QRAR Models for Central Nervous System Drugs using Biopartitioning Micellar Chromatography

C. Quiñones-Torrelo, Y. Martin-Biosca, J.J. Martínez-Pla, S. Sagrado, R.M. Villanueva-Camañas and M.J. Medina-Hernández*

Departamento de Química Analítica. Facultad de Farmacia. Universitat de Valencia. C/Vicente Andrés Estellés s/n, E-46100 Burjassot, Valencia, Spain

Abstract: The capability of biopartitioning Micellar Chromatography, BMC, to describe and estimate pharmacokinetic and pharmacodynamic parameters of central nervous system drugs is reviewed in this article. BMC is a mode of micellar liquid chromatography, MLC, that uses micellar mobile phases of Brij35 (polyoxyethilene(23) lauryl ether) prepared in physiological conditions (pH, ionic strength). The retention of a drug in this system depends on its hydrophobic, electronic and steric properties, which also determine its biological activity. The results of BMC studies suggest that this *in vitro* approach is an attractive useful tool to be implemented into the lead optimization step of drug development scheme.

Today with the development of combinatorial chemistry hundreds and hundreds of drugs that show potential biological activity are synthesized. The studies of drugs from discovery to market are very expensive and time consuming and include the selection of drug candidates and the study of their pharmacokinetic and pharmacodynamic properties. In the early stages of drug discovery, pharmacokinetic and pharmacodynamic studies have traditionally been conducted in living systems such as mice, rabbits, dogs, etc. For ethical and/or economic reasons, a great deal effort is currently being made to develop *in vitro* systems in order to avoid or reduce the use of experimental animals.

This article is not intended to be a comprehensive review of all the approaches proposed to obtain estimations of the pharmacokinetic and pharmacodynamic properties of drugs. Rather, the aim of this article is to take a critical look at the BMC approach, showing the results obtained in estimating and/or describing of the pharmacokinetic and pharmacodynamic properties of a wide set of drugs acting on the central nervous system.

2.-CENTRAL NERVOUS SYSTEM DRUGS

Drugs that exert their primary effects upon the central nervous system (CNS) comprise the most widely employed group of pharmacologically active agents. The majority of CNS drugs considered in this review, except barbiturates can be described as lipophilic organic bases. Broadly, CNS drugs can be classified according to their action mechanism as general or non-specific CNS drugs and selective modifiers of CNS functions [1]. Drugs whose mechanisms currently appear to be general or non-specific act by diverse molecular mechanisms affecting different target cells. They can have the ability to depress excitable tissue at all levels of the CNS or may stimulate the CNS (i.e. anaesthetics, hypnotics and sedatives). Drugs classified as selective modifiers of CNS functions produce the effects through an identifiable molecular mechanism unique to the target cells that bear receptors for them. These drugs can be classified more definitively according to their locus of action or specific therapeutic usefulness (i.e anticonvulsants, psychopharmacological agents such as antipsychotics, antidepressant, etc.)

Certain drugs not considered to be centrally acting may sometimes produce a profound effect on the CNS as part of their pharmacological actions. Many drugs administered for their peripheral action also produce side effects or toxic reactions that can affect the CNS (i.e. antihistamines)

3.- FUNDAMENTAL PROCESSES IN DRUG ACTION

The drug's overall activity can not be considered to result only from the specific interaction of a drug molecule at the action site (receptor) in a tissue or cellular substrate. Several fundamental processes determine the drug action: release of the active agent from dosage form, absorption into general circulation, binding to blood proteins, distribution to the various tissues where receptor site-drug interaction itself occurs, drug biotransformation into its metabolites and excretion of the unaltered drug or its biotransformation products.

The pharmacokinetics of a drug determines the amount of pharmacologically active agent in the target tissue and the time at which the drug is present at effective concentrations. In the pharmacodynamic phase the interaction of the drug with physiological receptors takes place, producing effective stimulation or blockade of a receptor and, consequently, the physiological effects characteristic of individual drugs. In all

*Address correspondence to this author at the Departamento de Química Analítica. Facultad de Farmacia. Universitat de Valencia. C/Vicente Andrés Estellés s/n, E-46100 Burjassot, Valencia, Spain; e-mail: Maria.J.Medina@uv.es

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these processes the chemical and physical properties of an individual drug are important [2, 3]. The most important properties are: hydrophobicity, solubility, acid-base properties, hydrogen bonding capacity, polarizability, and steric properties.

4.- QSAR MODELS

In computational QSAR studies, the physicochemical characteristics of compounds (organized into an ***X***-matrix) such as hydrophobic, electronic and steric properties are used as parameters to correlate to activity (typically a ***y***-vector) using different chemometric approaches. In classical QSAR based on MLR analysis, the number of samples required is preferably more than ten times the number of molecular descriptors needed to obtain both interpretative and predictive equations, which is the main purpose of QSAR studies. However, many times it is almost impossible to collect enough data for the QSAR studies of a family of drugs. Sometimes because there are few available compounds and other times because the biological activity data on most of them have not been determined or reported (we call them 'short data series' in this review). This makes the QSAR study unfeasible. In addition, in most cases the relationships between the dependent and independent variables are not linear, which implies that more complex QSAR models must be studied. There are two approaches to solve the problems associated with 'short data series': (i) the use of latent variables as an alternative to the original descriptors and activities; and (ii) the reduction of the number of descriptors.

The first alternative, involving the use of latent variables instead of the original descriptors, such as those obtained by Principal Component Regression, (PCR) or Partial least Squares (PLS) methods, is documented in the QSAR literature [4]. The use of ***x***-scores (***t***-vectors), and in the case of PLS **y**-scores (***u***-vector), instead of the original descriptors and activities, overcomes the limitations of the MLR-QSAR approach. Moreover, to solve the non-linearity situation, non-linear PLS algorithms based on non-linear functions (polynomial, splines, neural network) to fit the ***u-t*** inner relation or approaches like Locally Weighted Regression (LWR) or artificial neural networks (ANN) can be used. In spite of the usefulness of these approaches in QSAR studies, the descriptive ability (in a statistical sense) of the model may be devaluated to benefit the predictive ability.

The second alternative is simpler. The simplifying of the QSAR models, as occurs in the so-called 'Hansch analysis', [4] has proven to be acceptable for modeling some activities (i.e. effective dose or concentration, *C*) of congener compounds . Simple QSAR models of the type:

$$\log 1/C = b_0 + b_1 \log P \quad (1)$$

$$\log 1/C = b_0 + b_1 \log P + b_2 \log P^2 \quad (2)$$

would be ideal from a statistical point of view, because they only use one descriptor (the hydrophobicity parameter *log P*). The fitting parameter (***b***-vector) can be obtained using MLR. Unfortunately, for most situations other parameters that consider electronic or steric factors must be included in order to obtain adequate models [1]. However, the estimation of more coefficient (b_i) decreases or eliminates, in the case of 'short data series, the confidence level for coefficients and predicted activity.

5.- CHROMATOGRAPHY AS AN *IN VITRO* SYSTEM TO MODEL DRUG ACTION

The dynamic pharmacokinetic/pharmacodynamic processes of drug action are considered to have much in common with the processes that are the basis of chromatographic separations of drugs. The same basic properties -hydrophobic, electronic and steric- determine the behavior of chemical compounds in both the biological and chromatographic environment. In addition, none of the essential chromatographic or pharmacokinetic/pharmacodynamic processes except metabolism implies the breaking or the formation of bonds in the drug [5]. Therefore, chromatography can be used as a powerful technique for estimating physicochemical parameters and biological activities. In addition, chromatographic techniques are dynamic systems that permit the strict control of experimental conditions and the obtaining of very reproducible retention data.

The application of chromatographic parameters to quantitative structure-activity relationships gives rise to new field, quantitative retention-activity relationships (QRAR) [6-8]. This approach that uses a unique parameter as independent variable may be an alternative to QSAR models in order to obtain an estimate or at least useful qualitative information on drug activity.

The retention of compounds on reversed phase liquid chromatography using octadecyl silica (ODS) stationary phases was first used to correlate with biological activity. However, with this stationary phase, electronic interactions between solutes and, for example, the polar lipid head groups of biomembranes or blood protein interactions are not modeled [9]. Chromatographic systems have been developed in order to emulate the biological systems. Chromatographic surfaces have been synthesized by covalent immobilizing of phospholipids to propyl amide silica particles (IAM columns) [9-11]. Immobilized liposomes, proteoliposomes and biomembrane vesicles have been proposed as stationary phases for chromatographic analysis of membrane-solute interactions [12-14]. Special columns for quantifying the interaction of drugs with serum proteins (HSA and AGP-columns). keratin, collagen [15] and with specific pharmacological receptors [16] have also been developed.

