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Validated HPLC method for quantifying permethrin in pharmaceutical formulations

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Abstract

An isocratic HPLC method for permethrin determination in raw material and pharmaceutical presentations as lotion and shampoo has been developed and validated following ICH recommendations. *Cis* and *trans*- isomers, impurities and degradation products are well separated. The chromatographic analysis were performed on a 4 μm particle C-18 Nova-Pak (Waters, Madrid, Spain) column (15 × 0.39 cm) kept in a Biorad column oven at 35°C. Mobile phase consisted of methanol–water (78:22, v/v) at a flow rate of 1 ml/min. UV detection was performed at 272 nm and peaks were identified with retention times as compared with standards and confirmed with characteristic spectra using the photodiode array detector.

Keywords: Permethrin; Lotion; Shampoo; HPLC

1. Introduction

Permethrin is a synthetic pyrethroid, one of the two available as over-the-counter medications, approved by the Food and Drug Administration for the treatment of head lice, one of the most common diseases in the U.S. [1] and probably in most of the developed world. Moreover, the increase of opportunistic infections in HIV-infected patients, like scabies, has developed an increasing interest in formulations with this substance for therapy [2]. The use of permethrin has also been extended for the prevention of malaria in tropical areas. This compound has exhibited high light stability, low mammalian toxicity and has shown to be an effective insecticide against several major insect species [3]. Moreover, it is non-staining and odourless, acting through contact with the insect.

Permethrin (Fig. 1), presents two diastereomers with different chemical, physical and toxicological properties [4].

Several techniques have been reported for determining the value of permethrin, which relied upon thin layer chromatography [5,6], gas chromatography [7–10], HPLC [3,11–15] and more recently CG-MS [16]. Most of them are intended for analysis of multi-residue of pyrethroids in environmental matrices, crops and foods of ani-

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mal origin. Because of the complexity of matrices, several sample pre-treatment steps are needed.

A previous work exists for determination of permethrin in various pharmaceutical formulations [17] both by HPLC with a reversed phase C-8 column and by first derivative UV-spectrophotometry. RSD values in precision assay with HPLC (3.11%) are over usual criteria for pharmaceutical methods ($<2\%$) [20] and both isomers are not separated. Moreover, an increase of recovery with concentration is observed in the presence of degradation products.

This work describes an isocratic HPLC method for premethrin determination in raw material and pharmaceutical presentations as lotion and shampoo which have been validated following ICH recommendations and with *cis* and *trans*- isomers and impurities are well separated.

2. Experimental

2.1. Instrumentation and chromatographic analysis

A Beckman (Palo Alto, USA) HPLC system provided with a 126 pump, an automatic injector (507e), a 168 Diode Array detector and a Gold System data processor were used. The chromatographic analysis were performed on a 4 μm particle C-18 Nova-Pak (Waters, Madrid, Spain) column (15×0.39 cm) kept in a Biorad column oven at 35°C.

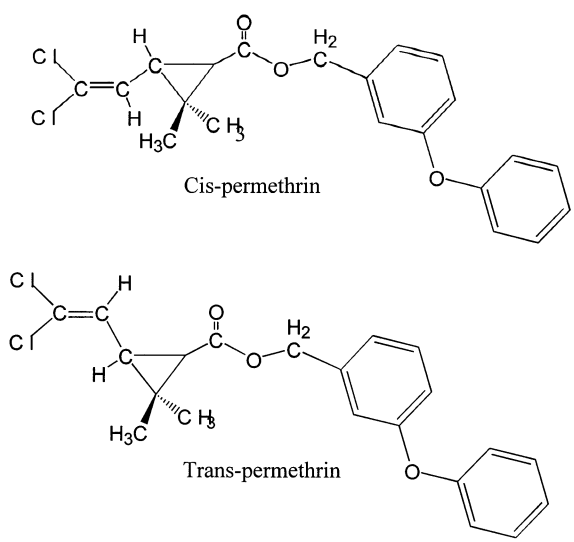

Fig. 1. Permethrin isomers.

Mobile phase consisted of methanol–water (78:22, v/v) at a flow rate of 1 ml/min and the injection volume was 20 μl. UV detection was performed at 272 nm and peaks were identified with retention times as compared with standards and confirmed with characteristic spectra using the photodiode array detector.

