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Article

Fast and Universal HPLC Method for Determination of Permethrin in Formulations Using 1.8-μm Particle-Packed Column and Performance Comparison with Other Column Types

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An HPLC method has been developed for the fast separation and quantification of permethrin using C18 column packed with 1.8 μm particles. The method is specific with good resolution to degradation products and to other present components. It has acceptable validation results. The run time is 4.5 min (or may be within 1.6 min is rapid resolution mode) with an organic solvent consumption of 3.6 mL per run. The method has been applied to samples of formulations for various uses: mattress cleaner, shampoo, and veterinary powder. The performance of the applied column is compared with other common column types. The relationships between linear velocity of the mobile phase (*u*) and resolution factor (*Rs*), back-pressure (Δ*P*), and efficiency (*H*) are presented. The experimental data shows the advantages of 1.8-μm particle columns to be a significant reduction in solvent consumption (by factor of 4.4 and 1.5) and a reduction in run-time (by factor 4.7 and 1.5), and the weaknesses are a high back-pressure and lower efficiency. Finally, it has been shown that use of 1.8-μm particle packed columns with conventional HPLC systems is possible, but with limitations in mobile phase flow-rate.

Introduction

The pyrethroids have been produced and used the last few decades. They became part of everyday life because of many various applications. The pyrethroid compound obtained as an ester of dichloro analog of chrysanthemic acid and 3-hydroxybenzoic alcohol is known as permethrin. According IUPAC it is: 3-phenoxybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (Figure 1). Permethrin has wide-ranging practical use in agriculture, which comes from its significant repellent action and sufficient photo stability (1). It is mostly used on industrial crops, vegetables, fruits, and stored grains. There are many permethrin formulations for the control of insects in the household, for forest pest control, as a fog in mushroom houses, as wood preservative, or for mosquito nets. Also, it has been found useful as ectoparasiticide; it has been approved by the Food and Drug Administration for treatment of head-lice (2); and recently, it has been applied for therapy of opportunistic infections such as scabies in human immunodeficiency virus (HIV) infected patients (3).

Permethrin in formulations is present as an isomers mixture (*cis* and *trans*) in various ratios (4). The cyclopropane ring in its structure (Figure 1) results in two asymmetrical carbon atoms giving four enantiomers (one pair of *cis* and one pair of *trans*) with respect to the plane of the cyclopropane ring (1). Permethrin's isomers differ in toxicity (3) and because of that, it is very important to have analytical methods for their identification and separate quantification.

Mainly, high-performance liquid chromatography (HPLC) and gas chromatography methods for permethrin analysis are reported (3, 5–9). Usually, every developed method is proposed specially for one formulation type (8, 9). So, there is no reported universal method proposed as applicable for analysis of formulations for various uses. On the other hand, the already reported methods for analysis of permethrin have various successes in separation of enantiomers, especially for the *trans*-forms (10–12). In these methods, the run-time may be 80 min (10), over 80 min (11), and according to recently reported method, 45 min (12). Such methods with long lasting runs where enantiomers are separated may be useful in kinetics or stability studies, but they are not suitable and practical for assay analysis or control of formulations because of the time consumed and higher costs per analysis. During control studies of formulations, acceptable separation of geometrical isomers (*cis*- from *trans*-form) is required.

Moreover, the continuous efforts in liquid chromatography are focused on improving the column's efficiency. The most important benefit arising from better column separation characteristics is in reduction of analysis time followed by reduced organic solvent consumption. The environmental aspect coming from this second mentioned segment is also important and must be taken into account. Reducing the analysis time may be achieved with a shorter column, which usually leads to a decreased number of theoretical plates and poorer chromatographic resolution in analysis of multi-component samples. One specific way of saving time with keeping and improving resolution at the same time has been introduced by HPLC monolithic columns (13–15). The new era in chromatography has begun by reducing the particle size of the packing materials, which has resulted in more efficient columns. Those techniques employing small particles (1.8 μm), packed into short columns, running at higher flow-rates, has been called rapid resolution liquid chromatography (RRLC) (16) and ultra high-performance liquid chromatography (UHPLC) (17–19). These techniques have proved their names in reported experimental data. Thus, it has been shown that those types of columns at the highest flow-rates and in isocratic mode may reduce the analysis time up to factor of 31, compared with conventional methods (16).

