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Opioid analgetics retention–pharmacologic activity models using biopartitioning micellar chromatography ☆

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Abstract

Opioids are drugs used in medicine for pain control. In this paper, retention–pharmacokinetics and retention–pharmacodynamics relationships of opioids are proposed and statistically validated. These models are based on the compound retention in the biopartitioning micellar chromatography system (BMC), a new methodology which has successfully been used to develop QRAR models for many other families of compounds. The obtained results are compared to the traditional QSAR models using lipophilicity data. The adequacy of QRAR models is due to the fact that the characteristics of the compounds such as the hydrophobicity, electronic charge and steric effects determine both their retention in BMC and their pharmacokinetic and pharmacodynamic behavior.

Keywords: Biopartitioning micellar chromatography; Retention–pharmacologic activity models; Opioids

1. Introduction

Much of medicine aims at the treatment of the symptoms of disease. One of the most important of these symptoms is pain, a syndrome of unpleasant sensations experienced inevitably by all of us. The pharmaceutical agents that produce insensibility to, or decreased awareness of pain are called analgetics or analgesics.

Until recently, analgetics were classified as narcotic (strong) or nonnarcotic (weak) being based on the differences in the development of tolerance and dependence and in analgetic potency. However, different facts like the demonstration that some separation of strong analgetic effect from dependence liability can be achieved, or the emergence of the narcotic antagonists as strong analgetics in man, have made it clear that analgetics be grouped into (a) peripherally acting, nonnarcotic, (b) centrally acting, nonnarcotic and (c) centrally acting, narcotic. Members of the second and third groups block synaptic transmission in the CNS [1]. The opioid receptor antagonists such as naloxone and naltrexone belong to the second group. The third group includes other analgetics considered in this work such as morphine, butorphanol, codeine, fentanyl, heroin, hydrocodone,

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hydromorphone, levorphanol, meperidine, methadone, nalbuphine, nalorphine, oxycodone and pentazocine.

Natural opioids (such as morphine or codeine) are derived from the poppy plant, which contains as many as 20 pharmacologically active alkaloids. In addition, semisynthetic (such as heroin or nalorphine) and totally synthetic (such as meperidine or fentanyl) analgetics have been developed [2].

Opioids are drugs which present selective affinity for opioids receptors (μ, κ, δ and σ) located throughout the brain and spinal cord. The most important receptors in pharmacology are μ (morphine-like) and κ (ketazocine-like), which provide analgesia. Other features of opioids include: mood elevation, respiratory depression, nausea and vomiting, alteration in hormonal levels etc, and also direct drug toxicity. For all these reasons it is essential to identify the potential risks of the treatment of acute or chronic pain and psychiatric disorders with opioids. Another area of potential risk is the predictable development of tolerance to physical dependence on the drug [3]. The drug used must be characterized by its lack of known active metabolites, high lipid solubility, good absorption and low cost.

In most patients, who are treated with opioid analgetics for pain control, the type of opioid needs to be changed at least once because of the presence of side effects or in order to avoid doses to toxic levels. Exhaustive pharmacokinetic and clinical studies are required to establish the adequacy of a compound [4].

It is difficult to relate the measured plasma concentrations to a therapeutic response for this kind of drugs. One major problem lies in the inability to establish a clearly defined therapeutic end-point. Also, the existence of active metabolites and altered plasma protein-binding. Another problem is that most of them are given to patients with diseases, which makes difficult, the differentiation of drug-related effects. In addition, for opioid analgetics it is necessary to consider their possible narcotic effect at the time of performing pharmacokinetics and pharmacodynamics studies.

As an alternative to ''in vivo'' measurements, structure–activity relationships have been proposed by modern medicinal chemistry. If the pharmacodynamic effect induced by a drug is the result of the interaction between the drug and part of the molecules composing the biological object, then there must be a relationship between the physicochemical properties of the drug and its biological action [5]. Different structure–activity relationships, QSAR, have been reported in order to describe the analgetic potency [6–9] or toxicity [10] of opioids.

Following QSAR studies, investigations have been made to obtain single parameters that provide adequate predictive and interpretative models to describe the biological behavior of drugs. The same molecular features — hydrophobicity, electronic charge and steric effects — affect not only transport processes and drug–biological target interaction, but also the compound retention in a chromatographic system under specific experimental conditions. This fact gave rise to the quantitative retention–activity relationship studies, QRAR. In this setting, our research group has demonstrated that the chromatographic system comprising a reversed stationary phase and saline solutions of Brij-35 micelles as mobile phase can be used as a drug biopartitioning system [11–13]. We have named this methodology biopartitioning micellar chromatography, BMC [14,15].

The success of QRAR models based on BMC could be attributed to the similarities between BMC systems and biological barriers and extracellular fluids [16,17]. Thus, the stationary phase modified by the hydrophobic adsorption of surfactant monomers (polyoxyethylene-23 lauryl ether monomers) resembles structurally the ordered array of the membrane hydrocarbon chains, the dual hydrophilic–hydrophobic character and the H-bounding groups of the adsorbed surfactant can provide different interaction types similar to the ones between the membrane components (phospholipids and proteins) and the compounds transported by the biological fluids. On the other hand, the saline micellar mobile phases present characteristics similar to the extracellular fluids. Extracellular fluids are basically composed by water, salts, glucose, proteins and lipids. The latter are amphiphilic molecules with aliphatic chains and polar heads that form micellar aggregates in aqueous solution if their concentration is over the critical micellar concentration ($cmc < 10^{-6}$ M) [18]. This methodology has been applied for describing and predicting the biological activity of different pharmacological kinds of drugs [19–25], permeability ac-

ross intestinal barriers [15], blood–brain barrier [26] and cornea [27].

