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Journal of Chromatography A, 952 (2002) 47–61

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Method for measuring the logarithm of the octanol–water partition coefficient by using short octadecyl–poly(vinyl alcohol) high-performance liquid chromatography columns

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Received 9 November 2001; received in revised form 14 January 2002; accepted 14 January 2002

Abstract

A simple, quick, versatile and inexpensive HPLC method to estimate the logarithm of the octanol–water partition coefficient (log P_{ow}) employing a methanol–water gradient and a short octadecyl–poly(vinyl alcohol) (ODP) column is described. This method is different from published HPLC-based log P_{ow} methods because it uses retention times from a rapid methanol–water gradient to directly generate log P_{ow} estimates, rather than from a series of isocratic mixtures extrapolated to 100% water. These HPLC log P_{ow} values have good precision and correlate well with traditional shake-flask log P_{ow} values. If necessary, the log P_{ow} determination (including replications) can easily be carried out using only a milligram of sample. By suppressing ionization of acids and bases by the use of a buffer in the aqueous phase, the method can measure the log P_{ow} of neutral organic molecules at any pH between 2 and 13. The method can be used with impure material and is rapid, 7 min per run and 4 min equilibration; it lends itself to and has been utilized for high-throughput hydrophobicity determinations (we have now carried out thousands of HPLC log P_{ow} measurements by this method).

Keywords: Octanol–water partition coefficients; Hydrophobicity; pH effects; Octadecyl–poly(vinyl alcohol) columns; Stationary phases, LC

1. Introduction

The most important physical property affecting the biological activity of substances is undoubtedly their lipophilicity (as a biphasic index) or hydrophobicity (as a chromatographic index). Hansch and Fujita developed a measurement of lipophilicity expressed as the logarithm of the octanol–water partition ratio (log P_{ow}) and then correlated the log P_{ow} with the biological activity [1,2]. Since that time many systems have been developed to estimate the lipophilicity. Lipophilicity, as expressed by the logarithm of the partition coefficient *P*, is a molecular parameter describing the distribution equilibrium of solute molecules between water and various water immiscible, lipid-like organic solvents. The most common log *P* measurement is the log P_{ow}, the logarithm of the partition ratio of a compound between octanol and water. Log P_{ow} values are of great importance in the study of pharmacological phenomena. These

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00064-X

lipophilicity values have been found to correlate very highly with a number of important biochemical and pharmacological phenomena [3–6]. For example, reports have been published on the relationship of pK_a and log P_{ow} to the mitochondrial uncoupling activity of insecticidal pyrroles [7,8]. An appropriate partition coefficient allows for an adequate drug concentration in the receptor phase. Thus, log P_{ow} finds numerous applications in quantitative structure–activity relationship (QSAR) studies [9–13]. The log P_{ow} has also been used in the estimation of environmental parameters [14–16]. Although partition coefficients have been measured in many different solvent–water systems, octanol–water is the most widely accepted reference system because of its correlation of physicochemical properties with biomembranes [17].

All too often, the log P_{ow} measurements of lipophilic compounds are not routinely determined experimentally due to the great difficulty and high cost inherent to the traditional shake-flask method. They can be estimated using such computational methods providing the following caveats are met: the values for all of the fragments of the molecule have been determined, the connectivity patterns are incorporated into the database, and the molecule can not be ionized. Two common methods for calculating log P_{ow} values involve the hydrophobic fragment approach of Rekker et al. [18,19], and the fragment approach of Leo and Hansch using the ClogP program [20,21]. (The Medchem Project ClogP program is available through Biobyte, Claremont, CA, USA; http://www.biobyte.com). Computational programs are very useful and provide valuable information for a large number of compounds. However, there are problems and limitations with these methods. For example, there are significant deviations between predicted and experimental values when the pattern of connectivity and non-bonded intramolecular interactions are unfamiliar to the database [22,23].

There is extensive literature describing the advantages, disadvantages, and problems of the traditional shake-flask method. A review on the measurement of partition coefficients delineates the factors that introduce error in these measurements [24]. The method suffers inaccuracies from several sources: impurities may adversely affect the results, it is often necessary to measure the pK_a, and accurate concentration measurement of highly lipophilic organic compounds in water is fraught with problems. The shake-flask method is also a tedious and time-consuming process. It is essential to pre-saturate the octanol with water and the water with octanol. It is also important to achieve complete separation of the layers, since any droplet of octanol in the aqueous phase will contain relatively large amounts of analyte. Assurance that concentrations are well below the critical micelle concentration (CMC) is necessary. The CMC of some compounds may be as low as 10^{-5} *M*. Log P_{ow} measurements need to be carried out at concentrations *below* the aqueous solubility limit. Even at the solubility limit in water, the concentration of a highly lipophilic compound (log P_{ow} 5–7) will require measurements in the parts per-billion range in the aqueous phase. In addition to these problems, when testing a highly lipophilic basic compound additional errors may be introduced, such as adsorption onto glass walls. These and other problems have led to a wide variation in reported log P_{ow} values.

The difficulties in directly measuring the octanol/water partition coefficient have inspired many researchers to develop alternative methods attempting to quantify lipophilicity. There have been a number of methods that use counter-current chromatography to estimate the log P_{ow} values [25–32]. The use of a hydrophobicity index from microemulsion electrokinetic chromatography (MEEKC) has shown some promise with the measurement of both acidic and basic analytes [33–36]. Immobilized artificial membranes (IAMs) are composed of lipids with polar headgroup and non-polar chains. They do not correlate highly with the log P_{ow} [37–40], but have been shown to better correlate with skin permeation [41] and bile salt/membrane interactions [42]. However, most alternative methods for estimating the octanol–water partition coefficient use an HPLC chromatographic procedure for which there are many papers and reviews on the subject [43–51]. The main advantages of these methods over direct partitioning are speed and simplicity. In addition, highly pure material is not needed and the solute analysis is not necessary. High-performance liquid chromatographic equipment is found in most laboratories and can be utilized for log P_{ow} determinations without much

modification. Most of these HPLC methods utilize the extrapolation of retention indices such as k' (the retention factor) to 100% water conditions [52–55]. These k' factors at 100% water are then correlated with known log P_{ow} values to give a regression equation for the column, solvent, and class of compound. In practice, the k' values are usually determined from 70 to 30% organic modifier and then extrapolated to 0%, but the extrapolation of the regression line from 30 to 0% organic is not linear [56]. Problems sometimes result when measuring highly hydrophobic compounds. Their low water solubility and strong attraction to reverse phase columns cause elution times at or below 50% organic solvent to be excessively long. Others have proposed extrapolation to 100% aqueous solvent is not recommended since the additional experimental effort is excessive compared to the gain in precision [57]. Another source of error occurs when the log P_{ow} of structurally unrelated analytes are measured [56,58]. In addition to the above, there are also HPLC methods that employ 50–60% organic modifiers that do not extrapolate the retention indices to 100% water conditions [59–63]. Besides isocratic methods, there are methods that use an acetonitrile gradient to measure hydrophobicity [64–66]. Of the two common organic solvents for HPLC, acetonitrile and methanol, the latter gives better correlations to log P_{ow} because of its hydrogen bonding capability [45,67,68].

There are non-silica based HPLC columns used to measure hydrophobicity, notably, the polystyrene–divinylbenzene (PS–DVB) columns. The PS–DVB columns have low efficiency and suffer from both shrinkage and swelling [69,70], are known to have specific interactions [71,72] due perhaps to the rich π orbitals [73–75] and correlate better to alkane–water partitions than to the octanol–water partitions [76]. For the purposes of QSAR, the alkane–water partition ratio correlates better to the blood–brain partition ratio than the octanol–water partition ratio [77].

An HPLC column consisting of an esterified poly(vinyl alcohol) copolymer [octadecyl groups bonded to a poly(vinyl alcohol) backbone, ODP] has previously been used to estimate log P_{ow} values [78–81]. Vallat et al. have shown that the ODP columns measure the log P_{ow} with good accuracy, even for strong hydrogen bonding compounds [82]. The chromatography is performed using a series of concentrations of organic modifier, followed by extrapolation of the retention indices to 100% water conditions. Four points from this work need to be stressed: (1) The poly(vinyl alcohol) columns demonstrated higher correlations with the log P_{ow} than silane based columns. (2) The use of methanol–water has been shown to be superior to acetonitrile–water for correlation to log P_{ow}. (3) The regression lines for the various functional groups are essentially coincident with each other. (4) Strong H-bond donating solutes ($\alpha \geq 0.56$) are not outliers. The ODP polymer takes on the role of octanol and the organic–water mobile phase takes on the role of water with methanol, not surprisingly, a better water mimic than acetonitrile. The regression lines being coincident with each other for diverse functional groups is not usually the case for silane based columns. Typically, one must carefully select standards of the same chemical class as the compound(s) of interest, and then calibrate the column for this class. This calibration is not necessarily valid for compounds with functional groups outside this class [56,58,83]. This leads to a very fundamental problem in HPLC log P methods; compounds of interest usually have many and varied functional groups. There are often no standards to use that match the exact combination of functional groups in the compound(s) of interest.

