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Development of predictive quantitative retention-activity relationship models of HMG-CoA reductase inhibitors by biopartitioning micellar chromatography

Shu-Rong Wang [a,c], Yu Chen [b], Li-Ping Wu [c], Wen-Juan Miao [c], Mei-Jin Xiong [c], Cong Chen [c], Zhi-Rong Zhong [c,d], Li-Ming Ye [c,*]

[a] *Department of Pharmacy, the Affiliated Hospital of Luzhou Medical College, Luzhou 646000, PR China*
[b] *West China Hospital, Sichuan University, Chengdu 610041, PR China*
[c] *West China School of Pharmacy, Sichuan University, Chengdu 610041, PR China*
[d] *Luzhou Medical College, Luzhou 646000, PR China*

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Abstract

Biological fluid cell membranes are barriers for the uptake of many kinds of drugs and their metabolites, along with passive transport across membranes and bioaccumulation. Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of Brij35 under adequate experimental conditions and can be useful to simulate the drug's passive absorption and the transport in biological systems. The use of micellar aqueous solutions of Brij35 as mobile phases in reversed-phase liquid chromatography has proven to be valid to predict the biological activities of barbiturates, benzodiazepines, catecholamines, local anesthetics, non-steriodal anti-inflammatory drugs and tricyclic antidepressants. In this study, the relationships between the capacity factor in BMC and some pharmacokinetic and pharmacodynamic parameters of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are studied. Predictive quantitative retention-activity relationship (QRAR) models describing some of the biological activities and pharmacokinetic properties of HMG-CoA reductase inhibitors are obtained. The results indicate that QRAR model may be a useful tool during the drug discovery process.

Keywords: Quantitative retention–structure relationship; Quantitative retention–activity relationship; HMG-CoA reductase inhibitors; Biopartitioning micellar chromatography

1. Introduction

Traditional pharmacokinetic and pharmacodynamic studies probably prevent the evaluation of many compounds in the early phase of drug discovery because of the difficulties and costs associated with experimental animals as well as the ethical problems. To circumvent the problems associated with screening new drugs in animal, many in vitro models for the prediction of pharmacokinetic and pharmacodynamic parameters have been set up including the use of physicochemical parameters of drugs, the permeability data from cell culture lines and chromatography models [1–4]. Quantitative structure–activity relationship (QSAR) studies play an important role in the research. The application of chromatographic parameters in QSAR gives rise to a new field, quantitative retention-activity relationship (QRAR) [5–7]. A great deal of efforts have been made to develop biological chromatographic models such as immobilized artificial membranes chromatography (IAMs chromatography [8]), immobilized liposomes chromatography (ILs chromatography) [9] and biopartioning micellar chromatography (BMC) [10].

BMC is a chromatographic modality that uses reversed stationary phases and polyoxyethylene (23) lauryl ether (Brij35) solution above the critical micellar concentration (CMC) as mobile phases under adequate experimental conditions [11]. BMC's system could describe the biological behavior of many kinds of drugs and simulate biopartitioning process. The success of BMC in describing drugs' biological behavior can be

* Corresponding author. Tel.: +86 28 8550 2305.
E-mail address: dryelimin@sohu.com (L.-M. Ye).

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attributed to the fact that the characteristics of the BMC systems are similar to biological barriers and extracellular fluids [12]. In the first place, the stationary phase modified by the hydrophobic adsorption of Brij35 surfactant monomers structurally resembles the ordered array of the membranous hydrocarbon chains. Meanwhile, the hydrophilic/hydrophobic character of the adsorbed Brij35 monomers resembles the polar membrane regions. Secondly, BMC micellar mobile phases prepared at physiological conditions could also mimic the environment of drug biological partitioning. The extracellular and intracellular fluids are basically composed of water, salts, glucose, amino acids, cholesterol, phospholipids, fatty acids and proteins. Phospholipids, cholesterol, fatty acids and triglycerides form micellar complexes with proteins (lipoproteins) (critical micelle concentration, CMC $< 10^{-6}$ M) [12].

