Validated RP-HPLC Method for Determination of Permethrin in Bulk and Topical Preparations Using UV–vis Detector

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Abstract

An isocratic reversed-phase high-performance liquid chromatographic method for the estimation of permethrin in raw materials and pharmaceutical topical preparations has been devised and validated. The chromatographic analysis was performed on a 5 μm particle C-18 Nucleosil (Macherey-Nagel, Germany) column (250 × 4.6 mm). Mobile phase consisted of methanol and 0.025 mM Phosphoric acid (85:15 v/v) at a flow rate of 1.5 mL/min. UV detection was performed at 272 nm and peaks were identified with retention times as compared with standards. The limit of detection was 1.782 μg/mL, while limit of quantitation was 48.0 μg/mL. The calibration was linear in a concentration range of 48.0–5000 μg/mL with correlation coefficient of 0.999978. Regression equation was absorbance = 2833.23 × concentration (μg/mL) + 19.1045 with variance of the response variable, S_{yx}^2, calculated to be 1.75328 (six degrees of freedom). The method was validated as per ICH guidelines and USP requirements and found advantageous for the routine analysis of the drug in pharmaceutical formulations and in pharmaceutical investigations involving permethrin.

Introduction

Permethrin (Figure 1), (3-phenoxyphenyl)-methyl(+)cis-trans-3-(2,2-dicloroethenyl)-2,2-dimethylcyclopropanecarboxylate, is a broad spectrum non-systemic synthetic pyrethroid insecticide. It is also used in healthcare, to eradicate parasites such as head lice and mites responsible for scabies. It acts on the nerve cell membrane to disrupt the sodium channel current by which the polarization of the membrane is regulated. Delayed repolarization and paralysis of the pests are the consequences of this disturbance. It is primarily a contact poison that depends on disturbance of axonic nerve impulse conduction, causing rapid paralysis followed by death (1).

Different methods including derivative spectrophotometric in shampoo (2), high-performance liquid chromatography (HPLC) in treated wood (3), in lotion and shampoo (4), in a forestry spray trial (5), and simultaneous spectrophotometric determination with cypermet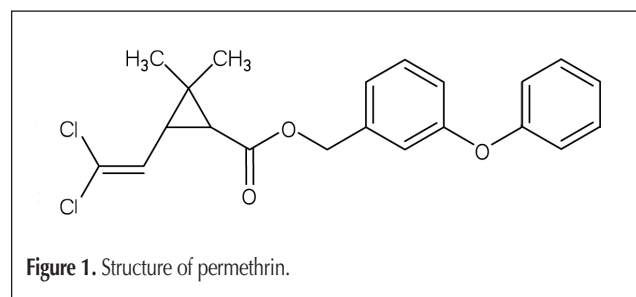hrin and tetramethrin (6) have been described for the determination of permethrin. It has no official (BP and USP) method for its determination in topical preparations.

Literature survey reveals less information about the quantitative determination of permethrin in topical formulations especially in cream matrix and no method have been previously reported. This led us to study the extraction of permethrin in topical preparation and to develop a simple and reliable reversed-phase (RP) HPLC method, being the only, for its determination in bulk and cream preparations. The developed method was validated as per ICH guidelines (7) and USP (8) requirements. Suitable statistical tests were performed on validation data (9–10).

Experimental

Apparatus

A UV-vis spectrophotometer (8453, Agilent Technologies, Santa Clara, CA) was used with quartz cells of 10 mm path length; the HPLC comprised a pump (1200 Series, Agilent), a variable wavelength UV-Visspectrophotometric detector (1200 Series, Agilent), and a degasser (1200 Series, Agilent Technologies) for de-aeration; Column Nucleosil C-18, 4.6 × 250 mm, 5 μm particle size (Macherey-Nagel, Germany); bath sonicator (LC-30 H, Elma, Germany); analytical balance (PL303 Mettler Toledo, USA); filtration assembly (Sartorius, Goettingen, Germany); filter papers 0.45 μm, 13 mm (Sartorius, Hannover, Germany); Sartorius Minisart RC 4 Syringe filters 0.45 μm, Whatman 41 filter paper circles of diameter 125 mm (Whatman, Maidstone, England).

Figure 1. Structure of permethrin.

