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Determination of octanol–water partition coefficient for terpenoids using reversed-phase high-performance liquid chromatography

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Abstract

Octanol–water partition coefficients (K_{ow}) for 57 terpenoids were measured using a RP-HPLC method. Sample detection was achieved with standard UV and refractive index detectors and required no special column treatment. Measured log K_{ow} values for the terpenoids ranged from 1.81 to 4.48 with a standard error of between 0.03 and 0.08 over the entire range. Partition coefficients determined by the RP-HPLC method were compared against shake flask, atom/fragment contribution, fragment and atomistic methods. The HPLC values were found to give the best correlation with shake flask results. Log K_{ow} values calculated by the atom/fragment contribution method gave the best correlation with the HPLC values when compared to fragment and atomistic methods.

Keywords: Octanol–water partition coefficient; Partition coefficients; Terpenoids

1. Introduction

Since the work of Hansch and Fujita [1] the octanol–water partition coefficient (K_{ow}) has been widely accepted as providing a good indication of the distribution of analytes into biological membranes [2–4]. This has made it one of the most commonly reported physical properties of drugs, pesticides and other chemicals [5,6]. This is not surprising since in order for compounds to have biological activity they must be able to traverse or at least partition into biological membranes [5].

The use of high-performance liquid chromatography (HPLC) methods for the determination of log K_{ow} is growing in use [7]. There are many examples in the literature of its successful use in predicting log K_{ow} for a wide range of compounds [2,8]. These predicted log K_{ow} values have also been successfully correlated with biological data in many instances. Several examples are given in a review by Carney (1985) [9].

Determination of log K_{ow} by reversed-phase (RP) HPLC is highly dependent on the retention of solutes and therefore the capacity factor (k'). The k' of a compound on a RP-HPLC column can be related to K_{ow} using the relationship between different partitioning systems derived by Collander as follows;

$$\log K_{ow} = a \log k' + b \quad (1)$$

where a and b are empirical constants which characterise the solvent system in question.

Even though k' can be related to K_{ow}, k' will, for a given solute and stationary phase, depend on the

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composition of the mobile phase mix used in elution [10]. Therefore it has been suggested that k' should be determined using pure water as eluent (k_w). In this case the capacity factor will be independent of any organic modifier effects and the polar–non-polar partitioning will be more similar to shake flask measurements and dependent on the solute's structure and polar functionalities [11]. Under most conditions, however, pure water cannot be used as the eluent due to inordinately long retention times. Hence an organic modifier must be added [2,12].

k_w can be calculated if k' is determined over a range of modifier concentrations and extrapolated back to 0% modifier (k_w) [1,5]. Experimentally it has been found that log k' is related to the volume fraction (φ) of the organic modifier added to water as follows [10,12];

$$\log k' = \log k_w + c\varphi + d\varphi^2 \quad (2)$$

where c and d are empirical constants characterised by the organic modifier in question.

This equation is quadratic and this can cause problems with extrapolation to 0% modifier due to the curvature which results when plotting log k' versus the volume fraction of the organic modifier [2]. This issue has been reviewed by Lambert [5] who suggested that methanol should be used as the modifier at a single volume fraction (above 25–30% but below 70% methanol) and without extrapolation. This is because most of the curvature for methanol occurs at volume fractions below 30% and above 70% and therefore extrapolated k' values determined at volume fractions within this range will deviate from the true k_w value and give poor correlations to K_{ow} [5]. In addition a volume fraction chosen within the range specified above will have a minimal effect on the stationary phase. This is important since it has been suggested that at high modifier levels the eluent is so unlike water that it becomes insensitive to hydrophobicity and can hence give poor correlations with biological data [5,7,12].

Alternatively, it has been suggested that polycratic extrapolation suffers from less problems than monocratic extrapolation since at only one modifier concentration there is a limitation to the range of hydrophobicity able to be measured because of the long retention times of highly lipophilic compounds. The opposite problem can also occur for highly hydrophilic compounds. Compounds either unretained or retained too long may therefore have to be measured using different column lengths or eluent composition. This retention data must then be cross correlated with the original conditions [13].

