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Gradient elution reversed-phase high-performance liquid chromatography for fractionation of complex mixtures of organic micropollutants according to hydrophobicity using isocratic retention parameters

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Abstract

Gradient elution RP-HPLC is used as a suitable method to infer the hydrophobicity values (log K_{ow}) of components in complex samples. In this study, the slope (S) and the intercept (log k_w) of the linear relationship between the logarithm of the retention factor (log k) and the percentage methanol of the eluent (φ) were first obtained by isocratic runs for a set of micropollutants with diverse structures, similar to the contaminants to which the method will be applied. Both S and log k_w obtained from isocratic runs could be related to log K_{ow}. Retention times in gradient elution, estimated from these isocratic parameters as a function of log K_{ow}, were in very good agreement with experimental values and an almost linear relationship can be established between log K_{ow} and the retention time. This makes gradient elution RP–HPLC a suitable method to fractionate complex mixtures according to hydrophobicity.

Keywords: Gradient elution; Hydrophobicity; Partition coefficient; Chlorobenzenes; Hydrocarbons; Phenols; Phthalates; Pesticides

1. Introduction

A widely used parameter in reversed-phase high-performance liquid chromatography (RP-HPLC) is the retention factor (k) of a compound in isocratic elution. Well-established relationships have been found between retention factors in RP-HPLC and hydrophobicity, e.g. [1–3], which is usually expressed as the octanol–water partition coefficient (K_{ow}). However, the relationship between log k and log K_{ow} is not a fixed one but depends on the structures of the investigated compounds, the composition of the eluent and the type of column [1].

In environmental chemistry, bioconcentration is mostly estimated from QSARs using log K_{ow}. The baseline toxicity by narcosis is directly related to bioconcentration, because this type of toxicity only depends on the concentration of accumulated micropollutants in biota [4]. Therefore, this baseline toxicity can also be estimated from log K_{ow}. In

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literature it is also indicated that RP-HPLC retention factors may even be better descriptors for membrane–water partition coefficients than log K_{ow} itself [1,2]. Consequently, for this purpose, log K_{ow} values calculated from RP-HPLC data can still be very useful.

Moreover, it is not possible to determine the hydrophobicity of complex mixtures, expressed as an octanol–water partition coefficient, by common techniques, such as the 'shake-flask' or 'slow-stirring' method. These methods are only valid for the determination of log K_{ow} of pure compounds [5]. However, RP-HPLC enables the separation of mixtures into fractions according to hydrophobicity.

Many chemical substances are actually complex mixtures of organic chemicals and environmental contamination is almost invariably caused by mixtures of compounds. Recently, several experimental methods were developed for which a fractionation according to hydrophobicity is required of complex mixtures, which contain organic micropollutants of very diverse structures. This fractionation can be used to perform tests for the presence of potentially bioconcentrating compounds in environmental samples [6] or to obtain information on the hydrophobicity distribution profile of complex mixtures of unknown composition [5].

If compounds, which cover a broad hydrophobicity range, have to be separated in the same run, gradient elution is applied in order to overcome the well-known disadvantages of isocratic elution. Recently, log K_{ow} has been correlated to retention in gradient elution empirically [6–8]. Alternatively, for single compounds, log K_{ow} could be easily derived from the retention time in a fast gradient run [9], which was related to a relationship between log K_{ow} and the percentage of organic modifier at which log $k=0$ (φ_0) is in isocratic elution [9,10].

Instead of optimising gradients experimentally, retention times in gradient elution can also be calculated from isocratic parameters, e.g. [11]. In this study, the retention times of very diverse compounds in gradient elution are described on the basis of these isocratic parameters, which can also be expressed in terms of log K_{ow}, and the applied gradient. The objective is to obtain an almost linear and well-defined relationship between the retention time in gradient elution RP-HPLC and log K_{ow} for a set of miscellaneous chemicals, because this has the advantage that for fractionation the fraction volume can be held constant with increasing hydrophobicity [6]. For this purpose, the capacity factors of 29 compounds are determined using isocratic elution at eluent compositions ranging from 80 to 100% methanol. From these isocratic data, retention in gradient elution is calculated, both on the basis of the isocratic parameters of each individual compound and on the basis of log K_{ow}. As a validation, the retention times are determined experimentally using a suitable solvent gradient. The criterion for the accuracy of the estimated retention times, is that the error in the corresponding log K_{ow} values is comparable to the error in the determination of log K_{ow} by conventional techniques, such as the 'shake-flask' method.