This nearly ''universal'' behavior of the ODP columns is what first drew our attention to these columns. Another attribute of these columns is their stability to acidic and strongly basic conditions. Manufactures of the ODP column claim that the recommended pH range is 2–13 [84]. Such basic conditions would destroy silane based reversed-phase columns. The stability of ODP columns at high pH values allows the log P of the neutral form of strong bases to be measured. Silanol based HPLC columns have about 50% of their solvent accessible silanol groups unprotected (or 3.5–5 $\mu mol/m^3$), even when ''heavily coated'' and end capped [85–92]. Silanol groups are weakly acidic with a pK_a of about 6.8 ± 0.2 [93]. In contrast, these ODP polymer columns have no unprotected Si–OH or Al–OH groups that may facilitate acid–base interactions, leading to an overestimation of the log P_{ow} of compounds with

basic groups [94,95]. The ODP polymer based columns have been found to strongly retain some aromatic compounds [96].

Leo suggests that when the methanol concentration is higher than 50%, the HPLC is relatively insensitive to hydrophobicity. Leo cites [97], as an example, the work of Spencer et al. who used 70–90% methanol for an HPLC–log P_{ow} method [98]. He found a poor correlation for an enzyme inhibition with the HPLC–log P_{ow} values. When Leo used calculated values, a positive correlation was found. In light of this finding, Lambert [46] recommends that investigators using the HPLC method minimize the percentage of organic solvents by possibly adjusting the column length. The suggestions of Leo to keep the methanol concentration low, of Lambert to shorten the columns, and the promising characteristics of ODP for HPLC log P_{ow} caused our attention to be immediately drawn to the very short ODP HPLC columns.

2. Experimental

2.1. Apparatus

The HPLC instrument consisted of Dual Gilson 306 HPLC pumps with 5 ml heads, a Gilson 811C solvent mixer with a 1.5 ml chamber, and controlled by a Gilson 714 HPLC program. The auto-injector was a Gilson 231XL sampling injector. In all cases, 2 μl were injected, usually a 2.00-ml/min flow-rate was used and a linear gradient was employed. The 20×4.0 mm, 5 μm 250 Å pore size, ODP-50 cartridge column was manufactured by Supelco (catalog number 59313C40, distributed through Sigma–Aldrich). The pH electrode was an Orion Ross semi-microelectrode, the pH meter was an Orion 720A, and was calibrated at pH values of 4.00, 7.00 and 10.00. The water was from deionized water that was further deionized and then distilled in glass by the Corning MP-12A MegaPure System. The Perkin-Elmer 235 diode array UV detector was used and the outputs from 260 to 285 nm were measured. The calculations were carried out using Excel 97-SR-2 manufactured by Microsoft, and the statistics were carried out using Table Curve 2D (version 4) manufactured by SPSS.

2.2. Reagents

OmniSolve grade methanol from EM Science was used as received. The compounds for analysis were obtained internally and from various commercial sources, and were used as received.

2.3. Chromatographic conditions

The columns were operated between 18 and 22 °C. The buffers were made from 0.01 *M* sodium phosphate adjusted to the required pH. The aqueous solution at pH 2 was 0.026 *M* trifluoroacetic acid. The 260 and 285 nm outputs from the Perkin-Elmer 235 diode array UV detector was measured. The standard conditions in this study (*see below*, method 2): were a 2.00-ml/min flow-rate, a linear gradient from 10 to 100% methanol in 7 min, and equilibration time between runs was 4 min. For lower pressure conditions and longer column life, the following conditions gave equivalent results (*see below*, method 13): a 1.50-ml/min flow-rate and a linear gradient from 10 to 100% methanol in 9.4 min. Equilibration time between runs was 6 min. A cocktail was prepared by adding 20 mg triphenylene to 2 ml of toluene, followed with the addition of 200 ml methanol. Approximately 1 mg of the unknown was added to 1 ml of this cocktail, then 2 μl was injected.

3. Results and discussion

We examined the retention behavior of a 150×4.6 mm Astec ODP-50 column using methanol–water gradients and quickly realized that it was highly retentive to lipophilic compounds. Lipophilic compounds would not elute until the methanol concentration was quite high. For the best correlation to log P_{ow} and other QSAR relationships, we wanted the lipophilic analytes to elute under as low a concentration of methanol as possible [97]. This led us to try very short ODP columns. We first tried 10×4.6 mm ODP-50 columns by Astec, and later found the 20×4.2 mm, 5 μm, ODP-50 cartridge columns by Supelco more convenient. With these columns even highly lipophilic compounds eluted without having to take the methanol concentration to

100%. A gradient of 10–100% methanol in 7 min gave an apparently linear relationship of the retention time with the literature log P_{ow} values as seen in Fig. 1.

We did not want to introduce yet another HPLC based hydrophobicity index, instead we have expressed the hydrophobicity scale in terms of log P_{ow}, making the results and implications of this study more accessible to a wider audience. Gradient retention times alone can not be used because of instrumental run-to-run variations and even greater laboratory-to-laboratory variations. Using such a short column and a rapid gradient necessitates the inclusion of at least two internal standards. By the term of internal standards, we mean that in each injection two standards must be included. This will allow for correction of such problems as subtle differences in flow-rate and percent composition from run-to-run. It will facilitate scaling of the gradient retention times. We chose toluene as one standard and triphenylene as the lipophilic standard. Both compounds are easy to detect at 260 nm. Toluene has the advantage of having little UV absorption higher than 285 nm. Setting one wavelength to 260 nm and another at 285 nm (or higher) on a UV diode array detector or a dual wavelength UV detector allows the measurement of analyte retention times that could be coincident with those of toluene. We took advantage of the extreme retention of triphenylene to avoid co-elution of the lipophilic standard with lipophilic analytes. By measuring the three retention times of toluene, triphenylene and the analyte, a hydrophobic index could be encoded relatively free of effects from instrumental run-to-run variations. Since it appeared that the plot of the log P_{ow} verses the retention time was a straight line, the log P_{ow} of an unknown could be easily determined using the known log P_{ow} values of toluene and triphenylene and the retention time of toluene, triphenylene and the unknown(s). Let (t_1, log P_1) and (t_2, log P_2) be (x, y) points on the straight line log $P = mt_R + b$, where log P is the log $P_{ow,}$ t_R is the retention time, and b is the y intercept:

$$\log P = mt_R + b \tag{1}$$

By the definition of slope and intercept:

$$\log P = \left(\frac{\log P_1 - \log P_2}{t_1 - t_2}\right) \cdot t_R + \left(\frac{t_1 \log P_2 - t_2 \log P_1}{t_1 - t_2}\right) \tag{2}$$

Simplification gives:

$$\log P = \frac{(\log P_1 - \log P_2) \cdot t_R + t_1 \log P_2 - t_2 \log P_1}{t_1 - t_2} \tag{3}$$

When toluene (tol) and triphenylene (triph) are chosen as the two internal log P_{ow} standards, the log P_{ow} of an unknown is simply found by substitution of the appropriate retention times and log P_{ow} values:

$$\log P_{\text{Unknown}} = \frac{(\log P_{\text{tol}} - \log P_{\text{triph}}) \cdot t_{\text{Unknown}} + t_{\text{tol}} \log P_{\text{triph}} - t_{\text{triph}} \log P_{\text{tol}}}{t_{\text{tol}} - t_{\text{triph}}} \tag{4}$$

The literature log P value and the log P* value (when available, and is also known as the preferred log P value or the value from the star list) from the Pomona College Database [99–102] were averaged. We found that the HPLC log P_{ow} and the average literature log P_{ow}, although linear, did not have a 1:1

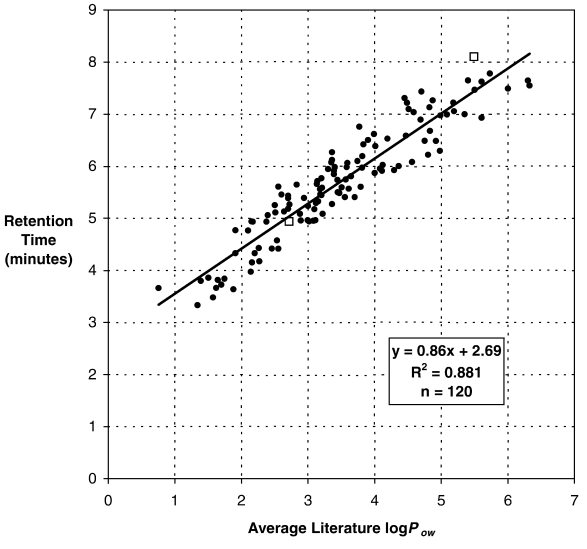

Fig. 1. Average literature log P_{ow} values compared to the HPLC retention time. Toluene and triphenylene used as the internal standards are represented by squares.

relationship, the slope was not one and the intercept was not zero. This was due mainly to triphenylene being very retentive. If the log P values of toluene and triphenylene were treated as variables, we could force both the slope to be one and the intercept to be zero as seen in Fig. 2. Based on this dataset, when the retention time for toluene is set to 2.605 and triphenylene set to 6.266, the desired slope of one and the intercept of zero were obtained. We tested some 120 diverse compounds that had well determined log P_{ow} values found in Table 1.