A simple, reproducible approach is micellar liquid chromatography (MLC). MLC is a mode of reversed-phase liquid chromatography, which uses a surfactant solution above the critical micellar concentration (cmc), as mobile phase [17, 18]. The use of micellar solutions produces the adsorption of surfactant monolayer to the stationary phase, thus providing it with both hydrophobic and electronic sites of interaction [19-21]. The retention of compounds in MLC

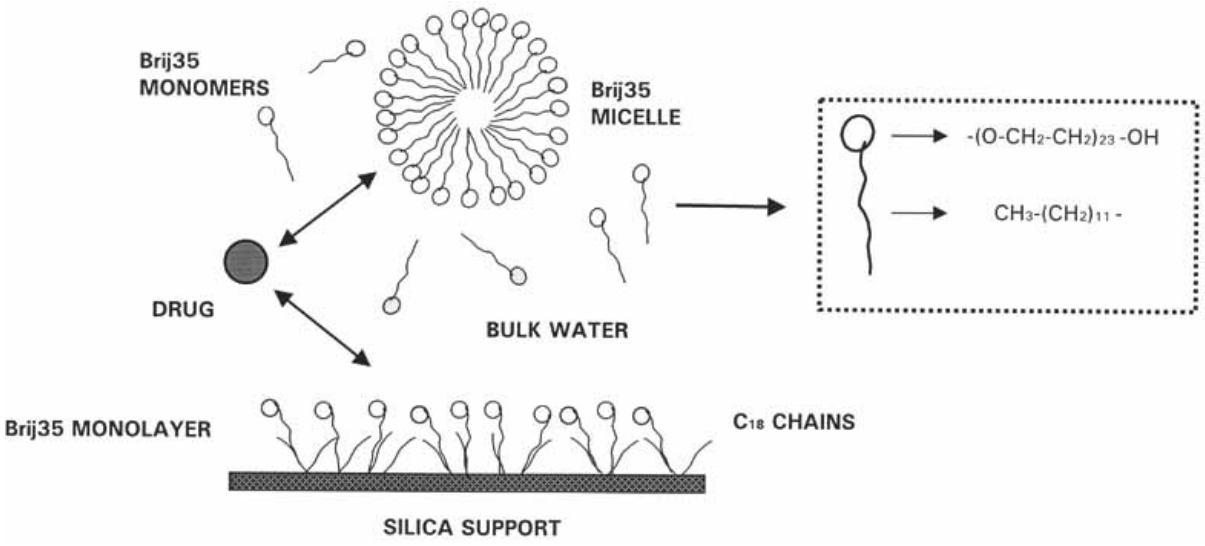

Fig. (1). Schematic representation of interactions drug-mobile phase and drug-modified stationary phase in BMC

depends on their interactions with the modified reversed stationary phase and micelles present in the mobile phase.

Recently, Micellar Electrokinetic Chromatography (MEKC), a mode of capillary electrophoresis that incorporates micelles acting as pseudo-stationary phases has also proven to be useful for describing the biological behavior of different kind of compounds, and successful applications [22-26] have been reported. MEKC can be viewed as a hybrid of MLC and capillary zone electrophoresis.

6.- BIOPARTITIONING MICELLAR CHROMATOGRAPHY

Our research group has demonstrated that the use of retention data obtained in a chromatographic system constituted by polyoxyethylene(23) lauryl ether (Brij35) micellar mobile phases and a C_{18} reversed stationary phase in adequate experimental conditions is helpful in describing the biological behavior of different kinds of drugs. We call this drug biopartitioning simulation chromatographic system Biopartitioning Micellar Chromatography (BMC). It is a MLC system optimized in order to describe the biological behavior of drugs.

The usefulness of BMC in constructing good pharmacokinetic and pharmacodynamic models could be attributed to the following:

(i) The BMC systems can be seen as similar to biological barriers and extracellular fluids (Fig. (**1**)). So the stationary phase modified by hydrophobic adsorption [19, 20] of Brij35 monolayer structurally resembles the ordered array of the membranous hydrocarbon chains and, the hydrophilic/hydrophobic character and the H-bonding groups of the adsorbed surfactant provides interaction sites similar to the membrane ones. In addition Brij35 micellar mobile phases prepared at physiological conditions (pH and ionic strength) have common characteristics to the physiological fluids. So, the extracellular and intracellular fluids are basically composed of water, salts, glucose, amino acids, cholesterol, phospholipids, triglycerides, fatty acids and proteins [27]. Phospholipids, cholesterol, fatty acids and triglycerides form micellar complexes with proteins (lipoproteins) (cmc<10^{-6} M) [28]

(ii) The retention of a drug in the BMC chromatographic system is mainly governed by its hydrophobic, electronic properties and, to a lesser extent, by its steric properties.

BMC has proven to be able to describe and predict different pharmacokinetic and pharmacodynamic properties of many different families of compounds: local anesthetics[28], barbiturates [30, 31], benzodiazepines [32], H_1–antihistamines [33], tricyclic antidepressants [34], anticonvulsants [35], phenothiazines [36] and butyrophenones [37], non steroidal antiinflammatories [38].

6.1.- Methodology in BMC

Experimental details about the use of the BMC technique are extensively reported in different papers [29-39] and are summarized in Table 1. The retention data were obtained from triplicate injections of compound solutions.

In this review two parabolic QRAR models, showed in Table 1, have been used. All the models were subjected to regression analysis via ANOVA. Since the p-value in the ANOVA table is less than 0.05 in all cases, there is a statistically significant relationship between the activity data and the retention at the 95% confidence level. This means that this relationship can be used as qualitative tool (i.e retention-activity relationship, RAR). In most cases, the regression coefficients are also significant (p-values are less

Table 1. Methodology in BMC

CHROMATOGRAPHIC SYSTEM		
Operational mode	Isocratic	
Injection	Manual (20µl)	
Column heater	Temperature fixed at 36.5 ºC	
Detection	UV-Vis detector variable wavelength, λ selected for each analyte.	
Chromatographic column	Kromasil C_{18} (5 or 15 cm length x 4.6 mm i.d.)	
Flow rate	1ml min^{-1}	
Mobile phases	0.02, 0.04 or 0.06 M Brij35 solutions Phosphate buffer 0.05 M, pH 7.4 9.2 g/l NaCl Ultrapure deionized water	
Data acquisition and processing	HP-Chemstation software	
DATA EXPRESSION		
Retention factors	$k = (t_r - t_o)/t_o$	
QRAR MODELS	(1) $log(activity) = a + b\ logk + c\ (logk)^2$ (2) $activity = a + b\ logk + c\ (logk)^2$	
EVALUATION OF PREDICTIVE ABILITY OF QRAR MODELS		
Parameter	**Equation**	**Characteristics**
RMSEC = root-mean-square error of calibration	$RMSEC = \sqrt{\frac{\sum_{i=1}^{n} (\bar{y}_i - y_i)^2}{n}}$	All the *n* molecules are included in the model construction. It has low predictive ability.
RMSECV = root-mean-square error of cross-validation, (leave-one-out approach)	$RMSECV = \sqrt{\frac{\sum_{i=1}^{n} (\bar{y}_i - y_i)^2}{n}}$	Each molecule (*i*) is used as test in turn for the model chosen on the remaining molecules, performing the procedure *n*-1 times It accounts for global predictive ability including interpolation and extrapolation information
RMSECVi = root-mean-square error of cross-validation	$RMSECVi = \sqrt{\frac{\sum_{i=2}^{n=1} (\bar{y}_i - y_i)^2}{n-2}}$	The same as for *RMSECV* but excluding the two extreme data (*i*=1, *n*), after ordering them by their *log k* values. It accounts only for interpolation information

t_0 dead time

t_r retention time

y_i predicted activity

y_i experimental activity

n number of molecules included in the model

than 0.05), which means that the models can be used also for quantitative purposes (QRAR), i.e, to predict activities of new drugs. In this cases, to evaluate the predictive ability of the models pointing out the difference between interpolated and extrapolated data, comparison between the fit error (RMSEC) and the prediction errors based on cross-validation (RMSECV and $RMSECV_i$) was used. From a qualitative point of view, large differences between RMSEC and RMSECV or RMSECVi indicate a lack of robustness of the QRAR models obtained and the need for greater caution in future predictions.