2. Theory

2.1. Retention in isocratic elution

In isocratic elution, retention can be described by the retention factor (k). The accuracy of k strongly depends on that of the hold-up time of the chromatographic system (t_0) [12,13]. Several methods are used to investigate the hold-up time. The most common methods to determine the hold-up time experimentally are the injection of a solution of an inorganic salt, a very polar organic compound or one of the components of the eluent, weighing the column with two different solvents or linearisation of the relationship between log k and the carbon number of a homologous series of compounds [13–16].

In RP-HPLC, a mixture of water and an organic solvent is mostly used as eluent. A relationship exists between log k of organic compounds and the volume fraction of the organic solvent (φ) of the eluent [17]:

$$\log k = A \cdot \varphi^2 + B \cdot \varphi + C \quad (1)$$

In this equation, A, B and C are constants dependent on the solute and the reversed-phase system. With methanol as modifier, the quadratic term in Eq. (1) can be neglected and an almost linear relationship exists between log k and φ [17–19]:

$$\log k = \log k_w - S \cdot \varphi \quad (2)$$

In this equation, k_w is the retention factor with pure water as eluent and S indicates the change in log k with changing solvent composition (φ). This linear relationship is only valid for a limited range of φ values [1,17,20]. Deviations from linearity occur especially for low percentages of organic modifier [20]. Therefore the extrapolated retention factor k_w has no distinct physical meaning [2,19]. This is also illustrated by the fact that extrapolated values of log k_w are significantly different if other organic modifiers are used [1].

2.2. Retention in gradient elution

By taking into account the fractional migration of a compound during the gradient and pre-elution prior to the gradient, the retention time in gradient elution (t_R) can be expressed as [21]:

$$t_R = \frac{t_0}{b} \log\left(2.3 \cdot b \cdot \left(k_0 - \frac{t_D}{t_0}\right) + 1\right) + t_0 + t_D \quad (3)$$

with

$$b = \frac{t_0 \cdot \Delta\varphi \cdot S}{t_G} \quad (4)$$

in which k_0 is the retention factor at the initial eluent composition of the gradient, t_D is the dwell time of the system, which is the time from the start of the gradient until it reaches the point of injection, t_G is the total time of the programmed gradient, $\Delta\varphi$ is the change of organic modifier in the eluent during the gradient programme and the factor 2.3, ln(10), is introduced by using log-base 10. For compounds that are initially strongly retained (large k_0), the term t_D/t_0 can be omitted. This analytical expression for the retention time in gradient elution consists only of isocratic elution parameters and parameters describing the applied gradient and chromatographic system. The parameters S and k_w can also be obtained analytically from gradient elution, if the retention times in at least two gradient programmes are available [22]. For compounds eluting after the gradient programme has reached the end of the column, the retention time is given by a simple equation [22], provided the retention factor at the final eluent composition (k_z), which is generally pure organic modifier, is negligible compared with the retention factor at the start of the gradient:

$$t_R = t_G + t_0 \cdot \left(k_z - \frac{1}{2.3 \cdot b} + 1\right) + t_D \quad (5)$$

2.3. Relationship between retention in RP-HPLC and K_{ow}

RP-HPLC has frequently been used to predict the log K_{ow} values of divergent classes of organic compounds, mostly using octadecylsilica as stationary phase and water–methanol mixtures as eluent. In most cases, the logarithm of the capacity factor extrapolated to 100% water (log k_w) is related to log K_{ow} [1,2,23], according to:

$$\log k_w = a_1 + a_2 \cdot \log K_{ow} \quad (6)$$

In this equation a_1 and a_2 are constants obtained by linear regression. In general, log k_w is considered as the best chromatographic parameter to estimate hydrophobicity [1]. However, the correlation between extrapolated values of log k_w and log K_{ow} is not exactly the same for all classes of compounds [23], with the slope being fairly constant (0.91–0.99) and the intercept varying from −0.22 to 0.28. Even experimentally determined log k_w values are not always suitable to estimate log K_{ow}, especially not in the case of polar solutes [1,2]. As mentioned above, recently, a new hydrophobicity index (φ_0) was proposed with the advantages that it is independent of the column used and the shape of the log k vs. φ plot [10].