The 90% confidence and prediction intervals are displayed. HPLC log $P_{ow} = 1.000$ (± 0.003) average literature log $P_{ow} + 0.00$ (± 0.12) $n = 120$, correlation coefficient = 0.94, fit standard error = 0.43, F statistic = 875.

We wanted to test the ruggedness of Eq. (1) to the effects of variations of pumping parameters. The instrumental parameters were systematically varied and the log P of benalaxyl still remained relatively constant. The variations shown are extreme examples; the minor fluctuations normally found in an instrument should be much less. Under routine conditions the *intra*-laboratory variations in the log P gave a run-to-run variation at or below 0.01 units as seen in Table 2.

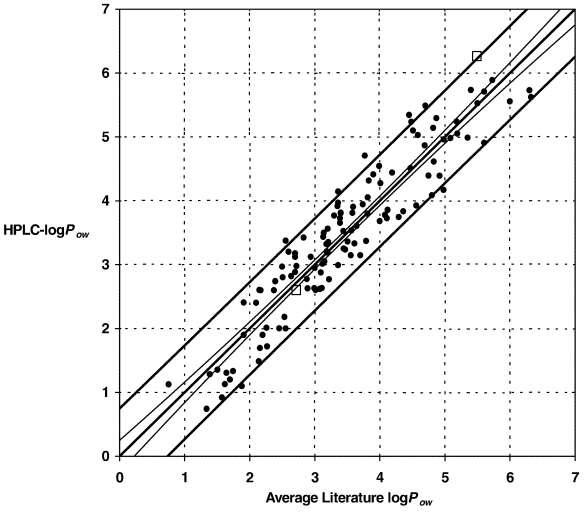

Fig. 2. Average literature log P_{ow} values compared to the HPLC log P_{ow} values. Ninety-percent confidence and prediction intervals displayed. Toluene and triphenylene used as the internal standards are represented by squares.

4. Determination of the log D of ionizable species

Knowing the log D (the effective log P at a particular pH) is crucial to QSAR. Direct interpretation of the log D of ions at a particular pH of interest by this (or any) HPLC method is fraught with difficulties. While this method will give reproducible results, the apparent value of the log D will be misleading if ionization of the analyte is taking place. A recent paper studied the chromatographic retention of acids and bases during gradient elution as a function of mobile phase pH [103]. The apparent pH may be different from the actual pH [104,105]. As the organic co-solvent concentration changes, both the pH and the pK_a will shift [106–108]. To avoid these difficulties, the log P was measured under conditions that suppress ionization. The pH of the aqueous phase was carried out at a pH of 2 for acids and a pH of 10 for bases. The stability of the ODP column allows the pH to be extended to 13 for very strong bases. To estimate the log D at the particular pH of interest, Eq. (5) for acids and Eq. (6) for bases derived by Horváth et al. were used [109]:

$$\log D = \log (P + P_i \cdot K_a/H) - \log(1 + (K_a/H)) \quad (5)$$

$$\log D = \log (P + H/P_i \cdot K_b) - \log(1 + (H/K_b)) \quad (6)$$

The equivalent Eqs. (7) for an acid and (8) for a base can also be used [110]:

$$\log D = \log (P \cdot 10^{pK_a} + P_i \cdot 10^{pH}) - \log(10^{pK_a} + 10^{pH}) \quad (7)$$

$$\log D = \log (P \cdot 10^{pH} + P_i \cdot 10^{pK_b}) - \log(10^{pK_b} + 10^{pH}) \quad (8)$$

where P is the partition coefficient for the neutral molecule. P_i is the partition coefficient for the ion, K_a is the equilibrium constant for acids, K_b is the equilibrium constant for bases, and H is the hydrogen ion concentration. We did not attempt to measure the log P of the ion, but assumed the log P_i was 3.15 less than the log P of the neutral molecule (a typical value), the actual difference between the log P and

Table 1
Data for the analytes tested

No.	Use	CAS No.	Name	ACD log P[a]	C log P[b]	Ref.	Literature log P	Log P [115]	Av. Lit. log P	pH	HPLC log P	Log P difference
1	Fungicide	118-74-1	Hexachlorobenzene	4.89	6.06		–	5.73	5.73	7	5.89	−0.16
2	Fungicide	94-26-8	Butyl paraben	3.43	3.57	[116]	3.32	3.57	3.45	2	3.25	0.19
3	Fungicide	82-68-8	Quintozene	4.16	4.93	[115]	5.1	4.64	4.87	7	5.30	−0.43
4	Fungicide	5234-68-4	Carboxin	3.00	2.10	[115]	2.2	2.14	2.17	7	2.60	−0.43
5	Fungicide	5259-88-1	Oxycarboxin	1.14	0.65	[115]	0.772	0.74	0.76	7	1.13	−0.37
6	Fungicide	10605-21-7	Carbendazim	1.46	1.71	[115]	1.49	1.52	1.51	7	1.35	0.15
7	Fungicide	66332-96-5	Flutolanil	4.63	3.48	[115]	3.7	3.7	3.70	7	3.15	0.55
8	Fungicide	2425-06-1	Captafol	3.02	3.74	[115]	3.8	3.83	3.82	7	4.05	−0.24
9	Fungicide	71626-11-4	Benalaxyl	3.87	4.50	[115]	3.54	3.4	3.47	7	3.24	0.23
10	Fungicide	106325-08-0	Epoxiconazole	2.87	3.22	[115]	3.44	NA	3.44	7	3.53	−0.09
11	Fungicide	57837-19-1	Metalaxyl	2.14	2.71	[115]	1.75	1.65	1.70	7	1.20	0.50
12	Fungicide	17804-35-2	Benomyl	2.61	1.79	[115]	1.37	2.12	1.75	7	1.33	0.41
13	Fungicide	74738-17-3	Fenpiclonil	2.68	3.66	[115]	3.86	4.3	4.08	7	3.78	0.30
14	Fungicide	143390-89-0	Kresoxim-methyl	4.34	4.72	[115]	3.4	–	3.40	2	3.81	−0.41
15	Fungicide	98730-04-2	Benoxacor	3.19	2.88	[115]	2.6	–	2.60	7	3.20	−0.60
16	Fungicide	43121-43-3	Triadimefon	3.02	3.34	[115]	3.11	2.77	2.94	7	3.12	−0.18
17	Fungicide	55219-65-3	Triadimenol	2.7	2.77	[115]	3.08	3.08	3.08	7	2.62	0.46
18	Fungicide	60207-90-1	Propiconazole	3.21	3.98	[115]	3.72	3.5	3.61	7	3.33	0.28
19	Fungicide	67747-09-5	Prochloraz	4.01	4.69	[115]	4.12	4.6	4.36	7	3.83	0.53
20	Fungicide	60168-88-9	Fenarimol	3.23	2.86	[115]	3.69	3.6	3.65	7	3.61	0.04
21	Fungicide	175013-18-0	Pyraclostrobin	3.3	4.99	[115]	3.99	–	3.99	7	4.54	−0.55
22	Fungicide	92-52-4	Biphenyl	3.98	4.03		–	4.01	4.01	7	4.27	−0.26
23	Fungicide	131860-33-8	Azoxystrobin	5.13	3.08	[115]	2.5	–	2.50	7	2.97	−0.47
24	Fungicide	131341-86-1	Fludioxonil	0.38	4.15	[115]	4.12	–	4.12	7	3.86	0.26
25	Herbicide	122-34-9	Simazine	0.69	2.19	[115]	2.1	2.18	2.14	7	1.49	0.65
26	Herbicide	3060-89-7	Metobromuron	2.43	2.46	[115]	2.41	2.38	2.40	7	2.74	−0.35
27	Herbicide	709-98-8	Propanil	3.49	3.33	[115]	3.3	3.07	3.19	7	3.21	−0.02
28	Herbicide	1194-65-6	Dichlobenil	2.46	2.74	[115]	2.7	2.74	2.72	7	2.98	−0.26
29	Herbicide	1582-09-8	Trifluralin	6.16	5.29	[115]	4.83	5.34	5.09	7	4.98	0.11
30	Herbicide	40487-42-1	Pendimethalin	5.56	4.88	[115]	5.18	5.18	5.18	7	5.24	−0.06
31	Herbicide	142459-58-3	Flufenacet	3.98	2.62	[115]	3.2	–	3.20	7	3.19	0.01
32	Herbicide	61213-25-0	Flurochloridone	3.37	3.85	[115]	3.36	3.36	3.36	7	3.97	−0.61
33	Herbicide	7287-19-6	Prometryn	3.37	3.29	[115]	3.36	3.36	3.36	7	2.99	0.37
34	Herbicide	42576-02-3	Bifenox	5.79	4.96	[115]	4.5	4.47	4.49	7	5.24	−0.75
35	Herbicide	21725-46-2	Cyanazine	0.33	1.39	[115]	2.1	2.22	2.16	7	1.70	0.46
36	Herbicide	19937-59-8	Metoxuron	1.92	1.78	[115]	1.6	1.64	1.62	7	1.13	0.49
37	Herbicide	15545-48-9	Chlorotoluron	2.46	2.49	[115]	2.5	2.41	2.46	7	2.00	0.45
38	Herbicide	51218-45-2	Metolachlor	2.9	3.25	[115]	2.9	3.13	3.02	7	2.60	0.41
39	Herbicide	42874-03-3	Oxyfluorfen	5.73	5.82	[115]	4.47	4.7	4.59	7	5.03	−0.44
40	Herbicide	51338-27-3	Diclofop-methyl	4.65	5.50	[115]	4.58	4.8	4.69	7	4.87	−0.18
41	Herbicide	27314-13-2	Norflurazon	1.94	2.89	[115]	2.45	2.3	2.38	7	2.60	−0.22
42	Herbicide	58011-68-0	Pyrazolynate	3.81	4.71	[115]	(2.58 rejected)	3.9	3.90	7	4.41	−0.51
43	Herbicide	64249-01-0	Anilofos	4.2	4.50	[115]	3.81	3.81	3.81	7	3.80	0.01
44	Herbicide	128639-02-1	Carfentrazone-ethyl	1.09	3.59	[115]	3.36	–	3.36	2	4.14	−0.78
45	Herbicide	117337-16-6	Fluthiacet-methyl	2.33	3.79	[115]	3.77	–	3.77	2	4.71	−0.94
46	Herbicide	142891-20-1	Cinidon-ethyl	3.92	5.18	[115]	4.51	–	4.51	2	5.10	−0.59
47	Herbicide	134605-64-4	Butafenacil-allyl	4.34	5.28	[115]	3.2	–	3.20	2	3.56	−0.36
48	Herbicide	1912-24-9	Atrazine	1.03	2.50	[115]	2.5	2.61	2.56	7	2.00	0.55