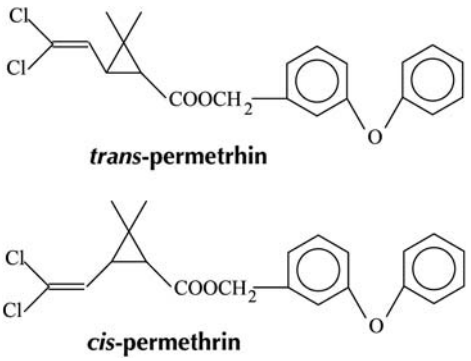

Figure 1. Permethrin isomers (*cis* and *trans*).

The main purpose of this study was to develop a fast and simple method for permethrin analysis, which will be universal at the same time, and so, applicable to formulations for various uses. For that purpose, the performance of the novel type of HPLC column, packed with 1.8-μm particles, has been tested. The optimized method has been validated and applied on samples of different, randomly chosen permethrin formulations: mattresses cleaner, shampoo, and veterinary powder. The method was also tested on different HPLC systems using the same column. Further, in this study a few practical questions were also tackled: are 1.8-μm particle packed columns compatible only with the specially designed HPLC systems, or can they be used with regular instruments? What is the benefit with regards to analysis time and solvent consumption when using columns packed with 1.8-μm particles compared with standard analytical columns? How do they compare to each other for resolution and efficiency? Are there other benefits from using of these columns and what are their limitations? For these reasons, the 1.8-μm particle packed columns have been compared with two other types of columns, usually applied in control laboratories: one conventional packed analytical column with 5-μm particle size and one monolithic rod-shaped column. The same chromatographic conditions and same analyte have been applied on all three columns. Evaluation of their performance has been done by testing resolution factor (R_s), efficiency (H, μm), and back-pressure (ΔP, bar) in correlation with linear velocity (u, mm/s), calculated for both isomers.

Experimental

Chemicals

The solvents used were HPLC grade (LiChrosolv) and obtained from Merck (Darmstadt, Germany). Purified water was obtained by TKA water purification systems (Bovenden, Germany). All solvents were filtered before application through nylon filters with 0.45 μm pores produced by Millipore Corporation (Billerica, MA).

Permethrin standard mixture (*cis*/*trans*, 94%, *m*/*m*) was supplied by Dr. Ehrenstorfer (Augsburg, Germany). Its *cis*/*trans* ratio (40/60%, *m*/*m*) has been calculated by comparison with other permethrin standard mixture (*cis*/*trans*, 60/39%, *m*/*m*) provided from Supelco (Chem Service, Bellefonte, PA).

Samples of formulations with different quantities of permethrin were purchased from the market: Mattress cleaner "Mites-ex" (0.1%, *m*/*m*) produced by Swiss Beauty AG (Abtwil, Switzerland); shampoo "Quit" (0.3%, *m*/*m*) from Z Cosmetics (Leskovac, Serbia); and veterinary powder "Tapilan" (2.0%, *m*/*m*) from Dorvet Ltd. (Holon, Israel).

Preparation of solutions

The first stock standard solution was prepared by dissolving 11 mg of permethrin standard in 50 mL ethanol by mixing on magnetic stirrer for 10 min. The second stock solution was obtained with tenfold dilution of the first stock solution (*cis*: 8.27 μg/mL and *trans*: 12.41 μg/mL).

Sample solutions were prepared from the three different permethrin formulations. Because of their different permethrin content, different sample quantities were taken (1 g mattress cleaner, 330 mg shampoo, and 60 mg veterinary powder) in 50-mL volumetric flasks. The samples were dissolved in ethanol by mixing on a magnetic stirrer for 10 min. All sample solutions were prepared in triplicate. Just before application, all solutions were filtered through nylon syringe filters with 0.45-μm pores from Agilent Technologies, Inc. (Germany). Volumes of 5 μL from each sample solution were injected into the HPLC system for analysis.

Chromatography

The rapid resolution chromatography tests was performed on an HPLC system 1200 series (Agilent, Darmstat, Germany) equipped with a binary pump SL, micro vacuum degasser, standard autosampler ALS SL, thermostatted column compartment TCC SL, diode array detector DAD SL, and data were analyzed with ChemStation software from Agilent Technologies. Data for comparison were obtained by applying the same method on two other HPLC systems. The first one consisted of a quaternary pump, autosampler, Diode Array Detector 235C, oven 101, supported by TotalChrom Navigator 6 software SERIES 200 from Perkin Elmer, Inc. (Norwalk, CT), and degasser Merck L-7614 from Merck. The second was Prominence HPLC system with LC-20AT quaternary pump, Degasser DGU-20A5, SIL-20AC auto sampler, SPD-M20A diode array detector, CTO-20AC Column Oven, software Shimadzu Client/Server, Version 7.3 from Shimadzu Corporation (Tokyo, Japan and Columbia, MA).