The correlation of the slope (S) of the linear relationship between log k and φ with hydrophobicity is strongly dependent on the organic modifier used. With acetonitrile as organic modifier, S appears to be a rather constant value for organic micropollutants, regardless of their hydrophobicity [19,21]. However, for methanol and tetrahydrofuran, the value of S is correlated with log k_w for compounds from homologous series according to [18]:

$$S = p + q \cdot \log k_w \quad (7)$$

With the same organic solvent, the differences in the slope (q) are small for different homologous series but the intercept (p) can vary considerably. For methanol–water eluents, these differences in p

are much smaller than for other organic solvents [18]. In addition, the retention factors can also be quite different for different types of column packings [24]. Data from the literature confirm that Eq. (7) provides a good correlation for methanol–water eluents, not only for homologous series of compounds but also for organic compounds with different functional groups, provided they are determined with the same column [17,19,25].

By means of Eq. (7), the parameters S and k_w can be calculated from a single gradient run if the regression parameters p and q are known, as for methanol [26], or by using a fixed value for S, as with acetonitrile [21]. For a set of compounds, for which Eq. (7) is valid, a linear relationship between S and $\log K_{ow}$ can be derived by combining Eqs. (6) and (7).

$$S = a_3 + a_4 \cdot \log K_{ow} \quad (8)$$

In this study, S and k_w are obtained by determination of the retention factors for a set of test compounds, which are micropollutants with many different functional groups, at different volume fractions of methanol. Next, the constants a_1, a_2, a_3 and a_4 are obtained by linear regression. By using S and k_w calculated from these constants in combination with Eqs. (2)–(5), $\log k$ and t_R in isocratic and gradient elution can be estimated from $\log K_{ow}$ and φ. Retention times in gradient elution RP-HPLC can then be assigned to fixed $\log K_{ow}$ values in order to select retention windows for the fractionation of a mixture of organic micropollutants according to hydrophobicity.

3. Experimental

3.1. Materials

Retention times were determined using a Varian (Walnut Creek, CA, USA) Star 9012 Solvent Delivery System and a Merck-Hitachi (Darmstadt, Germany) LiChroGraph® Model L-4000 UV detector operated at 254 nm. Output was recorded on a Varian Star Chromatography Workstation. In view of the purpose of fractionation, all analyses were carried out on a semi-preparative system consisting of a C_{18}-bonded silica column (Chrompack, Bergen op Zoom, The Netherlands, ChromSpher C18, particle size, 5 μm; L, 250 mm; I.D., 10 mm) with a guard column (Chrompack, Reversed Phase; L, 75 mm; I.D., 4.6 mm). A Spark Holland Marathon Autosampler (Emmen, The Netherlands) was used for the injections.

The eluent was a mixture of HPLC-grade methanol (J.T. Baker, Deventer, The Netherlands) and MilliQ water (Millipore, Bedford, MA, USA). An eluent flow of 4.7 ml/min was used during all experiments. All solutes were dissolved in pure methanol (J.T. Baker, Resi-analyzed grade). All chemicals used (see Table 1) were of high purity and obtained from either Fluka (Buchs, Switzerland), Aldrich-Chemie (Steinheim, Germany), Riedel-de Haën (Seelze, Germany), Merck (Darmstadt, Germany), Shell Nederland Chemie (Rotterdam, The Netherlands), J.T. Baker or Accu Standards (New Haven, CT, USA). All analytes were injected at a concentration of about 0.5–5 mM. The injection volume was 200 μl.