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Materials

Permethrin reference standard (99.45% potency on anhydrous basis) and Mitonil cream (5%, w/w) were supplied by Saffron Pharmaceuticals Pvt., Ltd., Faisalabad, Pakistan, and was used without any further purification; phosphoric acid of analytical grade (Merck, Darmstadt, Germany); methanol of HPLC grade, (J.T. Baker, Deventer, Holland)

Stock solution of permethrin (20 mg/mL) prepared by dissolving 1.0 g of permethrin in 50 mL of methanol using a bath sonicator.

Chromatographic system

Analysis was conducted on a Nucleosil CLC-ODS 4.6 × 250 mm, 5 μm particle size analytical reverse-phased column at 272 nm and 0.01 AUFS (absorbance units full scale). The samples were introduced through a Rheodyne injector valve (Erkerode, Germany) with a 20-μL sample loop. The mobile phase consisted of methanol and 0.025 mM phosphoric acid (85:15, v/v), filtered through a filter, degassed in ultrasonic bath, and pumped at a flow rate of 1.5 mL/min.

Analytical method development

Different media were investigated to develop a suitable RP-HPLC method for the analysis of permethrin in formulations. For selection of media the criteria employed was sensitivity of the method, ease of sample preparation, miscibility of the drug, cost of solvents and applicability of method to various purposes. Retention time and peak area of permethrin in the selected medium at respective wavelengths were determined and compared with the reference standard and with cream also.

Table I. Mean Absorbance Values and Regressed Values of the Calibration Curve for the Estimation of Permethrin

Conc. (mg/mL)	Mean peak area* (± S.E.)	Regressed values†
0.80	2309.672 ± 5.747002	0.9682
1.60	4528.063 ± 6.019064	0.3935
2.40	6824.862 ± 6.709286	0.9153
3.20	9065.095 ± 9.616009	0.1525
4.00	11371.57 ± 49.75530	0.3994

* Mean of six values.
† Using regression equation.

Table II. Analytical and Regression Parameters of the Proposed Methods

Characteristic	Values in cream
Absorption maxima (nm)	272*
Beer's law limits (μg/mL)	48–5000
Apparent molar absorptivity (L mol⁻¹ cm⁻¹)	2324.87
LOD (μg/mL)	1.782
LOQ (μg/mL)	48.00
Intercept (a)	19.1045
Slope (b)	2833.23
Correlation Coefficient (r)	0.999978

*Needs to be defined.

Preparation of calibration curve

Suitable aliquots (1–5 mL) of the stock solution were pipetted into 25 mL volumetric flasks and the volume was made up to 25 mL with methanol. The solutions were shaken well for proper mixing and injected after the filtration in the column through manual injector and measured their peak area at 272 nm.

The above procedure was repeated six times. Mean peak area values along with the regressed values (method of least squares) and statistical data for the method are shown in Table I. The optical characteristics for the solution of permethrin in methanol are given in Table II.

Stability

Stability of the solutions of permethrin, used for preparing the calibration curves in the method, was ascertained by observing for changes in the peak area at their respective analytical wavelengths over a period of 24 h.

Analytical validation

Specificity and selectivity

Permethrin solution (2.4 mg/mL) was prepared in methanol along with and without common excipients (cetostearyl alcohol, cetomacrogol 1000, liquid paraffin, white soft paraffin, propylene glycol, methyl paraben, propyl paraben, and purified water) separately. A placebo, comprising 2.0% w/w cetostearyl alcohol, 1.40% w/w cetomacrogol 1000, 4.0% w/w stearyl alcohol, 20.0% w/w liquid paraffin, 5.0% w/w propylene glycol, 0.4% w/w methyl paraben sodium, 0.1% w/w propyl paraben sodium, 0.56% w/w sodium metabisulphite, and 61.54% w/w purified water, was also prepared. Solutions were run over the HPLC at selected wavelength and checked for change in the retention time and peak area, which is shown in Figure 2.

Precision

In order to determine the precision of the method, solutions containing known amounts of pure drug were prepared and analyzed in three replicates and readings of area under curve (AUC) were recorded in six replicates to get the mean. The analytical results obtained from these investigations for the method are summarized in Table III.

