SIMULTANEOUS ESTIMATION AND VALIDATION OF CIS-/TRANS ISOMERS OF PERMETHRIN

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ABSTRACT
A sensitive and accurate high performance liquid chromatography assay (HPLC) technique was applied for determination of permethrin isomers. Cis-/trans-Permethrin isomers were separated at room temperature in raw material as well as pharmaceutical products such as lotions and creams without extraction. The calibration curve was found to be linear ($r \geq 0.9975$) over the concentration range of 0.80-1.66 µm/ml. The samples were measured at 280 nm detection wavelength as internal standard of quantitaion. Mean percentage (%) recovery ± % relative standard deviation (RSD) ranged from 97.50 ± 1.36 to 100.08 ± 0.39. Within-day and between-day precision were also in acceptable range below 2%. The reported method for the simultaneous estimation of cis-/trans-permethrin isomers provides several advantages e.g simplicity, highly specificity, accuracy and very short run-cycle time.
Key words: Permethrin, Cis/Trans Isomers, Estimation, Validation, High Performance Liquid Chromatography

INTRODUCTION

Permethrin is a synthetic analogue of an insecticide derived from the *pyrethrum chrysanthemum* daisy. Permethrin and its derivatives are commonly used in household insecticidal spray cans and for head lice treatments. It is readily broken down by soil bacteria and is non-persistent in the environment. It has highly specific insecticidal activity and is thought to be a contact repellent. This tends to discourage termites from destroying the enclosed treated wood Matsumoto , 2005. The structural formulas of two diastereomers of permethrin (having different chemical, physical and toxicological properties) are given in Fig. 1. Most of them are intended for analysis of multi-residue of pyrethroids in environmental matrices, wood, crops and foods of animal origin. Because of the complexity of Permethrin matrices, several sample pre-treatment steps are needed. Several techniques have been reported so far for the estimation of one of the isomers of Permethrin by thin layer chromatography, gas chromatography Matsumoto 2005, Garcia et. al, 2001, Noroozian et. al, 2004 & Liu et. al, 2005. Hence, it could be suggested that no simple and rapid chromatographic methods have been published till date for the simultaneous estimations and quantitation of cis-/trans-permethrin isomers. The present study reports a simple, rapid, specific, precise, and validated HPLC method for the simultaneous quantitative estimation of cis-/trans-permethrin isomers in various pharmaceutical formulations both by high performance liquid chromatographic assay technique with a reversed phase C-8 column and first derivative UV-spectrophotometry.

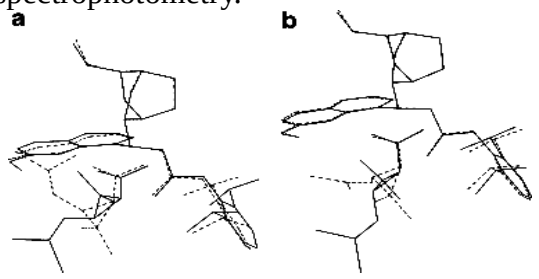

Figure 1: Cis (a) and Trans (b) isomers of permethrin

MATERIALS AND METHODS

Instrumentation

Isocratic Schimadzu SPD 20A high performance liquid chromatographic system was used for having UV detector with 20 µl injector, installed at Shiagon Pharma, Rawalpindi.

Methods

A mixture of methanol (90 ml) and water (10 ml) was prepared and mixed using mechanical shaker at 100 rpm for 30 minutes. The solution was then sonicated to remove air bubbles. It was filtered through nylon (47 mm diameter) filter paper (0.45 µm) using vacuumed filtration technique.

Permethrin (100 mg) reference standard containing known purity was accurately weighed and transferred into a 100 ml volumetric flask containing mobile phase. After the complete dissolution of permethrin, the volume was adjusted up to mark. This solution was filtered through nylon 0.2 µm before injecting to the column. Validity and suitability of the system was performed with five replicate injections.

Samples of permethrin in the bulk material, cream and lotion were prepared separately by the following methods. Sample of bulk material was prepared in the same manner as discussed under standard preparation. For cream sample preparation containing permethrin (100 mg) according to the labeled amount was weighed accurately and transferred to 100 ml volumetric flask dissolved in mobile phase, adjusted to volume and mixed. For lotion sample preparation cream containing permethrin (100 mg) according to the labeled amount was weighed accurately and transferred to a 100 ml volumetric flask dissolved in mobile phase, adjusted to the volume and mixed.

