Journal of Chromatographic Science, 2015, Vol. 53, No. 10, 1780–1785
doi: 10.1093/chromsci/bmv091
Advance Access Publication Date: 28 June 2015
Article

Article

Evaluation of Angiotensin-Converting Enzyme Inhibitor's Absorption with Retention Data of Micellar Thin-Layer Chromatography and Suitable Molecular Descriptor

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Received 20 November 2014; Revised 7 May 2015

Abstract

Twelve angiotensin-converting enzyme (ACE) inhibitors were studied to evaluate correlation between their absorption (ABS) data available in the literature (22–96%) and hydrophobicity parameters (k_m and $P_{m/w}$) obtained in micellar thin-layer chromatography (MTLC) using Brij 35. The theoretical considerations showed that the geometric molecular descriptor—volume value (Vol) should be considered as an independent variable simultaneously with calculated hydrophobicity parameters in multiple linear regression analysis to obtain reliable correlation between ACE inhibitor's absorption and lipophilicity (calculated KOWWINlog *P*) and that captopril should be excluded from further correlations. The results of MTLC confirmed that between the two hydrophobicity parameters k_m and $P_{m/w}$, for absorption prediction of 11 ACE inhibitors, the micelle–water partition coefficient $P_{m/w}$ provided higher correlation ($R^2 = 0.756$), while for the k_m parameter $R^2 = 0.612$ was obtained. The micelle–water partition coefficient $P_{m/w}$ could be considered as analogous to hydrophobicity parameter C_0 from reversed-phase thin-layer chromatography. Dissimilar retention behavior of lisinopril indicated its lowest non-polar interaction with micelle, because of its di-acid form. The proposed model which included ACE inhibitors on the opposite site of lipophilicity—lisinopril and fosinopril (KOWWINlog $P = -0.96$ and KOWWINlog $P = 6.61$, respectively), both with similar absorption values (25 and 36%, respectively), could indicate that absorption of investigated compounds occurs via two different mechanisms: active and passive transport.

Introduction

Angiotensin-converting enzyme (ACE) inhibitors are a significant group of drugs widely used in the treatment of hypertension and congestive heart failure. Although showing similar efficacy, ACE inhibitors are usually classified into three different groups (those with a sulfhydryl group—exemplified by captopril, those with a carboxyl group—exemplified by enalapril and those with the phosphinic acid group—exemplified by fosinopril) and exhibit different pharmacokinetic characteristics (1, 2).

For oral drug absorption the molecule's lipophilicity is the main determinant and the most frequently used parameter in the quantitative structure–activity relationship (QSAR) (3). Lipophilicity is characterized by the *n*-octanol/water partition coefficient ($\log P_{O/W}$) and can be determined by a traditional technique, the so-called *shake flask* method. Different chromatographic methods such as liquid chromatography (LC) or thin-layer chromatography (TLC) are well-established methods that can yield a significant amount of reproducible retention data for structurally different compounds, which can be correlated with their

physicochemical and biological properties principally lipophilicity (4–7). Furthermore, chromatographic lipophilicity data are frequently used in the modeling of a drug's activity or absorption, distribution, metabolism and excretion (ADME) studies. Micelle-mediated chromatographic techniques are very useful in these studies. The study of quantitative retention–structure and retention–activity relationship for local anesthetics by micellar liquid chromatography (MLC) was published (8). Also MLC was used for prediction of drugs transport (9). Biopartitioning micellar chromatography was used for modeling of quantitative retention–activity relationship of cephalosporins (10) as well as for prediction of oral absorption of 32 different drugs (11). The review of biopartitioning micellar separation methods in modeling drug absorption was also published (12). Furthermore, MLC methods were used for modeling the quantitative retention–activity relationship of drugs belonging to the ACE-inhibitor group (13, 14). According to a literature survey, micellar thin-layer chromatography (MTLC) is not as much utilized as MLC. Sumina *et al.* in their review paper reported application of surfactants as modifiers in TLC (15). For identification and quantification of ACE inhibitor lisinopril from urine samples (16) and for the lipophilicity study of 1,2,4-triazole (17) and *N*-phenyltrichloroacetamide derivatives (18), MTLC was used.

