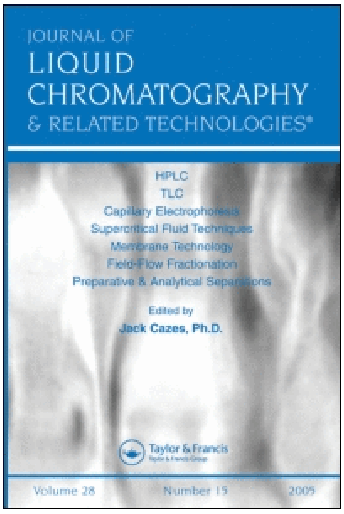

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CANONICAL CORRELATION ANALYSIS OF THE RELATIONSHIP BETWEEN PHYSICOCHEMICAL PARAMETERS OF STEROIDAL DRUGS AND THEIR RETENTION CHARACTERISTICS IN HPLC

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ABSTRACT

The retention of 13 steroidal drugs was determined on a polyethylene-coated silica column using methanol-water mixtures at various organic phase concentrations. Good linear relationships were found between the log k' values and the organic mobile phase concentration in the eluent. The slope and intercept values of the relationships were correlated with the physicochemical parameters of the steroidal drugs by means of canonical correlation analysis. Calculation proved that the retention characteristics significantly depend on the physicochemical parameters of the solute. The electronic parameters exerted a higher impact on the retention than the hydrophobicity did. This result, is probably due to the hydrophobic interactions between the polar substructures of drugs and the free silanol groups not covered by the hydrophobic ligand.

INTRODUCTION

Many efforts have been devoted to the elucidation of the relationship between the various physicochemical parameters of solutes and their retention behavior[1-3] in high performance liquid chromatography (HPLC). Various multivariate methods, such as, factor analysis,[4,5] principal component analysis,[6,7] stepwise regression analysis[8] and spectral mapping techniques,[9] have been frequently used for the calculation of various structure-retention relationships.

A more basic understanding of the structure-retention relationships may facilitate optimization processes[10] and the rational selection of stationary phases.[11]

Canonical correlation analysis (CCA) has been developed to find a relationship between two different data matrices.[12] The method of calculation is similar to those of principal component analysis[13] and factor analysis.[14]

Firstly, CCA extracts theoretical factors which explain the maximal variance in the matrix containing the lower number of variables (matrix II, i.e. more than one retention parameters of a set of solutes).

The number of factors explaining 100% of the variance in matrix II is equal to the number of original variables in matrix II. Then, CCA extracts from the other matrix (matrix I, i.e. various physicochemical parameters of the same solutes), variables which have the highest correlation with the factors extracted from matrix II.

Despite its obvious advantages, CCA found only limited application in chromatography. It has been used both in TLC[15] and HPLC,[16,17] for the evaluation of the influence of various physicochemical parameters on the retention behaviour of homologous series of solutes.

A wide variety of HPLC techniques has been developed for the separation and quantitative determination of various steroid drugs.[18-20] However, the relationship between the retention behavior of steroids and their physicochemical parameters has not been studied in detail.

The objectives of our investigation were to determine the retention behavior of some steroidal drugs on a polyethylene-coated silica column, and to elucidate the relationship between retention characteristics and physicochemical parameters of drugs by means of canonical correlation analysis.

Table 1

IUPAC Names of Steroidal Drugs

No. of Drug	IUPAC Name
1	17-Hydroxy-19-norpregn-4-en-3,20-dione
2	11β,17,21-Trihydroxy-19-norpregn-4-en-3,20-dione
3	11β,16α,17,21-Tetrahydroxy-19-norpreng-1,4-dien-3,20-dione-16,17-acetonide
4	17-Hydroxy-19-norpregn-4-en-20-yn-3-one
5	17,21-Dihydroxy-19-norpregn-4-en-3,20-dione
6	7-(Acetylthio)-17-hydroxy-3-oxo-pregn-4-ene-21-carboxylic acid τ-lactone
7	17α-Pregna-2,4-dien-20-yno[2,3-d]isoxazol-17-ol
8	13-Ethyl-17-hydroxy-18,19-donirpregn-4-en-20-yn-3-one
9	17β-Phenylacetyl-17-methylandrosta-1,4-dien-3-one
10	Estra-1,3,5(10)-triene-3,17-diol
11	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diacetate
12	19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol
13	3-Acetylestra-1,3,5,(10)-trien-17-one

MATERIALS AND METHODS

The IUPAC names of steroidal drugs are compiled in Table 1. The polyethylene-coated silica support was prepared in our laboratory; its retention characteristics were previously reported.[21] A 25 cm x 4 mm I.D. column was filled with a Shandon Analytical HPLC Packing Pump (Pittsburgh, USA) by the procedure normally used for the filling of reverse phase columns.[22] The HPLC equipment consisted of a Gilson gradient analytical system (GILSON Medical Electronics Villiers-le-Bell, France) with 2 piston pumps (Model 302), Detector (Model 116), Rheodyne injector with 20 μl sample loop (Cotati, California, USA), and a Waters 740 integrator (Milford, Massachusetts, USA). The flow-rate was 1.0 mL/min and the detection wavelength was 225 nm. Mixtures of methanol - water were used as eluents.