Sample and standard solutions were filtered through nylon 0.2 µm injection filter paper. These were injected separately to the column and the chromatograms were recorded Sajeev and Ranendra , 2008. Assay percentage was calculated using the equation 1.

$$\%Assay = {AU}/{AS} \times P \qquad \text{……..1}$$

AU = Peak area of sample, AS = Peak area of standard and P= Purity of reference standard.

Linearity

The linearity of the calibration curve was checked over the concentration range of 0.80-1.66 µg/ml.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of Detection (LOD) is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value, calculated using equation 2.

$$LOD = \frac{3\sigma}{S} \qquad \text{…….2}$$

Where σ is standard deviation of the intercept and S is slope of the calibration curve

LOQ is the lowest amount of analyte in a sample, which can be quantitatively determined in suitable precision and accuracy HAN et. al, 2008, using equation 3.

$$LOQ = \frac{10\sigma}{S} \qquad \text{……3}$$

Precision and accuracy

Intra-day and inter-day variabilities were determined by repeated injections of

quality control (QC) samples. The QC samples were prepared at 0.960, 1.152 and 1.382 mg/ml of permethrin reference standard having known purity, representing low, middle, and high controls, respectively. Accuracy was assessed by comparing the predicted concentrations of the QC samples with the nominal 0.960, 1.152 and 1.382 mg/ml concentrations

Specificity and placebo interference

By applying the same sample preparation method, placebo was prepared using all excipients of cream and lotion except Permethrin as the active ingredient and then was analyzed.

System suitability was performed before proceeding analysis. The statistical data about theoretical plates (N), Symmetry factor (As), and mass distribution ratio (capacity factor k) for replicate chromatograms (n=6) was calculated according to USP guideline.

RESULTS AND DISCUSSION

The system suitability parameters were found to be within acceptable limits (Table 1). An analytical run time of 15 min was optimized for each sample. The retention time of permethrin under the experimental conditions was 7.681 ± 0.013 min and well resolved from the formulation used. The following validation parameters were estimated and evaluated according to the ICH guidelines (ICH 1996). In order to achieve good sensitivity for quantitative determination, UV detection at 280 nm was selected for good separation and suitable retention time at ambient temperatures under the described chromatographic parameters using a mixture of water and methanol (1:9 v/v) as mobile phase.