Table 1. Continued

No.	Use	CAS No.	Name	ACD log P[a]	C log P[b]	Ref.	Literature log P	Log P [115]	Av. Lit. log P	pH	HPLC log P	Log P difference
49	herbicide	1836-77-7	Chlonitrofen	6.37	5.66		–	4.70	4.70	7	5.49	−0.79
50	Insecticide	56-38-2	Parathion	3.84	3.47	[115]	3.83	3.83	3.83	7	4.32	−0.49
51	Insecticide	121-75-5	Malathion	2.93	2.70	[115]	2.75	2.36	2.56	7	3.38	−0.82
52	Insecticide	298-02-2	Phorate	3.73	3.84	[115]	3.92	3.56	3.74	7	3.94	−0.20
53	Insecticide	333-41-5	Diazinon	3.44	3.50	[115]	3.3	3.81	3.56	7	3.15	0.41
54	Insecticide	51-03-6	Piperonyl butoxide	5.05	4.57	[115]	4.75	4.75	4.75	7	4.39	0.36
55	Insecticide	2032-65-7	Methiocarb	2.89	2.80	[115]	3.08	2.92	3.00	7	2.95	0.05
56	Insecticide	2921-88-2	Chlorpyrifos	4.77	4.49	[115]	4.7	5.27	4.99	7	4.96	0.03
57	Insecticide	1563-66-2	Carbofuran	1.76	2.47	[115]	1.52	1.63	1.58	7	0.92	0.65
58	Insecticide	13071-79-9	Terbufos	4.42	4.55	[115]	(2.77 rejected)	4.47	4.47	7	4.51	−0.04
59	Insecticide	6164-98-3	Chlordimeform	3.01	2.79	[115]	2.89	2.89	2.89	7	2.63	0.26
60	Insecticide	52315-07-8	Cypermethrin	6.53	6.61	[115]	6.6	6.05	6.33	7	5.62	0.70
61	Insecticide	120928-09-8	Fenazaquin	5.49	5.92	[115]	5.51	5.7	5.61	7	4.91	0.70
62	Insecticide	33089-61-1	Amitraz	5.64	5.50	[115]	5.5	5.5	5.50	7	5.53	−0.03
63	Insecticide	35400-43-2	Sulprofos	4.64	5.48	[115]	5.48	4.9	5.19	7	5.05	0.14
64	Insecticide	41198-08-7	Profenofos	5.09	4.66	[115]	4.44	4.68	4.56	7	3.93	0.63
65	Insecticide	112226-61-6	Halofenozide	3.09	3.36	[115]	3.22	–	3.22	7	2.77	0.45
66	Insecticide	82657-04-3	Bifenthrin	8.35	7.24	[115]	6	6	6.00	7	5.56	0.44
67	Insecticide	149877-41-8	Bifenazate	3.73	3.51	[115]	3.4	–	3.40	7	3.80	−0.40
68	Insecticide	112143-82-5	Triazamate	1.93	3.06	[115]	2.15	–	2.15	7	2.60	−0.45
69	Insecticide	52645-53-1	Permethrin	6.74	7.38	[115]	6.1	6.5	6.30	7	5.73	0.57
70	Insecticide	51630-58-1	Fenvalerate	7.45	6.85	[115]	5.01	6.2	5.61	7	5.71	−0.10
71	Insecticide	52918-63-5	Deltamethrin	6.86	6.79	[115]	4.6	6.2	5.40	7	5.74	−0.34
72	Insecticide	113507-06-5	Moxidectin	7.75	7.18	[115]	5.35	–	5.35	7	4.99	0.36
73	Insecticide	122453-73-0	Chlorfenapyr	5.54	5.42	[115]	4.83	–	4.83	7	4.61	0.22
74	Insecticide	120068-37-3	Fipronil	3.86	4.29	[115]	4	–	4.00	7	3.68	0.32
75	Miscellaneous	119-61-9	Benzophenone	3.18	3.18		–	3.18	3.18	7	3.32	−0.14
76	Miscellaneous	108-88-3	Toluene	2.68	2.64	[117]	2.69	2.74	2.72	7	2.61	0.11
77	Miscellaneous	100-02-7	4-Nitrophenol	1.57	1.85		–	1.91	1.91	2	2.41	−0.50
78	Miscellaneous	106-46-7	1,4-Dichlorobenzene	3.34	3.57		–	3.39	3.39	7	3.65	−0.26
79	Miscellaneous	90-47-1	Xanthone	3.16	2.98		–	3.39	3.39	7	3.73	−0.34
80	Miscellaneous	135-19-3	2-Naphthol	2.71	2.65		–	2.70	2.70	2	3.18	−0.48
81	Miscellaneous	122-59-8	Phenoxyacetic acid	1.34	1.35		–	1.34	1.34	2	0.74	0.60
82	Miscellaneous	99-94-5	4-Methylbenzoic acid	2.35	2.38		–	2.27	2.27	2	1.72	0.55
83	Miscellaneous	100-41-4	Ethylbenzene	3.21	3.17		–	3.15	3.15	7	3.05	0.10
84	Miscellaneous	93-99-2	Phenylbenzoate	3.59	3.62		–	3.59	3.59	7	3.90	−0.31
85	Miscellaneous	98-06-6	*tert*.-Butylbenzene	3.91	3.97		–	4.11	4.11	7	3.73	0.38
86	Miscellaneous	486-25-9	9-Fluorenone	3.58	3.05		–	3.58	3.58	7	3.81	−0.23
87	Miscellaneous	120-12-7	Anthracene	4.68	4.49		–	4.45	4.45	7	5.34	−0.89
88	Miscellaneous	217-59-4	Triphenylene	5.91	5.66		–	5.49	5.49	7	6.27	−0.78
89	Miscellaneous	91-20-3	Naphthalene	3.45	3.32		–	3.30	3.30	7	3.77	−0.47
90	Miscellaneous	108-70-3	1,3,6-Trichlorobenzene	4.04	4.28		–	4.19	4.19	7	4.44	−0.25
91	Miscellaneous	128-39-2	2,6-Di-*tert*.-butyl phenol	4.86	5.13		–	4.92	4.92	2	4.39	0.53
92	Miscellaneous	2243-42-7	2-Phenoxyacetic acid	2.84	3.55		–	3.11	3.11	2	2.63	0.48
93	Miscellaneous	831-82-3	4-Phenoxyphenol	3.39	3.57		–	3.35	3.35	2	3.92	−0.57
94	Miscellaneous	3558-69-8	2,6-Diphenylpyridine	4.82	4.84		–	4.82	4.82	7	5.14	−0.32
95	Pharma	40274-67-7	9-Oxo-9H-xanthene-2-carboxylic acid	2.43	2.85	[118]	3.16	3.12	3.14	2	3.50	−0.36
96	Pharma	22204-53-1	Naproxen	3.00	2.82	[101]	3.18	3.24	3.21	2	3.35	−0.14
97	Pharma	56-54-2	Quinidine	3.36	2.79		–	2.64	2.64	10	2.82	−0.18
98	Pharma	130-95-0	Quinine	3.36	2.79		–	2.88	2.88	10	2.77	0.11
99	Pharma	15687-27-1	Ibuprofen	3.72	3.68	[119]	3.51	3.50	3.51	2	3.36	0.14
100	Pharma	22071-15-4	Ketoprofen	2.81	2.76		–	3.12	3.12	2	3.01	0.11
101	Pharma	137-58-6	Lidocaine	2.36	1.95		–	2.26	2.26	10	2.01	0.25

