ELSEVIER

Journal of Chromatography B, 761 (2001) 13–26

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Retention pharmacokinetic and pharmacodynamic parameter relationships of antihistamine drugs using biopartitioning micellar chromatography

C. Quiñones-Torrelo, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández*

Departamento de Química Analítica, Facultad de Farmacia, Universidad de Valencia, C/ Vicente Andrés Estellés s/n, 46100 Valencia, Spain

Received 14 February 2001; received in revised form 30 May 2001; accepted 20 June 2001

Abstract

Antihistamines are drugs which act by competitive inhibition of the H_1 or H_2 histamine receptors. Little has been known about their clinical pharmacokinetics and biological responses until the last few years. In this paper, we propose quantitative retention–activity relationship, QRAR, models based on the retention data of antihistamines in a biopartitioning micellar chromatography (BMC) system using a Brij35 mobile phase for describing pharmacokinetic parameters such as half-life and volume of distribution, or the pharmacodynamic parameters, therapeutic plasma levels, lethal doses and drug-receptor dissociation constant. The predictive ability of these models is statistically validated. These results are compared to traditional quantitative structure–activity relationship, QSAR, models using lipophilicity data. The adequacy of QRAR models can be explained taking into account the fact that the retention of compounds in BMC depends on their hydrophobic, electronic and steric characteristics which are of great importance in pharmacokinetic and pharmacodynamic behavior.

Keywords: Quantitative retention–activity relationship; Pharmacokinetic parameters; Pharmacodynamic parameters; Antihistamines

1. Introduction

Antihistamines are drugs which antagonize the histamine effects by competitive inhibition of histamine receptors. They have an affinity for a specific receptor. The histamine receptors have been classified into subtypes H_1, H_2 and more recently, H_3. The major allergic responses are mediated through the H_1 receptor. H_2 effects include esophageal contraction, gastric acid secretion and increased lower airway secretion. The main function of the H_3 receptor seems to be to turn off histamine secretion but its exact physiologic role is currently not known [1].

Antihistamine drugs are classified into two categories: H_1 and H_2 antagonists [2]. The US Food and Drug Administration (FDA) classifies H_1 antihistamines as either ''sedating'', which cross the blood–brain barrier, or ''non-sedating''. The first ones block H_1 and cholinergic receptors in neural tissues of the CNS resulting in reduced physical and mental function [3]. This effect has made it possible

*Corresponding author. Tel.: +34-96-386-4899; fax: +34-96-386-4322.

E-mail address: maria.j.medina@uv.es (M.J. Medina-Hernández).

0378-4347/01/$ – see front matter
PII: S0378-4347(01)00294-8

that sometimes, H_1 antagonists are used as hypnotic agents in insomnia treatment. H_2 antihistamines are mainly used as antiulcer drugs.

Although H_1 and H_2 antihistamines have their own structural features (Fig. 1), in both cases three requirements are considered fundamental for the drugs to exert their action: an aromatic system, a flexible chain and an H-bonding group. For H_1 receptor antagonists, in most cases the aromatic system comprise two aromatic rings linked by short chain of carbon atoms (flexible chain) to a tertiary amino group (H-bonding group). These drugs have high $\log P$ values and they are also highly basic. These structures do not have a close chemical resemblance to histamine except the presence of an ammonium alkyl chain. It is presumed that they bind to the same anionic site at the receptor as does histamine, but that the aromatic rings bind to a nearby hydrophobic region.

Regarding the H_2 receptor antagonists, the aromatic system can be an imidazole ring (cimetidine, etintidine), a furan (ranitidine), a thiazole (famotidine, nizatidine) or a piperidinomethylphenoxy group (roxatidine). The flexible chain is generally a $-CH_2SCH_2CH_2$ group and the H-bonding group generally contains the system –NH–C–NH–. All these molecules have planar π-electron systems, are polar and hydrophilic with high dipole moments and low $\log P$ values. Early structure–activity studies have suggested that efficacy as H_2 receptor antagonists appears to be directly related to hydrophobicity, in fact, it was found that a 10-fold increase in P brought about a 100-fold increase in antagonist potency. In addition to hydrophobicity ($\log P$), differences in activity are accounted for by dipole orientation with respect to the side chain [4].

The same properties that condition antihistaminic activity (hydrophobicity, charge and steric effects) also describe the compound retention in a micellar chromatography system [5]. Our research group has demonstrated that, under adequate experimental conditions, the chromatographic system constituted by a reversed-phase stationary phase and saline micelle solutions of Brij35 as mobile phase can be used as an in vitro system to emulate drug biopartitioning. This methodology has been applied to describe the biological activity of different kinds of drugs [6–15]. We have named to this methodology biopartitioning micellar chromatography (BMC). The success of BMC in constructing QRAR models can be attributed to the similarities between BMC systems and biological barriers–extracellular fluid.

In this paper, QRAR models for describing pharmacokinetics (half-life and volume of distribution) and pharmacodynamics (therapeutic plasma levels, lethal doses and drug-receptor dissociation constant) of antihistamine drugs are proposed. These results are compared to analogous QSAR models obtained using $\log P_{app}$ values.

2. Experimental

2.1. Instrumental and measurement

A Hewlett-Packard 1100 chromatograph with an isocratic pump, a thermostat, an UV–Vis detector and a HP Vectra computer was used (Palo Alto, CA). Data acquisition and processing were performed on a HP Vectra XM computer (Amsterdam, The Netherlands) equipped with HP Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA) with a 20-μl loop. A Kromasil octadecyl silane C_{18} column (Scharlau, Barcelona, Spain, 5 μm; 50× 4.6 mm I.D.) was used. The mobile phase flow-rate was 1.5 ml/min. The detection was performed at 240 nm. All the assays were carried out at 36.5°C. The retention factor values were averages of at least triplicate determinations.

2.2. Reagents and standards

Micellar mobile phases of polyoxyethylene-23 lauryl ether (Brij35, Acros Chimica, Geel, Belgium) at pH 7.4 adjusted with 0.05 *M* phosphate buffer (analytical reagent, Panreac, Barcelona, Spain) were used. To reproduce the osmotic pressure of biological fluids, 9.20 g/l NaCl (purissim, Panreac) was added to the mobile phase.