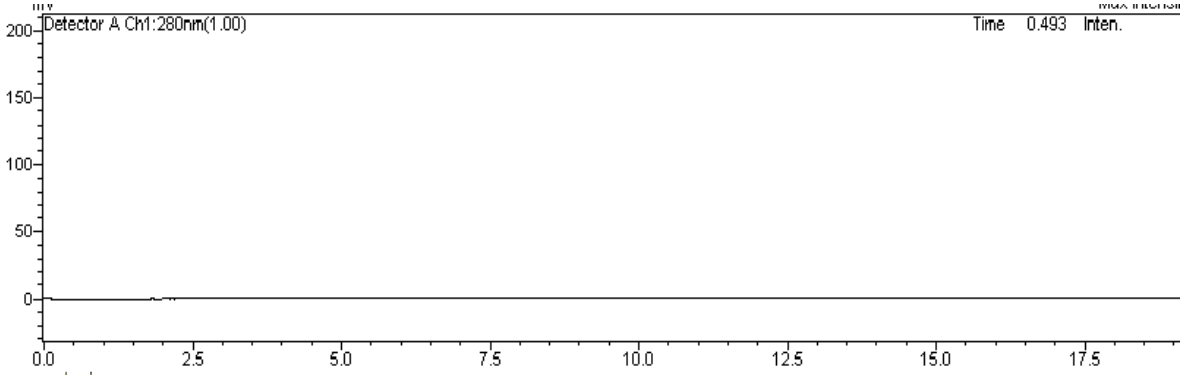

Figure 2: Chromatogram of simple mobile phase

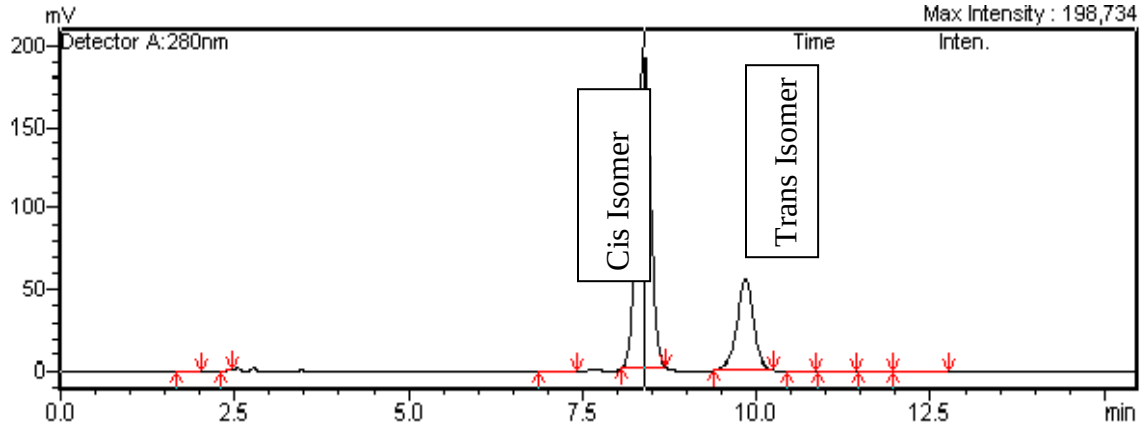

Figure 3: Chromatogram of Reference Material Spectra

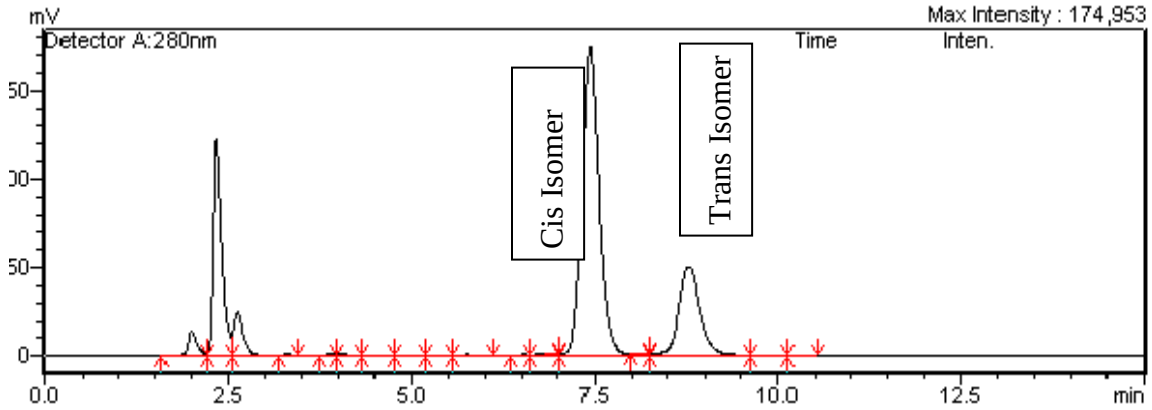

Figure 4: Chromatogram of cream

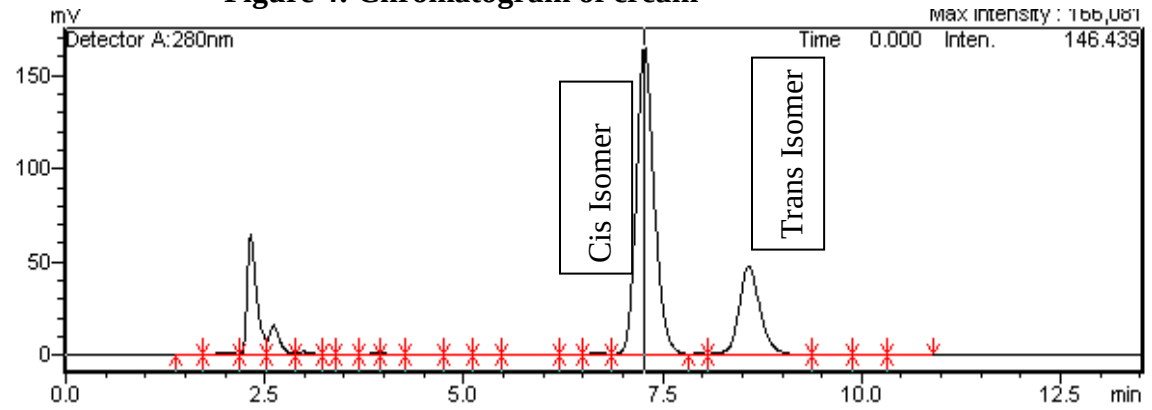

Figure 5: Chromatogram of lotion

Chromatogram of simple mobile phase is shown in figure 2. Figures 3, 4 and 5 illustrate the separation of permethrin in bulk material, cream and lotion respectively. Validation study of the analytical method was performed according to the USP requirements for the rapid quantification of major components (category 1). To insure the validity of analytical method, system suitability was studied before starting the sample analysis. The results for all critical parameters were found to be in the USP acceptable limits ISHIHAMA et. al, 2002.

