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QRAR Models for Diuretics using mixed Micellar Liquid Chromatography

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Abstract

In this article, the capability of traditional biopartitioning micellar chromatography (BMC), using the pure Brij35 solution and the mixed micellar system of Brij35/SDS (85:15) as mobile phase respectively, under adequate experimental conditions, to describe and estimate bioactivities of diuretics, has been focused. The $BMC_{Brij35/SDS}$–QRAR models can simulate the resting membrane potential and the conformation of the long hydrophilic polyoxyethylene chains remains unchanged. The predictive and interpretative ability of the chromatographic models was evaluated in terms of cross-validated data (RMSEC, RMSECV and RMSECVi). The obtained $BMC_{Brij35/SDS}$–QRAR were compared with the traditional BMC_{Brij35}–QRAR models, and better statistically models were obtained using Brij35-SDS retention data.

Keywords: Quantitative Retention–Activity Relationships (QRAR); Biopartitioning Micellar Chromatography (BMC); Polyoxyethylene (23) lauryl ether (Brij35); Sodium Dodecyl Sulfate (SDS); Diuretics

The demand to get a tool for biological parameter estimation of new compounds for clinical application, supports the postulation of predictive models as an alternative to conventional classical assays being no necessary the use of experimentation in animals. A lot of in vitro systems, including the use of physicochemical parameters of drugs, the permeability data from cell culture line and the chromatographic models [1-4], have been established. The study of the type of chemical structure of a foreign substance, which will interact to a living system and produce a well-defined biological endpoint, is commonly referred to as quantitative structure-activity relationship (QSAR). The application of chromatographic parameters in QSAR gives rise to a new field: quantitative retention-activity relationship (QRAR) [5-7]. Sagrado has named this methodology BMC [8,9]. Which is a chromatographic modality optimized in order to describe the biological behavior of drugs, comprises a hydrophobic stationary phase and saline solutions of Brij35 micelles as mobile phase, has been testified to be useful for describing and predicting the biological activities of different pharmacological kinds of drugs [10], permeability across the intestinal barrier [11,12], blood–brain barrier and cornea [13].

However, in the biopartitioning micellar chromatography, the resting membrane potential, which is essentially the result of the gradients of the ion concentrations that exist across the membrane of a cell, can not be simulated [14]. The resting state of a cell can be characterized by a large number of parameters: the ionic concentrations in the intra- and the extracellular electrolyte solution, the currents of the different kinds of ion transport across the cell membrane, and the membrane potential, which is the difference between the electric potentials in the bulk of the intra- and the extracellular medium [15]. In physiological conditions, the membrane potential has a value of about −60～−80mV [16]. The values of zeta potential in pure Brij35 solution and mixed micellar system have been studied, the results indicate that the value is about 0mV in pure Brij35 solution and the ratio of Brij35 and SDS (85:15) in mixed micellar system (zeta potential is about−45.0mV) is the best condition for modeling the physiological environment. Furthermore, in the mixed system the conformation of the long hydrophilic polyoxyethylene chains remains unchanged. Intermolecular interaction among the Brij35 molecules gradually weakens in the mixed micelles as the ratio Brij35/SDS decreases, but with even smaller changes when the ratio of Brij35 and SDS is 85:15. The hydrophobic chains of both Brij35 and SDS are involved in the mixed micellar core. The self diffusion coefficients, relaxation measurements, and 2D NOESY experiments show that the hydrophilic chains of Brij35 molecules in the mixed micelles remained unchanged with the variation in Brij35/SDS. Which means for the reagent, $BMC_{Brij35:SDS=85:15}$ is better than BMC_{Brij35} to emulate the solute partitioning into liposome/ water layer system [17,18].

The diuretics are ion transport inhibitors that decrease the reabsorption of Na^+ at different sites in the nephron, can induce a state of increased urine flow. We found many health products contain diuretics in China. For example, some of the weight-loss products were found to contain bumetainde or furosemide [19]. In this paper, we focused our attention on mixed micellar liquid chromatography methods, deriving the quantitative retention–activity relationship models based on the $BMC_{Brij35:SDS=85:15}$ and BMC_{Brij35} chromatographic retention to predict the biological parameters of diuretics. The aim of this article is to discuss the advantages and limitations of using mixed micellar solution of Brij35/SDS (85:15) as mobile phase to describe and estimate the bioactivities of diuretics.