In our previous studies, lipophilicity investigations of five ACE inhibitors by means of reversed-phase thin-layer chromatography (RP-TLC) (19) and salting-out thin-layer chromatography (SOTLC) (20) were reported. Recently we established good correlation between RP-TLC and ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS) (21) and SOTLC (22) hydrophobicity data for evaluation of absorption of an extended group of ACE inhibitors.

The aim of this study was correlation of classical MTLC hydrophobicity parameter k_m, the solute retention parameter at zero micellar concentration, with calculated log *P* values (KOWWINlog *P*) for 12 investigated ACE inhibitors. This hydrophobicity parameter (k_m), originally proposed by Foley for MLC (23), was successfully applied in MTLC (17, 18). Additionally, micelle–water ($P_{m/w}$) partition coefficients proposed by Tanaka *et al.* (24) in MLC as well as Čudina *et al.* (11) was checked and evaluated for MTLC in this study. The main topic was modeling of established hydrophobicity parameters with literature-available absorption data including an additional molecular descriptor as an independent variable [aqueous solubility (log *S*), electronic descriptor—polar surface area (PSA); constitutional parameter—molecular mass (Mr) and geometric descriptor—volume value (Vol)] as well as selection of an appropriate hydrophobicity parameter using multiple linear regression (MLR) analysis in predicting oral drug absorption.

Experimental

Reagents and materials

The following standards of ACE inhibitors were investigated: 1. *Enalapril maleate*, (*S*)-1-[*N*-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline maleate (Krka Research and Development Division; Novo Mesto, Slovenia); 2. *quinapril hydrochloride*, [3*S*-[2[*R**(*R**)],3*R**]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl] amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride (Hemofarm Stada Pharmaceutical Industry, Vrsac, Serbia); 3. *fosinopril sodium*, (4*S*)-4-cyclohexyl-1-[[(*R*)-[(1*S*)-2-methyl-1-(1-oxopropoxy)-propoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline, sodium salt (Bristol-Myers Squibb Pharmaceutical Research Institute; Princeton, NJ, USA); 4. *lisinopril dihydrate*, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate (Belupo Pharmaceutical Cosmetic Quality Control Department, Zagreb, Croatia); 5. *cilazapril monohydrate*, [1*S*-[1α,9α(*R**)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl] amino]octahydro-10-oxo-6*H*-pyridazino[1,2-a][1,2]diazepine-1-carboxylic acid monohydrate (Roche Pharmaceuticals; Paris, France); 6. *ramipril*, (2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydrocyclopenta[b] pyrrole-2-carboxylic acid (Hemofarm Stada Pharmaceutical Industry; Vrsac, Serbia); 7. *benazepril hydrochloride*, (3*S*)-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1*H*-1-benzazepine-1-acetic acid hydrochloride (EP Reference Standard; Strasbourg, France); 8. *perindopril erbumin*, 2-methylpropan-2-amine (2*S*,3a*S*,7a*S*)-1-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxopentan-2-yl]amino] propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid (EP Reference Standard; Strasbourg, France); 9. *moexipril*, (3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-6,7-dimethoxy-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid (Tocris Bioscience in an R and D Systems company); 10. *zofenopril calcium*, (2*S*,4*R*)-1-((*S*)-3-(benzoylthio)-2-methylpropanoyl)-4-(phenylthio)pyrolidine-2-calcium carboxilate (The Menarini Group, Florence, Italy), 11. *trandolapril*, [2*S*-[1[*R**(*R**)],2α,3aα,7aβ]]-1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid (EP Reference Standard; Strasbourg, France); 12. *captopril*, (*S*)-1-(3-Mercapto-2-methyl-1-oxopropyl)-L-proline (EP Reference Standard; Strasbourg, France).

Micellar thin-layer chromatography

The study of selected ACE inhibitors was performed on RP-TLC C_{18} plates, which are commercially available (Art. 5559, E. Merck, Germany). The plates were spotted with 1 μL aliquots of freshly prepared ethanolic solutions of enalapril, quinapril, fosinopril, cilazapril, ramipril, benazepril, perindopril, moexipril, trandolapril and captopril, and aqueous solutions of lisinopril and zofenopril (about 2 mg/mL). The mobile phase was composed of 20% tetrahydrofuran (THF) and 80% phosphate buffer (pH 6.8) with addition of polyoxyethylene (23) lauryl ether, Brij 35 (Sigma-Aldrich) in concentrations varying from 0.01 to 0.06 M (above the critical micellar concentration —$CMC_{BRIJ35} = 9 \times 10^{-5}$ M). The phosphate buffer (pH 6.8) as artificial intestinal medium was prepared according to the International Pharmacopoeia (25) by dissolving potassium dihydrogenphosphate (1.700 g) and sodium hydrogenphosphate (1.765 g) (analytical grade, Merck, Germany) in deionized water (500 mL). After development, by the ascending technique, the detection was performed under a UV lump (λ = 254 nm). All investigations were performed at room temperature (25 ± 2°C).

