Quantitative Retention-Activity Relationship Models of Angiotensin Converting Enzyme Inhibitors Using Biopartitioning Micellar Chromatography

Shu-Rong Wang[1,2], Cong Chen[2], Mei-Jin Xiong[2], Li-Ping Wu[2], and **Li-Ming Ye[2,*]**

[1]Department of Pharmacy, The Affiliated Hospital of Luzhou Medical College, Luzhou, 646000, PR China and [2]West China School of Pharmacy, Sichuan University, Chengdu, 610041, PR China

Abstract

Biopartitioning micellar chromatography (BMC) is a mode of micellar liquid chromatography that uses micellar mobile phases of Brij35 under adequate experimental conditions and can simulate biopartioning process of many kinds of drugs and describe their biological behavior. The capability of BMC to describe and estimate pharmacokinetic and pharmacodynamic parameters of angiotensin-converting enzyme inhibitors (ACEIs) had been studied in this paper. The correlation between retention factors of ACEIs obtained using BMC and bioactivity parameters (half-life, volume of distribution, clearance, and IC_{50}) was investigated utilizing a second-order polynomial model. The P-values obtained for half-life, volume of distribution, clearance, and IC_{50} models were less than 0.05, and the r^2 of those four models were 0.89, 0.98, 0.94, and 0.97, with r^2_{adj} (adjusted for freedom degrees) being 0.85, 0.98, 0.91, and 0.95, respectively. The predictive and interpretative ability of the chromatographic models was evaluated in terms of cross-validated data [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)]. The quantitative retention-activity relationship (QRAR) models of ACEIs developed in this paper may be a useful approach to screening new chemicals in the early stage of development.

Introduction

In the early steps of drug research, determing the pharmacokinetic and pharmacodynamic parameters of some drug candidates is very important. Traditional pharmacokinetic and pharmacodynamic studies have been conducted in living systems such as mice, rabbits, dogs, etc., but the experimental difficulty and costs associated with experimentation animals as well as the ethical problems prevent the evaluation of many compounds during the drug discovery process. To circumvent the problems associated with screening new drugs in animals, a lot of 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) (5–9). A great deal of effort has been made to develop biological chromatographic models such as immobilized artificial membranes chromatography (IAMs chromatography) (10), immobilized liposomes chromatography (ILs chromatography) (11), and biopartitioning micellar chromatography (BMC) (12).

BMC is a mode of reversed-phase liquid chromatography, which uses polyoxyethylene (23) lauryl ether (Brij35) solution above the critical micellar concentration (CMC) as a mobile phase under adequate experimental conditions (13). BMC's system can simulate the biopartioning process of many kinds of drugs and describe their biological behavior. The success of BMC in describing drug's biological behavior could be attributed to the fact that the characteristics of the BMC systems are similar to biological barriers and extracellular fluids (14). Firstly, 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. In addition, micellar mobile phases which are constituted by saline aqueous solutions of micelles in equilibrium with surfactant monomers resemble the extracellular fluids 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) (14).

Angiotensin-converting enzyme inhibitors (ACEIs) are medicines that block the conversion of the chemical in the blood angiotensin I to angiotensin II that increases salt and water retention in the body. ACEIs are used in the treatment of high blood pressure and of congestive heart failure. They make blood vessels relax, which helps lower blood pressure and allows more

*Author to whom correspondence should be addressed: email dryelimin@tom.com

oxygen-rich blood to reach the heart. And they may also be prescribed for other conditions. For example, captopril is used to treat kidney problems in people who take insulin to control diabetes.

Most of ACEIs are prodrugs, which are converted to active metabolites (diacids) in vivo except for captopril and ceronapril. In this paper, the QRAR models of the ACEIs studied using BMC were obtained, and the predictive abilities of the models were evaluated. The advantage and limitation of using a single parameter (i.e., the retention factor in BMC) to describe the activity of some ACEIs are discussed.

Experimental

Apparatus and operating conditions

An Agilent 1100 series high-performance liquid chromatograph (HPLC) (Agilent, Waldbronn, Germany) comprised of G1312A binary pump, G1313A auto sampler, G1314A variable wavelength UV detector, G1322A degasser, and G1316A thermostated column compartment was used. 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) with a 20-µL loop. The HPLC column was a Luna C_{18} (Phenomenex, Torrance, CA) (150 mm × 4.6 mm, 5 µm particle size) equipped with a 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 37°C by preheating the container of the eluent buffer in a thermostat-controlled waterbath (PolyScience, Niles, IL) for simulating human body temperature. Column temperature was also maintained at 37°C. The retention data in BMC were calculated as retention 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.

