

A simple reverse phase HPLC method for determination of partition coefficient of permethrin pesticide

Tentu.Nageswara Rao^{1*}, Tentu.Srinivasarao², Parvatamma.Botsa³

¹Department of Chemistry, Krishna University, Machilipatnam, A.P, (INDIA)

²Department of Analytical R&D, Johnson & Johnson R&D Centre, Mumbai, (INDIA)

³Department of Organic Chemistry, Gayathri PG College, Vizianagaram, A.P, (INDIA)

E-mail: tentu6581@rediffmail.com

ABSTRACT

The present study was conducted to determine the partition coefficient of permethrin. The log P_{ow} was calculated for the permethrin from the calibration curve generated from log k versus log P_{ow} (capacity factor) of seven known reference compounds. Reference compounds and test item k values were generated using an HPLC equipped with Phenomenox C_{18} column. The isocratic HPLC method used in the study consisted of a mobile phase of methanol/HPLC water (90:10) on a reverse phase Phenomenox C_{18} column. Since, test item resulted in two peak areas permethrin- P_1 and permethrin- P_2 , when analysed in HPLC, the calculation of weighted average log P_{ow} was adopted and the weighted average log P_{ow} value of test item was found to be 5.88 ± 0.001 .

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KEYWORDS

Permethrin;
HPLC;
Log P_{ow} ;
Partition coefficient.

INTRODUCTION

The partition coefficient is important to research scientists and product development staff in various branches of the pharmaceutical field^[1,2]. The principle and applications are involved in several different areas of current pharmaceutical interest^[3,4]. These include the techniques of extraction, preservation of oil-water systems, penetration through packaging materials, absorption and distribution of drugs in vivo, protein binding and hemodialysis, drug metabolism, enzyme inhibition, drug-receptor interactions, drug delivery systems and drug targeting^[5]. Because the partition coefficient is a measure of hydrophobic-bonding tendency, and all proteins (enzymes, membrane,

plasma and receptor proteins) contain 20 to 45% of amino acids with non-polar groups (e.g. leucine, isoleucine, phenylalanine, tyrosine, tryptophan etc.), continuing interest in using partition coefficients in correlating biological activity with molecular structure is expected. Juberma^[6]. Other areas of interest in the application of partition coefficient fall beyond the scope of this article. These include analytical chemistry, toxicology, forensic medicine, ecology and environmental protection^[7,8]. Permethrin is in class of compounds known as synthetic pyrethroids^[9]. Synthetic Pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from extracts of dried chrysanthemum flowers. Synthetic pyrethroids are more stable than natural pyrethroids,

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and therefore longer lasting in the field. Although synthetic pyrethroids are often thought of as “safe as chrysanthemums”, they are chemically engineered to be more toxic than natural pyrethroids. Permethrin is widely used as an insecticide in agriculture, homes, gardens and for treatment of ectoparasites (fleas, lice, scabies) on humans and animals. Worldwide, the dominant use of permethrin is for cotton, which accounts for over 60% of the permethrin used.

In the U.S., almost 70% of the permethrin used in agriculture is used on corn, wheat and alfalfa. Annually, over 100 million applications of permethrin are made each year in and around U.S. homes^[10]. Some common products containing permethrin as the active ingredient include: Nix, Elimite, Prelude, Combat, Ambush, Dagnet, Outflank, Pounce, Perthrine, Picket and Astro. Permethrin comes in many forms, including sprays, dusts, fogs, emulsifiable concentrates and creams. Additionally, in 2003, the EPA approved permethrin-impregnated clothing for public use.

EXPERIMENTAL

Materials and methods

Permethrin standard, DDT, Triphenyl amine and Phenanthrene were obtained from Sigma Aldrich. Acetonitrile and Water HPLC grade, were supplied from the Rankem. Naphthalene, Phenol, Methyl benzoate and Thiourea were purchased from Merck, India Limited.