6.2.- Scope of BMC

In order to identify the situations in which BMC is useful, it is necessary to know the advantages and limitations of BMC. The BMC technique is highly reproducible, the relative standard deviations of the retention factors were <1% for intra-day and <5% for inter-day assays. Moreover it is economical, timesaving and easily automated, the time-life of columns is long (in our laboratory we have used in a continuous way a chromatographic column for 3 years without stability problems. BMC can be very useful in describing drug passive permeability across biological

membranes and drug-biological entity interactions governed by hydrophobic, electronic and steric properties.

However, BMC has some drawbacks that it may fail: (i) in describing active and metabolic processes, (ii) in determining enantioselective differences between the enantiomers of a quiral drug and (iii) obviously, when the biological response is not determined by hydrophobic, electronic or steric properties.

7.- PHARMACOKINETIC-RETENTION RELATIONSHIPS

The term pharmacokinetics is employed to designate the dynamics of the absorption, distribution, metabolism and elimination processes that occur when a drug is administered to a living system [40]. Each of these individual steps has a decisive influence on drug overall effect. Various aspects of the pharmacokinetic profile of drugs (absorption, metabolism, protein binding) can be reproduced with *in vitro* techniques and computational approaches [41].

The main pharmacokinetic parameters are: absorption, bioavailability, volume of distribution, protein binding, clearance and renal elimination. Absorption is the process by which a drug comes into the systemic circulation after being administered [42], while bioavailability is the fraction of the administered dose that reaches systemic circulation and consequently is available to produce its action. The volume of distribution is the hypothetical fluid volume that would be required to dissolve the total amount of available drug at the same concentration as that found in the plasma or blood [42]. Not all the absorbed drug will be able to reach its active sites in the body because a fraction of it is bound to plasma-proteins (mainly albumin or α_1-acid glycoprotein). If a drug is highly bound to plasma-proteins the volume of distribution is huge and renal elimination is low, as it can not be filtered into the glomerule in the kidneys and so remains in the systemic circulation for a longer time. Clearance is related to the ratio of the overall elimination of a drug to its concentration in the reference fluid (generally plasma). Finally, renal elimination is the percentage of the bioavailable drug that is excreted by the kidneys with no modification. Therefore, when renal elimination of a drug is low, it is because this drug has been metabolized and only its metabolites can be found in the urine. [40]

Molero-Monfort *et al.* performed exhaustive study on the applicability of BMC to describe and predict the oral absorption of a large set of compounds, including drugs that do not act on the SNC [39, 43]. The results of this study support the idea that BMC can be used to describe and predict oral drug absorption and yield results similar to those obtained with Caco-2 cell lines.

In this review the relationships between some of the most important pharmacokinetic parameters (volume of distribution, clearance and renal elimination) of drugs acting on the central nervous system listed in Table 2 and retention in BMC are shown [44]. The compounds come from unrelated families like: barbiturates, benzodiazepines, butirophenones, tricyclic antidepressants, H_1-antihistamines and anticonvulsants. These compounds have been grouped

Table 2. Retention and Pharmacokinetic data used to construct QRAR models.

Compound	Therapeutic category [a]	*logk* [b]*BMC*	Vd (l/kg)	Cl (ml/min/kg) [c]	Renal elimination (%) [c]
Alprazolam	1	1.20	1	0.95	20
Amitriptyline	4	1.76	15.3	12.1	0
Bromazepam	2	1.17	1.2	0.7	0
Butabarbital	1	0.96	0.8	--	0
Caffeine	5	0.30	0.61	1.4	1.1
Carbamazepine	4	0.99	1.2	0.9	0
Clemastine	1	2.11	13	8.3	0
Clobazam	1	1.25	1.6	0.49	0
Clomipramine	4	1.79	15.05	8.1	1
Clonazepam	1, 3	1.25	3.2	1.55	0
Clorazepate	1	1.42	0.33	1.8	0
Clozapine	2, 4	1.95	3.7	6.1	0
Chlordiazepoxide	1, 3	1.29	0.4	0.395	0
Chlormethiazole	1, 3	1.19	4.4	20.1	1.5
Chlorpheniramine	1	1.79	3.4	1.7	--
Chlorpromazine	2	1.92	21	8.6	0

(Table 2). contd.....

Compound	Therapeutic category [a]	*logk* [b] *BMC*	Vd (l/kg)	Cl (ml/min/kg) [c]	Renal elimination (%) [c]
Desipramine	4	1.35	22.4	20	2
Diazepam	1, 3	1.43	1.5	0.395	0
Diphenhydramine	1	1.55	4.5	11.2	2
Dothiepin	2, 4	1.60	44.5	43	0
Doxepine	2, 4	1.55	20	14	0
Ethosuximide	3	0.31	0.7	0.17	25
Felbamato	3	0.83	0.76	0.5	45
Flunitrazepam	1	1.33	3.3	3.5	0
Haloperidol	2, 4	1.65	18.7	11.8	0
Hexobarbital	1	0.99	1.2	3.9	0
Hydroxyzine	1	1.36	17.75	13.15	0
Imipramine	4	1.67	21	13.8	0
Lamotrigine	3	0.93	1.175	0.425	5.5
Lorazepam	1	1.29	1.225	1.025	0
Maprotiline	2, 4	1.35	36.75	15.1	0
Methapyrilene	1	1.48	3.9	28	0
Mianserin	4	1.78	11.15	6.6	5
Midazolam	1, 3	1.48	1.15	6.5	0
Nortriptyline	4	1.40	21	8.35	0
Oxazepam	1	1.28	1	1.45	0
Pentobarbital	1	1.29	1.1	0.455	--
Perphenazine	2	1.41	--	25.5	--
Pimozide	2	2.01	28	4.1	--
Primidone	3	0.78	0.6	0.55	40
Promethazine	1	1.77	13.25	16.1	0
Sulpiride	2, 4	0.43	1	2	100
Trimipramine	4	1.75	31	15.95	0
Zopiclone	1, 3	1.06	1.4	3.4	2.25

[a] (1) Hypnotics; (2) Antipsychotics; (3) Anticonvulsants; (4) Antidepressants; (5) SNC stimulants

[b] Retention data obtained using a mobile phase containing Brij35 0.06M

[c] The values for the pharmacokinetic parameters presented in this table are the median values obtained from the literature

into four therapeutic categories (TC): anxiolytics, sedatives and hypnotics (TC 1), antipsychotics (TC 2), anticonvulsants (TC 3) and antidepressants (TC 4).

Figure **2** shows the plots of pharmacokinetic parameter vs the retention data of the drugs studied using a mobile phase containing 0.06M Brij35. The relationship between retention in BMC and renal elimination was clearer when the retention factors of the drug (k) were used as independent variables, while for volume of distribution and clearance the representation vs logk was selected.

As can be observed, although there is no quantitative relationship between pharmacokinetic parameters (Fig.(**2A-C**) and retention, important qualitative information can be obtained from these plots. Compounds with logk values below 1.25 (k <18) present low volumes of distribution, while for more retained compounds these values are generally higher and more variable (Fig. (**2A**)). This agrees with the large variations in the reported data found in the bibliography for highly hydrophobic compounds; for these compounds the pharmacokinetic parameters also depend on individual anthropometrical properties [40, 45-47].