The antihistamines were obtained from several sources: chlorpheniramine, brompheniramine, doxylamine, chloropyramine, clemastine, antazoline, carbinoxamine, chlorcyclizine, cinnarizine, ketotifen, methapyrilene, orphenadrine, pyrilamine, prometha-

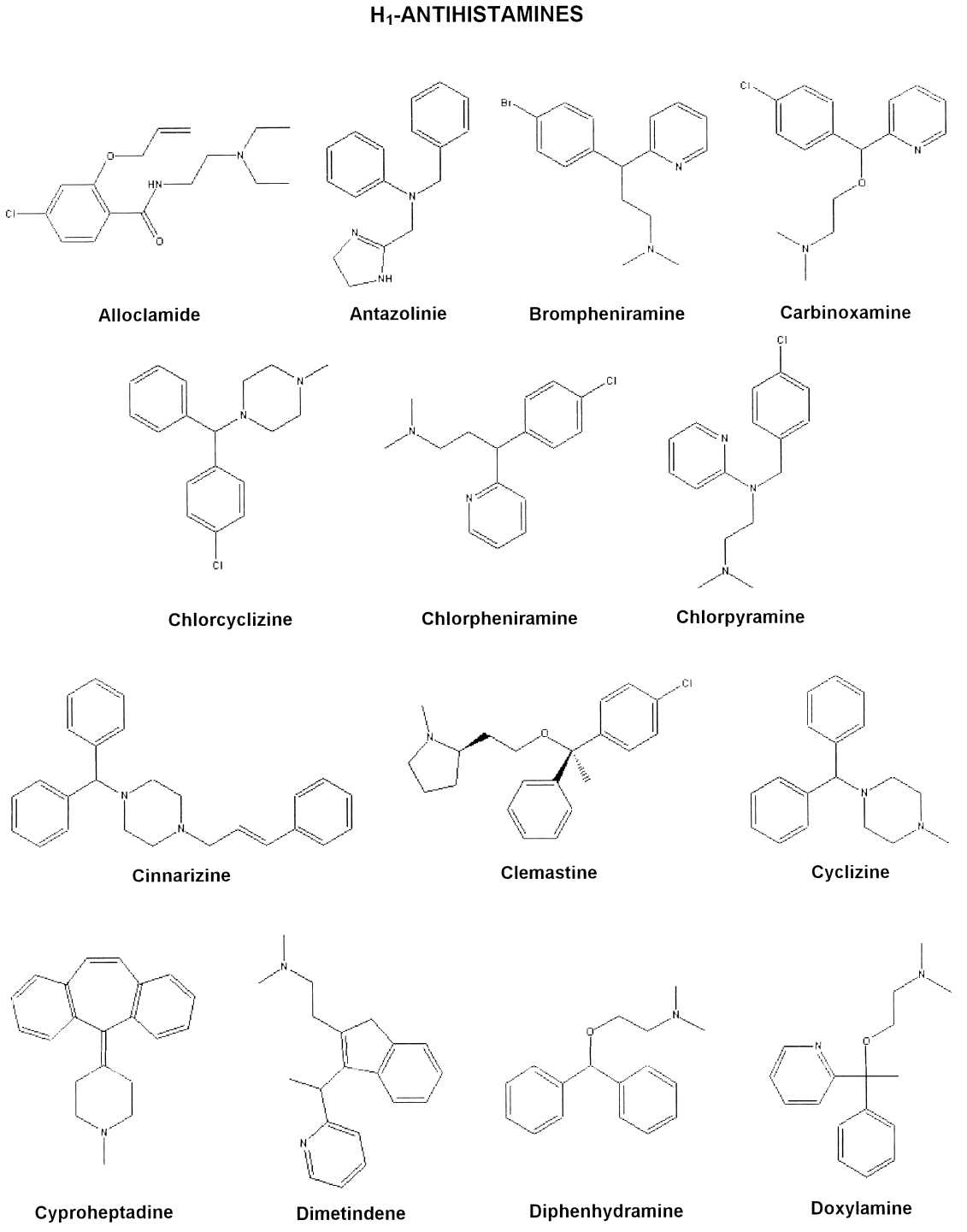
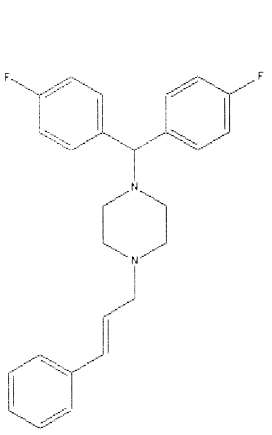
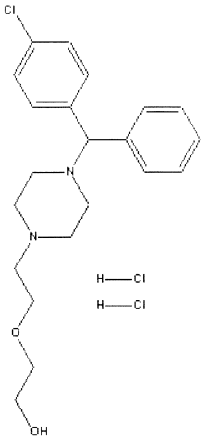


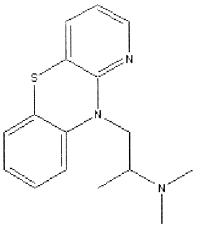
Fig. 1. Chemical structure of the antihistamine drugs studied.



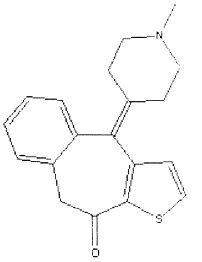
Flunarizine



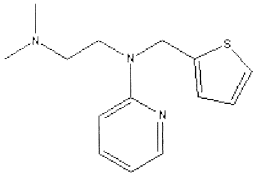
Hydroxyzine



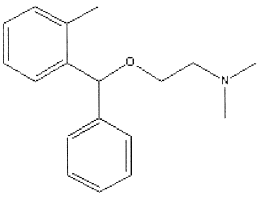
Isothipendyl



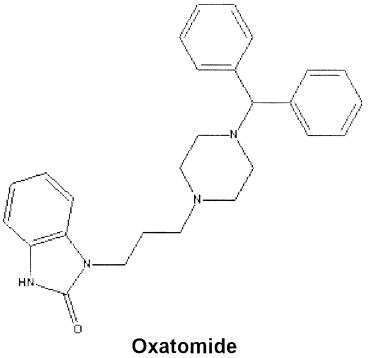
Ketotifen



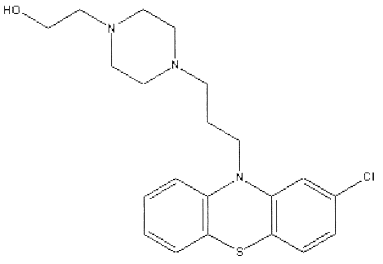
Methapyrilene



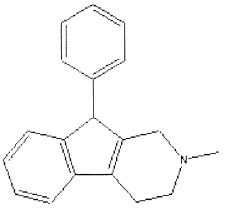
Orphenadrine



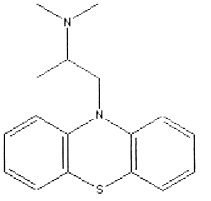
Oxatomide



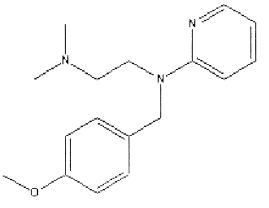
Perphenazine



Phenindamine

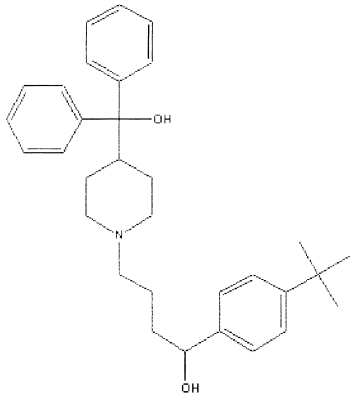


Promethazine

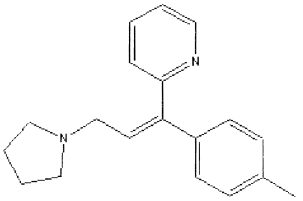


Pyrilamine

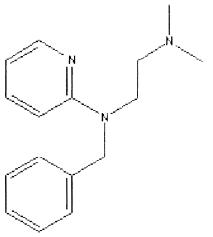
Fig. 1. (*continued*)



