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Journal of Chromatography B, 757 (2001) 89–99

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Retention–property relationships of anticonvulsant drugs by biopartitioning micellar chromatography

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Received 10 November 2000; received in revised form 8 February 2001; accepted 8 February 2001

Abstract

Epilepsy may be considered as a group of disorders with only one thing in common: the fact that recurrent anomalous electrochemical phenomena appear in the central nervous system. Different classes of drugs are included under the generic term of anticonvulsant drugs. All of them work by decreasing discharge propagation in different ways. Biopartitioning micellar chromatography (BMC) is a mode of reversed-phase liquid chromatography, which can be used as an in vitro system to model the biopartitioning process of drugs when there are no active processes. In this paper, relationships between the BMC retention data of anticonvulsant drugs, their pharmacokinetics (oral absorption, protein binding, volume of distribution, clearance, and renal elimination) and their therapeutic parameters (therapeutic, toxic and comatose-fatal concentration, and LD_{50}) are studied and the predictive ability of models is evaluated.

Keywords: Retention–activity relationships; Biopartitioning micellar chromatography; Anticonvulsant drugs

1. Introduction

Epilepsy is not be considered to be a unique pathology entity. In fact, it is widely considered to be a group of disorders with only one thing in common: the fact that recurrent anomalous electrochemical phenomena appear in the central nervous system. Epileptic fits occur when a high frequency electric discharge takes place. Anticonvulsant drugs 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-aminobutyric acid GABA (valproic acid, vigabatrin, gabapentine, barbiturates and benzodiazepines), or by inhibiting neuroexcitatory aminoacids like glutamic acid (lamotrigine and felbamate).

In drug development, the processes that occur from drug administration to its excretion are called ADME (absorption, distribution, metabolism and excretion). Physicochemical properties of drugs (i.e. hydrophobicity and electrical charge) play an important role in these processes. However, there are other specific metabolic and active processes where these properties become less important. For example,

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0378-4347/01/$ – see front matter
PII: S0378-4347(01)00124-4

for the vast number of drugs absorption and excretion are due to passive permeability, which is highly correlated with hydrophobicity and charge. On the other hand, for a significant number of drugs, active processes play an equivalent or dominant role. These processes are mediated by transporters and metabolic enzymes.

Distribution is another process that is mediated by carrier proteins (mainly serum albumin and α_1-glycoprotein). The percentage of a drug bound to these proteins strongly depends on the hydrophobicity and electrical charge of the drug [1]. However, stereochemistry also plays a role in drug–protein binding.

A number of different attempts have been made to establish relationships between physicochemical characteristics of drugs and their pharmacokinetics, pharmacodynamics, and biological properties. In quantitative structure–activity relationships (QSAR), mathematical models that include measurements of the partition coefficient in the biphasic octanol–water solvent system, log *P*, together with other molecular descriptors of drugs, are used [2,3]. In general, these models require a large number of compounds in order to obtain statistically significant models.

Chromatography is a powerful technique for the measurement of physicochemical parameters. The application of chromatographic parameters in structure–activity relationships gives rise to a new field, quantitative retention–activity relationships, QRAR [4,5]. This approach has the advantage that it requires only one parameter (k' or $\log(k')$) to construct the QRAR models and consequently it is possible to obtain validated models from a smaller number of compounds than the QSAR approach. Different strategies have been developed in order to emulate the biological membranes, including the immobilization of phospholipids onto silica propyl amide particles [6–8] and immobilization of liposomes [9–11], rat liver microsomes [12] and nicotinic receptors [13] onto chromatographic surfaces.

Our research group has demonstrated that in adequate experimental conditions the use of micellar solutions of Brij35 as surfactant, and reversed stationary phases can be very useful to construct predictive retention–property models. This simple and reproducible approach we have called biopartitioning micellar chromatography (BMC). The success of this methodology can be attributed to the similarities between the modified stationary phase–mobile phase in BMC and the membrane–water interfaces [14].

