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Development of predictive retention–activity relationship models of non-steroidal anti-inflammatory drugs by micellar liquid chromatography: comparison with immobilized artificial membrane columns

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Abstract

The predictive and interpretative capability of quantitative chromatographic retention–biological activity models is supported by the fact that under adequate experimental conditions the solute partitioning into chromatographic system can emulate the solute partitioning into lipid bilayers of biological membranes, which is the basis for drug and metabolite uptake, passive transport across membranes and bioaccumulation. The use of micellar solutions of Brij35 as mobile phases in reversed-phase liquid chromatography has proven to be valid to predict some biological activities of different kinds of drugs. In this study, quantitative retention–activity relationship (QRAR) models to describe some of the biological activities and pharmacokinetic properties of non-steroidal anti-inflammatory drugs (NSAIDs) with predictive and interpretative ability are obtained. These models are compared with those obtained using immobilized artificial membrane (IAM) column data taken from the literature. For NSAIDs, the statistical characteristics of the micellar liquid chromatography (MLC) QRAR models were better than or at least comparable to those of the IAM-QRAR models.

Keywords: Quantitative retention–structure relationship; Quantitative retention–activity relationship; Immobilized artificial membrane surfaces; Non-steroidal anti-inflammatory drugs

1. Introduction

The development of predictive methodologies that can decrease the cost and experimental effort of drug development is of great importance in pharmaceutical research and medicinal chemistry. The use of quantitative structure–activity relationships, QSARs, can serve as an alternative to in vivo methodologies. The application of chromatographic parameters in QSARs gives rise to a new field, quantitative retention–activity relationships, QRARs [1,2]. Extensive studies have been performed to develop stationary phases that can emulate the biological barriers [3–7].

Immobilized artificial membranes (IAMs) are chromatographic surfaces prepared by covalently immobilizing cell membrane phospholipid molecules to solid surfaces at monolayer densities. IAMs have

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been proposed to purify membrane proteins [8,9], immobilize enzymes [10,11], determine enzyme–ligand binding constants for drugs [10] and obtain hydrophobic parameters [12]. Several successful studies correlating IAM parameters with biological data of several drugs have also been performed [13–16].

Micellar liquid chromatography (MLC) has been shown to be useful for describing the biological behavior of different kinds of drugs [17–23]. MLC is a chromatographic modality that uses reversed stationary phases and surfactant solution above the critical micellar concentration (CMC) as mobile phases [24–27]. The retention of a drug in MLC depends on its hydrophobic, electronic and steric properties. The success of the MLC in constructing QRAR models could be attributed to the fact that MLC systems present similarities with the biological barriers and extracellular fluids. Firstly, the stationary phase modified by the hydrophobic adsorption of surfactant monomers [26,27] structurally resembles the ordered array of the membranous hydrocarbon chains. In addition, the hydrophilic/hydrophobic character of the adsorbed surfactants resembles the polar membrane regions. On the other hand, 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 [28]. Phospholipids, cholesterol, fatty acids and triglycerides form micellar complexes with proteins (lipoproteins) (CMC$<10^{-6}$ M) [29].

Non-steroidal anti-inflammatory drugs (NSAIDs) are agents that in addition to having anti-inflammatory action also have analgesic, antipyretic and platelet-inhibitory properties. They are used primarily in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation. They act by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides, precursors of prostaglandins. Inhibition of prostaglandin synthesis accounts for their analgesic, antipyretic and platelet-inhibitory actions; while other mechanisms may contribute to their anti-inflammatory effects. Certain NSAIDs may also inhibit lipooxygenase enzymes or phospholipase C or may modulate T-cell function [30].

The capability of a drug to reach the receptor site can strongly depend on its membrane affinity. As penetration of the cell membrane by NSAIDs has been reported to be an important aspect of their activity [31], differences in chromatographic retention could be expected to relate to biological activities. In this paper, the usefulness of MLC to establish QRARs of NSAIDs is studied. The advantages and limitations of using a single parameter as the retention factor in MLC to describe the activity of a small number of NSAIDs are discussed. Finally, the ability of MLC retention data to describe different biological activities of NSAIDs is compared with the results obtained using IAM column retention data.

2. Experimental

2.1. Instrumental and measurement

A Hewlett-Packard 1100 chromatograph with an isocratic pump, a UV–visible detector and an HP Vectra computer was used (Palo Alto, CA, USA). Data acquisition and processing were performed on an 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, USA), with a 20 μl loop. A Kromasil octadecyl-silane C_{18} column (5 μm, 50×4.6 mm I.D.) and a guard column of similar characteristics (35×4.0 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow-rate was 1 ml min^{-1}. Detection was performed in UV at 220 nm. All the assays were carried out at room temperature. The retention data in MLC 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 means of triplicate injections.