Terfenadine

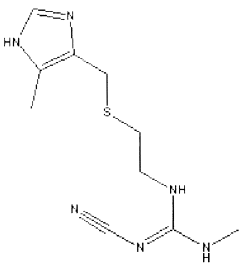


Tripelennamine

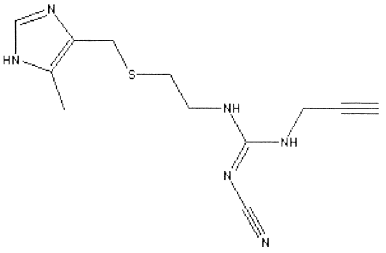


Triprolidine

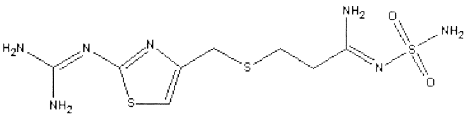
H_2-ANTIHISTAMINES



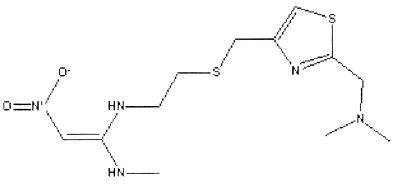
Cimetidine



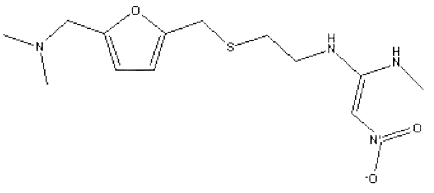
Etintidine



Famotidine



Nizatidine



Ranitidine

Fig. 1. (*continued*)

zine, cyclizine, triprolidine, terfenadine, flunarizine and perphenazine from Sigma–Aldrich S.A. (Madrid, Spain); cimetidine, ranitidine, famotidine, hydroxyzine, tripelennamine, cyproheptadine and diphenhydramine from Guinama (Valencia, Spain). Other drugs were kindly donated by different laboratories: Nizatidine (Lilly, Mayagüez, Puerto Rico), dimethindene (Novartis, Nyon, Switzerland), etin-

tidine and isothipendyl (Bristol-Myers Squibb, Princeton, NJ, USA).

Stock standards (400 mg/l) of antihistamines in methanol (HPLC, Reagent grade, Scharlau, Barcelona, Spain) were prepared. Working solutions were prepared by dilution of the stock standard ones using 0.04 *M* Brij35 (pH 7.4).

Barnstead E-pure deionized water (Sybron, Boston, MA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45 and 0.22 μm nylon membranes, respectively (Micron Separations, Westboro, MA).

2.3. Software and data processing

Excel 7.0 Microsoft Office software was used to perform the statistical analysis of the multiple linear regression (MLR).

2.4. Evaluation of the QRAR models predictive ability

To evaluate the adequacy of the models, the fit error (i.e. root-mean-square error of calibration, RMSEC), the prediction error based on cross-validation (i.e. root-mean-square error of cross-validation, RMSECV), parameter which includes both interpolation and extrapolation information [16] and the RMSECVi [11,12] for measuring only interpolation information, were compared.

2.5. Data sources

Table 1 contains the log *P* [17,18] and pK_a [17,19–22] values and the pharmacokinetics and pharmacodynamics available and reported data [23–38] for the antihistamine drugs used in this study. The gaps in the table indicate that these values were not found in literature. The pK_a values for alloclamide, clemastine and dimethindene were experimentally obtained by potentiometric titration in Brij35 0.04 *M* medium for this work.

The criteria used to select, from those found in bibliography, the pharmacokinetic and pharmacodynamic value to be used to construct the QRAR models were the following. When an unique source of data was available, the reported value or the mean value from the reported range has been used. When the data for a compound activity is provided by several sources the median value has been used.

3. Results and discussion

3.1. Retention behavior of antihistamines

The retention of the compounds included in Fig. 1 was measured using 0.02, 0.04 and 0.06 *M* Brij35 mobile phases. In all cases, the pH was adjusted to 7.4 to obtain experimental conditions as close as possible to physiological ones. All the H_1 antihistamines studied are tertiary amines, present policyclic structures and their molecular mass range between 255 (diphenhydramine) and 472 (terfenadine). These features give them a high hydrophobicity: their corresponding log *P* values vary between 2.17 (doxylamine) and 6.42 (flunarizine) [17]. At physiological pH they are positively charged.

The H_2 antihistamines used in this work are highly polar compounds, therefore low hydrophobic character: log *P* values ranged from –0.62 (nizatidine) to 0.61 (etintidine) [17]. Consequently, they will be weakly retained in the BMC system.

As can be checked in Table 1, for the H_1 antihistamines the different Brij35 concentrations in the mobile phase produce large changes in the relative retention and even inversion in the elution order for some of the compounds, while the H_2 antihistamines retention was scarcely modified with the surfactant concentration.

3.2. Retention–log P_{app} relationships

Retention in MLC is not linearly related to log *P* [39]. In this work, the nonlinear dependence formulated by a second-order expression was checked, using the antihistamines retention factors obtained with 0.02, 0.04 and 0.06 *M* Brij35 mobile phases:

$$\log k = a + b(\log P_{app}) + c(\log P_{app})^2 \quad (1)$$

where log P_{app} is the apparent octanol–water partition coefficient calculated at physiological pH [39].