Accuracy

The accuracy of the method for the estimation of the drug in presence of various cream excipients such as cetostearyl
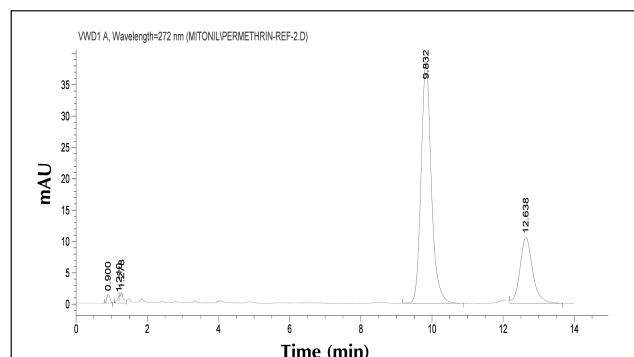

Figure 2. Chromatogram of permethrin reference standard.

aclcohol, cetomacrogol 1000, liquid paraffin, white soft paraffin, propylene glycol, methyl paraben, propyl paraben, and purified water was investigated.

Blends of drug and placebo at different concentrations were not possible due to manufacturing in different phases (i.e., water phase and oil phase and the emulsification of both phases), so six samples of 100% concentration were prepared and recorded in six replicates to get the mean. The drug was then extracted from the sample using methanol. The methanol extract was filtered through Whatman 41 filter paper circles of diameter 125 mm (Whatman, Maidstone, England) and peak area of the filtrates, appropriately diluted, degassed in ultrasonic bath, and injected in the column, was measured at 272 nm. Results of these determinations are included in Table IV.

Linearity

To establish the linearity of the proposed method, five separate series of solutions of the drug (0.8–4.0 mg/mL in methanol) were prepared and analyzed. Least square regression analysis was done for the obtained data (Table I). An ANOVA test (one-way) was performed based on the absorbance values observed for each pure drug concentration during the replicate (six readings) measurement of the standard solutions are given in Table II.

Limit of detection

The limit of detection (LOD) of permethrin by the proposed method was determined by preparing and analyzing three separate series of solutions of the drug (0.096–0.288 mg/mL in methanol) and calculated by the following formula and shown in Table II.

$$y = y_b + 3s_b \qquad \text{Eq. 1}$$

where y, y_b and s_b are signal of sample at LOD, blank signal and standard deviation of the blank respectively. According to the equation of line,

$$y = mx + b \qquad \text{Eq. 2}$$

Where y is the peak area, m is the slope of the line, x is the concentration of sample and b is the y-intercept of the line. In order to calculate the LOD, it was necessary to calculate, all the associated values, that is, the values of y, m, b, r (correlation coefficient), and s_b ($s_{y/x}$).

Where:

$$r = \Sigma_i \frac{[(x_i - x_{avg})(y_i - y_{avg})]}{\{[\Sigma_i(x_i - x_{avg})^2][\Sigma_i(y_i - y_{avg})^2]\}^{1/2}} \qquad \text{Eq. 3}$$

$$m = \Sigma_i \frac{[(x_i - x_{avg})(y_i - y_{avg})]}{\Sigma_i(x_i - x_{avg})^2} \qquad \text{Eq. 4}$$

$$s_{y/x} = \left[\frac{\Sigma_i(y_i - \hat{y})^2}{(n-2)} \right]^{1/2} \qquad \text{Eq. 5}$$

Results and Discussion

Permethrin in methanol yields a characteristic UV spectrum when scanned in the ultraviolet wavelength range between 200 and 400 nm. The scan showed absorption maxima at 272 (Table II) and absorptivity at 272 nm was found to be 2324.87 L/mol cm^{-1} and this wavelength was chosen as the analytical wavelength for HPLC studies.

Figure 3. Chromatogram of permethrin spiked sample.

Figure 4. Chromatogram of Mitonil cream sample from a real determination.

Table III. Evaluation of Precision of the Proposed Method in Pure Drug Substance

Amount of drug Added (mg)	Individual amounts found (mg) mean (S.D.)*	CV†	RME‡	Confidence Limits§
1.0170	1.090729	0.53844	0.31087	1.08139–1.11071
1.0220	1.102396			
1.0190	1.095015			
	1.096047 (0.00590)			
1.0030	1.00074	0.53161	0.30693	0.98679–1.01321
1.0034	1.00491			
1.0023	0.99435			
	1.00000 (0.00532)			
0.9042	0.89255	0.16094	0.09292	0.88951–0.89665
0.9040	0.89198			
0.9048	0.89470			
	0.89308 (0.00144)			

