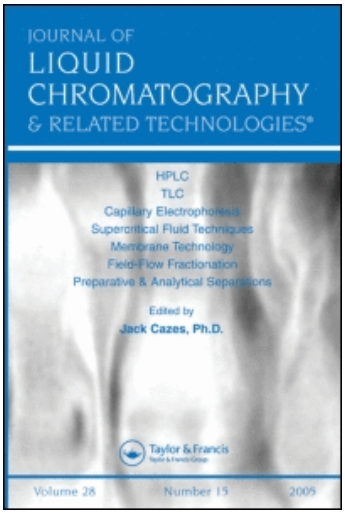

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Klára Valkó[ab]; Péter Slégel[ab]
[a] Central Research Institute for Chemistry Hungarian Academy of Sciences, Budapest, Hungary [b] EGIS Pharmaceuticals, Budapest, Hungary

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MOLECULAR MODELING FOR MOBILE PHASE OPTIMIZATION IN RP-HPLC

KLÁRA VALKÓ[1]* AND PÉTER SLÉGEL
[1]*Central Research Institute for Chemistry*
Hungarian Academy of Sciences
P.O. Box 17
H-1525 Budapest, Hungary
[2]*EGIS Pharmaceuticals*
P.O. Box 100
H-1475 Budapest Hungary

ABSTRACT

The applicability of molecular parameters calculated on the bases of molecular mechanics have been investigated for the prediction of reversed-phase retention behavior of structurally unrelated series of drug molecules. Non-polar, non-polar unsaturated and polar surface areas, surface energies, dipole moments, van der Waals radii and hydrophobicity values expressed by the logarithm of the octanol/water partition coefficients have been calculated from the molecular structure. The reversed-phase retention behavior was described by the slope and the intercept of the straight lines obtained by plotting the log k' values against the acetonitrile concentration of the mobile phase. The acetonitrile concentration ($OP\%_0$) which was needed for the log k'=0 retention was also calculated from the slope and intercept values. Step-wise linear regression analyses have been applied for revealing the correlations between the investigated parameters. The slope values could be described by the difference of the non-polar and non-polar accessible surface areas or by the total surface

energy values and the van der Waals radii. The intercept values could be described by the hydrophobicity parameter, the slope and the reciprocal values of dipole moment. The acetonitrile concentration for the log k'=0 retention ($OP\%_0$) could have been calculated from the hydrophobicity and the non-polar unsaturated surface area values of the investigated compounds.

INTRODUCTION

Optimization of mobile phase composition for the separation of a given mixture of compounds by high performance liquid chromatography is an exciting research field [1]. The HPLC method development is time-consuming and rather expensive procedure, therefore many attempts have been taken to reduce the number of experiments before optimization by simulating chromatograms by computers [2]. All of the optimization strategies are based more or less on the physico/chemical considerations of solute retention which is governed by the interaction energies with the stationary and mobile phase molecules. In the early work of Horváth et. al. [3] the retention was attributed to a reversible association of the solutes with the hydrocarbonaceous ligand of the reversed-phase stationary phase. The energetics of the association process was also analyzed and the dependence of the capacity factors on the ionic strength of the eluent and the hydrophobic surface of the solute were revealed. The correlations of the octanol/water partition coefficients, molecular surface areas, and reversed phase capacity ratios were studied by Funasaki et. al. [4]. The importance of the molecular cavity surface area and various connectivity indices, which can be calculated from the chemical structure of the compounds was pointed out also by Funasaki et. al. [5]. Eng et. al. [6] showed the application of holistic conformation and total surface area calculations for the prediction of chromatographic

retention parameters for triphenyl derivatives. Möckel et. al. [7] investigated the effect of the molecular surface type and area to the retention of various hydrocarbon classes. It cannot be questioned that molecular parameters play an important role in the retention but the extent of possible generalization of the found equations and the predictive power of it for the chromatographic retention have not been explored yet.

Many methods have been devised for the determination of molecular structures. In more recent years it has been proven to be possible to determine accurate structures by computational methods, typically ab initio calculations for small molecules and by molecular mechanics calculations [8] for large molecules. In this study molecular mechanics calculations have been carried out to determine the three dimensional structures of molecules having pharmaceutical importance. The relations of the calculated molecular parameters have been investigated to the reversed-phase retention parameters obtained with changing gradually the acetonitrile concentration of the mobile phase.