Preparation of diluent

Methanol (HPLC grade) and water (HPLC grade) was taken in the ratio of 95: 10 v/v respectively. This sample was sonicated and used as a diluent.

Preparation of reference standard solutions

The reference solutions of Thiourea (Purity 98.0 %), DDT (Purity 98.5 %) Triphenyl amine (Purity 97.8 %), Phenanthrene (Purity 97.2 %), Naphthalene (Purity 99.0 %), Phenol (Purity 99.4 %) and Methyl benzoate (Purity 98.5 %) were prepared by weighing approximate amount into different 10 mL volumetric flasks. The flasks were diluted with diluent solution, sonicated and made up to the mark with the same diluent. The weighing details are given in TABLE 1.

TABLE 1 : Details of weight of reference compounds

S.No.	Name of the reference standard	Weight of reference standard (mg)
1	Thiourea	2.56
2	DDT	2.38
3	Triphenyl amine	3.11
4	Phenanthrene	3.74
5	Naphthalene	2.63
6	Phenol	3.56
7	Methyl benzoate	3.96

Preparation of Permethrin sample solution

A 2.59 mg of Permethrin (Purity 96.12 %), sample was weighed into a 10 mL volumetric flask. The flask was diluted with diluent solution, sonicated and made up to the mark with the same diluent.

Dead Time (t_0)

The dead time was determined in duplicate based on the retention time of the unretained reference item, Thiourea. Due to the properties of Thiourea, it was not retained on the column and, therefore, eluted at 2.680 minutes. This retention time, or dead time (t_0), of 2.680 minutes was used in equation to determine k' for the reference standards and test item.

Partition coefficient ($\log P_{ow}$) of permethrin technical

The capacity factor, k' for each reference standard and test item can be calculated for the HPLC system from their respective retention times. Office of Prevention, Pesticides, and Toxic Substances (7101) Guideline^[11]. EEC A.8^[12].

$$k' = \frac{(t_R - t_0)}{t_0}$$

TABLE 2 : Literature p_{ow} values, $\log P_{ow}$, measured retention times (T_R) and calculated $\log k'$ for reference standards

REFERENCE COMPOUND	LOG P_{ow} VALUES ^A	T_R (MIN)	LOG K' VALUES
Phenol	1.5	3.13	-0.7735
Methylbenzoate	2.1	3.62	-0.4548
Naphthalene	3.6	4.47	-0.1743
Phenanthrene	4.5	5.87	0.0751
Triphenyl amine	5.7	7.04	0.2109
DDT	6.2	7.14	0.2208

^aLiterature value - Slope : 4.6085; Intercept : 4.6214

Where k' = Capacity Factor
 t_R = retention time (min)
 t_0 = dead time (min)

The dead time, t_0 , can be measured by using the retention time of Thiourea, an un retained organic compound. The duplicate injections of Thiourea were

TABLE 3 : Retention Times (TR), measured log pow and calculated pow values for permethrin using 95/5 Methanol/Milli-Q Water

TEST ITEM	REPLICATION	T _R (MIN)	K'	LOG K'	LOG P _{ow}	WEIGHTED AVERAGE LOG POW
Permethrin	1	Permethrin - P ₁	7.320	1.7313	0.2384	5.88
		Permethrin - P ₂	8.266	2.0843	0.3190	
	2	Permethrin - P ₁	7.316	1.7299	0.2380	5.87
			Permethrin - P ₂	8.264	2.0836	
Average					5.94	5.88
Standard Deviation					0.21	0.001