Low correlation coefficients were obtained: $R^2 =$

Table 1
Log*P*, pK_a, retention data, pharmacokinetics and pharmacodynamics of the antihistamine drugs

Antihistamine	log *P* [17]	pK_a [17]	log *k*/Brij35 (*M*)			Pharmacokinetics				Pharmacodynamics					
			0.02	0.04	0.06	$T_{1/2}$ (h)	$T_{1/2}$ (*)	V_d (l/kg)	V_d (*)	TPL [23] (μg/ml)	TPL (*)	LD_{50} (H_1) (mg/kg)	LD_{50} (*)	K_d [37] (H_1) (n*M*)	ED_{50} [38] (mg/kg)
H_1-antagonists															
Alloclamide	3.59 [18]	8.57^+	1.89	1.55	1.45							740 [35]	740		
Antazoline	4.25	2.5, 10.1 [19]	1.35	1.04	0.94										
Brompheniramine	2.88	9.79	2.19	1.88	1.86	2–20 [23] 15.6–34.2 [24] 25 [25]	–	11.7 [25] 8.6–14.8 [24]	11.7	0.008–0.015	0.0115			4.7	
Carbinoxamine	2.17	8.10	1.93	1.80	1.66	10–15 [23]	12.5			0.02–0.04	0.03			2.3	
Chlorcyclizine	4.68	2.12, 8.15	2.48	2.07	1.88									9	13.5
Chlorpheniramine	2.73	9.16	2.06	1.77	1.77	11.2–18 [24] 15–25 [26] 14–24 [25] 13–20 [2] 20 [27] 20 [23]	19.5	5.9–11.7 [25] 3–10 [2] 5.4–9.6 [24] 2.9–3.5 [26] 3.4 [27]	6.5	0.003–0.017	0.01	162 [35] 121 [36]	141.5	8	27.1
Chloropyramine	3.56	8.76 [20]	2.18	1.86	1.73										
Cinnarizine	6.14	7.80 [20]	2.54	2.26	2.01	20.4–26.8 [28]	23.6								
Clemastine	5.05	6.8^+	2.58	2.22	2.10	9.6–32.8 [29] 9–35 [26]	21.6	8.3–14.5 [29] 13–23 [26]	14.7			730 [35]	730		
Cyclizine	3.97	2.54, 8.32	2.29	1.96	1.77					0.1–0.25	0.175	147 [35,36]	147		
Cyproheptadine	4.92	8.87	2.47	2.08	1.88	16 [24]	16					74.2 [35] 123 [36]	98.5	3.1	
Dimethindene	3.42	6.58^+	2.24	1.94	1.85									8	
Diphenhydramine	3.36	9.00	1.95	1.73	1.61	7 [23] 2.9–3.9 [24] 5 [27] 5.3–11.7 [26] 3–5 [2] 3.4–9.3 [25]	–	3.3 [24] 1.7–7.3 [26] 3.3–6.8 [25] 4 [27] 3–7 [2]	4.5	0.05–0.1	0.075			17	33.8
Doxylamine	2.28	4.4, 9.20	1.49	1.39	1.24	10 [23] 10 [25]	10			0.05–0.2	0.125	470 [35]	470		
Flunarizine	6.42	7.80 [20]	2.65	2.36	2.08					0.025–0.2	0.1125				
Hydroxyzine	4.16	2.1, 7.10	1.91	1.60	1.36	14–20 [25] 15.9–24.1 [24] 13.5 [23] 7–20 [2] 7.1 [24]	13.5	16–19.5 [25] 20 [2]	–	0.05–0.1	0.075	480 [36]	480		
Isothipendyl	3.93	8.66 [20]	2.34	2.09	1.83							204–240 [35] 222 [36]	222		
Ketotifen	3.56	8.24 [20]	1.97	1.59	1.5	12 [30]	12								45.5
Methapyrilene	2.50	3.7, 8.90	1.86	1.69	1.48	1.6 [25] 1.1–2.1 [31]	–	3.9 [25] 2.14–6.61 [31]	4.1			169–195 [35] 182 [36]	182	4.5	
Orphenadrine	3.86	8.40	2.28	2.05	1.81	16 [23] 16 [25]	16			0.1–0.2	0.15	150 [36]	150		
Oxatomide	5.42	8.00 [20]	2.1	1.82	1.57	14 [27] 20 [25]	17								21.0
Perphenazine	5.57	7.80	1.94	1.57	1.38	10 [23]	10			0.001–0.02	0.0105	120 [36]	120		
Phenindamine	3.74	8.30	2.33	1.95	1.79							280 [35]	280		

Table 1. Continued

Antihistamine	log *P* [17]	pK_a [17]	log *k*/Brij35 (*M*)			Pharmacokinetics				Pharmacodynamics					
			0.02	0.04	0.06	$T_{1/2}$ (h)	$T_{1/2}$ (*)	V_d (l/kg)	V_d (*)	TPL [23] (μg/ml)	TPL (*)	LD_{50} (H_1) (mg/kg)	LD_{50} (*)	K_d [37] (H_1) (n*M*)	ED_{50} [38] (mg/kg)
Promethazine	4.65	9.10	2.25	1.88	1.75	14 [23] 12 [25] 12 [27] 10.3–15.1 [24] 7–13 [2] 10–14.4 [24]	12.1	13 [27] 13 [2] 13.5 [25] 9.8–17 [24]	13.2	0.05–0.2	0.125			2.9	
Pyrilamine	2.77	4.02, 8.92	1.94	1.59	1.58							338 [35]	338		
Terfenadine	6.09	9.50 [20]	2.62	2.33	2.05	20 [27] 18.5 [23] 16.1–22.7 [24] 16–20 [2] 16–23 [25]	19			<0.01	0.01	2000 [35]	2000		24.3
Tripelennamine	2.85	4.2, 8.71	1.95	1.61	1.56	3–4.5 [25]	–	10 [25]	10			235 [36]	235	35	
Triprolidine	3.47	6.50, 9.5	2.3	2.05	2.04	5 [25] 3–6 [2]	–							5.6	
H_2-antagonists															
Cimetidine	0.21	6.80	0.5	0.41	0.3	2 [27] 2.75 [23] 1.7–2.3 [26] 1–5 [25] 1.5–2.3 [2]	2	1.3 [25] 0.8–2.1 [2] 1.3 [27] 0.8–1.2 [26]	1.3	0.25–3	–				
Etintidine	0.61 [18]	9.85 [20]	0.81	0.68	0.53	1.2–1.6 [25]	1.4	2 [25]	2						
Famotidine	−0.57	6.89 [21]	0.73	0.54	0.45	3 [27] 2.5–4 [25] 1.6–3.6 [26] 3.25 [23] 2.5–4 [2]	3.25	0.8–1.4 [25] 1.1–1.5 [26] 1.2 [27] 1.1–1.4 [2]	1.2	0.02–0.2	0.11				
Nizatidine	−0.62	−0.8, 1.95, 6.67 [22]	−0.07	−0.08	−0.15	1.3–1.6 [25] 1.4 [23] 1–1.6 [26] 1.7 [32] 1.4 [27]	1.45	1.2–1.4 [34] 0.8–1.3 [25] 0.8–1.5 [2] 0.7–1.7 [26] 1.3 [27]	1.2	0.05–1	0.525				
Ranitidine	0.27	2.3, 8.2	−0.14	−0.15	−0.22	1.5–2.5 [25] 1.9–2.3 [26] 1.6–2.4 [2] 4 [23] 2 [27] 2.6–3 [33]	2.05	1.2–2.5 [25] 0.9–1.7 [26] 1.2–1.9 [2] 1.5 [27] 1.6–2.4 [33]	1.55	0.05–1	0.525				

Data taken from Refs. [2,17–38]. Individual references are beside data in the table. + Experimental value. * Value used to construct the QRAR model. (–) Not used to construct the QRAR model due to the big variability in the reported data or its behaviour as outlier.