HMG-CoA Reductase is a natural compond that helps the liver to produce cholesterol. The HMG-CoA reductase inhibitors, commonly referred to as "statins", get in the way of that process, thus reducing the amount of cholesterol being produced [13–16]. All statins can effectively lower LDL cholesterol (LDL-C or "bad cholesterol"), total cholesterol, and triglycerides; and each drug in this class can also raise HDL cholesterol (HDL-C or "good cholesterol"), which is desirable [16]. Statins are considered as a first-line therapy for the treatment of hypercholesterolemia and have showed remarkable activity in preventing cardiovascular morbidity and mortality [13–15].

2. Experimental

2.1. Instrumental and measurement

An Agilent 1100 series HPLC from Agilent Technologies (Waldbronn, Germany) comprised of G1312A binary pump, G1313A auto sampler, G1314A variable wavelength UV detector, G1322A degasser, G1316A thermostatted column compartment. Data acquisition and processing were performed on HP-Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 μl loop. The HPLC column was a Luna C_{18} (phenomenex, Torrance, CA, USA) 150 mm × 4.6 mm, 5 μm particle size, with a phenomenex securityGuard™ C_{18} guard cartridge. The mobile phase flow rate was 1.0 ml/min, and the detective wavelength was 240 nm. Temperature of the eluent was maintained at 36.5 °C by pre-heating the container of the eluent buffer in a thermostat-controlled water bath (PolyScience, Niles, USA). Column temperature was also maintained at 36.5 °C for simulating human body temperature. The retention data in BMC were calculated as capacity factors, $k = (t_r - t_0)/t_0$, where t_r is the retention time of the test compound and t_0 is the column dead time. The k values used in this study were the average value of triplicate injections.

2.2. Materials and methods

Mobile phases were aqueous solutions of polyoxyethylene (23) lauryl ether (Brij35, Acros, New Jersey, USA). The pH value of mobile phases was adjusted to 7.4 with 0.05 M phosphate buffer, which was prepared with sodium dihydrogenphosphate and sodium hydroxide (analytical-reagent grade, Kelong, Chengdu, China). NaCl (analytical-reagent grade, Kelong, Chengdu, China) was added to mobile phases for simulating the osmotic pressure of biological fluids.

Fluvastatin sodium, mevastatin, lovastatin and simvastatin were kindly donated by Sichuan Industrial Institute of Antibiotics, Chengdu, China. Other HMG-CoA reductase inhibitors were obtained in terms of bulk drug or pharmaceutical preparations as follows: rosuvastatin calcium (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China), pravastatin sodium tablet (Squibb, Shanghai, China), atorvastatin calcium tablet (Godecke GmbH, Freiburg, Germany), cerivastatin sodium tablet (Bayer, Leverkusen, Germany).

Atorvastatin, cerivastatin, fluvastatin, pravastatin and rosuvastatin are administered as active compounds (acid form), whereas lovastatin and simvastatin are applied as inactive forms (lactone), which have to be enzymatically hydrolyzed to generate active forms [17].

Stock standard solutions were prepared by dissolving 10 mg of the bulk compound in 10 ml of mobile phase solution. Working solutions were prepared by dilution of the stock standard solutions using mobile phase solution. For pharmaceutical preparations, working solutions were prepared as follows: tablet powders of the HMG-CoA reductase inhibitors studied and mobile phase were taken into a mortar, and ground thoroughly, then sonicated for 10 min in a sonxi CQ-250 sonicator (Shanghai, China). The mixtures were transferred to a brown volumetric flask. The samples were centrifuged at 4000 × g for 5 min, and the supernatant was filtered through 0.45 μm microporous membrane. The solutions were stored at 4 °C before injection.

Water was from a Millipore (Billerica, MA, USA) synergy™ 185 system and was degassed before HPLC. The mobile phase and the solutions injected into the chromatograph were filtered through 0.45 μm microporous membrane.