The separation was carried out on the following HPLC column: Rapid Resolution Zorbax SB-C18 with dimensions: 5 cm × 4.6 mm i.d., packed with 1.8 μm particles from Agilent; Chromolith Performance RP-18e 10 cm × 4.6 mm i.d., monolith rod column from Merck KGaA, and Zorbax SB-C18 with dimensions: 25 cm × 4.6 mm i.d., packed with 5 μm particles from Agilent (Santa Clara, CA).

The optimized chromatographic conditions are: mobile phase acetonitrile–water in ratio 75:25%, V/V, flow-rate 1 mL/min, column temperature at 45°C, and UV-detection at 215 nm. The flow-rate suggested when using the RRLC mode is 3 mL/min.

Method validation

For selectivity testing, a portion of the standard solution was stored at room temperature for ~ 8 months in order to derive permethrin degradation products.

The linearity has been tested using seven permethrin working standard solutions. They have been prepared by dilution of the first stock solution with ethanol. The concentration ranges of these solutions were 1.65–33.09 μg/mL for the *cis*- and 2.48–49.64 μg/mL for the *trans*-isomer. During analysis, 5 μL of each solution was injected, which correspond to an injected mass range from 8.27–165.44 ng for the *cis*-isomer and 12.41–248.16 ng for the *trans*-isomer. Linearity was evaluated by calibration curves presented by equation:

$$A = \mathrm{a}\ m + \mathrm{b} \qquad \text{Eq. 1}$$

The linearity is obtained by plotting the peak areas (A, mAU) of isomers versus injected permethrin mass (m, μg) onto column and by the value of their correlation coefficients (R^2).

In order to test the method precision, three working solutions were prepared by diluting the first stock solution to concentrations of 12.41 μg/mL, 20.68 μg/mL, and 41.36 μg/mL. The intra-day (instrumental) and the inter-day (intermediate) precision were tested by analyzing those three permethrin solutions. Every solution was freshly prepared and injected five times for four days.

The accuracy of the method was tested by recovery studies. The prepared sample stock solution of one of the permethrin formulations (veterinary powder) was spiked with different portions of standard solution of permethrin. For that purpose, a sample solution was prepared from 60 mg sample in ethanol and filled to 50 mL in a volumetric flask. This solution was mixed in a magnetic stirrer for 10 min. Then, 5-mL portions of this sample solution were spiked with permethrin first stock solution (0.25 mL, 0.5 mL, and 0.75 mL) in 10 mL volumetric flasks, filled up to volume with ethanol, and filtered. The test was performed in triplicate.

Five permethrin working solutions in the low concentration range were prepared for determination of the limit of detection (LOD) and limit of quantification (LOQ). The concentration range of the prepared solutions was: 0.50–3.31 μg/mL for the *cis*- and 0.78–4.96 μg/mL for the *trans*-isomer. Here, also, 5 μL from each of these solutions were injected. Linear calibration curves in shape of Equation 1 were obtained by plotting the peak areas (A, mAU) of both isomers versus permethrin quantity (m, ng) onto column. The LOD and LOQ were calculated by regression analysis, by SD-value of the line obtained by analyzing these five low-concentration solutions and following equations:

$$\mathrm{LOD} = 3.3\ \mathrm{SD/a} \qquad \text{Eq. 2}$$

$$\mathrm{LOQ} = 10\ \mathrm{SD/a} \qquad \text{Eq. 3}$$

All solutions used were filtered through nylon syringe filters with 0.45 μm pores from Agilent Technologies, Inc. before injection.