In this paper, retention–pharmacokinetics and retention–pharmacodynamics models for opioids are proposed. These results are compared to analogous QSAR models obtained using $\log P_{app}$ as independent variable.

2. Experimental section

2.1. Instruments and measurements

The retention of opioids was measured using a Hewlett-Packard 1100 chromatograph comprised of an isocratic pump, a manual sample injector (Rhoedyne valve with a 20-μl loop; Cotati, CA), a thermostat, a variable wavelength UV absorbance detector operated at 250 nm, and a reversed-phase column packed with 5 μm kromasil octadecyl silane, C_{18} (50×4.6 mm I.D.). The mobile phase was pumped at a flow-rate of 1.5 ml/min. Data acquisition and processing were performed on an HP-Chemstation software (A0402, 1996).

All the assays were carried out at 36.5°C. The retention factor values were averages of at least triplicate determinations. The relative standard deviations of $\log k$ values ranged between 0.1 and 0.9%.

2.2. Chemicals

The mobile phases were 0.02, 0.04 and 0.06 *M* solutions of polyoxyethylene-23 lauryl ether (Brij-35, Acros Chimica, Geel, Belgium) at pH 7.4 adjusted with 0.05 *M* phosphate buffer (analytical reagent, Panreac, Barcelona, Spain). In order to reproduce the osmotic pressure of biological fluids, 9.2 g/l NaCl (purissim, Panreac) was added to the mobile phases. All of them were filtered through a membrane filter (0.45 μm; Micron Separations, Westboro, MA) before use.

Drug solutes were purchased from Sigma–Aldrich S.A. (Madrid, Spain) except of pentazocine, which was obtained from the pharmaceutical preparation ''Sosegon'' (Sanofi, Barcelona, Spain). Stock standard solutions of opioids were prepared by dissolving 1 mg amount of drug solute in 2 ml of methanol. The solutions were injected onto the column after filtering through a 0.22-μm nylon membranes (Micron Separations, Westboro, MA). Barnstead E-pure deionized water (Sybron, Boston, MA) was used throughout.

2.3. Software and data processing

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

2.4. Evaluation of the QRAR models predictive ability

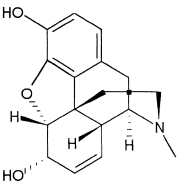
To evaluate the adequacy of the models, the fit error (i.e. root-mean-square error of calibration, RMSEC), the prediction error based on cross-validation (i.e. root-mean-square error of cross-validation, RMSECV), parameter which includes both interpolation and extrapolation information [28] and the RMSECVi [19,20] for measuring only interpolation information, were compared.

3. Results and discussion

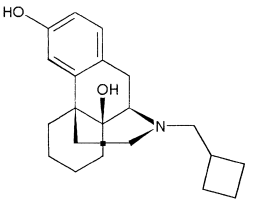
3.1. Retention behavior of opioid analgetics

The retention of the studied compounds (see Fig. 1) was measured using 0.02, 0.04 and 0.06 *M* Brij-35 pH 7.4 mobile phases. All the opioids studied are tertiary amines with polycyclic structure. The presence in the molecule of other functional groups such as hydroxyl or epoxy groups (in morphine-like compounds) provides a hydrophilic character to these compounds. They have $\log P$ values ranged between −0.15 (naloxone) and 4.45 (pentazocine) (see Table 1). At physiological pH they are positively charged. Fig. 2 contains the chromatograms (in 0.02 *M* Brij-35 mobile phase) of some of the compounds studied. As can be observed, the baseline drift, width and shape of analyte peak and signal-to-noise ratio are adequate under the experimental conditions.

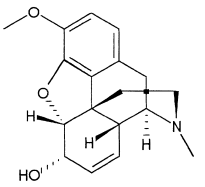
Fig. 3 shows the effect of the Brij-35 concentration in the mobile phase on the opioid analgetics retention. As could be expected, for the more hydrophobic compounds studied large changes in the retention were obtained upon increasing the surfac-



