DETERMINATION OF OCTANOL-WATER PARTITION COEFFICIENT FOR DDTS USING RP-HPLC

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Abstract

A reversed phase-high performance liquid chromatographic (RP-HPLC) method is applied for determining octanol-water partition coefficient (K_{ow}) of DDTs. For this purpose, a semi-experimental equation is established by plotting a set of shake-flask K_{ow} values against the corresponding corrected retention times (CRT) of 10 benzene homologues, which are chosen as model compounds in this study. The physicochemical data K_{ow} for 3 DDTs (p,p'-DDD, o,p'-DDT, and p,p'-DDE) and Dicofol were calculated by using this equation. In addition, the measured log K_{ow} values for verification compounds (p,p'-DDT and HCB) determined by RP-HPLC method and software-computed method were compared against shake-flask data respectively, and the RP-HPLC values were found to give better consistency with shake-flask results.

Introduction

The octanol-water partition coefficient (K_{ow}) has been widely accepted as providing a good indication of the distribution of analytes into biological membranes[1-3] since the work of Hansch and Fujita[4]. This has made it one of the most commonly reported physical properties of drugs, pesticides and other chemicals [5,6]. Up to now, K_{ow} is the most widely used parameter for predicting the bioaccumulation potential of persistent organic pollutants (POPs). It is generally assumed that POPs accumulate in lipids and equilibrium partitioning of these chemicals between organism lipid and water can be estimated by using partitioning to octanol as a measure of their hydrophobicity.

The correlation between chromatography retention time (t_R) and K_{ow} is a classic and widely used strategy for determining log K_{ow} values for a wide range of compounds [7-9]. These indirectly measured log K_{ow} values have also been successfully correlated with biological data in many instances. In this paper, a semi-experimental equation derived from RP-HPLC method is proposed to predict K_{ow} values of p,p'-DDD, o,p'-DDT, p,p'-DDE and Dicofol, which are found to exist extensively in soil and water as a result of organochlorine pesticides abuse beforetime. In addition, the corrected retention time (CRT) was proposed to calibrate t_R of DDTs and resulted in a better linear correlation with log K_{ow}, as well as a better repeatability when it is applied to predict K_{ow} values of the analytes.

As we know, one of the greatest obstacles to estimated K_{ow} by commercial calculation software is the difficulty in determining the reliability of the results, and this difficulty is more significant when software calculation results are obtained for POPs because of lack of the corresponding shake-flask data for verification. The method proposed in this study offered a powerful and dependable protocol for validating the accuracy of the software computed data for POPs.

Materials and Method

Materials

Methanol (HPLC grade) was from Merck (Darmstadt, Germany). Wahaha purified water (Wahaha Group Ltd., Hangzhou, China) was used throughout the experiment. Tables 1 and 2 list the compounds investigated. These compounds were all with the purity of 98% or greater. Their purities were checked by HPLC and then used without further purification. Stock solutions of compounds studied (ca. 1.0 mg/mL) were respectively prepared in methanol and stored in refrigerator before injection.

***Table* 1**. Model and Verification Compounds

Compounds	log K_{ow}	Compounds	log K_{ow}
Anisole	2.04 [1]	1,2,4-Trichlorobenzene	4.02 [7]
Benzyl chloride	2.30 [2]	1,3,5-Trichlorobenzene	4.19 [8]
Toluene	2.61 [3]	1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT)	6.36 [9]
Ethylbenzene	3.15 [4]	Hexamethylbenzene	4.75 [10]
1,2,3-Trichlorobenzene	4.05 [5]	Pentachlorobenzene	5.17 [11]
2-Methylnaphthalene	4.00 [6]	Hexachlorobenzene (HCB)	5.73 [12]

Log K_{ow}: [1] from [10]; [2] from [11]; [3] from [12]; [4] from [13]; [5,7,11] from [14]; [6,10] from [3]; [7] from [15]; [9] from [16]; [12] from [17]. Only values determined by the shake-flask method were used.

***Table* 2**. Sample compounds

Compounds	Ab.	log K_{ow}*
2,2-bis(p-Chlorophenyl)-1,1-dichloroethane	p,p'-DDD	5.81
2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol	Dicofol	5.66
2-(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1-trichloro-ethan	o,p'-DDT	6.39
2,2-bis(p-Chlorophenyl)-1,1-bischloroethene	p,p'-DDE	5.84

*Calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994-2007 ACD/Labs).

