Journal of Chromatography A, 1218 (2011) 6356–6368

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Lipophilicity assessment of basic drugs ($\log P_{o/w}$ determination) by a chromatographic method

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ARTICLE INFO

Article history:
Received 11 May 2011
Received in revised form 30 June 2011
Accepted 1 July 2011
Available online 8 July 2011

Keywords:
Hydrophobicity of basic drugs
log *P*o/w of basic drugs
HPLC
Polarity model
QSPR model

ABSTRACT

A previously reported chromatographic method to determine the 1-octanol/water partition coefficient ($\log P_{o/w}$) of organic compounds is used to estimate the hydrophobicity of bases, mainly commercial drugs with diverse chemical nature and pK_a values higher than 9. For that reason, mobile phases buffered at high pH to avoid the ionization of the solutes and three different columns (Phenomenex Gemini NX, Waters XTerra RP-18 and Waters XTerra MS C_{18}) with appropriate alkaline-resistant stationary phases have been used. Non-ionizable substances studied in previous works were also included in the set of compounds to evaluate the consistency of the method. The results showed that all the columns provide good estimations of the $\log P_{o/w}$ for most of the compounds included in this study. The Gemini NX column has been selected to calculate $\log P_{o/w}$ values of the set of studied drugs, and really good correlations between the determined $\log P_{o/w}$ values and those considered as reference were obtained, proving the ability of the procedure for the lipophilicity assessment of bioactive compounds with very different structures and functionalities.

1. Introduction

The ability to cross biological membranes affects the pharmacokinetic behaviour of drugs and their capability to access the receptor site. The reference property to predict passive diffusion of drugs through biological barriers is the hydrophobicity, commonly expressed as the 1-octanol/water partition coefficient ($\log P_{o/w}$) [1–6].

The reference procedure for determining the $\log P_{o/w}$ is the shake-flask method [7,8]. However, it is highly time-consuming, narrow in applicability range and needs relatively high amounts of sample. Potentiometric methods [9–12] are a good alternative in the case of acidic or basic compounds, but they also require relatively high quantities of sample. It is also possible to get estimations of the $\log P_{o/w}$ from several software packages (e.g., ACD-Labs, ClogP, AlogPs) but different values can be obtained, depending on the software, because each one uses a different algorithm [13].

Reversed-phase high-performance liquid chromatography (RP-HPLC) came to be a good alternative to the methods mentioned above because of its high throughput ability (essential in the pharmaceutical industry because of the high number of potential drug candidates), insensitivity to impurities or degradation products, low sample consumption, good accuracy, broad dynamic range, and on-line detection. In RP-HPLC, the lipophilicity indexes are commonly derived from the logarithm of the retention factor, $\log k$:

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right) \quad (1)$$

where t_r and t_0 are the retention times of the solute and an unretained compound, respectively.

Isocratic $\log k$ has been considered as a direct measure of the hydrophobicity, although most investigators use the retention factors extrapolated to pure water ($\log k_w$) in order to eliminate the effects of organic solvent and make them comparable between chromatographic systems. Several correlations between $\log k_w$ and $\log P_{o/w}$ have been also reported [14]. Some researchers have studied the performance of the addition of 1-octanol to the mobile phase in order to mimic the 1-octanol/water partitioning and improve the correlations mentioned before [15–22]. Another lipophilicity parameter described in the literature is the chromatographic hydrophobicity index (CHI) [23,24], which permits a convenient estimation of the hydrophobicity for non-ionizable compounds, although a right interpretation of the results is more difficult in the case of acids and bases [25,26].

Nowadays, most of the commercial drugs are bases, and lipophilicity assessment is essential in their development. However, the determination of 1-octanol/water partition coefficients can be difficult because of a high pK_a, which affects the applicability of potentiometric methods and also the use of HPLC methods. In this

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doi:10.1016/j.chroma.2011.07.002

last case, the lipophilicity assessment requires the use of columns stable in alkaline media [20,22,27]. Moreover, the pH value of the buffered mobile phase and the pK_a of the solutes in the mobile phase have to be taken into account [28], in both isocratic and gradient elution measurements. The control of pH is especially critical in gradient elution [16] because of the continuous change in the composition and pH of the mobile phase, which favours the existence of different fractions of acidic and basic forms during the chromatographic run [26].

The purpose of this work is to extend the previously proposed method [29] to the determination of the 1-octanol/water partition coefficient ($\log P_{o/w}$) of strongly basic compounds (p$K_a > 9$), because most of pharmaceuticals belong to this kind of chemicals. Thus, it is of great interest to provide a useful tool to measure the hydrophobicity of these basic drugs. The careful selection of experimental conditions plays here a very important role because they have to ensure that the solutes are in neutral form in the measurement conditions.

1.1. The chromatographic method used to determine $\log P_{o/w}$

The reported method [29] is based on the polarity model [30–34] that describes retention in RP-HPLC as a function of the polarity of the neutral solute, p, the polarity of the mobile phase (P_m^N) and the polarity of the stationary phase (P_s^N), according to Eq. (2):

$$\log k = (\log k)_0 + p(P_m^N - P_s^N) \quad (2)$$

where $(\log k)_0$ stands for the intercept of the $\log k$ vs. $(P_m^N - P_s^N)$ correlation. P_m^N is calculated through expressions that depend only on the organic solvent fraction in the mobile phase. In this work, acetonitrile was used as organic modifier, so the polarity of the mobile phase is calculated according to Eq. (3):

$$P_m^N = 1.00 - \frac{2.13\varphi}{1 + 1.42\varphi} \quad (3)$$

where φ stands for the acetonitrile volume fraction in the mobile phase. The p value can be easily determined in a characterized chromatographic system (i.e. when $(\log k)_0$ and P_s^N are known [32,33]) with a single isocratic run. Thus, the p values in the working system (column, organic modifier) have to be transferred to the reference chromatographic system (Waters Spherisorb ODS-2 column and acetonitrile as organic modifier), by means of the linear relationships:

$$p_{working} = a p_{reference} + b \quad (4)$$

Once this p value is expressed in the reference system, $p_{reference}$, the 1-octanol/water partition coefficient is determined by applying a Quantitative Structure–Property Relationship model [35] that relates it to the polarity of the solute ($p_{reference}$) and four descriptors directly derived from the structure of the compound:

$$\log P_{o/w} = 1.22 p_{reference} + 1.89(\text{HDCA-2}) - 0.17(\text{HOMO–LUMO}) + 1.98(\text{pol/d}^2) - 1.27 \times 10^3(\text{DPSA-1}) - 0.99 \quad (5)$$

These descriptors encode different information. HDCA-2 is the hydrogen bond acidity descriptor, pol/d^2 is related to the molecular polarity, HOMO–LUMO belongs to the molecular polarizability and DPSA-1 encodes the features responsible for polar interactions between molecules.

2. Experimental

2.1. Apparatus and columns

For the chromatographic measurements, a Shimadzu (Kyoto, Japan) HPLC system equipped with two LD-20AD isocratic pumps, a SIL-10Avp autoinjector, a DGU-20A$_5$ degasser, a CTO-10ASvp oven thermostatized at 25 °C, a SPD-M20A diode array detector and a CBM-20Alite controller was used.

pH measurements were taken with a combined Crison 5014 electrode (Crison Instruments, Alella, Spain) in a Crison pH meter GLP 22 potentiometer. The electrode system was standardized with the ordinary aqueous buffers of pH 7.00 and 9.21 also from Crison.

Retention data were obtained using three columns: a Gemini NX column from Phenomenex (Torrance, CA, USA), a XTerra RP-18 and a XTerra MS C_{18} columns from Waters (Milford, MA, USA). Their characteristics are given in Table 1.

2.2. Chemicals

Acetonitrile HPLC gradient grade was purchased from VWR (West Chester, PA, USA). Water was purified by the Milli-Q® plus system from Millipore (Billerica, MA, USA) with a resistivity of 18.2 MΩ. The solutes studied were reagent grade or better, and were obtained from Merck (Darmstadt, Germany), Fluka (Steinheim, Germany), Sigma–Aldrich (Steinheim, Germany), Baker (Deventer, Netherlands), Riedel de Haën (Seelze, Germany) and Carlo Erba (Milano, Italy). For buffer preparation, pyrrolidine, redistilled (>99.5%) from Sigma–Aldrich (Steinheim, Germany) and ammonium hydrogencarbonate, p.a., from Fluka (Steinheim, Germany) were used.