Experimental

Instrumental and measurement

The retention of drugs was measured using an LC-6A chromatograph with an LC-6A pump, an SPD-6AV UV-visible detector and a CTO-6A column thermostat (Shimadzu, Japan). Data were collected and processed on a Compaq computer installed with Alltech-chromstation software. The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20 μL loop. The HPLC

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column was a Kromasil C_{18} column (5um, 150 mm × 4.6 mm i.d.) with a phenomenex security Guard™ C_{18} guard cartridge. Mobile phase flow-rate was 1.0 mL/min. Detection was performed at 300 nm. All the assays were carried out at 37.0°C for imitating body temperature.

The retention data in BMC were calculated according to the IUPAC approach [20], based on the extra-column time correction, for any chemical can be estimated as:

$$k=(t^g_R-t^g_m)\ /\ (t^g_m-t_{ext}) \quad (1)$$

Where t^g_m is the gross hold up times; t^g_R is the gross retention time and t_{ext} is the extra-column time. All retention values used in this study were the averages of at least triplicate determinations. The relative standard deviations of *k* values ranged between 0.1 and 0.9%.

Reagents and standards

All diuretics were control articles except torasemide: furosemide(National institute for control of pharmaceutical and biological products, China, 100544-200501), hydrochlorothiazide(National institute for control of pharmaceutical and biological products, China, 00309-200001), bumetanide(National institute for control of pharmaceutical and biological products, China, 173-9301), etacrynic acid (National institute for control of pharmaceutical and biological products, China, 0259-9701), amiloride hydrochloride (National institute for control of pharmaceutical and biological products, China, 100310-200201), triamterene(National institute for control of pharmaceutical and biological products, China, 100429-200401), torasemide tablets (Nanjing Xingang Union Pharma. CO.,LTD, Nanjing, China).

Mobile phases of $BMC_{Brij35:SDS=85:15}$ and BMC_{Brij35} were prepared with aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35,Acros, New Jersy, USA) and sodium dodecyl sulfate (SDS, New Jersy, USA). Micellar eluent pH was adjusted with 0.05 M phosphate buffer, which was prepared with disodium hydrogen phosphate and potassium dihydrogen phosphate (analytical-reagent grade, Kelong, Chengdu, China). In order to reproduce the osmotic pressure of biological fluids, NaCl (9.20 g/L, analytical-reagent grade, Kelong, Chengdu, China) was added to the micellar mobile phase. This NaCl concentration was close to physiological concentration of biological fluids. Water was from a Millipore (Billerica,MA,USA) synergy™ 185 system and was degassed before HPLC. The mobile phases injected into the chromatograph were filtered through 0.45 μm microporous membrane.

Stock standard solutions of reference substance of the diuretics were prepared by dissolving 5 mg of the compound in 5 mL of mobile phase solution. Working solutions were prepared by dilution of the stock standard ones using the Brij35 solution. For the pharmaceutical preparation, working solutions were prepared by dissolving 10 mg of the tablet powders of the drugs in 10 mL of mobile phase solution, then centrifuged at 1000g for 5 minutes. The working solutions injected into the chromatograph were filtered through 0.45 um microporous membranes (Xinya, Shanghai, China), respectively. All the solutions were stored under refrigeration at 4°C before injection.

Software and data processing

Matlab® for Windows (version 7.1, The Math Works Inc.) was used for multivariate analysis, and Microsoft® Excel 2003 (Microsoft Corporation) was used to perform statistical analysis of regression models.

Evaluation of the QRAR models predictive ability

To evaluate the predictive ability of the QRAR models, the comparison between the root mean squared error of calibration (RMSEC), the root mean squared error of cross-validation (leave-one-out) (RMSECV), and the root mean squared error of cross-validation (leave-one-out) for interpolated data (RMSECVi) [21] was used. Table 1 shows the equations and the characteristics. From a qualitative point of view, large differences between RMSEC, RMSECV and RMSECVi indicate a lack of robustness of the QRAR models obtained and the need for greater cautions in future predictions.