Apparatus

A LabTech 600 LC (Lab-Tech Instru. Co. Ltd., Beijing, China) consisting of a Rheodyne 7725i injector valve equipped with a 10-μL loop (Cotati, CA, USA), a HB-230A incubator (Hanbon Sci. & Tech. Co. Ltd., Huai'an, China) and a UV-vis 600 Detector (Lab-Tech) set at the maximum absorption wavelength for each test compound was employed for experiments. All retention data for the studied compounds were measured on a Kromasil C_{18}, 5 μm, 150 mm × 4.6 mm i.d. column (Hanbon) at 30°C. Data acquisition and processing were performed on a LC workstation 2006 (Lab-Tech). All experimental retention times were obtained by averaging the results of at least two independent injections at 1.0 mL/min mobile phase flow rate.

Method

Determination of log K_{ow} by RP-HPLC is highly dependent on the retention of solutes, that is, the capacity factor (k'). Even though k' can be related to K_{ow}, k' will, for a given solute and stationary phase, depend on the composition of the mobile phase used in elution[18]. 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. The linear relationship between log K_{ow} and log k_w known as Collander equation has been illustrated experimentally and theoretically[19]

$$\text{Log } K_{ow} = m \text{ log } k_w + n \quad (1)$$

where m and n are empirical constants which characterize the solvent system in question.

Under most conditions, however, pure water cannot be used as the eluent due to inordinately long retention time. Thus k_w can be calculated if k' is determined over a range of organic modifier concentrations and extrapolated back to 0% (k_w)[4,5]. General Snyder-Soczewinski equation relating k' with volume fraction of organic modifier (Φ) in binary hydroorganic mobile phase for liquid chromatography can be expressed in terms of [20]:

$$\text{Log } k' = \text{log } k_w - S\Phi \quad (2)$$

where S is the entropy function of an adsorbed solute[21], reflecting the interactions between analytes and eluent molecules[22]. It is considered as constant for a given solute-eluent combination.

The analytes studied in this work were eluted by the mobile phase composed of methanol and water. Methanol content in binary eluent ranged from 40% to 95%, and all compounds were analyzed at a minimum of four methanol - water ratios. Then t_R for these compounds were recorded at each methanol - water ratio along with the holdup time t_0 of the chromatographic system, where t_0 was determined by using $NaNO_3$ as the holdup time marker. For each solute the logarithm of k' can be plotted against the volume fraction of methanol, Φ, and log k_w of the analytes were subsequently obtained by extrapolation via Eq. 2. Thus equations that relate log K_{ow} with log k_w of the solutes for model compounds were derived.

Results and Discussions

Correction of retention time

Silica-based stationary phase often collapses by constant flushing of mobile phase during the course of using, which is called aging of column, and this leads to the inconsistency of retention for the same analyte under the same chromatographic conditions in different measuring periods. Therefore, t_R of solutes should be rectified to reduce this deviation. The commonly used method is to simply employ the different retention time of $NaNO_3$ at each measuring situation as t_0 to adjust t_R. It actually is a single point correction method, and means large random error if the measurement for t_0 of $NaNO_3$ is not accurate enough. In fact, this random error must be large because t_0 is too short to be measured accurately.

Another correction method is proposed by C. G. Huber in DNA retention behavior study[23]:

$$t_{normalize} = (t_{measured} - t_{(dC)14}) \times \frac{\overline{t}_{(dT)26} - \overline{t}_{(dc)14}}{t_{(dT)26} - t_{(dC)14}} + \overline{t}_{(dC)14} \quad (3)$$

where t and $\overline{t}$ representing the retention time and average retention time, respectively, of the oligonucleotides. The analogical method was employed in this paper by choosing a real column used in a certain day as "standard column" (usually the day a column first used which describes the truest retention behavior for analyte) instead of "imaginary average column" employed in Huber's work. t_R of the solutes measured on a changed column in any other day should be adjusted to the ones equivalent to be obtained on "standard column" by a correction parameter. In order to get this parameter, t_R of 10 model compounds were collected at the mobile phase of 80% of methanol by volume in two different days, then these 2 groups of vectors were fitted to a linear equation ($r^2 \geqslant$ 0.999). Its slope is just the correction parameter. Because of its good linearity, only 2 compounds were chosen as "regulated substance" to obtain the parameter in the following experiments that day for the purpose of shortening experiment time and improving efficiency. Therefore, this method should be called two points correction method.