Table 1. Continued

No.	Use	CAS No.	Name	ACD log P[a]	C log P[b]	Ref.	Literature log P	Log P [115]	Av. Lit. log P	pH	HPLC log P	Log P difference
102	Pharma	525-66-6	Propranolol	3.10	2.75	[120]	3.21	2.98	3.10	10	2.88	0.22
103	Pharma	91-64-5	Coumarin	1.39	1.41		–	1.39	1.39	7	1.28	0.11
104	Pharma	81-81-2	Warfarin	3.47	2.89		–	2.70	2.70	2	3.12	−0.42
105	Pharma	50-49-7	Imipramine	4.47	5.04		–	4.80	4.80	10	4.09	0.71
106	Pharma	1229-29-4	Doxepin	5.06	4.09	[121]	4.29	–	4.29	10	3.75	0.54
107	Pharma	50-48-6	Amitriptyline	6.13	4.85	[122]	4.92	5.04	4.98	10	4.17	0.81
108	Pharma	52-53-9	Verapamil	5.02	4.47		–	3.79	3.79	10	3.37	0.42
109	Pharma	13655-52-2	Alprenolol	2.88	2.65	[101]	2.89	3.10	3.00	10	2.63	0.36
110	Pharma	37350-58-6	Metoprolol	1.79	1.35		–	1.88	1.88	10	1.10	0.78
111	Pharma	42399-41-7	Diltiazem	4.53	3.65	[123]	2.70	–	2.70	10	2.88	−0.18
112	Pharma	114-07-8	Erythromycin	2.49	1.47	[101]	2.48	2.54	2.51	10	2.80	−0.29
113	Pharma	50-22-6	Corticosterone	2.74	2.62	[101]	2.46	1.94	2.20	7	1.90	0.30
114	Pharma	50-23-7	Hydrocortisone	1.58	2.22	[101]	1.68	1.61	1.65	7	1.31	0.34
115	Pharma	50-02-2	Dexamethason	2.09	1.75	[101]	1.99	1.83	1.91	7	1.90	0.01
116	Pharma	57-83-0	Progesterone	4.04	3.77	[124]	3.26	3.87	3.57	7	3.54	0.03
117	Pharma	50-04-4	Cortisone acetate	1.82	1.83		–	2.10	2.10	7	2.40	−0.30
118	Pharma	53-16-7	Estrone	3.69	3.38		–	3.13	3.13	7	3.44	−0.31
119	Pharma	143-62-4	Digitoxigenin	3.08	2.68	[125]	2.43	2.64	2.54	7	2.18	0.35
120	Pharma	71-63-6	Digitoxin	2.73	3.05		–	2.83	2.83	7	3.42	−0.59

[a] ACD log P Version 4.56 from Advanced Chemistry Development Inc., Toronto, Canada.
[b] C log P Version 4.0 from Biobyte Corporation, Claremont, CA, USA.

the log P_i can be from 1.5 to 4.5 depending on structure and ionic strength [110–112].

5. Source of deviations from literature values

As stated in the introduction, there are many reasons for the difficulty in obtaining accurate partition ratios from the shake flask technique. These problems have given rise to many different values for a particular analyte. Selecting which particular log P_{ow} value to use from a list of many literature values is often very subjective. A variation of 0.3 in the reported log P_{ow} for a compound is typical. A 0.3 difference in the log P_{ow} equates to a two-fold difference in the partition coefficient, a very clear indicator of the difficulties inherent in the method. It was the hope that by using a large enough number of analytes in the dataset, the effects of errors intrinsic to the literature log P_{ow} values would be minimized. To aid in the assessment of the HPLC log P_{ow} values with respect to the literature log P values, the calculated values from the ClogP and ACD programs are also listed in Table 1. We purposefully did not limit the dataset to a particular structural type. Had we done that, the correlation would undoubtedly be much better. We wanted this to be a general method; therefore, in the dataset there are such structurally diverse compounds as aromatic, heteroaromatic, cyclic, heterocyclic, macrocyclic lactones, steroids and many others. The diversity also extends to acidic, basic and neutral compounds; from molar volumes ranging from 106 to 575 ml; and from molecular masses varying between 92 and 765 g/mol. The functional groups include the types most often found in agrochemical and pharmaceutical usage. Some of the diversity of the dataset is depicted in Fig. 3.

Another factor for consideration is the apparent large deviation from a literature value may be mitigated because the HPLC log P_{ow} may correlate better to QSAR than the true shake flask log P_{ow} value. There are a number of reports from investigators who believe that HPLC based log P_{ow} derived values can and should correlate better to biological QSAR than the shake flask log P_{ow} derived values [113,114]. This is not just because of possible errors in the shake flask technique, but

Table 2
Variations in the pump parameters to demonstrate the ruggedness of the HPLC log *P* method[a]

Method	t_R (min)			log *P*	Average log *P*	Standard deviation	pH	Buffer concentration (m*M*)	Solvent flow (ml/min)	MeOH gradient (%)	Gradient delay (min)	Period of gradient (min)
	Benalaxyl	Toluene	Triphenylene									
1	5.362	4.711	7.998	3.330								
	5.371	4.736	8.007	3.316	3.322	0.007	7	7.4	2.25	10–100	0	7.0
	5.374	4.736	7.996	3.321								
2[b]	5.565	5.037	8.267	3.203								
	5.508	4.961	8.293	3.206	3.203	0.003	7	7.4	2.00	10–100	0	7.0
	5.464	4.910	8.314	3.201								
3	5.693	5.200	8.629	3.131								
	5.692	5.202	8.623	3.129	3.127	0.005	7	7.4	1.75	10–100	0	7.0
	5.696	5.209	8.660	3.122								
4	5.999	5.587	9.106	3.034								
	6.004	5.591	9.105	3.035	3.041	0.011	7	7.4	1.50	10–100	0	7.0
	5.996	5.568	9.059	3.054								
5	5.826	5.399	8.477	3.113								
	5.830	5.415	8.492	3.099	3.106	0.007	7	7.4	2.00	0–100	0	7.0
	5.852	5.434	8.485	3.107								
6	5.191	4.480	8.082	3.328								
	5.159	4.434	8.065	3.336	3.334	0.005	7	7.4	2.00	20–100	0	7.0
	5.155	4.428	8.060	3.338								
7	6.519	5.904	9.291	3.270								
	6.532	5.901	9.320	3.281	3.275	0.005	7	7.4	2.00	10–100	1.0	7.0
	6.523	5.893	9.335	3.275								
8	4.945	4.494	7.541	3.147								
	4.935	4.481	7.520	3.152	3.151	0.004	7	7.4	2.00	10–100	0	6.0
	4.932	4.476	7.517	3.154								
9	6.088	5.332	9.199	3.321								
	6.108	5.345	9.212	3.327	3.327	0.006	7	7.4	2.00	10–100	0	8.0
	6.134	5.370	9.218	3.332								
10	5.542	5.047	8.183	3.183								
	5.542	5.056	8.191	3.173	3.182	0.009	7	15	2.00	10–100	0	7.0
	5.543	5.042	8.176	3.190								
11	5.514	4.996	8.164	3.204								
	5.530	5.019	8.171	3.199	3.200	0.003	neat distilled water	0	2.00	10–100	0	7.0
	5.536	5.025	8.181	3.198								
12	5.539	5.030	8.174	3.198								
	5.535	5.022	8.170	3.202	3.199	0.002	2	26	2.00	10–100	0	7.0
	5.524	5.014	8.163	3.198								
13[c]	7.495	6.842	10.944	3.188								
	7.498	6.849	10.943	3.185	3.186	0.002	2	26	1.50	10–100	0	9.4
	7.517	6.871	10.950	3.185								
14	7.515	6.884	10.937	3.175								
	7.509	6.879	10.946	3.172	3.173	0.001	7	7.4	1.50	10–100	0	9.4
	7.513	6.881	10.952	3.173								