Calculations

Microsoft Office Excel 2003 was used for the statistical regression analysis and for the 3D graph, OriginLab 8 software was applied by using the option "Non-linear surface fitting" and function "plane". The software packages used for calculation of ACE inhibitors' molecular descriptors, lipophilicity (log *P* values), aqueous solubility (log *S*), electronic descriptor—polar surface area (PSA); constitutional parameter—molecular mass (Mr) and geometric descriptor—volume value (Vol) as well as textbooks used to collect human oral absorption data of examined drugs have been cited in detail in our previously published articles (21, 22).

Results

In this research, 12 ACE inhibitors were studied by using MTLC to evaluate the correlation between their hydrophobicity parameters and the literature-obtained oral absorption data. The calculated

KOWWINlog P values varied from −0.94 to 6.61; Mr values from 217.3 to 563.7 and Vol values from 195.6 to 538.7, while oral absorption (ABS) data were in the range of 22–96% (Table I). The ACE inhibitor's solubility data (log S values) ranged from −4.7 to −1.7 and the PSA values from 57.6 to 132.9 Å^2.

According to Janicka *et al.* (17) and Foley (23) in MLC the following relationship (Equation (1)) was established between retention parameter $k\cdot$ and micelle (surfactant) concentration in eluent

$$\frac{1}{k'} = \frac{1}{k_m} + \frac{K_{AM}}{k_m}[M]. \quad (1)$$

The parameter $[M]$ may represent either the concentration of surfactant or the concentration of micelle (23) and when noting that micelle concentration corresponds to the total surfactant concentration minus the CMC; K_{AM} (or K_{sm}) is the constant describing solute–micelle binding and k_m is the solute retention parameter at zero micellar concentration (or k'_S defined as retention factor for the free solute) (17, 23, 26). The parameters K_{AM} and k_m can be evaluated from the slope and intercept of experimental $1/k'$ versus $[M]$ relationships (23). For this study, the retention data from MTLC were used as k' (also noted as k). The micellar log k_m parameter is considered as analogous to obtained R_M^0 in RP-TLC and different authors showed relationship between k_m and log k_m lipophilicity descriptors with log Pvalues (17, 18).

Micelle–water partition coefficient ($P_{m/w}$) in MLC was proposed as a useful hydrophobicity descriptor of antiplatelet agents (24) as well as for absorption prediction of 32 drugs from different pharmacological groups (11). According to Čudina *et al.* the retention parameter k was represented by the following equation:

$$\frac{1}{k} = b \cdot C_m + a, \quad (2)$$

where the intercept $a = 1/(P_{s/w}\,\Phi)$; slope $b = (P_{m/w} - 1)V/(P_{s/w}\,\Phi)$ and V is the partial molar volume (V = 1.18 l/mol for Brij 35); $P_{s/w}$ is the partition coefficient between stationary phase and water; C_m is the concentration of micelle in the mobile phase (total surfactant concentration minus CMC) and Φ is the chromatographic phase ratio (11). The CMC of Brij 35 (9×10^{-5} M) is not of significance for further calculations. The values of slope (b) and intercept (a) were calculated from the linear equation (Equation (2)) by simple regression analysis with MTLC experimental data (C_m and k). The obtained slope and intercept permitted calculation of $P_{m/w}$

$$P_{m/w} = \frac{b}{aV} + 1. \quad (3)$$

In this study, following the replacement of k values (from MLC) with retention data obtained in MTLC, the values of intercept (a) and slope (b) were determined from linear regression analysis between retention data and used micelle concentration range, from which $P_{m/w}$ data were calculated according to Equation (3). The obtained hydrophobicity parameters k_m and $P_{m/w}$ are listed in Table I.