3.2. Methods

All determinations were carried out at 22 (±0.2)°C. The hold-up time of the eluent (t_0) was determined by injecting a water–methanol mixture containing 1% more methanol than the eluent itself at eluent compositions ranging from 50 to 95% (v/v) methanol by steps of 5%.

The retention times of all compounds were determined separately at eluent compositions of 80, 85, 90, 95 and 100% (v/v) methanol. Further, the retention times of all compounds were determined separately using linear gradient elution, programmed from 50% methanol at the start of each run to 100% methanol after 50 min. The dwell time of the chromatographic system was determined by recording the signal of a blank gradient run at 254 nm, both with and without the column.

4. Results and discussion

4.1. Retention times in isocratic elution

By injecting a mixture of water and methanol that

Table 1
Data on log K_{ow}, and log k_w and S (according to Eq. (2)) and their standard errors and log k vs. correlation coefficients (r) for the set of test compounds

Compound	log K_{ow} [a]	log k_w [b]	±s.e.	S	±s.e.	r^2
Thiourea	−1.02					
Benzothiazol-2-one	1.76	1.66	0.04	2.77	0.05	0.999
2-Chloroaniline	1.90	2.17	0.09	3.32	0.10	0.997
3-Chlorophenol	2.50	2.45	0.08	3.62	0.09	0.998
Atrazine	2.61	2.36	0.05	3.37	0.06	0.999
2,3-Dichlorophenol	2.84	2.50	0.04	3.52	0.05	0.999
4-Chloronitrobenzene	2.39	2.41	0.06	3.28	0.06	0.999
Benzene	2.13	2.14	0.03	2.92	0.03	1.000
Chlorobenzene	2.89	2.66	0.05	3.36	0.06	0.999
Toluene	2.73	2.64	0.05	3.27	0.06	0.999
2,4,5-Trichlorophenol	3.72	3.19	0.04	3.89	0.04	1.000
1,2-Dichlorobenzene	3.43	2.99	0.04	3.58	0.04	1.000
1,4-Dichlorobenzene	3.44	3.17	0.06	3.75	0.06	0.999
1,3-Dichlorobenzene	3.53	3.24	0.04	3.76	0.05	1.000
Dibutylphthalate	4.72	4.32	0.08	5.00	0.09	0.999
1,2,3-Trichlorobenzene	4.14	3.46	0.06	3.88	0.06	0.999
1,2,4-Trichlorobenzene	4.02	3.68	0.04	4.07	0.04	1.000
Dibenzo-1,4-dioxin	4.38	3.82	0.06	4.14	0.06	0.999
Phenanthrene	4.47	3.86	0.06	4.14	0.07	0.999
1,3,5-Trichlorobenzene	4.19	3.96	0.05	4.22	0.05	1.000
Endrin	5.20	4.55	0.05	4.96	0.05	1.000
1,2,3,4-Tetrachlorobenzene	4.64	4.06	0.04	4.29	0.05	1.000
1,2,4,5-Tetrachlorobenzene	4.60	4.26	0.03	4.45	0.03	1.000
1,2,3,5-Tetrachlorobenzene	4.66	4.32	0.02	4.47	0.02	1.000
Fluoranthene	5.16	4.20	0.06	4.33	0.07	0.999
Pentachlorobenzene	5.18	4.80	0.02	4.78	0.03	1.000
Aldrin	6.50	5.72	0.05	5.91	0.06	1.000
Hexachlorobenzene	5.73	5.45	0.02	5.21	0.02	1.000
Di(2-ethylhexyl)phthalate	7.45	7.96	0.09	8.06	0.10	1.000

[a] Values taken from [28].
[b] log k_w and S and their standard errors obtained from linear regression of log k vs. φ plot according to Eq. (2) ($\varphi = 0.8–1$); r, correlation coefficient of this equation.

contains slightly more methanol than the eluent itself, a hold-up time (t_0) of 2.72 min was determined. This method, which is similar to that used by Hafkenscheid and Tomlinson [27], causes a disturbance of the baseline signal as a small narrow peak. The hold-up time was constant over the tested range of eluent compositions, which is necessary for the calculation of the retention times in gradient elution [19,26]. The dwell time of the chromatographic system (t_D) could also be accurately determined by a slight change in eluent composition. By means of the blank gradient run, a chromatographic dwell time of 0.67 min was determined.