Chemicals, reagents, and standards

Mobile phases were aqueous solutions of polyoxyethylene (23) lauryl ether (Brij35, Acros, Morris Plains, New Jersey). 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 the BMC mobile phase for simulating the osmotic pressure of biological fluids.

Captopril, perindopril, imidapril, spirapril, ramipril, cilazapril, benazepril, and fosinopril sodium were kindly donated by a pharmaceutical laboratory (West China School of Pharmacy, Chengdu, China). Other ACEIs were obtained in terms of reference substance or bulk drug as follows: enalapril maleate (National Institue for the Control of Pharmaceutical and Biological Products, Beijing, China), quinapril hydrochloride (National Institue for the Control of Pharmaceutical and Biological Products, Beijing, China), and zofenopril (Wenbo, Mianyang, China).

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. All the solutions were stored at 4°C before injection.

Water was from a Millipore (Billerica, MA) 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.

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).

Evaluation of the QRAR models predictive ability

To estimate the predictive ability of the QRAR models, three important parameters were proposed, which were the RMSE, RMSECV, and RMSECVi (14), respectively.

RMSEC displays the fit error whereas RMSECV and RMSECVi indicate the prediction error. RMSEC value informs us about the average deviation of the model from the data:

$$\mathrm{RMSEC} = \sqrt{\frac{\sum_{i=1}^{n} (\overline{Y}_i - Y_i)^2}{n}} \qquad \text{Eq. 1}$$

where $\overline{Y}_i$ 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 $\overline{Y_i}$ are predictions for other ACEIs 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):

$$\mathrm{RMSECVi} = \sqrt{\frac{\sum_{i=2}^{n-1} (\overline{Y}_i - Y_i)^2}{n-2}} \qquad \text{Eq. 2}$$

From a qualitative point of view, the more differences between RMSEC and RMSECV or RMSECVi exist, the lower the QRAR model's robustness is and then more cautions must be taken in future predictions (14).

Results and Discussion

Retention behavior of angiotensin-converting enzyme inhibitors

Table I shows the structure, the logarithm of the protonation constants (pKa) and the log P values of the ACEIs in the litera-

ture (15–17). Most of ACEIs are diprotic compounds with the exception of fosinopril with only one proton. At physiological pH 7.4, most of the ACEIs are negatively charged with an ionization degree of more than 99%. The use of anionic surfactant (e.g., sodium dodecylsulphate, SDS) mobile phases enormously shortens the time of retention of the ACEIs 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 greatly lengthens the retention time of 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.

The effect of the Brij35 concentration (0.01, 0.02, 0.04, 0.05, 0.06 M) in the mobile phases on the retention of the ACEIs is shown in Figure 1. As can be observed, for the highly retained compounds (fosinopril and zofenopril), the retention was enormously decreased upon increasing the Brij35 concentration in the mobile phase. In addition, for moderately retained compounds (ramipril and spirapril), the retention was slowly decreased. Finally, for the rarely retained compound (captopril), the retention was slightly increased.

This fact could be explained by the chemical structure of the compounds. For the highly retained compounds, such as fosinopril, they have the structure of lactone, ketone, phosphoryl group with a high molecular weight, so they have high liposolubility and high retention in the chromatographic column. When the concentration of Brij35 in the eluent is increased, more and more Brij35 micelle come into being, and more drug molecules come into Brij35 micelle. Then drug molecules are rapidly taken out of column along with Brij35 micelle, so the retention is enormously decreased. For those moderately retained compounds, such as ramipril, they have the structure of lactone with a moderate molecular weight, 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 lightly decreased upon increasing the Brij35 concentration. Finally, for the rarely retained compound (captopril), it has sulfhydryl with low molecular weight, so it has high

