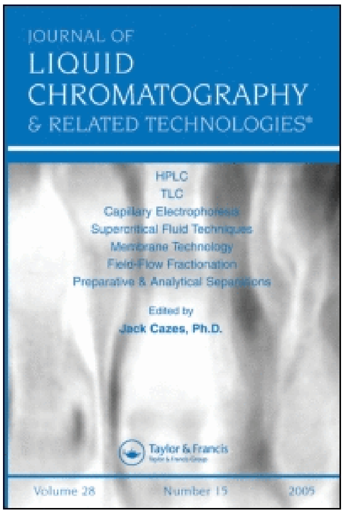

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Manadas, R. , Veiga, F. , Sousa, J. J. and Pina, M. E.(1999) 'DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF *CIS*- AND *TRANS*- PERMETHRIN AND PIPERONYL BUTOXIDE IN PHARMACEUTICAL DOSAGE FORMS', Journal of Liquid Chromatography & Related Technologies, 22: 12, 1867 — 1876

To link to this Article: DOI: 10.1081/JLC-100101772
URL: http://dx.doi.org/10.1081/JLC-100101772

J. LIQ. CHROM. & REL. TECHNOL., 22(12), 1867–1876 (1999)

DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF *CIS*- AND *TRANS*-PERMETHRIN AND PIPERONYL BUTOXIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous and separate determination of cis and trans-permethrin and piperonyl butoxide in a shampoo formulation is described and fully validated. This method entails the quantification of both components by external standard and ultraviolet detection at two wavelengths (201 and 287nm). A C8 Lichrosorb column and a three component mobile phase, isocratically delivered was used.

Results for process validation parameters – selectivity, precision, linearity, and recovery - were in agreement with international specifications. This is a simple method that requires no sample clean up prior to its injection in the system.

INTRODUCTION

Permethrin (PER) is a light-stable, synthetic, type II pyrethroid, important in veterinary medicine, agriculture, forestry, household, industrial, stored products, and public health.[1,2] In human preparations PER has been used both as an insecticide (in Pediculosis capitis,[2,3] Pediculosis pubis, and scabies[3]) and as a repellent directly in the skin, in clothes or in bed nets.[4,5] It is active against a wide range of pests including lice, ticks, fleas, mites, mosquitoes, and black flies.[4] PER has also been used as grain, fruit, and vegetable protector.[6,7]

The world-wide uses of PER are due to its selective insecticide activity, fast biotransformation and excretion by the mammalian catabolic system, minimal percutaneous absorption in humans, low mammalian and bird toxicity, and its non-persistence in the environment.[4,8,9]

Piperonyl butoxide (PBO), a synthetic methylenedioxyphenyl inhibitor of cytochrome(s) P450, is an important synergist in several insecticide formulations that acts by delaying metabolic breakdown or by enhancing the penetrability of cell membranes,[10-11] without having any insecticidal properties of its own.[6]

There are several methods for the determination of PER or PBO in different matrices. These include, for PER, gas chromatography,[7,8,12,13] HPLC,[7,9,14-17] and aqueous capillary electrophoresis.[18] Detection of PER may also be achieved by the Beilstein test[5] and, more recently, by enzyme immunoassays.[19] For the determination of PBO the following methods have been described: RP-HPLC,[10,11,20-22] UV-spectroscopy,[23] differential pulse voltammetry,[22] and GC.[24] However the simultaneous determination of PER and PBO has only been developed by RP-HPLC on paddy rice matrix[6] and spray solutions,[25] and by derivative ultraviolet spectrophotometry in insecticide formulations.[26]

The present study aimed to develop a simple, direct, and time saving method for the quantification of PER and PBO in a shampoo formulation used for the treatment of Pediculosis capitis, and quantify cis and trans isomers of PER separately, since these isomers have different activity against certain species of insects.[27]

This study describes the development and the optimisation of the analytical parameters for the validation of a simple, rapid, specific, precise, and sensitive method that allows the separation of the cis and trans-PER and PBO in a shampoo formulation by RP-HPLC, using a isocratic solvent elution and its quantification with external standard and UV detection.

EXPERIMENTAL

Chemicals

Permethrin standard (96.5% p/p, 22.3% cis, and 77.7% trans-isomers – as mentioned by the manufacturer and confirmed in our lab), piperonyl butoxide standard (94.3% p/p), and shampoo (1% PER and 2% PBO) were supplied by SmithKline Beecham. Acetonitrile, HPLC-grade, was purchased from Merck. Methanol, HPLC-grade, was purchased from Sigma. Water was obtained by a Millipore Elix system.