2.3. Software and data processing

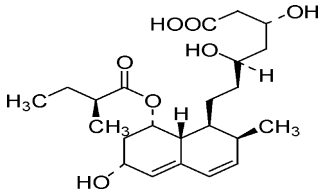
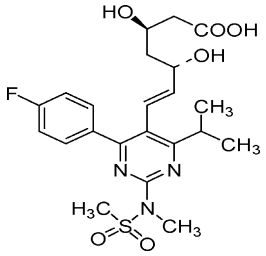
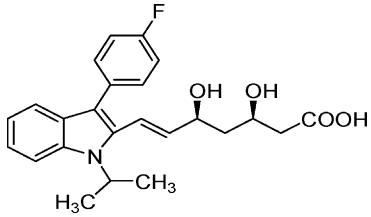
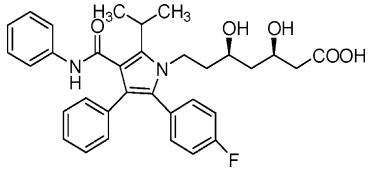
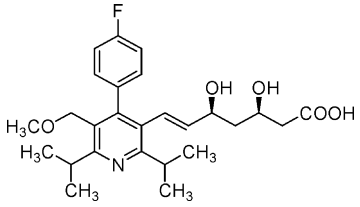
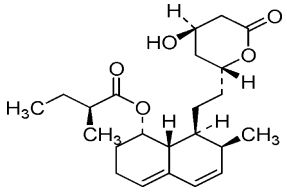
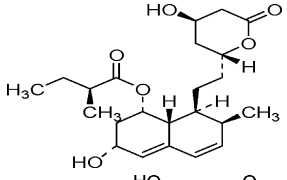
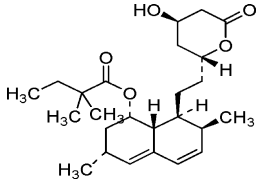
Matlab 6.0 of the MathWorks Incorporation and Excel 2003 of Microsoft office software were used to accomplish the statistical analysis of the multiple linear regression (MLR).

2.4. Evaluation of the QRAR models predictive ability

To estimate the predictive ability of the QRAR models, three important parameters were proposed, which were root mean squared error of calibration (RMSEC), root mean squared error of cross-validation (leave-one-out) (RMSECV), and root mean squared error of cross-validation (leave-one-out) for interpolated data (RMSECVi) [12], respectively.

RMSEC displays the fit error, whereas RMSECV and RMSECVi indicate the prediction error. RMSEC value informs

Table 1
Structure, pK_a[a], log P[b] (for the non-ionic forms) and log D[c] values of the statins studied

Statins	Structure	pK_a [18]	Log P [18]	Log D [19]
Pravastatin		4.31	1.44	−0.8
Rosuvastatin		4.25	0.42	−0.3
Fluvastatin		4.27	3.62	1.3
Atorvastatin		4.29	4.13	1.1
Cerivastatin		4.24	3.70	1.7
Mevastatin		13.49	3.57	
Lovastatin		13.49	4.07	
Simvastatin		13.49	4.42	1.6

[a] pK_a = logarithm of the protonation constant.
[b] log P = logarithm of the partition coefficient in the biphasic octanol–water solvent system.
[c] log D = log P value at pH 7.4.

us about the average deviation of the model from the data:

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n}(\bar{Y}_i - Y_i)^2}{n}} \quad (1)$$

where $\bar{Y}_i$ is the predicted activity when all the n molecules are included in the model construction. In contrast, the RMSECV value is a measure of the model's ability of predicting pharmacokinetic and biological parameters of new compounds. RMSECV is defined as RMSEC in Eq (1) except that now $\bar{Y}_i$ are predictions for other HMG-CoA reductase inhibitors not included in the model formulation (e.g., each one of the calibration molecules is used as a test in turn for the model chosen on the remaining molecules, performing the procedure $n-1$ times, which is referred to as the leave-one-out cross-validation). That is to say, the RMSECV parameter includes both interpolation and extrapolation information. However, the RMSECVi parameter only includes the interpolation information (e.g., excluding the two extreme data, after ordering them by their log k values):

$$\mathrm{RMSECVi} = \sqrt{\frac{\sum_{i=2}^{n-1}(\bar{Y}_i - Y_i)^2}{n-2}} \quad (2)$$

From a qualitative point of view, the more differences between RMSEC and RMSECV or RMSECVi exist, the lower the QRAR model's obtained robustness is and the more cautions must be taken in future predictions.