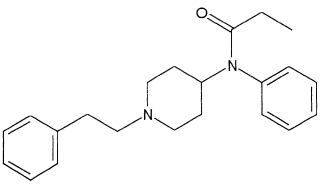
Morphine



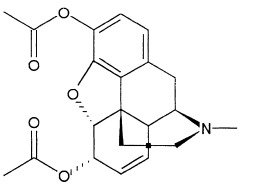
Butorphanol



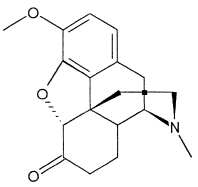
Codeine



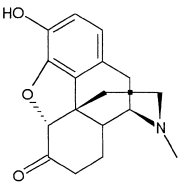
Fentanyl



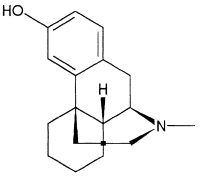
Heorin



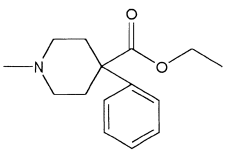
Hydrocodone



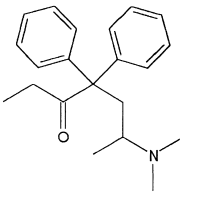
Hydromorphone



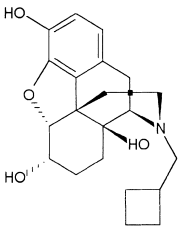
Levorphanol



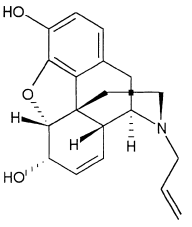
Meperidine



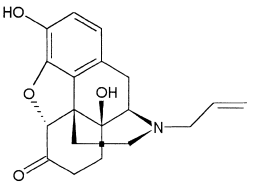
Methadone



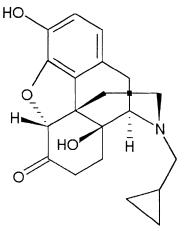
Nalbuphine



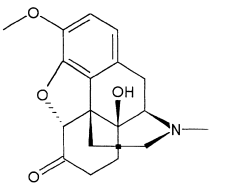
Nalorphine



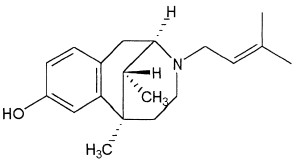
Naloxone



Naltrexone



Oxycodone



Pentazocine

Fig. 1. Chemical structure of the opioid analgetics studied.

Table 1
Log *P*, pK_a, pharmacokinetics and pharmacodynamics values of the opioids analgetics

Opioid analgetic	Log *P* [31]	pK_a [31,32]	Pharmacokinetics			Pharmacodynamics			
			$T_{1/2}$ (h) [33–39]	CL_M (ml/min/kg) [31,34,35,37,40–44]	V_d (l/kg) [34,35,37,45–48]	T.C. (ng/ml) [36]	LD_{50} (mice, s.c.) (mg/kg) [1,49]	IC_{50} (μ-receptor) (n*M*) [50]	IC_{50} (*Tetrahymena pyriformis* (m*M*) [51]
Butorphanol	3.72	9.2[a]	–	–	–	–	–	–	–
Codeine	1.14	8.2	3.5 (2.8–4)	15 (11–23.3)	3.5 (2.6–4)	140 (30–280)	241	20 000	59.8 (44.3–75.3)
Fentanyl	3.66	7.8, 8.4	16.5 (16–17)	11 (10.8–13)	3.81 (3–4)	300 (300)	62	–	–
Heroin	1.14	7.8	0.275 (0.05–0.5)	–	–	–	262	–	7.01 (5.75–8.27)
Hydrocodone	1.13	8.3	4.0 (4)	–	–	20 (10–30)	–	–	–
Hydromorphone	0.55	8.2	2.5 (2.4–3.1)	–	2.06 (1.22–2.9)	–	84	–	–
Levorphanol	3.40	9.2	–	–	5.2 (5.2)	–	187	2	–
Meperidine	2.42	8.7	6.9 (3.5–7)	14.53 (9.75–17)	4.4 (4–4.7)	–	185	1000	3.72 (3.35–4.09)
Methadone	2.97	8.3	24.5 (23–29)	1.72 (1.64–1.8)	3.7 (3.6–4)	400 (50–750)	35	–	0.27 (0.21–0.33)
Morphine	0.18	8.0, 9.9	2.5 (1.9–3.1)	20.75 (17.1–33.5)	3.4 (3–5.16)	55 (10–100)	360	7	23.0 (17.89–28.11)
Nalbuphine	1.05	7.8[b]	3.25 (3–4.5)	22 (22)	5.65 (5.65)	110 (20–220)	–	–	6.32 (5.54–7.10)
Nalorphine	0.69	7.8	1.24 (1.06–1.41)	–	–	–	500	3	–
Naloxone	−0.15	7.9	1.5 (1.1–1.5)	23.4 (22–24.8)	5 (5)	20 (10–30)	368	10	–
Naltrexone	0.31	7.9[c]	2.7 (2.7)	21.2 (20–22.4)	–	5 (5)	–	–	–
Oxycodone	−0.08	8.5, 10.0	4.25 (3.5–5)	11.1 (11.1)	2.6 (2.6)	35 (20–50)	–	30 000	–
Pentazocine	4.45	8.9	4.3 (3–5)	18.5 (17–18.8)	6 (5.6–7.1)	105 (10–200)	175	50	0.93 (0.74–1.12)

Data taken from reference [31], [31,32], [33–39], [31,34,35,37,40–44], [34,35,37,45–48], [36], [1,49], [50], [51] as indicated above. Assigned values for structural similarities with: [a] levorphanol, [b] nalorphine and [c] naloxone.