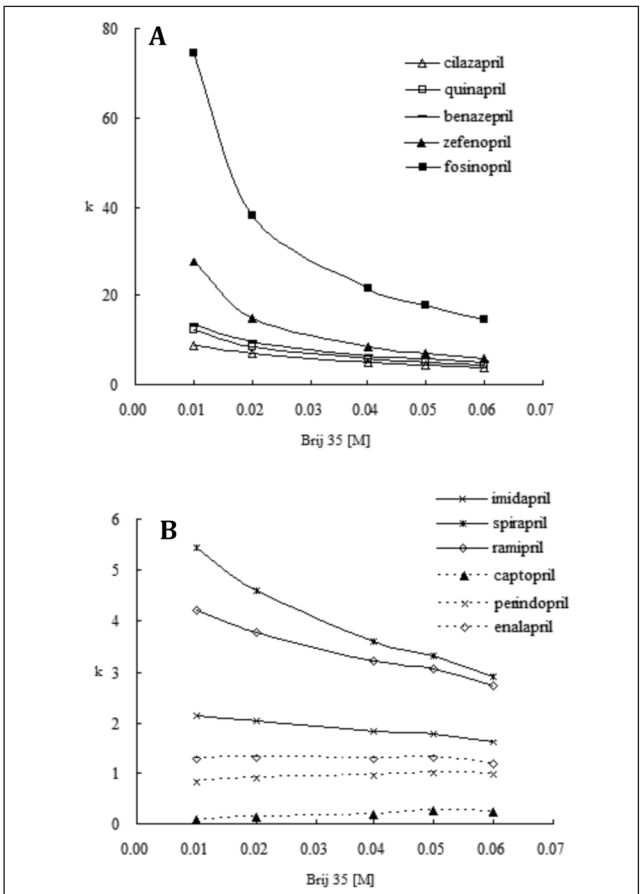
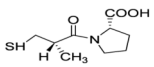
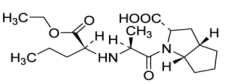
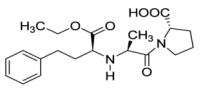
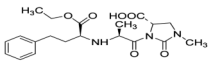
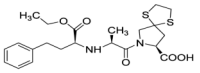
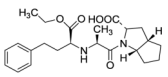
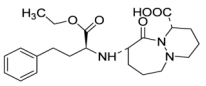
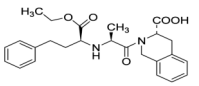
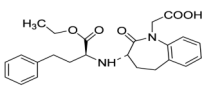
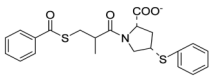
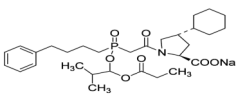


Figure 1. Effect of Brij35 concentration at pH 7.4 in the mobile phase on the retention of ACEIs.

Table I. Structures, pKa, and log P Values for the Non-Ionic Forms of the ACEI Studied

ACEIs	Structure	pKa	LogP
Captopril		3.7 (15) 9.8 (15)	0.546 (17)
Perindopril		3.7 ± 0.4 (16) 5.7 ± 0.4 (16)	
Enalapril		3 (15) 5.4 (15)	2.271 (17)
Imidapril			
Spirapril			
Ramipril		3.7 ± 0.4 (16) 5.5 ± 0.4 (16)	3.149 (17)
Cilazapril		3.3 ± 0.4 (16) 5.9 ± 0.4 (16)	
Quinapril		3.3 ± 0.4 (16) 5.4 ± 0.4 (16)	3.384 (17)
Benazepril		3.7 ± 0.1 (16) 5.0 ± 0.4 (16)	3.217 (17)
Zofenpril			
Fosinopril Sodium		3.8 ± 0.6 (16)	6.61 (17)

hydrophilicity and lowest retention in the chromatographic column in this study. The lowest retention of captopril was slightly increased due to its high hydrophilicity, which makes for little interaction with the tenside as well as viscosity of the mobile phase increased which kept the drug molecules from moving forward rapidly .

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 coefficients (r_2) were 0.86, 0.85, 0.86, 0.88, and 0.87 for 0.01, 0.02, 0.04, 0.05, and 0.06 M Brij35 concentrations, respectively. While the molar total charge of compounds was added into the novel model (Eq. 3) (4), the log *k*-log P relationships obtained become better.

$$\log k = a\log P + b\alpha + c \qquad \text{Eq. 3}$$

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

$$a = \sum_{j=0}^{n} a_j \delta_j \qquad \text{Eq. 4}$$

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 ACEIs 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 charge of the compounds at this pH value were adjusted to Eq. 3. Table II 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 ACEIs (r^2 > 0.96; r^2_{adj} > 0.93).