2.2. Reagents

All solvents were HPLC grade quality purchased from Merck (Darmstadt, Germany) and standard of permethrin (21.8% and 75.5% of *cis* and *trans*- isomers, respectively) was from Riedel de Häen (Madrid, Spain). Lotion and shampoo containing the active compound were from CINFA S.A. (Pamplona, Spain).

2.3. Quantitation procedure

Through the analytical procedure a 100% standard is used for quantitation. It will be run five times. If the C.V. is under the 2.7%, the series will be processed with three samples between each 100% standards all of them injected by duplicate.

2.4. Specifications for limits of the active

$100 \pm 5\%$ of the theoretical permethrin (sum of both isomers).

2.5. Validation

Once chromatographic conditions were established, method validation was performed following ICH recommendations [18,19]. Standard and samples linearity was verified by analysis in triplicates of five points in the range 32–96 mg/l which corresponded to 50–150% of the expected sample values. Solutions of standards were prepared from a well known purity permethrin with certificate of the content in each isomer and samples in this step were synthetic mixtures of the product components to which known quantities of the substance to be analysed have been added.

For this, a stock standard solution of permethrin was prepared by transferring an accurately weighed amount of 40.0 mg permethrin into a 100.0 ml volumetric flask and dilution to volume with methanol. This solution was stable for at least three days at room temperature. For standards linearity 2, 3, 4, 5 and 6 ml of the stock solution were diluted to 25 ml with methanol in volumetric flask and solutions were filtered to the vials for injection. The same procedure was followed for permethrin raw material, but it was previously melted at 50°C in water bath and homogenised. For samples linearity and accuracy the appropriate quantity of placebo in each formulation was spiked with 2, 3, 4, 5 and 6 ml of the same stock of permethrin.

Recoveries were evaluated with the same method, by comparing through the linearity range the theoretical and measured concentrations.

Instrumental precision was determined by processing two six-sample series of injections of the same standard, or the same sample (raw material, lotion and shampoo), corresponding to the mid point in linearity range, on different days. Results are reported as % RSD (C.V.).

Method precision (repeatability and intermediate precision) applied to the final product were determined by processing two series of six samples, prepared independently as afterwards, on different days and with the corresponding standards for quantification. Results are reported as %RSD (C.V.).

Sample treatment: 1000 mg of the sample (lotion or shampoo) were added to 20 ml of methanol, sonicated for 15 min and then the volume was completed with the same methanol in a 25 ml flask. Four millilitre of this solution were diluted to 25 ml in flask with the methanol. The samples were filtered through nylon membranes with a pore size of 0.45 μm before passing to injection vials.

2.6. Accelerated degradation

Alkali-induced degradation was previously described [17] for permethrin, so working standard solutions of permethrin, lotion and shampoo were prepared with NaOH 0.1 M added. After 48 h they were injected together with solutions freshly prepared and without NaOH added.

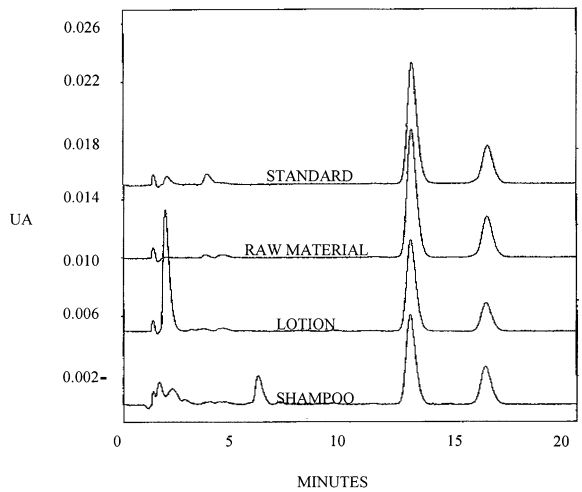

Fig. 2. Chromatograms of standard permethrin, raw material, lotion and shampoo. For conditions see the text.