3. Results and discussion

3.1. Retention behavior of HMG-CoA reductase inhibitors

Table 1 shows the structure, the logarithm of the protonation constants (pK_a), the log P values for the non-ionic form, and log D at pH 7.4 of the statins studied. At physiological pH 7.4, most of the statins are negatively charged with an ionization degree of above 99%. Mevastatin, lovastatin and simvastatin, however, are neutral. The use of anionic surfactant (e.g., sodium dodecylsulphate, SDS) mobile phases shortens enormously the time of retention of the statins because of the solutions' electrostatic repulsions with monomers of surfactant adsorbed into the stationary phase. However, the use of cationic surfactant (e.g., cetyltrimethyl ammonium bromide, CTAB) mobile phases lengthens greatly the retention time due to the existence of strong electrostatic attractions between the compounds and the modified stationary phase. A non-ionic surfactant (Brij35) was used to prepare micellar mobile phases. The mobile phase pH was adjusted to 7.4 in order to obtain conditions as close as possible to the physiological pH.

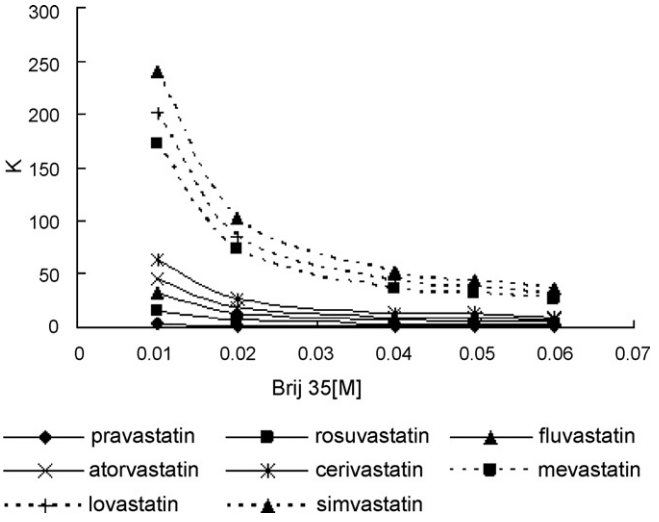


Fig. 1. Effect of Brij35 concentration at pH 7.4 in the mobile phase on the retention of Statins.

Table 2
Retention data of the statins using different Brij35 concentrations

No.	Statins	$k_{0.01M}$	$k_{0.02M}$	$k_{0.04M}$	$k_{0.05M}$	$k_{0.06M}$
1	Pravastatin	4.05	2.77	1.99	2.07	1.81
2	Rosuvastatin	14.96	7.98	4.66	4.36	3.69
3	Fluvastatin	32.73	13.88	7.18	6.48	5.32
4	Atorvastatin	46.81	20.06	10.39	9.24	7.64
5	Cerivastatin	63.78	27.48	14.21	12.72	10.32
6	Mevastatin	171.39	72.96	37.13	32.61	26.28
7	Lovastatin	201.16	85.85	43.66	38.27	30.51
8	Simvastatin	239.95	101.94	51.52	44.99	35.91

The effect of the Brij35 concentration (0.01, 0.02, 0.04, 0.05 and 0.06 M) in mobile phases on the retention of the statins is shown in Fig. 1 and Table 2. As can be observed, for the highly retained compounds (mevastatin, lovastatin and simvastatin), the retention was enormously decreased upon increasing the Brij35 concentration in the mobile phase, whereas for the less-retained compounds (i.e., pravastatin and rosuvastatin), the retention was scarcely modified.

Table 3
Statistical analysis of the QSRR models log $k = a$ log $P + b\alpha + c$ at different Brij35 concentrations for statins