0.65, 0.60 and 0.51 for 0.02, 0.04 and 0.06 *M* Brij35 mobile phases, respectively. These results corroborate the fact that, in BMC, the compound hydrophobicity at the pH considered is not the only determining factor controlling its retention; other electronic interactions and steric factors are also important [40].

3.3. Retention–activity relationships

The molecular features of drugs, mainly hydrophobicity, electronic and steric properties, condition the drug-carrier protein (in biological fluids) and drug-receptor interactions and consequently their biological activity [41]. These same molecular features determine the BMC drug retention, therefore, it could be expected that retention–activity relationships exist.

The possibility of establishing relationships between antihistamine drugs retention data, log *k*, and their corresponding pharmacokinetic and pharmacodynamic parameters was studied. Pharmacokinetics and pharmacodynamics shown in Table 1 were used for the construction of QRAR models.

3.3.1. Retention–pharmacokinetics relationships

Most of the antihistamines are well absorbed following oral administration and metabolized in the liver via the cytochrome P-450 system. The half-lives of antihistamines vary greatly from about 2 h (H_2 antagonists) to days. They have relatively large apparent volumes of distribution: over 4 l/kg for H_1 antagonists and approximately 2 l/kg for H_2 antihistamines.

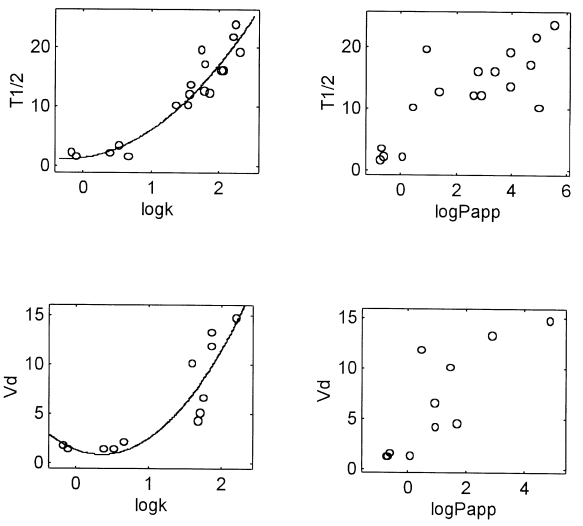


Fig. 2. Pharmacokinetics vs. log *k* (obtained using 0.04 *M* Brij-35 mobile phase) (left) and log P_{app} (right) relationships.

Relationships between antihistamines retention and their half-lives ($T_{1/2}$), and volume of distribution (V_d) have been established. Data were adjusted to a second order polynomial model, which agrees with the type of dependence that had been proved to be usual in reported QSAR and QRAR models [42,12,13]. Fig. 2 shows the relationship between the pharmacokinetics of antihistamines and their retention data when a 0.04 *M* Brij-35 mobile phase was used. In all cases, a random distribution of the residuals was found and they were statistically equal to zero, which suggested that, from a qualitative point of view, there is an adequacy of the polynomial model obtained to data.

Table 2 shows the statistical analysis and the predictive features of the QRAR models obtained when 0.02, 0.04 and 0.06 *M* Brij35 mobile phase were used. For all models, the *P* values were lower than 0.01 indicating that the relationships between the $T_{1/2}$ or V_d and the log *k* values were statistically significant at the 99% confidence level. The R^2 statistic values mean that the models, as fitted, explain between 89–91, and 82–88% of the variability in $T_{1/2}$ and V_d data, respectively. The *P* value on the highest order term is less than 0.05 for all models, indicating that this term is statistically significant at the 95% confidence level; consequently, the order of the polynomial is appropriate. As can be seen in Table 2, the Durbin–Watson values are greater than 1.4, which means that there is probably not any serious autocorrelation in the residuals.

3.3.2. Retention–pharmacodynamics relationships

Pharmacodynamics is the study of the molecular interaction between the drug and the site of action, which characterizes the pharmacological response. Histamine produces its effects through actions at two types of receptors, H_1 and H_2. Activation of the first one produces such effects as bronchoconstriction and contraction of the gut. The H_1 antihistamines antagonize these effects. In addition, these agents frequently have antagonist actions at other receptors.

Table 2
Statistical analysis and predictive features of the QRAR models (pharmacokinetic parameter) = $a + b(\log k) + c(\log k)^2$ corresponding to the retention data obtained using different Brij-35 mobile phases

[Brij-35] (M)	Pharmacokinetic parameter (n)	$a \pm La$ (P value)	$b \pm Lb$ (P value)	$c \pm Lc$ (P value)	R^2 $(R_{adj})^2$	SE	F (P value)	DW	RMSEC	RMSECV	RMSECVi
0.02	$T_{1/2}$ (18) (h)	1±3 (0.3874)	1±5 (0.7148)	2.5±2.1 (0.0223)	0.89 (0.87)	3	59 (0.0000)	2.1	2.4	2.7	2.8
	V_d (12) (l/kg)	1±3 (0.2460)	−3±5 (0.2102)	3±2 (0.0086)	0.88 (0.85)	2	32 (0.0001)	1.6	1.7	2.0	2.2
0.04	$T_{1/2}$ (18) (h)	1±3 (0.3265)	1±6 (0.5821)	3±2 (0.0164)	0.90 (0.89)	2	69 (0.0000)	2.1	2.2	2.6	2.5
	V_d (12) (l/kg)	1±3 (0.3497)	−3±8 (0.4220)	4.0±3.7 (0.0396)	0.82 (0.77)	2	20 (0.0005)	1.7	2.1	2.5	2.7
0.06	$T_{1/2}$ (18) (h)	2±2 (0.1949)	1±5 (0.5365)	4±3 (0.0082)	0.91 (0.90)	2	80 (0.0000)	2.3	2.1	2.4	2.5
	V_d (12) (l/kg)	1±3 (0.3376)	−2±7 (0.4585)	4.1±3.7 (0.0338)	0.83 (0.79)	2	22 (0.0003)	1.7	2.0	2.4	2.5

n: number of available activities; L: 95% confidence interval for coefficients estimates; $(R_{adj})^2$: R^2 adjusted for degrees of freedom; SE: standard error of the estimate; F: F-ratio; DW: Durbin–Watson statistic; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation (leave-one-out); RMSECVi: root mean square error of cross-validation (leave-one-out) for interpolate data.