2.3. Chromatographic procedure

The mobile phases used were mixtures of acetonitrile and aqueous buffers adjusted at pH 11.0 in order to have the solutes in their neutral form. Acetonitrile/buffer mixtures with a volume fraction of 40 or 50% of organic modifier were used. Two different buffers were prepared at pH 11.0: pyrrolidine 0.01 M, which is a cationic buffer ($PyrH^+$/Pyr), and ammonium hydrogencarbonate 0.01 M, which is an anionic buffer (HCO_3^-/CO_3^{2-}), to compare the performance of the buffers in the same chromatographic conditions.

The compounds were solved in methanol, and the injection volume was 10 μL. Potassium bromide was used as the void volume marker. The flow rate was 1 mL min^{-1} for all the measurements. Retention data were expressed as the logarithm of the retention factor as defined by Eq. (1). All measurements were taken in triplicate.

2.4. Structural descriptors and calculations

Descriptors were calculated from the structure of each compound. The structures were drawn using HyperChem Lite software (HyperCube, Gainesville, USA). The geometrical optimization of the structures was done with MOPAC 6.0 and AuxQSPR program in order to obtain the energy minimum. These programs provided files prepared to calculate the numeric values of the structural descriptors using CODESSA software (University of Florida, USA). Microsoft Excel was used to perform all the calculations involved in this work.

3. Results and discussion

3.1. Physico-chemical data and molecular descriptors of the selected compounds

The set of 58 studied compounds comprises 20 non-ionizable and 38 basic compounds, including 26 commercial drugs which are shown in Fig. 1. Some of the substances were already studied in the previous work [29] and have been included in this set in order to verify the consistency of the method by comparison of the values obtained before. The studied compounds, their pK_a and the reference $\log P_{o/w}$ values, as well as their structural descriptors values are shown in Table 2. The reference $\log P_{o/w}$ values were

Table 1
Characteristics of the studied columns.

Features	Phenomenex Gemini NX	Waters XTerra RP-18	Waters XTerra MS C_{18}
Structure type	Particulate	Particulate	Particulate
Particle size (μm)	5	5	5
Average pore diameter (Å)	105	127	125
Surface area ($m^2\ g^{-1}$)	372	178	179
Pore volume ($cm^3\ g^{-1}$)	1.10	0.70	0.69
Total carbon (%)	14.0	14.6	15.4
Endcapped	Yes	Yes	Yes
Column length (mm)	150	150	150
Column diameter (mm)	4.6	4.6	4.6
pH stability range	1–12	2–12	1–12

Table 2
Physico-chemical data of the studied compounds: reference log $P_{o/w}$, aqueous pK_a ($^{w}_{w}pK_a$) and molecular descriptor values.

Compound	#	CAS	Reference log $P_{o/w}$	$^{w}_{w}pK_a$	Molecular descriptors			
					pol/d^2	HDCA-2	HOMO–LUMO	DPSA-1
1,2,4-Trimethylbenzene	**1**	95-63-6	**3.63**	–	0.01	0.00	9.48	193.12
1,4-Dimethylbenzene	**2**	106-42-3	**3.15**	–	0.00	0.00	9.55	149.12
1-Phenylethylamine	**3**	64-04-0	**1.41**	9.74	0.17	0.22	9.95	162.03
1-Phenylpiperazine	**4**	92-54-6	**1.11**	9.02	0.16	0.30	8.99	199.18
2,4-Dinitroaniline	**5**	97-02-9	**1.90**	4.53	0.03	0.18	8.45	21.33
2,6-Dimethylaniline	**6**	87-62-7	**1.84**	3.95	0.16	0.19	8.95	161.05
2-Aminopyridine	**7**	504-29-0	**0.49**	6.68	0.16	0.50	9.06	49.92
3,4-Dichloroaniline	**8**	95-76-1	**2.69**	2.90	0.01	0.23	8.74	170.81
4-Aminopyridine	**9**	504-24-5	**0.32**	9.11	0.17	0.45	9.31	43.03
4-Chloroaniline	**10**	106-47-8	**1.83**	3.98	0.17	0.23	8.79	−7.62
4-Nitrotoluene	**11**	99-99-0	**2.37**	–	0.02	0.00	9.26	35.88
Acebutolol	**12**	37517-30-9	**1.71**	9.41	0.28	0.95	8.31	332.95
Acenaphthene	**13**	83-32-9	**3.92**	–	0.00	0.00	8.28	110.83
Acetophenone	**14**	98-86-2	**1.58**	–	0.11	0.10	9.58	52.26
Allylamine	**15**	107-11-9	**0.07**	9.49	0.17	0.23	10.85	128.53
Alprenolol	**16**	13655-52-2	**2.89**	9.60	0.04	0.51	9.63	284.37
Amitriptyline	**17**	50-48-6	**4.92**	9.42	0.03	0.09	9.02	315.78
Aniline	**18**	62-53-3	**0.90**	4.60	0.17	0.22	8.97	81.48
Atenolol	**19**	29122-68-7	**0.16**	9.60	0.04	1.00	9.27	254.85
Atropine	**20**	51-55-8	**1.83**	9.60	0.28	0.49	9.37	234.36
Benzamide	**21**	55-21-0	**0.64**	–	0.11	0.51	9.73	25.85
Benzene	**22**	71-43-2	**2.13**	–	0.00	0.00	10.20	51.14
Benzofuran	**23**	271-89-6	**2.67**	–	0.05	0.00	8.95	23.51
Benzophenone	**24**	119-61-9	**3.18**	–	0.11	0.00	9.44	36.98
Benzylamine	**25**	100-46-9	**1.09**	9.33	0.17	0.23	9.77	113.81
Biphenyl	**26**	92-52-4	**4.01**	–	0.00	0.00	8.55	27.34
Bromobenzene	**27**	108-86-1	**2.99**	–	0.01	0.00	9.66	110.11
Bupivacaine	**28**	2180-92-9	**3.41**	8.10	0.03	0.29	9.33	358.21
Butylbenzene	**29**	104-51-8	**4.38**	–	0.00	0.00	9.83	200.53
Butyrophenone	**30**	495-40-9	**2.66**	–	0.11	0.09	9.58	141.07
Chlorobenzene	**31**	108-90-7	**2.89**	–	0.05	0.00	9.72	−34.53
Chlorpromazine	**32**	50-53-3	**5.35**	9.21	0.00	0.24	7.66	176.40
Chrysene	**33**	281-01-9	**5.81**	–	0.00	0.00	7.70	11.05
Clonidine	**34**	4205-90-7	**1.43**	8.05	0.02	0.59	8.83	124.94
Cyproheptadine	**35**	129-03-3	**4.92**	8.87	0.03	0.10	8.30	267.18
Diphenhydramine	**36**	58-73-1	**3.27**	9.02	0.05	0.11	9.50	250.95
Ephedrine	**37**	299-42-3	**0.93**	9.59	0.28	0.50	9.90	193.57
Imipramine	**38**	50-49-7	**4.80**	9.40	0.01	0.14	8.84	311.09
Lidocaine	**39**	137-58-6	**2.21**	7.84	0.03	0.30	9.27	285.83
Maprotiline	**40**	10262-69-8	**4.85**	10.20	0.17	0.19	9.49	300.26
Mepivacaine	**41**	96-88-8	**1.95**	7.92	0.03	0.42	9.38	278.55
Metoprolol	**42**	37350-58-6	**1.88**	9.56	0.00	0.52	9.30	352.47
N,N-dimethylbenzylamine	**43**	103-83-3	**1.98**	8.80	0.03	0.10	9.64	192.43
Nadolol	**44**	42200-33-9	**0.71**	9.39	0.01	1.28	9.63	301.22
Naphthalene	**45**	91-20-3	**3.28**	–	0.00	0.00	8.45	44.33
Nortriptyline	**46**	72-69-5	**4.04**	10.11	0.17	0.19	8.91	277.05
Oxprenolol	**47**	6452-71-7	**2.10**	9.32	0.01	0.51	9.18	280.89
Penbutolol	**48**	36507-48-9	**4.06**	9.40	0.01	0.53	9.36	387.58
Phenanthrene	**49**	85-01-8	**4.47**	–	0.00	0.00	8.21	12.83
Pilocarpine	**50**	92-13-7	**0.00**	7.07	0.12	0.35	9.75	121.28
Procainamide	**51**	51-06-9	**0.98**	9.38	0.01	0.71	8.78	278.22
Propiophenone	**52**	93-55-0	**2.19**	–	0.11	0.10	9.58	110.56
Propranolol	**53**	525-66-6	**2.98**	9.47	0.04	0.46	8.27	228.52
Propylbenzene	**54**	103-65-1	**3.72**	–	0.00	0.00	9.83	171.48
Quinine	**55**	130-95-0	**2.64**	9.01	0.01	0.71	8.43	251.34
Toluene	**56**	108-88-3	**2.73**	–	0.11	0.11	9.77	173.88
Trazodone	**57**	19794-93-5	**3.80**	6.73	0.02	0.67	7.96	181.08
Trimethoprim	**58**	738-70-5	**0.91**	7.12	0.00	0.85	8.70	250.54