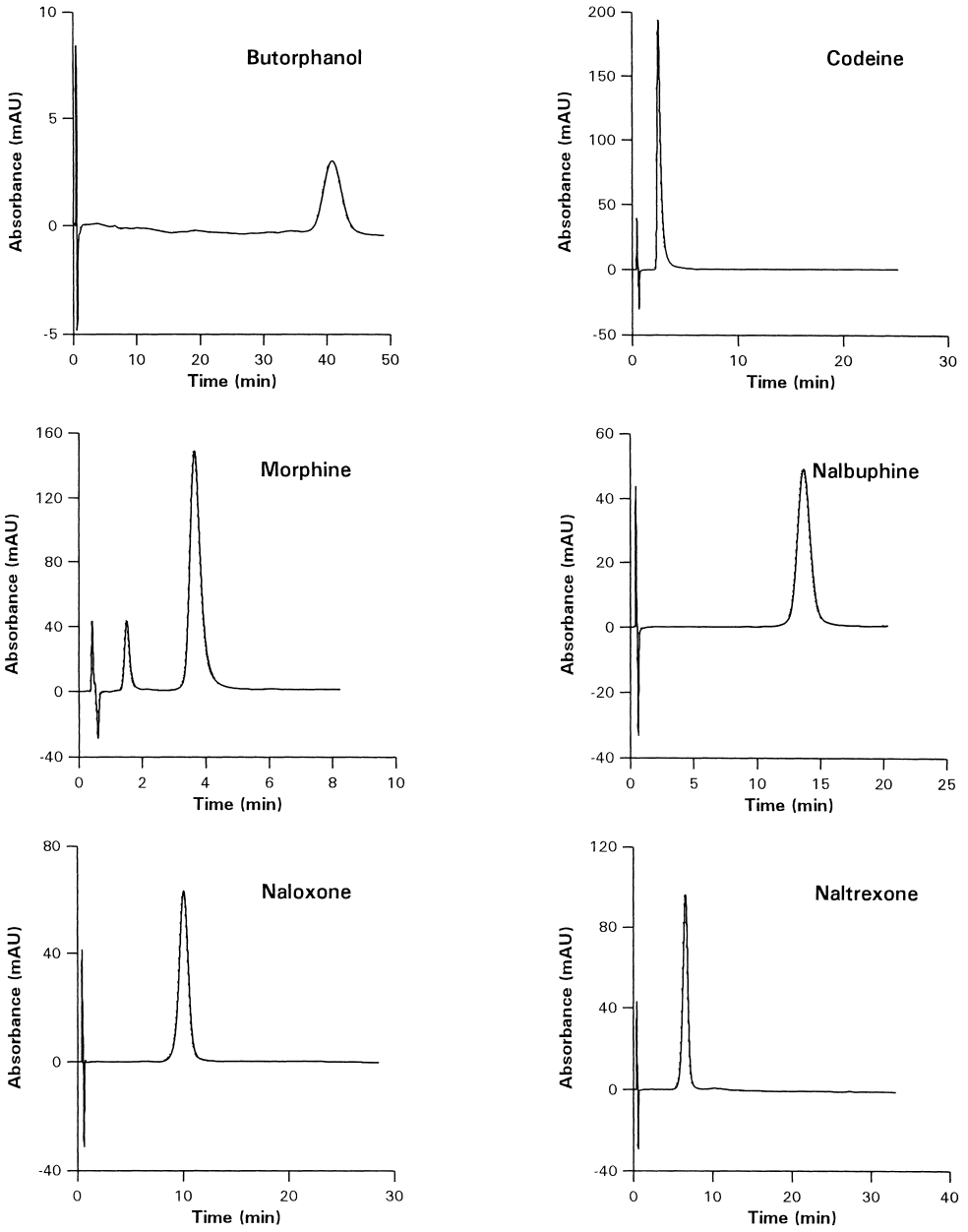


Fig. 2. Representative chromatograms (obtained in 0.02 *M* Brij-35 mobile phase) of some of the opioid analgetics studied.

tant concentration in the mobile phase, while for the hydrophilic compounds the retention is scarcely modified.

The retention in MLC is not linearly related to log *P* [29]. In this work, the nonlinear dependence formulated by a second-order expression was checked using the opioids retention factors obtained with 0.02, 0.04 and 0.06 *M* Brij-35 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 parti-

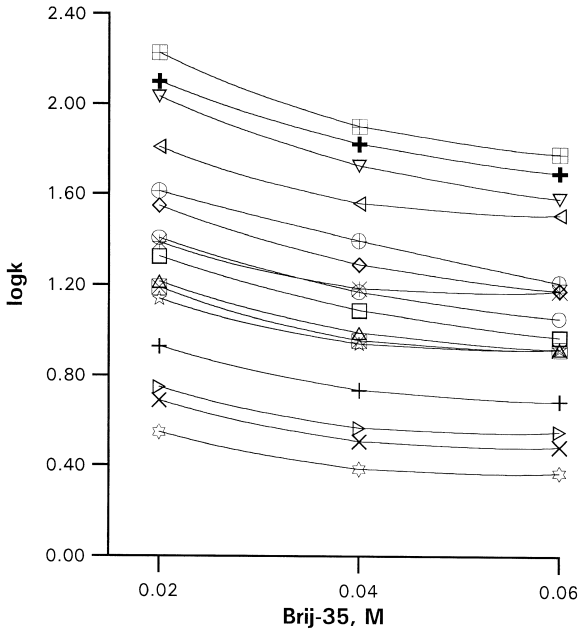


Fig. 3. Effect of Brij35 concentration in the mobile phase on the opioid analgetics retention: (▽) butorphanol, (▷) codeine, (+) fentanyl, (*) heroin, (☆) hydrocodone, (✡) hydromorphone, (⊠) levorphanol, (◁) meperidine, (⊞) methadone, (+) morphine, (◇) nalbuphine, (□) nalorphine, (○) naloxone, (△) naltrexone, (×) oxycodone, and (⊕) pentazocine.

tion coefficient calculated according to Ref. [30] at pH 7.4. Low correlation coefficients were obtained $R^2 = 0.54$, 0.53 and 0.50 for the three mobile phases mentioned above, respectively. These results corroborate the fact that, in BMC, the compound hydrophobicity at the pH considered is not the only determinant factor over its retention: other electronic interactions and steric factors are also important.