Data on log K_{ow} [28], retention factors extrapolated to 100% water (log k_w) and the slope of log k vs. φ plot (S) for all test compounds are presented in Table 1. As can be seen from Table 1, strong linear correlations between log k and φ were found for all compounds, or, in other words, the change in log k in the range of 80 to 100% methanol can be accurately described by Eq. (2).

For the present set of organic compounds with their divergent chemical structures, a strong correlation was observed between log k_w and S according to Eq. (7) (Fig. 1). The resulting relationship between log k_w and S is very similar to that reported by Schoenmakers et al. [19,26], who also used compounds of very diverse structures. Because of the

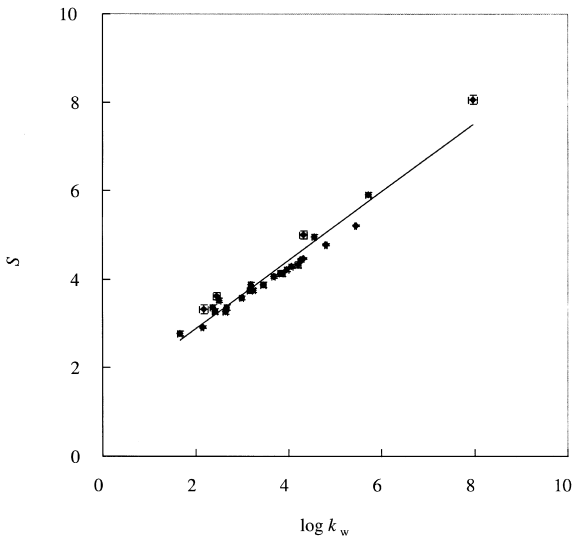

Fig. 1. Correlation between S and log k_w: $S=0.77$ (±0.03)·log $k_w+1.34$ (±0.13), $r^2=0.954$, $n=28$.

strong correlation between log k_w and S, both parameters could be adequately correlated to log K_{ow}:

$$\log k_w = 0.93(\pm0.04)\cdot\log K_{ow} - 0.05(\pm0.17)$$
$$r^2 = 0.951 \quad (n = 28) \tag{9}$$

and

$$S = 0.70(\pm0.06)\cdot\log K_{ow} + 1.37(\pm0.23)$$
$$r^2 = 0.860 \quad (n = 28) \tag{10}$$

Summarising, for the set organic micropollutants used in this study, the retention in isocratic elution as a function of log K_{ow} is represented by Eqs. (9) and (10).

4.2. Calculated versus experimental retention times in gradient elution

To be able to apply the values of S and log k_w, estimated from log K_{ow}, for gradient elution, it is necessary that the retention times of the individual compounds can be described accurately by Eq. (3). This condition is indeed met for all compounds, as can be seen from Table 2. The standard deviation of the calculated retention times from the experimental retention times is equal to 1.1% of the gradient time ($n=28$). The relatively largest differences are found for compounds that elute very rapidly, i.e. when the eluent contains 50–60% methanol. Because they have low retention factors, the errors in the determination of the retention time and hold-up time become more important. Most markedly, for three of these compounds (2-chloroaniline, 3-chlorophenol and benzene) the experimental retention times are lower than calculated. This cannot be explained by the neglection of the quadratic term in Eq. (1), which would lead to experimental retention times that are higher than the calculated values [19]. An imperfect gradient profile can also cause these differences: the lower experimental retention times are probably due to gradient rounding at the beginning of the gradient [22], which was observed by running a blank gradient run with the column disconnected.