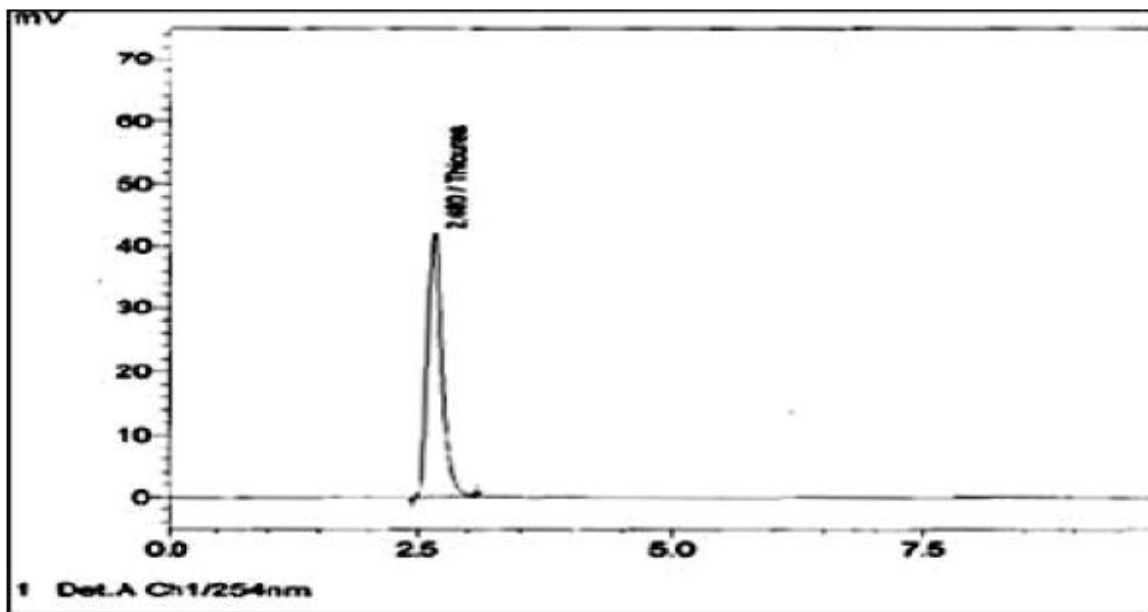


Figure 1 : Representative chromatogram - dead time analysis using thiourea

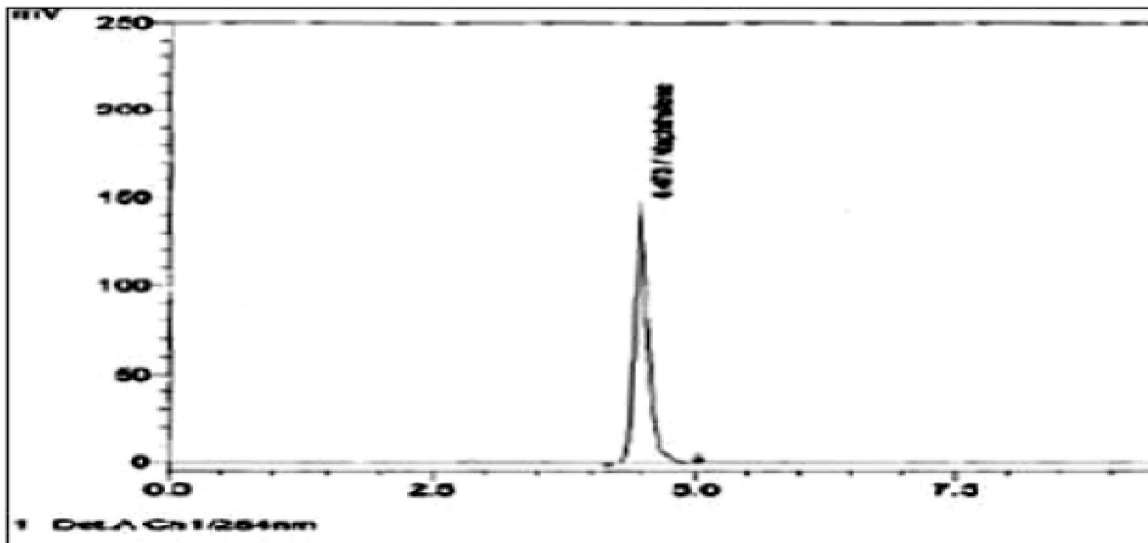