3.2. Retention–activity relationships

The molecular features of drugs, mainly hydrophobicity, molar total charge and steric properties, condition the pharmacokinetics and pharmacodynamics of drugs and consequently their biological activity [5]. These same features determine the BMC drug retention, therefore it could be expected that retention–activity relationships exist. The relationships between opioid analgetics retention data, log *k*, and their corresponding pharmacokinetic and pharmacodynamic parameters have been obtained. Table 1 contains the available data used in the QRAR models development. In the case of the pharmacokinetics, due to the high number of data sources found and their variability, the value chosen to construct the corresponding QRAR models was the median value (in Table 1 the minimum and maximum values are shown in brackets). In the case of the therapeutic blood-plasma/serum concentration in man, the value used was the mean value from the reported range in the only data source available.

3.2.1. Retention–pharmacokinetics relationships

Relationships between opioids log *k* values and their half-life ($T_{1/2}$), clearance (*CL*) and volume of distribution (V_d) have been established. In all cases, data could be fitted to a second order polynomial model, which agrees with the type of the dependence that have been proved to be usual in previous QSAR models [52]. It has been also demonstrated in previous QRAR studies that this is the usual retention–activity relationship for pharmacokinetics and biological responses of other families of drugs [19–25].

Nevertheless, we have found a remarkable difference in opioids *CL* and V_d models with respect to our previous studies: convex instead of concave parabolas were obtained. This difference could be attributed to characteristics of analgetic opioids such as: the hydrophilic character, low plasma protein binding [33], multi-compartmental distribution behavior, and extensive metabolism that produces active/toxic metabolites [53].

Fig. 4 shows the relationship between the pharmacokinetics of different opioids and their retention data in 0.02 *M* Brij35 mobile phase. Similar graphical models were obtained from retention data in 0.04 and 0.06 *M* Brij35 mobile phase, but obviously, as a consequence of the diminution of the compound retention when the Brij35 concentration in the mobile phase was increased, different coefficients in the QRAR models were obtained. A random distribution of the residuals was found in all cases. The residuals were statistically equal to zero, which suggested that, from a qualitative point of view, there is an adequacy of the polynomial model selected to data. Table 2 contains the statistical analysis and the predictive features of the QRAR models obtained when 0.02, 0.04 and 0.06 *M* Brij-35 mobile phases were used.

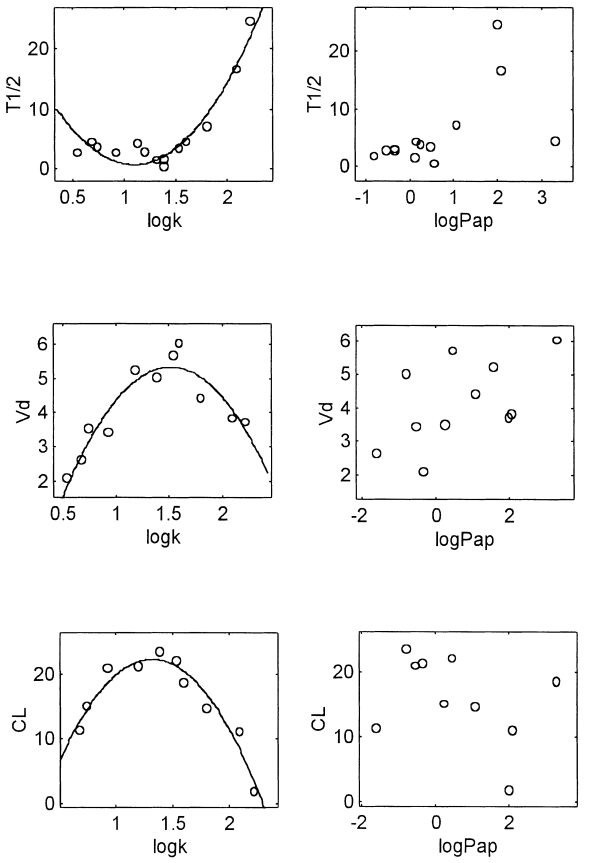


Fig. 4. Pharmacokinetics vs. log *k* (obtained using 0.02 *M* Brij35 mobile phase) (left) and log P_{app} (right) relationships.

Since for all models the *P* value is less than 0.01, there is a statistically significant relationship between $T_{1/2}$, *CL* or V_d and log *k* values at the 99% confidence level. The *R*-squared statistic values indicate that the models as fitted explain between 89 and 93, 92–93 and 88–91% of the variability in $T_{1/2}$, *CL* and V_d data, respectively. The *P* value on the highest order term is less than 0.01, which means that this term is statistically significant at the 99% confidence level. The Durbin–Watson statistic is greater than 1.4 indicating that there is probably not any serious autocorrelation in the residuals.

3.2.2. Retention–pharmacodynamics relationships

Pharmacodynamics studies the molecular interaction between the drug and the site of action, which characterizes the drug pharmacological response. Most of the pharmacodynamic parameters considered in this work are related to the opioid receptors. Nevertheless, the anesthetic effect is due to a hydrophobic interaction of chemicals with lipid and proteins components of the cell membrane, causing a depressant effect. This membrane-mediated toxicity may contribute to the fatal outcome in overdose of drugs.

Table 1 shows the pharmacodynamic parameters studied: the therapeutic blood-plasma/serum concentration in man (T.C.), the acute toxicity (expressed as the LD_{50}, subcutaneous in mice), the IC_{50} values for the μ-opiate receptor in rat brain and the IC_{50} values for the *Tetrahymena pyriformis* motility to evaluate the anesthetic toxicity of opioids agents

Fig. 5 shows the relationships between the pharmacodynamics of different opioids and their retention data in a 0.02 *M* Brij-35 mobile phase. As in the pharmacokinetic–retention relationships, similar graphical models were obtained using 0.04 and 0.06 *M* Brij35 retention data. Data were fitted to a second order polynomial model, but for the LD_{50} in mice and the IC_{50} values for the μ-opiate receptor better correlation were obtained when the logarithm of these values were considered. A random distribution of the residuals was found in all cases.