The experimentally obtained hydrophobicity parameters k_m, logk_m or $P_{m/w}$ were correlated with selected log P values in order to evaluate k_m, log k_m and $P_{m/w}$ data as measure of lipophilicity.

Among numerous *in silico* obtained log P values of ACE inhibitors, in our previous studies (21, 22) the selection of KOWWINlog P as appropriate log P was evaluated on the basis of its agreement with chromatographic hydrophobicity parameters R_M^0 or C_0 obtained with RP-TLC and SOTLC, respectively. Also, an additional approach based on algorithm and validation for the sum of ranking differences between numerous log P values (27) was applied for evaluation of log P value selection in our recently published SOTLC study (22), indicating that only four log P values (Aclog P, Xlog P2, Alog P or KOWWINlog P) are acceptable.

In this study the value of KOWWINlog P was selected on the basis of its best agreement with MTLC hydrophobicity parameters k_m, log k_m and $P_{m/w}$. Lower values of R^2 were obtained for other three log P values: Aclog P, Xlog P2, Alog P. Parameter k_m showed higher R^2 in comparison with $P_{m/w}$ for selected KOWWINlog P (Table II).

The correlations between all calculated molecular descriptors and hydrophobicity parameters k_m, log k_m and $P_{m/w}$ obtained in MTLC showed that the geometric descriptor—volume value (Vol) or constitutional parameter—molecular mass (Mr) could be considered as independent variables in MLR analysis with k_m or $P_{m/w}$ values, due to their low correlations ($R^2 < 0.15$), while only Mr values could be considered as independent variable with log k_m. In the MLR analysis applied for modeling of ACE inhibitors' absorption as Y values, literature-available ABS data were used, while two independent variables were used as X values, lipophilicity values (calculated or obtained in MTLC) Vol values as X_1 or Mr values as X_2. The preliminary theoretical MLR analysis of all 12 studied ACE inhibitors with application of calculated KOWWINlog P values and values of Vol or Mr, as independent variables, indicate variable Vol value as a more suitable descriptor for correlation between predicted and literature-available absorption data since a higher correlation (R^2 = 0.463) was obtained with Vol than with Mr (R^2 = 0.417). The MLR analysis also indicated that captopril should be excluded from further correlations since considerably lower R^2 were obtained with captopril than after its exclusion (R^2 = 0.685) for KOWWINlog P and Vol values.

This MLR analysis of 11 ACE inhibitors, with application of Vol values and KOWWINlog P values as independent variables, provides the following correlation (Equation (4)) with acceptable R^2 as well as probability value ($P < 0.05$):

$$\begin{aligned} ABS_{predicted} &= 10.951\,(\pm 3.252)\,KOWWINlogP \\ &\quad - 0.451\,(\pm 0.111)\,Vol + 203.291\,(\pm 39.565) \\ n &= 11, R^2 = 0.685, S = 13.841, F = 8.686 \end{aligned} \quad (4)$$

In further modeling, substitution of KOWWINlog P with MTLC hydrophobicity parameters, k_m and $P_{m/w}$ values, was evaluated. The application of Vol and k_m or $P_{m/w}$ values as independent variables in MLR provides correlations (Equations (5) and (6)) with probability value $P < 0.05$ (Figure 1)

$$\begin{aligned} ABS_{predicted} &= -83.429\,(\pm 30.037)\,k_m - 0.306\,(\pm 0.099)\,Vol \\ &\quad + 194.721\,(\pm 43.367) \\ n &= 11, R^2 = 0.612, S = 15.353, F = 6.310 \end{aligned} \quad (5)$$

$$\begin{aligned} ABS_{predicted} &= -3.513\,(\pm 0.852)P_{m/w} - 0.331\,(\pm 0.079)\,Vol \\ &\quad + 166.258\,(\pm 31.055) \\ n &= 11, R^2 = 0.756, S = 12.172, F = 12.401 \end{aligned} \quad (6)$$

The obtained correlations could be evaluated as good as proposed by Asuero *et al.* (R range 0.70–0.89, which corresponds to an R^2 range 0.49–0.79) (28). Figure 1 represents MLR models of an ACE inhibitor's predicted absorption with values of molecular descriptor Vol and k_m or $P_{m/w}$ hydrophobicity parameters.