2.2. Reagents and standards

Mobile phases were prepared using aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35, Acros, Geel, Belgium). Micellar eluent pH was adjusted to 7.4 with 0.05 M phosphate buffer, which

was prepared with disodium hydrogenphosphate and sodium dihydrogenphosphate (analytical-reagent grade, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, NaCl (9.20 g l^{-1}, purissim, Panreac) was added to the micellar mobile phase.

Some non-steroidal anti-inflammatory agents were kindly donated by several pharmaceutical laboratories: acematazin (A) (Laboratorios Fher, Barcelona, Spain), diclofenac (DI) (Novartis, Barcelona, Spain), ibuproxam (IBX) (Ferrer, Barcelona, Spain), indomethacin (IND) (Laboratorio Llorens, Barcelona, Spain), ketoprofen (KE) (Rhône-Poulenc Rorer, Madrid, Spain), nabumetone (NA) (Smithkline Beechman, UK), naproxen (NX) (Syntex Latino, Madrid, Spain), piketoprofen (PK) (Laboratorios Farmacéuticos Almirall, Barcelona, Spain), and tolmetin (TO) (Laboratorio Estedi, Barcelona, Spain). Other NSAIDs in pharmaceutical preparations were used: fenbufen (FEN) (Cincopal, Cyanamid Ibérica, Madrid, Spain), fentiazac (Donorest 100, Wyeth-Orfi, Barcelona, Spain), flurbiprofen (FLUR) (Froben 50, Laboratorios Knoll, Madrid Spain), ibuprofen (IB) (Nurofen 400, Laboratorios Boots Healthcare, Madrid, Spain) and sulindac (SU) (Sulindal, Merck Sharp&Dohme, Madrid, Spain). Acetylsalicylic acid (ASA) was from Panreac (purissimum).

Stock standard solutions of NSAIDs were prepared by dissolving 10 mg of the compound in 10 ml of mobile phase solution. Working solutions were prepared by dilution of the stock standard solutions using Brij35 solution. The solutions were stored at 4°C.

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

2.3. Software and data processing

The logarithm of octanol–water partition coefficient values, log *P*, for the non-ionic forms of NSAIDs were taken from the literature [32]. Excel 7.0 Microsoft Office software was used to perform the statistical analysis of the multiple linear regression (MLR).

2.4. Predictive ability of the QRAR models

To evaluate the predictive ability of the models [21], the comparison between the fit error (e.g., the root mean squared error of calibration, RMSEC), the prediction error based on cross-validation (e.g., root mean squared error of cross-validation, RMSECV) parameter that includes both interpolation and extrapolation information [33] and the RMSECVi parameter [21] for measuring only the interpolation information, was used.

The lower the differences are between RMSEC, RMSECV and RMSECVi parameters, the greater the robustness of the QRAR model obtained is.

3. Results and discussion

3.1. Retention behavior of non-steroidal anti-inflammatory agents

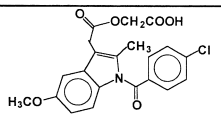
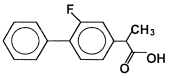
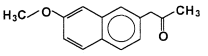
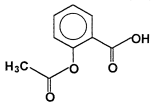
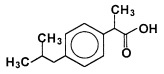
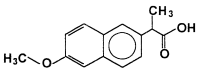
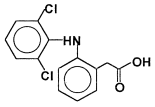
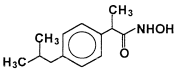
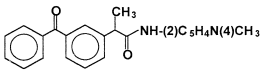
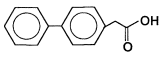
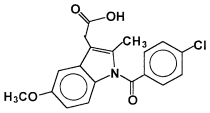
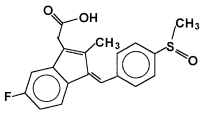
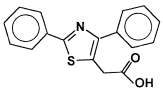
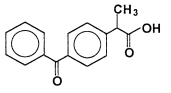
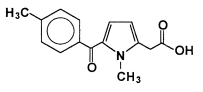
Table 1 shows the structures, the logarithm of the protonation constants (log *K*) and the log *P* values for the non-ionic form of the NSAIDs studied. At physiological pH, 7.4, most of the NSAIDs are negatively charged with an ionization degree of over 99.9%. Ibuproxam, nabumetone and piketoprofen, however are neutral.

The use of anionic surfactant mobile phases does not favor the retention of the NSAIDs because of the electrostatic repulsions with monomers of surfactant adsorbed into the stationary phase. However, the use of cationic surfactant produces an excessive increase in the retention 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 to obtain experimental conditions as close as possible to the physiological pH.

