic parameter logK on partition coefficient (logP) for the mobile phase 60–40.

(2) The chromatographic behaviour of a series of progesterone derivatives, eluted with different aqueous mobile phases, appear to be well correlated with the hydrophobic character of these chemicals, as determined in the standard system 1-octanol-water.
(3) There exist linear relationships between the partition coefficient and the logK chromatographic capacity factor, and also between the former and the Rq parameter. This is true for all the distribution systems employed, but particularly for the mobile phase with a methanol content of 70% v/v.

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