the ones recommended from the experimental data compiled in BioLoom on-line database [36], and they are between 0.00 and 5.81. The pK_a values of the compounds were obtained from the same database and they are in the range from 2.90 to 10.20, including 23 basic compounds with pK_a > 9.

3.2. Chromatographic system characterization

The characterization of a chromatographic system, i.e., the determination of $(\log k)_0$ and P_s^N parameters, is widely described in the literature [29,32–34]. The systems are characterized from

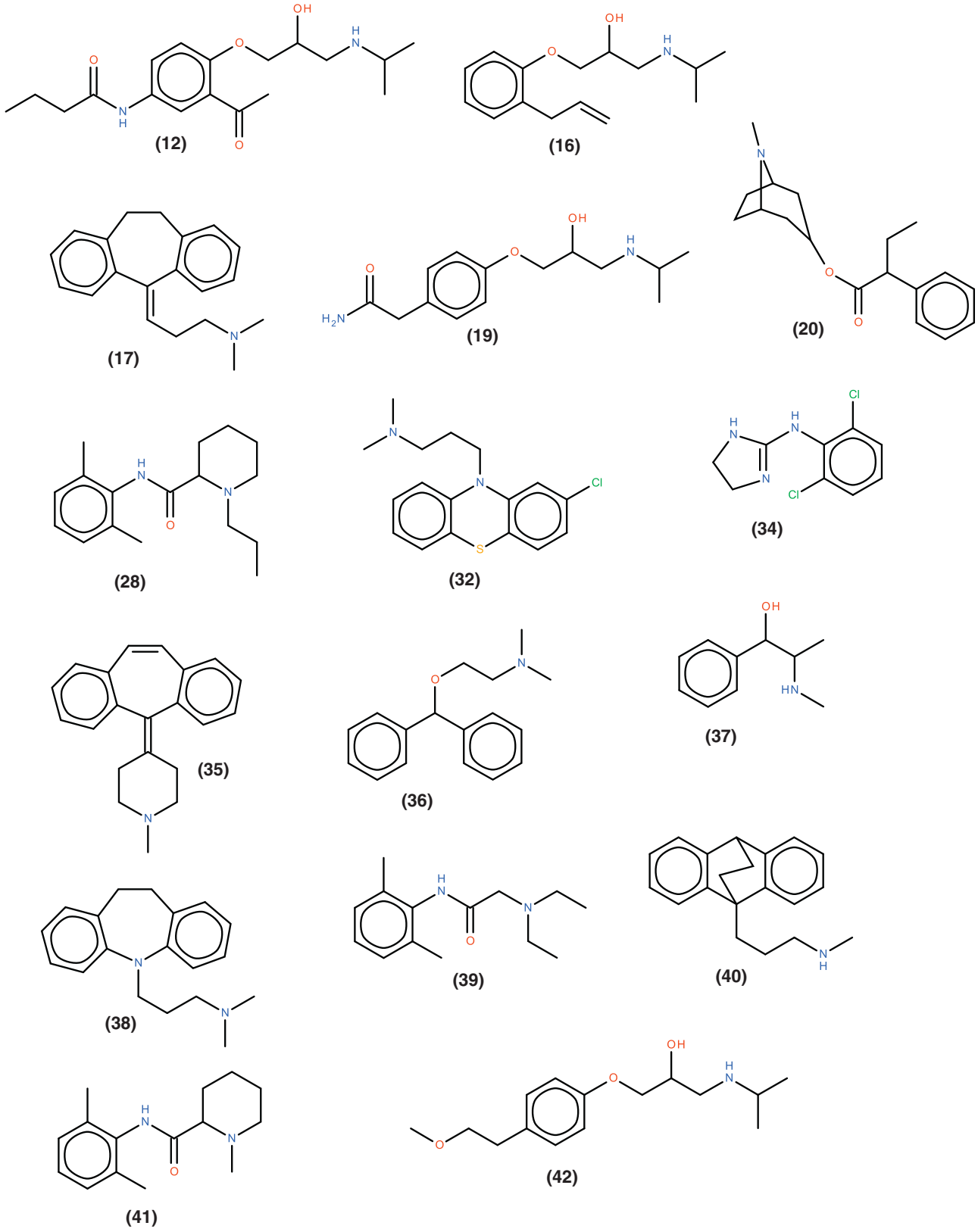

Fig. 1. Structures of the new 26 drugs studied in this work. Numbers at the bottom refer to compounds in Table 2.

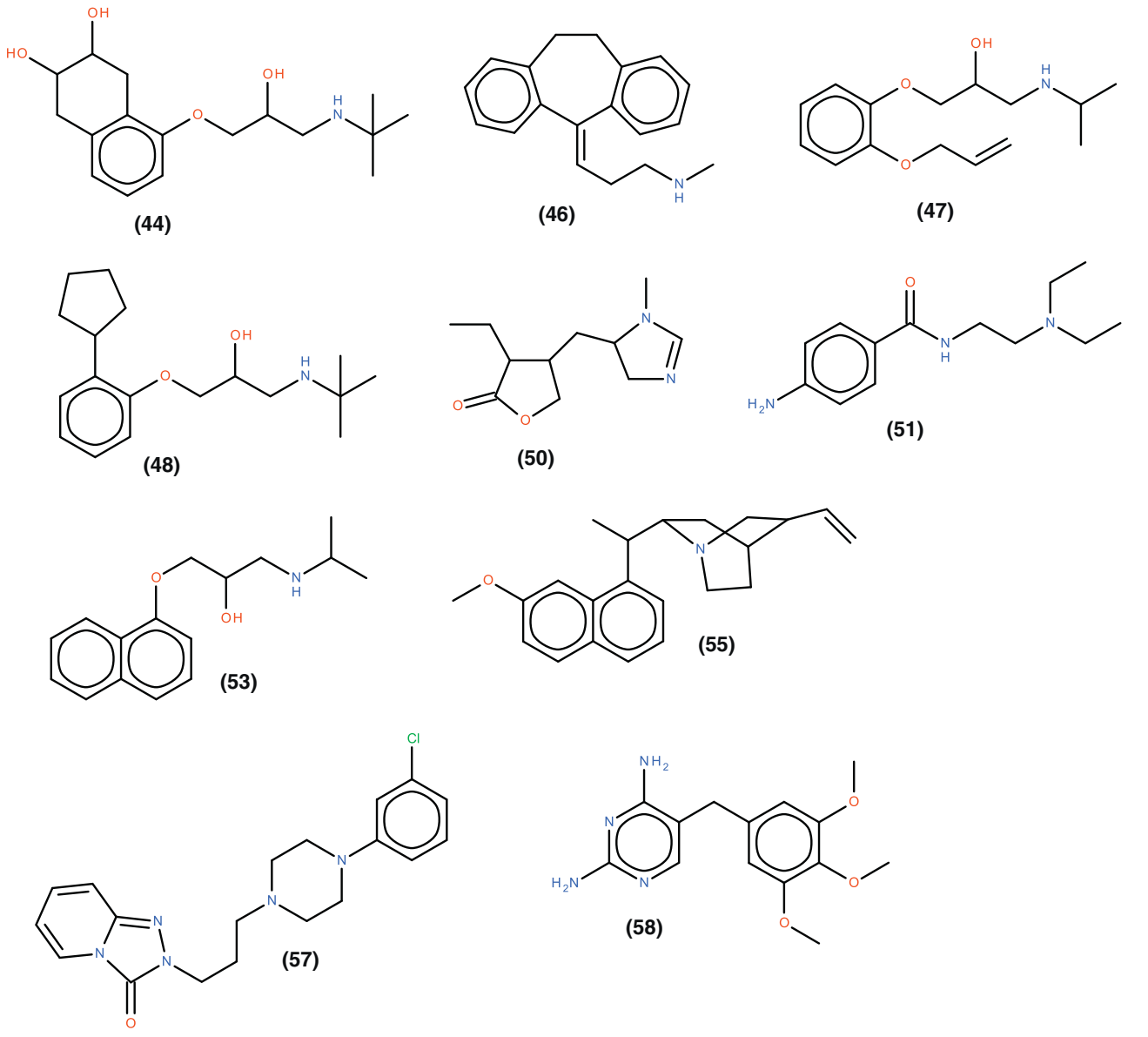

Fig. 1. (Continued)