Table 1: Results showing the system suitability

Peak area: (Mean ± SD)	2558245 ± 5685
Relative standard deviation (RSD)	0.75%
Retention time Rt: (Mean ± SD)	7.681 ± 0.013 min`
Theoretical plats N: (Mean ± SD)	648962 ± 15459
Symmetry Factor As:	1.32 ± 0.02
Capacity factor K:	1.51 ± 0.07

Linearity

A linear detector response was obtained for concentration ranging from 0.8 to 1.66 mg/ml. The results of the studies for recovery and accuracy across the above mentioned range of the analytical method showed 99.41 ± 0.5% recovery. Percent RSD for the intra-day and inter-day

precision studies were found to be less than 2 %. The values comply with the acceptance criteria of the ICH guidelines NAGWA et. al, 1994, ICH 2005. Based on results of five concentrations, replicate of each concentration (n=5), the Peak area (mean ± SD) was plotted against concentration and was found to be linear with linearity of 0.999. Slope and correlation coefficient (R^2) were determined (Figure 6) and (Table 2).

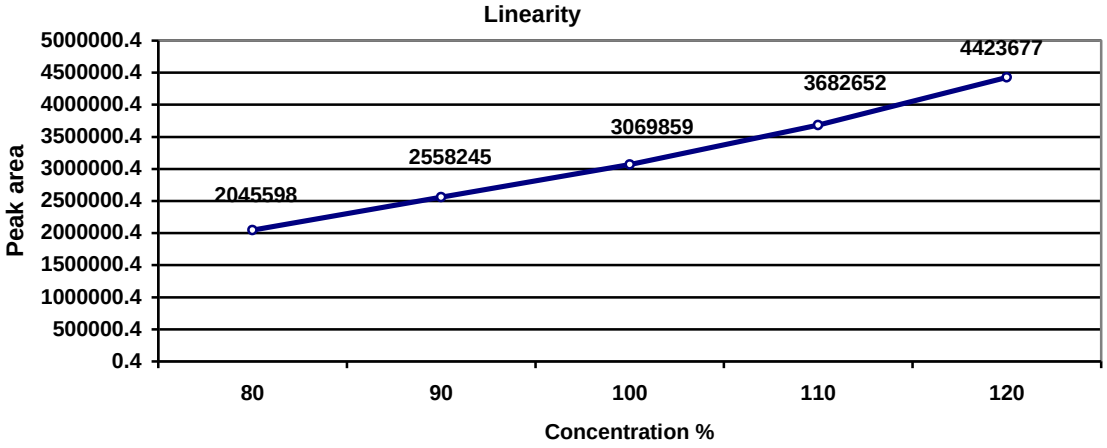

Figure 6: Mean standard HPLC-Calibration Curve for permethrin (n=5)

Table 2: Reverse predicted concentrations, % recovery and % RSD

Concentrations Percentage	Concentrations Injected (mg/ml)	Concentrations recovered mg/ml (mean ± SD)	% Assay (Mean ± SD)	% RSD n=5
80	0.80	0.78 ± 0.003	97.50 ± 1.36	1.37
90	0.960	0.95 ± 0.005	99.00 ± 1.05	1.05
100	1.152	1.16 ± 0.004	100.8 ± 0.39	0.39
110	1.382	1.38 ± 0.013	99.85 ± 0.86	0.86
120	1.66	1.65 ± 0.016	99.93 ± 0.81	0.81
Slope		2527271		
R^2 Value		0.999073		

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ (table 3) (determined from slope of calibration and standard deviation of recovery studies) were found to be 0.0123 and 0.0372 mg/ml, respectively. The application of the method was checked by analyzing different dosage forms of different manufacturers available in the market (Table 6) and results for assay were found to be complying with the label claim ranging 99.71 to 101.91 KRULL et. al, 1997, as shown in Table 6.

Table 3: Characteristics of the analytical method derived from the calibration curve

Slope	R^2 Value	LOD (mg/ml)	LOQ (mg/ml)
2527271	0.999073	0.0123	0.03727

Accuracy and Precision

A known concentration of permethrin sample in bulk material corresponding to five concentrations levels were prepared ranging from 0.8 to 1.66 mg/ml and was analyzed for accuracy and Six replicate for precision studies. Results were obtained using replicate of each concentration (n=6). The relative standard deviation was found to be below 2 %. The results of accuracy and precision studies are presented in Tables 4 and Table 5.