3. Results and discussion

The method development began with a stationary phase described in the literature as medium in hydrophobicity and silanol activity (Nova-Pak C18), with 4 μm of particle diameter, which improves resolution. Results showed, as can be seen in Fig. 2, that with the mobile phase described above, resolution was good enough, not only for the two isomers but for impurities that appeared in the raw material and components of the commercial formula. Moreover, peak purity tests was performed to show that the analyte chromatographic peak is not attributable to more than one component with the diode array. When comparing the spectra of standards and sample corresponding to permethrin peaks a correlation of over 0.9999 was obtained.

Concentrations were diminished in order to avoid the negative effects of surfactants, coming from shampoo samples, and other excipients in the column.

Fig. 3 shows that degradation of permethrin solutions in standards and samples was complete in the assayed time, and no interference appeared

Table 1
Summary of method validation parameters[a]

		Linearity and range				Precision RSD (%)						Accuracy	
						Instrumental			Method				
						One day ($n=6$)		Different days	Repeatability ($n=6$)		Intermediate precision		
		r	$a \pm$ C.L.	$b \pm$ C.L.	Range (mg/ml^{-1})	Day 1	Day 2		Day 1	Day 2		Mean recovery $n=15$	RSD
Standard	*trans*	0.9998	0.01 ± 0.06	0.096 ± 0.001	32–96	0.22	0.39	0.61 ($n=24$)	0.20	0.36	0.44 ($n=24$)	99.98	0.74
	cis	0.9998	-0.01 ± 0.01	0.091 ± 0.001	32–96	1.81	1.22	1.39 ($n=24$)	1.81	1.21	1.43 ($n=24$)	100.02	0.93
Raw Material	*trans*	0.9998	-0.08 ± 0.09	0.100 ± 0.002	32–96	0.30	0.49	1.00 ($n=12$)	0.64	1.02	0.86 ($n=12$)	98.84	0.66
	cis	0.9991	-0.04 ± 0.05	0.131 ± 0.003	32–96	1.02	0.71	1.73 ($n=12$)	0.68	1.23	1.17 ($n=12$)	98.44	0.84
Lotion	*trans*	0.9998	-0.08 ± 0.09	0.098 ± 0.002	32–96	0.60	0.63	1.12 ($n=12$)	0.40	0.73	0.71 ($n=12$)	100.12	0.80
	cis	0.9990	-0.03 ± 0.06	0.094 ± 0.004	32–96	1.61	1.49	1.25 ($n=12$)	1.36	1.16	1.86 ($n=12$)	100.21	1.86
Shampoo	*trans*	0.9998	-0.00 ± 0.08	0.098 ± 0.001	32–96	0.28	1.11	0.94 ($n=12$)	0.44	1.94	1.34 ($n=12$)	100.12	0.66
	cis	0.9996	-0.01 ± 0.04	0.09 ± 0.02	32–96	0.87	1.49	1.29 ($n=12$)	1.24	2.39	1.89 ($n=12$)	100.12	1.03

[a] a, intercept; b, slope; C.L, confidence limits.

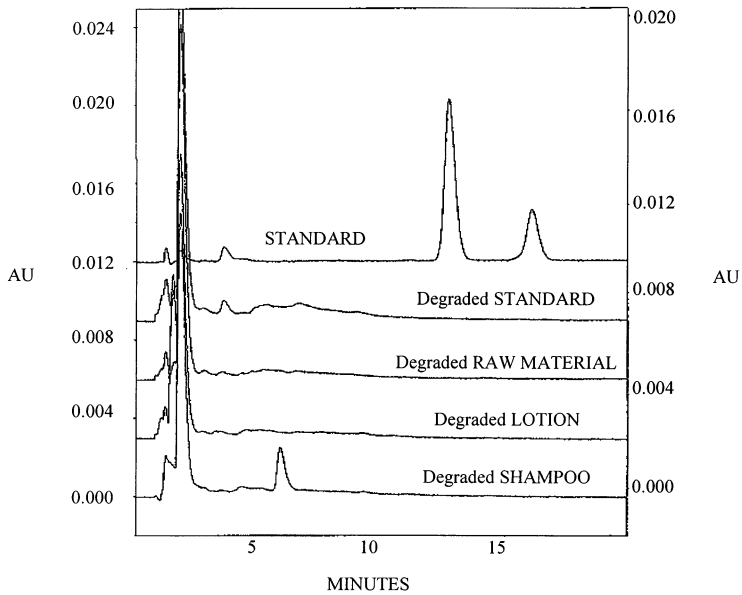

Fig. 3. Chromatograms of standard permethrin as compared with the same disolution and the raw material, lotion and shampoo after degradation in basic media. For conditions see the text.

in the retention time of permethrin in such conditions.