Fig. 1 shows the effect of the Brij35 mobile phase concentration (0.02, 0.04 and 0.06 *M*) on the retention of compounds. As can be observed, for the highly retained compounds studied (IBX, NA and PK), large changes in retention were obtained upon increasing the Brij35 concentration in the mobile phase, while for the less-retained compounds (i.e., ASA and SU) the retention was scarcely modified.

When the log *k* values of the compounds obtained

Table 1
Structures, log *K* and log *P* values for the non-ionic forms of the NSAIDs studied

NSAIDs	logK	logP	NSAIDs	logK	logP	NSAIDs	logK	logP
 Acemetazin (A)	4	4	 Flurbiprofen (FLUR)	4.27	4.16	 Nabumetone (NA)	-	2.77
 Acetylsalicylic acid (ASA)	3.5	1.23	 Ibuprofen (IB)	5.2	3.68	 Naproxen (NX)	4.2	2.82
 Diclofenac (DI)	4.5	4.77	 Ibuproxam (IBX)	-	2.61	 Piketoprofen (PK)	-	4.24
 Fenbufen (FEN)	4.51	3.21	 Indomethacin (IND)	4.5	4.23	 Sulindac (SU)	4.5	2.77
 Fentiazac (FTZ)	3.6	4.63	 Ketoprofen (KE)	4.6	2.79	 Tolmetin (TO)	3.5	2.79

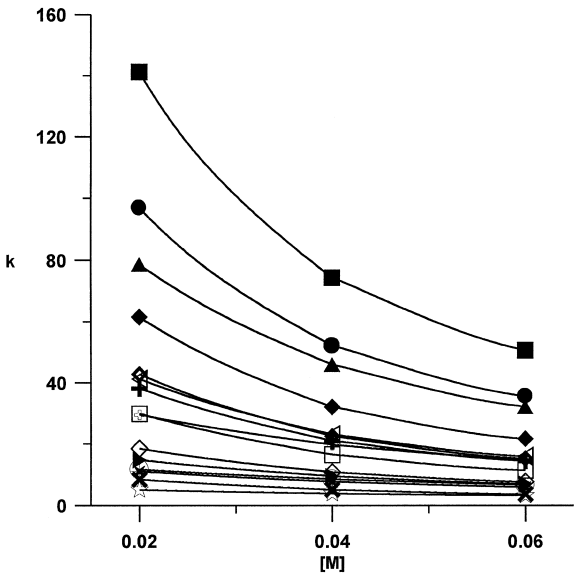


Fig. 1. Effect of Brij35 concentration in the mobile phase on the retention of non-steroidal anti-inflammatory drugs: azemetazin (✚), acetylsalicylic acid (☆), diclofenac (◈), fenbufen (◇), fentiazac (⧫), flurbiprofen (◘), ibuprofen (✙), ibuproxam (▲), indomethacin (◁), ketoprofen (○), nabumetone (●), naproxen (▶), piketoprofen (■), sulindac (✖), tolmetin (✶).

for a certain mobile phase were correlated with the corresponding log *P* values for the non-ionic forms of the compounds, very poor correlation coefficients were obtained; r^2=0.43, 0.38 and 0.35 for 0.02 *M*, 0.04 *M* and 0.06 *M* Brij35 concentrations, respectively. This behavior may suggest that the NSAID retention depends not only on the hydrophobic interactions but also on the compounds ionization degree. In order to overcome these limitations for ionic compounds, we proposed in a previous paper [34] the use of a novel retention model (Eq. (1)) which includes the hydrophobicity and the ionization degree.

$$\log k = a \log P + b\alpha + c \quad (1)$$

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

$$\alpha = \sum_{j=0}^{n} a_j \delta_j \quad (2)$$

where a_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 NSAIDs obtained with 0.02, 0.04 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 {$\alpha=0$ for IBX, NA and PK; and $\alpha=-1/(1+K[H^+])$ for the other NSAIDs} were adjusted to Eq. (1). Table 2 shows the results obtained from regression analysis of the data. As can be observed, the quantitative structure–retention relationship (QSRR) models obtained with the three mobile phases were adequate to describe the retention behavior of NSAIDs ($r^2 \geq 0.92$; $r^2_{adj.} \geq 0.91$).

3.2. Retention–activity relationships for NSAIDs in MLC

The molecular features of drugs (mainly hydrophobicity, ionization and steric properties) determine their membrane affinity and the drug–receptor interaction, and, consequently their biological activity. Since these molecular features also determine the retention of compounds in MLC, retention–activity relationships could be expected.