In particular, they present anticholinergic properties as well as are used clinically to treat such conditions as motion sickness and vertigo [24].

Table 1 shows some pharmacodynamic parameters of the antihistamine drugs reported in bibliography, such as the H_1-antihistamines dissociation constant values (K_d), calculated by inhibition of [^{3}H]mepyramine binding to H_1-histamine receptor in rat brain membranes (equilibrium experiments), the effective dose values, ED_{50}, for IgE-mediated biphasic cutaneous reaction, the therapeutic plasma level (TPL) and the toxicity expressed as the drug mice oral LD_{50} value.

Fig. 3 shows the relationship between these pharmacodynamic parameters and the antihistamine's retention data obtained using 0.04 M Brij35. As can be observed, the polynomial models were adequate to model the data. Table 3 contains the results of the statistical analysis and the prediction features of the QRAR models obtained for TPL, LD_{50} and K_d from the retention data in 0.04 M Brij35. The P value of the models is less than 0.05 indicating that there is a statistically significant relationship at the 95% confidence level.

When the retention data in 0.02 and 0.06 Brij35 mobile phases were used, not statistically adequate QRAR models of the pharmacodynamics LD_{50} and K_d were obtained. It was due to the fact that the compounds involved in the corresponding QRAR models show large relative change on the retention and even inversion in the elution order when the Brij35 concentration was modified.

There are other effects of antihistamines, which are not related to the antagonism of histamine at H_1 or H_2 receptor. They result either from stabilization of the mast cell membrane, from competitive inhibition of the binding of calcium to membrane phospholipids, or from binding of the drug to calmodulin and preventing calcium from activating it [1]. Allergy, particularly asthmatic attacks are solely mediated by a sudden release of copious quantities of histamine. It is recognized that mast cells are needed for this histamine release following an IgE response [38]. The relationship between the ED_{50} values of various antihistamines for IgE-mediated biphasic cutaneous reaction and their BMC retention data (in 0.04 M Brij35) was examined (Fig. 3). It was found a second order polynomial model which explained 97% of the data variability, in addition the statistical analysis justified the adequacy of this polynomial

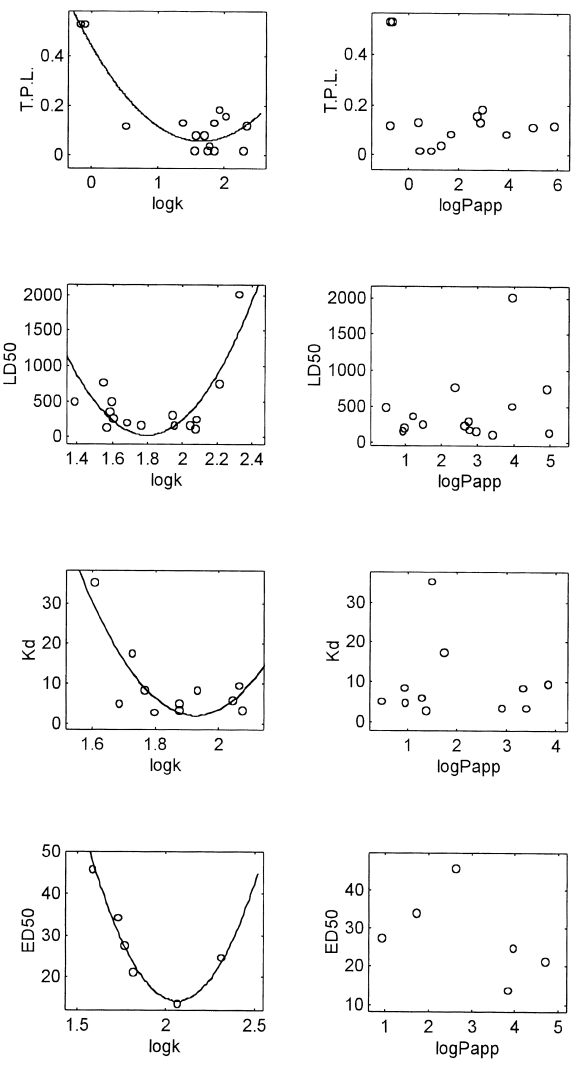


Fig. 3. Pharmacodynamics vs. log k (obtained using 0.04 M Brij-35 mobile phase) (left) and log P_{app} (right) relationships.

model to data (P value <0.01). Due to the limited data series at our disposal ($n=6$), the prediction of new ED_{50} values was not considered.

3.4. Predictive ability of QRAR models

As can be observed in Tables 2 and 3, except for LD_{50} and K_d, the RMSEC, RMSECV and RMSECVi values for the QRAR-models obtained are comparable, which suggests that prediction based on interpolations and extrapolations in these models should be reasonably adequate. However, for LD_{50} and K_d models some cautions must be taken with extrapolated predicted values.

Table 4 shows the pharmacokinetics and pharmacodynamics predicted values along with their 95% confidence intervals calculated from the standard deviation of the residuals for several compounds whose data were not available in bibliography. Although these values could not be corroborated, they could be useful in the clinical practice.

3.5. Comparison with traditional QSAR model based on log P_{app}

In order to compare the quality of the QRAR models obtained using the experimental log k parameters to those QSAR models obtained from the traditional lipophilicity parameter, relationships between both pharmacokinetic and pharmacodynamic parameters of antihistamines and their apparent octanol–H_2O partition coefficient at pH 7.4, log P_{app}, were performed. Two second-order polynomials models, one proposed by Hansch [43] (Eq. (2)) and other that consider the non-logarithmic form of the dependent variable (Eq. (3)), were assayed:

$$\log(1/C) = a + b(\log P_{app}) + c(\log P_{app})^2 \quad (2)$$

$$C = a + b(\log P_{app}) + c(\log P_{app})^2 \quad (3)$$

where C are the pharmacokinetic and pharmacodynamic parameters studied. The statistical analysis for both models are summarized in Table 5. As can be seen, the results obtained are not good. In general, models and coefficients were not statistically significant ($P>0.05$) and the correlation coefficients were lower than 0.75. Comparing the statistical analysis of the QRAR (Tables 2 and 3) and QSAR models (Table 5), we can conclude that the QRAR models obtained are more adequate than the classical QSAR models, using log P_{app} values as independent variable, for describing pharmacokinetic and pharmacodynamic behavior of antihistamine drugs.