[Brij 35]	n	$a \pm ts_a$ (P-value)	$b \pm ts_b$ (P-value)	$c \pm ts_c$ (P-value)	r^2 ($r^2_{adj.}$)	SE	F (P-value)
0.01 M	8	0.22 ± 0.22 (0.0430)	0.65 ± 0.59 (0.0371)	1.41 ± 0.95 (0.0124)	0.8559 (0.7982)	0.2751	14.85 (0.0079)
0.02 M	8	0.18 ± 0.18 (0.0466)	0.64 ± 0.49 (0.0201)	1.21 ± 0.79 (0.0111)	0.8755 (0.8257)	0.2292	17.58 (0.0055)
0.04 M	8	0.15 ± 0.15 (0.0460)	0.63 ± 0.42 (0.0116)	1.02 ± 0.67 (0.0113)	0.8938 (0.8513)	0.1946	21.03 (0.0037)
0.05 M	8	0.14 ± 0.14 (0.0435)	0.62 ± 0.38 (0.0086)	1.01 ± 061 (0.0082)	0.9036 (0.8650)	0.1774	23.43 (0.0029)
0.06 M	8	0.14 ± 0.13 (0.0445)	0.61 ± 0.37 (0.0079)	0.93 ± 0.59 (0.0095)	0.9054 (0.8675)	0.1710	23.92 (0.0028)

Table 4
Retention factors in 0.01 M Brij35 mobile phase and biological activities values for the Statins studied

No.	Statins	log *k* (0.01 M Brij35)	$t_{1/2}$ (h)	V_d (l kg^{-1})	Cl (ml min^{-1} kg^{-1})	IC_{50} (nmol l^{-1})
1	Pravastatin	0.61	77 [20]	11.6 [21]	13.50 [22]	44.1 [23]
2	Rosuvastatin	1.17	20.8–21.4 [23]	1.9 [24]		
3	Fluvastatin	1.51	2.3 ± 0.9 [25]	0.49 [26]		27.6 [23]
4	Atorvastatin	1.67	11–14 [24]		4.17 [22]	8.2 [23]
5	Cerivastatin	2.23	2.5 [26]	0.3 [27]	3.33 [22]	10.00 [23]
6	Lovastatin	2.30	1.9–2.0 [28]	1.1–1.7 [29]	4.33–18.33 [22]	
7	Simvastatin	2.38	3.3 [30]		7.50 [22]	11.2 [23]

This fact could be explained by the chemical structure of the compounds. The highly retained compounds, such as mevastatin, lovastatin, simvastatin are lactones with high liposolubility and high retention in the chromatographic column. When the concentration of Brij35 in the eluent is increased, more and more Brij35 micelles come into being, and more drug molecules come into Brij35 micelle, and then drug molecules are rapidly taken out of column along with Brij35 micelle, so the retention is enormously decreased. The less-retained compounds, such as pravastatin and rosuvastatin, contain carboxyl group, so they have low liposolubility and low retention in the chromatographic column at pH 7.4. The retention of these drugs in the chromatographic column is slightly decreased upon increasing the Brij35 concentration.Drug's retention depends not only on the hydrophobic interactions but also on the molar total charge and steric properties of the compounds. In fact, when the log *k* values of the compounds obtained for a certain mobile phase were correlated with the corresponding log *P* values, correlation coef-

