ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 497–503

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Effect of molecular parameters on the retention of steroid drugs on alumina support

Tibor Cserháti *, Esther Forgács [1]

Central Research Institute of Chemistry, Hungarian Academy of Sciences, P.O.Box 17, H-1525 Budapest, Hungary

Received 15 May 1998; received in revised form 30 July 1998; accepted 16 August 1998

Abstract

The retention of 18 steroids was determined on an alumina HPLC column and in TLC carried out on alumina layers using dichloroethane-dioxane mixtures as eluents. R_{M0}-values of steroids decreased linearly with increasing concentration of dioxane in the eluent. The adsorption capacity and specific hydrophilic surface area of steroids were not correlated indicating the inhomogenous character of steroids as solutes. The prediction power of TLC for HPLC was low probably due to the different pH of alumina surface. The hydrogen donor and hydrogen acceptor capacities of steroids have the highest impact on the retention. Steroids with free –OH group differ in their retention behavior from the other derivatives. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Molecular parameters; Steroid drugs; Alumina support

1. Introduction

The use of silica or modified silica sorbents in adsorption and reversed-phase high performance liquid chromatography is hampered by their low stability at alkaline pH [1,2]. Due to it higher pH stability alumina support is a promising substituent of silica [3,4]. The retention behaviour of alumina [5] and alumina based sorbents [6] has been recently evaluated. Some new experimental processes have been developed for coating the alumina surface with hydrophobic ligands [7–9], and these sorbents have been used for the separation of organic bases [10], imidazol(in)e drugs [11], proteins [12] and peptides [13].

Many HPLC methods have been developed for the separation of bioactive steroids [14] using various sorbents such as silica [15], amino-propyl silica [16] ion-exchanger [17], porous graphitic carbon [18], cyclodextrin bonded silica [19], etc. however, according to our knowledge alumina support has not been frequently used for the HPLC separation of steroid derivatives.

The objectives of our investigations were to study the retention behaviour of alumina supports using biologically active steroids as model compounds, to find the physicochemical parameters of solutes governing the retention and to evaluate the prediction power of TLC data for HPLC analysis [20,21].

* Corresponding author. Tel.: + 36-1-3257900; fax: + 36-1-3257554.

[1] E-mail: forgacs@cric.chemres.hu

PII: S0731-7085(98)00204-0

2. Experimental

The structures of steroid drugs are listed in Table 1. The steroids were dissolved in dioxane at concentration of 5 and 0.1 mg ml^{-1} for TLC and HPLC investigations, respectively.

2.1. High performance liquid chromatography

A 25 cm × 4 mm I.D. alumina column was used in each experiment. The retention characteristics of the column have been previously reported [22]. The HPLC equipment consisted of a Liquopump Type 312 (Labor MIM, Budapest, Hungary, a Cecil CE-212 spectrophotometer (Cambridge, England) used as the detector, a Valco 20-μl injector (Houston, TX), and a Waters 740 integrator (Milford, MA). The eluent was dichloroethane:dioxane 95:5 v/v. The flow rate was 1 ml min^{-1}, and the detection wavelength was set to 240 nm. The column was not thermostated. Each HPLC measurement was run in triplicate. The capacity factor and the coefficient of variation of capacity factor was calculated for each solutes showing acceptable mobility in the eluent.

2.2. Thin-layer chromatography

DC-Alufolien Aluminiumoxide F_{254} (Merck, Darmstadt, Germany) plates were used without any pretreatment. The developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, and the running distance was ca. 15 cm. The use of sandwich chambers was motivated by the fact that their void volume is lower than that of normal TLC chambers resulting lower solvent comsumption. Dichloroethane-dioxane mixtures were used as eluents in the concentration range of 0–15 vol.% dioxane in steps of 2.5 vol.%. The change of the dioxan concentration was always linear. The chambers were not presaturated. After development the plates were dried at 105°C, and the spots were detected under UV light or with iodine vapour. Each determination was run in quadruplicate. When the coefficient of variation between the parallel determinations was higher than 6%, the data were omitted from the calculations.