the retention factors of 12 standard substances at several mobile phase compositions. Phenomenex Gemini NX and both Waters XTerra columns have been characterized and the obtained parameters are shown in Table 3, which also shows the transference parameters between chromatographic systems according to Eq. (4).

Table 3
Polarity parameters of the chromatographic systems and parameters of the transference between the working systems to the reference system, where *a* is the slope and *b* the intercept of the linear regression (Eq. (4)).

	Phenomenex Gemini NX	Waters XTerra RP-18	Waters XTerra MS C_{18}
Polarity parameters (acetonitrile)			
$(\log k)_0$	−0.88	−0.35	−0.48
P_N^S	−0.03	−0.06	−0.01
Transference parameters			
a	0.86	0.78	0.85
b	0.21	−0.25	−0.22
R^2	0.98	0.99	0.99

3.3. Mobile phase pH and compound pK_a

The variation of the pH of the mobile phases and the pK_a of the solutes with the organic modifier fraction has been widely discussed [28,37]. The pH variation depends on the type of the buffer and the organic modifier. Thus, the pH of mobile phases increases with the addition of organic modifier for neutral or anionic buffers but it decreases, in a minor degree, when cationic buffers are used, and the same occurs with the pK_a of compounds. The pK_a of any compound in a specific acetonitrile-water mixture (until 60% of acetonitrile) can be easily calculated from the equations already proposed [28]. These pH and pK_a variations have to be taken into account in order to make sure that there is no ionization of the solutes in the chromatographic conditions of the measurements. Two different buffers were used: pyrrolidine and carbonate, both at aqueous pH 11. The variation of the pH of the buffered mobile phases with the acetonitrile fraction is shown in Table 4. As expected, the pH of mobile phases buffered with pyrrolidine shows a slight decrease when increasing the acetonitrile content, while the pH of those buffered with carbonate increases with the

Table 4
pH variation of the aqueous buffers used in this work with the acetonitrile fraction in the mobile phase.

Buffer	${}^{s}_{w}$pH at several acetonitrile volume fractions				
	0%	30%	40%	50%	60%
Pyrrolidine	10.99	10.75	10.63	10.57	10.45
Carbonate	11.00	11.36	11.73	11.85	11.88

addition of acetonitrile. It must be pointed out that the symbol ${}^{s}_{w}$pH refers to the mobile phase pH measured after the addition of organic modifier (in this case, acetonitrile) to the aqueous buffer by means of a glass electrode standardized with ordinary aqueous buffers.

3.4. Determined log $P_{o/w}$ values

The 1-octanol/water partition coefficients of the compounds shown in Table 2 have been determined according to the method explained in the introduction section using the Phenomenex Gemini NX, Waters XTerra RP-18 and XTerra MS C_{18} columns. Mobile phases containing 40% or 50% of acetonitrile were used since they provide the best results in terms of accuracy and run time [29]. As explained before, mobile phases were buffered at aqueous pH 11 with pyrrolidine or carbonate. No significant difference between the results obtained with each buffer was observed, despite the diversity in the behaviour of the pH of the buffered solutions with the addition of acetonitrile (Table 4). This means that the pH of the different mobile phases is high enough to have the bases completely in their neutral form, regardless of the different buffer pH variation.

The correlations between the determined and reference log $P_{o/w}$ values are illustrated in Figs. 2–4. For each column, mobile phase composition and buffer, the number of compounds, the average of the differences between the determined and reference log $P_{o/w}$ values (residual average), as well as the number of outliers, are given in Table 5. As in the previous work, absolute values of residuals higher than 0.6 units have been taken as outliers. It should be pointed out that, in all instances, most outliers are hydrophilic compounds, whose retention times are close to the void time. In this instance, slight errors in the experimental measurements may involve significant errors in log k calculations and, hence, in $p_{reference}$ and log $P_{o/w}$ calculated values.

The covered log $P_{o/w}$ range obtained depends mostly on the retention time of the most hydrophobic substances, as reten-

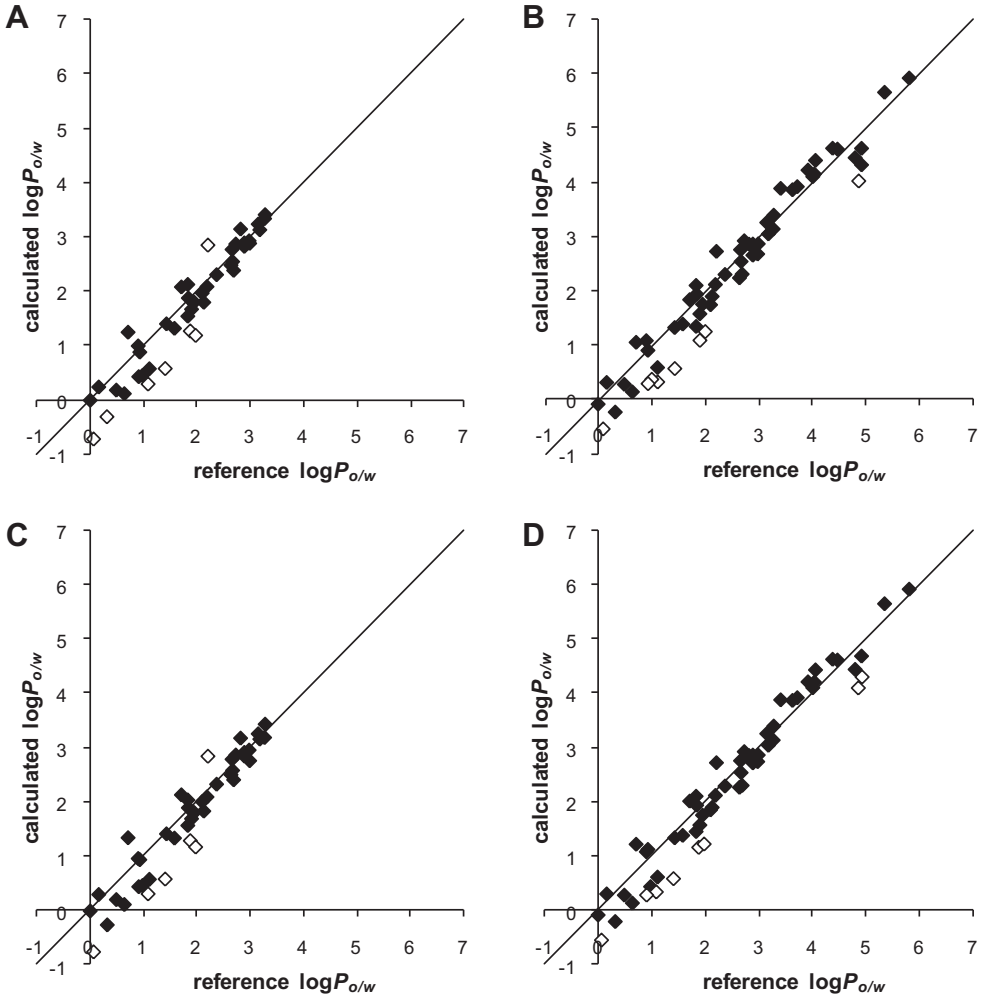

Fig. 2. Plots of calculated log $P_{o/w}$ vs. reference log $P_{o/w}$ values for Phenomenex Gemini NX column. (A) MeCN/carbonate buffer 40%; (B) MeCN/carbonate buffer 50%; (C) MeCN/pyrrolidine buffer 40%; (D) MeCN/pyrrolidine buffer 50%. Symbols: (♦) compounds; (◊) outliers.