[a] The HPLC log *P* of Benalaxyl remains relatively constant. Outlined items denote changes from Method 2.
[b] Method 2 is the standard conditions for rapid runs and for this publication.
[c] Method 13 is the standard conditions for lower pressure runs (longer column life).

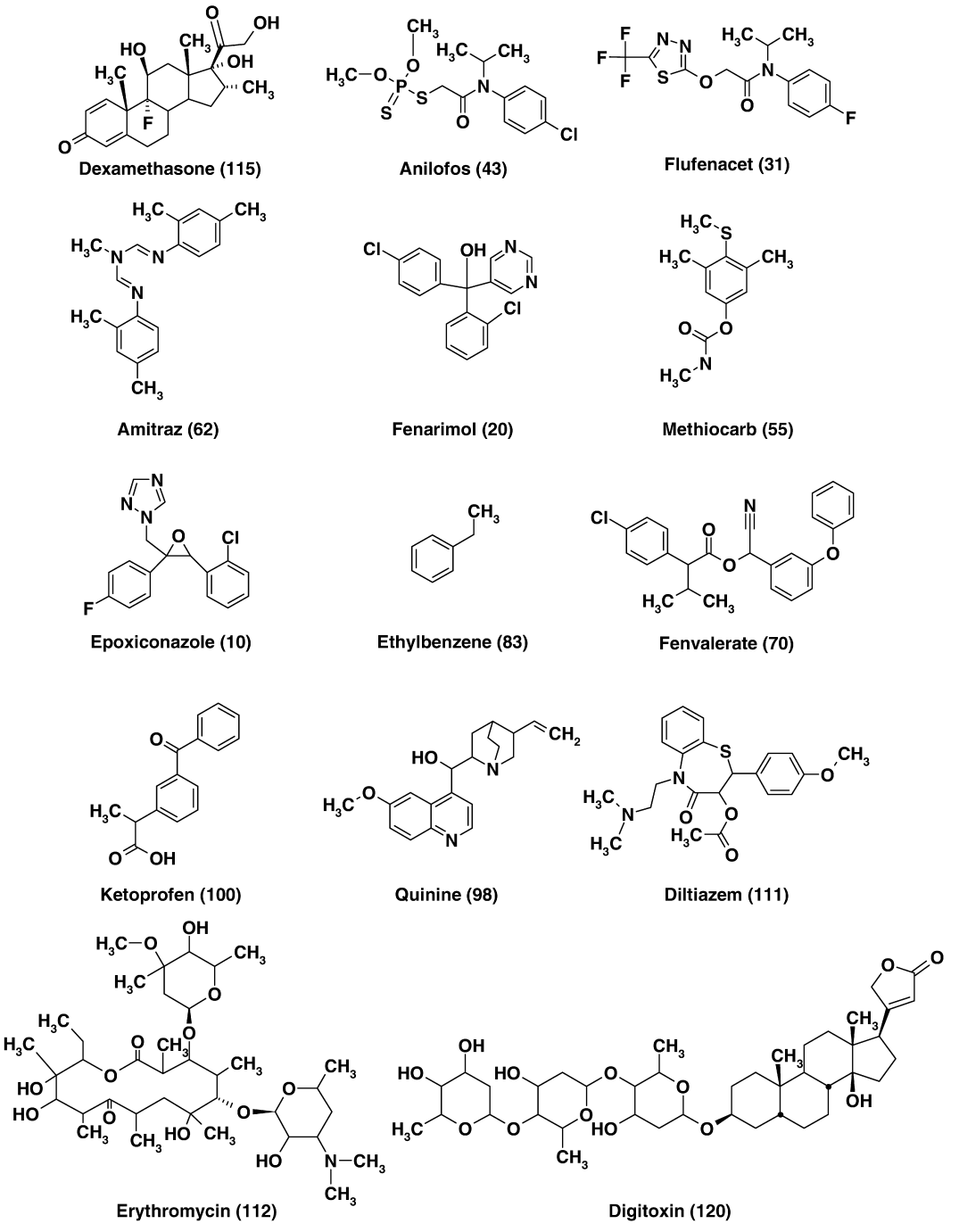

Fig. 3. Some representative structures from the dataset showing great diversity.

rather due to the nature of the technique itself. Processes underlying biological QSAR more closely resemble the processes in HPLC rather than in bulk phase partitioning; efforts aimed at modeling the octanol–water process are quite possibly not the best approach to the problem [47].

6. Recommendations

For this work we used a flow-rate of 2 ml/min (method 2 in Table 2) to expedite the results. For our routine work we prefer 1.5 ml/min to reduce the operating pressure and increase the column life (method 13 in Table 2). When we see peak broadening, reversing the direction of the column usually restores adequate resolution at least temporarily; if not, then the column is discarded because these columns are not expensive. Since this is a very small column, overloading the capacity of the column is an issue. We tend to inject as little compound as feasible; this gives more reproducible results than when using larger amounts. Not surprisingly, the short ODP-50 column has less resolution than a traditional column. Nevertheless, we have been able to measure the retention times of 14 peaks in a single run that simultaneously determined the log P_{ow} of 12 analogs. Besides obtaining log P values in our high-throughput HPLC log P_{ow} screening of active compounds, we also have observed impurities and decomposition products. Knowledge that a compound is impure or can easily decompose gives research direction that might not be realized until a much later in the discovery and development process. This early knowledge of an active chemistry also saves time and money.

When using this method in the high-throughput mode for a large series of compounds, we suggest sequentially evaluation at pH values of 2, 7 and 10. In this way neutral, acidic and basic compounds can be easily identified. Those compounds deemed interesting can then be subjected to further scrutiny using the standard methods of pK_a measurement. Again, as mentioned previously, direct interpretation of the log P_{ow} of ionized or partially ionized compounds by HPLC is difficult.

7. Conclusions

The method reported here of estimating the log P_{ow} from HPLC differs from usual published methods in a number of ways: it uses a very fast methanol gradient instead of a series of isocratic runs, a very short polymeric ODP column instead of a regular length C_{18} silica column, and the use of two internal standards in each injection. A simple, quick, versatile and inexpensive method employing an HPLC with a methanol–water gradient and a short polymer based column has been demonstrated to directly estimate log P_{ow} values with fair accuracy and very good precision. Only a milligram (usually much less) of sample is needed for a log P_{ow} determination (including replications). A single injection will give the log P_{ow} value and repeated injections will assure the precision of the measurement. The method has the capacity to measure the log P_{ow} at any given pH between 2 and 13, can be carried out on impure material and does not require a radiolabeled compound. The majority of compounds of interest for this study were compounds with a log P_{ow} between 2 and 6.

The suggestions to keep the methanol concentration low and shorten the HPLC column were followed [46,97]. A short column of 20×4.2 mm will have many times greater void volumes passing through it in a given time than could pass through a traditional length HPLC column. The percentage of methanol need not be as high as for analyte elution from a standard length column. The use of very short (20×4.20 mm) ODP-50 columns helps keep the methanol concentration low as possible. Another benefit of keeping the methanol concentration low is that the effects on organic ions caused by shifting pH and pK_a will also be minimized. There are some limitations on the type of compounds that can be measured using this HPLC log P_{ow} method. Since a solvent gradient is used, the use of a refractive index detector is not possible. Since UV detectors are typically used on an HPLC, the presence of a chromophore is essential for detection. There has been an increasing use of evaporative light-scattering detectors to monitor the effluent from gradient methods. These detectors may permit the use of this method to measure the log P_{ow} values of compounds

that are not strong UV chromophores, such as amino acids, sugars, and aliphatic compounds. Use of a mass spectrometry detector has been used with this method, but it is not a routine detector in most laboratories.