Results and Discussion

Retention–activity relationships for the diuretics in BMC

Table 2 shows the structure of the diuretics. The retention of the compounds was measured using 0.02 M Brij35 and 0.02 M Brij35/SDS (85:15) mobile phase, respectively. The pH was adjusted to 7.4 to obtain experimental conditions as close as possible to physiological ones. In order to obtain predictive and interpretative models, the retention data of diuretics and the corresponding biological responses were adjusted to a second-order polynomial model (Eqn.2), which has also been demonstrated in previous QRAR studies that this is the usual retention–activity relationship for pharmacokinetics and biological response of drugs [20,21].

$$\text{Bioactivity parameter} = \alpha(\log k)^2 + b\log k + c \quad (2)$$

Where bioactivity parameter includes pharmacokinetic parameters. [e.g., half-life time ($t_{1/2}$), volume of distribution (V_d), plasma clearance (Cl), and time after administration of a drug when the maximum plasma concentration is reached (tmax)].

Table 3 shows the bioactivities of diuretics reported in the literature and the retention data (log *k*) obtained in 0.02 M Brij35 and Brij35/SDS (85:15) mobile phase. In the case of the pharmacokinetics, due to the large number of data sources found and their variability, the values chosen to construct the corresponding QRAR models were the median

Parameter	Equation	Characteristics
RMSEC	$RMSEC=\sqrt{\frac{\sum(\bar{Y}-Y)}{n}}$	All the *n* molecules are included in the model construction.
RMSECV (leave-one-out approach)	$RMSEC=\sqrt{\frac{\Sigma(\bar{Y}-Y)}{n}}$	Each molecule (*i*) is used as test in turn for the model chosen on the remaining molecules, performing the procedure *n-1* times
RMSECVi (leave-one-out approach)	$RMSECVi=\sqrt{\frac{\sum(\bar{Y}-Y)}{n-2}}$	The same as for RMSECV but excluding the two extreme data (*i*=1, *n*), after ordering them by their log *k* values.

Table 1: Equations and characteristics of RMSEC, RMSECV and RMSECVi.

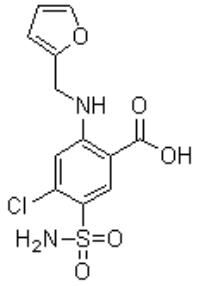
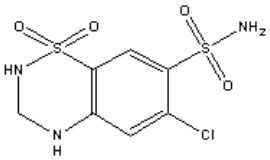
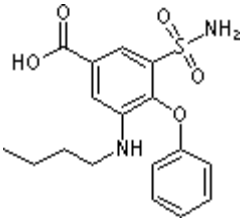
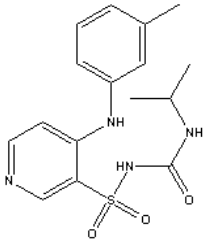
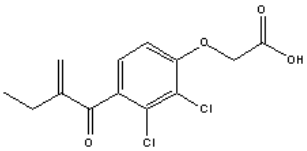
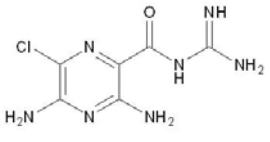
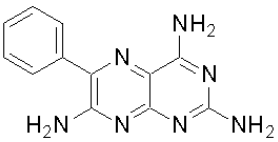
	
Furosemide	Hydrochlorothiazide
	
Bumetanide	Torasemide
	
Ethacrynic acid	Amiloride
	
Triamterene	

Table 2: Structures of the diuretics studied.

values. Table 4 summarizes the statistical analysis of the second-order polynomial models using BMC_{Brij35} and $BMC_{Brij35:SDS=85:15}$ systems. In all cases, the equations and statistics for the QRAR models are adjusted to the format recommended by Sagrado and Cronin [22,23]. The p-values were less than 0.01, which indicated that there were statistically significant relationships between these parameters and log *k* values obtained in the BMC_{Brij35} and $BMC_{Brij35:SDS=85:15}$ systems at the 99% confidence level. The r^2 statistic values indicated that the models, as fitted, explain 0.9323–0.9453 and 0.9742–0.9824% of the variability in BMC_{Brij35} system and $BMC_{Brij35:SDS=85:15}$ system at pH 7.4, respectively. The standard error of the estimate shows the standard deviation of the residuals to be 0.2598 and 0.9047 (for BMC_{Brij35} system at pH 7.4), 0.1853–0.5133 (for $BMC_{Brij35:SDS=85:15}$ system at pH 7.4), which can be used to construct prediction limits for new observations. As can be deduced by comparing the r^2, r^2_{adj}, SE, F and p-values, the $BMC_{Brij35/SDS}$–QRAR models provide either better or at least comparable statistical results than the BMC_{Brij35}–QRAR models.