2.3. Mathematical methods for the evaluation of retention behaviour

Linear correlations were calculated between R_M-value and the dioxane concentration (C) in the eluent Eq. (1) separately for each steroid:

$$R_M = R_{M0} + b \cdot C \quad (1)$$

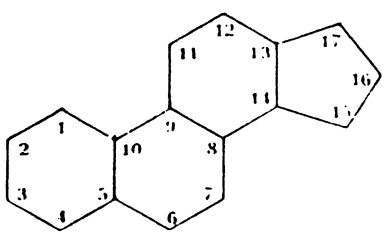
where R_M is the actual R_M-value of a steroid determined at C vol.% dioxane concentration, R_{M0} (intercept) is the theoretical R_M-value extrapolated to zero dioxane concentration, and b (slope) is related to the specific hydrophilic surface area of steroid derivatives [23,24].

To elucidate the validity of the hypothesis that in the case of homologous series of solutes the slope and intercept values are strongly intercorrelated [25,26], the homologous or inhomogenous character of steroids as solutes in adsorption chromatography was assessed by calculating linear correlations between the slope (b) and intercept values (R_{M0}) of Eq. (1). To find the relationship between the HPLC and TLC retention data, $\log k'$-values were correlated with the slope and intercept values of Eq. (1).

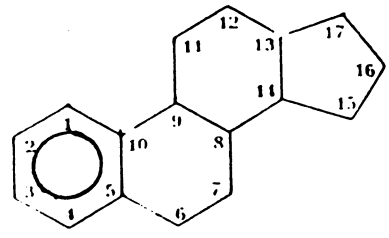
To find the physicochemical parameters of steroids influencing their retention on alumina supports, stepwise regression analysis was applied [27]. Parameters included in the calculation were: π = Hansch-Fujita's substituent constant characterizing hydrophobicity; H-Ac and H-Do are the indicator variables for proton acceptor and proton donor properties, respectively; M-RE is the molar refractivity; F and R are the Swain-Lupton's electronic parameters characterizing inductive and resonance effect, respectively; σ is Hammett's constant, characterizing electron-withdrawing power of substituent; Es is Taft's constant, characterizing steric effects of substituent; $B1$ and $B4$ are the Sterimol width parameters determined by distance of substituent at their maximum point perpendicular to attachment bond axis [28]. The parameters of the steroidal drugs were calculated by using the fragmental constants and the additivity rule.

Stepwise regression analysis was carried out three times, the $\log k'$, R_{M0} and b (Eq. (1)) values being separately the dependent variables, whereas

Table 1
Chemical structures of steroid drugs



A



B

General structures

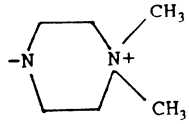
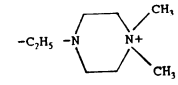
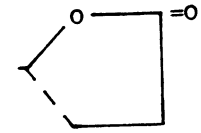
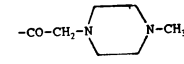
No. of Compound	Structure	Substituent position						
		2	3	7	11	13	16	17
1	A		–O–$COCH_3$	–	–			–O–CO–CH_3
2	A^a	–	=O	–	–	–	–	—OH –CO–CH_3
3	A^a	–	=O	–	–OH	–	–	—OH –CO–CH_2OH
4	A^a	–	=O	–	–	–	–	–OH —C≡CH
5	A^a	–	=O	–	–	–	–	—OH –CO–CH_2OH
6	A^a	–	–	–	–	–	–	–OH –CH_2–C≡CH
7	A^a	–	=O	—S–SO–CH_3–	–	–	–	
8	A^a	–	=O	–	–	$-C_2H_5$	–	–OH —C≡CH
9	A^a	–	=O	–	–	–	–	–O–CO–CH_2–C_6H_5
10	A^a	–	–O–CO–CH_3	–	–	–	—C≡CH	–O–CO–CH_3
11	A^a	–	=O	–	–	–	–	–O–CO–CH_3 —C≡CH
12	A^b	–	=O	–	–OH	–		–CO–CH_3 C_{16}—O–$C(CH_3)_2$–O—C_{17}
13	A^b	–	=O	–	–OH	–	–	