This HPLC log P_{ow} method was developed for generating log P_{ow} values for biological activity–physical property related QSAR studies. It has fair accuracy and very good precision. The precision is typically equal to or better than ± 0.01 log P_{ow} units, which is far superior to the traditional shake-flask log P_{ow} method. Our interest in log P_{ow} values is not for analytical chemistry purposes, but rather to develop a simple-to-use tool to better understand the biological activity and environmental fate of our compounds. It is not suggested that this method supersede the traditional shake flask log P_{ow} method for registration purposes. However, even in those cases, it can be used as a quick check of the validity of classically measured log P_{ow} values.

References

[1] C. Hansch, T. Fujita, J. Am. Chem. Soc. 86 (1964) 1616.
[2] C. Hansch, P.P. Maloney, T. Fujita, Nature 194 (1962) 178.
[3] A. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525.
[4] A. Leo, Chem. Rev. 93 (1993) 1281.
[5] C. Hansch, A. Leo, Exploring QSAR: Fundamentals and Applications in Chemistry and Biology (ACS Professional Reference Book), American Chemical Society, Washington, DC, 1995.
[6] C. Hansch, A. Leo, D. Hoekman, Exploring QSAR: Hydrophobic, Electronic and Steric Constants (ACS Professional Reference Book), American Chemical Society, Washington, DC, 1995.
[7] B. Black, R. Hollingworth, K. Ahammadsahib, C. Kukel, S. Donovan, Pesticide Biochem. Physiol. 50 (1994) 115.
[8] D.M. Gange, S.F. Donovan, R.J. Lopata, K. Henegar, in: C. Hansch, T. Fujita (Eds.), Classical and 3-D QSAR in Agrochemistry and Toxicology (ACS Symposium Series), American Chemical Society, Washington, DC, 1995.
[9] H. Van de Waterbeemd, B. Testa, Adv. Drug Res. 16 (1987) 85.
[10] C. Yamagami, H. Takami, K. Yamamato, K. Miyoshi, N. Takao, Chem. Pharm. Bull. 32 (1979) 4994.
[11] H. Kubinyi, Prog. Drug Res. 23 (1979) 97.
[12] H. Walter, D.E. Brooks, D. Fisher, in: Partitioning in Aqueous Two Phase Systems, Academic Press, London, 1985.
[13] R. Kaliszan, in: J.D. Winefield (Ed.), Quantitative Structure–Chromatographic Retention Relationships, Wiley, New York, 1987, p. 232.
[14] D.J.W. Blum, R.E. Speece, Ecotoxicol. Environ. Saf. 22 (1981) 198.
[15] R.L. Lipnick, Environ. Toxicol. Chem. 4 (1985) 255.
[16] W.J. Lyman, W.F. Reehl, D.H. Rosenblatt, in: Handbook of Chemical Property Estimation Methods, McGraw-Hill, New York, 1990.
[17] H. Walter, D.E. Brooks, D. Fisher, in: Partitioning in Aqueous Two Phase Systems, Academic Press, London, 1985.
[18] G.G. Nys, K. Dross, Eur. J. Med. Chem. 9 (1974) 361.
[19] R.E. Rekker, The Hydrophobic Fragment Constant, Elsevier, Amsterdam, 1976.
[20] A. Leo, P.Y.C. Jow, A. Vittoria, C. Hansch, J. Med. Chem. 18 (1975) 865.
[21] A. Leo, Chem. Rev. 93 (1993) 1281.
[22] N. Bodor, Z. Gabanyi, C.K. Wong, J. Am. Chem. Soc. 111 (1989) 3783.
[23] R.S. Tsai, B. Testa, N. El Tayar, P.A. Carrupt, J. Chem. Soc., Perkin Trans. 2 (1991) 1797.
[24] J.C. Dearden, G.M. Bresnen, Quant. Struct.-Act. Relatsh. 7 (1988) 133.
[25] A. Berthod, M. Bully, Anal. Chem. 63 (1991) 2508.
[26] N. El Tayar, R.S. Tsai, P.A. Carrupt, B. Testa, J. Chem. Soc., Perkin Trans. 2 (1992) 79.
[27] C. Altomare, R.S. Tsai, N. El Tayar, B. Testa, A. Carotti, S. Cellamare, P.G. De Benedetti, J. Pharm. Pharmacol. 43 (1990) 191.
[28] C. Altomare, P.A. Carrupt, B. Testa, T. Nagatsu, Helv. Chim. Acta 74 (1991) 290.
[29] N. El Tayar, R.S. Tsai, P. Vallat, C. Altomare, B. Testa, J. Chromatogr. 556 (1991) 181.
[30] P. Vallat, N. El Tayar, B. Testa, I. Slacanin, A. Marston, K. Hostettmann, J. Chromatogr. 504 (1990) 411.
[31] R.S. Tsai, N. El Tayar, B. Testa, Y. Ito, J. Chromatogr. 538 (1991) 119.
[32] A. Berthod, R.A. Menges, D.W. Armstrong, J. Liq. Chromatogr. 15 (1992) 2669.
[33] Y. Ishihama, Y. Oda, N. Asakawa, Anal. Chem. 68 (1996) 1028.
[34] M. Greenaway, G. Okafo, D. Manallack, P. Camilleri, Electrophoresis 15 (1994) 1284.
[35] A. Schmutz, W. Thormann, Electrophoresis 15 (1994) 1295.
[36] S.J. Gluck, M.H. Benko, R.K. Hallberg, K.P. Steel, J. Chromatogr. A 744 (1996) 141.
[37] R. Kaliszan, A. Kaliszan, I.W. Wainer, J. Pharm. Biomed. Anal. 11 (1993) 505.
[38] R. Kaliszan, A. Nasal, Eur. J. Med. Chem. 29 (1994) 163.
[39] A. Ducarme, M. Neuwels, S. Goldstein, R. Massingham, Eur. J. Med. Chem. 32 (1997) 226.
[40] F. Barbato, M.I. La Rotonda, F. Quaglia, J. Pharm. Sci. 86 (1997) 225.
[41] A. Nasal, M. Sznitowska, R. Kaliszan, J. Chromatogr. A 692 (1995) 83.