Successful applications of BMC in constructing QRAR models that describe the anaesthetic potency of local anaesthetics [15], the hypnotic activity of barbiturates [16], the α- and β- andrenergic activity of catecholamines [17], the toxicity and anxiolytic activity of benzodiazepines [18], the pharmacokinetics parameters and biological responses of triclyclic antidepressants [19], the pharmacokinetics, preclinical pharmacology, and therapeutic efficacy parameters of phenothiazines [20], the activity of non-steroidal anti-inflammatory drugs [14], and the preclinical pharmacology and therapeutic efficacy parameters of barbiturates [21] have been reported. Recently, a model to predict the oral absorption of drugs from the retention data in BMC [22], β-adrenoceptor blocking agents pharmacokinetic and pharmacodynamic properties [23], the preclinical pharmacology and therapeutic parameters of butirophenones [24] and the pharmacokinetics and pharmacodynamics of antihistamines [25] have been developed.

Although BMC has demonstrate its capability of predicting different properties of many different families of compounds, it is necessary to note its limitations in order to clarify the situations where it is useful. BMC can neither describe active and metabolic processes nor determine enantioselective differences between the enantiomers of a chiral drug.

In this paper, quantitative relationships between the BMC retention data of anticonvulsants using Brij35 as surfactant and their pharmacokinetics and therapeutic data are studied and the predictive ability of models is evaluated.

2. Experimental

2.1. Instrumental and measurement

The chromatographic system was composed of a Hewlett-Packard 1100 chromatograph equipped with an isocratic pump, a UV-visible detector and a column heater (Palo Alto, CA, USA). Data acquisition and processing were performed on an HP Vectra

XM computer (Amsterdam, The Netherlands) equipped with HP-Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA), with a 20-μl loop. A Kromasil octadecylsilane column (5 μm, 150×4.6 mm I.D., Scarlab, Barcelona, Spain) was used. The mobile phase flow-rate was 1.0 ml/min. The detection was performed in UV at 220 nm, except for allobarbital, amobarbital, carbamazepine, chlormethiazole, diazepam, lamot-

Table 1
Structure, log *P* and logarithm of the protonation constants (log *k*) of the anticonvulsants studied

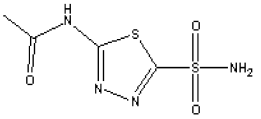
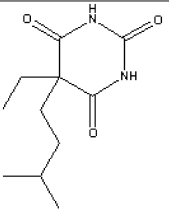
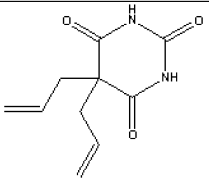
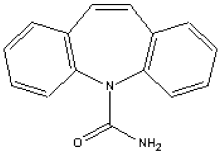
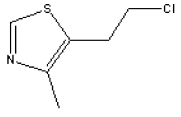
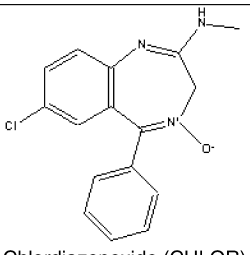
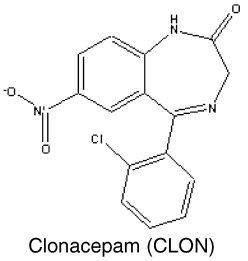
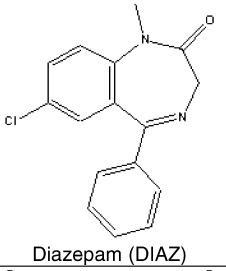
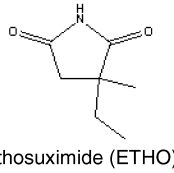
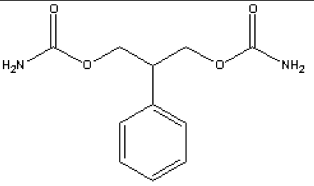
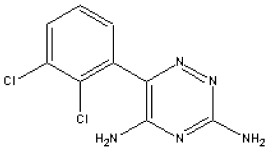
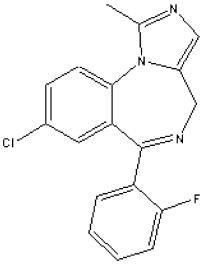
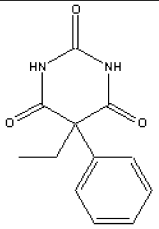
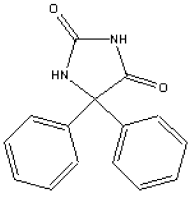
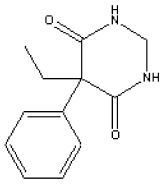
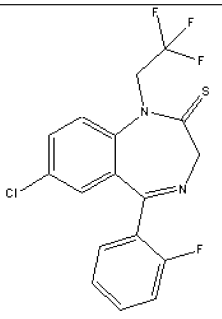
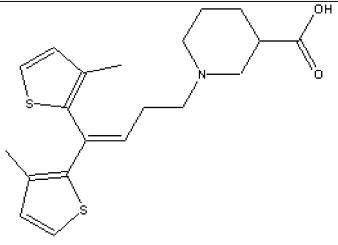
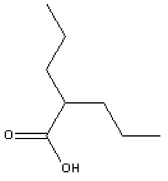
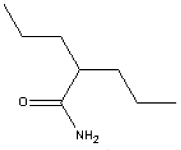
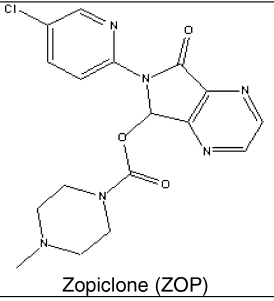
Drug	logP	logK	Drug	logP	logK
 Acetazolamide (ACET)	-0.3	7.2, 9	 Allobarbital (ALLO)	0.63	7.7
 Amobarbital (AMO)	2.11	7.9	 Carbamacepine (CARB)	2.5	--
 Chlomethiazole (CHLOM)	1.56	3.2	 Chlordiazepoxide (CHLOR)	2.4	4.8
 Clonacepam (CLON)	2.84	1.5, 10.5	 Diazepam (DIAZ)	3.18	3.3
 Ethosuximide (ETHO)	-0.33	9.5	 Felbamate (FEBL)	--	--