The greatest shortcoming of the HPLC method is when the interaction of analytes with the stationary phase are significantly different. This occurs especially when analytes have large differences in hydrogen bonding capability. Where this occurs correlations between k_w and K_{ow} can be improved by separating compounds into classes according to their hydrogen bonding properties [13]. Effects such as this can occur especially where free silanol groups occur on columns [2]. Thus highly endcapped columns are recommended for the RP-HPLC method [5,14,15]. Alternatively the addition of a masking agent such as *n*-decylamine to the mobile phase may suppress the necessary interactions [2]. However this can introduce other variables into the method since the masking agent can have selective effects on retention times. Masking agents such as *n*-decylamine can also ion pair with acidic solutes [5].

Another method of avoiding specific interactions of solutes with the stationary phase of the column is to saturate the column with octanol and use as the eluent octanol saturated water. It has been suggested that this method provides a much more realistic estimate of log K_{ow}. One limitation of this method however is that compounds of very limited solubility tend to give anomolously low log K_{ow} values [14]. Also when using water–methanol eluents the required dispersive interactions and hydrogen bonding exists in the HPLC system thus precluding the need for coating the stationary phase with octanol [12].

One of the greatest obstacles to estimating K_{ow} is the difficulty in determining the reliability of the results [7]. Hence estimation of K_{ow} via calculation can be advantageous. Two of the more commonly used calculation methods are fragment and atomistic methods.

In fragment methods the molecule is regarded as being a combination of a number of chemically recognisable and common atoms or groups of atoms. One of the more commonly used fragment methods was developed by Leo and Hansch [10].

Rather than considering fragments, atomistic

methods are based on contributions of single atoms. Again these single atom contributions depend on the local environment within the molecule. Ghose and Grippen deduced contributions for 110 atom types which was updated to 120 types by Viswanadhan et al. [16].

Comparison of atomistic and fragment methods lead to the conclusion by Mannhold et al. [17] that the Hansch and Leo fragment technique and another called the Rekker method generally gave more accurate log K_{ow} estimations than the other types. In general however, all calculation methods perform better on simple rather than complicated molecules.

In this study log K_{ow} values were determined for 57 terpenoids using a simple RP-HPLC method and compared with calculated values using both fragment and atomistic methods.

Table 1
Standards used for determination of log K_{ow} for terpenoids

	Log K_{ow} [a]
Non-phenolic standards	
Benzyl alcohol	1.10
Benzaldehyde	1.48
Benzene	2.03
Toluene	2.61
Cyclohexene	2.86
p-Cymene	4.10
Phenolic standards	
Phenol	1.48
Eugenol	2.99
Thymol	3.30

[a] K_{ow} values are averages of values taken from Refs. [10] and/or [18]. Only values determined by the shake-flask method were used to calculate averages.

2. Experimental

2.1. Terpenoids and standards

Standard compounds and terpenoids were purchased from Aldrich (Castle Hill, Australia) and Fluka (Castle Hill, Australia). The standard compounds and terpenoids were of 95% purity or greater, as determined by gas chromatography (GC), with the following exceptions; α-terpinyl acetate (90%), 1,4-cineole (75%), α-terpinene (85%), α-terpinolene (89%), piperitone (92%), α-ionone (91%). Each of these exceptions was the highest purity available at the time of purchase. Stock solutions of standards and terpenoids were made up in HPLC grade methanol to a concentration of approximately 1 mg/ml.

2.2. Determination of log K_{ow} by RP-HPLC

Nine compounds (Table 1) of known log K_{ow} and of similar chemical structure to that of the terpenoids were used as standards in the determination of log K_{ow} values. The three phenolic standards were used for determination of log K_{ow} for phenolic and amine terpenoids.

HPLC analysis of samples and standards was carried out using a Hewlett-Packard 1090 HPLC system fitted with an Alltech Altima C_{18} column (150 mm×4.6 mm I.D., pore size: 5 μm), diode array detector and auto-injector. Detection of compounds using the diode array was done at 215 nm with 500 nm as the reference wavelength. Where compounds were unable to be detected using diode array an Erma ERC-7510 refractive index (RI) detector was fitted. Instrument conditions for all analyses was as follows; oven temperature: 37°C, flow-rate: 1 ml/min, injection volume: 10 μl.

Each analysis was performed isocratically using a range of methanol–water mixtures between 75 and 30% methanol with the water portion buffered to pH 7.2 using 0.02 *M* 4-morpholinepropanesulphonic acid (MOPS). All compounds were analysed at a minimum of four different methanol–water ratios, however the lower limit of methanol able to be used was governed by the polarity of the compounds tested. Three injections were made for each terpenoid and five injections for the standards at each methanol–water ratio. Retention times for the all compounds were recorded at each methanol–water ratio along with the hold time of the column as estimated by the retention times of methanol used to dissolve each sample.