4.3. Calculation of retention times in gradient elution from K_{ow}

By substituting values for log k_w and S derived from log K_{ow} (by means of Eqs. (9) and (10)) into Eq. (3), retention times in gradient elution can be estimated from log K_{ow}. The full-drawn curve in Fig. 2 represents the calculated retention times based on Eqs. (3)–(5). As is to be expected, the differences between the experimental retention times and the retention times calculated from log K_{ow} are larger than for the retention times calculated from log k_w and S for each individual compound (Table 2). However, the slope of the relationship between the experimental and calculated retention times is still close to unity:

$$t_{R,cal.} = 0.989(\pm0.017)\cdot t_{R,exp.}$$
$$r^2 = 0.958 \quad (n = 29) \tag{11}$$

The experimental retention times of the highly hydrophobic compounds differ most markedly from the calculated values. This can be explained by the decreasing correlation between capacity factor and log K_{ow} with increasing methanol content of the eluent. However, only for thiourea and four compounds having log $K_{ow}>5$, the difference between the value of log K_{ow} calculated from the retention

Table 2
Experimental retention times for the applied gradient, retention times calculated from log k_w and S for each individual compound, retention times calculated from log k_w and S based on log K_{ow} and log K_{ow} calculated from the experimental retention times

Compound	t_R [a]	$t_R(S, k_w)$ [b]	$t_R(K_{ow})$ [c]	log $K_{ow}(t_R)$ [d]
Thiourea	2.78		2.85	−1.53
Benzothiazol-2-one	7.70	7.28	7.46	1.80
2-Chloroaniline	8.20	9.70	8.27	1.89
3-Chlorophenol	10.02	11.28	12.83	2.16
Atrazine	12.47	12.02	13.85	2.46
2,3-Dichlorophenol	13.21	12.79	16.11	2.54
4-Chloronitrobenzene	13.23	13.59	11.87	2.54
Benzene	11.72	12.47	9.81	2.37
Chlorobenzene	17.28	17.44	16.62	2.95
Toluene	17.60	17.99	15.01	2.98
2,4,5-Trichlorophenol	22.25	21.76	25.47	3.42
1,2-Dichlorobenzene	22.24	21.56	22.38	3.42
1,4-Dichlorobenzene	23.37	23.19	22.48	3.52
1,3-Dichlorobenzene	24.75	24.73	23.45	3.65
Dibutylphthalate	30.82	30.03	35.11	4.25
1,2,3-Trichlorobenzene	28.51	27.90	29.76	4.01
1,2,4-Trichlorobenzene	30.14	30.02	28.57	4.18
Dibenzo-1,4-dioxin	32.90	32.25	32.06	4.47
Phenanthrene	33.86	33.19	32.80	4.58
1,3,5-Trichlorobenzene	33.81	34.05	30.25	4.57
Endrin	35.51	35.14	38.99	4.77
1,2,3,4-Tetrachlorobenzene	35.32	35.02	34.42	4.74
1,2,4,5-Tetrachlorobenzene	37.07	37.07	34.06	4.96
1,2,3,5-Tetrachlorobenzene	37.77	37.86	34.59	5.04
Fluoranthene	38.41	37.78	38.69	5.12
Pentachlorobenzene	42.93	42.96	38.84	5.76
Aldrin	42.89	42.86	47.35	5.75
Hexachlorobenzene	48.53	48.61	42.75	6.72
Di(2-ethylhexyl)phthalate	48.34	48.45	51.95	6.69

[a] Experimental retention times in gradient elution, programmed from methanol–water (50:50 v/v) at the start of the run to 100% methanol after 50 min.
[b] Calculated retention times in gradient elution from log k_w and S for each individual compound (Eq. (3)).
[c] Calculated retention times in gradient elution from log k_w and S estimated from log K_{ow} (Eqs. (3), (9) and (10)).
[d] Calculated log K_{ow} corresponding to experimental retention time (Eqs. (3), (9) and (10)).

time and the literature value for log K_{ow} [28] was more than 0.5 log K_{ow} unit and for all the compounds studied, the difference was within one unit of log K_{ow} [28]. The standard deviation of the estimated log K_{ow} values was 0.38 ($n=29$) and for compounds with log K_{ow} in the range of 1–5, it was only 0.24 log K_{ow} unit ($n=22$). From the literature [29], it can be concluded that the error in log K_{ow}, determined by other techniques, is in the same range. One may, therefore, conclude that a complex mixture of organic micropollutants can be separated according to hydrophobicity, with an accuracy comparable to that of conventional log K_{ow} determinations.