Table 4: Accuracy data of various samples (Results were expressed as mean values, n = 6)

Concentrations (mg/ml)	Retention Time (mean ± SD)	Peak Area (Mean ± SD)	% RSD n=6
0.80	7.672 ± 0.033	2045598 ± 2231	0.317
0.960	7.681 ± 0.036	2558245 ± 5685	0.540
1.152	7.694 ± 0.014	3069859 ± 6735	0.639
1.382	7.669 ± 0.015	3682652±6574	0.463
1.66	7.675 ± 0.018	4423677±4765	0.692

Table 5:Precision data of Six replicate samples (Results were expressed as mean values,n = 5)

Standard Concentration (%)	Mean Assay %
Replicate 1	99.89 %
Replicate 2	100.0 %
Replicate 3	100.00 %
Replicate 4	100.22 %
Replicate 5	100.00 %
Replicate 6	100.11 %
Mean	100.04 %
Standard Dev	0.001136
Relative Standard dev (RSD)	0.113566

Table 6: Results of permethrin dosage form collected from market

Product	Dosage	% Assay (Mean ± SD)	% RSD
Permethrin bulk material	-	100.07± 0.43	0.43
Cream A	50mg/g	99.71 ± 0.66	0.66
Cream B	50mg/g	100.85 ± 0.96	0.95
Cream C	50mg/g	100.46 ± 0.52	0.52
Lotion A	50mg/ml	100.21 ± 0.67	0.67
Lotion B	50mg/ml	101.91 ± 0.49	0.48

Specificity and placebo interference

No peak was obtained at retention time 7.68±2 minutes for permethrin. The representative chromatograms are shown in figure 3. Specificity was also determined by spiking the sample with appropriate level of excipients that showed no significant alteration in peak area. The results showed that there was no chromatographic interference of other excipients present in Permethrin dosage forms. Thus the developed method was found to be very specific for the analysis of permethrin in the cream and lotion Jerome et. al, 1999. Robustness of the method was checked by small changes in flow rate and mobile phase ratio. The peak area of Permethrin was not adversely affected by these changes which could be the evident for robustness of the method. The low values of standard deviation also indicate the robustness of the method developed. The results of robustness are presented in Table 7.

Table 7: Robustness of analytical method

Parameter	Change	Retention time (Mean ± SD)	Peak Area (Mean ± SD)	% RSD
Mobile phase ratio	Normal Condition	7.681 ± 0.011	2558245± 5374	0.81
	92:8	8.15± 0.043	2559128± 5169	0.78
	88:12	6.81± 0.034	2558435± 5256	0.75
Flow Rate	1.1 ml/min	6.80.± 0.004	2557925± 5451	0.79
	0.9 ml/min	8.42±0.067	2558455±4948	0.68

For ruggedness of the analytical method, permethrin sample were analyzed by two different analysts, using different HPLC systems and columns of different manufacturers but using same chromatographic parameters. Repeated results were obtained and the relative standard deviation RSD was found below 2% as shown in table 8.

Table 8: Ruggedness of analytical method used

Analyst	System	Column	Peak Area (Mean ± SD)	% RSD
Analyst A	System A	Kromasil ODS C18 5µm	2558245±4578	0.65
		Hypersil ODS C18 5µm	2559135±5117	0.73
		Mediterranea ODS C18 5µm	2558678±3031	0.43
Analyst B	System B	Kromasil ODS C18 5µm	2559745±3570	0.50
		Hypersil ODS C18 5µm	2559443±6153	0.86
		Mediterranea ODS C18 5µm	2558876±4447	0.62

List of different brands collected from market

Product Code	Company	Brand name
Cream A	Wilsons	Scabiderm
Cream B	Stiefel	Nedax plus
Cream C	Valor	Lice O mite
Cream D	Shaigan	Skab
Lotion A	Shaigan	skab
Lotion B	Bio scab	Bio Lab

CONCLUSION

A simple, rapid, and a specific high-performance liquid chromatographic assay for permethrin has been developed and validated practically. The isocratic flow rate of 1 ml gave the shortest retention time 7.681 ± 0.013 min, which was well resolved from the formulation used under the experimental conditions to confirm its practical applicability in bulk and pharmaceutical formulations such as cream and lotion.

Note: The authors have no conflict of interest.

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