MATERIALS AND METHODS

The 23 compounds listed in Table 1. were obtained from the Drug Store of Semmelweis University Medical School, Budapest, Hungary and they are registered drug substances in the 6th Edition of Hungarian Pharmacopoeia complying its requirement regarding the purity. The compound series have a wide range of hydrophobicity and chemical structure. The reversed-phase chromatographic measurements were carried out on LiChrosorb RP-18 10 μm columns with the dimensions of 250 x 4.6 mm (Merck, Darmstadt, F.R.G.). The mobile phase was various composition of the acetonitrile and 0.05M phosphate buffer (pH=4.6). Lower pH (pH=2) was used for the retention

Table 1.
The chromatographic retention data and hydrophobicity data of the investigated compounds.

Compound	log P_c	slope	int	$OP\%_0$
1. sulfadimidine	1.644	-0.0280	0.854	30.50
2. sulfamerazine	0.612	-0.0283	0.892	31.52
3. barbital	-1.050	-0.0402	1.063	26.44
4. phenobarbital	-0.430	-0.0319	1.341	42.04
5. chloramphenicol	0.464	-0.0414	1.625	39.25
6. salicylamide	0.236	-0.0255	0.871	34.16
7. phenacetin	1.128	-0.0226	1.002	44.34
8. vanillin	1.119	-0.0244	0.866	35.49
9. benzaldehyde	1.535	-0.0303	1.575	51.98
10. acetanilide	0.529	-0.0270	1.021	37.81
11. nicotinamide	-0.690	-0.0382	0.251	6.57
12. benzoic acid	1.769	-0.0284	1.252	44.08
13. salicylic acid	2.140	-0.0301	1.425	47.34
14. acetyl sal. acid	1.037	-0.0272	1.077	39.60
15. caffeine	-0.912	-0.0299	0.552	18.46
16. hydrochlorothiazide	-1.717	-0.0456	0.887	19.45
17. dexamethasone	-0.472	-0.0139	0.568	40.86
18. deoxycorticosterone	3.795	-0.0147	1.120	76.19
19. isoniazide	-2.003	-0.0382	0.060	1.57
20. methyl salicylate	2.528	-0.0244	1.727	70.78
21. hydrocortisone	0.053	-0.0129	0.436	33.80
22. progesterone	4.002	-0.0192	1.831	95.36
23. testosterone	4.874	-0.0143	1.085	75.87

measurements of the acidic compounds. The detailed instrumentation of the measurements and also the obtained retention data have been published earlier [9]. The retention of the compounds was expressed by the logarithmic values of the capacity ratio (log k') and it was plotted against the applied acetonitrile concentration. On the bases of three to five points the slope and the intercept values of the straight lines have been calculated and listed in Table 1. It has been also published earlier that the $OP\%_0$ values, namely the acetonitrile concentration necessary for obtaining log k'=0 retention showed excellent correlation to the logarithmic values of octanol/water partition coefficients (log P). These values are also presented in Table 1.

PC Model approach was used to determine the three dimensional structure of compounds based on energy minimization. After setting up the geometries of the molecules having the smallest mmx-energy the non-polar (nopol), non-polar unsaturated (nupol) and polar surface (polsa) areas, their energies have been calculated. The water solvation shell was considered also in the calculations of the accessible polar (polac) and non-polar (npac, nupac) surface areas. The calculated total surface energy (tfcal) was expressed in kcal/mole. The dipole moment (dm) values and van der Waals radii (vdw) of the molecules were also calculated.

The log P_c values were estimated by the help of ProLogP™ expert system made by Compudrug Ltd., Budapest, Hungary.

Step-wise linear regression analysis has been carried out by the Drugidea™ program system developed for drug design (Chemicro Ltd., Budapest, Hungary). All of the programs were run on an IBM AT compatible personal computer.

Table 2.
The calculated molecular parameters for the investigated compounds. (nopol=non-polar surface area, Å^2, nupol=non-polar unsaturated surface area, Å^2, dm=dipole moment, vdw=van der Waals radius, npac=non polar accessible surface area, Å^2, tfcal=total surface energy, kcal/mole)