Table 3
Statistical analysis and predictive features of the QRAR models (pharmacodynamic parameter) $= a + b(\log k) + c(\log k)^2$ corresponding to the retention data obtained using Brij-35 mobile phase

[Brij-35] (*M*)	Pharmacodynamic Parameter (*n*)	*a*±*La* (*P* value)	*b*±*Lb* (*P* value)	*c*±*Lc* (*P* value)	R^2 $(R_{adj})^2$	SE	*F* (*P* value)	DW	RMSEC	RMSECV	RMSECVi
0.04	TPL (15)	0.44±0.09	−0.47±0.18	0.14±0.08	0.84	0.07	32	2.3	0.06	0.08	0.09
	(μg/ml)	(0.0000)	(0.0001)	(0.0025)	(0.82)		(0.0000)				
	LD_{50} (15)	17 000±8000	−19 000±9000	5000±2000	0.70	300	14	1.8	260	470	240
	(mg/kg)	(0.0008)	(0.0007)	(0.0005)	(0.65)		(0.0008)				
	K_d (11)	1000±700	−1000±800	300±200	0.67	6	8.1	2.3	5.2	9.3	6.5
	(n*M*)	(0.0134)	(0.0161)	(0.0188)	(0.59)		(0.0120)				

n: number of available activities; *L*: 95% confidence interval for coefficients estimates; $(R_{adj})^2$: R^2 adjusted for degrees of freedom; SE: standard error of the estimate; *F*: *F*-ratio; DW: Durbin–Watson statistic; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation (leave-one-out); RMSECVi: root mean square error of cross-validation (leave-one-out) for interpolate data.

Table 4
Pharmacokinetics and pharmacodynamics predicted (95% confidence interval) by applying the corresponding QRAR models for other antihistamine drugs whose data were not found in literature

Antihistamine	Pharmacokinetics		Pharmacodynamics		
	$T_{1/2}$ (h)	V_d (l/kg)	TPL (μg/ml)	LD_{50} (H_1) (mg/kg)	K_d (H_1) (n*M*)
Alloclamide	9–13	4–9	0–0.11	–	21–58
Antazoline	4–9	0–6	0.03–0.19	1500–4600	71–340
Brompheniramine	–	–	–	0–300	–
Carbinoxamine	–	7–11	–	0–260	–
Chlorcyclizine	16–20	9–15	0.02–0.13	180–630	–
Chlorpyramine	14–16	8–12	0.01–0.11	0–290	0–10
Cinnarizine	–	11–19	0.03–0.18	770–1500	0.9–60
Clemastine	–	–	0.03–0.17	–	0.4–48
Cyclizine	15–18	9–13	–	–	0–8
Cyproheptadine	–	10–16	0.02–0.13	–	–
Dimetindene	15–18	8–13	0.02–0.11	0–370	–
Diphenhydramine	–	–	–	0–270	–
Doxylamine	–	2–8	–	–	34–120
Etintidine	–	–	0.11–0.27	–*	–*
Hydroxyzine	–	–	–	–	17–43
Isothipendyl	16–20	10–16	0.02–0.14	–	0–18
Ketotifen	–	5–9	0–0.11	30–460	18–46
Methapyrilene	–	–	0.003–0.11	–	–
Orphenadrine	–	9–15	–	–	0–13
Oxatomide	–	7–11	0.01–0.10	0–260	0–11
Perphenazine	–	4–9	–	–	19–52
Phenindamine	15–18	8–13	0.02–0.11	–	0–8
Promethazine	–	–	–	0–300	–
Pyrilamine	10–13	5–9	0–0.11	–	18–46
Terfenadine	–	11–21	–	–	2–86
Tripelennamine	–	–	0–0.11	–	–
Triprolidine	–	9–15	0.02–0.13	120–580	–

The gaps correspond to values used to construct the QRAR model. * H_2 antihistamine. Not predicted because the QRAR model is referred to H_1 antihistamines.

Table 5
Statistical analysis of the QSAR models developed: (I) log(activity) = $a + b(\log P_{app}) + c(\log P_{app})^2$ and (II): (activity) = $a + b(\log P_{app}) + c(\log P_{app})^2$

Model	Activity (n)	$a \pm La$ (P value)	$b \pm Lb$ (P value)	$c \pm Lc$ (P value)	R^2 $(R_{adj})^2$	SE	F (P value)	DW
(I)	$T_{1/2}$ (18)	0.6±0.1	0.3±0.1	−0.03±0.02	0.80	0.2	30	1.6
	(h)	(0.0000)	(0.0000)	(0.0114)	(0.77)		(0.0000)	
	V_d (12)	0.5±0.2	0.2±0.2	−0.02±0.05	0.65	0.3	8	2.6
	(l/kg)	(0.0008)	(0.0147)	(0.4682)	(0.57)		(0.0051)	
	TPL (15)	−1.0±0.4	−0.2±0.4	0.05±0.07	0.16	0.5	1	1.6
	(μg/ml)	(0.0003)	(0.1871)	(0.1781)	(0.09)		(0.3802)	
	LD_{50} (15)	2.4±0.8	−0.05±0.65	0.02±0.11	0.04	0.4	0.2	1.7
	(mg/kg)	(0.0000)	(0.8592)	(0.7457)	(0.0)		(0.7985)	
	K_d (11)	0.6±1.3	0.3±1.4	−0.1±0.3	0.04	0.4	0.2	2.2
	(nM)	(0.3190)	(0.6432)	(0.6174)	(0.0)		(0.8614)	
(II)	$T_{1/2}$ (18)	7±3	3±2	−0.2±0.5	0.69	4.3	17	1.5
	(h)	(0.0004)	(0.0045)	(0.3205)	(0.65)		(0.0001)	
	V_d (12)	4±2	2±2	0.01±0.52	0.68	3.2	10	2.1
	(l/kg)	(0.0038)	(0.0294)	(0.9721)	(0.61)		(0.0058)	
	TPL (15)	0.2±0.1	−0.1±0.1	0.02±0.02	0.37	0.1	3	1.2
	(μg/ml)	(0.0011)	(0.0353)	(0.0847)	(0.25)		(0.0803)	
	LD_{50} (15)	200±1000	50±800	8±150	0.08	500	0.5	1.3
	(mg/kg)	(0.6674)	(0.8974)	(0.9075)	(0.0)		(0.5960)	
	K_d (11)	−2±30	15±37	−4±8	0.12	10	0.6	2.7
	(nM)	(0.8748)	(0.3744)	(0.3432)	(0.0)		(0.5939)	

n: number of available activities; L: 95% confidence interval for coefficients estimates; $(R_{adj})^2$: R^2 adjusted for degrees of freedom; SE: standard error of the estimate; F: F-ratio; DW: Durbin–Watson statistic.

