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Hydrophobicity of β-adrenoceptor blocking agents: Study of correlations between retention in reversed-phase HPLC systems and octanol-water partition constants

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Abstract

The capacity factors (k') of seven β-adrenoceptor blocking agents in six different reversed-phase HPLC systems have been determined. Octanol-aqueous buffer (pH 7.4) partition constants (P) for these blocking agents were also obtained. By using target factor analysis (TFA), good empirical correlations between the log k' and log P were derived. The resulting hydrophobicity order agrees well with the metabolic elimination pathways of these drugs.

Keywords: β-Blocking agent; RP-HPLC retention; Octanol-water partition constant; Target factor analysis; Hydrophobicity

1. Introduction

About the end of the last century, from the landmark works of Overton (1899) and Meyer (1899), it was recognized that the hydrophobic properties of drugs play an important role in their pharmacological activity. The evaluation of hydrophobicity of organic compounds is of great interest in the design of new pharmaceutical formulations. The hydrophobicity of drugs is most commonly characterized by their octanol-water partition constants (P) (Hansch and Fujita, 1964; Dearden, 1985). Consideration of this parameter in structure-activity and structure-toxicity studies might substantially reduce drug development costs.

Owing to the difficulties in performing log P measurements by the conventional 'shake-flask' method, several chromatographic approaches have been proposed (Kaliszan, 1992; Valkó and Slégel, 1993). Among others, the use of logarithmic capacity factors values (log k') obtained from reversed-phase (RP) HPLC columns at a given mobile phase composition is extensively used (Nowotnik et al., 1993).

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β-Adrenoceptor blocking agents are commonly used in the treatment of hypertension, angina pectoris and cardiac arrhythmias, the metabolic elimination pathway being dependent on their lipophilicity (hydrophobicity).

In the present paper, we have studied the correlations between log k' and log P for seven β-blocking agents (atenolol, alprenolol, oxprenolol, metoprolol, acebutolol, pindolol and propranolol) on six RP-HPLC columns (five octadecylsilane type columns and one macroporous polymeric type column).

2. Theory

Empirical correlations between log k' and log P invoking the Collander equation have been studied since 1950 (Bate-Smith and Westall, 1950), the model equation being

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

Models are often used without previous checking for testing their adequacy to the data to be processed. This is a typical misconception claimed by Deming (1984) who states that some researchers still 'fit the data to a model' instead of 'fit the model to the data', which suggests a lack of scientific integrity. Thus, the blind application of conventional regression techniques based on least squares without a discussion of the model adequacy is not advisable. Target factor analysis (TFA) is a powerful technique that easily enables us to fit a model to the data, by taking the model variables as the number of proper underlying factors compatible with the data matrix. Once the number of true factors is selected, the possible factors (model variables) are tested for data adequacy. Only the accepted factors are taken as proper model variables. Finally, the coefficients involved in the model equation fitted to the data are evaluated as the factor loadings (Sindreu et al., 1994).

In order to perform all the mentioned TFA calculations, we have used the program HOLMES, developed by González-Arjona et al. (1994), utilizing an IBM compatible PC with an 80386 processor.

3. Materials and methods

3.1. Apparatus

Retention measurements were carried out using the following instrumentation: an isocratic pump Shimadzu LC-9A, a Rheodyne type injector with a 20 μl loop, a Waters 486 tunable absorbance detector and a Hewlett Packard HP 3394A integrator. The utilized RP-HPLC columns were: Tracer 150 × 4.0 mm Spherisorb ODS2 5 μm (I), LKB Super Pac Cartridge 250 × 4.0 mm Spherisorb ODS 5 μm (II), Scharlau 250 × 4.0 mm Spherisorb ODS 5 μm (III), Waters Novapak 150 × 3.9 mm C-18 4 μm (IV), Waters μ-Bondapak 150 × 3.9 mm C-18 10 μm (V) as well as a Hamilton PRP-1 150 × 4.1 mm poly(styrene-divinylbenzene) 5 μm (VI).

A low-cost home-made water jacket (built from a suitable reflux condenser) assembled with a Techne C-100 circulator thermostat was utilized for controlling the column temperature within 25 ± 1° C.

Operational conditions were: flow rate, 1 ml/min. Each β-blocking agent was detected at its maximum wavelength closest to visible zone: 265 nm (pindolol), 290 nm (propranolol), 274 nm (oxprenolol), 326 nm (acebutolol), 271 nm (alprenolol) and 275 nm (atenolol and metoprolol) (the spectra were measured using as solvent the corresponding mobile phase). Detection was carried out with AUFS = 0.1.

Absorbance measurements were performed on a Hewlett Packard 8452A diode array spectrophotometer using matched silica cuvettes of 10 mm path length.

A Techne Tempette TE-8D thermostat assembled to a Techne Refrigerated bath circulator was utilized for controlling the temperature of solutions.

3.2. Reagents

1-Octanol (Merck, extra pure grade) was used as received without the need for prior purification. Moreover, the solvent did not show any UV-absorbing contaminant.