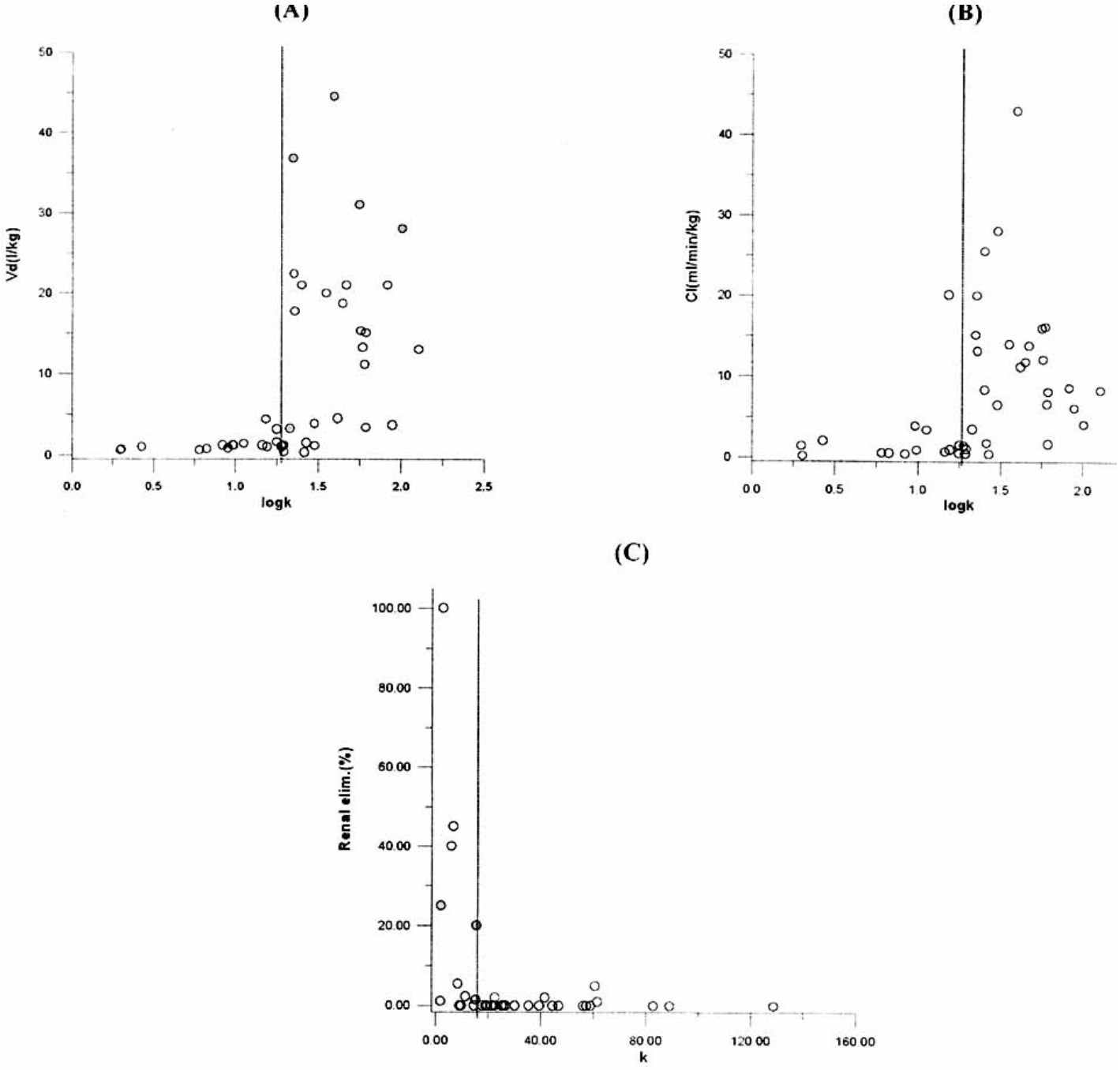

Fig. (2). Retention-activity relationships for pharmacokinetic parameters: a) volume of distribution, b) Clearance, c) renal elimination. Mobile phase: 0.06M Brij35, pH 7.4.

As can be observed in Fig. (**2B**), the relationship between the clearance and retention data of drugs is quite similar to that obtained for volume of distribution. However, some caution would be taken when describing clearance from the retention data since this pharmacokinetic parameter is related to the elimination step, which is influenced by metabolic processes that can not be explained by BMC. Finally, the plot of renal elimination of drugs vs. their corresponding k values is shown in Fig. (**2C**). In this case, the least retained compounds have variables renal elimination values, while those with k values higher than 18 present renal elimination values close to 0%. This is

consistent with the fact that the most hydrophobic compounds and those with an electrical charge close to 0 present renal elimination values very close to 0% [40].

For these three pharmacokinetic parameters it seems that a k value of approximately 18 in 0.06M Brij35 marks a transition between two different behaviors: from compounds with low distribution across the biological tissues and high elimination unaltered via urine, those with log k lower than 1.25 (k<18), to drugs that are extensively distributed and eliminated mostly as metabolites of the original drug (more retained compounds).

The results presented show that BMC can describe the pharmacokinetic properties (volume of distribution, clearance and renal elimination) of an unrelated set of drugs. This supports the idea that BMC successfully mimics some of the phenomena involved in the pharmacokinetic behaviour of drugs and that determine their overall activity.

8.- PHARMACODYNAMIC PARAMETERS-RETENTION MODELS

The parameters used to evaluate pharmacodynamic responses indicate relationships between drug concentration at its site of action and the resulting effect, including the time and the intensity of the therapeutic and toxic effects. The pharmacodynamic parameters most widely used are: IC_{50} and K values to estimate the interaction between drug and a specific receptor; the minimum effective doses, ED_{50} and ratios of ED_{50} used as predictors of different clinical and side effects; onset and duration of action, potency to evaluate the relative therapeutic efficacy; toxic and comatose concentration and LD_{50} to evaluate the drug toxicity.

Statistically significant relationships between pharmacodynamic responses and retention in BMC for different therapeutic families: hypnotics and sedatives, antipsychotics, anticonvulsants and antidepressants were obtained (Table 3). In all cases for obtaining QRAR models for a set of compounds, all available pharmacodynamic data were used with the unique restriction of using homogeneous pharmacodynamic data, such as same experimental conditions and bibliographic source.

8.1.- Hypnotics and Sedatives

Hypnotics fall into different categories, including the barbiturates, benzodiazepines and H_1-antihistamines with sedative activity.

The hypnotic and sedative properties of barbiturates are mainly due to their effect on GABA. These drugs both enhance and mimic postsynaptic responses to GABA, probably by increasing the open-life time of GABA-activated chloride channels. From the retention data of 13 barbiturates good QRAR were obtained for: the occupancy of the barbiturate site at the concentration causing general anesthesia in tadpoles expressed as %[GA] [48], hypnotic activity expressed as minimum effective hypnotic dose in rabbits [49], onset and duration of action, hypnotic doses in humans [50] and ability of barbiturates to induce general anesthesia measured by loss of righting reflex as EC_{50}-LRR [51] (Table 3). Similar QRAR models were observed for the displacement of [^{3}H]-acetylcholine and [^{14}C]-amobarbital to their binding sites by barbiturates, expressed as IC_{50}[4] [48].

Table 3. Statistical Analysis and Predictive Features of the Parmacodynamic Parameters-Retention Models

Activity	Brij35 (M)	Model [a]	*n* [b]	*a* ± *ts* [c]	*b* ± *ts*	*c* ± *ts*	r^2	*SE* [d]	*F* [e]	*RMSEC* [a]	*RMSECV* [a]	*RMSECVi* [a]
Barbiturates	0.06	1										
%GA			6	-5 ± 2	10 ± 4	-5 ± 2	0.96	0.02	40.4	-	-	-
(1/C) in rabbits, (Kg/mol)			10	3.0 ± 0.2	-	0.6 ± 0.2	0.85	0.12	47.1	0.10	0.12	0.13
Duration of action, (h)			9	2.1 ± 0.5	-2.7 ± 1.2	1.1 ± 0.7	0.95	0.05	55.0	0.04	0.19	0.06
Onset of action, (h)			8	1.0 ± 0.4	-3.5 ± 1.1	1.9 ± 0.6	0.94	0.04	38.5	0.03	0.08	0.05
Hypnotic dose, (g)			9	0.3 ± 0.3	-2.0 ± 0.8	0.9 ± 0.4	0.94	0.03	49.8	0.02	0.07	0.03
EC_{50}-LRR, (μM)			6	-3.0 ± 1.4	12 ± 4	-6 ± 2	0.98	0.11	77.7	-	-	-
IC_{50} (^{14}C-amobarbital), (μM)			7	-4.3 ± 1.3	15 ± 3	-7 ± 2	0.98	0.13	103	0.10	0.36	0.15
Benzodiazepines	0.02	1										
ED_{50}, orally in mice, (mg/Kg)			7	-13 ± 9	19 ± 11	-6 ± 3	0.98	0.04	86.2	0.03	0.10	0.04
H_1-antihistamines	0.04	2										
K_D (H_1 receptor), (nM)			10	1100 ± 500	-1100 ± 500	290 ± 130	0.88	3.9	25.9	3.25	5.09	4.05

(Table 3). contd.....