4. Conclusions

In this paper it has been shown that the retention of compounds in a BMC system, is an adequate parameter to obtain an estimate or, at least, useful qualitative information about antihistamine activity. In addition, the results obtained were much better than the ones obtained from QSAR studies, which suggests the greater ability of the BMC retention factor, than the classical descriptor, log P, for describing the pharmacokinetics and pharmacodynamic behavior of antihistaminic drugs. This can be due to the fact that log k values depend not only on the hydrophobic characteristics of compounds, but also on the other molecular features, mainly electronic and steric, which condition the drug's biochemical behavior.

The results shown in this paper encourage the idea that the use of models based on structural features of compounds, as the QRAR models developed, could be considered as an alternative for reducing the number of drugs for which animal tests are required.

Acknowledgements

The authors acknowledge the Interministerial Commission of Science and Technology (CICYT) of Spain (Project SAF99-0110), and Generalitat Valenciana (support for research groups, GR-0055) for financial support.

References

[1] N.J. Withers, A.J. Chauhan, S.T. Holgate, Pulmonary and Critical Care Pharmacology and Therapeutics, McGraw-Hill, New York, 1996.
[2] J. Flórez, Farmacología Humana, Masson, Barcelona, 1997.
[3] B.A. Wroblewski, J. Head Trauma Rehabil. 13 (1998) 83.
[4] D.G. Cooper, R.C. Young, G.J. Durant, C.R. Ganellin, in: C. Hansch (Ed.), Comprehensive Medicinal Chemistry, Vol. 3, Pergamon Press, New York, 1990.
[5] M. Cuenca-Benito, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Chromatogr. A 814 (1998) 121.
[6] L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Anal. Chem. 70 (1998) 28.

[7] J.M. Sanchis-Mallols, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Chromatographia 46 (1997) 605.
[8] M. Molero-Monfort, Y. Martín-Biosca, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Chromatogr. A 870 (2000) 1.
[9] Y. Martín-Biosca, M. Molero-Monfort, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Biomed. Chromatogr. 13 (1999) 478.
[10] M. Molero-Monfort, Y. Martín-Biosca, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Biomed. Chromatogr. 13 (1999) 394.
[11] C. Quiñones-Torrelo, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Med. Chem. 42 (1999) 3154.
[12] L. Escuder-Gilabert, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Chromatogr. B 740 (2000) 59.
[13] Y. Martín-Biosca, M. Molero-Monfort, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Quant. Struct.–Act. Relat. 19 (3) (2000) 247.
[14] C. Quiñones-Torrelo, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, Biomed. Chromatogr. 15 (2001) 31.
[15] J. Martinez-Plá, S. Sagrado, R.M. Villanueva-Camañas, M.J. Medina-Hernández, J. Chromatogr. B 757 (2001) 89.
[16] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[17] C. Hansch, Comprehensive Medicinal Chemistry, Vol. 6, Pergamon Press, New York, 1990.
[18] ACD/log *P* Method Software (demo version), Advanced Chemistry Development Inc., Toronto, 1996.
[19] J.E.F. Reynolds (Ed.), Martindale's, The Extra Pharmacopeia, 28th ed., Pharmaceutical Press, London, 1982.
[20] www.compudrug.com (demo version).
[21] M.S. Islam, M.M. Narukar, J. Pharm. Pharmacol. 45 (1993) 682.
[22] D. Dumanovic, I. Juranic, D. Dzeletovic, V.M. Vasic, J. Jovanovic, J. Pharm. Biomed. Anal. 15 (1997) 1667.
[23] M. Schulz, A. Schmoldt, Pharmazie 12 (1997) 895.
[24] D.M. Paton, D.R. Webster, Clin. Pharmacokinet. 10 (1985) 477.
[25] W.G. Clark, D.C. Brater, Goth: Farmacología Médica, Mosby, Madrid, 1992.
[26] J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A. Goodman-Gilman, Goodman & Gilman's: Las Bases Farmacológicas de la Terapéutica, McGraw-Hill, Mexico, 1996.
[27] N.H.G. Holdford, Clinical Pharmacokinetics: Drug Data Handbook, Adis, Auckland, 1998.
[28] G. Castaneda-Hernández, Y. Vargas-Alvarado, F. Aguirre, F.J. Murrieta-Flores, Arzneimittelforschung 43 (1993) 539.
[29] H.F. Schran, L. Petryk, C.T. Chang, R. O'Connor, M.B. Gelbert, J. Clin. Pharmacol. 26 (1996) 911.
[30] A. Grahnen, A. Lonnebo, O. Beck, S.A. Eckernas, B. Dahlstrom, B. Lindstrom, Biopharm. Drug Dispos. 13 (1992) 255.
[31] E.P. Calandre, N. Alferez, K. Hanssanein, D.L. Azarnoff, Clin. Pharmacol. Ther. 29 (1981) 527.
[32] P. Villani, M.B. Regazzi, M. Pecorini, M. Tizzoni, D. Savio, Eur. J. Drug Metab. Pharmacokinet. 3 (1991) 205.
[33] D.C. Garg, F.N. Eshelman, D.J. Weidler, J. Clin. Pharmacol. 25 (1985) 437.
[34] G.R. Aronoff, R.F. Bergstrom, R.J. Bopp, R.S. Sloan, J.T. Callaghan, Clin. Pharmacol. Ther. 43 (1988) 688.
[35] S. Budavari et al., The Merck Index, 12th ed., Merck Research Laboratories, Whitehouse Station, NJ, 1996.
[36] ecdin.etomep.net
[37] www.cmbi.kun.nl/7tm/ligand/Hista.html
[38] N. Inagaki, T. Sakurai, T. Abe, K. Musoh, H. Kawasaki, M. Tsunematsu, H. Nagai, Life Sci. 63 (1998) 145.
[39] L. Escuder-Gilabert, J. M Sanchis-Mallols, S. Sagrado, M.J. Medina-Hernández, R.M. Villanueva-Camañas, J. Chromatogr. A 823 (1998) 549.
[40] L. Escuder-Gilabert, Y. Martín-Biosca, R.M. Villanueva-Camañas, M.J. Medina-Hernández, S. Sagrado, Chromatographia 50 (1999) 325.
[41] J.J. MacKichan, in: W.E. Evans, J.J. Schentag, W.J. Jusko, M.V. Relling (Eds.), Applied Pharmacokinetics. Principles of Therapeutic Drug Monitoring, Applied Therapeutics, Vancouver, 1992.
[42] A. Gringauz, Introduction to Medicinal Chemistry, Wiley–VCH, New York, 1997.
[43] T. Fujita, in: C. Hansch (Ed.), Comprehensive Medicinal Chemistry, Vol. 4, Pergamon Press, New York, 1990.