Table 3 contains the statistical analysis and the predictive features of the QRAR models obtained when 0.02, 0.04 and 0.06 *M* Brij-35 mobile phases were used. Since for all models the *P* value is less than 0.05, there is a statistically significant relationship between the pharmacodynamic parameters studied and log *k* values at the 95% confidence level. The *R*-squared statistic values indicate that the models as fitted explain between 91 and 93, 84–87, 83–91 and 93–94% of the variability in the therapeutic blood-plasma/serum concentration in man, the acute toxicity in mice, the inhibitory concentration on the μ-opiate receptor in rat brain, and the anesthetic effect, respectively. In all cases, the coefficients are also statistically significant at the 95% confidence level (*P* value less than 0.05). The Durbin–Watson statistic values, greater than 1.4, indicate that there is probably not any serious autocorrelation in the residuals.

3.3. Predictive ability of QRAR models

To evaluate the predictive ability of the models in term of cross-validated data, the RMSEC, RMSECV

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	RMSECV1	RMSECV1i
0.02	$T_{1/2}$ (14)	21±8	−36±13	17±5	0.92	2.0	67.1	2.6	1.78	2.77	1.79
	(h)	(0.0002)	(0.0001)	(0.0000)	(0.91)		(0.0000)				
	V_d (11)	−3±2	11±4	−3.6±1.3	0.88	1.0	29.3	2.3	0.41	0.54	0.55
	(l/kg)	(0.0120)	(0.0001)	(0.0002)	(0.85)		(0.0002)				
	CL_M (10)	−19±13	63±19	−24±7	0.93	2.0	45.3	1.7	1.70	2.53	2.37
	(ml/min/kg)	(0.0088)	(0.0001)	(0.0001)	(0.91)		(0.0001)				
0.04	$T_{1/2}$ (14)	16±8	−36±15	20±6	0.90	2.3	51.2	2.6	2.01	3.16	1.99
	(h)	(0.0007)	(0.0002)	(0.0000)	(0.89)		(0.0000)				
	V_d (11)	−1.8±1.7	11±4	−4.5±1.6	0.89	0.5	31.0	2.1	0.40	0.51	0.54
	(l/kg)	(0.0461)	(0.0001)	(0.0002)	(0.86)		(0.0002)				
	CL_M (10)	−12±11	63±20	−29±8	0.92	2.1	40.1	2.0	1.80	2.88	2.58
	(ml/min/kg)	(0.0356)	(0.0002)	(0.0001)	(0.90)		(0.0001)				
0.06	$T_{1/2}$ (14)	16±9	−37±17	22±8	0.89	2.4	44.0	2.4	2.16	3.27	2.23
	(h)	(0.0015)	(0.0006)	(0.0001)	(0.87)		(0.0000)				
	V_d (11) (l/kg)	2.1±1.6	13±3	−5.4±1.6	0.91	0.4	42.0	2.2	0.35	0.46	0.47
	(l/kg)	(0.0156)	(0.0000)	(0.0000)	(0.89)		(0.0001)				
	CL_M (10)	−12±11	70±20	−33±10	0.92	2.1	42.5	1.8	1.75	2.72	2.42
	(ml/min/kg)	(0.0334)	(0.0002)	(0.0001)	(0.90)		(0.0001)				

n: number of available activities; *L*: 95% confidence interval for coefficients estimates; R^2: square of the product–moment correlation coefficient; $(R_{adj})^2$: *R*-squared 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.

and RMSECVi values for the QRAR models were obtained (see Tables 2 and 3). Except for the IC_{50} (*Tetrahymena pyriformis)* model, the three values are comparable then both interpolations and extrapolations should be reasonably adequate. For the mentioned IC_{50} model, RMSECV is larger than RMSECVi indicating that some cautions must be taken with extrapolated data. Fig. 6 shows the predicted (fitted and cross-validated) versus actual activity for the available data.

Using the QRAR models obtained, the pharmacokinetics and pharmacodynamics parameters of other compounds whose data were not available in bibliography could be predicted.

3.4. Comparison with traditional QSAR models based on log P

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 data of opioids and their octanol–H_2O partition coefficient at pH 7.4, log P_{app}, were performed. Two second-order polynomials models that consider the logarithmic and non-logarithmic form of the dependent variable (see Figs. 4 and 5) were assayed.

The statistical analysis for both models are summarized in Table 4. As can be observed, the statistics obtained indicate that these QSAR models are not as adequate as the corresponding QRAR models. It has been reported [54] that the use of receptor binding affinity together with the lipophilicity as independent variables, or the ratio of intraventricular to intravenous analgetic potency as dependent variable, is necessary in order to obtain good QSAR correlations for describing the analgetic potency of opioids.