Methanol concentration ranged from 40 to 70 vol.% in steps of 5 vol.%. The drugs were dissolved in the eluents at a concentration of 0.05 mg/mL. The average retention time of each compound was determined by three consecutive determinations. Linear correlation was used to describe the dependence of the log k' value on the methanol concentration:

$$\log k' = \log k'_0 + b \cdot C \qquad (1)$$

where log k' is the logarithm of capacity factor; log k'_0 is the logarithm of capacity factor extrapolated to zero methanol concentration in the eluent (intercept); b is the change of log k' value caused by unit change (1 vol%) of methanol concentration (slope); and C is the methanol concentration in the eluent (vol %).

To find the molecular parameters significantly influencing the retention, the intercept and slope values of eq.1 were correlated with the physicochemical characteristics of steroidal drugs using canonical correlation analysis. The physicochemical parameters included in the calculation were: π = Hansch - Fujita's substituent constant characterizing hydrophobicity; H - Ac and H - Do = indicator variables for proton acceptor and proton donor properties, respectively; M - RE = molar refractivity; F and R = electronic parameters characterizing the inductive and resonance effects, respectively; σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent; Es = Taft's constant, characterizing steric effects of the substituent; B_1 and B_4 = Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachement.

Canonical correlation analysis was carried out on the data matrices consisting of the slope and intercept values of eq.1 (matrix II containing the retention characteristics of steroidal drugs) and of the physicochemical parameters listed above (matrix I).

Calculations were carried out with an IBM AT computer. Canonical correlation analysis software was prepared by Dr. Barna Bordás, Plant Protection Institute of Hungarian Academy of Sciences, Budapest, Hungary.

RESULTS AND DISCUSSION

The parameters of eq.1 are compiled in Table 2. The correlation between the methanol concentration in the eluent and the logarithm of the capacity factor is, in each instance, significant, indicating the regular retention behavior of the solutes.

The slope and intercept values of the steroidal drugs differ considerably, suggesting that the drugs can be sucessfully separated on this column by an appropriate mixture of methanol - water. The values of standard deviation are low, indicating the good reproducibility of the retention time on polyethylene-coated silica column.

Table 2

Parameters of Linear Correlations Between the Logarithim of Capacty Factor and Methanol Concentration in the Eluent.

$$\log k' = \log k'_0 + b \cdot c$$

Compound No.	$\log k'_0$	$-b \cdot 10^2$	$s_b \cdot 10^3$	r_{calc}
1	2.76	4.49	0.9	0.9990
2	1.79	3.81	1.7	0.9953
3	2.26	4.50	1.2	0.9981
4	2.79	4.76	1.13	0.9980
5	2.34	4.35	1.4	0.9973
6	3.07	5.09	2.4	0.9945
7	3.88	5.74	2.25	0.9971
8	2.83	4.69	0.7	0.9995
9	6.37	5.12	0.4	0.9999
10	2.72	4.40	0.8	0.9994
11	4.09	5.34	1.7	0.9990
12	2.90	4.99	1.1	0.9991
13	3.49	5.03	1.5	0.9983

s_b = standard deviation of the slope ‘b’ value.

The standard and weighted canonical coefficients of the relationships corresponding to the two columns in data matrix II (retention parameters) are compiled in Tables 3 and 4.

The relationships found by CCA indicate that the retention behavior of steroidal drugs on polyethylene-coated silica column considerably depends on the physicochemical parameters included in the calculation. The high r^2 values suggest that this set of physicochemical parameters can be successfully used for the prediction of the retention behavior of steroidal drugs on polyethylene-coated column.