Table 1 (Continued)

14	A[c]	C_2–CH=N–O–C_3		–	–	–	—C≡CH	–OH
15	B	–	–OH	–	–	–	–	–OH
16	B	–	–O–CO–C_6H_5	–	–	–	–	–OH
17	B	–	–O–CO–CH_3	–	–	–	–	=O
18	B	–	–OH	–	–	–	—C≡CH	–OH

[a] Double bond between C_4 and C_5.
[b] Double bonds between C_1–C_2.
[c] Double bonds between C_2–C_3 and C_4–C_5.

the physicochemical parameters listed above were in each case the independent variables. The acceptance level for the individual independent variables was set to a 99% significance level.

To assess the similarities or dissimilarities between the retention characteristics and physicochemical parameters of steroids, principal component analysis (PCA) was applied [29]. The log k', the adsorption capacity (intercept values of Eq. (1)), the specific hydrophilic surface area (slope values of Eq. (1)), and the physicochemical parameters of steroids were taken as variables and the steroids were the observations. The two dimensional non-linear map of PC loadings and variables was also calculated [30]. Iteration was carried out to the point when the differences between the two last iterations was lower than 10^{-8}.

3. Results and discussion

Some chromatograms are shown in Figs. 1 and 2. The peaks are relatively symmetric, however, the peak widths are sometimes high. This finding makes probable that the activity of the adsorption centers on the alumina surface are different resulting in the modification of the energy of solute-support interaction.

The log k'-values and the coefficient of variation are listed in Table 2 (compounds 1, 12, 13, 15 and 18 were very strongly retained under the experimental conditions in HPLC). The log k'-values show high diversity indicating that the steroids can be successfully separated on the alumina column. The coefficients of variation are low (in most cases under 1%) showing the good reproducibility of HPLC measurements.

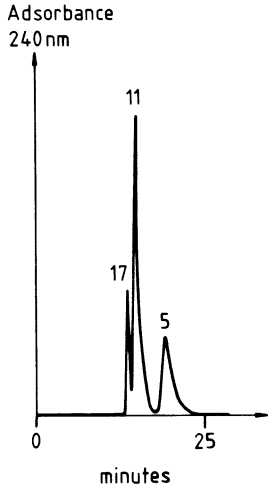


Fig. 1. Separation of some steroid drugs on alumina HPLC column. Eluent: dichloroethane:dioxane 95:5 v/v. Flow rate 1 ml min^{-1}. Numbers refer to steroids in Table 1.

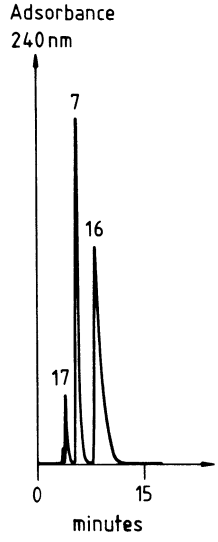


Fig. 2. Separation of some steroid drugs on alumina HPLC column. Eluent: dichloroethane:dioxane 95:5 v/v. Flow rate 1 ml min^{-1}. Numbers refer to steroids in Table 1.

Table 2
Log k'-values of bioactive steroids on alumina column[a]

No. of steroid drugs	Log k'	
	Mean	Coefficient of variation %
2	0.236	0.23
3	0.150	0.17
4	0.764	0.36
5	0.226	0.14
6	−0.125	0.38
7	0.176	0.22
8	0.690	1.14
9	9.00×10^{-3}	0.49
10	0.084	0.73
11	0.115	0.16
14	0.768	1.03
16	0.554	0.84
17	−1.048	0.63

[a] Numbers refer to steroid drugs in Table 1.