The use of the relationship between the retention times in gradient elution, calculated from isocratic parameters and log K_{ow}, has some advantages over that of a relationship derived from a linear regression analysis of the experimental retention times in gradient elution and log K_{ow}. First, retention times calculated from isocratic parameters give a physical description of the retention behaviour in gradient elution. This is most evident for analytes of either low or high hydrophobicity. For lower log K_{ow} values, the calculated retention time approximates the hold-up time, whereas an empirical linear fit cannot be extrapolated to this range (Fig. 2). At higher values of log K_{ow}, the slope of the retention time vs. log K_{ow} plot decreases. It is also possible, by

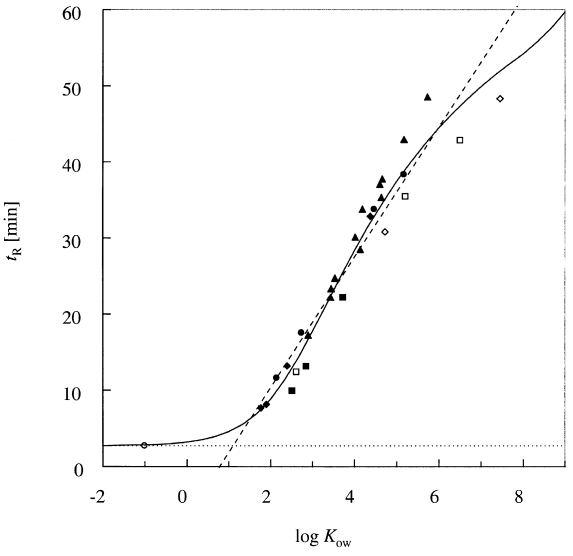

Fig. 2. Retention time as a function of log K_{ow}: ▲ chlorobenzenes, ● aromatic hydrocarbons, □, pesticides, ■ phenols, ◇ phthalates, ♦ other compounds, ○ thiourea (hold-up time marker); ——— theoretical curve, - - - best linear fit, · · · hold-up time.

using Eq. (5), to estimate the retention times for highly hydrophobic compounds that elute after completion of the gradient run.

Another advantage of the method is that no extensive gradient optimisation is required because the retention times for each gradient can be calculated from log K_{ow}, once the isocratic parameters log k_w and S have been determined for a set of reference compounds. In this way, a suitable gradient can be selected. As was mentioned by Schoenmakers et al. [26], the 50–100% gradient is suitable for not too polar analytes. The use of such a gradient provided a good separation for the selected set of compounds, which included both low- and high-hydrophobic analytes. For log K_{ow} values between 1 and 6, the calculated retention time vs. log K_{ow} plot is almost linear for such a gradient. This means that for the fractionation of mixtures, the volume of fractions representing log K_{ow} ranges of fixed length remains almost constant.

Finally, from a comparison between retention times, calculated from log k_w and S for each individual compound, and retention times, calculated from log k_w and S based on log K_{ow}, the correlation between experimental retention times and log K_{ow} can be estimated. For the set of compounds used in this study, the correlation between experimental retention times and log K_{ow} cannot be improved significantly, unless the gradient time is allowed to increase dramatically. Moreover, it is not possible to achieve a comparable correlation between log k and log K_{ow} in the same time by using isocratic elution.

5. Conclusions

Gradient elution RP-HPLC is a suitable technique to separate a mixture of structurally very diverse micropollutants according to hydrophobicity. This was validated by a set of 28 compounds, with similar structures as the contaminants, expected in the mixtures to which the method will be applied. The retention times of in gradient elution RP-HPLC could be calculated from isocratic parameters. Using the relationship between isocratic parameters and log K_{ow}, the retention times in gradient elution could also be related to log K_{ow}. In this way, the log K_{ow} of unknown compounds can be estimated using gradient elution, and appropriate retention windows for mixture fractionation according to hydrophobicity can be chosen. The deviation of the calculated log K_{ow} values from the experimentally determined literature values was less than one unit for all compounds, and the standard deviation of the estimated values was only 0.38 log K_{ow} unit.

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