* $n = 6$.
† CV = Coefficient of Variation.
‡ RME = Relative mean error.
§ Confidence limits at $P = 0.95$ and two degrees of freedom.
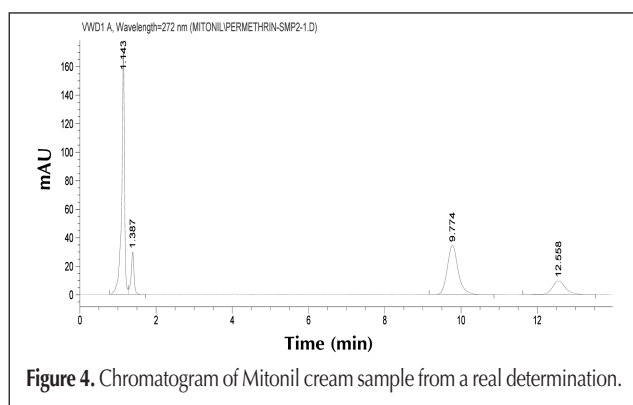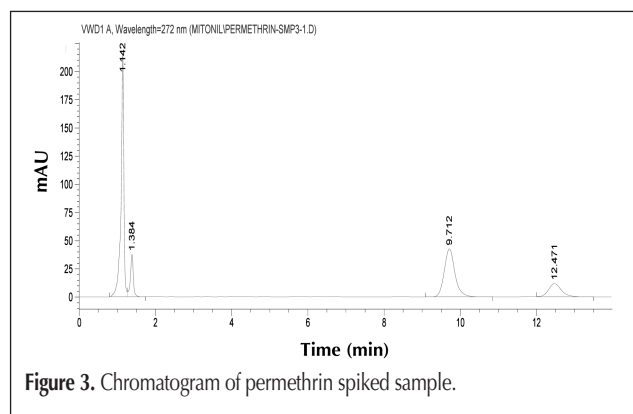
The ultraviolet spectra in this case can be attributed mainly to the phenoxy phenyl nucleus in the permethrin molecule. Correlation coefficient was found to be 0.999978, signifying that a linear relation existed between area under curve (AUC) and concentration of the drug. The limit of detection of permethrin at 272 nm was 1.782 μg/mL and Beer's law was found to be obeyed between 48–5000 μg/mL. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is shown in Table I. The regression equation was $y = 2833.23x + 19.1045$. The variance of the response variable, S_{yx}^2, was calculated to be 1.75328 (six degrees of freedom). This low value indicates the closeness of the experimental points to the least squares line. The fact is in concurrence with the low values of the standard error of the mean   AUCs of the solutions used for preparing the calibration curve. To examine whether the intercept was significantly different from zero, the intercept was subjected to a "t" test. The value of "t" was obtained as 0.00655 (five degrees of freedom) whereas the corresponding tabulated value of t was 2.571 at five degrees of freedom at the 1% level. Thus, acceptance of the null hypothesis indicates that the intercept was not significantly different from zero. Therefore, there is no interference from the solvent used in the method (i.e., methanol).

The stability of permethrin in methanol was monitored over a period of 24 h. ANOVA studies of the mean absorbance values of the solutions of different concentrations at pre-selected time intervals indicated that no significant difference existed between the readings. Thus, permethrin is stable over a period of 24 h in methanol.

The precision of the method was carried out using known amounts of pure drug that were subjected to recovery studies in triplicate and evaluated using the S.D. of the results, the coefficient of variation and confidence limits. Table III summarizes the results of these investigations. The lower S.D. of the results and the lower coefficient of variation make the method more precise. This is also reflected in the larger confidence limits (Table III) for different levels when the method is used for estimation.

In order to determine the accuracy of method, estimation of

permethrin was carried out in the drug product with six 100% dilutions because spiking to the placebo was not possible due to cream manufacturing complexity. From Table IV, it can be seen that there is no significant difference between the amount present in the drug product and the amount recovered. Thus, excipients like cetostearyl clcohol, cetomacrogol 1000, liquid paraffin, white soft paraffin, propylene glycol, methyl paraben, and propyl paraben did not interfere with the estimation. Also, the filtration medium did not absorb the drug to any extent. Accuracy of method was ascertained by using the "t" test (computed "t" 0.0093 and calculated $t = 4.303$ at two degrees of freedom) P 0.05. These values are lower than the tabulated "t" value of 4.303 ($P = 0.05$) indicating no significant difference between the added and the estimated quantity. To establish the accuracy of method the relative mean error (RME) of method was calculated and is shown in Table IV. The relative mean error is less at each level signifying that the method is accurate.