Both chromatographic parameters have similar weights in the second equation (but not in the first one), indicating that these parameters are related but they are not identical. This observation indicates that the information content of both retention parameters is different: therefore, they can be separately included in future structure-retention relationship studies. The

Table 3

Relationship Between the Physicochemical Parameters and Retention Characteristics of Steroidal Drugs. Results of Canonical Correlation Analysis. First Equation

Variables	Canonical Coefficients	
	Standard	Weighted %
Chromatographic Parameters		
$\log k'_0$	-0.31	12.27
b	0.95	87.73
Physicochemical Parameters		
π	-0.53	2.77
H-Ac	1.56	14.69
H-Do	-0.73	11.99
M-RE	-1.02	0.73
F	0.55	21.54
R	0.16	3.67
σ	-0.57	26.22
Es	0.58	1.35
B_1	-1.92	11.34
B_4	1.64	5.70

$r^2 = 0.9824$; $Chi^2 = 35.37$.

impact of the various physicochemical parameters on the retention behavior is similar in both equations. Surprisingly, the calculated molecular hydrophobicities of drugs exert a negligible influence on the retention; however, both the column and the eluents are typical for reverse phase chromatographic system. This result, can be explained by the supposition that the apolar polyethylene chains lie parallel to the silica surface; therefore, the possibility of hydrophobic interactions between the stationary phase and the solutes is lower than in the case of octade-cylsilica columns.

It can be further assumed, that the polyethylene layer does not entirely cover the silica surface, and the hydrophilic interactions occurring between the active adsorption centers not covered by the hydrophobic ligand and the polar substructures of drugs may be involved in the retention mechanism. This assumption, is supported by the finding that the electron-withdrawing power of the substituents, their inductive and resonance effect and proton acceptor capacity have the highest weights in the equations.

Table 4

Relationship Between the Physicochemical Parameters and Retention Characteristics of Steroidal Drugs. Results of Canonical Correlation Analysis. Second Equation

Variables	Canonical Coefficients	
	Standard	Weighted %
Chromatographic Parameters		
$\log k'_0$	0.80	50.59
b	-0.78	49.41
Physicochemical Parameters		
π	0.03	0.31
H-Ac	-1.42	17.47
H-Do	0.70	8.55
M-RE	0.05	0.56
F	-1.47	18.01
R	-1.50	18.39
σ	2.09	25.64
Es	-0.05	0.62
B_1	0.60	7.31
B_4	-0.26	3.14

r^2 = o.9068; Chi^2 = 13.05

The results of canonical correlation analysis are supported by the fact that some significant correlations were found between the individual retention characteristics and the physicochemical parameters having high weights in the equations ($n = 13$; $r_{95\%} = 0.5529$):

$$\log k'_0 = 2.51 - (1.26 \pm 0.34)\,.\,R \qquad r_{calc} = 0.7492 \qquad (2)$$

$$b = 4.55 - (0.46 \pm 0.17)\,.\,R \qquad r_{calc} = 0.6329 \qquad (3)$$

$$b = 5.48 - (0.91 \pm 0.36)\,.\,\sigma \qquad r_{calc} = 0.6075 \qquad (4)$$

The canonical variates calculated from matrix II (related to the relative importance of the individual steroidal drugs in the determination of the retention parameters) are compiled in Table 5. The data in Table 5 clearly show that the contributions of the drugs to the determination of the

Table 5

Contribution of Individual Steroidal Drugs to the Relationships Between Retention Characteristics and Physicochemical Parameters. Canonical Variates Calculated from Matrix II.

No. of Drugs	First Equation	Second Equation
1	-0.46	-0.09
2	-1.51	-0.33
3	-0.32	-0.50
4	-0.03	-0.28
5	-0.62	-0.32
6	0.60	-0.36
7	1.62	-0.17
8	-0.10	-0.19
9	-0.24	2.31
10	-0.62	-0.06
11	0.80	0.31
12	0.45	0.38
13	0.37	0.07

chromatographic parameters is highly different. We assume that similar data can be used for the selection of solute sets - with the number of solutes as low as possible-which are suitable for characterisation of chromatographic systems.

It can be concluded from the results, that canonical correlation analysis is a suitable method for the assessment of the relationships between the retention behavior of steroidal drugs on polyethylene-coated silica support and their physicochemical parameters. Calculation indicated, that the retention characteristics of this support differ from that of traditional octadecylsilica support and the electronic parameters of drugs exert a considerable influence on the retention, suggesting the involvement of hydrophilic forces in the solute - support interaction.

REFERENCES

1. R. Kaliszan, "Quantitative Structure-Retention Relationships Applied to Reverse Phase High Performance Liquid Chromatography, " J. Chromatogr. A, **656**, 417 (1993).

2. R. M. Smith, "Functional Group Contributions to the Retention of Analytes in Reverse Phase High Performance Liquid Chromatography," Chromatogr. A, **656**, 381, (1993).