Table II. Statistical Analysis and Predictive Features of the QSRR Models at Different Brij35 Concentration*

(Brij35)	*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	6	0.50 ± 0.19 (0.0035)	–0.64 ± 0.74 (0.0704)	–2.05 ± 1.49 (0.0220)	0.9605 0.9342	0.2618	36.48 (0.0079)
0.02 M	6	0.42 ± 0.16 (0.0033)	–0.56 ± 0.61 (0.0604)	–1.74 ± 1.23 (0.0203)	0.9621 0.9369	0.2161	38.12 (0.0074)
0.04 M	6	0.35 ± 0.12 (0.0029)	–0.44 ± 0.48 (0.0595)	–1.39 ± 0.96 (0.0195)	0.9654 0.9424	0.1693	41.87 (0.0064)
0.05 M	6	0.31 ± 0.11 (0.0029)	–0.37 ± 0.43 (0.0719)	–1.17 ± 0.87 (0.0237)	0.9647 0.9412	0.1534	41.01 (0.0066)
0.06 M	6	0.31 ± 0.10 (0.0023)	–0.38 ± 0.39 (0.0528)	–1.24 ± 0.79 (0.0156)	0.9701 0.9501	0.1392	48.61 (0.0052)

* logk = a logP + ba + c.

Retention-activity relationships for the ACEIs in BMC

The molecular features of drugs (such as hydrophobicity, ionization and steric properties) determine their membrane affinity, the drug-enzyme, or drug-receptor interaction and their biological activity. Because 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 ideal fit equation was a second-order polynomial model (Eq. 5) (5).

$$\text{Bioactivity parameter} = a(\log k)^2 + b\log k + c \qquad \text{Eq. 5}$$

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

Table III. Retention Factors in 0.01 M Brij35 Mobile Phases and Biological Aactivities Values for the ACEIs

No.	ACEIs	logk (0.01M (Brij35)	$t_{1/2}$ (h)	V_d (l/kg)	Cl (mL/min/kg)	IC_{50} (nmol/L)
1	Captopril	–1.09	2.2 (17)	–	12 (18)	29
2	Perindopril	–0.09	0.2 (19)	4.2 (19)	2.2 (20)	2.4
3	Enalapril	0.11	–	2.7 (21)	2.2 (22)	3.1
4	Imidapril	0.33	1.4 (23)	–	–	–
5	Spirapril	0.74	0.9 (24)	0.39 (25)	0.65 (24)	–
6	Ramipril	0.62	0.67 (26)	–	1.1 (27)	2.85
7	Cilazapril	0.95	1.5 (28)	0.24 (29)	–	0.61
8	Quinapril	1.08	1.9 (29)	0.03 (30)	2 (31)	3
9	Benazepril	1.12	1.1 (30)	0.12 (32)	0.35 (32)	2
10	Zofenopril	1.44	5.5 (30)	–	–	–
11	Fosinopril	1.87	11.3 (33)	0.13 (33)	0.54 (30)	11

Table IV. Statistical Analysis and Predictive Features of the QRAR Models Obtained Using BMC*

Parameter	n	$a \pm ts_a$ (P-value)	$b \pm ts_b$ (P-value)	$c \pm ts_c$ (P-value)	r^2_{adj}.	SE	F (P-value)	RMSEC	RMSECV	RMSECVi
$t_{1/2}$	10	3.04 ± 1.22 (0.0006)	0.00 ± 1.52 (0.9976)	-0.72 ± 1.47 (0.2869)	0.8861 (0.8536)	1.2880	27.24 (0.0005)	1.0758	3.2878	1.2972
V_d	7	1.89 ± 0.57 (0.0008)	–5.29 ± 1.02 (0.0001)	3.48 ± 0.44 (0.0000)	0.9878 (0.9816)	0.2245	161.33 (0.0001)	0.1675	0.913	0.2447
Cl	8	2.23 ± 1.23 (0.0055)	–5.13 ± 1.54 (0.0004)	3.18 ± 1.45 (0.0025)	0.9362 (0.9107)	1.1537	36.71 (0.0010)	0.9045	3.3886	1.2071
IC_{50}	8	8.60 ± 2.28 (0.0002)	-12.11 ± 2.79 (0.0001)	4.62 ± 2.72 (0.0072)	0.9652 (0.9513)	2.1018	69.36 (0.0002)	1.6419	5.4323	2.3503

* Bioactivity parameter = a (log k)^2 + b logk + c; 0.01 M Brij35 + 0.05 M phosphate buffer, pH 7.4 mobile phase.

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 III shows the retention data (log k) in 0.01 M Brij35 mobile phase and the bioactivity parameters of the ACEIs in the literature (17–33). In the case of the pharmacokinetics, owing to the large number of data sources found and their variability, the values chosen to construct the corresponding QRAR models were the median values.

Figure 2 shows the relationships between the pharmacokinetic parameters or pharmacodynamic parameter and the retention data in BMC of the ACEIs as well as the corresponding residual plots. There is a random distribution of the residuals, and practically all were statistically close to zero. From a qualitative point of view, this suggested the adequacy of the models to the data.