Table 1. Continued

Drug	logP	logK	Drug	logP	logK
 Lamotrigine (LAM)	2.08	--	 Midazolam (MIDA)	2.7	6.2
 Phenobarbital (PHEN)	1.5	7.4	 Phenytoin (PHENY)	2.5	8.3
 Primidone (PRIM)	0.9		 Quazepam (QUAZ)	4.2	--
 Tiagabine (TIAG)	1.59	3.3, 9.4	 Valproic acid (VALPA)	--	4.8
 Valpromide (VALPM)	1.72	--	 Zopiclone (ZOP)	1.54	--

rigine, mephobarbital, midazolam and quazepam, which were detected at 240 nm. All the assays were carried out at 36.5°C.

2.2. Reagents and standards

Mobile phases were prepared by aqueous solutions of polyoxyethylene(23) lauryl ether (Brij35, Acros Chimica, Geel, Belgium). Micellar eluent pH was adjusted to 7.4 with 0.05 *M* phosphate buffer, which was prepared with sodium di-hydrogen phosphate (analytical reagent, Panreac, Barcelona, Spain).

Anticonvulsants were obtained from several sources: valproic acid and tiagabin from the pharmaceuticals Depakine® and Gabitril®, respectively (Sanofi Winthrop, Barcelona, Spain); acetazolamide from Diamox® (Lederle, Madrid, Spain); carbamacepine was obtained from Carbamazepina 200 Alter EFG (Alter, Madrid, Spain); chlordiazepoxide from Omnalio® (Estedi, Barcelona, Spain); clonazepam from Rivotril® (Roche, Madrid, Spain); chlormethiazole from Distraneurine® (Astra, Barcelona, Spain); ethosuximide from Etosuximida Faes® (Faes, Bilbao, Spain); felbamate from Taloxa® (Schering-Plough, Madrid, Spain); and lamotrigine from Labileno® (Glaxo Wellcome, Burgos, Spain). Allobarbital, and amobarbital were obtained from Sigma–Aldrich (Madrid, Spain). Some of the anticonvulsants were kindly donated by several pharmaceutical laboratories: phenobarbital (Bayer, Barcelona, Spain), zopiclone (Aventis Pharma, Madrid, Spain), primidone (Zeneca Farma, Porriño, Pontevedra, Spain), and phenytoin (Rubió, Barcelona, Spain).

Stock standard solutions of anticonvulsants of 1000 mg/l were prepared using buffered 0.04 *M* Brij35 as solvent. Working solutions were prepared by dilution of stock standard solutions using Brij35 of the same concentration as the mobile phase in each case. Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected in the chromatograph were vacuum filtered through 0.45- and 0.22-μm nylon membranes, respectively (Micron Separations, Westboro, MA, USA). All retention factors were obtained by triplicate injections of compounds solutions. The dead time value (average t_0=0.98 min) was determined for each injection as the first perturbation in the chromatogram.