Table I. The Oral Absorption Data, Calculated Molecular Descriptors and MTLC Hydrophobicity Parameters of the Selected ACE Inhibitors

ACE inhibitor	ABS	AC log *P*	Xlog *P*2	Alog *P*	KOWWINlog *P*	Vol	Mr	k_m	$P_{m/w}$
1. Enalapril maleate	60	1.52	2.11	2.46	2.45	356.8	376.5	0.262 (±0.005)	−4.799 (±0.484)
2. Quinapril hydrochloride	60	2.08	3.16	3.66	3.72	411.4	438.6	0.136 (±0.007)	−6.727 (±1.433)
3. Fosinopril sodium	36	3.05	6.31	5.44	6.61	538.7	563.7	0.040 (±0.005)	−11.769 (±4.458)
4. Lisinopril dihydrate	25	0.53	1.24	1.78	−0.94	384.4	405.5	0.668 (±0.013)	6.151 (±0.311)
5. Cilazapril monohydrate	60	0.25	2.15	2.69	2.27	392.5	417.6	0.138 (±0.008)	−6.941 (±1.682)
6. Ramipril	54	2.07	2.89	3.31	3.32	396.4	416.6	0.126 (±0.005)	−7.734 (±1.296)
7. Benazepril hydrochloride	37	2.09	3.28	3.73	3.5	394.8	424.5	0.112 (±0.003)	−7.072 (±0.686)
8. Perindopril erbumin	70	1.58	2.58	2.73	2.59	358.3	368.5	0.289 (±0.004)	−3.789 (±0.363)
9. Moexipril	22	1.87	2.72	3.62	3.36	462.4	498.6	0.206 (±0.003)	−5.518 (±0.458)
10. Zofenopril calcium	96	3.83	4.07	4.02	3.94	376.1	429.6	0.070 (±0.008)	−11.898 (±3.929)
11. Trandolapril	70	2.39	3.45	3.76	3.81	413.2	430.5	0.123 (±0.006)	−9.443 (±1.641)
12. Captopril	65	0.90	0.64	0.75	0.84	195.6	217.3	0.286 (±0.009)	−8.435 (±1.023)

Table II. The Selection of Appropriate log *P* Based on Correlation Coefficients for Relationships Between Experimental MTLC (k_m, log k_m and $P_{m/w}$) and calculated (KOWWINlog *P*, Aclog *P*, Xlog *P*2, Alog *P*) hydrophobicity parameters of the selected ACE Inhibitors

	R^2			
	KOWWINlog *P*	Aclog *P*	Xlog *P*2	Alog *P*
Parameter				
k_m	0.758	0.409	0.460	0.478
log k_m	0.843	0.566	0.773	0.709
$P_{m/w}$	0.620	0.438	0.365	0.295

Discussion

The results of an MTLC study with Brij 35 showed that an increase of the micelle concentration in the mobile phase led to a decrease of compounds retention (with the exception of lisinopril) and that order of retention of examined ACE inhibitors are in accordance with the order obtained for k_m values. According to Ruiz-Angel *et al.* (29) as well as Komsta *et al.* (30) compounds can be classified into three groups, according to their elution behavior with a micellar mobile phase: compound binding to micelles (micelles increase R_F values), non-binding compounds (no trend) and anti-binding ones (decrease R_F) (29, 30). Almost all of investigated ACE inhibitors can be considered as compounds binding to micelles (micelles increase R_F values) with the exception of lisinopril (micelles decrease R_F values). Retention behavior of lisinopril could be additionally explained with the structure of lisinopril where no ester functional group exists, hence the lowest log *P* values as well as the lowest non-polar interactions with Brij 35.

Although the application of pure micellar solutions is usual in micellar chromatography, the addition of a small amount (20%) amount of organic solvent, THF, was needed to improve spots visualization and to achieve adequate retention. The addition of a smaller amount of THF (10%) provides lower values of R_F and hydrophobicity parameters, k_m, log k_m and $P_{m/w}$ that could not be utilized for ACE inhibitor absorption modeling.