Activity	Brij35 (M)	Model [a]	*n* [b]	*a* ± *ts* [c]	*b* ± *ts*	*c* ± *ts*	r^2	*SE* [d]	*F* [e]	*RMSEC* [a]	*RMSECV*[a]	*RMSECVi*[a]
Phenothiazines	0.06	1										
IC_{50} (^{3}H-haloperidol) in rats, (nM)			8	-30 ± 10	40 ± 12	-11 ± 3	0.93	0.15	40.8	0.12	0.16	0.17
IC_{50} (^{3}H-haloperidol) in calves, (nM)			8	-30 ± 10	40 ± 11	-12 ± 3	0.95	0.10	45.5	0.08	0.98	0.12
IC_{50} (D_1 receptor) in rats, (μM)			8	-50 ± 20	50 ± 20	-14 ± 6	0.90	0.16	22.9	0.13	0.28	0.18
Potency [f]			10	-48 ± 11	60 ± 13	-18 ± 4	0.94	0.13	60.0	0.11	0.17	0.16
ED_{50} (AP), s.c. in dogs, (mg/Kg)			9	-50 ± 20	60 ± 20	-18 ± 7	0.88	0.29	21.7	0.24	0.32	0.31
Duration, s.c. in dogs, (h)			9	-19 ± 10	24 ± 12	-7 ± 4	0.84	0.12	15.2	0.09	0.15	0.17
ED_{50} (AP)p.e., s.c. in dogs, (mg/Kg)			9	-50 ± 30	70 ± 30	-20 ± 9	0.91	0.28	25.4	0.22	0.33	0.34
ED_{50} (AM), s.c. in rats, (mg/Kg)			10	-34 ± 10	46 ± 13	-12 ± 4	0.90	0.17	60.1	0.14	0.18	0.20
ED_{50} (AP), s.c. in rats, (mg/Kg)			10	-70 ± 20	80 ± 20	-23 ± 7	0.89	0.32	29.8	0.27	0.38	0.38
ED_{50} (JB), s.c. in rats, (mg/Kg)			9	-40 ± 20	50 ± 30	-14 ± 6	0.84	0.26	15.8	0.21	0.33	0.36
ED_{50} (JB), s.c. in dogs, (mg/Kg)			9	-25 ± 15	30 ± 20	-8 ± 5	0.80	0.23	12.1	0.19	0.30	0.24
ED_{50} (NE), s.c. in rats, (mg/Kg)			9	20 ± 11	-25 ± 14	8 ± 4	0.90	0.18	26.7	0.15	0.21	0.20
ED_{50} (CA), s.c. in rats, (mg/Kg)			9	-40 ± 20	40 ± 20	-12 ± 7	0.98	0.26	11.1	0.21	0.34	0.30
Butyrophenones	0.06	2										
IC_{50} (^{3}H-spiperone) in rats, (nM)			9	1000 ± 200	-1200 ± 300	340 ± 80	0.96	10.2	64.8	8.35	21.14	11.97
IC_{50} (^{3}H-haloperidol) in calves, (nM)			11	380 ± 90	-430 ± 110	120 ± 30	0.93	4.8	49.5	4.09	12.18	4.96
min. eff. dose , s.c. in rats, (mg/Kg)			8	4.8 ± 1.0	-5.7 ± 1.3	1.7 ± 0.4	0.98	0.04	103	0.031	0.124	0.043
min. eff. dose, s.c. in dogs, (mg/Kg)			8	1.2 ± 0.3	-1.4 ± 0.4	0.4 ± 0.1	0.97	0.01	78.6	0.009	0.021	0.013
ED_{50} (NE/AM)			8	1300 ± 1100	-2100 ± 1400	800 ± 400	0.93	42	33.5	33.3	111.4	45.3
ED_{50} (PP/CA)			9	50 ± 50	-80 ± 70	40 ± 20	0.96	2.6	67.9	2.11	2.74	2.98
Anticonvulsants	0.06	2										
Therap. Conc. in man, (mg/L)			13	80 ± 20	-120 ± 40	40 ± 20	0.88	5.2	35.8	4.54	13.51	5.04
Toxic conc. in man, (mg/L)			10	270 ± 40	-440 ± 90	180 ± 50	0.98	7.9	145	6.62	22.89	8.42
Comatose-fatal conc. in man, (mg/L)			9	420 ± 140	-600 ± 300	300 ± 200	0.90	27	27.3	22.3	87.17	28.75
Tricyclic antidepressants	0.04											
IC_{50} (NA), (10^{-8} M)		1	6	-50 ± 30	60 ± 30	-20 ± 10	0.97	3.3	49.1	0.09	0.22	0.31
IC_{50} (5-HT), (10^{-8} M)		1	6	-40 ± 60	50 ± 70	-15 ± 20	0.89	2.2	12.5	0.20	0.46	0.30

(Table 3). contd.....

Activity	Brij35 (M)	Model [a]	*n* [b]	*a* ± *ts* [c]	*b* ± *ts*	*c* ± *ts*	r^2	*SE* [d]	*F* [e]	*RMSEC* [a]	*RMSECV* [a]	*RMSECVi* [a]
IC_{50} (α_1 receptor), (nM)		2	9	8000 ± 5000	-10000 ± 6000	3000 ± 2000	0.87	31.3	19.91	25.6	39.5	36.0
K_D (H_1 receptor) (nM)		2	10	3600 ± 1200	-4100 ± 1400	1100 ± 400	0.94	9.3	55.7	7.8	10.9	8.5
Toxicity data	0.02	2										
LD_{50} in mice (mg/Kg)			53	20000 ± 3000	-18000 ± 3000	4000 ± 800	0.83	230	121	223	236	239
LD_{50} in rats (mg/Kg)			32	21000 ± 5000	-19000 ± 5000	4000 ± 1000	0.81	280	63	262	315	257

[a] See Table 1.

[b] *n*= number of used data.

[c] *ts* = 95% confidence interval for coefficients estimates.

[d] *SE* = standard error of the estimate.

[e] *F* = F-ratio.

[f] Relative potency with respect to chlorpromazine.

In all cases except the minimum effective hypnotic dose in rabbits (where the maximum activity had probably not been reached) parabolic relationships were obtained. In addition, in all the cases studied maximum or minimum pharmacodynamic parameter value was achieved at the same logk value (mean logk value 1.1 in 0.06M Brj35). This indicates that the hypnotic and sedative characteristics of barbiturates seem to be governed by hydrophobic, electronic and steric factors, features that also determine retention in BMC.

Retention in BMC satisfactorily describes the interaction of barbiturates on GABA receptor, and it constitutes a good tool for predicting the hypnotic and sedative effects of these drugs [48-50].

Benzodiazepines exert anxiolytic, sedative, anticonvulsant, and muscle-relaxant effects. All these actions result from augmenting the activity of inhibitory neurons and are mediated by specific benzodiazepine receptors that form an integral part of the $GABA_A$ receptor-chloride channel complex [52]. A parabolic relationship between the clinical activity of benzodiazepines expressed as ED_{50} (dose of drug causing activity in 50% of mice treated orally tested against maximum electroshock) [50] and the retention of 11 benzodiazepines in micellar mobile phases of 0.02 M Brij35 was obtained [32]

The hypnotic and sedative actions of H_1-antihistamines seem to be related to the blockade of the H_1 histamine and the muscarinic receptors in neural tissues of the CNS. [33, 53]. A relationship between the drug-H_1-receptor dissociation constant [54] (calculated by inhibition of ^{3}H-mepyramine binding to H_1-histamine receptor in rat brain membranes) and the retention of 10 antihistamines was obtained (Table 3).

8.2.- Antipsychotic Drugs

Antipsychotic agents produce calm in severely disturbed psychiatric patients and relieve them of the symptoms of their disease. Unlike hypnotics and sedatives, they do not cloud consciousness or depress vital centres, nor do they produce coma and anesthesia even at large doses. Their main application is in the treatment of functional psychoses, especially schizophrenia. Among the different drugs acting as antipsychotics, phenothiazines and butyrophenones are the most widely used in medical practice.