2.3. Software and data processing

Table 1 shows the structures, log *P* and pK_a values for the anticonvulsants studied. The logarithm of octanol–water partition coefficient values, log *P*, for the non-ionic forms of the anticonvulsants were taken from the literature [3]. Excel 7.0 Microsoft Office was used to perform the statistical analysis of the multiple linear regression (MLR).

2.4. Predictive ability of the QRAR models

To evaluate the predictive ability of the models [18,19], the comparison between the fit error (e.g. the root-mean-squared error of calibration, RMSEC), the prediction error based on cross-validation (e.g. root-mean-squared error of cross-validation, RMSECV) parameter that includes both interpolation and extrapolation information, and the RMSECVi parameter for measuring only the interpolation information, was used. The lower the differences are between the RMSEC, RMSECV and RMSECVi parameters, the greater is the robustness of the QRAR model obtained.

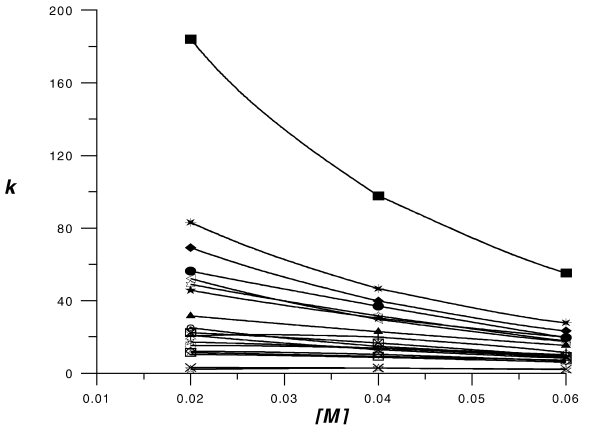


Fig. 1. Effect of Brij35 concentration in the mobile phase on the retention of anticonvulsants: quazepam (■), midazolam (✳), diazepam (◆), chlordiazepoxide (●), phenytoin (◁), clonazepam (✡), amobarbital (★), chlormethiazole (▲), carbamazepine (☉), tiagabine (⊠), lamotrigine (✳), zopiclone (+), phenobarbital (⋈), valpromide (○), felbamate (◇), allobarbital (□), primidone (△), azetazolamide (×), and ethosuximide (☆).

3. Results and discussion

3.1. Retention behaviour of anticonvulsant drugs

The retention of anticonvulsants was measured using Brij35 as micellar mobile phase at physiological pH. Fig. 1 shows the effect of the Brij35 mobile phase concentration (0.02, 0.04 and 0.06 *M*) on the retention of anticonvulsants. As can be observed, for most hydrophobic compounds (quazepam, midazolam, diazepam and clonacepam), large changes in the retention were obtained upon increasing the Brij35 concentration in the mobile phase, while for the less hydrophobic compounds the retention was scarcely modified.

As can be observed in Table 1, drugs which present anticonvulsant activity do not belong to an unique chemical family. They are structurally unrelated compounds and present variable hydrophobicity ($\log P$ values ranged between -0.3 and 4.2) and molar total charge (α values ranged from -1 to $+1$).

3.2. Retention–property relationships

In the absence of active processes, the capability of a drug to reach the receptor site strongly depends on its membrane affinity. The molecular features of drugs (mainly hydrophobicity, ionization and steric properties) determine their membrane affinity and the drug–receptor interaction, and consequently their biological activities. Since these molecular features also determine the retention of compounds in BMC, retention–activity relationships could be expected.

Tables 2 and 3 show the pharmacokinetic and therapeutic data of anticonvulsant drugs reported in the literature [23–30]. In order to obtain predictive