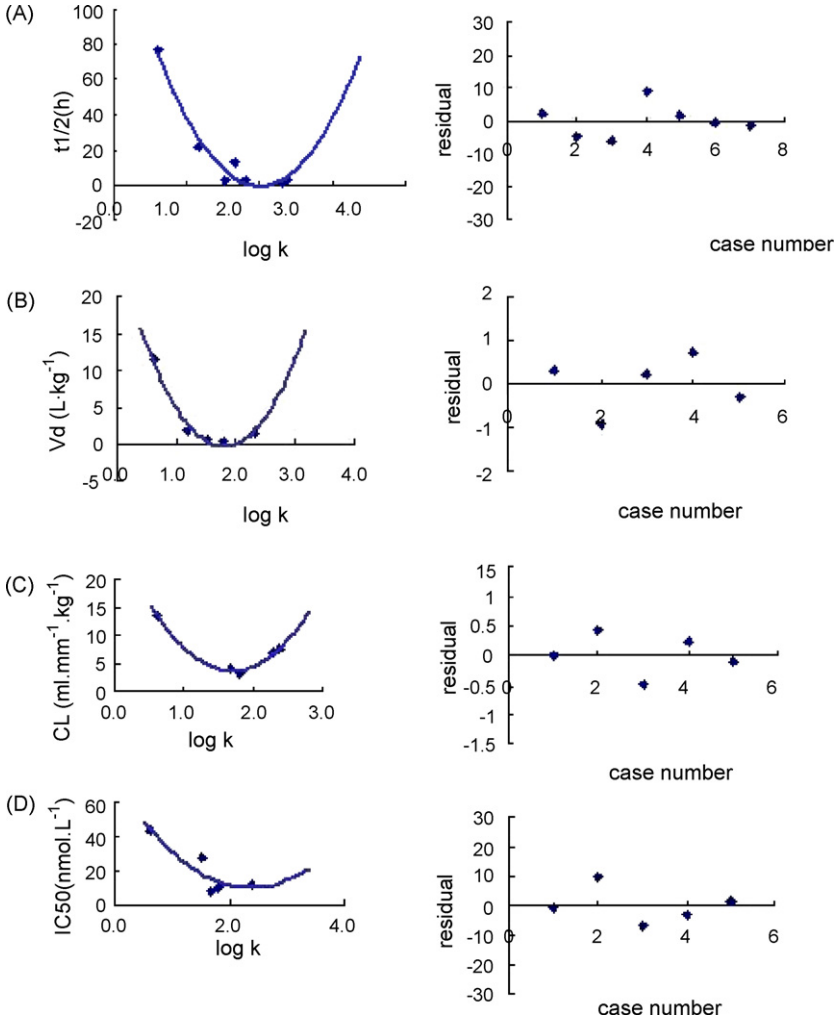


Fig. 2. Bioactivity parameter-retention data relationships for different Statins (left part) and the corresponding residuals plots (right part) obtained using BMC (0.01 M Brij35 and 0.05 M phosphate buffer at pH 7.4 mobile phase).

ficients (r^2) were 0.52, 0.53, 0.55, 0.58 and 0.59 for 0.01, 0.02, 0.04, 0.05 and 0.06 M Brij35 concentrations, respectively. While the molar total charges of compounds were added into the novel model [see Eq. (3)] [10], the log *k*–log *P* relationships obtained become better.

$$\log k = a \log P + b\alpha + c \quad (3)$$

where the variable α measures the molar total charge of compounds at a given pH value. For polyprotic compounds the α value can be calculated as follows:

$$\alpha = \sum_{j=0}^{n} a_j \delta_j \quad (4)$$

where α_j and δ_j are the values of the net charge and the molar fraction, respectively, of the considered species at the fixed pH.

The log *k* values for the statins acquired with 0.01, 0.02, 0.04, 0.05 and 0.06 M Brij35 mobile phases at pH 7.4, the log *P* values and the molar total charges of the compounds were adjusted to Eq. (3). Table 3 shows the regression analysis results. As can be observed, the quantitative structure–retention relationship (QSRR) models obtained with the five mobile phases were adequate to describe the retention behavior of HMG-CoA reductase inhibitors.

3.2. Retention–activity relationships for the HMG-CoA reductase inhibitors in BMC

The molecular features of drugs (such as hydrophobicity, ionization and steric properties) determine their membrane affinity and the drug–enzyme or drug–receptor interaction and their biological activity. Since these molecular properties also determine the retention of compounds in BMC, retention–activity relationship could be expected.

In order to obtain predictive and interpretative models, the retention data of statins and the corresponding biological responses were adjusted to a second-order polynomial model (see Eq. (5)) [10].

$$\text{bioactivity parameter} = a(\log k)^2 + b \log k + c \quad (5)$$

where bioactivity parameter includes pharmacokinetic parameters [e.g., half-life time ($t_{1/2}$), volume of distribution (V_d) and plasma clearance (Cl)] and pharmacodynamic parameters [e.g., the concentration of drug required to give 50% inhibition of Angiotensin converting enzyme(IC_{50})].

Relationships between the biological activities and the log *P* and ionization degree values were not adequate or were statistically not as good as the relationships obtained for the QRAR models shown below. The results given in this paper were obtained using a 0.01 M Brij35 mobile phase. Similar QRAR models were achieved using the retention data corresponding to 0.02, 0.04, 0.05 and 0.06 M Brij35 mobile phases.

Table 4 shows the retention data (log *k*) in 0.01 M Brij35 mobile phase and the bioactivity parameters of the HMG-CoA reductase inhibitors in the literature [20–30].