Antipsychotic drugs are thought to modulate catecholamine functions in the CNS by blocking dopamine receptors. The therapeutic efficacy of antipsychotics and also the appearance of extrapyramidal effects is a consequence of their effect on these receptors, especially on the D_2 defined herein as the dopaminergic site. To measure the blockade of the dopaminergic site (D2) by phenothiazines, the inhibition of 2 nM of ^{3}H-haloperidol binding in caudate nucleus homogenate (IC_{50} values) [55] is used. The inhibition of the dopamine effect on adenylate cyclase (D1) by these drugs is also evaluated as IC_{50} [56, 57].

Table 3 shows the QRAR models obtained by relating the retention of 18 phenothiazines in micellar mobile phases of 0.06M Brij35 and their corresponding IC_{50} values.

On the other hand, the relationships between the retention data of phenothiazines and the ED_{50} values [58] related to onset and duration of neuroleptic action (ED_{50}(AP) (onset) and ED_{50}(AP)p.e. in dogs, and effect duration), antispychotic effects (ED_{50}(AM), ED_{50}(AP) and ED_{50}(JB) in rats, and ED_{50}(JB) in dogs) and side effects (ED_{50}(NE) and ED_{50}(CA) in rats) were also examined. Table 3 shows the statistical analysis and the predictive features of the corresponding QRAR models obtained.

In all cases, parabolic models were obtained that showed the minimum or maximum in pharmacodynamic response at the same $\log k_0$ with a mean value of 1.78.

The therapeutic efficacy of butyrophenones is mainly a consequence of their effect on the D_2 receptor. The IC_{50} values (expressed as the concentration of drug required to give 50% inhibition of 2 nM of ^{3}H-haloperidol or 0.02-0.3 nM of ^{3}H-spiperone binding in caudate nucleus homogenate) used to evaluate the therapeutic efficacy of butyrophenones [55] were employed to construct the corresponding QRAR models from the retention data of 11 butyrophenones in Brij35 mobile phases [37]

Other butyrophenone QRAR models were also obtained for the minimum effective doses in rats and dogs [59] related to antipsychotic effects, and ED_{50}(PP/CA) and ED_{50}(NE/AM) ratios in rats [58] related to relative sedative and relative adrenolytic effects, respectively (Table 3).

All QRAR relationships for butyrophenones are parabolic. The parabolic models obtained for the parameters related to the antipsychotic effects (IC_{50} and minimum effective doses) show a minimum for $(\log k)_0$=1.75.

8.3.- Anticonvulsants

Epilepsy is considered a group of disorders with only one thing in common: the fact that recurrent anomalous electrochemical phenomena appear in the CNS. Anticonvulsants suppress epileptic seizures by depressing the CNS selectively without impairing the latter and without depressing respiration. The many classes of drugs that are used as anticonvulsants work by decreasing the discharge propagation in different ways, for example by controlling the sodium and calcium ion channels (phenytoin, ethosuximide), by enhancing the action of neuroinhibitory aminoacids such as γ-aminobuthiric acid GABA, (valproic acid, vigabatrin, gabapentine, barbiturates and benzodiazepines), or by inhibiting neuroexcitatory aminoacids like glutamic acid (lamotrigine and felbamate).

In [35]. the dependence between retention in BMC and therapeutic parameters of a heterogeneous set of 14 anticonvulsant compounds was studied. Parabolic models showing the minimum at $(\log k)_0$ = 1.2 and good correlations between therapeutic, toxic and comatose-fatal blood-plasma/serum concentrations in man [60] and the retention data were obtained.

8.4.- Tricyclic Antidepressant

The tricyclic antidepressant drugs have achieved widespread clinical use in the treatment of depression. Although blockade by antidepressants of biogenic amine uptake into nerve endings is one of the cornerstones of the biogenic amine hypothesis of affective illness, the exact mechanism of action of antidepressants remains uncertain [61]. Many antidepressants are potent inhibitors of the uptake of the biogenic amine neurotransmitters, noradrenaline (NA) and serotonin (5-HT) [62]. Moreover, tricyclic antidepressants show an accentuated antagonist effect on α_1-adrenergic and H_1 histamine receptor sites [63.].

In Table 3 the models that relate the retention data of the compounds to the antidepressant activity, expressed as the IC_{50} values for NA and 5-HT reuptake in rat brain and the IC_{50} values on the α_1-adrenergic receptor (in calf frontal cortex), and the drug H_1 receptor dissociation constant (in rat brain), are shown. [64]. All the models are statistically significant, although the coefficients in the QRAR model for the 5-HT uptake inhibition were statistically non significant; nevertheless, the information obtained may be useful from a qualitative point of view.

8.5.- Drug Toxicity

LD_{50} determination is the main way to measure acute toxicity of all types of substances. The species used in traditional LD_{50} studies are laboratory mice and rats [65]. At the present time, however, there is an increasing opposition to the use of living animals in research and testing activities from animal rights groups as well as some scientists. The need to have a tool for estimating the potential toxicity of new compounds for human consumption and chemicals in general, has encouraged to the development of alternative methods as *in vitro* cytotoxicity methods and computer-based structure-activity models [66].

Our research group has demonstrated that the compound retention in the BMC system is an adequate parameter to describe and predict the toxicity of drugs acting at the CNS, expressed as the corresponding LD_{50} value [67]. All of the CNS depressants may induce hypotension and hypothermia, respiratory distress, coma and in some instances, death. To construct the LD_{50}-retention QRAR models, 9 Triyclic antidepressants, 6 butyrophenones, 14 benzodiazepines, 14 phenothiazines and 10 H_1-antihistamines were used [68-72]. Barbiturates have not been included because they are structurally quite different than the other families of compounds and their toxic effects induce death by respiratory failure [40].

Figure **3d** shows the relationship between the psychotropic drug retention data and the available mice or rats oral LD_{50} values. Table 3 summarizes the statistical analysis and the predictive features, in cross-validation terms, of the QRAR models obtained when 0.02 M Brij-35 mobile phase was used.

9.- RELIABILITY OF QSAR AND QRAR MODELS

The main purpose of QSAR or QRAR models is to predict the activity of new molecules, thus helping to select candidate drugs in preliminary steps of drug development. However, for this selection step, a simple qualitative indication (SAR or RAR estimation) of the molecule activity would in most cases be sufficient.

On the other hand, some questions arise when dealing with QSAR or QRAR models. Are they reliable?, do they reflect the real activity-descriptors relationships?. To