The main subject of this study was to establish possible correlations between MTLC hydrophobicity data, k_m or log k_m and $P_{m/w}$ values, and oral absorption data of examined ACE inhibitors. The obtained hydrophobicity parameters k_m, log k_m or $P_{m/w}$ were evaluated as measures of the lipophilicity of ACE inhibitors via their correlation with selected KOWWINlog *P* values. Our previous RP-TLC and SOTLC studies pointed out the significance of MLR analysis using a selected molecular descriptor, log *S* (21) or PSA (22), as an independent variable to obtain reliable correlation between the absorption and lipophilicity of ACE inhibitors. The preliminary MLR analysis of all investigated ACE inhibitors indicated that the variable Vol value can be used in addition to KOWWINlog *P* as a suitable descriptor for correlation between predicted and literature-available absorption data as well as that captopril should be excluded from further correlations. It can be assumed that captopril was excluded from the proposed model since this ACE inhibitor belongs to a different (sulfhydryl) group, with a notably lower Vol value of 195.6.

The results presented by Equations (5) and (6) and obtained using MLR analysis after substitution of KOWWINlog *P* with values of MTLC hydrophobicity parameters k_m and $P_{m/w}$ make it obvious that MTLC hydrophobicity parameters k_m or $P_{m/w}$, accompanied with molecular descriptor Vol are closely related to ACE inhibitors absorption data and could be considered for their absorption modeling. Better correlation between predicted and literature-available oral absorption data were established for micelle–water partition coefficients $P_{m/w}$.

The micelle–water partition coefficient $P_{m/w}$, previously proposed by Čudina *et al.* (11) as a potential biopartitioning micellar hydrophobicity descriptor in prediction of oral drug absorption, was confirmed in this MTLC study. In comparison with parameter k_m, which was calculated with one regression parameter, intercept, the micelle–water partition coefficient ($P_{m/w}$) was calculated using both parameters slope and intercept of regression analysis. This indicates that the micelle–water partition coefficient $P_{m/w}$ can be considered as analogous to C_0 in RP-TLC, initially introduced as a more reliable measure of lipophilicity by Bieganowska *et al.* (31) and successfully applied (21, 32).

In the current study fosinopril retention behavior could be screened due to increase of elution force (R_F values were increased) by incorporating the surfactant Brij 35 to the mobile phase. In previously proposed models for ACE inhibitors absorption evaluation based on SOTLC (22), fosinopril was excluded due to disagreement with "Lipinski's rule of five" (33). In comparison with RP-TLC and SOTLC studies, MTLC resembles more to biological barriers since solute retention behavior, which is mostly controlled by non-polar, dipole–dipole and proton donor–acceptor interactions (29), in a micellar system with physiological conditions (pH 6.8) could be used to mimic drug biological partitioning similar to biopartitioning micellar chromatography.

The relationship between different drugs biopartitioning processes and chromatographic data has already been shown and thin-layer chromatographic methods have been to be proved as suitable for