No.	nopol	nupol	dm	vdw	npac	tfcal
1.	261.8	62.5	5.44	10.43	246.7	-3.7
2.	194.7	68.4	6.81	11.60	183.5	-8.3
3.	193.3	0.0	0.84	5.11	182.2	-7.0
4.	206.7	27.6	0.68	9.84	195.3	-6.3
5.	246.1	27.9	6.3	11.01	205.9	-6.9
6.	103.1	50.8	3.14	5.60	97.4	-7.2
7.	286.4	53.3	4.83	9.95	269.7	4.1
8.	164.1	40.2	2.89	7.33	154.6	-3.6
9.	151.2	54.1	2.81	6.38	142.9	0.5
10.	196.6	40.7	3.03	8.01	185.4	1.5
11.	106.3	36.9	2.16	6.48	100.4	-5.4
12.	125.5	54.3	1.51	6.88	118.6	-2.4
13.	108.7	53.8	1.93	6.88	102.7	-5.5
14.	182.6	42.4	2.58	7.01	172.0	-3.7
15.	254.0	18.3	1.87	8.03	239.0	1.9
16.	124.2	15.8	6.42	5.26	103.7	-14.8
17.	370.2	12.2	4.92	17.36	348.8	-1.7
18.	383.3	6.9	3.91	13.52	361.7	0.8
19.	108.9	38.3	2.47	6.73	103.0	-7.6
20.	201.2	31.9	2.22	7.86	189.4	0.2
21.	356.6	6.8	4.36	15.21	336.4	-2.7
22.	410.2	6.8	2.28	13.00	387.0	4.2
23.	371.9	6.6	2.09	13.13	351.1	3.4

RESULTS AND DISCUSSIONS

The studied retention data and molecular parameters are listed in Table 1. and Table 2, respectively.

As it is well known, the reversed-phase retention is governed mostly by hydrophobicity of the compounds. Therefore first the correlations were studied between the retention parameters and the calculated log P_c values. As it was published earlier [9] when structurally unrelated compounds are investigated neither the slope nor the intercept values show significant correlation to the log P_c values. However, significant correlation has been found when both the slope and the intercept were considered as two independent variables. It also means that knowing only the hydrophobicity values of the molecules is not enough to calculate both the slope and intercept values. For the mobile phase optimization it is necessary to predict both the slope and intercept values because one can calculate the log k' values at any acetonitrile concentration of the mobile phase only from these two values. If the log k' vs. acetonitrile concentration function is known for each compound by the help of a simple algorithm the optimum mobile phase concentration can be calculated for the minimum resolution.

As it also has been published earlier [10] the $OP\%_0$ values, namely the acetonitrile concentration for obtaining retention time exactly double of the dead time can be calculated from equation 1.

$$OP\%_0 = 11.09(\pm 1.21)*\log P_c + 31.28 \quad (1)$$

n=23 r=0.895 s=10.10 F=84.3

The mathematical statistical parameters could be slightly increased by introducing the non-polar unsaturated surface area (nupol) of the compounds according to equation 2.

$$OP\%_0 = 11.00(\pm 1.06)*\log P_c - 0.25(\pm 0.92)*nupol + 39.58 \quad (2)$$

n=23 r=0.924 s=8.85 F=58.7

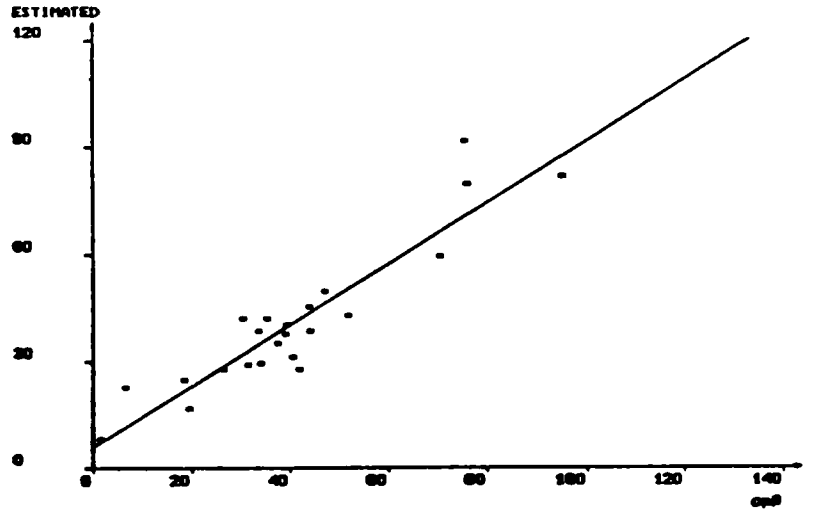


Figure 1.
The plot of the measured and calculated $OP\%_0$ values according to equation 2.

The plot of the measured and calculated $OP\%_c$ values can be seen in Fig. 1.