Table IV shows the statistical analysis and the predictive features of the QRAR models with 0.01 M Brij 35 mobile phase. The P-values obtained for $t_{1/2}$, V_d, Cl, and IC_{50} 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 V_d, Cl, and IC_{50} models were also significant ($P < 0.05$) at the same confidence level except for coefficient *b* and *c* in the $t_{1/2}$ QRAR model, which were 0.9976 and 0.2869, respectively. But for the four QRAR models, the r^2 ($0.88 < r^2 < 0.99$), r^2_{adj} (adjusted for freedom degrees, $0.85 < r^2_{adj} < 0.99$) were adequate. In addition, the standard errors of the estimate (SE) were also low. In a word, the four models can be used to predict pharmacokinetics and pharmacodynamics values for new ACEIs in the early stage of development.

Predictive ability of QRAR models for the ACEIs in BMC

Table IV also shows the RMSEC, RMSECV, and RMSECVi values for the QRAR models obtained. The QRAR models for $t_{1/2}$, V_d, Cl, and IC_{50} showed comparable RMSEC and RMSECVi values while the RMSECV values of these models were much larger than the RMSEC or the RMSECVi values. This indicated that some cautions might be taken with the extrapolated parameter data. Nevertheless, the qualitative information obtained may be useful to the studies of pharmacokinetic and pharmacodynamic properties of new ACEIs. The ability of log *k* values in BMC to describe and predict the biological responses and pharmacokinetic parameters of ACEIs in terms of cross-validated data was adequate.

Conclusions

The need to develop some tools in vitro for pharmacokinetic and pharmacodynamic properties estimation of drug candidates facilitates the development of many predictive models and makes these models an alternative to the traditional studies. Biopartitioning chromatographic systems, which mimic the main interactions between drug and biological membrane, show the intrinsic advantages of HPLC measurements, such as economy, speed, reproducibility and easy automation. The retention of compound in BMC could predict in vitro bioactivity parameters of ACEIs to a certain extent. The QRAR model seems to be an attractive tool for estimating the potential activity of the new ACEIs in the early stage of development.

In order to obtain better QRAR models for ACEIs, further research would be necessary. Firstly, the number of the ACEIs studied should be increased for increasing statistical power. Meanwhile, more biological activities values for the ACEIs studied should be collected. Secondly, we should control the confounding factor about drugs' bioactivity parameters (e.g., age, gender, race of subjects, health status, software of data processing) bceause 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 the previously mentioned efforts do not work well, maybe another novel QRAR model formulation should be proposed.

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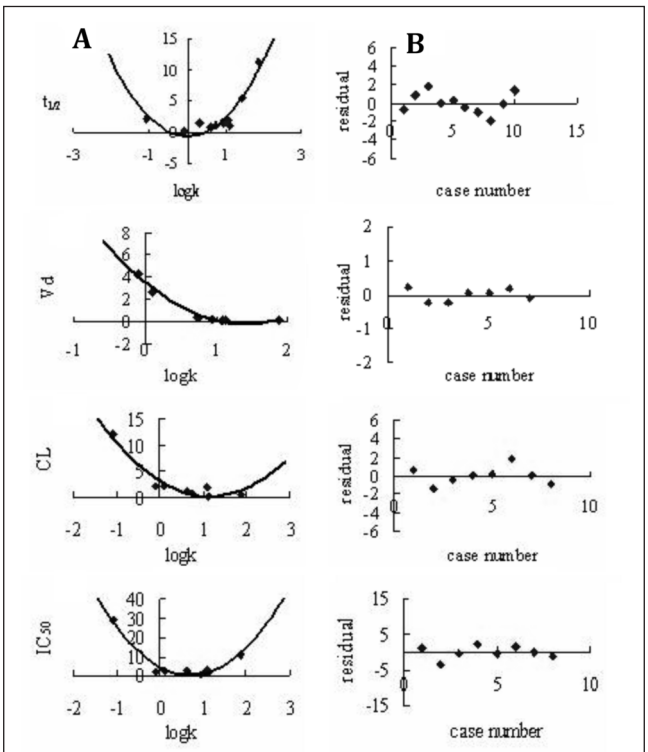

Figure 2. Bioactivity parameter-retention data relationships for different ACEIs (column A) and the corresponding residuals plots obtained using BMC (column B) (0.01 M Brij35 + 0.05 M phosphate buffer, pH 7.4 mobile phase).

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