From the results, we could see for the BMC_{Brij35}–QRAR models,

No.	Diuretics	$t_{1/2}$ (h)	t_{max} (h)	logk_{BMC} (pH7.4)	log$k_{BMC\text{-}SDS}$ (pH7.4)
1	Furosemide	0.5-1.0[25]	1.0-2.0 [26]	0.90	0.85
2	Hydrochlorothiazide	7.0-12.2[27]	1.4-3.8 [27]	1.26	8.60
3	Bumetanide	1.0-1.5[28]	1.0-2.0 [29]	0.82	1.00
4	Torasemide	3.5[30]	------	0.68	3.50
5	Ethacrynic acid	1.0-3.0[31]	2.0 [30]	0.75	2.00
6	Amiloride	6.0-9.0[30]	3.0-4.0 [32]	1.30	7.50
7	Triamterene	1.5-3.0[30]	2.0-5.0 [29]	1.12	2.70

Table 3: The bioactivities, retention data (log *k*) obtained in 0.02 M Brij35 and Brij35/SDS (85:15) mobile phase, respectively.

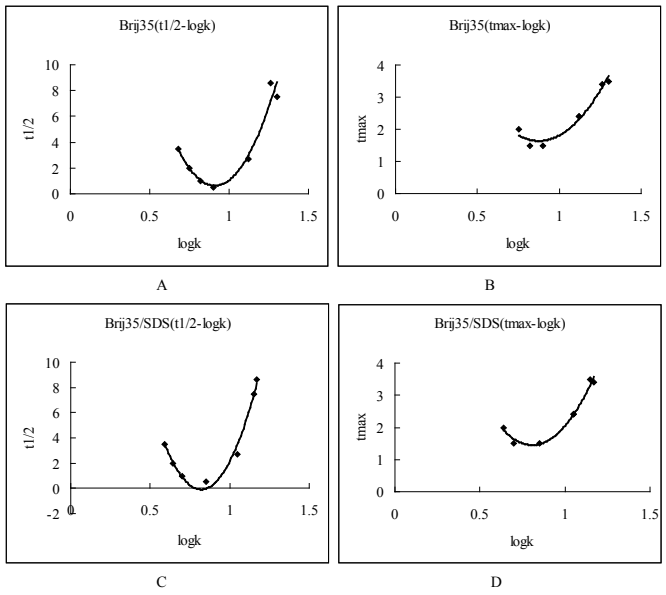


Figure 1: (A~D) The plots of biological activity parameters vs. the retention data studied in the BMC_{Brij35} and $BMC_{Brij35:SDS=85:15}$ system.

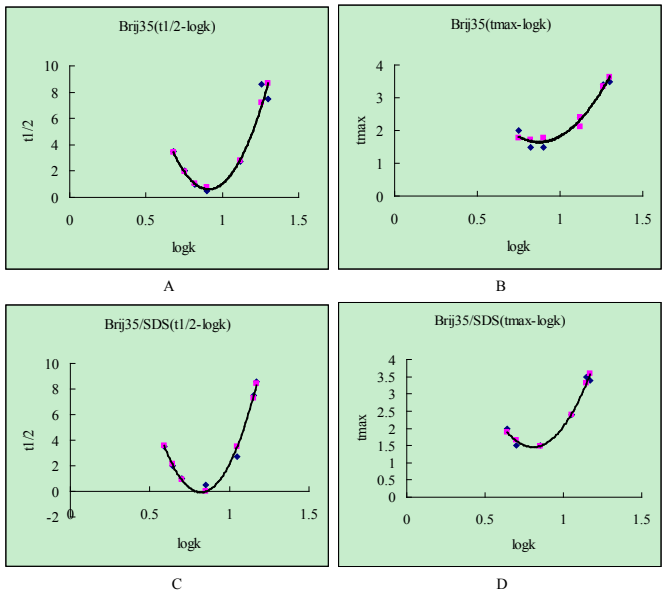


Figure 2: (A~D) The actual versus predicted (fitted and cross-validated) activities in the BMC_{Brij35} and $BMC_{Brij35:SDS=85:15}$ system.