Equation 1 and 2 help in estimating mobile phase composition but one cannot know how the retention of the compounds would change by changing the mobile phase composition. Therefore the slope values of the investigated compounds were also investigated as the function of the molecular parameters. Two significant equations could be set up as it is shown by equation 3 and 4.

$$slope = 1.41(\pm 0.35)*10^{-3}*vdw + 9.29(\pm 0.26)*10^{-3}*tfcal - 0.04 \quad (3)$$

$$n=23 \quad r=0.845 \quad s=5.07*10^{-3} \quad F=25.1$$

$$slope = 6.98(\pm 1.73)*npac - 5.95(\pm 1.64)*nopol - 0.04 \quad (4)$$

$$n=23 \quad r=0.857 \quad s=4.90*10^{-3} \quad F=27.6$$

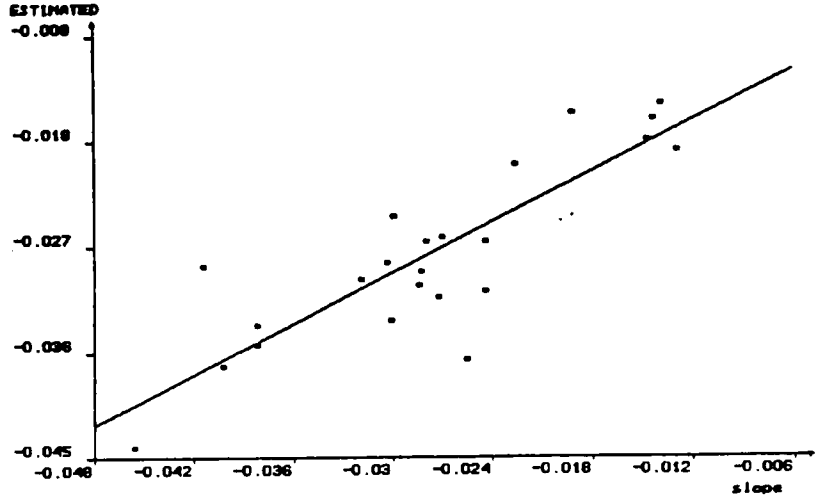


Fig. 2.
The plot of the measured and estimated slope values according to eqn. 3.

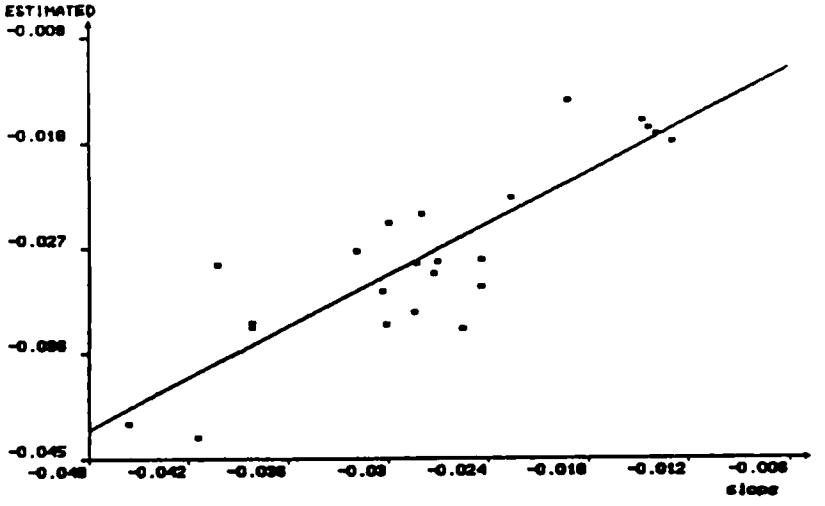
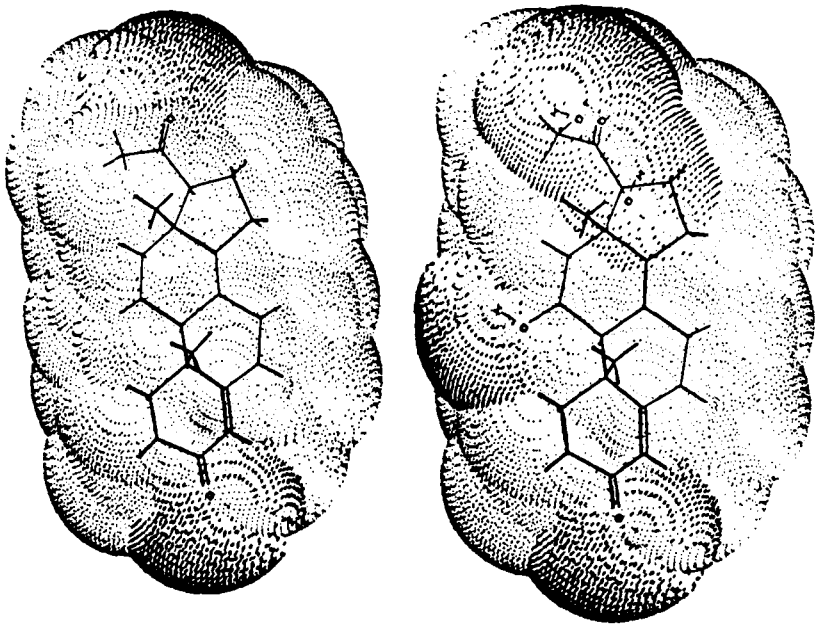