In order to obtain predictive and interpretative models, the retention data of NSAIDs and the corresponding biological responses were adjusted to a second-order polynomial model. Relationships between the biological activities studied and the log P and ionization degree values were not adequate or were statistically not as good as the relationships obtained for the QRAR models shown below. The results given in this paper were obtained using a 0.02 M Brij35 mobile phase. Similar QRAR models were achieved using the retention data corresponding to 0.04 and 0.06 M Brij35 mobile phases.

NSAIDs mainly exhibit analgesic, anti-inflammatory and antipyretic activity. They are potent inhibitors of the prostaglandin synthesis by inactivating cyclooxygenase. The IC_{50} values (expressed as the concentration of drug required to give 50% inhibition of cyclooxygenase-2, COX-2) determined in free enzymes and intact cells can be considered as the indices of the NSAIDs overall effect including their intrinsic activity, their affinity and access capability to the receptor site. Table 3 shows the retention data (log k) obtained in 0.02 M Brij35 and the IC_{50} values of some of the NSAIDs reported in the literature [16].

Fig. 2A shows the relationships between the IC_{50} values and the retention data in MLC of some NSAIDs together the corresponding residual plots. As can be observed, there is a random distribution of the residuals and practically all were statistically equal to zero. From a qualitative point of view, this suggests the adequacy of the models to the data. Table 4 shows the statistical analysis and the predictive features of the second-order polynomial model. Since the P-value was less than 0.05, there is a statistically significant relationship between the IC_{50} and log k values at the 95% confidence level. The coefficients were also significant ($P<0.05$) at the same confidence level. As can be observed in the statistical analysis, the r^2, $r^2_{adj.}$ (adjusted for degrees of freedom) and the F-ratio values were adequate. The standard error of the estimate (S.E.) for the IC_{50} model can be used to construct prediction limits for new observations.

In general, NSAID absorption occurs mainly by passive diffusion of the unionized molecules across the gastrointestinal tract following oral administration. NSAIDs are rapidly distributed throughout the

Table 2
Statistical analysis of the QSRR model log $k=a$ log $P+b\alpha+c$ for non-steroidal anti-inflammatory drugs

[Brij35]	n	$a\pm ts_a$ (P-value)	$b\pm ts_b$ (P-value)	$c\pm ts_c$ (P-value)	r^2 $r^2_{adj.}$	S.E.	F (P-value)
0.02 M	16	0.29±0.06 (<0.0001)	0.76±0.15 (<0.0001)	1.1±0.2 (<0.0001)	0.94 0.93	0.11	100.7 (<0.0001)
0.04 M	16	0.25±0.06 (<0.0001)	0.71±0.15 (<0.0001)	1.0±0.2 (<0.0001)	0.93 0.92	0.11	80.7 (<0.0001)
0.06 M	16	0.23±0.07 (<0.0001)	0.69±0.15 (<0.0001)	0.9±0.3 (<0.0001)	0.92 0.91	0.11	68.1 (<0.0001)

Table 3
Retention factors in 0.02 *M* Brij35 mobile phase and biological activities values for the QRAR models studied

NSAID	Log *k* (0.02 *M* Brij35)	IC_{50} (mmol l^{-1}) [16]	Cl (ml min^{-1} kg^{-1}) [35]	$t_{1/2}$ (h) [36]	V_d (l kg^{-1}) [35]
ASA	0.70	$278 \cdot 10^{-3}$	9.3	–[a]	0.2
DI	1.63	$1.1 \cdot 10^{-3}$	3.7	1.5	0.12
FEN	1.26	–	–	10	0.1
FLUR	1.47	$0.102 \cdot 10^{-3}$	0.3	4	0.1
IB	1.47	$72.8 \cdot 10^{-3}$	0.75	2.25	0.1
IND	1.61	$1.68 \cdot 10^{-3}$	1.5	6	0.12 [36]
KE	1.07	–	1.15	–	0.11
NX	1.17	$5.65 \cdot 10^{-3}$	0.07	14	0.1 [36]
SU	0.92	$112 \cdot 10^{-3}$	–	67.5	0.15 [36]
TO	1.04	$27.2 \cdot 10^{-3}$	1.8	–	0.097

[a] Dose dependent.

extracellular fluid and into most body tissues and fluids, with high concentrations in the liver and kidneys, and their volume of distribution is generally 0.15–0.2 l kg^{-1} at the usual therapeutic concentrations. NSAIDs are rapidly and almost completely excreted in the urine of patients with normal renal function; 80–100% of a single dose is excreted in the urine within 24–72 h [37].