Validation results appear in Table 1. Both standards and samples show a good linearity: RSDs of slopes (s_b 100/b) ranging from 0.51 to 1.95% in standards and samples and RSDs of factors of response ranging from 0.62 to 2.10% (data not shown in the table), showing the good fit of individual points to the regression line, as it could be also observed with the narrow limits of confidence of the slopes. In all cases the intercept plus its confidence limits include the zero value showing that there is no bias. Correlation coefficients over 0.999.

Instrumental precision shows RSDs ranging from 0.22 to 1.81%, being always poorer for the *cis*-isomer.

RSD of the values are $\leq 2\%$ for repeatability (0.2–2.39%) and $\leq 5\%$ (0.44–1.87%) for intermediate precisions of the method.

Recoveries do not statistically differ from 100% (t-test, $P < 0.05$; $n = 15$) in any case, having RSD values ranging from 0.66 to 1.86%.

In previous assays in the laboratory, recoveries in the raw material exceeded 100% for *trans*-isomer while they were clearly under for *cis*-isomer. Permethrin melting point is near room temperature and probably slight differences between both isomers produced the separation. When the product was melted and homogenised before weighing the difference in recoveries disappeared.

In conclusion, the method developed is reliable for quantifying separately *cis* and *trans* isomers of permethrin in raw material and pharmaceutical preparations as lotions or shampoos.

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References

[1] C.V. Bainbridge, G.L. Klein, S.I. Neibart, et al., Clin. Pediatr. 37 (1998) 17–22.
[2] E. Baldrich i Moix, A. Capola i Cusí, Farmacéutiques 21 (1996) 53–54.
[3] E.J. Kikta, Jr., J.P. Shierling, J. Chromatogr. 150 (1978) 229–232.

[4] P.E. Darren, G.F. Martyn, J. Chromatogr. A 777 (1997) 363–369.
[5] E. Polygo, F. Zakar, J. Assoc. Off. Anal. Chem. 66 (1983) 1013–1017.
[6] M.A. Kisenko, D.B. Girenko, Zh. Anal. Khim. 39 (1984) 1132–1134.
[7] R.B. Lubs, J. Hamann, W. Heeschen, Milchwissenshaft. 38 (1983) 597–600.
[8] T. Okada, M. Uno, M. Nozawa, K. Taniwaka, Shokuhim Eiseigaku Zasshi. 24 (1983) 147–154.
[9] D.A. George, J. Assoc. Off. Anal. Chem. 66 (1985) 1160–1163.
[10] J.F. Tyler, J. Assoc. Off. Anal. Chem. 70 (1987) 53–55.
[11] M.S. Sundaram, C. Feng, J. Broks, J. Liquid Chromatogr. 8 (1985) 2607–2624.
[12] R.J. Rando, Y.Y. Hammad, J. Liquid Chromatogr. 8 (1985) 1869–1880.
[13] R.B. Leidy, T.J. Sheets, L.A. Nelson, Beit. Tabk-forsh Int. 13 (1986) 191–203.
[14] U. Nehmer, N. Dimov, J. Chromatogr. 288 (1984) 227–229.
[15] M. Martínez Galera, J.L. Martínez Vidal, A. Garrido, M.D. Gil, J. Chromatogr. A 727 (1996) 39–46.
[16] G.A. Bonwick, C. Sun, P. Abdul-Latif, P.J. Baugh, C.J. Smith, R. Armitage, D.H. Davies, J. Chromatogr. A 707 (1995) 293–302.
[17] M. Sharaf El-Din, M.A. Abuirjeie, Acta Pharmaceutica Fennica. 101 (1992) 111–118.
[18] ICH Topic Q 2 A, Validation of Analytical Procedures: Definitions and Terminology CPMP/ICH/281/95.
[19] ICH Topic Q 2 B, Validation of Analytical Procedures: Methodology. CPMP/ICH/281/95.
[20] J.M. Green, Analytical Chemistry news & features. 305A–309A (1996).