Study of the Interaction of Structurally Similar Bioactive Compounds by Thin-Layer Chromatography

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Abstract

The interaction between steroids and cholesterol is studied by reversed-phase thin-layer chromatography (TLC) using TLC plates impregnated by cholesterol and methanol–water mixtures as the eluents. The R_M values obtained are in linear correlation with the methanol concentration of the eluent. The slope obtained from the linear regression analysis, which is characteristic of the strength of interaction, is determined. Stepwise regression and principal component analysis are carried out to find a relationship between the physicochemical parameters of steroid drugs and the strength of the interaction, which is followed by nonlinear mapping and cluster analysis to visualize the obtained results. The results show that steric and electronic parameters of the steroid drugs have a significant effect on the strength of the interaction between these structurally similar molecules.

Introduction

Thin-layer chromatography (TLC) has been a widely used technique for the simultaneous qualitative analysis of numerous biomolecules. Its main advantages are its quickness and simplicity. In normal phase, the surfaces of the most commonly used TLC plates and high-performance liquid chromatography (HPLC) columns are silica gel or aluminium-oxide. In reversed-phase, modified silica gels are also used. The number of homemade HPLC columns and TLC plates with a special coating has recently increased. A bonded cholesterol stationary phase for HPLC was prepared by Siouffi et al., for which the temperature effects on the retention and selectivity of polyaromatic hydrocarbons were studied (1). Buszewski et al. reported studies on retention and separation performance of a cholesterol–silica stationary phase for HPLC (2–7). Kaliszan et al. reviewed molecular interactions using silica-based human serum albumin (8,9), keratin (10), collagen (11), melanin (12,13), and cholesterol (14) stationary phases.

Steroids play an important role in the human body, and most of them are anti-inflammatory drugs and contraceptive agents (15). Cholesterol is an important component of biological membranes. It constitutes the third group of polar membrane lipid sterols after phospholipids and glycolipids. Cholesterol molecules decrease the permeability of the phospholipid bilayer and increase its stability (16). The degree of the absorption of several drugs (e.g., apolar steroid drugs) can depend on the strength and nature of the interaction between cholesterol and steroid drugs. A simple determination of steroids by reversed-phase (RP) TLC is a method of general use (17–23). TLC plates impregnated by cholesterol developed by us can be used to estimate the molecular interactions of these structurally similar compounds.

Multivariate mathematical–statistical methods such as principal component analysis (PCA) and stepwise regression analysis (SRA) have been developed to evaluate the information coming from complex data matrices (24,25). Using these methods, it is possible to map the relationship between physicochemical parameters and the chemical behavior of the studied molecules. SRA automatically eliminates the insignificant independent variables from the selected equation, which increased, in this manner, the information power of the calculation. PCA reduces the dimension of the data matrices and has the further advantages of being able to both extract one or more background variables that have concrete physicochemical meaning and decrease the number of variables to the minimum necessary for the solution of a problem. Two-dimensional nonlinear mapping and cluster analysis facilitate the evaluation of the results of PCA. With the help of these methods, we can classify compounds on the strength of their chemical behavior.

The aim of our work was to establish the existence of an interaction between cholesterol and steroid drugs. Furthermore, we focused on the applicability of the TLC method for the determination of the interaction between structurally similar compounds.

Experimental

TLC

Five grams of cholesterol (commercially avaliable) was dissolved in a chloroform–acetone mixture (50:50 mL). DC-

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Aluminiumoxide F_{254} plates 20 × 20 cm, (Merck, Darmstadt, Germany) were impregnated overnight in this cholesterol solution. Fourteen steroid drugs were separately dissolved in methanol at a concentration of 2 mg/mL, and 5 µL of the solutions were plotted on the plates. The chemical structures of the drugs are shown in Figure 1. Methanol–water mixtures were used as the mobile phases in the concentration range of 10–60%. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at ambient temperature; the distance of development was approximately 15 cm. After development, the plates were dried at room temperature, and the spots were detected under UV light.