The possibility of establishing relationships between the retention data of NSAIDs and their

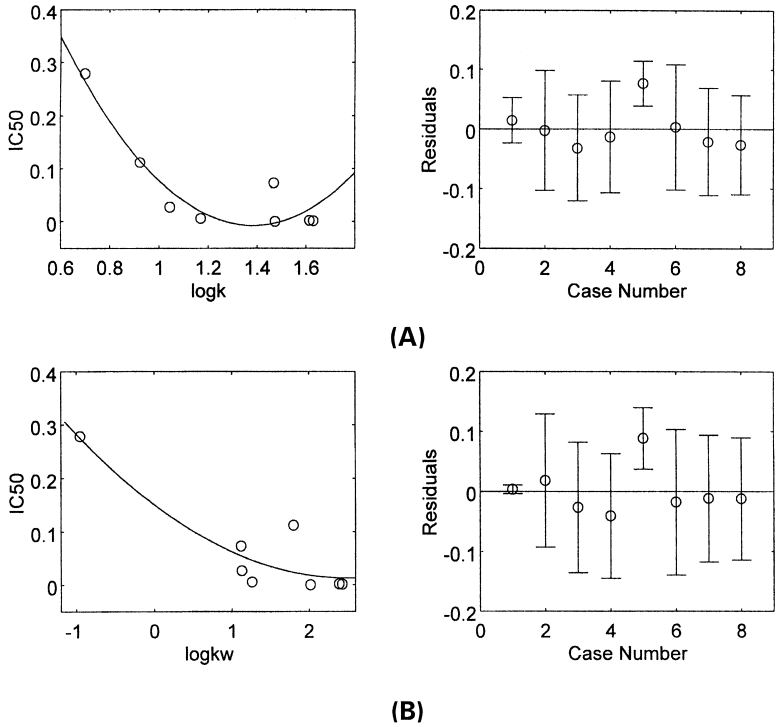


Fig. 2. IC_{50} retention data relationships for different non-steroidal anti-inflammatory drugs (left part) and residuals plots (right part): (A) using a 0.02 *M* Brij35 mobile phase, (B) using an IAM column.

Table 4
Statistical analysis and predictive features of the QRARs models $IC_{50} = a + b(\log k^a) + c(\log k^a)^2$ [a]

Chromatographic technique	n	$a \pm ts_a$ (P-value)	$b \pm ts_b$ (P-value)	$c \pm ts_c$ (P-value)	r^2 $r^2_{adj.}$	S.E.	F (P-value)	RMSEC	RMSECV	RMSECVi
MLC	8	1.1±0.6 (0.0064)	−1.6±1.1 (0.0139)	0.6±0.5 (0.0239)	0.87 0.81	0.04	16.8 (0.0061)	0.032	0.059	0.046
IAM	8	0.15±0.08 (0.0048)	−0.11±0.07 (0.0103)	0.02±0.04 (0.1902)	0.83 0.76	0.05	12.0 (0.0122)	0.037	0.309	0.053

[a] Log k^a for MLC, retention factor obtained using a 0.02 M Brij35+0.05 M phosphate buffer at pH 7.4 mobile phase and, for IAM, retention factors extrapolated to 100% aqueous phase obtained using different concentrations of an acetonitrile+0.1 M phosphate buffer mobile phase. n, Number of available activities. ts, 95% Confidence interval for coefficient estimates. $r^2_{adj.}$, r^2 adjusted for degrees of freedom. S.E., Standard error of the estimate. F, F-ratio. 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 interpolated data.

pharmacokinetic parameter values [clearance (Cl), half-life time ($t_{1/2}$) and volume of distribution (V_d)] (see Table 3) was evaluated.

Fig. 3A–C show the relationships between the pharmacokinetic parameter values and the retention data of some NSAIDs obtained using MLC, together with the corresponding residual plots. As can be observed, the experimental points are well adapted to the model.

Table 5 shows the statistical analysis and the predictive features of the quantitative retention–pharmacokinetic parameters relationship (QRPkRs) models obtained. As can be observed, the P-values obtained for clearance, half-life time and volume of distribution models were less than 0.05; which indicates that the relationship between these parameters and the log k was statistically significant at the 95% confidence level. The coefficients were also significant ($P<0.05$) at the same confidence level. The r^2 and $r^2_{adj.}$ values were higher than 0.95 and 0.93, respectively, and the S.E. values were also low.

3.3. MLC-QRAR models vs. IAM-QRAR models

Barbato et al. [16] reported a study on the influence that different experimental conditions have on the IAM chromatographic behavior of NSAIDs. The retention factors extrapolated to a 100% aqueous phase ($\log k_w^{IAM}$) of NSAIDs, obtained using different concentrations of an acetonitrile and 0.1 M phosphate buffer at pH 7.0 and an IAM PC MG (15×4.6 mm) column, were taken from these authors and were used to compare the MLC- and IAM-QRAR models. The same NSAIDs were included in the study.

In Fig. 2B and Fig. 3D–F the relationships between the IC_{50} values, clearance, half-life time and volume of distribution and the retention data in IAM of some NSAIDs, respectively are shown.