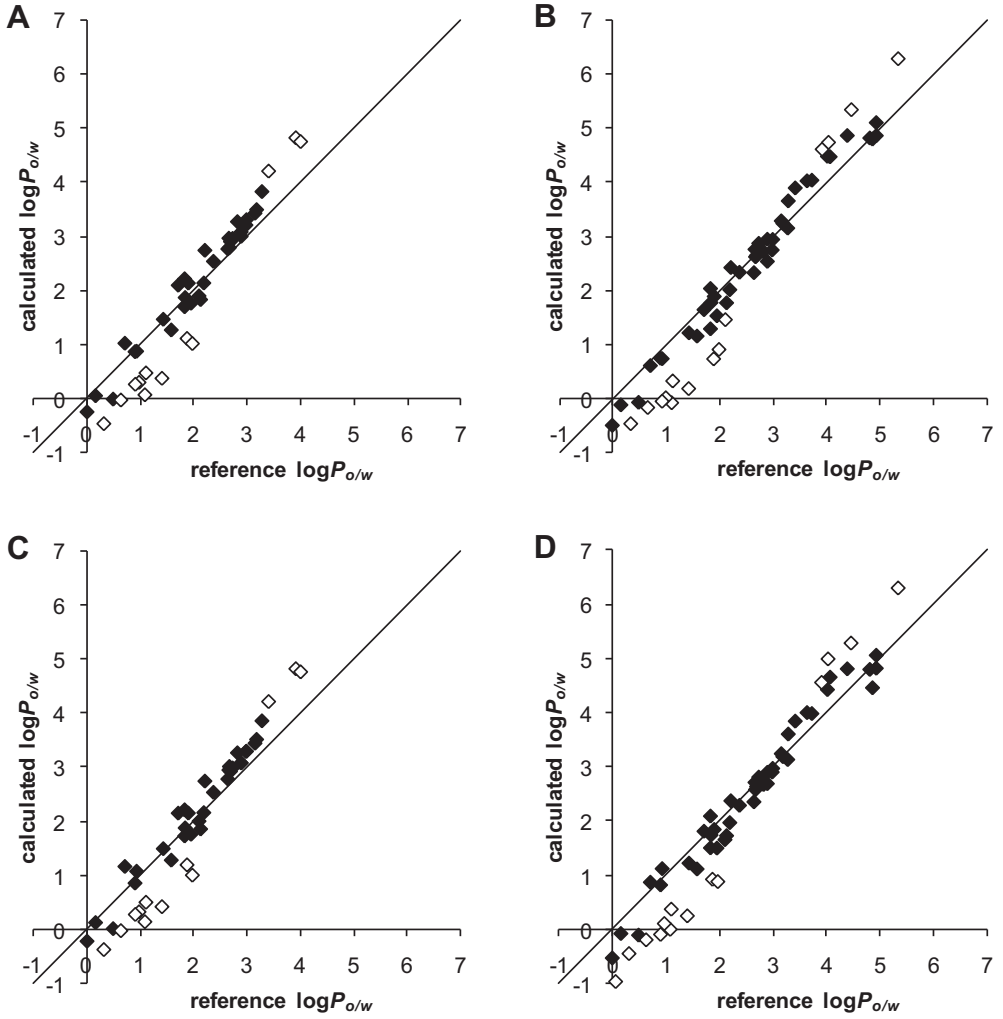

Fig. 3. Plots of calculated $\log P_{o/w}$ vs. reference $\log P_{o/w}$ values for Waters XTerra RP-18 column. (A) MeCN/carbonate buffer 40%; (B) MeCN/carbonate buffer 50%; (C) MeCN/pyrrolidine buffer 40%; (D) MeCN/pyrrolidine buffer 50%. Symbols: (♦) compounds; (◊) outliers.

tion times of approximately 45 min have been considered as long enough in these experiments. This has led to the following $\log P_{o/w}$ ranges: for mobile phases containing 40% of acetonitrile it is approximately between 0 and 3.5 and working with 50% of acetonitrile it is between 0 and 6.

Table 5 shows similar results for all the tested columns without any difference attributable to the buffer used in the mobile phase preparation. However, the number of outliers is higher for XTerra RP-18 column. According to the results shown in Table 5 and Figs. 2–4, Phenomenex Gemini NX column has been selected as typical column for further studies. For instance, in this column, the parameters of the correlation between calculated and reference $\log P_{o/w}$ values for the entire set of 58 compounds, working with 50% of acetonitrile and pyrrolidine buffer, are the following: slope, 1.06 (±0.03); intercept, −0.29 (±0.09); with $r^2 = 0.95$, the standard deviation is 0.37 and $F = 1158$.

In Table 6, the comparison of the final calculated $\log P_{o/w}$ values for a set of compounds previously studied with

Table 5
Number of compounds studied, average of the residuals ($\log P_{o/w}$ (calc.) − $\log P_{o/w}$ (ref.)) and number of outliers for column, buffer and acetonitrile fraction.

Column	Buffer	Acetonitrile fraction	n	Residual average	Outliers
Gemini NX	Carbonate	40%	43	−0.15	7
		50%	58	−0.14	8
	Pyrrolidine	40%	43	−0.16	6
		50%	58	−0.14	8
X Terra RP-18	Carbonate	40%	45	−0.05	13
		50%	58	−0.15	16
	Pyrrolidine	40%	45	−0.02	13
		50%	58	−0.11	15
X Terra MS C_{18}	Carbonate	40%	45	−0.06	5
		50%	58	−0.03	6
	Pyrrolidine	40%	45	−0.07	5
		50%	58	−0.08	11