BMC BMC-SDS BMC BMC-SDS *Vd* BMC	mobile phase BMC BMC-SDS CL BMC BMC-SDS BMC BMC-SDS	n	$a \pm ts_a$	$b \pm ts_b$	$c \pm ts_c$	r^2	S.E.	F	RMSEC	RMSECV	RMSECVi
			(p-value)	(p-value)	(p-value)	r^2_{adj}		(p-value)			
$t_{1/2}$	Brij35	7	52.42±5.225	-95.56±4.737	44.28±4.579	0.9453	0.9047	34.590	0.6968	0.7388	0.5425
			0.0064	0.0091	0.0102	0.9180		0.0029			
	Brij35-SDS	7	65.93±10.35	-107.6±9.506	43.94±9.279	0.9824	0.5133	111.68	0.3880	0.4191	0.3733
			0.0004	0.0007	0.0008	0.9736		0.0003			
tmax	Brij35	6	8.586±2.601	-14.26±2.088	7.647±2.231	0.9323	0.2598	27.546	0.1808	0.5053	0.1980
			0.0499	0.1050	0.0895	0.8985		0.0046			
	Brij35-SDS	6	15.29±5.319	-24.52±4.673	11.31±4.953	0.9742	0.1853	56.583	0.3583	0.3928	0.4249
			0.0129	0.0185	0.0158	0.9570		0.0041			

Table 4: The statistical analysis and the predictive features of the second-order polynomial models using BMC_{Brij35} and $BMC_{Brij35: SDS=85:15}$ systems, respectively.

all the fitting parameters of models were significant (p-values were less than 0.05), while for the tmax QRAR model at pH 7.4 the fitting parameter b and c were not. Since the p-values were larger than0.05, the square of the product–moment correlation coefficient ($r^2 = 0.9323$) and r^2 for degrees of freedom($r^2_{adj} = 0.8985$) of the relationships testified that the BMC_{Brij35}–QRAR model was adequate. On the other hand, for the $t_{1/2}$ and tmax QRAR models, using mixed micellar as mobile phase, the fitting parameters were all significant. Figure 1(A–D) includes the plots of biological activity parameters vs. the retention data studied in the BMC_{Brij35} and $BMC_{Brij35:SDS=85:15}$ system. In all cases, data can be fitted to a second-order polynomial model, which has been proved to be usual in QSAR models [24]. It has also been demonstrated in previous QRAR studies that this is the usual retention–activity relationship for pharmacokinetics and biological responses of drugs. As can be observed in Figure 1, the relationship obtained using BMC_{Brij35} system at pH 7.4 was quite similar to that obtained using the $BMC_{Brij35:SDS=85:15}$ system. From a qualitative point of view, both systems provide the same information at physiological pH, the use of mixed mobile phases did not improve the results obviously.

Predictive ability of QRAR models

To compare the predictive ability of the models in terms of crossvalidated data, but pointing out the difference between interpolated data and extrapolated data, three parameters (the RMSEC, RMSECV and RMSECVi values) for the BMC_{Brij35}–QRAR and $BMC_{Brij35/SDS}$–QRAR models were obtained (Table 4). For the BMC_{Brij35}–QRAR models, the RMSECV values were larger than RMSECVi values, and were larger than those corresponding to the $BMC_{Brij35/SDS}$–QRAR models. Which indicated that for the BMC_{Brij35}–QRAR models, some caution should be exercised with the extrapolated parameter data. For $BMC_{Brij35/SDS}$–QRAR models the RMSEC, RMSECV and RMSECVi values were similar, which suggested that both interpolations and extrapolations of parameters based on the current $BMC_{Brij35/SDS}$–QRAR models should be reasonably adequate. Figure 2 (A~D) includes the actual versus the predicted (fitted and cross-validated) activities for diuretics. Apparently, there were slight differences between these figures when using the $BMC_{Brij35:SDS=85:15}$ system, visual differences mainly located in the smaller values ($0.50<\log k<1.00$) in pure Brij35 solution. However, from the qualitative perspective, the ability of log *k* values in describing the biological responses and pharmacokinetic parameters of diuretics in terms of cross-validated data was adequate. Using the QRAR models obtained, the biological parameters of other compounds whose data were not available in bibliography could be predicted.