The proposed method was also applied to another drug product namely Lotrix cream to observe the ruggedness of the method and efficiency of extraction of proposed method in other cream matrix. Results of the determination in triplicate are given in Table V. From the results, it can be seen that the proposed method may also be used as an official method in terms of accuracy and precision.

Conclusion

Permethrin can be estimated using the method at 272 nm. It has the advantages of simplicity, stability, reproducibility, and accuracy and is associated with higher sensitivity and precision. The non-interference of cream excipients makes the method suitable for the estimation of the drug in cream and hence can be used for routine quality control of permethrin formulations of all potencies and in forensic sciences involving the estimation of permethrin.

Results of the above study indicate the suitability of the method to estimate permethrin in bulk as well as in dosage formulations. The developed method is comparable to any other method elaborated in the literature.

Table IV. Evaluation of Accuracy of the Proposed Method in Drug Product (Mitonil Cream)

Amount of drug Added (mg)	Individual amounts found (mg) mean (S.D.)*	CV†	RME‡	Confidence Limits§
1.2241	1.2974	2.7710	1.1314	1.2780–1.3545
1.2350	1.3050			
1.2534	1.3161			
1.3165	1.3865			
1.2481	1.3111			
1.2241	1.2816			
	1.3163 (0.0365)			

* $n = 6$.
† CV = Coefficient of Variation.
‡ RME = Relative mean error.
§ Confidence limits at P = 0.95 and two degrees of freedom.

Table V. Ascertainment of the Proposed Method in Other Drug Product (Lotrix Cream)

Amount of Drug Added (mg)	Individual amounts found (mg) mean (S.D.)*	CV†	RME‡	Confidence Limits§
1.2241	1.2250	1.4745	0.9872	1.3396–1.2758
1.2350	1.2713			
1.2534	1.2683			
	1.2549 (0.0185)			

* $n = 6$.
† CV = Coefficient of Variation.
‡ RME = Relative mean error.
§ Confidence limits at P = 0.95 and two degrees of freedom.

Acknowledgement

References

1. P.J. Linnett. Permethrin toxicosis in cats. *Austr. Vet. J.* **86(1–2):** 32–35 (2008).
2. M. Kazemipour, E. Noroozian, M.S. Tehrani, and M. Mahmoudian. A new second-derivative spectrophotometric method for the determination of permethrin in shampoo. *J. Pharm. Biomed. Anal.* **30(4):** 1379–1384 (2002).
3. K. Tumirah, M.A. Mohamad Nasir, S. Salamah, H. Shaharuddin, J. Mohd Dahlan, Y.H. Lee, and A. Musa. Validated HPLC method for quantifying permethrin in treated wood. pp. 201–205 in Norhara, H., Nik Zanariah, N.M., Aminah, H., How, S.S. & Norhayati, N. (Eds.). Highlights of FRIM's MOSTI Projects 2007, 21 to 22 January 2008, Ayer Keroh, Melaka.
4. E. García, A. García, and C. Barbas. Validated HPLC method for quantifying permethrin in pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **24(5–6):** 999–1004 (2001).
5. K.M.S. Sundaram, C. Feng, and J. Broks. High performance liquid chromatographic method for the determination of permethrin deposits in a forestry spray trial. *J. Liq. Chrom. Rel. Tech.* **8(14):** 2607–2624 (1985).
6. A. Niazi, M. Goodarzi, and A. Yazdanipour. A comparative study between least-squares support vector machines and partial least squares in simultaneous spectrophotometric determination of cypermethrin, permethrin and tetramethrin. *J. Braz. Chem. Soc.* **19(3):** 536–542 (2008).
7. The European Agency for the Evaluation of Medical/Products. ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology CPMP/ICH281/95, 2005.
8. United States Pharmacopoeia. Validation of Compendial Procedures, 32nd ed. Pharmacopoeial Convention Inc., Rockville, MD, 2009, pp. 733–736.
9. S. Bolton. *Pharmaceutical Statistics: Practical and Clinical Application*, 4th ed. Marcel Dekker, New York, 2004, pp. 428–434.
10. L. Huber. Validation of analytical methods. Validation and Qualification in Analytical Laboratories, 2nd ed. New York: Informa Healthcare, 2007, 125–54 p.
11. M.S. Arayne, N. Sultana, and F. Hussain. Spectrophotometric method for quantitative determination of Montelukast in bulk, pharmaceutical formulations and human serum. *J. Anal. Chem.* **64(7):** 690–695 (2009).