Methanol (Romil, Super purity solvent of HPLC quality) was used as received.

Milli-Q (Millipore) treated water was used throughout.

Alprenolol hydrochloride, oxprenolol hydrochloride and metoprolol tartrate (Sigma), acebutolol hydrochloride, propranolol hydrochloride and pindolol (ICN) were used. Atenolol was kindly provided by Roig-Farma (Barcelona). They showed high HPLC purity. Tris and potassium iodide (Merck analytical-reagent grade) were used as received.

3.3. Procedures

3.3.1. Preparation of mobile phase

In order to mimic the physiological pH of blood, both mobile phase (in RP-HPLC retention measurements) and aqueous layer (in partition constant determinations) were buffered at pH 7.4. Tris buffer is the best choice for avoiding drawbacks of typical phosphate buffers (Perrin and Dempsey, 1974).

The mobile phase was prepared by mixing 80% v/v of methanol with 20% v/v of aqueous 0.1 M Tris buffer. Before use, the mobile phase must be (i) filtered through a 0.45 μm filter in a solvent filtration apparatus and (ii) degassed by means of a flow of He. This eluent leads to short retention times (even for strongly RP-HPLC retained blocking agents such as propranolol) and avoids broad chromatographic peaks. Considering that the pK_a values of all the blocking agents studied are very close to 9.5 (Wang and Lien, 1980), they practically behave as neutral species. The degree of ionization $\alpha = 1/(1 + \text{antilog}(pK_a - pH)) = 1/(1 + \text{antilog}(9.5 - 7.4) = 0.00788$, is a negligible amount for all accounts.

3.3.2. Determination of partition constants

The β-blocking agents were partitioned between 1-octanol saturated with 0.01 M Tris buffer of pH 7.4 and the same aqueous phase saturated with 1-octanol. The procedure was according to the papers of Wong and McKeown (1988) and Wang and Lien (1980): bottles (50 or 100 ml) with ground-glass stoppers were used. The volume ratio of aqueous phase/1-octanol was based on a precalculated log P value for the drug based on the theory of fragmentation constants (Rekker and Mannhold, 1992). The amount of solute was weighed directly in the partitioning bottle, and this was followed by the introduction of a volume of 1-octanol (25–50 ml at 25° C), allowing a drainage time of 20 min and a volume of aqueous phase (25–50 ml at 25° C). The partitioning bottle was stoppered, hand shaken for 15 min, and left standing in a water bath (25° C) for 8 h. It was hand shaken for 5 min every 30 min during the first 2 h. A portion of aqueous phase, separated from the 1-octanol, was centrifuged at 300 rpm for 30 min. Then the aqueous sample was adequately diluted to perform the spectrophotometric analysis. Calibration graphs of each drug at its maximum wavelength were previously established. According to the negligible ionization of the studied drugs, the partition coefficient was calculated from

$$P = \frac{(y - x)}{x} \cdot \frac{\text{volume of aqueous phase}}{\text{volume of } 1 - \text{octanol}} \qquad (2)$$

where y is the total mass of drug used and x denotes the mass of solute in the aqueous phase (in all cases referred to the neutral drug, i.e., propranolol instead of propranolol hydrochloride). At least quadruplicate determinations were made in all cases and the standard deviation in log P was less than 0.03.

3.3.3. Determination of capacity factors

Solutions of each β-blocking agent at concentrations adequate to give suitable chromatographic peaks (30–80 ppm) under the conditions cited above were prepared in the mobile phase and then filtered through a 0.4 μm disposable syringe filter unit. The hold-up time of each column, t_0, was established by using potassium iodide dissolved in the mobile phase detected at 245 nm (Csokán et al., 1993).

If t is the retention time of the studied drug, the capacity factor at 25° C for each β-blocking agent is given by:

$$k' = \frac{(t - t_0)}{t_0} \qquad (3)$$

Table 1
Log k' values for the β-blocking agents in the different columns (I–VI) using the 80:20 mobile phase (methanol-0.1 M Tris, pH 7.4)

	I	II	III	IV	V	VI
Pindolol	−0.308	−0.051	0.531	−0.708	−0.510	−0.865
Propranolol	0.294	0.772	0.988	−0.051	−0.080	0.207
Oxprenolol	0.119	0.584	0.862	−0.280	−0.078	−0.160
Acebutolol	−0.176	0.122	0.648	−0.541	−0.415	−0.643
Alprenolol	0.280	0.725	0.976	−0.060	0.068	0.038
Atenolol	−0.479	−0.195	0.428	−0.885	−0.671	−1.332
Metoprolol	−0.009	0.409	0.740	−0.400	−0.242	−0.448

Taking into consideration that retention measurements are more precise than partition constant determinations, triplicate experiments were performed only.