The capacity factors (k') for terpenoids and standards were calculated at each methanol:water ratio from retention times using the formula below;

$$k' = (t_R - t_0)t_0 \quad (3)$$

where t_R is the sample retention time and t_0 is the mobile phase hold time as estimated by retention

time of methanol. For the each standard and terpenoid a plot of k' versus proportion of organic modifier (i.e., methanol) was generated and extrapolated back to 0% modifier to determine the capacity factor of each compound as if the eluent were 100% water (k_w).

In the case of the standards k_w was then plotted against the known log K_{ow} of the compounds to form a standard curve. Two separate standard curves were plotted, one for non-amine and non-phenolics (log $K_{ow}=1.43$ log $k_w-0.60$, $r^2=0.950$, $n=6$, S.E.=0.27) and the other for amine and phenolic compounds (log $K_{ow}=1.41$ log $k_w-0.15$, $r^2=0.989$, $n=3$, S.E.=0.148). k_w of the terpenoids was then used to determine their log K_{ow} from these standard curves.

2.3. Determination of log K_{ow} by shake flask

Solutions of known terpenoid concentration were prepared using type I water, pre-saturated with 1-octanol (Aldrich ACS spectroscopic grade) for 24 h prior to use. For those terpenoids suspected to have log K_{ow} values of less than 3, equivalent volumes of the terpenoid and octanol (pre-saturated with type I water for 24 h before use) were added together. For those terpenoids with a log K_{ow} suspected to be greater than 3, a ratio of 1 ml of octanol to 10 ml of water was used. The resulting two-phase mixture was repeatedly inverted for 1 h using a slow rotating wheel (approx. 1 revolution/10 s) to which the samples were attached.

After mixing, samples were centrifuged for 30 min (6000 rpm) to ensure any possible emulsions were removed. Both fractions of the sample were analysed using a 6890 Hewlett-Packard GC system fitted with an SGE BPX5 capillary column (50 m×0.25 mm I.D., 1 μm film thickness) and a flame ionization detection (FID) system. The GC operating parameters for the analysis were as follows: inlet temperature: 240°C, carrier gas: hydrogen at 40 cm/s, injection size 1 μl, detector temperature: 280°C, initial oven temperature 100°C for 5 min, increased at 4°C/min to 160°C then increased at 45°C/min to 250°C and held for 5 min. Injector split ratios were varied according to the sensitivity required. Quantitation was achieved by use of external standards of each of the terpenoids. External standards were run at each of the split ratios and in each of the solvents (i.e., water and octanol) used. Octanol fractions were diluted in methanol before analysis to reduce column overloading by octanol.

2.4. Molecular modelling and calculation of molecular parameters

Compounds were constructed using the molecular modelling program Chemsite version 2.0. These structures were then transferred to the molecular modelling program Molecular Modelling Pro where log K_{ow} values were calculated using an atomistic method developed by Ghose and Crippen [19] and a fragment addition method developed by Hansch and Leo [20]. These structures where then imported to KOWWIN version 1.54 (Syracuse Research Corp.) where the log K_{ow} values were calculated using an atom/fragment contribution method as described in Meylan and Howard [21].

3. Results and discussion

3.1. Precision of RP-HPLC method

The log K_{ow} values of 57 terpenoids examined in this study, determined using HPLC, shake flask, atomistic, fragment and atom/fragment contribution methods, are shown in Table 2. Table 2 also shows the standard error (S.E.) for the HPLC and shake flask methods. It can be seen from this data that the standard error in the HPLC measurements lies between 0.03 and 0.08 while the standard error for the shake flask results lies between 0.01 and 0.06. Hence the HPLC method has a comparable level of precision to the shake flask technique over the entire log K_{ow} range (1.81–4.48) examined. Such consistent precision at higher log K_{ow} values is advantageous when compared to other HPLC methods which use octanol treated columns since such methods are restricted to log K_{ow} values of around 3 due to problems with peak detection [22].