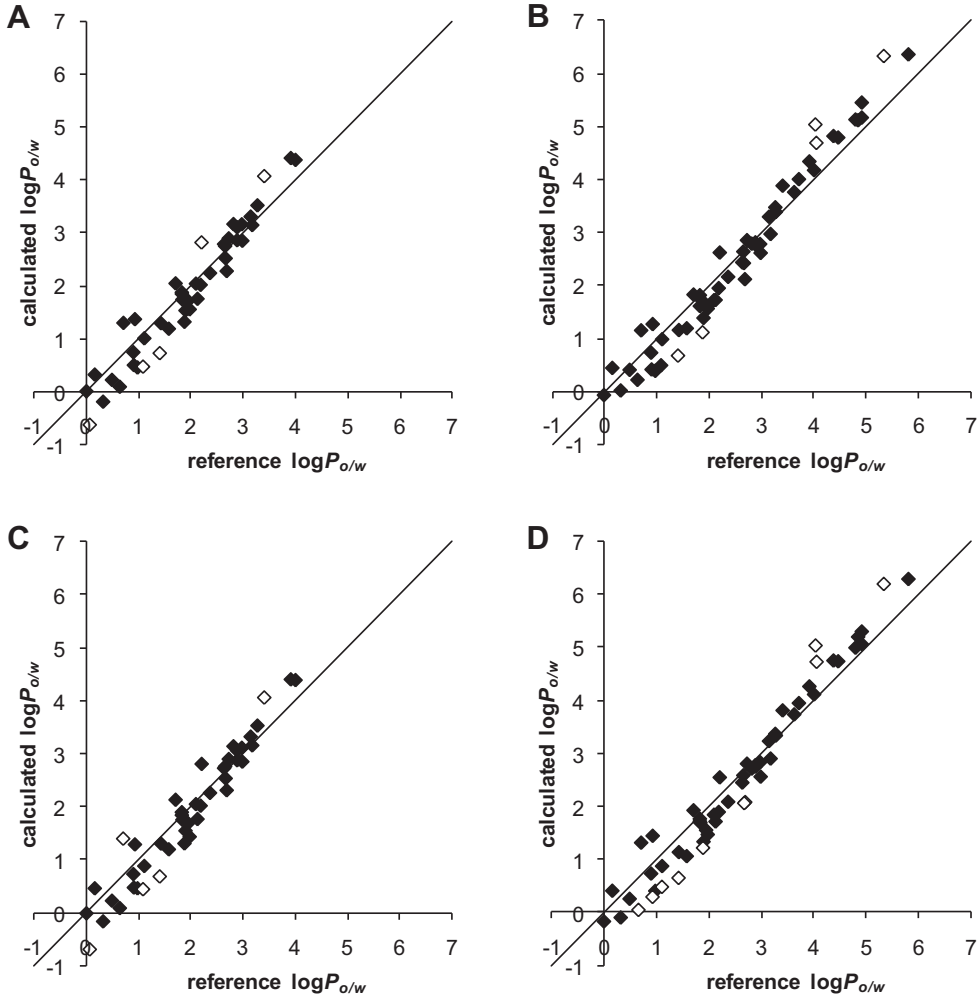

Fig. 4. Plots of calculated log $P_{o/w}$ vs. reference log $P_{o/w}$ values for Waters XTerra MS C_{18} column. (A) MeCN/carbonate buffer 40%; (B) MeCN/carbonate buffer 50%; (C) MeCN/pyrrolidine buffer 40%; (D) MeCN/pyrrolidine buffer 50%. Symbols: (♦) compounds; (◊) outliers.

Phenomenex Luna C_{18} (2) and Merck Chromolith Performance RP-18 columns [29] shows the consistency of results and, hence, the suitability of the Phenomenex Gemini NX column for log $P_{o/w}$ determinations.

3.5. Lipophilicity assessment of the studied drugs

Tables 7 and 8 illustrate the steps to obtain the log $P_{o/w}$ from the retention factor (log k) for the 26 drugs included in this study,

Table 6
Comparison between log $P_{o/w}$ values obtained in three different chromatographic systems.

Compound	Luna C_{18} (2), MeCN/water 50%	Chromolith RP-18, MeCN/water 40%	Gemini NX, MeCN/water 50%
1,2,4-Trimethylbenzene	–	3.70	3.86
1,4-Dimethylbenzene	3.16	3.27	3.26
2,4-Dinitroaniline	1.53	–	1.57
2,6-Dimethylaniline	1.93	1.88	1.93
3,4-Dichloroaniline	2.27	2.29	2.30
4-Chloroaniline	1.98	2.29	2.09
4-Nitrotoluene	2.15	1.99	2.30
Aniline	1.06	0.97	1.07
Benzamide	0.09	0.42	0.13
Benzene	1.79	1.86	1.89
Biphenyl	3.96	4.18	4.10
Butylbenzene	4.52	4.61	4.63
Butyrophenone	2.60	2.73	2.75
Chlorobenzene	2.73	2.84	2.86
Chrysene	–	6.22	5.92
Naphthalene	3.28	3.33	3.39
Phenanthrene	4.63	4.64	4.61
Propiophenone	1.98	2.09	2.11
Propylbenzene	3.80	3.89	3.92
Toluene	2.48	2.56	2.85

Table 7
$\log P_{o/w}$ calculation from retention factor $\log k$. Phenomenex Gemini NX column, mobile phase MeCN/pyrrolidine buffer 40%. Residual column: $\log P_{o/w}$ (calc.) − $\log P_{o/w}$ (ref.).

Compound	$\log k$	$p_{reference}$	calc. $\log P_{o/w}$	Residual
Atenolol	−0.36	0.99	0.28	0.12
Trimethoprim	−0.22	1.32	0.43	−0.48
Pilocarpine	−0.12	1.55	−0.02	−0.02
Nadolol	−0.12	1.56	1.33	0.62
Procainamide	−0.11	1.58	0.45	−0.53
Ephedrine	0.04	1.92	0.92	−0.01
Acebutolol	0.12	2.12	2.11	0.40
Clonidine	0.23	2.37	1.40	−0.03
Atropine	0.25	2.42	1.55	−0.28
Metoprolol	0.37	2.71	1.27	−0.61
Quinine	0.56	3.17	2.48	−0.16
Mepivacaine	0.57	3.18	1.79	−0.16
Oxprenolol	0.58	3.21	1.99	−0.11
Trazodone	0.75	3.63	3.15	0.33
Propranolol	0.84	3.83	2.93	−0.05
Alprenolol	0.90	3.97	2.89	0.00
Lidocaine	1.00	4.20	2.82	0.61
Diphenhydramine	1.22	4.74	3.16	−0.11

being the retention data lower than 45 obtained with mobile phases containing 40 or 50% of acetonitrile with pyrrolidine buffer at pH 11 and using the Gemini NX column. From these data, the following correlations between the determined and the reference $\log P_{o/w}$ values were obtained (Eq. (6) from data given in Table 7 and Eq. (7) from those in Table 8):

$$\log P_{o/w}(\text{calc.}) = 1.01(\pm 0.05)\log P_{o/w}(\text{ref.}) - 0.04(\pm 0.18),$$
$$n = 18; \quad r^2 = 0.89; \quad \text{SD} = 0.36; \quad F = 126 \qquad (6)$$

$$\log P_{o/w}(\text{calc.}) = 0.98(\pm 0.05)\log P_{o/w}(\text{ref.}) - 0.06(\pm 0.15),$$
$$n = 26; \quad r^2 = 0.94; \quad \text{SD} = 0.39; \quad F = 399 \qquad (7)$$

The slope of the correlations is 1 and the intercept is 0. Thus, the accuracy of the method is very good and it provides directly the $\log P_{o/w}$ values of the drugs, with a precision lower than 0.4 logarithmic units, shown as the standard deviation of the correlations. These correlations are plotted in Fig. 5.

To evaluate, from a general point of view, the validity, the reproducibility and the robustness of the proposed chromatographic method to estimate the hydrophobicity of drugs, the determined $\log P_{o/w}$ values of bioactive compounds published in the previous work [29] were added to those obtained in this study, and they are all shown in Table 9. The set of bioactive compounds include compounds with different structures and functional groups: non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or naproxen, anesthesics like bupivacaine or mepivacaine, antidepressants such as maprotiline or notriptyline, antihistaminics like cyproheptadine, β-blockers like alprenolol or propranolol and others. The correlation obtained by comparison of the calculated values vs. the reference values for each compound,

Table 8
$\log P_{o/w}$ calculation from retention factor $\log k$. Phenomenex Gemini NX column, mobile phase MeCN/pyrrolidine buffer 50%. Residual column: $\log P_{o/w}$ (calc.) − $\log P_{o/w}$ (ref.).

Compound	$\log k$	$p_{reference}$	calc. $\log P_{o/w}$	Residual
Atenolol	−0.44	0.99	0.28	0.12
Trimethoprim	−0.37	1.20	0.28	−0.63
Nadolol	−0.28	1.45	1.20	0.49
Pilocarpine	−0.27	1.48	−0.11	−0.11
Procainamide	−0.24	1.55	0.42	−0.56
Acebutolol	−0.08	2.02	1.99	0.28
Ephedrine	−0.06	2.07	1.10	0.17
Clonidine	0.02	2.31	1.32	−0.11
Atropine	0.03	2.32	1.43	−0.40
Metoprolol	0.13	2.62	1.16	−0.72
Quinine	0.26	2.98	2.25	−0.39
Oxprenolol	0.30	3.08	1.83	−0.27
Mepivacaine	0.31	3.13	1.73	−0.22
Trazodone	0.40	3.36	2.83	0.01
Propranolol	0.50	3.66	2.72	−0.26
Alprenolol	0.55	3.81	2.70	−0.19
Lidocaine	0.66	4.11	2.70	0.49
Diphenhydramine	0.87	4.70	3.12	−0.15
Bupivacaine	1.03	5.15	3.86	0.45
Nortriptyline	1.05	5.21	4.19	0.15
Maprotiline	1.06	5.23	4.09	−0.76
Penbutolol	1.08	5.30	4.41	0.35
Cyproheptadine	1.17	5.57	4.29	−0.63
Imipramine	1.24	5.76	4.42	−0.38
Amitriptyline	1.34	6.04	4.66	−0.26
Chlorpromazine	1.43	6.31	5.63	0.28

Table 9
Summary of the log $P_{o/w}$ determination of bioactive compounds.