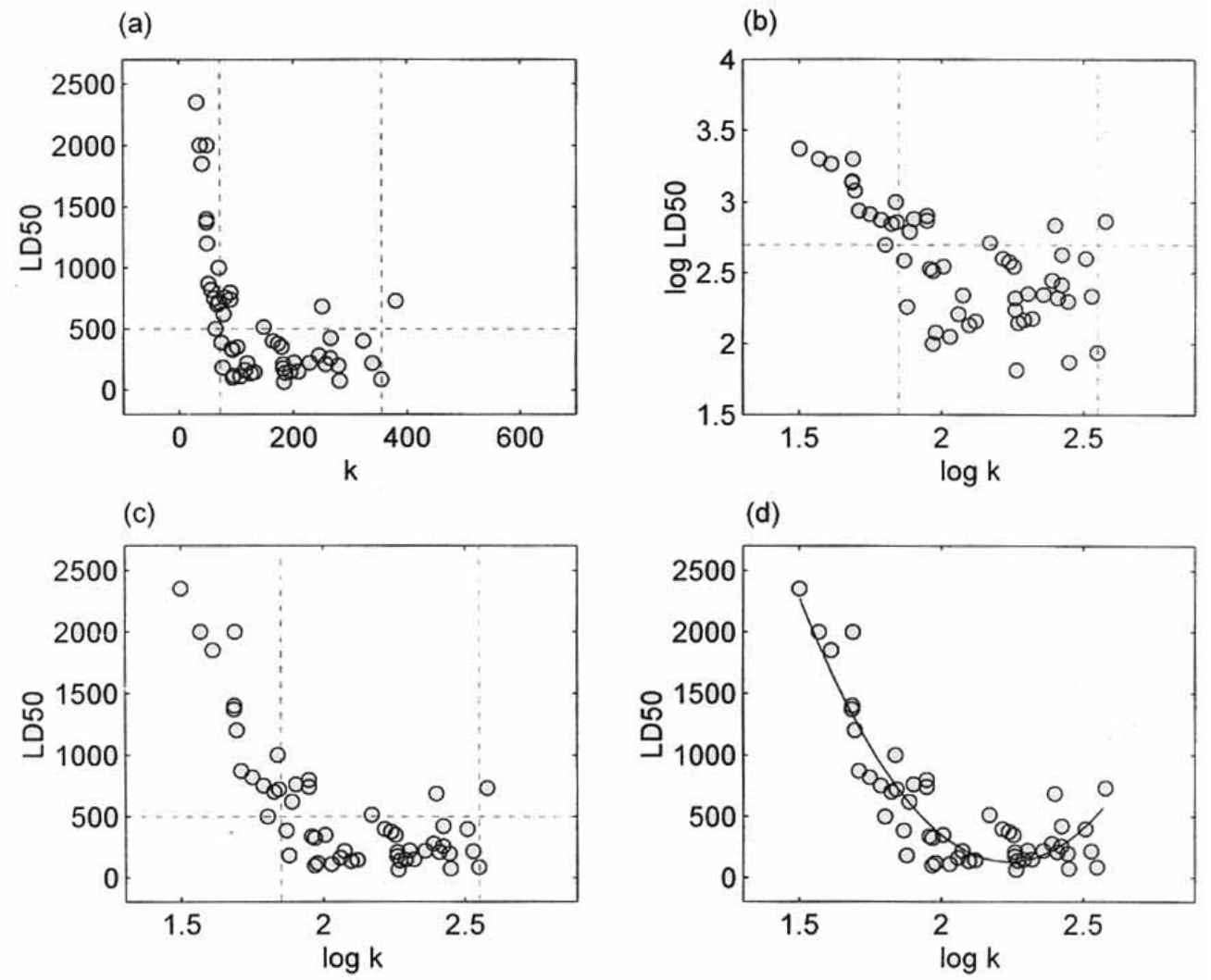

Fig. (3). Retention-activity relationships for LD50: (a, b, c) RAR obtained from different transformations of data. d) QRAR model based on polynomial regression. (see text and also Table 3 for details).

demonstrate the validity of the QSAR or QRAR models authors have traditionally used regression statistics, usually the coefficient of determination, r^2, the residual standard deviation, SE, and the modeled-to-residual variance ratio, F. But, even when these statistics are adequate, are they sufficient to assure the quality of further activity estimations?. In other words, can the estimations be reliable in a quantitative sense or must they be considered a qualitative indication of the molecule activity?.

Table 4 shows the mice and rats oral toxicity predicted values together with their confidence intervals calculated from the standard deviation of the residuals, for other psychotropic drugs whose data were not found in the literature.

In order to improve the models reliability, some statistics can be added to the regression analysis. To assure the significance of the coefficients it seems to be reasonable to use their confidence limits. [29]. To improve the information about the predictive ability of the model the RMSECV and RMSECVi (see Table 1) can be used [32]. These criteria increase the statistical confidence of estimations obtained using these models.

Although QSAR or QRAR models satisfy the statistical requirements, two considerations should , in our opinion , be taken into account to judge whether the models are reliable for quantitative or only for qualitative estimations. Firstly, the uncertainty of the dependent variable, activity, is not considered, because in most cases it is not available. Uncertainty values, which may be high due to the difficulties associated with the *in vivo* experimental conditions, influence the uncertainty of the estimations. However, it is important to bear in mind that in most cases qualitative or semiquantitative information about new drug activity is valuable and sufficient.

Let us consider, for example, the QRAR model developed to describe/predict the toxicity (LD_{50}) of 50 drugs

Table 4. Mice and Rats Oral LD_{50} Values, and their 95% Confidence Interval, Predicted from the QRAR Models for CNS Drugs whith non-Available LD_{50} Bibliographic Data

Compound	LD_{50} / mice (mg/Kg)	LD_{50} / rats (mg/Kg)	Compound	LD_{50} / mice (mg/Kg)	LD_{50} / rats (mg/Kg)
ANTIDEPRESSANTS			Dimetindene	130 ± 90	–[1]
Amineptine	3000 ± 400	3300 ± 500	Diphenhidramine	410 ± 90	500 ± 140
Amoxapine	–[1]	380 ± 140	Hydroxyzine	500 ± 90	–[1]
Clomipramine	150 ± 80	–[1]	Isothipendyl	–[1]	240 ± 140
Dothiepin	210 ± 90	290 ± 140	Ketotifen	410 ± 90	500 ± 140
Loxapine	–[1]	180 ± 130	Methapyrilene	–[1]	740 ± 140
Melitracen	210 ± 90	260 ± 140	Orphenadrine	–[1]	210 ± 130
Mianserin	–[1]	190 ± 130	Oxatomide	190 ± 90	260 ± 140
Nortriptyline	–[1]	770 ± 140	Pyrilamine	–[1]	520 ± 140
Quinupramine	150 ± 90	210 ± 140	Tripelennamine	370 ± 90	460 ± 140
Trimipramine	130 ± 80	180 ± 130	Triprolidine	140 ± 80	190 ± 130
ANXIOLYTICS-SEDATIVES			**NEUROLEPTICS**		
BENZODIAZEPINES			*BUTYROPHENONES*		
Bentazepam	–[1]	710 ± 140	Azaperone	430 ± 90	520 ± 140
Clobazam	–[1]	1900 ± 200	Fluanisone	2300 ± 200	2600 ± 400
Clonazepam	–[1]	1470 ± 190	Fluspirilene	1450 ± 130	1600 ± 200
Chlordiazepoxide	–[1]	1210 ± 160	Pipamperone	2800 ± 300	3000 ± 500
Lorazepam	–[1]	1420 ± 180	Spiperone	430 ± 90	520 ± 140
Lormetazepam	–[1]	1470 ± 190	*PHENOTHIAZINES*		
Midazolam	–[1]	680 ± 140	Ethopromazine	–[1]	200 ± 130
Oxazepam	–[1]	1470 ± 190	Methotrimeprazine	–[1]	210 ± 140
Tetrazepam	–[1]	420 ± 140	Pericyazine	1230 ± 110	–[1]
H_1-ANTIHISTAMINES			Pimozide	490 ± 160	–[1]
Alloclamide	–[1]	550 ± 140	Prochlorperazine	–[1]	500 ± 200
Antazoline	3000 ± 400	3300 ± 500	Promethazine	–[1]	190 ± 130
Brompheniramine	140 ± 90	200 ± 130			
Carbinoxamine	500 ± 90	600 ± 140			
Chlorciclyzine	400 ± 140	400 ± 200			
Chloropyramine	140 ± 90	200 ± 130			
Ciclyzine	–[1]	190 ± 130			
Cinnarizine	520 ± 80	600 ± 300			
Ciproheptadine	–[1]	360 ± 170			
Clemastine	–[1]	600 ± 300			

[1] The LD_{50} prediction was not performed because these data were used to construct the QRAR model.

that belong to five therapeutic families from retention data. Since all these compounds have a similar toxicity mechanism, a general QRAR model could be expected. Figures **3a** to **3c** show three different toxicity-retention relationships from the same data. (LD_{50} vs k, $logLD_{50}$ vs logk and LD_{50} vs logk). Apparently, there are visual differences between these figures, mainly located in the lower LD_{50} values (specially visible in Fig. (**3b**)), and they are due to the data logarithmic transformation. However, the three plots lead to the same qualitative conclusions. Most of the compounds with logk values ranging from 1.50 to 1.85 ($32<k< 70$) present LD_{50} values between 500-2500 mg kg^{-1} and are classified as "moderately toxic" according to the Gosselin criteria [73]. Most of the compounds located in the 1.85-2.55 logk range ($71<k<355$) exhibit LD_{50} values below 500 $mg.kg^{-1}$ and they are considered "very toxic" compounds. Consequently, from a practical point of view, there are no differences between using Fig. (**3a** or **3c**) to reach qualitative estimations on which a decision in the drug candidate selection scheme can be based.

However, if we want to obtain quantitative information, not all the relationships can be easily modeled. In this case, only the representation LD50 vs. log k (Fig. (**3d**) can be adequately fitted to a polynomial QRAR model (See Table 3). As can be observed, the statistical analysis of the model make the quantitative use of this QRAR model possible (See Table 4). In this example, both RAR/QRAR observations are supported by a relative large number of compounds thus giving a reasonable confidence for the estimations.