Fig. 3.
The plot of the measured and estimated slope values according to eqn. 4.



Progesterone Hydrocortisone

Figure 4.
The three dimensional plots of the electron clouds for progesterone and hydrocortisone.

Equation 3 means that the higher is the van der Waals radius and the higher is the total surface energy, the more sensitive is the compound retention by changing the acetonitrile concentration.

Equation 4 expresses the retention sensitivity of the compound to the organic phase concentration as the function of the difference between the non-polar and the non-polar accessible surface areas.

The plots of the calculated and measured slope values according to eqn. 3 and 4 are shown in Fig. 2 and 3, respectively.

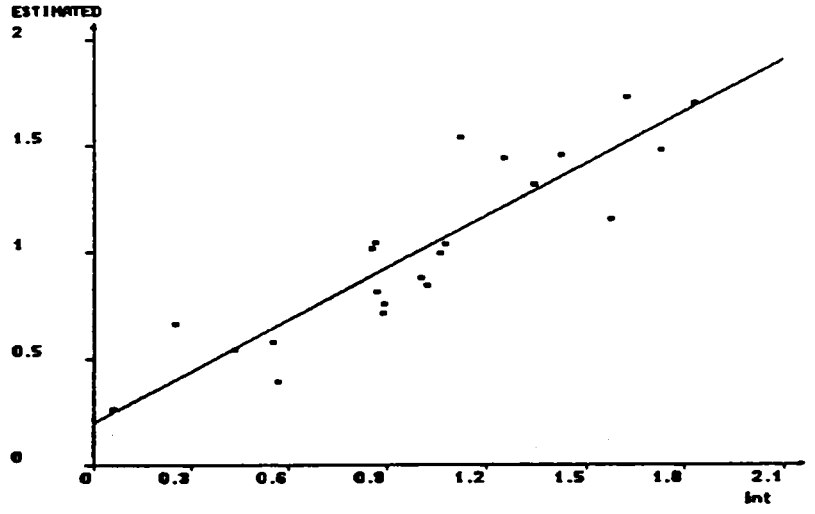


Figure 5.
The plot of the measured and estimated intercept values according to eqn. 5.

The three dimensional plots of hydrocortisone and progesterone are presented in Fig. 4. The polar surface regions seem to be darker. As the figure demonstrates the non-polar surface of progesterone is interrupted by polar hydroxyl groups in the case of hydrocortisone. Also the slope values of the two compounds differs markedly, -0.0192 and -0.0129 for progesterone and hydrocortisone, respectively.

The most significant equation for describing the variance of the intercept values was obtained when the log P_c values, the molecular parameters describing the slope values and the reciprocal value of the dipole moment were taken as independent variables.

$$int=0.268(\pm0.036)*\log P_c + 0.037(\pm0.008)*nopol - -0.040(\pm0.009)*npac +0.652(\pm0.165)*1/dm +39.58 \quad (5)$$

n=23 r= 0.896 s=0.230 F=17.4

The plot of the measured and estimated intercept values is shown in Fig. 5.

In conclusion the investigated retention parameters could be described by molecular parameters obtained from molecular modeling for structurally unrelated compounds. The obtained linear regression equations were significant in the mathematical statistical point of view, but the standard deviations seem higher than would be enough for mobile phase optimization without any experimental trials. The relatively low correlation coefficients can be explained by neglecting in the molecular mechanics force field calculations which are based on ab initio calculations with the assumption that the calculated force fields are transferable to larger molecules. Neglecting of special interactions (the presence of free silanol groups) in the chromatographic systems also might resulted in higher standard deviations.

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