Compound		Reference log $P_{o/w}$	Column	MeCN Fraction (%)	Buffer pH	Calculated log $P_{o/w}$	Residual
1-Acetyl-2-isonicotinoil-hydrazine[a]	Antitubercular	**−0.87**	Luna C_{18} (2)	40	7	**−1.13**	−0.26
			Luna C_{18} (2)	50	7	**−1.14**	−0.27
1-Ethyl-2-ethylthio-1*H*-benzo[*d*]imidazole[a]	Antitubercular	**3.36**	Chromolith RP-18e	40	6	**2.82**	−0.54
			Chromolith RP-18e	50	6	**2.77**	−0.59
			Luna C_{18} (2)	40	7	**2.78**	−0.57
			Luna C_{18} (2)	50	7	**2.87**	−0.49
2-(2-Cyclohexylethylthio)-1*H*-benzo[*d*]imidazole[a]	Antitubercular	**4.94**	Luna C_{18} (2)	50	7	**4.55**	−0.38
			Chromolith RP-18e	40	6	**4.65**	−0.29
			Chromolith RP-18e	50	6	**4.40**	−0.54
4-Methylcyclohexanone-isonicotinoil-hydrazine[a]	Antitubercular	**0.63**	Luna C_{18} (2)	40	7	**0.57**	−0.06
			Luna C_{18} (2)	50	7	**0.57**	−0.06
Acebutolol	β-Blocker	**1.71**	Gemini NX	40	11	**2.11**	0.40
			Gemini NX	50	11	**1.99**	0.28
Aldrin	Insecticide	**6.50**	Chromolith RP-18e	60	3	**6.71**	0.21
Alprenolol	β-Blocker	**2.89**	Gemini NX	40	11	**2.89**	0.00
			Gemini NX	50	11	**2.70**	−0.19
Amitriptyline	Antidepressant	**4.92**	Gemini NX	50	11	**4.66**	−0.26
Atenolol	β-Blocker	**0.16**	Gemini NX	40	11	**0.28**	0.12
			Gemini NX	50	11	**0.28**	0.12
Atropine	Alkaloid	**1.83**	Gemini NX	40	11	**1.55**	−0.28
			Gemini NX	50	11	**1.43**	−0.40
Bupivacaine	Anesthesic	**3.41**	Gemini NX	50	11	**3.86**	0.45
Chlorpromazine	Antipsicotic	**5.35**	Gemini NX	50	11	**5.63**	0.28
Clonidine	Analgesic	**1.43**	Gemini NX	40	11	**1.40**	−0.03
			Gemini NX	50	11	**1.32**	−0.11
Cyclohexanone-isonicotinoil-hydrazine[a]	Antitubercular	**0.27**	Luna C_{18} (2)	40	7	**0.00**	−0.27
			Luna C_{18} (2)	50	7	**0.06**	−0.21
Cyclopentanone-isonicotinoil-hydrazine[a]	Antitubercular	**−0.62**	Luna C_{18} (2)	40	7	**−0.27**	0.35
			Luna C_{18} (2)	50	7	**−0.30**	0.32
Cyproheptadine	Antihistaminic	**4.92**	Gemini NX	50	11	**4.29**	−0.63
Diphenhydramine	Antihistaminic	**3.27**	Gemini NX	40	11	**3.16**	−0.11
			Gemini NX	50	11	**3.12**	−0.15
Ephedrine	Decongestant	**0.93**	Gemini NX	40	11	**0.92**	−0.01
			Gemini NX	50	11	**1.10**	0.17
Flurbiprofen	NSAID	**4.16**	Chromolith RP-18e	40	3	**3.90**	−0.26
			Chromolith RP-18e	50	3	**3.42**	−0.74
			Luna C_{18} (2)	40	3	**4.15**	−0.01
			Luna C_{18} (2)	50	3	**3.71**	−0.45
Ibuprofen	NSAID	**3.97**	Chromolith RP-18e	40	3	**4.48**	0.51
			Chromolith RP-18e	50	3	**4.10**	0.13
Imipramine	Antidepressant	**4.80**	Gemini NX 50	50	11	**4.42**	−0.38
Isoniazid	Antibiotic	**−0.65**	Luna C_{18} (2)	40	7	**−1.04**	−0.39
			Luna C_{18} (2)	50	7	**−0.73**	−0.08
Ketoprofen	NSAID	**3.14**	Chromolith RP-18e	40	3	**3.48**	0.34
			Chromolith RP-18e	50	3	**3.09**	−0.05
			Luna C_{18} (2)	40	3	**3.68**	0.54
			Luna C_{18} (2)	50	3	**3.36**	0.22
Lidocaine	Anesthesic	**2.21**	Gemini NX	40	11	**2.82**	0.61
			Gemini NX	50	11	**2.70**	0.49
Maprotiline	Antidepressant	**4.85**	Gemini NX	50	11	**4.09**	−0.76
Mepivacaine	Anesthesic	**1.95**	Gemini NX	40	11	**1.79**	−0.16
			Gemini NX	50	11	**1.73**	−0.22
Metoprolol	β-Blocker	**1.88**	Gemini NX	40	11	**1.27**	−0.61
			Gemini NX	50	11	**1.16**	−0.72
Metoxuron	Herbicide	**1.64**	Chromolith RP-18e	40	6	**1.23**	−0.41
			Chromolith RP-18e	50	6	**1.19**	−0.45
			Luna C_{18} (2)	40	6	**1.21**	−0.43
			Luna C_{18} (2)	50	6	**1.13**	−0.51

Table 9 (*Continued*)

Compound		Reference log $P_{o/w}$	Column	MeCN Fraction (%)	Buffer pH	Calculated log $P_{o/w}$	Residual
Nadolol	β-Blocker	**0.71**	Gemini NX	40	11	**1.33**	0.62
			Gemini NX	50	11	**1.20**	0.49
Naproxen	NSAID	**3.34**	Chromolith RP-18e	40	3	**3.50**	0.16
			Chromolith RP-18e	50	3	**3.09**	−0.25
			Luna C_{18} (2)	40	3	**3.70**	0.36
			Luna C_{18} (2)	50	3	**3.38**	0.04
Nortriptyline	Antidepressant	**4.04**	Gemini NX	50	11	**4.19**	0.15
Oxprenolol	β-Blocker	**2.10**	Gemini NX	40	11	**1.99**	−0.11
			Gemini NX	50	11	**1.83**	−0.27
Paracetamol	Analgesic	**0.51**	Luna C_{18} (2)	40	6	**0.55**	0.04
			Luna C_{18} (2)	50	6	**0.59**	0.08
Penbutolol	β-Blocker	**4.06**	Gemini NX	50	11	**4.41**	0.35
Pilocarpine	Alkaloid	**0.00**	Gemini NX	40	11	**−0.02**	−0.02
			Gemini NX	50	11	**−0.11**	−0.11
Procainamide	Antiarrhytmic	**0.98**	Gemini NX	40	11	**0.45**	−0.53
			Gemini NX	50	11	**0.42**	−0.56
Propranolol	β-Blocker	**2.98**	Gemini NX	40	11	**2.93**	−0.05
			Gemini NX	50	11	**2.72**	−0.26
Quinine	Antimalarial	**2.64**	Gemini NX	40	11	**2.48**	−0.16
			Gemini NX	50	11	**2.25**	−0.39
Trazodone	Antidepressant	**2.82**	Gemini NX	40	11	**3.15**	0.33
			Gemini NX	50	11	**2.83**	0.01
Trimethoprim	Antibiotic	**0.91**	Gemini NX	40	11	**0.43**	−0.48
			Gemini NX	50	11	**0.28**	−0.63
Warfarin	Anticoagulant	**3.25**	Chromolith RP-18e	40	3	**3.25**	0.00
			Chromolith RP-18e	50	3	**2.88**	−0.37

[a] Drug candidates, whose reference log $P_{o/w}$ values have been submitted for publication.

column and mobile composition tested, is illustrated in Eq. (8):

$$\log P_{o/w}\,(\text{calc.}) = 0.98(\pm 0.02)\log P_{o/w}(\text{ref.}) - 0.07(\pm 0.07),$$
$$n = 84;\quad r^2 = 0.96;\quad \text{SD} = 0.35;\quad F = 1828 \qquad (8)$$

The substances included, the reference log $P_{o/w}$ values, the experimental conditions of the measurements, the determined log $P_{o/w}$ as well as the differences between the determined and reference values are shown in Table 9. In spite of the diversity of columns, mobile phases and buffers that were used, the correlation between the determined and reference values is really good. As mentioned before, absolute values of residuals higher than 0.6 units were considered outliers. Again, this correlation shows that the method provides accurate log $P_{o/w}$ values with a precision lower than 0.4 log $P_{o/w}$ units. This standard deviation (0.35) is similar to those observed in the literature [16,18,22,24,27] although most of those correlations are between a variety of hydrophobicity parameters such as log k, log k_w or CHI, and not directly between calculated and reference log $P_{o/w}$ values as in the methodology discussed here.