4. Conclusions

The main problem to get models with predictive

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 different Brij-35 mobile phases

[Brij-35] (*M*)	Pharmacodynamic 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	RMSECV1	RMSECV1i
0.02	T.C. (10)	400±300	−600±400	280±140	0.91	45.0	35.0	1.9	37.67	63.52	54.17
	(ng/ml)	(0.0116)	(0.0075)	(0.0018)	(0.88)		(0.0002)				
	LD_{50} (11)	1.0±0.7	2.5±1.1	−1.0±0.4	0.86	0.1	25.0	1.3	0.13	0.17	0.16
	(mice, subcutaneous) (mg/Kg)	(0.0181)	(0.0008)	(0.0003)	(0.83)		(0.0004)				
	IC_{50} (8)	19±6	−29±11	11±5	0.91	0.6	24.4	1.5	0.48	0.84	0.74
	(μ-receptor) (n*M*)	(0.0006)	(0.0011)	(0.0014)	(0.87)		(0.0026)				
	IC_{50} (7)	150±60	−160±90	40±30	0.94	6.7	30.3	2.2	5.03	16.08	7.24
	(*Tetrahymena pyriformis*) (m*M*)	(0.0028)	(0.0087)	(0.0204)	(0.91)		(0.0038)				
0.04	T.C. (10)	300±200	−700±400	370±150	0.93	39.0	47.9	1.5	32.62	52.55	43.56
	(ng/ml)	(0.0046)	(0.0028)	(0.0006)	(0.91)		(0.0001)				
	LD_{50} (11)	1.2±0.6	2.5±1.1	−1.2±0.5	0.87	0.1	25.8	1.5	0.13	0.17	0.16
	(mice, subcutaneous) (mg/kg)	(0.0015)	(0.0007)	(0.0003)	(0.83)		(0.0003)				
	IC_{50} (8)	15±5	−28±11	13±5	0.90	0.6	22.4	1.6	0.50	0.80	0.80
	(μ-receptor) (n*M*)	(0.0006)	(0.0013)	(0.0017)	(0.86)		(0.0032)				
	IC_{50} (7)	130±50	−160±90	50±40	0.94	6.6	30.5	2.0	5.01	14.41	7.26
	(*Tetrahymena pyriformis*) (m*M*)	(0.0026)	(0.0092)	(0.0235)	(0.91)		(0.0038)				
0.06	T.C. (10)	300±200	−700±400	410±180	0.92	41.4	42.1	1.6	34.62	55.32	47.40
	(ng/ml)	(0.0092)	(0.0056)	(0.0011)	(0.90)		(0.0001)				
	LD_{50} (11)	1.2±0.7	2.7±1.3	−1.4±0.6	0.84	0.2	20.3	1.8	0.14	0.19	0.18
	(mice, subcutaneous) (mg/kg)	(0.0035)	(0.0017)	(0.0007)	(0.79)		(0.0008)				
	IC_{50} (8)	14±6	−26±14	13±7	0.83	0.8	12.13	1.3	0.65	1.79	1.98
	(μ-receptor) (n*M*)	(0.0023)	(0.0047)	(0.0059)	(0.76)		(0.0119)				
	IC_{50} (7)	130±60	−180±110	60±50	0.92	7.4	24.3	1.5	5.57	13.94	8.18
	(*Tetrahymena pyriformis*) (m*M*)	(0.0038)	(0.0123)	(0.0290)	(0.89)		(0.0058)				

n: number of available activities; *L*: 95% confidence interval for coefficients estimates; R^2: square of the product–moment correlation coefficient; $(R_{adj})^2$: *R*-squared 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; RMSECV1: root mean square error of cross-validation (leave-one-out); RMSECV1i: root mean square error of cross-validation (leave one-out) for interpolate data.

Table 4
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]	$T_{1/2}$ (14)	0.4±0.3	0.1±0.3	0.03±0.14	0.21	0.5	1.5	2.4
	(h)	(0.0143)	(0.5091)	(0.6244)	(0.07)		(0.2733)	
	V_d (11)	0.6±0.1	0.1±0.1	−0.00±0.05	0.31	0.1	1.8	1.4
	(l/kg)	(0.0000)	(0.2174)	(0.8998)	(0.13)		(0.2304)	
	CL_M (10)	1.2±0.3	−0.1±0.3	0.01±0.13	0.12	0.4	0.5	2.2
	(ml/min/kg)	(0.0001)	(0.4888)	(0.8944)	(0.0)		(0.6377)	
	T.C. (10)	1.6±0.5	0.3±0.5	−0.0±0.2	0.46	0.6	3.0	3.0
	(ng/ml)	(0.0002)	(0.1276)	(0.7724)	(0.30)		(0.1179)	
	LD_{50} (11)	2.4±0.3	−0.2±0.4	0.04±0.15	0.31	0.3	1.8	2.7
	(mice, subcutaneous) (mg/kg)	(0.0000)	(0.1907)	(0.5236)	(0.14)		(0.2197)	
	IC_{50} (8)	2±2	−0.6±1.7	0.2±0.6	0.13	1.9	0.4	2.2
	(μ-receptor) (nM)	(0.0678)	(0.4261)	(0.5775)	(0.0)		(0.7040)	
	IC_{50} (7)	1.3±0.8	−0.9±1.2	0.1±0.4	0.73	0.5	5.5	1.1
	(*Tetrahymena pyriformis*) (mM)	(0.0083)	(0.1025)	(0.3790)	(0.60)		(0.0710)	
[II]	$T_{1/2}$ (14)	4±4	3±4	−0.1±1.9	0.30	6.1	2.3	2.3
	(h)	(0.0459)	(0.1594)	(0.8862)	(0.17)		(0.1448)	
	V_d (11)	4±1	0.4±0.9	0.0±0.4	0.30	1.2	1.7	1.2
	(l/kg)	(0.0000)	(0.2702)	(0.9500)	(0.13)		(0.2352)	
	CL_M (10)	17±7	−1±6	−0±3	0.12	5.0	0.5	1.7
	(ml/min/kg)	(0.0006)	(0.5880)	(0.9446)	(0.0)		(0.6436)	
	T.C. (10)	100±100	80±90	−10±40	0.49	109.9	3.3	3.0
	(ng/ml)	(0.0502)	(0.0659)	(0.4221)	(0.34)		(0.0966)	
	LD_{50} (11)	280±110	−100±160	16±60	0.36	127.5	2.3	2.7
	(mice, subcutaneous) (mg/kg)	(0.0004)	(0.1711)	(0.5499)	(0.20)		(0.1651)	
	IC_{50} (8)	5000±12000	−7000±10000	2000±4000	0.38	10987.8	1.5	1.6
	(μ-receptor) (nM)	(0.3026)	(0.1462)	(0.3504)	(0.13)		(0.3017)	
	IC_{50} (7)	30±30	−20±50	3±16	0.37	21.2	1.2	1.4
	(*Tetrahymena pyriformis*) (mM)	(0.0733)	(0.3477)	(0.5984)	(0.05)		(0.3993)	