[42] D.E. Cohen, M.R. Leonard, J. Lipid Res. 36 (1995) 2251.
[43] R. Kaliszan, in: N.P. Cheremisinoff, P.N. Cheremisinoff (Eds.), Handbook of Advanced Materials Testing, Marcel Dekker, New York, 1995, pp. 87–103.
[44] R. Kaliszan, Structure and Retention in Chromatography—a Chemometric Approach, Harwood Academic, Amsterdam, 1997.
[45] E. Forgács, T. Cserháti, Molecular Basis of Chromatographic Separations, CRC Press, Boca Raton, FL, 1977.
[46] W.J. Lambert, J. Chromatogr. A 656 (1993) 469.
[47] J.G. Dorsey, M.G. Khaledi, J. Chromatogr. A 656 (1993) 485.
[48] H. van de Waterbeemd, M. Kansy, B. Wagner, H. Fishcher, in: V. Pliška, B. Testa, H. van de Waterbeemd (Eds.), Lipophilicity in Drug Action and Toxicology, VCH, Weinheim, 1996, pp. 73–87.
[49] P. Carr, Microchem. J. 48 (1993) 4.
[50] C. Poole, A. Gunatilleka, S.K. Poole, Adv. Chromatogr. 40 (2000) 159.
[51] R. Kaliszan, J. Chromatogr. B 717 (1998) 125.
[52] K. Belsner, M. Pfeifer, B.J. Willert, J. Chromatogr. 629 (1993) 123.
[53] K.P. Dross, R. Mannhold, R.F. Rekker, Quant. Struct.-Act. Relatsh. 11 (1992) 36.
[54] A. Hulshoff, J.H. Perrin, J. Chromatogr. 120 (1976) 65.
[55] G.L. Biagi, M.C. Guerra, A.M. Barbaro, S. Barbieri, M. Recanatini, P.A. Borea, M.C. Pietrogrande, J. Chromatogr. 498 (1990) 179.
[56] M.H. Abraham, H.S. Chadha, A.J. Leo, J. Chromatogr. A 685 (1994) 203.
[57] E. Klein, W. Kördel, M. Weiβ, H.J. Poremski, Chemosphere 17 (1988) 361.
[58] K. Valkó, in: H. Kalász, L. Ettre (Eds.), Chromatography—The State of the Art, Akadémia, Budapest, 1995, p. 739.
[59] M.I. La Rotonda, G. Amato, F. Barbato, C. Silipo, A. Vittoria, Quant. Struct.-Act. Relatsh. 2 (1983) 168.
[60] M.C. Pietrogrande, P.A. Borea, G.L. Biagi, J. Chromatogr. 447 (1988) 404.
[61] M.C. Pietrogrande, F. Dondi, P.A. Borea, C. Bighi, J. Chromatogr. 471 (1989) 407.
[62] M. Kuchař, V. Rejholec, E. Kraus, V. Miller, V. Rábek, J. Chromatogr. 280 (1983) 279.
[63] C. Yamagami, K. Araki, K. Ohnishi, K. Hanasato, H. Inaba, M. Aono, A. Ohta, J. Pharm. Sci. 88 (1999) 1299.
[64] K. Valkó, M. Plass, C. Bevan, D. Reynolds, M.H. Abraham, J. Chromatogr. A 797 (1998) 41.
[65] K. Valkó, C. Bevan, D. Reynolds, Anal. Chem. 69 (1997) 2022.
[66] C.M. Du, K. Valkó, C. Bevan, D. Reynolds, M.H. Abraham, Anal. Chem. 70 (1998) 4228.
[67] L.A. Cole, J.G. Dorsey, Anal. Chem. 64 (1992) 1324.
[68] G. Cimpan, F. Irimie, S. Gocan, H.A. Claessens, J. Chromatogr. B 714 (1998) 247.
[69] F. Denotes-Mainard, C. Jarry, J. Thomas, P. Dallet, J. Liq. Chromatogr. 14 (1991) 795.
[70] K. Jinno, Y. Yokoyama, J. Chromatogr. 550 (1991) 325.
[71] V. DeBiasi, W.J. Lough, J. Chromatogr. 353 (1986) 279.
[72] K. Miyake, F. Kitaura, N. Mizuno, H. Terada, Chem. Pharm. Bull. 35 (1987) 377.
[73] J.R. Benson, D.J. Woo, J. Chromatogr. 464 (1984) 400.
[74] A.V. Kiselev, J. Colloid. Interf. Sci. 28 (1968) 430.
[75] J. Paloes, J. Colloid. Interf. Sci. 31 (1969) 7.
[76] M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kalisan, A. Nasal, P. Haber, J. Chromatogr. 14 (1977) 35.
[77] R.C. Young, R.C. Mitchel, T.H. Brown, C.R. Ganellin, R. Griffiths, M. Jones, K.K. Rana, D. Saunders, I.R. Smith, N.E. Sore, T.J. Wilks, J. Med. Chem 31 (1988) 656.
[78] K. Yasukawa, Y. Tamura, T. Uchida, Y. Yanagihara, K. Noguchi, J. Chromatogr. 410 (1987) 129.
[79] Y. Arai, M. Hirukawa, T. Hanai, J. Liq. Chromatogr. 10 (1987) 635.
[80] A. Bechalany, T. Röthlisberger, N. El Tayar, B. Testa, J. Chromatogr. 473 (1989) 115.
[81] A. Bechalany, A. Tsantili-Kakoulidou, N. El Tayar, B. Testa, J. Chromatogr. 541 (1991) 221.
[82] P. Vallat, W. Fan, N. El Tayar, P.A. Carrupt, B. Testa, J. Liq. Chromatogr. 15 (1992) 2133.
[83] C. Yamigami, N. Takao, Chem. Pharm. Bull. 40 (1992) 925.
[84] Supelco 2001 catalog, Bellefonte, PA, USA 16823 (http://www.sigmaaldrich.com)
[85] F. Gobet, E. sz. Kovats, Adsorpt. Sci. Technol. 9 (1968) 77.
[86] P. Roumeliotis, K.K. Unger, J Chromatogr. 149 (1978) 211.
[87] W.G. Tramposch, S.G. Weber, Anal. Chem. 56 (1984) 2567.
[88] L.C. Sander, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 299.
[89] A. Berthod, J. Chromatogr. 549 (1991) 1.
[90] S.M. Staroverov, A. Yu Fadeev, J. Chromatogr. 544 (1991) 77.
[91] K. Albert, E.J. Bayer, J. Chromatogr. 544 (1991) 345.
[92] R.P.W. Scott, Silica Gel and Bonded Phases—Their Production, Properties and Use in LC, Wiley, Chichester, 1993.
[93] P. Schindler, H.R. Kamber, Helv. Chim. Acta 51 (1969) 1781.
[94] N. El Tayar, A. Kakoulidou, T. Rothlisberger, B. Testa, J. Chromatogr. 439 (1988) 237.
[95] C. Altomare, A. Carotti, S. Cellamare, F. Ferappi, Int. J. Pharm. 56 (1989) 273.
[96] J. Yamaguchi, T. Hanai, Chromatographia 27 (1989) 371.
[97] A.J. Leo, J. Pharm. Sci. 76 (1987) 166.
[98] R.W. Spencer, L.J. Copp, J.R. Pfister, J. Med. Chem. 28 (1985) 1828.
[99] Medchem Project log *P* database, available through Biobyte, Claremont CA. (http://www.biobyte.com/)
[100] C. Hansch, A.J. Leo, D. Hoekman, Exploring QSAR; Hydrophobic, Electronic, and Steric Constants (ACS Professional Reference Book), American Chemical Society, Washington, DC, 1995.
[101] C. Hansch, A.J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979.
[102] A.J. Leo, C. Hansch, D. Elkins, Chem. Rev. 71 (1971) 525.
[103] I. Canals, K. Valkó, E. Bosch, A.P. Hill, M. Roses, Anal. Chem. 73 (2001) 4937.

[104] F. Szokoli, Z. Nemeth, J. Inczedy, Chromatographia 29 (1990) 265.
[105] C. Herrenknecht, D. Ivanovic, E.G. Nivaud, M. Guernet, J. Pharm. Biomed. Anal. 8 (1990) 1071.
[106] E. Bosch, C. Rafols, M. Rosés, Ann. Chim. Acta 302 (1995) 109.
[107] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[108] M. Rosés, I. Canals, H. Allemann, K. Sigur, E. Bosch, Anal. Chem. 68 (1996) 4094.
[109] Cs. Horváth, W. Meländer, I. Molnár, Anal. Chem. 49 (1977) 865.
[110] R.A. Scherrer, (ACS Symposium, Series No. 255) in: P.S. Magee, G.K. Kohn, J.J. Menn (Eds.), Pesticide Synthesis Through Rational Approaches, American Chemical Society, Washington, DC, 1984, pp. 225–246.
[111] R.A. Scherrer, in: B. Testa, H. van de Waterbeemd, G. Folkers, R. Guy (Eds.), Pharmacokinetic Optimization in Drug Research-Biological, Physicochemical, and Computational Strategies, Wiley–VCH, Zurich, 2001.
[112] H. Kubinyi, in: R. Mannhold, P. Krogsgaard-Larsen, H. Timmerman (Eds.), QSAR: Hansch Analysis and Related Approaches, VCH Publishers, Weinheim, Germany, 1993, pp. 80–81.
[113] T. Baumann, J. Chromatogr. 373 (1986) 191.
[114] M.-M. Hsieh, J.G. Dorsey, Anal. Chem. 67 (1997) 48.
[115] C. Tomlin (Ed.), 12th ed, The Pesticide Manual-A World Compendium, British Crop Protection Council/Royal Society of Chemistry, Bath, 2000.
[116] L. Lacko, B. Wittke, G. Zimmer, Biochem. Pharmacol. 30 (1981) 1425.
[117] T. Fugita, J. Iwasa, C. Hansch, J. Am. Chem. Soc. 86 (1964) 5175.
[118] A. Hersey, R.M. Hyde, D.J. Livingstone, J. Pharm. Sci. 80 (1991) 333.
[119] W.J. Dunn, J. Med. Chem. 16 (1973) 484.
[120] R.D. Schoenwald, H.-S. Huang, J. Pharm. Sci. 72 (1983) 1266.
[121] G. Burgot, J.-L. Burgot, Int. J. Pharm. 94 (1993) 135.
[122] P. Ritter, M. Jerman, Arzneim. Forsch. 16 (1966) 1647.
[123] L. Illum, W. Bundgaard, S.S. Davis, Int. J. Pharm. 17 (1983) 183.
[124] W.M. Pardridge, L.J. Mietus, J. Clin. Invest. 64 (1979) 145.
[125] N. Dzimiri, U. Fricke, K. Uwe, W. Klaus, Br. J. Pharmacol. 91 (1987) 31.