Conclusions

Since the revolutionary development of combinatorial chemistry, the bottleneck in drug discovery has shifted to pharmacokinetic and pharmacodynamic optimization of lead compounds. Chromatographic retention-based approaches (QRAR) system which encompasses the main interactions between a drug and biological membrane, can offer a rapid, simple pharmacokinetic/pharmacodynamic profiling of compounds. The main problem to get models with predictive ability of biological parameters of drugs is the limited number of available data of compounds because they have not been studied or been reported. This problem is special important for multivariate models based on the use of molecular descriptors.

In this study, $BMC_{Brij35-SDS}$ provides results that are better than, or at least comparable to, BMC_{Brij35} in the QRAR models involving the diuretics reported. This mixed micellar liquid chromatography approach can be very useful in pharmaceutical research of new diuretics and helpful in the study of adding banned diuretics in health products.

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References

1. Outinen K (1995) Optimization of the HPLC: Analysis of biogenic amines in Peucedanum palustre plants and cell culture lines. Planta Med 61: 259-263.
2. Detroyer A, Vander Heyden Y, Cambre I, Massart DL (2003) Chemometric comparison of recent chromatographic and electrophoretic methods in a quantitative structure–retention and retention–activity relationship context. J Chromatogr A 986: 227-238.
3. Escuder-Gilabert L, Sagrado S, Villanueva-Camanas RM (2000) Development of predictive retention–activity relationship models of non-steroidal anti-inflammatory drugs by micellar liquid chromatography: comparison with immobilized artificial membrane columns. J Chromatogr B 740: 59-70.
4. Hilgers AR, Conradi RA, Burton PS (1990) Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. Pharm Res 7: 902-910.
5. Ma T, Ye LM, Chen Y (2004) The application of Quantitative retention-activity relationship models in prediction of oral drug absorption and biological activity. Asian J Drug Metab Pharmacok 4: 251-260.

6. Wang S, Yang G, Zhang H (2007) QRAR models for cardiovascular system drugs using biopartitioning micellar chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 846: 329-333.

7. Ye LM, Ma T, Chen C, Chen Y (2005) Study on predictive quantitative retention-activity relationship models of non-steroidal anti-inflammatory drugs by micellar liquid chromatography. J Chin Pharm 40: 1737-1741.

8. Escuder-Gilabert L, Mart´ın-Biosca Y, Sagrado S, Villanueva-Camañas RM, Medina-Hernández MJ (2001) Biopartitioning micellar chromatography to predict ecotoxicity. Anal Chim Acta 448: 173-185.

9. Cuenca-Benito M, Sagrado S, Villanueva-Camanas RM, Medina-Hernandez MJ (1998) Quantitative retention–structure and retention–activity relationships of barbiturates by micellar liquid chromatography. J Chromatogr A 814: 121-132.

10. Quiñones-Torrelo C, Sagrado S, Villanueva-Camañas RM, Medina-Hernández MJ (1999) Development of predictive retention-activity relationship models of tricyclic antidepressants by micellar liquid chromatography. J Med Chem 42:3154-3162.

11. Balon K, Riebesehl BU, Muller BW (1999) Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. Pharm Res 16: 882-888.

12. Molero-Monfort M, Escuder-Gilaber L, Villanueva-Camanas RM, Sagrado S, Medina-Hernandez MJ (2001) Biopartitioning micellar chromatography: an in vitro technique for predicting human drug absorption. J Chromatogr B Biomed Sci Appl 753: 225-236.

13. Wu LP, Cui Y, Xiong MJ, Wang SR, Chen C, Ye LM (2008) Mixed micellar liquid chromatography methods: modelling quantitative retention–activity relationships of angiotensin converting enzyme inhibitors. Biomed Chromatogr 22: 1243–1251.