Tables 4 and 5 show the statistical analysis for the IAM-QRAR models. As can be deduced by comparing the r^2, $r^2_{adj.}$, S.E. and F values, in all cases the MLC-QRAR models provide either better or at least comparable statistical results than the IAM-QRAR models. For the IC_{50} IAM-QRAR model, the fitting parameter c was non-significant ($P=0.1902$). This suggests that a linear IC_{50}–$\log k_w^{IAM}$ relationship may exist. However, when the linear model was built a very poor correlation coefficient was obtained ($r^2=0.75$). On the other hand, the IAM-QRAR model obtained for half-life time was non-significant ($P=0.46$, $r^2=0.32$ and $r^2_{adj.}=0.00$, $F=1$) whereas when the retention data obtained using Brij35 as mobile phase were used to construct the MLC-QRAR model, a significant pharmacokinetic model was obtained ($P=0.0006$, $r^2=0.98$ and $r^2_{adj.}=0.96$, $F=82$).

3.4. Predictive ability of QRAR models

Tables 4 and 5 show the RMSEC, RMSECV and RMSECVi values for the QRAR models obtained. As can be observed, the MLC-QRAR models for IC_{50}, Cl and V_d showed comparable RMSEC, RMSECV and RMSECVi values. This indicates the

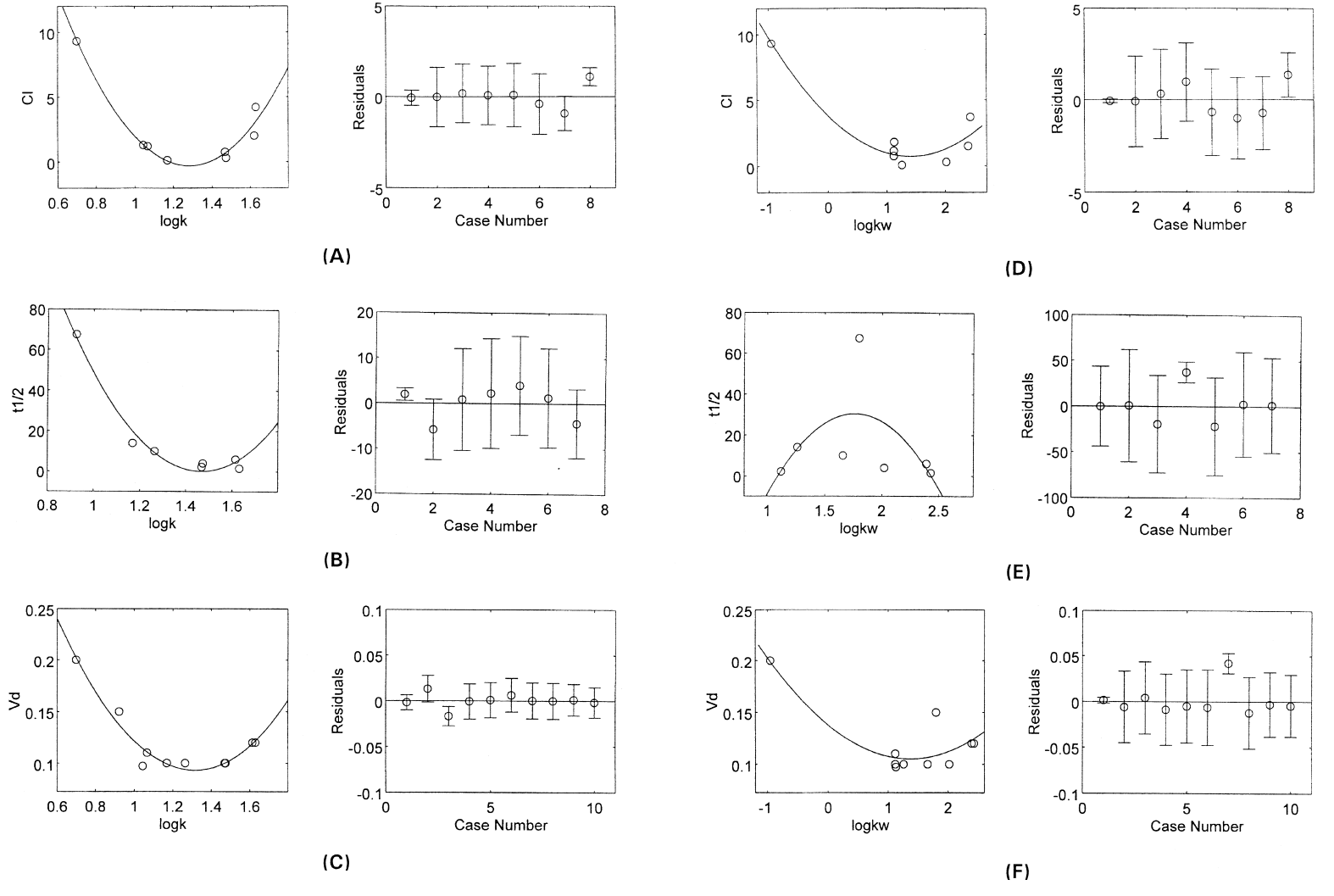


Fig. 3. Pharmacokinetic parameter–retention data relationships for different non-steroidal anti-inflammatory drugs and residuals plots obtained using MLC (0.02 *M* Brij35+0.05 *M* phosphate buffer, pH 7.4 mobile phase) (left part) and an IAM column (acetonitrile+0.1 *M* phosphate buffer, pH 7.0) (right part): (A) clearance, (B) half-life time and (C) volume of distribution MLC-QRAR models; (D) clearance, (E) half-life time and, (F) volume of distribution IAM-QRAR models.