The parameters of Eq. (1) are compiled in Table 3 (compound 1 was very strongly retained in TLC). The retention of steroids decreases linearly with increasing concentration of dioxane in the eluent, and no anomalous retention behaviour was observed.

No significant correlation was found between the slope and intercept values of Eq. (1). This finding proves that from chromatographic point of view, these steroid derivatives cannot be regarded as a homologous series of solutes. The log k'-values were not correlated neither with R_{M0} nor with b-values. This discrepancy can be tentatively explained by the supposition that the surface pH and the adsorption capacity of active centers on the alumina surface may be different due to the different production procedures. This finding also indicates that in our case the predictive power of TLC for HPLC is fairly low.

Stepwise regression analysis selected only one significant correlation:

$$R_{M0} = -0.46 + (0.86 \pm 0.16).H - Do \quad (2)$$

$n = 17$; $r_{calc.} = 0.8062$; $r_{99\%} = 0.7247$.

Eq. (2) suggests that the hydrogen-donor capacity of steroids has the highest impact on their retention (it accounts for about 65% of total variance). This finding support our previous conclusions that the pH-value of the surface of alumina support has a considerable influence on the retention.

The results of principal component analysis are compiled in Table 4. The first four components account for about 80% of the total variance. This means that four background variables include the majority of the information content of the 13 chromatographic and physicochemical parameters. It must be emphasized that the four hypothetical variables need not to have any concrete physical (or chromatographical) meaning. PC analysis only proves their mathematical possibility. The high loadings of retention parameters are distributed between the principal components supporting our previous conclusions that the HPLC and TLC results are different, and TLC is not suitable for the prediction of retention behaviour in HPLC. We have to emphasize that this conclusion is valid only for the special case investigated, and we regard the extrapolation of the results to other TLC-HPLC pairs as inadmissible.

Table 3
Correlations between R_M-values of steroid drugs and the concentration of dioxane (C) in the eluent[a]

No. of steroids	R_{M0}	$-b$	S_b	$r_{calc.}$
2	0.98	11.80	1.53	0.9602
3	1.91	1.52	0.20	0.9830
4	0.60	10.14	1.91	0.9213
5	1.47	3.54	0.77	0.8993
6	−0.94	2.24	0.27	0.9790
7	0.41	11.89	1.69	0.9530
8	0.36	9.40	1.58	0.9358
9	−0.27	7.86	1.88	0.9020
10	−0.73	5.76	1.29	0.9121
11	−0.04	10.26	1.73	0.9356
12	1.67	3.44	0.59	0.9462
13	2.08	2.86	0.40	0.9635
14	0.20	8.81	1.57	0.9291
15	1.28	10.89	1.61	0.9492
16	0.13	9.20	1.77	0.9186
17	−0.74	5.76	1.01	0.9388
18	0.90	6.48	1.01	0.9248

[a] Numbers refer to steroid drugs in Table 1. S_b, standard deviation of slope.

$R_M = R_{M0} + b \cdot C$

Table 4
Relationship between the retention characteristics and physicochemical parameters of steroids[a]

No. PC components	Eigenvalues	Variance explained (%)	Total variance explained (%)		
1	3.66	28.13	28.13		
2	2.63	20.23	48.36		
3	2.35	18.10	66.46		
4	1.64	12.63	79.09		
5	0.89	6.88	85.97		
Parameters			Principal component loadings		
	1	2	3	4	5
π	−0.01	0.50	0.28	−0.06	0.65
H-Ac	0.71	0.48	0.44	−0.11	−0.09
H-Do	0.46	0.82	−0.19	−0.20	−0.16
M-RE	−0.29	0.22	0.53	0.54	0.31
F	0.82	−0.42	−0.07	−0.04	0.11
R	0.24	−0.50	0.68	−0.09	−0.24
σ	0.28	−0.18	0.86	0.29	−0.03
Es	−0.12	0.18	−0.18	0.70	−0.36
*B*1	0.91	−0.33	−0.07	−0.05	−0.01
*B*4	0.70	−0.24	−0.36	−0.14	0.29
log k'	0.41	0.31	−0.55	0.55	0.07
R_{M0}	0.64	0.67	0.26	0.10	−0.16
b_{TLC}	0.39	−0.50	−0.14	0.61	0.15

[a] Results of principal component analysis.