It has to be pointed out the significance of the descriptors contribution in log $P_{o/w}$ evaluation and, consequently, the

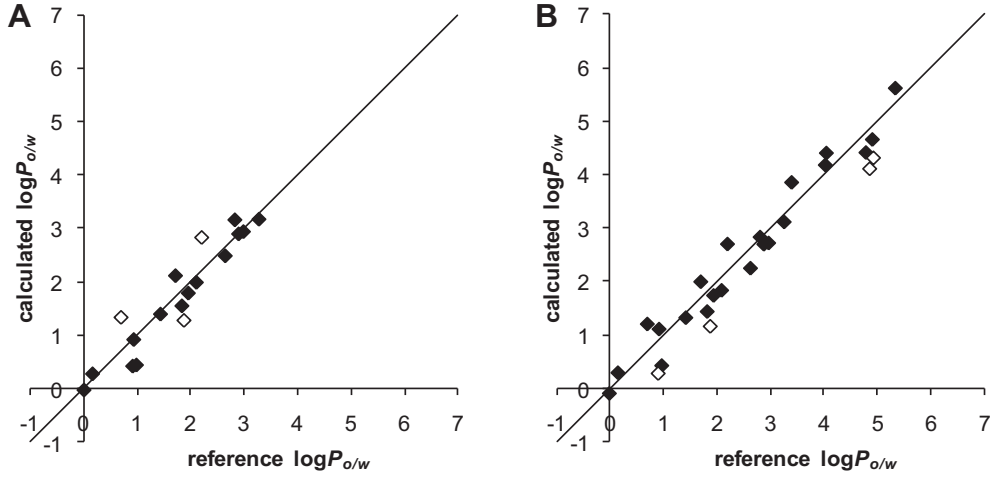

Fig. 5. Plots of calculated log $P_{o/w}$ vs. reference log $P_{o/w}$ values. (A) Plot for the studied drugs, Gemini NX column, mobile phase MeCN/pyrrolidine buffer 40%; (B) plot for the studied drugs, Gemini NX column, mobile phase MeCN/pyrrolidine buffer 50%. Symbols: (♦) compounds; (◊) outliers.

Table 10
Contribution of the descriptor terms in the calculated $\log P_{o/w}$ according to Eq. (5) of some representative compounds.

Compound	MeCN fraction	$\log k$	1.22 $p_{reference}$	1.99 pol/d^2	1.89 HDCA-2	−0.17 HOMO–LUMO	-1.27×10^{-3} DPSA-1	calc. $\log P_{o/w}$
Acebutolol	50	−0.08	2.47	0.56	1.80	−1.42	−0.42	**1.99**
Ephedrine	50	−0.06	2.52	0.56	0.95	−1.69	−0.25	**1.10**
Quinine	40	0.56	3.87	0.02	1.34	−1.44	−0.32	**2.48**
Mepivacaine	40	0.57	3.88	0.06	0.79	−1.60	−0.35	**1.79**
Oxprenolol	40	0.58	3.92	0.02	0.96	−1.57	−0.36	**1.99**
Bupivacaine	50	1.03	6.29	0.06	0.55	−1.59	−0.45	**3.86**
Nortriptyline	50	1.05	6.35	0.34	0.36	−1.52	−0.35	**4.19**
Maprotiline	50	1.06	6.38	0.34	0.36	−1.62	−0.38	**4.09**
Penbutolol	50	1.08	6.47	0.02	1.00	−1.60	−0.49	**4.41**

inadequacy of chromatographic retention solely to predict $\log P_{o/w}$ partition coefficients properly when compounds of different structures and hydrogen bond capabilities are considered. Abraham et al. [38,39] analysed the dependence of the $\log P_{o/w}$ from the main property descriptors for a wide set of 613 compounds and no significant dependence of $\log P_{o/w}$ on the hydrogen bond acidity of the compounds have been noticed. However, the chromatographic retention in C_{18} columns is really influenced by the hydrogen bond acidity of the solutes, which significantly decreases the retention of the solute [19,20,22,32,34,40,41]. According to this fact, when $\log P_{o/w}$ is estimated from chromatographic measurements, the hydrogen bond acidity of the solute has to be taken into account, because two compounds with the same chromatographic retention do not necessarily have the same hydrophobicity. This fact was already shown by Valkó et al. [24], who established a nice correlation between $\log P_{o/w}$ and the CHI parameter, where the chromatographic index is complemented with a molecular descriptor of the solute which expresses its hydrogen bond acidity. In this work, the hydrogen bond acidity is expressed by the HDCA-2 descriptor, which is highly correlated with the A (effective hydrogen bond acidity) term of the Abraham's general solvation equation [35,42]. Abraham's descriptors are continuously validated through a wide variety of chemical and biological systems. Nevertheless, they are usually determined experimentally and this is a drawback in the case of new compounds, while HDCA-2 and the other descriptors used in this work can be easily calculated solely from the structure of the compound.

Table 10 shows the contribution of each term of Eq. (5) in the calculated $\log P_{o/w}$ values of some representative compounds. It can be observed that HDCA-2 is the descriptor term which has the strongest influence in the $\log P_{o/w}$ values discrimination. For example, acebutolol and ephedrine have the same chromatographic retention ($\log k \approx -0.07$) and the contribution of the descriptors is really similar with the exception of HDCA-2. The calculated $\log P_{o/w}$ values are 1.99 and 1.10, respectively, and they have the same difference between them (≈0.9) as the contribution of HDCA-2 term for each substance. The same can be observed for quinine, mepivacaine and oxprenolol. They have similar retention factors ($\log k \approx 0.57$) but significantly different $\log P_{o/w}$ values (2.48, 1.79 and 1.99, respectively), and the difference between them comes again from the difference in the contribution of HDCA-2 term, which is the biggest for quinine, followed by oxprenolol and finally by mepivacaine. Similar reasoning can be applied to bupivacaine, nortriptyline, maprotiline and penbutolol, which have $\log k \approx 1$. In this example, the contribution of pol/d^2 term is also significant and calculated $\log P_{o/w}$ for nortriptyline and maprotiline, which have the same pol/d^2 term, is very similar because the HDCA-2 term is the same for both compounds. However, the $\log P_{o/w}$ of bupivacaine and penbutolol, which also shows the same value for the pol/d^2 term, differs in 0.55 $\log P_{o/w}$ units, according to the difference between their HDCA-2 terms.

4. Conclusions

The described method allows the determination of the 1-octanol-water partition coefficient ($\log P_{o/w}$) of neutral substances and, specifically, drugs with very different structures, hydrophobicity, functional groups and acid-base properties from a single isocratic run, working with a characterized chromatographic system. The only requirement of the method is an accurate control of the pH of the mobile phase in order to avoid the ionization of the solutes, and for this purpose it is advisable a previous estimation of their pK_a (for example, with an appropriate commercial software). The structural descriptors can be easily calculated from the structure of the solutes using CODESSA software. The results obtained, as well as their comparison with the reference $\log P_{o/w}$ for the solutes demonstrate that the method provides good estimations (with a precision of 0.4 logarithmic units) of the hydrophobicity of the compounds. The applicability of the method for drugs is especially remarkable, because of its interest for drug research and development.

Acknowledgements

The authors thank the Ministerio de Ciencia e Innovación of the Spanish Government and the Fondo Europeo de Desarrollo Regional (FEDER) of the European Union (Project CTQ2010-19217/BQU) for financial support. JMP also thanks the Ministerio de Ciencia e Innovación for a FPI grant supported by the Project CTQ2007-61623/BQU.

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