Table 2
Retention and pharmacokinetic data of the anticonvulsants studied

Compound	Log k'[h]	Oral absorption (%)	V_d[i] (l/kg)	Protein binding (%)	Cl[j] (ml/min per kg)	Renal elimination (%)
Acetazolamide	0.3501	–	0.2[c]	–	0.64[b]	
Allobarbital	0.8605	–	–	–	–	
Amobarbital	1.3017	95[e]	–	–	–	
Carbamacepine	0.9943	–	1.2[b]	75[b]	0.26[b]	2–3[d]
Chlordiazepoxide	1.2932	100[e]	–	96[b]	0.24[b]	0[d]
Chlomethiazole	1.1863	–	–	64[c]	17.1[c]	<3[d]
Clonazepam	1.2515	98[b]	3[b]	85[b]	1.43[b]	0[d]
Diazepam	1.3677	100[f]	2.5[c]	98[b]	0.43[b]	0[d]
Ethosuximide	0.3085	–	–	2.5–9[a]	0.17[b]	17–40[d]
Felbamate	0.8285	–	0.8[a]	22.5[a]	–	45[a]
Lamotrigine	0.9246	98[b]	1.15[b]	55[b]	0.45[b]	<7[d]
Midazolam	1.4437	–	–	95[b]	17.1[c]	<1[d]
Phenobarbital	0.9396	100[g]	0.7[b]	50[b]	0.07[b]	20[d]
Phenytoin	1.2458	98[b]	–	90[b]	–	–
Primidone	0.7837	90[b]	0.6[b]	20[a]	0.48[b]	40[d]
Quazepam	1.7428	100[b]	6.8[b]	>95[b]	–	0[d]
Tiagabin	0.9611	–	1[a]	96[a]	–	–
Valpromide	1.0060	–	–	–	–	–
Zopiclone	1.0551	100[e]	1.4[b]	45[c]	3.52[b]	4–5[d]

[a] From Ref. [27].
[b] From Ref. [28].
[c] From Ref. [29].
[d] From Ref. [30].
[e] From Ref. [31].
[f] From Ref. [32].
[g] From Ref. [33].
[h] Retention data in Brij35 0.06 *M*.
[i] Volume of distribution.
[j] Clearance.

Table 3
Therapeutic data of the anticonvulsants studied

Compounds	Therapeutic conc. (mg/l)[a]	Toxic conc. (mg/l)[a]	Comatose conc. (mg/l)[a]	LD_{50} oral in mice (mg/kg)[b]
Acetazolamide	–	–	–	–
Allobarbital	2–5	10	20	–
Amobarbital	1–5	5–6	10	345
Carbamacepine	–	10	20	529
Chlordiazepoxide	1.7	11.5	–	820
Chlomethiazole	0.7–2	4–15	50	–
Clonacepam	0.01–0.08	0.1	–	2000
Diazepam	0.2–2	3–5	–	481
Ethosuximide	40–60	100–200	250	1750
Lamotrigine	1–5	–	–	–
Midazolam	0.04–0.1	1–1.5	1.5–3	1600
Phenobarbital	–	–	50–60	137
Phenytoin	5–15	–	50	490
Primidone	4–12	20–50	65	280
Quazepam	0.01–0.05	–	–	5000
Zopiclone	<0.1	–	–	–

[a] From Ref. [34].
[b] From Ref. [35].

and interpretative models, the retention data of anticonvulsants and their corresponding properties were adjusted to a polynomial QRAR model:

$$\text{Property} = a + b(\log k) + c(\log k)^2$$

The results given in the paper were obtained using the retention data of compounds in 0.06 *M* Brij35 mobile phase. Similar QRAR models were obtained for 0.02 and 0.04 *M* Brij35 mobile phases.

Anticonvulsant drugs are generally well absorbed after oral administration (oral absorption higher than 80%), although they present large variations in bioavailability (ranging between 40 and 100%). Large variations in other pharmacokinetics properties such as protein binding (0–100%), volume of distribution (0.3–7.2 l/kg), clearance (0.2–72 l/h) and renal elimination (0–45%), expressed as % of drug eliminated by the kidneys without further modifications, are also observed.

Fig. 2 shows the plots of pharmacokinetic parameters versus the retention data of the anticonvulsant drugs studied (in solid symbols). In order to check the trends observed, in the same figure the retention–property plots of other families of compounds (benzodiazepines [18], barbiturates [21], antidepressants [19], non-steroidal anti-inflammatory drugs [14], local anesthetics [15], β-blockers [23], butirophenones [24] and antihistamines [25]) (in dotted symbols) have been included. As can be observed, although there is no quantitative relationships between oral absorption, protein binding, volume of distribution, clearance, renal elimination (Fig. 2A–E, respectively) and retention, important qualitative information can be obtained from these plots. In a previous paper [22] it was reported that compounds with *k* values lower than 5 present variable oral absorption (from 20 to 95%) while for more retained compounds the absorption is close to 100%. In Fig. 2A it can be observed that the inclusion of anticonvulsant drugs into the previous model does not change the general trend previously described [22]. Fig. 2B shows the plot of the protein binding percentage of anticonvulsants and how it increases with retention until a *k* value of 25 is reached where protein binding remains constant near 100%. From Fig. 2C it can be concluded that compounds with log *k* values below 1 present relatively low values of volume of distribution, while for more retained compounds these values are generally higher and more variable. For highly hydrophobic compounds, the pharmacokinetic parameters can also depend on individual anthropometrical properties, therefore large variations in the reported data are