Mathematical–statistical methods

The R_M value, which characterized the molecular hydrophobicity in RP-TLC, was calculated for each solute in each eluent as follows:

$$R_M = \log(1/R_F - 1) \qquad \text{Eq. 1}$$

where R_F is the retention factor value of the analyte.

Linear correlations were calculated between R_M and the methanol concentration (c) in the eluent for each steroid:

$$R_M = R_{M0} + bc \qquad \text{Eq. 2}$$

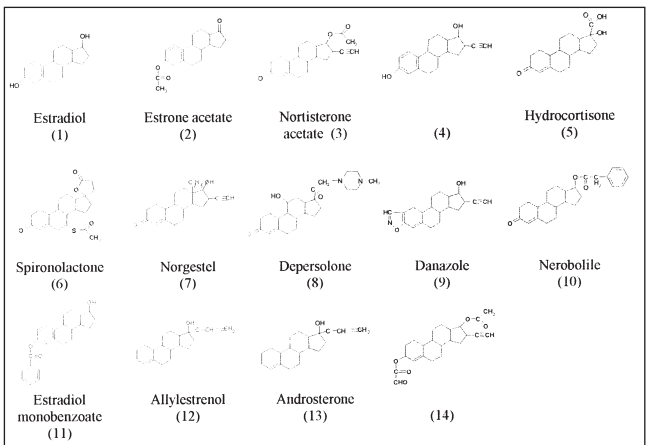

Figure 1. Chemical structures and names of steroid drugs.

Table I. Correlations Between the R_M Values of Steroid Drugs and the Concentration of Methanol* in the Methanol–Water Eluent†

Number of steroids	R_{M0}	b	*r*
1	2.9836	–0.043	0.9701
2	2.280	–0.0378	0.9761
3	2.416	–0.045	0.9883
4	2.524	–0.044	0.9914
5	1.438	–0.035	0.9941
6	1.9967	–0.0438	0.9882
7	2.248	–0.043	0.9816
8	1.3616	–0.034	0.9878
9	2.6419	–0.0427	0.9480

* c, in the range of 0–50%.
† From equation 2.

where R_M is the actual R_M value of a steroid determined at c volume %methanol concentration, R_{M0} (intercept) is the theoretical R_M value extrapolated to zero methanol concentration, and b (slope) is characteristic of the strength of the molecular interaction between the steroids and cholesterol (26).

SRA and PCA were applied to find the significance of the physicochemical parameters of steroids influencing their binding capacity to cholesterol. Parameters included in the calculation were: (*a*) π was the Hansch-Fujita's substituent constant, which characterized hydrophobicity; (*b*) H-Ac and H-Do were the indicator variables for proton acceptor and donor properties; (*c*) M-Re was the molar refractivity; (d) F and R were the Swain-Lupton's electronic parameters, which characterized inductive and resonance effects, respectively; (*e*) σ was the Hammet constant, which characterized the electron-withdrawing power of the substituent; (*f*) Es was the Taft's constant, which characterized the steric effects of the substituent; and (*g*) B1 and B4 were the Sterimol width parameters determined by the distance of the substituents at their maximum point perpendicular to the attachment bond's axis (27). The parameters of the steroid drugs were calculated by using the fragmental constants and additivity rule.

SRA was carried out; the dependent variable was the slope (b), and the independent variables were the physicochemical parameters of the drugs. The number of steps was not limited. The acceptance level for the individual independent variables was 90%.