14. Escuder-Gilabert L, Sanchis-Mallols JM, Sagrado S, Medina-Hernández MJ, Villanueva-Camañas RM (1998) Chromatographic quantitation of the hydrophobicity of ionic compounds by the use of micellar mobile phases. J Chromatogr A 823: 549-559.

15. Bonet Domingo E, Medina Hernández MJ, Ramis Ramos G, García Alvarez-Coque MC(1992) High-performance liquid chromatographic determination of diuretics in urine by micellar liquid chromatography. J Chromatogr B 582: 189-194.

16. Jäckle J (2007) The causal theory of the resting potential of cells. J Theor Biol 249: 445-463.

17. Ebrahimi P, Hadjmohammadi MR (2006) Simultaneous optimization of resolution and analysis time in mixed micellar liquid chromatography of coumarins by use of a utility function. Anal Bioanal Chem 384:851-858.

18. Hongchang Gao, Sui Zhao, Shizhen Mao, Hanzhen Yuan, Jiayong Yu, Lianfang Shen, You-Ru Du (2002) Mixed Micelles of Polyethylene Glycol (23) Lauryl Ether with Ionic Surfactants Studied by Proton 1D and 2D NMR. J Colloid Interface Sci 249: 200-208.

19. Wu LP, Sun CJ, Chen C, Ye LM (2009) Determination of diuretic in functional foods by HPLC with tandem MS. West China J Pharm Sci 24: 90-92.

20. Bermúdez-Saldaña JM, Escuder-Gilabert L, Villanueva-Camañas RM, Medina-Hernández MJ, Sagrado S (2007) On the measurement of consistent long-term retention factor values in micellar liquid chromatography. Anal Chim Acta 595: 19-27.

21. Quiñones-Torrelo C, Martin-Biosca Y, Martínez-Pla JJ, Sagrado S, Villanueva-Camañas RM, Medina-Hernández MJ (2002) QRAR Models for Central Nervous System Drugs using Biopartitioning Micellar Chromatography. Mini-Rev Med Chem 2: 145-161.

22. Escuder-Gilabert L, Molero-Monfort M, Villanueva-Camañas RM, Sagrado S, Medina-Hernández MJ (2004) Potential of biopartitioning micellar chromatography as an *in vitro* technique for predicting drug penetration across the blood–brain barrier. J Chromatogr B 807: 193-201.

23. Molero-Monfort M, Martin-Biosca Y, Sagrado S, Villanueva-Camanas RM, Medina-Hernandez MJ (2000) Micellar liquid chromatography for prediction of drug transport., J Chromatogr A 870: 1-11.

24. Patrick GL (2009) An introduction to Medicinal Chemistry. Oxford University Press, New York.

25. Abdel-Hamid ME (2000) High-performance liquid chromatography–mass spectrometric analysis of furosemide in plasma and its use in pharmacokinetic studies. IL Farmaco 55: 448-454.

26. Andreasen F, Tietze I, Hansen FA, Petersen JS, Christensen S,Steiness E (1991) Furosemide kinetics and dynamics in rats and humans. Comp Biochem Physiol C: Comp Pharmacol 100: 635-641.

27. Hsien JK, Lin C, Matuszweski BK, Dobrinska MR (1994) Fully automated methods for the determination of hydrochlorothiazide in human plasma and urine. J Pharm Biomed Anal 12: 1555-1562.

28. Wells TG, Fasules JW, Taylor BJ, Kearns GL (1992) Pharmacokinetics and pharmacodynamics of bumetanide in neonates treated with extracorporeal membrane oxygenation. J Pediatr 121: 974-980.

29. Hamed E, Gerson MC, Millard RW (2003) A study of the pharmacodynamic differences between immediate and extended release bumetanide formulations. Int J Pharm 267: 129-140.

30. Weast RC (1989) CRC Handbook of Chemical and Physics. CRC Press, Boca Raton.

31. Kirkendall WM, Stein JH (1968) Clinical pharmacology furosemide and ethacrynic acid. Am J Cardiol 22: 162-167.

32. Lee C, Tannock I (1996) Pharmacokinetic studies of amiloride and its analogs using reversed-phase high-performance liquid chromatography. J Chromatogr B 685: 151-157.