3. N. Chen, Y. Zhang, P. Lu, "Effects of Molecular Structure on the Log k'_w Index and Linear S-log k'_w Correlation in Reverse Phase High Performance Liquid Chromatography," J. Chromatogr., **633**, 31, (1993).

4. M. Righezza, J. R. Chretien, "Factor Analysis of Experimental Design in Chromatography," Chromatographia, **36**, 125, (1993).

5. U. Haldna, J. Pentchuk, M. Righezza, J. R. Chretien, "Factor Analysis in Ion Chromatography of Carboxylate Ions," J. Chromatogr. A, **670**, 51, (1994).

6. P. Karsnas, T. Lindblom, "Characterization of Hydrophobic Interaction and Hydrophobic Interaction Chromatography Media by Multivariate Analysis," J. Chromatogr., **599**, 131, (1992).

7. R. Gami-Yilinkou, A. Nasal, R. Kaliszan, "Application of Chemometrically Processed Chromatographic Data for Pharmacological Relevant Classification of Antihistamine Drugs," J. Chromatogr., **633**, 57, (1993).

8. R. Kaliszan, K. Osmialowski, "Correlation Between Chemical Structure of Non-Congeneric Solutes and their Retention on Polybutadiene-Coated Alumina," J. Chromatogr., **506**, 3, (1990).

9. T. Hamoir, F. Cuesta Sanchez, B. Bourgignon, D. L. Massaart, "Spectral Mapping Analysis: A Method for the Characterization of Stationary Phases," J. Chromatogr. Sci., **32**, 488, (1994).

10. J. R. Torres-Lapasió, R. M. Villanueva-Camanas, J. M. Sanchis-Mallols, M. J. Medina-Hernandez, M. C. Garcia-Alvarez-Coque, "Interpretative Strategy for Optimization of Surfactant and Alcohol Concentration in Micellar Liquid Chromatography," J.Chromatogr. A, **677**, 239, (1994).

11. R. J. M. Vervoort, M. W. J. Derksen, F. A. Maris, "Selection of Stationary Phases for the Liquid Chromatographic Analysis of Basic Compounds Using Chemometric Methods," J. Chromatogr. A, **678**, 1, (1994).

12. L. Orloci, C. R. Rao, W. M. Stitiler, **Multivariate Methods in Ecological Work**, International Cooperative Publishing House, Fairland, Maryland, USA, 1979.

13. K. V. Mardia, J. T. Kent, J. M. Bibby, **Multivariate Analysis**, Academic Press, London, 1979.

14. E. R. Malinowski, D. C. Howery, **Factor Analysis in Chemistry**, Wiley, New York, 1980.

15. T. Cserháti, E. Forgács, "Use of Canonical Correlation Analysis for the Evaluation of Chromatographic Retention Data," Chemom. Int. Lab. Syst., **28**, 305, (1995).

16. E. Forgács, T. Cserháti, B. Bordás, "Application of Multivariate Mathematical-Statistical Methods for the Comparison of the Retention Behaviour of Porous Graphitized Carbon and Octadecyl-Silica Columns," Anal. Chim. Acta, **279**, 115, (1993).

17. E. Forgács, T. Cserháti, B. Bordás, "Comparison of the Retention Behaviour of Phenol Derivatives on Porous Graphitized Carbon and Octadecyl-Silica Columns," Chromatographia, **36**, 19, (1993).

18. J. Novakovic, E. Tvrzicka, V. Pacakova, "High-Performance Liquid Chromatographic Determination of Equine Estrogens with Ultraviolet Absorbance and Electrochemical Detection," J. Chromatogr. A, **678**, 359, (1994).

19. J. Lykkesfeldt, S. Loft, H. E. Poulsen, "Simultaneous Determination of Urinary Free Cortisol and 6ß-Hydroxycortisol by High Performance Liquid Chromatography to Measure Human CYP3A Activity," J. Chromatogr. B, **660**, 23, (1994).

20. S. M. R. Stanley, B. S. Wilhelmi, J. P., Rodger, A. Guthrie, "Detection of Corticosteroids by High Performance Liquid Chromatography/Negative Ion Chemical Ionization Mass Spectrometry Using a Particle Beam Interface," Biol. Mass Spectrom., **23**, 483, (1994).

21. E. Forgács, "Retention Behavior of Some Ring-Substituted Aniline Derivatives on Polyethylene-Coated and Octadecylsilica Columns," J. Liquid Chromatogr., **16**, 3757, (1993).

22. **Instruction Manual HPLC Packing Pump,** Shandon, Cheshire, UK, 1989.

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