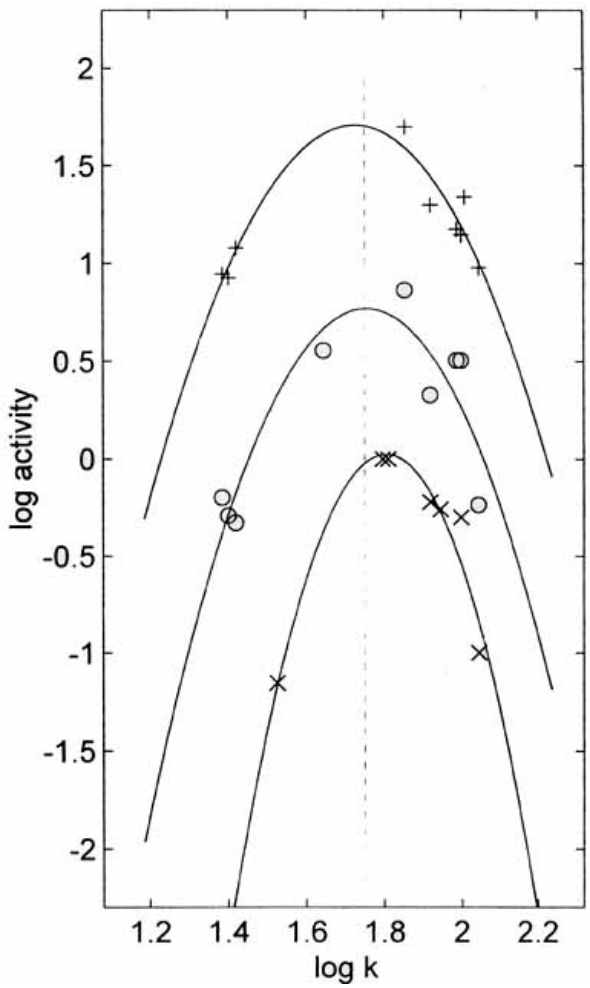

Fig. (4). QRAR models for the effect duration , ED_{50} (JB) in dogs and IC_{50} of phenothiazines. Maximum activity is approximately located at the same *log* k_{BMC} value.

The second factor introducing uncertainty in the estimations is the above-mentioned use of 'short data series'. In our opinion, in such situations MLR-QSAR models must be avoided and QRAR models may be the only option even though in some cases they can only be used to obtain qualitative or semi-quantitative information. In these cases extra-information could increase the confidence with respect to the proposed relationship. For instance, let us examine three BMC QRAR models for phenothiazines obtained at 0.06 M Brij35 [36] related in this case to the logarithm of three activities: (neuroleptic effect) *duration*, *ED_{50} (JB)* in dogs and *IC_{50}* and *log k_{BMC}* (see Table 3 for details). Although the retention data was measured for 18 phenothiazines, a smaller number of activities are available (n = 7-9). Fig. (**4**) shows the experimental points for the neuroleptic effect duration (+), ED_{50} (JB) in dogs (o) and IC_{50} (x). With the data in each series (independent examination) it is possible to obtain qualitative information, but it is more difficult to establish the type of quantitative relationship. However, when each data series was fitted to a polynomial model the fact that in all cases the parabolic relationship showed a maximum located close to $logk_{BMC}$ = 1.75 gives more confidence to each individual QRAR model.

Then, any statistically consistent QRAR model could be clearly used to reach at least reliable qualitative estimations and is, therefore, interesting from an *in vivo* vs.*in vitro* and cost/benefit point of view.

10.- CONCLUSIONS

Since the revolutionary development of combinatorial chemistry the bottleneck in drug discovery has shifted to pharmacokinetic and pharmacodynamic optimization of lead compounds. Lead optimization, a vital point in pharmaceutical decisions about which compounds should be developed, requires collaborative decision-making. At this stage data complexity increases with the inclusion of *in vivo* assays, SAR/QSAR assessments and medicinal or physicochemical studies. *In vitro* approaches have ethical and practical advantages because they permit a reduction in animal experimentation, cost and time.

Chromatographic retention-based approaches (RAR/QRAR) can offer a rapid, simple pharmacokinetic/ pharmacodynamic profiling of compounds. Although BMC is not the only HPLC-based methodology, it is probably the most accessible, economical, robust, and stable. Reproducible results are obtained, and it preserves the intrinsic advantages of HPLC measures. Due to its experimental simplicity, BMC has the advantage (i.e. over the use of cell-lines) of closely controlling the uncertainty of

the independent variable. A low ratio descriptor/activity variances is a prerequisite for correct application of conventional (type I) regression models (assuming errors only in the dependent variable). The use of only one descriptor, the retention in BMC, also has statistical benefits. This is particularly useful for 'short data series'.

From the results shown in this review, the BMC approach seems to be an attractive tool for estimating the potential activity of new molecules, e.g. a newly synthesized compound from a generic molecular structure, which justifies the development of predictive QRAR models. It has been shown that under adequate conditions the chromatographic system can reproduce the drug biopartitioning. The use of the retention in BMC, which encompasses the main interactions between a drug and its corresponding biological target (hydrophobic, electronic and steric contributions to the free energy change in the biological response), should guarantee a progressive incorporation of BMC into the drug discovery scheme.

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GLOSSARY

- AGP: α_1-acid glycoprotein.
- BMC: Biopartitioning micellar chromatography.
- Brij35: Polyoxyethylene(23)lauryl ether.
- Cl: Clearance. Ration between the elimination rate of a drug over its concentration in plasma in the steady state.
- Comatose-fatal concentration: Plasmatic concentration of a drug from which lethal intoxication or coma state has been induced.
- EC_{50}-LRR: Concentration of drug required to induce general anesthesia, measured as loss of righting reflex, in the fifty per cent of the population administered.
- ED_{50} (Δw): Dose of drug causing activity for the food-intake inhibition test in the 50% of the population.
- ED_{50} (AM): Dose of drug causing activity for the amphetamine antagonism test in the 50% of the populations.
- ED_{50} (AP): Dose of drug causing activity for the apomorphine antagonism test at the onset time in the 50% of the animals treated.
- ED_{50} (AP)p.e.: Dose of drug causing activity for the apomorphine antagonism test measured at the time of the maximum peak effect in the 50% of the animals treated.
- ED_{50} (CA): Dose of drug causing activity for the catalepsy test in the 50% of the population treated.
- ED_{50} (JB): Dose of drug causing activity for the jumping box test in the 50% of the animals treated.
- ED_{50} (NE): Dose of drug causing activity for the norepinephrine antagonism test in the 50% of the animals treated.
- ED_{50} (NE/AM): Dose of drug that produces norepinephrine antagonism (NE) over apomorphine antagonism (AM). The ratio of NE to AM can be used as an index for the relative adrenolytic activity versus neuroleptic activity.
- ED_{50} (PP/CA): Effective dose to produce palpebralptosis (PP) over catalepsy (CA) in the 50% of the population. It serves as a useful index of relative sedative versus neurological effects.
- ED_{50}: Dose of drug causing activity in the 50% of the animals treated.
- HSA: Human serum albumin.
- IAM columns: Immobilized artificial membranes.
- IC_{50}: Concentration of drug required to fifty per cent displacement of a marker from a binding site or receptor.
- K_D: Drug-receptor dissociation constant.
- LD_{50}: The statistically derived dose that is expected to cause death in 50% of the treated animals in a given period, when administered in an acute toxicity test
- LogP: Logarithm of the partition coefficient in the bifasic octanol-water solvent system. This is a widespread measurement of the hydrophobicity of chemical compounds.
- Min. eff. dose: Minimum effective dose.
- MLC: Micellar liquid chromatography.
- Potency: Therapeutic efficacy of a drug relative to the efficacy of a prototype compound from the same family.
- QRAR: Quantitative retention-activity relationships.
- QSAR: Quantitative structure-activity relationships.
- Renal elimination: Percentage of the bioavailable fraction of drug that is eliminated unaltered by the kidneys.

- RMSEC: Root-mean-square error of calibration.
- RMSECV: Root-mean-square error of cross-validation.
- $RMSECV_i$: Root-mean-square error of cross-validation for interpolated data.
- Therapeutic concentration: Plasmatic concentration of a drug required to obtain the desired therapeutic effect in humans.
- Toxic concentration: Plasmatic concentration of a drug from which toxic effects have been observed in humans.
- V_d: Volume of distribution. Volume of body fluid that would be required to dissolve the total amount of drug at the same concentration than in the blood. It is a measurement of the distribution of a drug all over the body

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