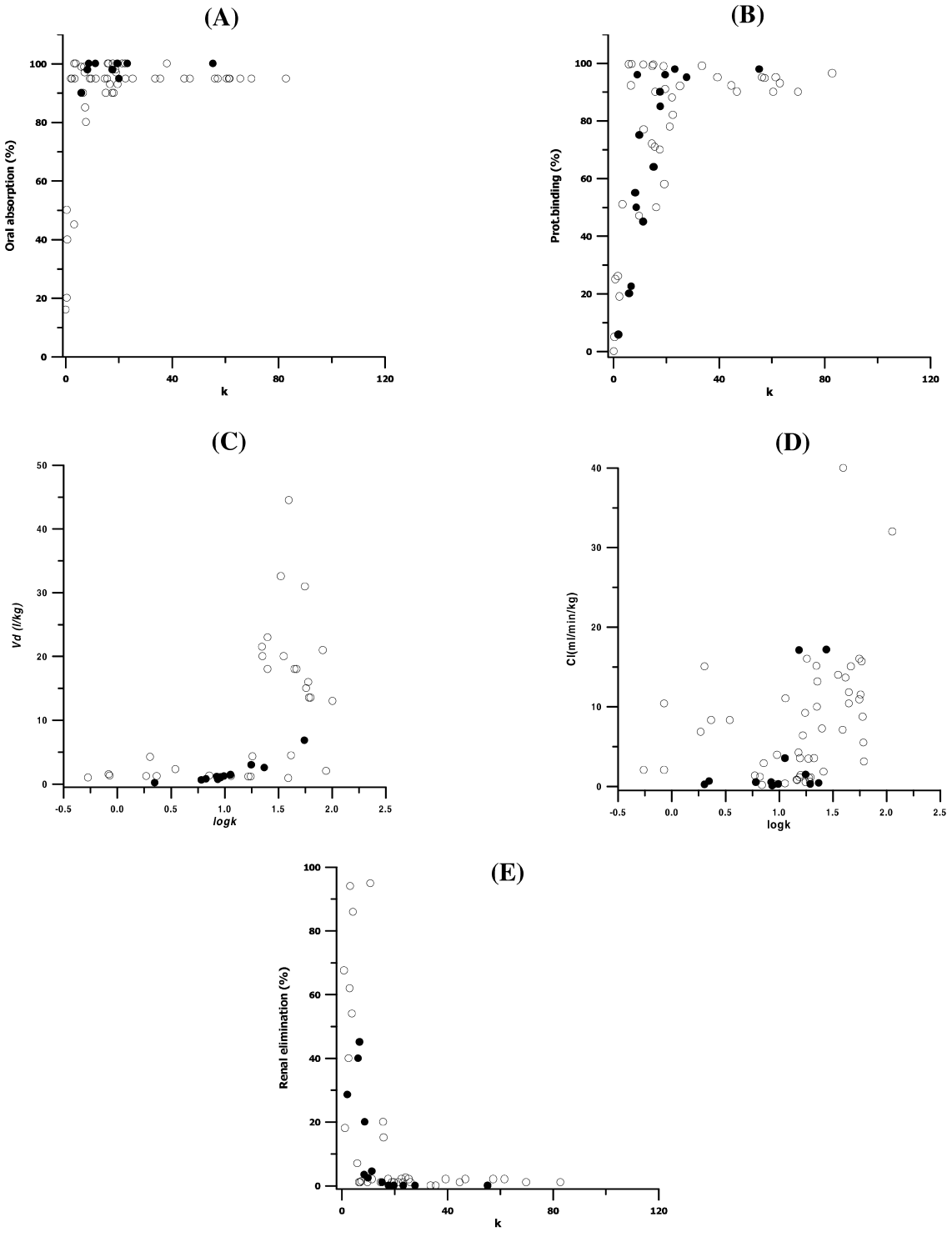


Fig. 2. Relationships between oral absorption (A), protein binding (B), volume of distribution (C), clearance (D) and renal elimination (E) parameters and retention data relationships at 0.06 *M* Brij35 mobile phase for the anticonvulsants (●) and other families of compounds (○).