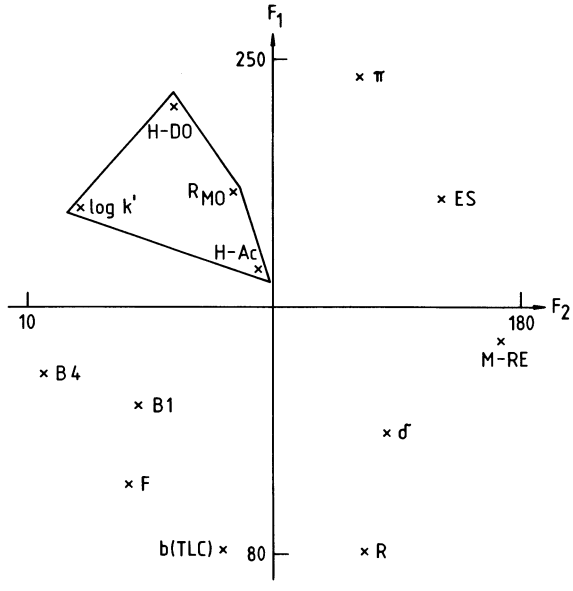


Fig. 3. Similarities and dissimilarities between the chromatographic parameters and physicochemical characteristics of steroid drugs. Two-dimensional nonlinear map of principal component loadings. No of iterations: 181; max. error: 6.23×10^{-2}. Signs refer to chromatographic parameters and physicochemical characteristics of steroid drugs in Experimental.

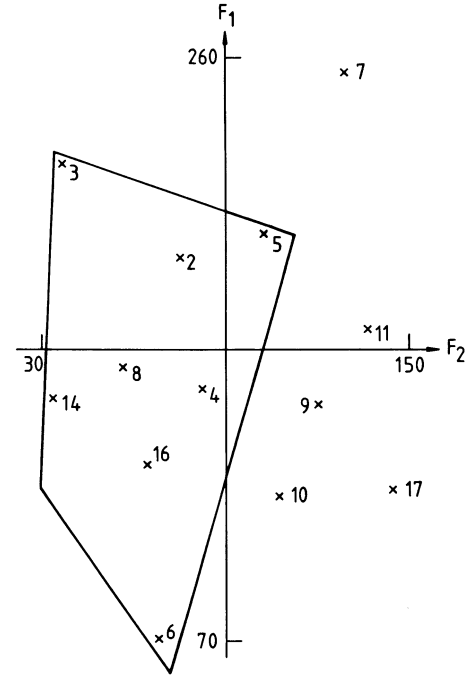


Fig. 4. Similarities and dissimilarities of steroid drugs. Two-dimensional nonlinear map of principal component variables. No of iterations: 90; max. error: 5.80×10^{-2}. Numbers refer to steroid drugs in Table 1.

Capacity values of alumina supports ($\log k'$ and R_{M0}) and the hydrogen-donor and hydrogen-acceptor capacities of steroids form a common cluster on the two-dimensional nonlinear map of PC loadings (Fig. 3). This finding is in accordance with the results of stepwise regression analysis, and indicates that acidic or basic character of support surface and/or solute exert a considerable impact on the retention.

Steroids with free –OH group form separate cluster on the two-dimensional nonlinear map of principal component variables (Fig. 4). This result supports again the previous conclusions about the important role of the hydrogen donor and hydrogen acceptor capacities.

Acknowledgements

This work was supported by the grant OTKA T 023422.