The comparison between log k_w values obtained from both two correction methods was made, and relative standard deviation (R.S.D.) of log k_w measured in three different experiment periods are ranged from 0.05% to 1.82% and from 0.14% to 3.27%, respectively, which indicated the better reproducibility by using two points method for correction.

Precision of RP-HPLC method

10 benzene homologues of known log K_{ow} and similar chemical structure to that of POPs were used as standards in the determination of K_{ow}. The availability and accuracy for correlation between log K_{ow} and corrected log k_w presented here (see Table 3) was checked by 2 POPs, p,p'-DDT and HCB with known shake-flask K_{ow} values. The error between predicted K_{ow} and shake-flask ones are shown in Table 4, which indicated this correlation obtained by RP-HPLC has a comparable level of precision to the shake-flask technique for determination of K_{ow} for POPs with similar structure. Simultaneously, software-computed log K_{ow} were obtained for 10 model compounds by using ACD/Labs Software computed technique. The result showed that the deviation between shake-flask measured and software-calculated K_{ow} increases with the growing of K_{ow} value. Furthermore, the software calculated log K_{ow} 5.92 ± 0.40 for p,p'-DDT and 4.89 ± 0.36 for HCB had much larger error than the ones obtained by RP-HPLC method presented in Table 4.

***Table* 3**. The relationships between log K_{ow} and log k_w for 10 model compounds (r represents the regression correlation coefficient)

Group	log K_{ow} (y) – log k_w (x)	
	Relation equation	r^2
1	y = -0.78 + 1.15 x	0.978
2	y = -0.75 + 1.14 x	0.981
3	y = -0.75 + 1.14 x	0.980

***Table* 4.** The validation for RP-HPLC method

Compounds	Average RP-HPLC measured log K_{ow}	Shak-flask log K_{ow}	S.E.
p,p'-DDT	6.52	6.36	0.16
HCB	5.93	5.73	0.20

Prediction log K_{ow} for sample compounds

As the correlation between log K_{ow} and log k_w obtained in this work has been validated reliable to calculate K_{ow} of POPs with similar structure to the model compounds, in this work log K_{ow} values were determined for 3 DDTs and Dicofol using the RP-HPLC method and compared with calculated values using fragment method to check out the accuracy of calculation techniques. The prediction results were collected in Table 5 and compared with software-computed ones. The error shown in Table 5 suggested that the software computation method had a comparable level of precision to the RP-HPLC method for p,p'-DDD, but had certain deviation while employed for calculating K_{ow} values of o,p'-DDT and Dicofol, and had an even larger error for p,p'-DDE with the biggest log K_{ow} value.

The disparity between calculated and RP-HPLC values as seen in this study may be a reflection of the complex factors such as solute aggregation of Dicofol, solvation or complex functional groups to the fragment methods predictive capability when compared with more simple hydrocarbons.

***Table* 5.** Prediction of log K_{ow} for sample compounds

Group	Compounds	log K_{ow} (RP-HPLC)	log K_{ow} (software-calculated)	Error
1	p,p'-DDD	5.81	5.81	0.00
	Dicofol	6.14	5.66	0.48
	o,p'-DDT	6.82	6.39	0.43
	p,p'-DDE	7.38	5.84	1.54
2	p,p'-DDD	5.58	5.81	-0.23
	Dicofol	6.15	5.66	0.49
	o,p'-DDT	6.85	6.39	0.46
	p,p'-DDE	7.50	5.84	1.66
3	p,p'-DDD	5.59	5.81	-0.22
	Dicofol	6.15	5.66	0.49
	o,p'-DDT	6.85	6.39	0.46
	p,p'-DDE	7.49	5.84	1.65

Acknowledgments

This work was supported by National Basic Research Program of China (973 program, No. 2009CB421601), National Natural Science Foundation of China (20575027), National Science Funds for Creative Research Groups (20821063), and the Analysis & Test Fund of Nanjing University.

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