Piraprez et al. [23] have also shown the RP-HPLC technique has consistent precision in a study of log k_w values of 96 aroma and flavour compounds. The main difference in the method used by Piraprez et al. [23] and our method was their addition of de-

Table 2
Log K_{ow} values of terpenoids determined using HPLC, fragment and atomistic methods

Compound	Measured log K_{ow}				Calculated log K_{ow}		
	RP-HPLC	S.E.	Shake flask	S.E.	Fragment method	Atom/fragment contribution method	Atomistic method
p-Ment-6-ene-2,8-diol	1.81	0.03	1.11	0.01	0.94	2.29	1.44
(−)-*cis*-Myrtanylamine	2.05	0.03			3.22	2.88	1.81
(1*s*)-(−)-Verbenone	2.23	0.04			2.22	3.21	2.52
Car-3-en-2-one	2.42	0.05			2.35	2.85	2.64
Linalool oxide	2.43	0.04			0.85	1.53	1.27
(±)-Camphor	2.74	0.05			2.34	3.04	2.95
(*R*)-(−)-Carvone	2.74	0.05	2.71	0.01	1.86	3.12	2.33
1,8-Cineole	2.84	0.05	2.74	0.06	2.80	3.13	1.69
Piperitone	2.85	0.06			2.76	3.07	3.05
1,4-Cineole	2.97	0.04			5.05	3.13	1.83
Myrtenal	2.98	0.06			2.71	2.78	2.08
(−)-Borneol	3.01	0.05			2.71	2.85	2.42
(*cis, trans*)-Isoeugenol	3.04	0.06			2.36	2.65	2.51
(−)-Menthone	3.05	0.07			2.68	2.87	3.15
Dihydrocarvone	3.08	0.06	2.85	0.01	2.14	2.86	2.76
(+)-Pulegone	3.08	0.06			2.76	3.20	2.77
(−)-Carveol	3.12	0.06	2.85	0.03	2.19	3.23	2.27
(−)-Perilla aldehyde	3.13	0.06			2.73	3.34	2.22
(*S*)-*cis*-Verbenol	3.16	0.06			2.04	2.73	1.95
(*S*)-(−)-Perilla alcohol	3.17	0.06			2.38	3.36	1.93
(+)-Fenchol	3.17	0.06			2.58	2.85	2.57
(+)-Isomenthol	3.19	0.07			3.14	3.38	2.78
Limonene oxide	3.20	0.06			3.05	3.43	1.84
Dihydrocarveol	3.21	0.06			2.60	3.37	2.39
(1*R*)-(−)-Myrtenol	3.22	0.07			2.36	2.80	1.79
(±)-Isoborneol	3.24	0.06			2.71	2.85	2.42
Terpinen-4-ol	3.26	0.06	2.80	0.01	2.60	3.33	2.16
α-Terpineol	3.28	0.06			2.70	3.33	2.02
(−)-Menthol	3.40	0.06			3.14	3.38	2.78
(±)-Menthol	3.40	0.06			3.14	3.38	2.78
(1*S*2*S*5*S*)-(−)-Myrtanol	3.41	0.08			3.03	2.89	2.16
Methyleugenol	3.45	0.07			2.75	3.03	2.59
Nerol	3.47	0.07			2.65	3.47	2.46
Carvacrol	3.49	0.07	3.75	0.01	3.83	3.52	3.42
Linalool	3.50	0.07			2.43	3.38	2.52
(1*R*)-(−)-Fenchone	3.52	0.08			2.56	3.04	3.41
Geraniol	3.56	0.07			2.65	3.47	2.46
(*R*)-(+)-Citronellal	3.83	0.08			3.32	3.53	2.72
β-Ionone	3.84	0.08			3.78	4.42	3.37
α-Ionone	3.85	0.08			3.78	4.29	3.55
β-Citronellol	3.91	0.08	4.04	0.01	3.19	3.56	2.75
Linalyl acetate	3.93	0.07			5.45	4.39	2.21
α-(±)-Terpinyl acetate	3.96	0.06			5.72	4.34	1.71
Neryl acetate	3.98	0.07			3.56	4.48	2.59
Menthyl acetate	4.00	0.05			4.27	4.39	2.91
Geranyl acetate	4.04	0.07			3.56	4.48	2.59
β-Pinene	4.16	0.05			4.34	4.35	2.84
(+)-Camphene	4.22	0.05			4.99	4.80	3.27
α-Terpinolene	4.24	0.05			4.36	4.88	2.71
α-Terpinene	4.25	0.05			4.36	4.75	2.89
γ-Terpinene	4.36	0.05			4.36	4.75	2.89
(+)-Limonene	4.38	0.05			4.36	4.83	2.94
(−)-Limonene	4.38	0.05			4.36	4.83	2.94
Car-3-ene	4.38	0.05			4.47	4.61	2.80
Car-2-ene	4.44	0.06			4.47	4.61	2.80
(+)-α-Pinene	4.44	0.06			4.34	4.27	2.80
(−)-α-Pinene	4.48	0.06			4.34	4.27	2.80

cylamine to the mobile phase as a masking agent to combat silanotrophic effects on thiazole derivatives, pyrazine derivatives, phenols and sulphur-containing compounds which made up the majority of the compounds tested. Although no masking agent was used in our experiments, comparison of log k_w for compounds common to both studies showed good agreement.