Instrumentation

The HPLC system consisted of a solvent-delivery pump (Shimadzu LC-10AD) equipped with a variable-dual wavelength SPD-10AVvp UV/VIS detector, a quaternary FCV-10AL solvent delivery system, and an injector with 20µL loop. Data were recorded and analysed in a Pentium computer by the LC-WORKSTATION (Class LC-10 Version 1) interfaced with Shimadzu's CBM-10A communications bus module.

Chromatographic Conditions

A reversed-phase column C-8 Lichrosorb (250 x 4.6mm i.d.) 5 µm. (Jones Chromatography) was used at room temperature (20-23ºC). The detector was set at two wavelengths: 201nm for quantification of PER and 287nm for quantification of PBO. Analyses were carried out isocratically using a three-component mobile phase: acetonitrile:methanol:water (56:20:24) (v/v). The mobile phase was filtered and degassed prior to use. Injection volume was 20µL and flow rate was of 1mL/min.

Calibration Curve

Five standard solutions corresponding to PER (2-12,5µg/mL) and PBO (4-25µg/mL) were prepared from a stock solution containing PER (100µg/mL) and PBO (200µg/mL) in acetonitrile (Table 1). Based on the relative percentage of each isomer of PER, respectively 77.7% of trans and 22.3% of cis, we have weighed an amount of PER taking into account its 96.5% purity, and the drug content of each isomer has been calculated in accordance to that relation.

Table 1

Preparation of Standard Solutions

Standard	Drug Content of Each Standard (μg/mL) **PBO**	 **Trans-PER**	 **Cis-PER**
1	4	1.554	0.446
2	10	3.885	1.115
3	16	6.216	1.784
4	20	7.770	2.230
5	25	9.713	2.788

Table 2

Results of the Calibration Curve

Compound	**Regression Equation**	**Correlation Coefficient**
PBO	$Y = 14909 \times + 413.92$	$R^2 = 0.9997$
Trans-PER	$Y = 155477 \times + 2966.2$	$R^2 = 0.9999$
Cis-Per	$Y = 196599 \times - 142.53$	$R^2 = 0.9999$

A 20μL volume was then injected in the chromatograph and the calibration curve was calculated by linear regression of the peaks' areas versus concentrations of PER and PBO. Unknown PER and PBO concentrations from samples were determined using the regression equations (Table 2).

Sample Preparation

An aliquot of 1g of the shampoo was dissolved in acetonitrile and the volume was brought up to 100 mL. From this stock solution, several dilutions were prepared, filtered, directly injected into the chromatographic system, and analysed.

RESULTS AND DISCUSSION

Optimisation of the Method

Separation, resolution, and retention times were optimised by modifying the mobile phase constitution and the flow rate until the attaining of a good resolution (R) of the two cis and trans isomers of PER ($R \cong 2.2$) and a relatively short run time for each injection (18 minutes).

Selectivity

In Figure 1 typical chromatograms can be seen at 201nm and 287nm from standard solution containing PER and PBO, from sample solution, and from a sample fortified with PER and PBO. In samples, identification of peaks has been done by the comparison of the retention times of constituents to those of standard solutions and by scanning each peak at its maximum height, between 190-350 nm, and comparing the absorption spectra to those of standards. Because at 201 nm (maximum absorbance of PER) the peak of PBO shows interferences, the quantification has been carried out at 287 nm for PBO, where no interferences were detected.

We can conclude that the method is selective because it provides responses, for the chemical entities of interest, which may be distinguished from each other.[28]

Precision

The repeatability of the method, concerning the retention times and the areas under the peaks of PBO and cis and trans-PER, was determined and results are shown in Table 3.

Intermediate precision of the method, defined as the results from within-lab variations due to random events such as differences in experimental period, analysts, equipment, and so forth, was also determined over a four weeks period and the results are also presented in Table 3.

According to the AOAC manual for the peer-verified methods program, and comparing with the values of the relative standard deviation (RSD) obtained, we can conclude that the method has acceptable precision for the referred compounds' quantification.[28]