Figure 2 : Representative chromatogram of reference compound- naphthalene

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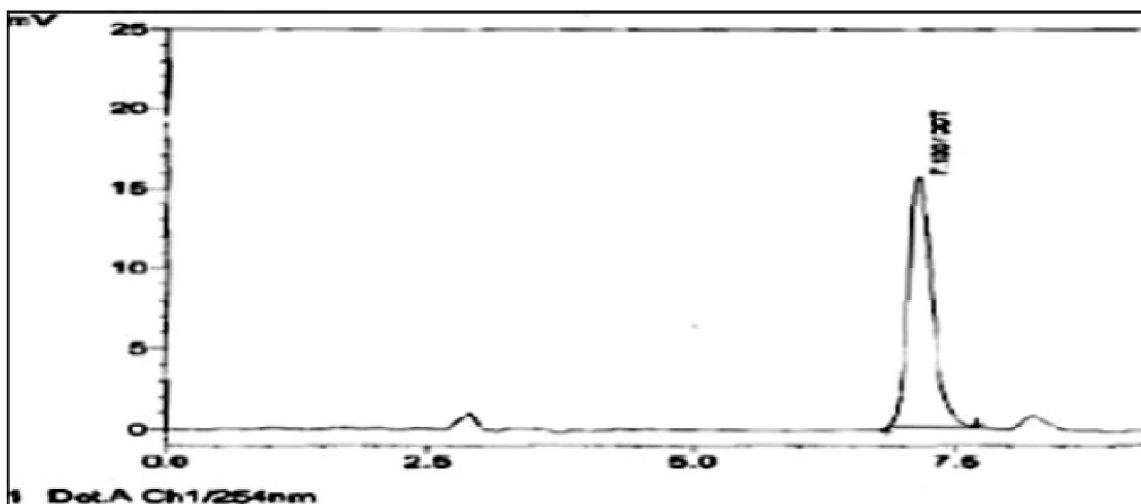