3.2. RP-HPLC method accuracy and comparison to calculation techniques

To determine the accuracy of the HPLC method and calculation techniques shake flask results were plotted against each of the data sets. The resulting correlation coefficients showed that the HPLC method was the most closely correlated ($r^2=0.94$) to the shake flask data followed by the atom/fragment contribution ($r^2=0.87$), fragment ($r^2=0.80$) and atomistic ($r^2=0.66$) techniques.

Examination of the data in Table 2 shows that the log K_{ow} values derived by the three calculation methods do not always match that obtained by HPLC or shake flask. Additionally the different methods of calculation do not always give the same result. When the values from each of the calculation methods were plotted against the HPLC data it was found that the atom/fragment contribution method gave the best correlation ($r^2=0.76$) followed by the fragment ($r^2=0.61$) and then the atomistic methods ($r^2=0.26$).

The fact that the atomistic method had the lowest correlation with the HPLC method coupled with the fact that this method also showed poor correlations with the other two calculation methods (data not shown) indicates that the atomistic method is not appropriate for the estimation of log K_{ow} values for terpenoids.

Despite its statistically significant correlation with the atom/fragment contribution method it is important to examine where the HPLC data deviates from the calculated values. While Fig. 1 shows that the atom/fragment contribution method has a reasonably consistent variability throughout the log K_{ow} range studied, Fig. 2 shows that this is not the case

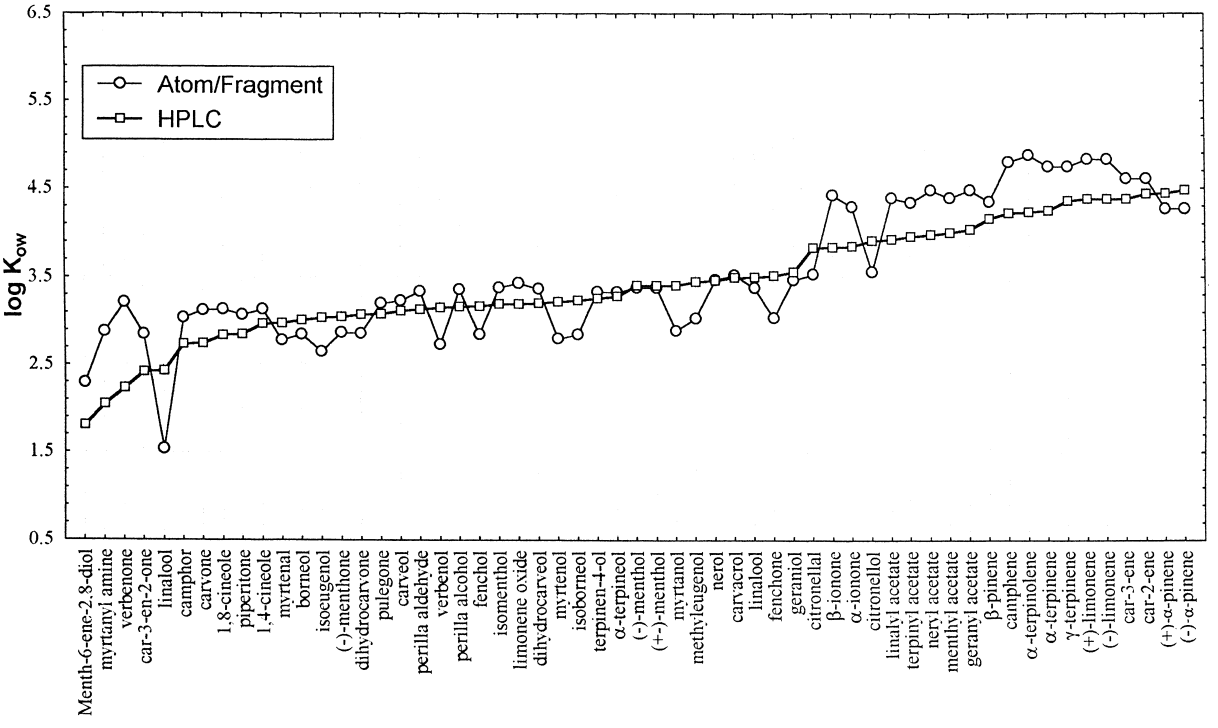

Fig. 1. Comparison of log K_{ow} values measured by RP-HPLC and calculated by the atom/fragment contribution method.