Fig. 2 A–D shows the relationships between the pharmacokinetic parameters or pharmacodynamic parameter and the

Table 5
Statistical analysis and predictive features of the QRAR models *bioactivity parameter* = $a(\log k)^2 + b \log k + c$ obtained using BMC (0.01 M Brij35 and 0.05 M phosphate buffer at pH 7.4 mobile phase)

Parameter	*n*	$a \pm ts_a$ (*P*-value)	$b \pm ts_b$ (*P*-value)	$c \pm ts_c$ (*P*-value)	r^2 ($r^2_{adj.}$)	SE	*F* (*P*-value)	RMSEC	RMSECV	RMSECVi
$t_{1/2}$	7	38.99 ± 19.04 (0.0047)	−155.53 ± 59.47 (0.0019)	155.53 ± 42.97 (0.0006)	0.9661 (0.9491)	6.1614	56.98 (0.0011)	4.7469	12.8799	7.6403
V_d	5	8.27 ± 5.28 (0.0213)	−29.72 ± 15.57 (0.0145)	26.32 ± 10.46 (0.0084)	0.9830 (0.9660)	0.8800	57.90 (0.0170)	0.5729	3.1894	1.1009
Cl	5	8.26 ± 2.85 (0.0063)	−28.03 ± 8.58 (0.0050)	27.50 ± 5.65 (0.0023)	0.9923 (0.9845)	0.4970	128.40 (0.0077)	0.3160	5.6586	0.7850
IC_{50}	5	11.38 ± 46.95 (0.4064)	−53.89 ± 139.56 (0.2385)	73.47 ± 96.67 (0.0822)	0.8366 (0.6733)	8.8320	5.12 (0.1634)	5.5585	64.9772	11.2392

retention data in 0.01 M Brij35 mobile phase of the statins, as well as the corresponding residual plots.

Table 5 shows the statistical analysis and the predictive features of the QRAR models with 0.01 M Brij35 mobile phase. The P values obtained for $t_{1/2}$, V_d and Cl models were less than 0.05, which indicated that the relationships between these parameters and the log k were statistically significant at the 95% confidence level. The coefficients obtained for those models were also significant ($P < 0.05$) at the same confidence level. On the other hand, the QRAR model obtained for IC_{50} was non-significant ($P = 0.16$, $r^2 = 0.84$, $r^2_{adj.} = 0.67$, $F = 5$).

In order to obtain better QRAR models for IC_{50}, further research would be necessary. Firstly the number of the statins studied should be increased for increasing statistical power. Secondly, we should control the confounding factor about drugs' bioactivity parameters (e.g., age, gender, race of subjects, health status, software of data processing) since the parameters may vary with those factors. The best solution for controlling the confounding factor is that all the bioactivity parameters be determined in the same laboratory on healthy non-smoking young male volunteers of the same race. Thirdly, the chromatographic condition should be optimized via changing pH value, the concentration of phosphate and Brij35 of the mobile phase. Finally, if above-mentioned efforts do not work, maybe another novel QRAR model formulation should be proposed.

3.3. Predictive ability of QRAR models for the HMG-CoA reductase inhibitors in BMC

The RMSEC, RMSECV and RMSECVi values for the QRAR models obtained are shown in Table 5. The QRAR models for $t_{1/2}$, V_d, and Cl showed comparable RMSEC and RMSECVi values, while the RMSECV values of these models were much larger than the corresponding RMSEC or RMSECVi values. This indicated that caution should be taken with the extrapolated parameter data. Nevertheless, the qualitative information obtained may be useful to the studies of pharmacokinetic and pharmacodynamic properties. The ability of BMC log k values to describe and predict pharmacokinetic parameters of HMG-CoA reductase inhibitors in terms of cross-validated data was adequate.

4. Conclusions

The development of in vitro tools for the estimation and prediction of pharmacokinetic and pharmacodynamic properties of drug candidates is an alternative to the traditional studies. The approach in this paper (i.e., QRAR models) could reduce experiment efforts and costs, and facilitates the screening of drug candidates for their pharmacokinetic and pharmacodynamic properties. The retention of compounds in BMC could predict in vitro human bioactivity parameters of HMG-CoA reductase inhibitors. This approach could be helpful in the development of the new HMG-CoA reductase inhibitors in the early stage of the studies.

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