PCA was applied to determine similarities and differences between the retention characteristics and physicochemical parameters of the steroids. The b values and physicochemical parameters of the steroids were taken as variables, and the

Table II. Similarities and Differences Between the Physicochemical Parameters of Steroids and the Force of the Interaction Between Steroids and Cholesterol

Results of PCA

Number of PC	Eigen value	Variance explained (%)	Total variance explained (%)
1	4.7569	43.24	43.24
2	3.3849	30.77	74.02
3	1.1302	10.27	84.29
4	0.9364	8.51	92.80
5	0.9403	4.46	97.26

PC loadings

	Number of PCs				
Parameter	1	2	3	4	5
b	0.1625	0.7769	–0.3045	–0.0523	–0.5090
π	–0.4011	0.7419	0.4487	–0.0642	0.2284
H-Ac	0.4161	0.7006	–0.4672	0.1419	0.3046
H-Do	–0.6459	0.3815	0.1131	0.6248	–0.0911
M-Re	0.9410	–0.0658	0.1784	0.1808	0.1655
F	–0.5926	0.7502	–0.0315	–0.0424	0.1340
R	0.8678	0.1434	–0.2171	–0.4119	0.0669
σ	0.6628	0.6727	–0.1687	0.1982	0.0350
Es	–0.8743	–0.1866	–0.2978	–0.2501	0.1121
B1	0.8704	–0.2463	0.2930	0.2366	–0.0615
B4	0.1678	0.6774	0.5675	–0.3963	–0.1028

steroids were the observations. The two-dimensional nonlinear map of principle component (PC) loadings and variables and cluster analysis of PC variables were also calculated.

Results and Discussion

The parameters of equation 2 are compiled in Table I. Three of the steroids (12, 13, and 14) did not migrate at all. Furthermore, no significant correlation was found between the R_M and c values of two steroid drugs (10 and 11), which were also omitted from the series. We found significant correlation by nine steroids; the values of the regression coefficients were over 0.9. We had applied SRA and PCA for only the first nine steroid drugs. SRA selected two significant correlations shown in equation 3:

$$B = -0.04 + 1.0(\pm 4.26 \bullet 10^{-3})\sigma - 0.63(\pm 4.71 \bullet 10^{-5})\text{M-Re} \quad \text{Eq. 3}$$

where n (9) is the number of the steroids included in the calculation, r (0.8829) is the multiple correlation coefficient, and $F_{99.9}$ (5.85) is the calculated value of the Fisher significance test.

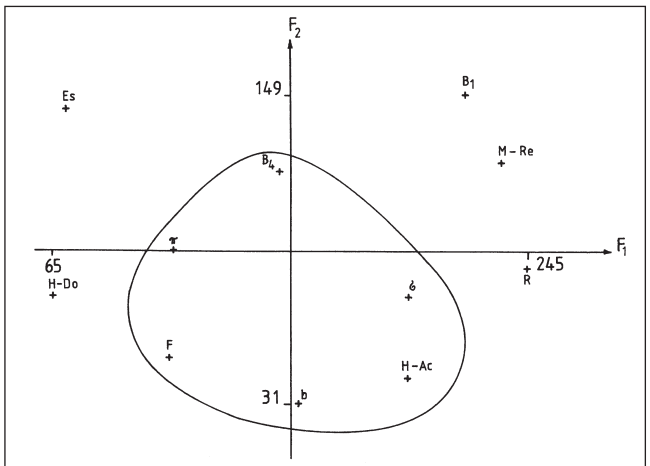

Figure 2. Two-dimensional nonlinear map calculated from the original PC loadings. The number of iterations was 129, and the maximum error was 3.42 × 10^{-2}.

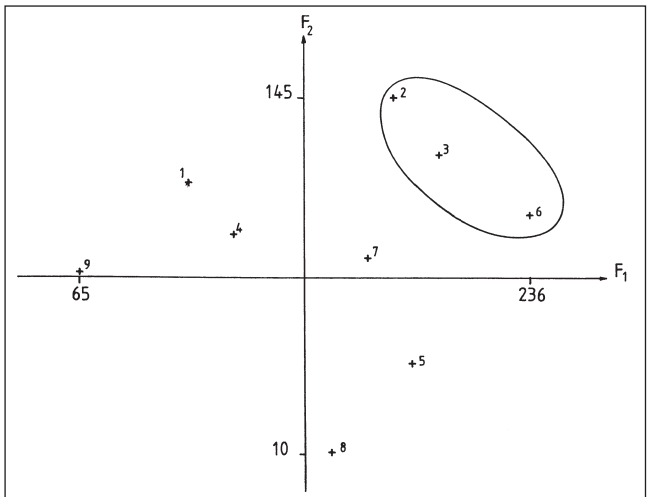

Figure 3. Two-dimensional nonlinear map of PC variables. The number of iterations was 37, and the maximum error was 2.69 × 10^{-2}. For the symbols, see the Experimental section.