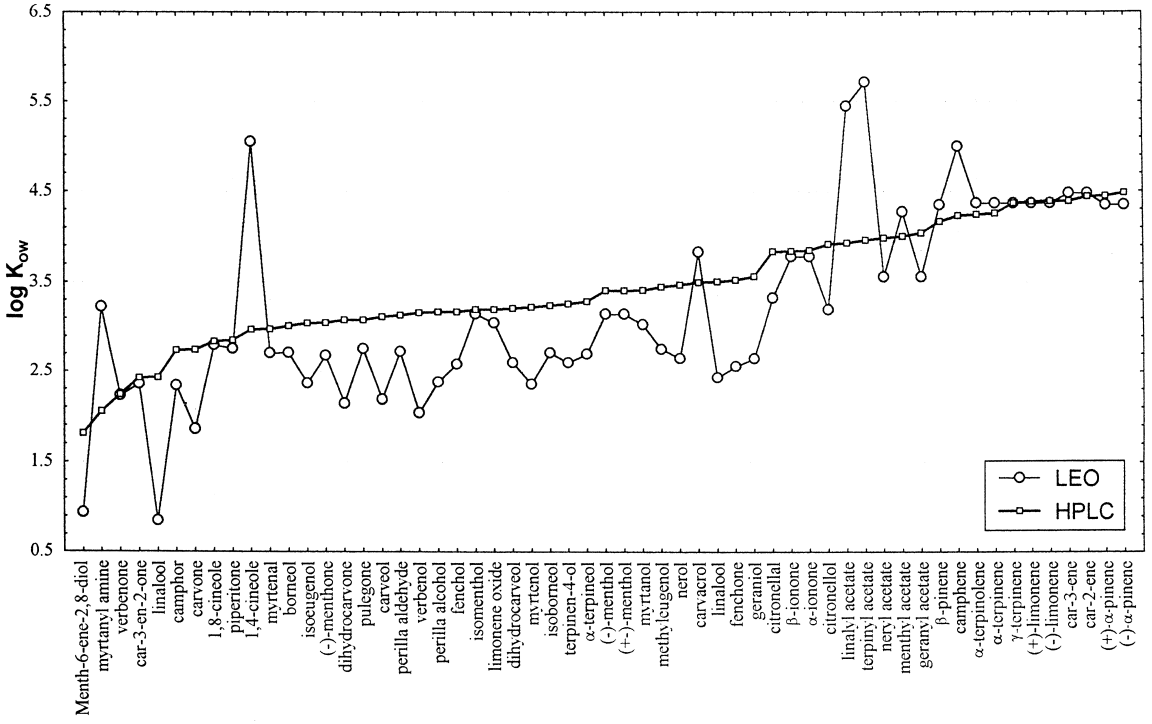

Fig. 2. Comparison of log K_{ow} values measured by RP-HPLC and calculated by the Leo fragment method.

for the fragment method. It can be seen in Fig. 2 that most of the deviation occurs with terpenes that have an alcohol, aldehyde, acetate or ketone functional group.

Within the oxygenated compounds, aldehyde and ketone terpenoids ($r^2 = 0.66$, range = 1.86–3.78, $n = 14$) were found to have a better correlation with the fragment method values than alcohols ($r^2 = 0.21$, range = 1.79–3.42, $n = 20$). Valid correlations for acetates and hydrocarbons were unable to be determined due to clustering of the data.

The disparity between calculated and RP-HPLC values as seen in this study may be a reflection of the complicating factors that oxygenated functional groups introduce to the fragment methods predictive capability when compared with more simple hydrocarbons.

4. Conclusions

The RP-HPLC method used in this study was found to be able to accurately determine log K_{ow} values for terpenes without any column treatment. It operated with a consistent precision over a range of log K_{ow} values from 1.8–4.48 and showed good correlation with the shake flask technique. When estimation techniques were used to calculate log K_{ow} values the atom/fragment contribution method was found to be the most accurate.

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