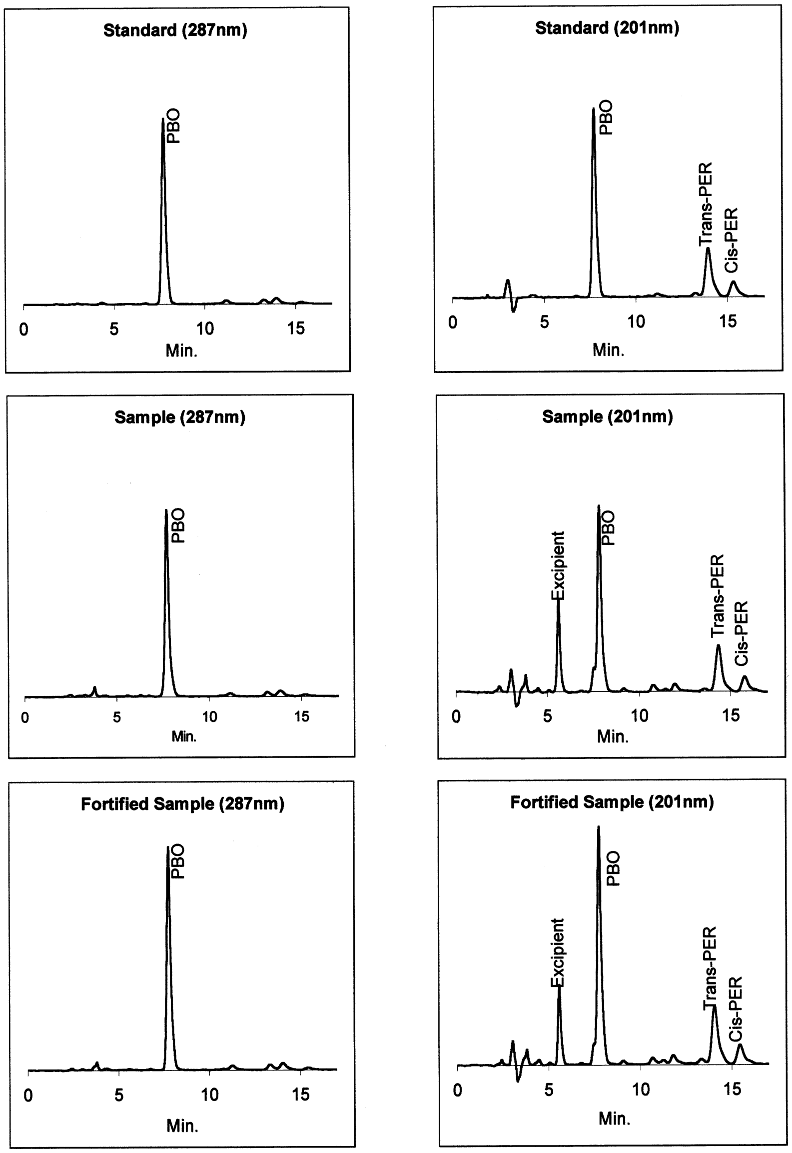

Figure 1. RP-HPLC chromatograms of analytical standards, shampoo samples and fortified samples, at 201 and 287 nm, in the described chromatographic conditions.

Table 3

Precision Data from Standards and Samples

Compound	Ret. Time (Minutes) (± SD)	Concentration Range (μg/mL)	Precision Mean RSD* (%)	Intermed Prec. of Stds: Mean RSD (%)**	RSD of Smpl. (Shampoo) (%)***
PBO	7.73 (± 0.04)	4 - 25	0.31	1.16	0.15
Trans-PER	13.86 (± 0.07)	1.554 - 9.713	1.07	2.07	1.53
Cis-PER	15.22 (± 0.24)	0.456 - 2.788	2.70	3.20	3.58

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* Relative Standard Deviation (n = 5).
** (n = 15).
*** (n = 5).

Table 4

Percentage of Drug Recovery from Fortified Shampoo

Compound	Added Amount (μg/mL)	Recovered Amount (%)
PBO	4	99.24
	10	99.21
	16	101.60
Trans-PER	1.554	97.98
	3.885	98.89
	6.216	100.26
Cis-PER	0.446	105.8
	1.115	102.5
	1.784	101.8

Linearity

The linearity of the method was verified under the range of concentrations described in Table 3. The regression equations obtained, and respective correlation coefficients, are shown in Table 2 and proved excellent linearity between peak-area and concentration.

Recovery

The recovery of known PER and PBO concentrations added to the shampoo was analysed. The results (Table 4) varied between 95-105% which indicates (according to the AOAC manual for the peer-verified methods program) good effectiveness.[28]

CONCLUSION

It was demonstrated that the procedure developed is simple, sensitive, precise, and accurate. According to the validation parameters, we can conclude that this is a rapid method for the routine control of cis and trans-PER and PBO presented in a shampoo formulation and may be further studied for the application in other matrices with similar complexity.

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Received August 15, 1998
Accepted October 19, 1998
Manuscript 4874

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