Table 5
Statistical analysis and predictive features of the QRPkR models (pharmacokinetic property) $= a + b(\log k^a) + c(\log k^a)^{2a}$

Pharmacokinetic property (n)		$a \pm ts_a$ (P-value)	$b \pm ts_b$ (P-value)	$c \pm ts_c$ (P-value)	r^2 $r^2_{adj.}$	S.E.	F (P-value)	RMSEC	RMSECV	RMSECVi
Clearance (Cl), ml min^{-1} kg^{-1}	MLC	46±10	−73±17	28±7	0.97	0.68	69.7	0.534	0.989	0.657
		(0.0001)	(0.0001)	(0.0002)	0.95		(0.0002)			
(8)	IAM	3.8±1.6	−4.4±1.5	1.6±0.7	0.92	0.99	30.7	0.779	11.539	0.966
		(0.0015)	(0.0006)	(0.0028)	0.89		(0.0016)			
Half-life time ($t_{1/2}$), h	MLC	480±150	−650±240	220±90	0.98	4.46	81.7	3.370	13.889	4.944
(7)		(0.0010)	(0.0017)	(0.0027)	0.96		(0.0006)			
	IAM	−180±420	238±500	−70±140	0.32	23.76	1.0	17.962	27.538	32.556
		(0.3047)	(0.2542)	(0.2453)	0.00		(0.4602)			
Volume of distribution (V_d), l kg^{-1}	MLC	0.59±0.11	−0.76±0.19	0.29±0.08	0.95	0.008	62.5	0.0071	0.0097	0.0099
		(<0.0001)	(<0.0001)	(0.0001)	0.93		(<0.0001)			
(10)	IAM	0.14±0.03	−0.05±0.02	0.017±0.012	0.78	0.017	12.1	0.015	0.078	0.019
		(<0.0001)	(0.0018)	(0.0101)	0.71		(0.0053)			

[a] Log k^a for MLC, retention factor obtained using a 0.02 M Brij35 + 0.05 M phosphate buffer at pH 7.4 mobile phase and, for IAM, retention factors extrapolated to 100% aqueous phase obtained using different concentrations of an acetonitrile + 0.1 M phosphate buffer mobile phase. n, Number of available activities. ts, 95% Confidence interval for coefficient estimates. $r^2_{adj.}$, r^2 adjusted for degrees of freedom. S.E., Standard error of the estimate. F, F-ratio. 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 interpolated data.