The path coefficient (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable) was 61.3% for σ and 38.7% for M-Re. The highest impact on the strength of the interaction between the steroid drugs and cholesterol was exhibited by the steroid substituent's electron withdrawing power (σ) and the molar refractivity (M-Re) as shown by equation 3. To explore every important parameter in the interaction, SRA was carried out again, but this time σ was not included in the independent variables. In this case, SRA showed the following correlation between H-Ac and b:

$$B = -0.052 + 0.59(\pm 2.52 \bullet 10^{-3})\text{H-Ac} \quad \text{Eq. 4}$$

where n = 9, r = 0.5979, and F = 3.89.

Equation 4 confirmed the results of equation 3 (i.e., that σ and H-Ac, which characterized the polarity of the molecules, were the determinant factors in the interaction). Equations 3 and 4 fit the experimental data well; the significance levels in each were over 99.9% (see calculated $F_{99.9}$ values).

The results of PCA are shown in Table II. Five PCs explained more than 98% of the total variance, thus, the physicochemical parameters can be substituted by five background variables with only a 2% loss of information. Because the loading of b in the second PC was reasonably high (see Table II), five of the independent variables (π, H-Ac, F, σ, and B4) had high loading in the second PC studied as a major influencing parameter. This fact was also supported by two-dimensional nonlinear mapping of the PC loadings (Figure 2). The following parameters are close to the position of b: σ, H-Ac, π, F, and B4. This finding was in accordance with the results of SRA, and it showed that steric and electronic effects had a great influence on the strength of the steroid–cholesterol interaction.

The results of two-dimensional nonlinear mapping and cluster analysis of the PC variables are shown in Figures 3 and 4. These two methods gave similar results, but the two-dimensional nonlinear mapping had higher dimensionality. Both figures show that drugs containing acetyl and more polar OH groups form a cluster, which gave evidence of the major role of polarity in the formation of interaction. These clusters are not well separated,

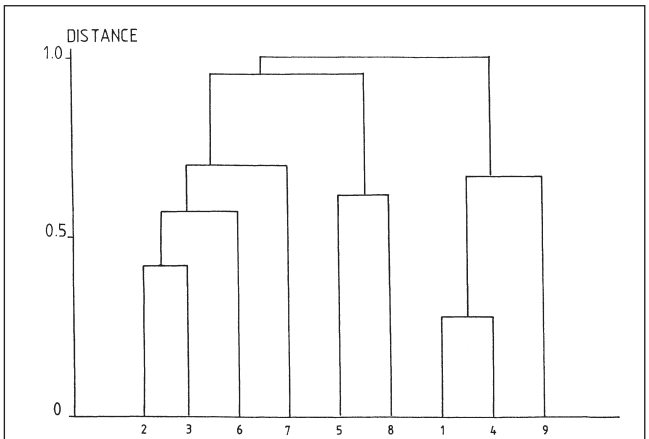

Figure 4. Cluster dendrograms calculated from the PC variables. For the symbols, see the Experimental section.

which could have been caused by the few number of the studied molecules.

Conclusions

In our work, an RP-TLC method using cholesterol impregnation was successfully applied for the examination of the strength of interaction between cholesterol and steroid drugs. SRA and PCA, followed by two-dimensional nonlinear mapping and cluster analysis found the same results; the strength of interaction was mainly influenced by both the electronic parameters and polar properties of the substituents of the steroids. Further investigations are necessary to study the effect of steric and polar properties on the stregth of the interaction in case of steroids not involved in this work.

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