found in the literature [27–30]. As can be observed in Fig. 2D, there is no clear relationship between clearance and retention data. This result is not surprising taking into account that this pharmacokinetic parameter is related to the elimination step, which is influenced by metabolic processes that can not be explained by BMC as has been discussed previously. Finally, the plot of renal elimination of drugs versus their corresponding k values is shown in Fig. 2E. In this case, the less retained compounds have variable renal elimination values, while those with k value higher than 15 present renal elimination values close to 0%. As can be observed for all of these five plots, the inclusion of other groups of drugs does not change the trend observed for anticonvulsants.

Fig. 3 shows the dependence between therapeutic parameters (therapeutic, toxic and comatose-fatal blood-plasma/serum concentrations (μg/ml) in man and LD_{50} values) and the retention data of anticonvulsant drugs together the corresponding residual plots. As can be observed the experimental points are well adapted to the model.

Table 4 shows the statistical analysis of the QRAR models obtained using 0.06 M Brij35 mobile phase. As can be observed, since the P-values of these models are less than 0.05, there are statistically significant relationships between therapeutic, toxic, comatose-fatal concentrations, and LD_{50} and $\log k$ at the 95% confidence level. In all cases, the coefficients were also significant at the same confidence level ($P<0.05$) and the r^2 values were adequate. The standard error of the estimate (SE) can be used to construct prediction limits for new observations.

As can be observed, the RMSEC and RMSECVi values were similar, but lower than RMSECV values, which suggests that interpolation parameters based on these QRAR models should be reasonably adequate. However caution should be exercised as regards extrapolation values.

The predictive ability of the QRAR models can be also evaluated from the validation plots (Fig. 4), which show the predicted (fitted and cross-validated) versus actual activities for the available data. As can be seen, the ability of $\log k$ values to describe therapeutic, toxic and comatose-fatal concentrations and LD_{50} is adequate, except for extreme data in cross-validation.

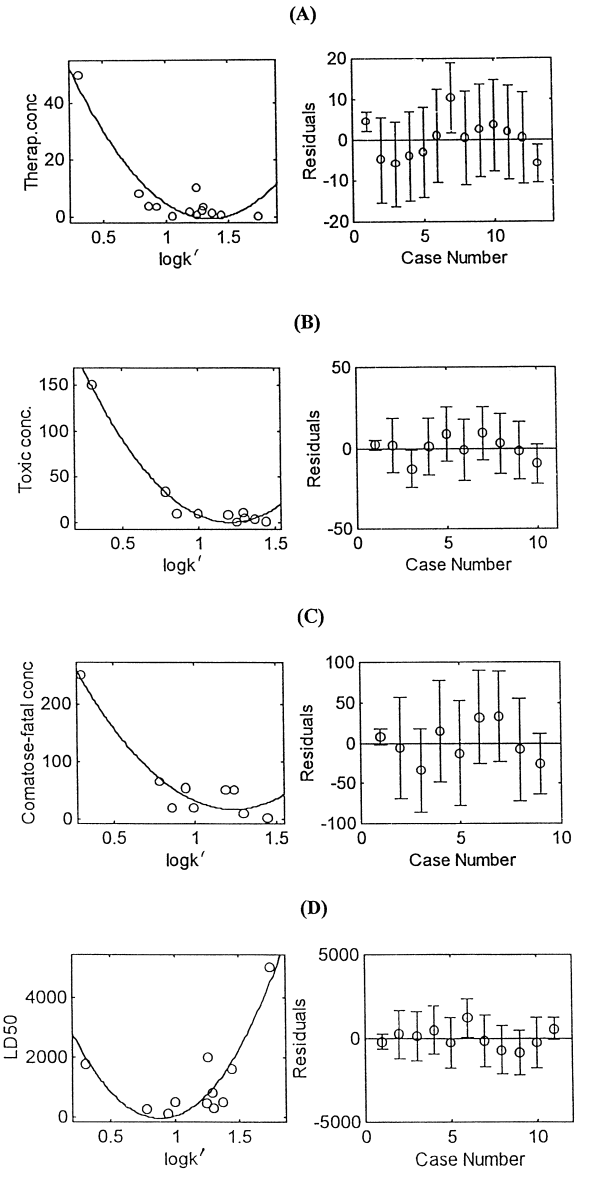


Fig. 3. Therapeutic–retention data relationships for different anticonvulsants at 0.06 M Brij35 mobile phase (left). Residual plots (right).