References

[1] R. Kaliszan, J. Petrusewitz, R.W. Blain, R. Hartwick, J. Chromatogr. 458 (1988) 395–404.
[2] A. Berthod, J. Chromatogr. 549 (1991) 1–28.
[3] J.E. Haky, S. Vemulapalli, L.F. Wieserman, J. Chromatogr. 505 (1990) 307–318.
[4] J.E. Haky, S. Vemulapalli, J. Liq. Chromatogr. 13 (1990) 3111–3131.
[5] K. Cabdera, D. Lubda, G. Jung, Kontakte (Darmstadt) (1992) 12–15.
[6] K. Cabdera, D. Lubda, G. Jung, Kontakte (Darmstadt) (1992) 32–35.
[7] J.J. Pesek, H.D. Lin, Chromatographia 28 (1989) 565–568.
[8] J.E. Haky, N.D. Ramdial, A.R. Raghani, L.F. Wieserman, J. Liq. Chromatogr. 14 (1991) 2859–2874.
[9] J.J. Pesek, J.E. Sandoval, M. Su, J. Chromatogr. 630 (1993) 95–103.
[10] G. Yilinkou, R. Kaliszan, Chromatographia 30 (1990) 277–282.
[11] R.-G. Yilinkou, R. Kaliszan, J. Chromatogr. 550 (1991) 573–584.
[12] J.E. Haky, A. Raghani, B.M. Dunn, J. Chromatogr. 541 (1991) 303–315.
[13] J.E. Haky, N.D. Ramdial, B.M. Dunn, L.F. Wieserman, J. Liq. Chromatogr. 15 (1992) 1831–1852.
[14] A. Lagana, A. Marino, J. Chromatogr. 588 (1991) 89–98.
[15] H. Chiba, Y. Ito, K. Matsuno, K. Kobayashi, T. Kurosawa, S. Ikegawa, R. Mahara, M. Toma, J. Chromatogr. 613 (1993) 132–136.
[16] V.K. Boppana, C. Miller-Stein, G.R. Rhodes, J. Chromatogr. 631 (1993) 251–254.
[17] V. Legrand-Defretin, C. Juste, R. Henry, T. Corting, Lipids 26 (1991) 578–583.
[18] B.J. Clark, Adv Steroid Anal '90. Proc. 4th Symp. Anal. Steroids 1990, in: S. Görög; E. Heftmann (eds.), Akadèmimiai Kiadó Budapest, 1991, pp. 129–137.
[19] A.H. Ahmed, S.M. El-Gizany, N.M. Omar, Anal. Lett. 24 (1991) 2207–2216.
[20] J.K. Rozylo, M. Jaroniec, Fresenius J. Anal. Chem. 321 (1985) 371–373.
[21] J.K. Rozylo, M. Janicka, J. Liq. Chromatogr. 14 (1991) 3197–3212.
[22] T. Cserháti, Chromatographia 29 (1990) 593–596.
[23] T. Cserháti, K. Magyar, J. Biochem. Biophys. Methods 24 (1992) 249–264.
[24] T. Cserháti, K. Valkó, J. Biochem. Biophys. Methods 20 (1990) 81–95.
[25] T. Cserháti, Chromatographia 18 (1984) 18–20.
[26] K. Valkó, J. Liq. Chromatogr. 7 (1984) 1405–1424.
[27] H. Mager, Moderne Regressionsanalyse. Salle, Sauerlander, Frankfurt/Main, 1982, pp.135–157.
[28] R. Franke, in: J.K. Seydel (Ed.), QSAR and Strategies in the Design of Bioactive Compounds, VCH Verlagsgesellschaft, Wienheim, 1985, pp. 59–67.
[29] A.R. Dillon, M. Goldstein, Multivariate Analysis, Wiley, New York, 1984, pp. 157–208.
[30] J.W. Sammon Jr., IEEE Trans. Comput. C18 (1969) 401–407.