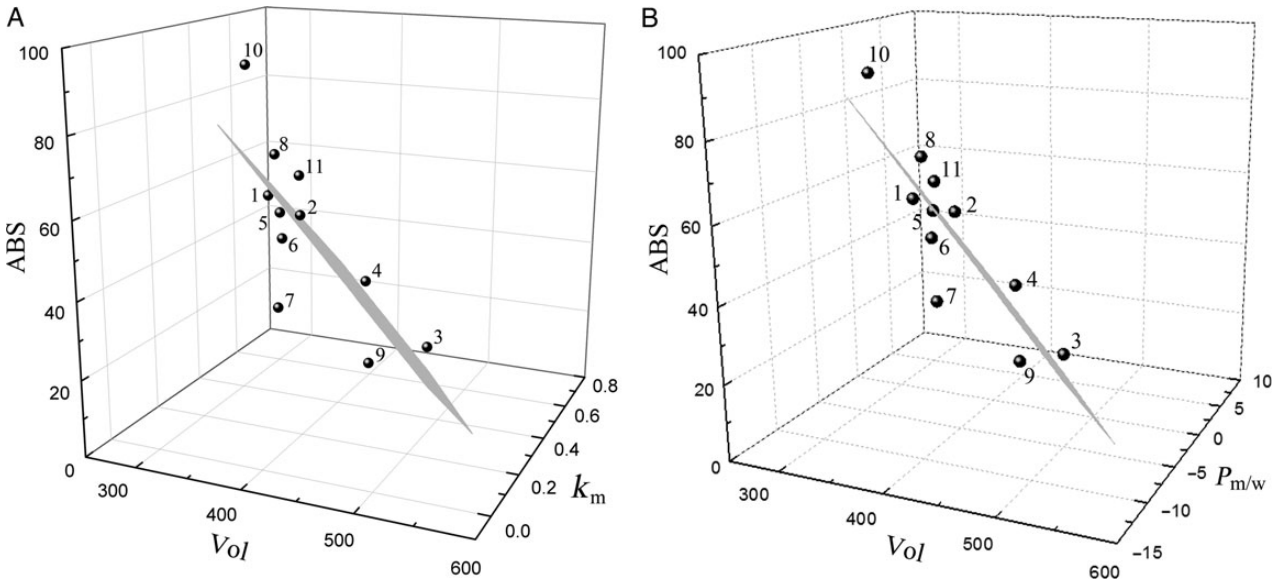

Figure 1. Multiple linear regression models of ACE inhibitor's predicted absorption with molecular descriptor Vol and (A) k_m and (B) $P_{m/w}$.

fast and easy estimation of biological properties (34). In Sherma's review paper different planar chromatographic systems were described, from a medicinal chemistry point of view, as useful tools capable of simulating biological processes (35). Karelson *et al.* (36) developed QSAR models for parallel artificial membrane permeability assay (PAMPA) permeabilities ($\log P_{app}$) at different pH values (pH 5.5 and 7.4). The obtained linear (multilinear regression) and non-linear (artificial neural network) models link the drug structures to their reported permeabilities. In the linear approach the best MLR provided R^2 ranging from 0.688 to 0.789, while better correlations were established using a non-linear approach. Also, Hou *et al.* (37) predicted human intestinal absorption for a set of 648 different chemical compounds by correlation with several important molecular properties. The best prediction model was established with four molecular descriptors and was able to predict the absorption of all compounds with $R = 0.84$ (corresponding to $R^2 = 0.705$) while for a reduced, 98-compound test set R was 0.90 (corresponding to $R^2 = 0.810$). Since Hou proposed the criteria $|R| \geq 0.6$ for correlation between important molecular properties and intestinal absorption (37), the results obtained in our study can be considered as sufficiently good, especially for MLR analysis with application of the micelle–water partition coefficient $P_{m/w}$ with $R^2 = 0.756$ (corresponding to $R = 0.869$).

The ACE inhibitors investigated in current paper applying MTLC included the compounds with the lowest, 22% (moexipril) and highest, 96% (zofenopril calcium) oral absorption. Also, according to - Figure 1 the most hydrophilic (lisinopril dihydrate; KOWWINlog $P = -0.96$) and most lipophilic (fosinopril; KOWWINlog $P = 6.61$) compounds were successfully included in the proposed absorption model and their similar (25 and 36%, respectively) absorption values indicate that a higher extent of ACE inhibitors' absorption proceeds via active transport. Consequently, although the correlations obtained in MTLC were slightly lower than previously established in SOTLC modeling, they still should be considered as sufficiently reliable and significant. In the previous proposed model (SOTLC) established in our study (22), fosinopril could not be included due to its highest lipophilicity. The main advantage of the presented MTLC model in comparison with our previously published SOTLC study of modeling ACE inhibitors absorption is the applicability of the proposed model obtained with MTLC hydrophobicity parameters on an extended group of ACE inhibitors in which the most lipophilic (fosinopril) could be included simultaneously with the whole group of investigated drugs.

Conclusion

The results of MTLC (using non-ionic surfactant Brij 35) confirmed that between two hydrophobicity parameters k_m or $P_{m/w}$, for absorption prediction of investigated 11 ACE inhibitors, micelle–water partition coefficient $P_{m/w}$ provides higher R^2 (0.756) and can be evaluated as a better MTLC parameter based on MLR analysis including the geometric descriptor—Vol as an independent variable. Moreover, micelle–water partition coefficient $P_{m/w}$, established in the MTLC study, could be considered as analogous to C_0 in RP-TLC. The proposed model which included compounds on the opposite site of lipophilicity—lisinopril (KOWWINlog $P = -0.96$) and fosinopril (KOWWINlog $P = 6.61$), both with similar absorption values (25 and 36%, respectively)—could indicate that absorption of investigated compounds occurs via two different mechanisms: active as well as passive transport.

Acknowledgments

This work was partially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia as a part of Project No. 172041. The Menarini Group, Florence, Italy, is kindly acknowledged for having supplied zofenopril calcium.

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