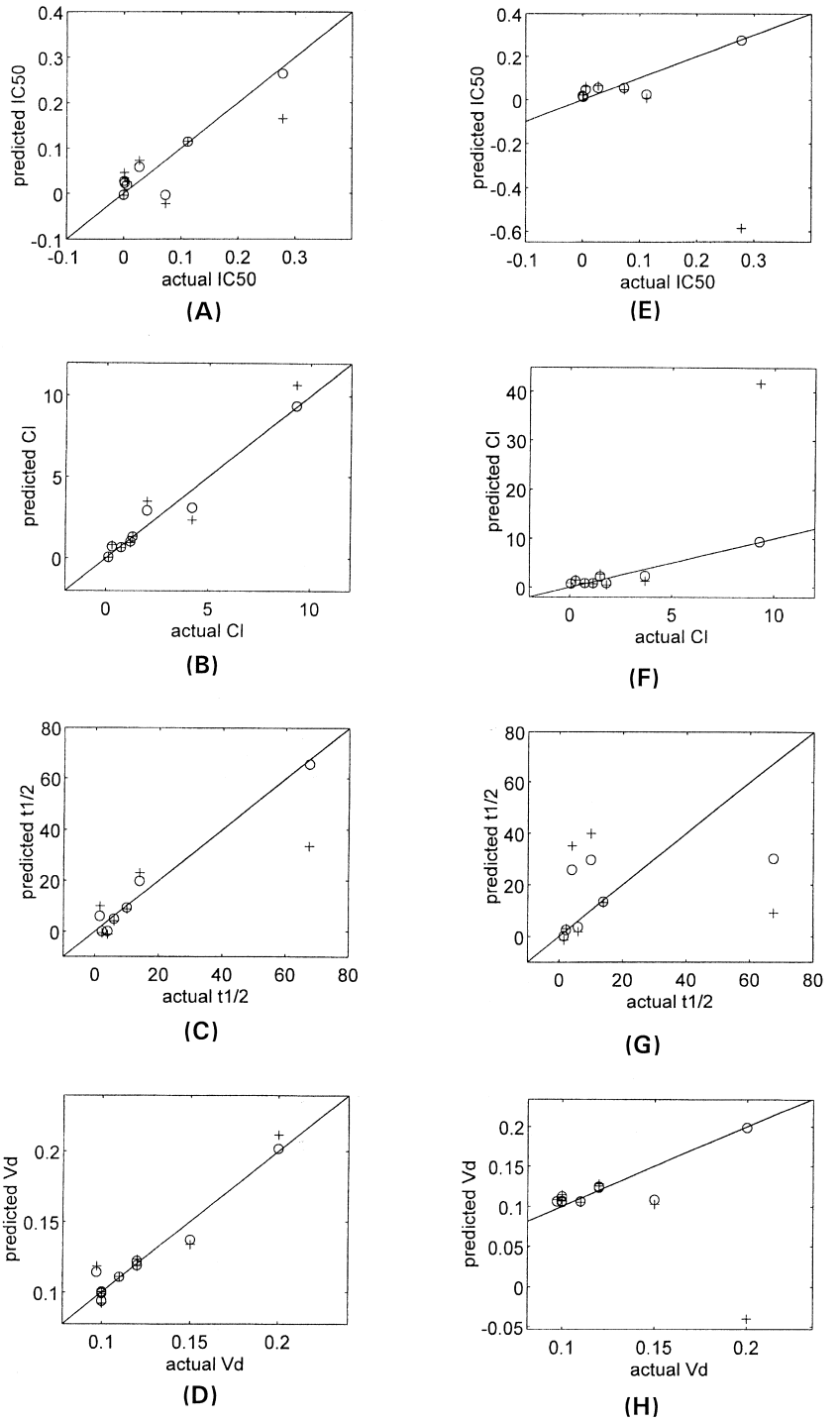


Fig. 4. Validation plots for QRAR models: predicted parameters versus actual values. (A) IC_{50}, (B) clearance, (C) half-life time and (D) volume of distribution MLC-QRAR models; (E) IC_{50}, (F) clearance, (G) half-life time and (H) volume of distribution in IAM-QRAR models. Fitted (○) and cross-validated (+) results are shown.

stability of these models and suggests that both interpolations and extrapolations of parameters based on the current QRAR models should be reasonably adequate. In contrast, for the $t_{1/2}$ model, the RMSECV value was much larger than the RMSECVi value, which indicates that some caution must be exercised with extrapolated parameter data. Nevertheless, the qualitative information obtained can be useful from a practical point of view.

For the IAM-QRAR models, the RMSECV values were larger than RMSECVi values, and both were larger than those corresponding to the MLC-QRAR models. This indicates that the results obtained by interpolation and extrapolation in the model could not be certain.

Fig. 4 shows the predicted (fitted and cross-validated) vs. actual activities for the MLC-QRAR models (Fig. 4A–D) and for IAM-QRAR models (Fig. 4E–H). As can be observed, for the available data, the ability of MLC log k values to describe and predict the biological responses and pharmacokinetic parameters of NSAIDs in terms of cross-validated data was adequate and better than the log k_w^{IAM} values.

4. Conclusions

To satisfy the need for a tool that would make it possible to estimate the pharmacokinetic and biological parameters of new compounds for clinical application, new predictive models must be developed to complement conventional classical assays, and permit a reduction in experimentation with animals. The approach proposed in this paper, involving QRARs, is less expensive than in vivo models, minimum experimental effort is required and, it facilitates the screening of drugs for their potential biological activities. The retention of compounds in MLC using Brij35 as surfactant is able to describe and predict in vitro the pharmacokinetic parameters and biological responses of NSAIDs. This approach can be very useful in medicinal chemistry and pharmaceutical research for the development of new NSAIDs.

On the other hand, MLC provides better, or at least comparable, results than IAM in the QRAR models involving the NSAIDs reported in this study.

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