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

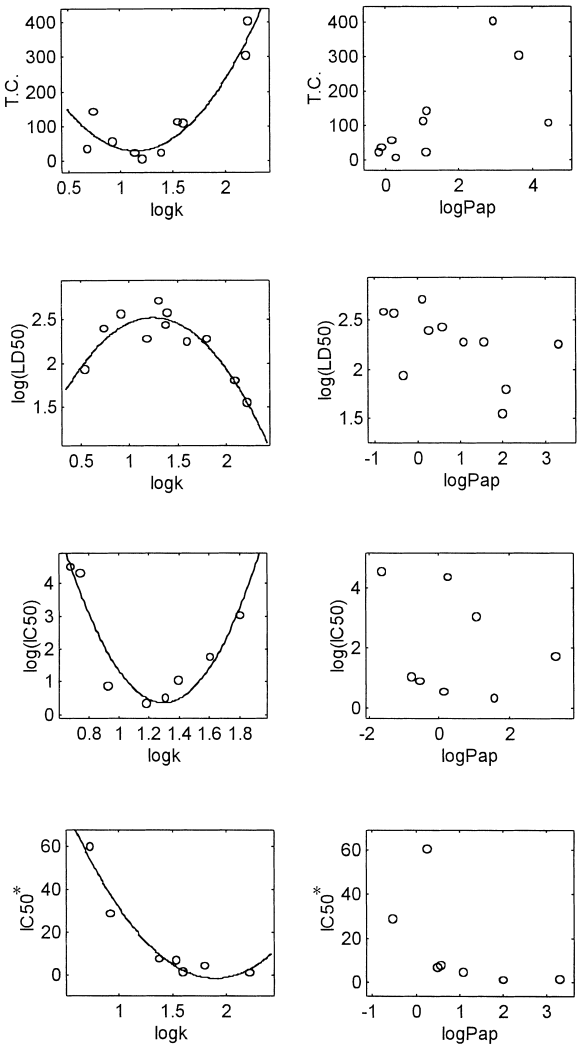


Fig. 5. Pharmacodynamics vs. $\log k$ (obtained using 0.02 *M* Brij35 mobile phase) (left) and $\log P_{app}$ (right) relationships. (T.C. = therapeutic blood-plasma serum concentration; LD_{50} = lethal dose, subcutaneous in mice; IC_{50} = inhibitory concentration on μ-receptor; IC^{*}_{50} = effect on the *Tetrahymena pyriformis* motility).

ability of pharmacological responses of drugs is, from a statistical point of view, the limited number of available data of compounds because they have not been studied or because this information has not been reported. This problem is specially important for multivariate models based on the use of molecular descriptors. In this paper it has been shown that the retention of compounds in a BMC system is an

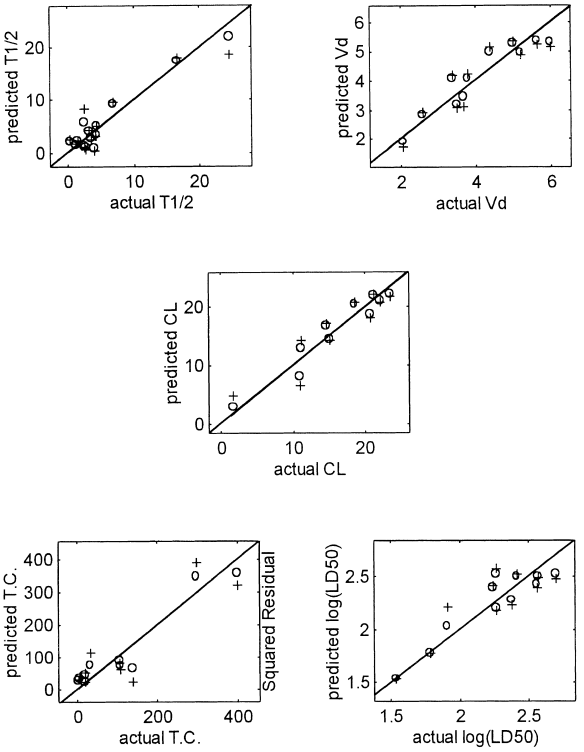


Fig. 6. Validation plots for QRAR models: predicted vs. actual values. Fitted (○) and cross-validated (+) results are shown.

adequate parameter to obtain estimation, or at least qualitative information, about opioids pharmacological responses. In addition, the results obtained were better than the ones obtained form QSAR studies, which suggests the adequacy of the BMC retention factor for describing pharmacodynamics and specially pharmacokinetics of opioid analgetics.

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