4. Conclusions

There is an ongoing campaign to eliminate most of the animal based pharmacokinetic and therapeutic studies used in the drug discovery process. Thus, there is a need to develop new in vitro models to predict these parameters.

Table 4
Statistical analysis and predictive features of therapeutic and pharmacokinetic data–retention models (activity = $a + b(\log k) + c(\log k)^2$ for anticonvulsants)

Activity (n)	$a \pm ts_a$ (P-value)	$b \pm ts_b$ (P-value)	$c \pm ts_c$ (P-value)	r^2 $r^2_{adj.}$	SE	F (P-value)	RMSEC	RMSECV	RMSECVi
Therap. conc. (13) (mg/l)	80±20 (0.0000)	−120±40 (0.0001)	43±19 (0.0005)	0.88 0.85	5.175	35.8021 (0.0000)	4.5386	13.5090	5.0418
Toxic conc. (10) (mg/l)	270±40 (0.0000)	−440±90 (0.0000)	180±50 (0.0001)	0.98 0.97	7.914	144.6897 (0.0000)	6.6214	22.8935	8.4207
Comatose-fatal conc. (9) (mg/l)	420±140 (0.0004)	−600±300 (0.0029)	300±200 (0.0124)	0.90 0.87	27.372	27.2636 (0.0010)	22.3495	87.1731	28.7502
LD_{50} in mice oral (11) (mg/kg)	5000±3000 (0.0040)	−11000±7000 (0.0018)	6000±3000 (0.0007)	0.82 0.78	662.933	12.2178 (0.0000)	624.1	1345.9	810.2

F, F-ratio; n, number of available data; r^2_{adj}, r^2 adjusted for degrees of freedom; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross-validation (leave-one-out); RMSECVi, root mean square error of cross-validation (leave-one-out) for interpolated data; SE, standard error of of the estimate; ts, 95% confidence interval for coefficients estimates.

The approach proposed in this paper, involving qualitative or quantitative retention–activity relationships (QRAR), may be a useful tool to obtain estimations of anticonvulsant drug properties such as oral absorption, protein binding, volume of distribution, clearance, renal elimination, and LD_{50}, therapeutic, toxic and comatose-fatal concentrations. This approach can be very useful in the development of new anticonvulsant drugs, thereby avoiding the use of experimentation animals.

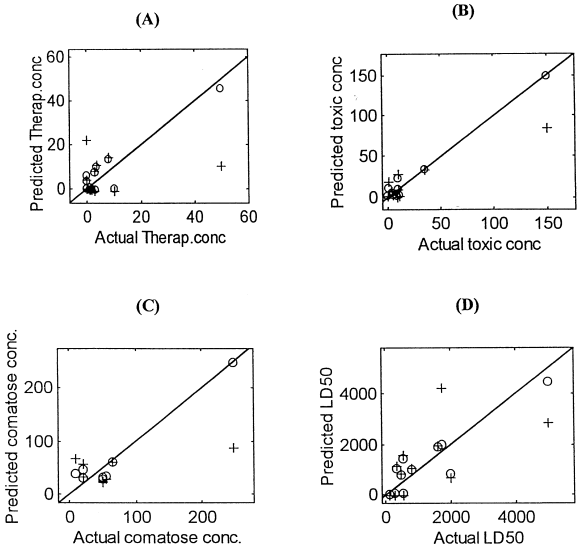


Fig. 4. Validation plots for QRAR models: (A) therapeutic concentration, (B) toxic concentration, (C) comatose concentration and (D) LD_{50}. Fitted (○) and cross-validated (+) results are shown.

The results presented in this paper support the idea that BMC is an adequate in vitro system to predict the biopartitioning process and biological activities of drugs. Moreover, it is important to take into account that BMC can describe the properties of a very unrelated set of drugs. This fact can be explained if we assume that BMC successfully mimics some of the phenomena that produce pharmacological responses irrespective of the chemical nature or pharmacological application of the compounds.

Acknowledgements

The authors acknowledge the financial support of the Interministerial Commission of Science and Technology (CICYT) of Spain (Project SAF99-0110). J.J. Martínez-Pla is grateful to the Ministerio de Educación y Cultura for the FPU grant (FPU-AP99).

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