Figure 3 : Representative Chromatogram of Reference compound- DDT

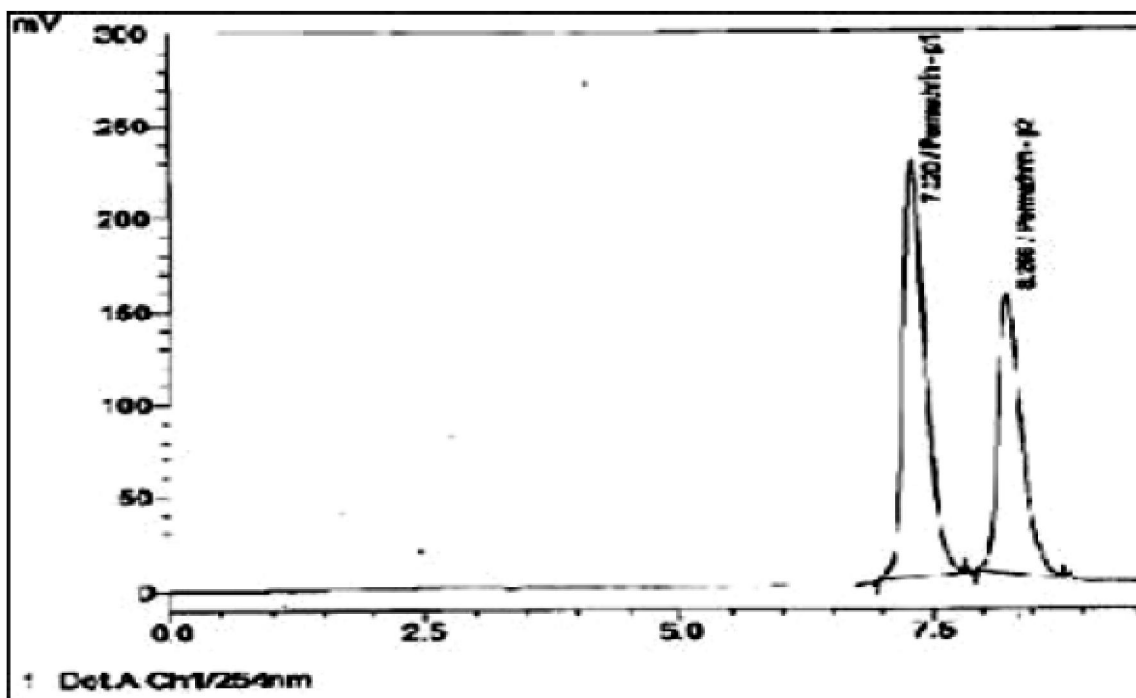


Figure 4 : Representative chromatogram of permethrin

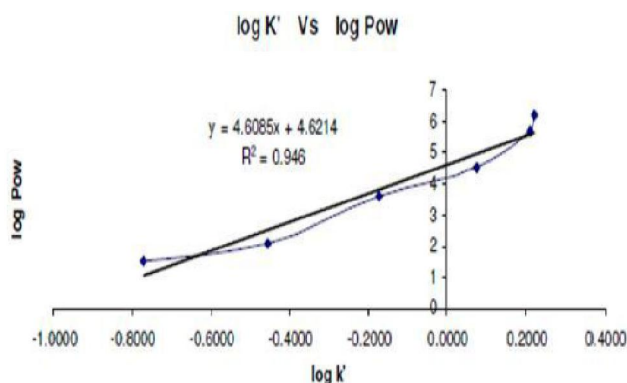


Figure 5 : Calibration curve of known reference standard log Pow vs. LogK2 at 254 nm

averaged to determine the t_0 .

A correlation graph was generated of $\log k'$ versus $\log P_{ow}$ for reference standard solutions, with replicate injections plotted on the same graph. Literature $\log P_{ow}$ values were obtained from OECD Guideline 117^[13]. The $\log P_{ow}$ for Permethrin was then interpolated from the correlation graph.

Instrumentation

• Chromatographic separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT

pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 μ m (Phenomenex Luna-C18) Column oven temperature was maintained at 25°C. The injected sample volume was 10 μ L. Mobile Phases A and B was Methanol and HPLC Grade Water (90:10 (v/v)). The flow-rate used was kept at 1.0 mL/min with a detector wavelength at 254 nm.

RESULTS AND DISCUSSION

The experimental method described in this study uses HPLC to determine Pow. Analysis of the unretained reference item, Thiourea provided a Dead Time analysis (t₀) of 2.68 minutes. A representative chromatogram of a Dead Time analysis are provided in Figure 1. Measured retention times (t_R) of the standards and test items are shown in TABLE 2 and TABLE 3. Example chromatograms of the reference compounds and the permethrin are shown in Figure 1 to Figure 4. Linear regression of reported log P_{ow} values and measured retention times of the calibration standards gave a correlation coefficient of 0.946 (Figure 5), and an equation describing the line of: $\log P_{ow} = 4.6085 (\log k') + 4.6214$

Using this regression, log P_{ow} of the test item was calculated.

Since, test item resulted in two peak areas permethrin-p₁ and permethrin-p₂, when analysed in HPLC, the calculation of weighted average log Pow was adopted by considering following formula:

Weighted average log Pow = $\Sigma[\log \text{Pow} (p_1) \times \text{Peak area of } (p_1) + \log \text{Pow} (p_2) \times \text{Peak area of } (p_2)] / \text{Total peak area} [\text{peak area } (p_1) + \text{peak area } (p_2)]$

CONCLUSION

Partition coefficient of Permethrin technical was determined by HPLC Method. The weighted average value of partition coefficient of Permethrin technical was found to be 5.88 ± 0.001.

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