4. Results and discussion

The logarithmic partition constants of propranolol, metoprolol and atenolol were taken from the literature (Wang and Lien, 1980), or otherwise determined according to section 3.3.2. The corresponding results are: atenolol (0.27), pindolol (1.02), acebutolol (1.55), metoprolol (2.04), oxprenolol (2.68), alprenolol (3.20) and propranolol (3.39).

The log k' values determined are presented in Table 1 as a data matrix where the rows are the β-blocking agents and the columns, the different columns used.

As can be observed, the sequence of β-blocking agents according to their log k' values is in all cases: atenolol < pindolol < acebutolol < metoprolol < oxprenolol < alprenolol < propranolol, which is the same order found according to the log P values. This is at first sight a symptom of correlation.

In order to explore the factors or independent variables which govern retention, factor analysis (FA) techniques were applied to the data matrix of Table 1. Four different tests were applied for this purpose: the indicator function (IND) proposed by Malinowski (1991), Malinowski's F-test (Malinowski, 1991), the cross-validation technique (Deane, 1992) and the RSD F-test (Sindreu et al., 1994). In Table 2, the values of IND, the cross-validation ratio R (Deane, 1992), Malinowski's F-test (M-F) and RSD F-test (RSD-F) are displayed against the number k of selected factors.

A minimum corresponding to two factors can be seen from the IND function. The M-F test leads to a minimum significance level (SL) for two factors ($F = 212.34$, SL% = 0.07), as well as the RSD-F test ($F = 128.20$, SL% = 0.102). The cross-validation technique also yields two significant factors (with ratio $R < 1.00$). Accordingly, all the methods give two underlying factors. Target

Table 2
Results of testing the true number of factors using different procedures

k [a]	IND	R	M-F	RSD-F
1	0.013833	0.136	3.23	-
2	0.001909	0.964	212.34	128.20
3	0.002036	1.007	2.70	2.78
4	0.002786	1.003	2.04	2.70
5	0.005185	1.001	2.74	4.62

[a] k denotes the number of the factor being tested.

Table 3
Target testing for unity and log P

Drug	Unity		Log P	
	Input	Prediction	Input	Prediction
Pindolol	1	0.949	1.02	0.90
Propranolol	1	0.981	3.39	3.34
Oxprenolol	1	1.024	2.68	2.72
Acebutolol	1	0.968	1.55	1.44
Alprenolol	1	1.011	3.20	3.22
Atenolol	1	1.042	0.27	0.37
Metoprolol	1	1.018	2.04	2.14
	SPOIL = 0.751		SPOIL = 0.816	
	Successful targeting		Successful targeting	

Table 4
Factor loadings for unity (a) and log P (b) based on covariance, for the correlation log $k' = a + b$ log P in each chromatographic system

Column	a	b
I	−0.56(1) [a]	0.256(7)
II	−0.33(4)	0.33(2)
III	0.36(1)	0.188(5)
IV	−0.97(2)	0.273(9)
V	−0.76(2)	0.253(9)
VI	−1.40(4)	0.46(2)

[a] Values between parentheses refer to the standard deviation associated with the last figure.

factor analysis (TFA) (González-Arjona et al., 1994) was then utilized to decide whether the model equation, Eq. 1, is adequate. Therefore, two target factors were selected: unity to account for the unity coefficient multiplier of the a term in Eq. 1, and the log P values obtained. The criterion for accepting a target is based on the similarity between the target factor and the predicted one. The SPOIL function (Malinowski, 1991) is the most reliable proof for this purpose. A value of SPOIL less than 3.0 indicates an excellent prediction. Table 3 lists the results of target testing for the targets unity and log P. As can be observed both targets were accepted. The reproduction of the data matrix with these two factors gives an RMS < 0.03, an excellent precision value.

These results imply that Eq. 1 is accomplished, and accordingly, a strong correlation between log k' and log P exists. The final result of the treatment is to obtain the values of a and b of Eq. 1 for each column. However, they should not be calculated from a series of individual regression models, but the matrix of factor loadings based on covariance (Sindreu et al., 1994). The factor loadings for unity and log P are collected in Table 4. For the sake of information, the correlation coefficient of any individual correlation was higher than 0.99.

5. Conclusions

From this study, the log k', a readily measurable quantity, in RP-HPLC stationary phases (octadecylsilane and poly(styrene-divinylbenzene) materials) gives a good indication of the lipophilicity/hydrophobicity of the studied β-blockers, an excellent distinguishing property for them. Very hydrophilic ones are eliminated from kidney and exert long-lasting action. In contrast, lipophilic β-blocking agents are rapidly metabolized in liver. The latter are prone to causing sleeplessness, nightmares and other central secondary effects as a result of the ability to penetrate the haematoencephalic barrier easily. The hydrophobic sequence obtained from retention is in good agreement with the metabolic elimination pathway of the β-blockers: Thus, propranolol, alprenolol, oxprenolol and metoprolol with relative high values of log k' are eliminated from liver and conversely, acebutolol, pindolol and atenolol with low log k' values, are eliminated from kidney.

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