Results and Discussion

There is a need of fast and high-efficient separations in many HPLC applications, especially in the quality control of food and pharmaceuticals, and in environment trace analysis. To meet this demand, high-pressure and high-speed columns filled up with particles of 1.8 μm were introduced (17–19). Another type of modern high-speed efficient columns is the monolithic silica because of the higher permeability compared with particle packed columns (20–21). These approaches to a high-efficiency column are mainly related to reducing the resistance against mass transfer of a solute based on a small skeleton size or particle size, or even a thin porous shell (22). The relationship among column separation efficiency, the mobile phase linear velocity and particle size has been studied, and the relation between parameters is given by the van Deemter equation (23):

$$H = \mathrm{A} + \mathrm{B}/u + \mathrm{C}u \qquad \text{Eq. 4}$$

where H is a theoretical plate height; u is linear velocity of the mobile phase; A, B, C are coefficients.

The challenges that arise on how to control the A-term (eddy diffusion) and the C-term, which are related to mass transfer in the mobile and stationary phase, have already been described (22). These are directly related to the pore size and the skeleton size or particle size, as well as the structure homogeneity. Because the B-term corresponds to the longitudinal diffusion it hardly affects a plate height under practical HPLC conditions (24–26).

The comparisons of columns packed with small particles and monolithic columns have been reported (27–32). The only condition to completely use the ability of the 1.8-μm particle packed columns is an HPLC system with column back pressure up to 600 bars. There are many questions about those recently introduced columns. In this study, some of these questions have been treated as a practical analytical problem: analysis of permethrin isomers using one method for various formulations.

Optimization of analytical conditions

For higher sensitivity, the method detection was chosen to be at 215 nm because of high absorption of permethrin at that wavelength, according its UV spectrum (12). The experiment was run in a controlled oven temperature for better reproducibility of experimental data. Elevated column temperatures of 45°C is considered to be appropriate to all types of HPLC columns and at the same time, it will reduce the back-pressure. As the goal was to create a simple method, the use of buffer solutions has been avoided. Better peak shapes for permethrin isomers were obtained using acetonitrile–water in comparison with a methanol–water mobile phase. So, further optimization of chromatographic working conditions was done by varying the mobile phase composition within the acetonitrile/water pair.

During optimization of the chromatographic conditions, the dependence of resolution (R_S) of permethrin isomers (Figure 2), column back-pressure (ΔP, Figure 3) and efficiency of the 1.8 μm particle packed column expressed as height of the theoretical plates (H, Figure 4) on the linear velocity (u) of the mobile phase were tested. According to the plot presented in Figure 2, values of up to 3 mm/s for u are still appropriate because at that value, the R_S is 1.6 and, therefore, still acceptable. But, by using 3 mm/s for the mobile phase, the ΔP value for this column is very high (451 bar, Figure 3) and isomers elute at 1.3 min and 1.5 min, respectively, and the run time is within 1.6 min. These chromatographic conditions are suitable for RRLC mode of the method, and they are not applicable on

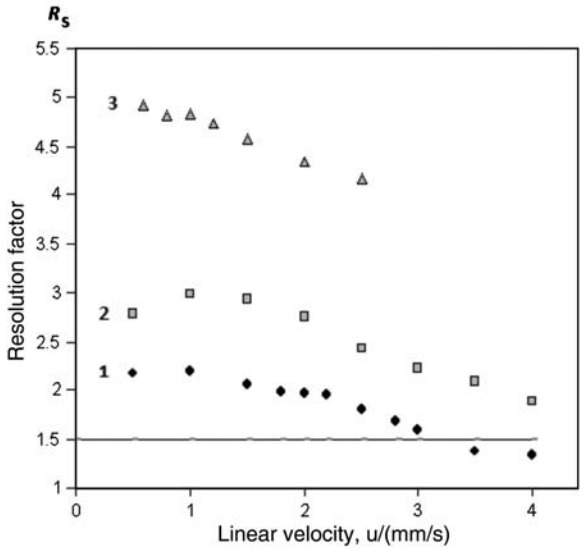

Figure 2. Comparison of data for resolution factor (R_s) versus linear velocity (u, mm/s) using: Rapid Resolution Zorbax SB-C18 column (5 cm × 4.6 mm i.d., 1.8 μm particle-size) (1); Chromolith Performance RP-18e column (10 cm × 4.6 mm i.d., rod) (2); and analytical Zorbax column SB-C18 (25 cm × 4.6 mm i.d., 5 μm particle-size) (3). Chromatographic conditions used are: mobile phase acetonitrile–water ratio 75:25 % (V/V), linear velocity in range: 0.5–2.5 or 4.0 mm/s, column temperature 45°C and UV-detection at 215 nm.

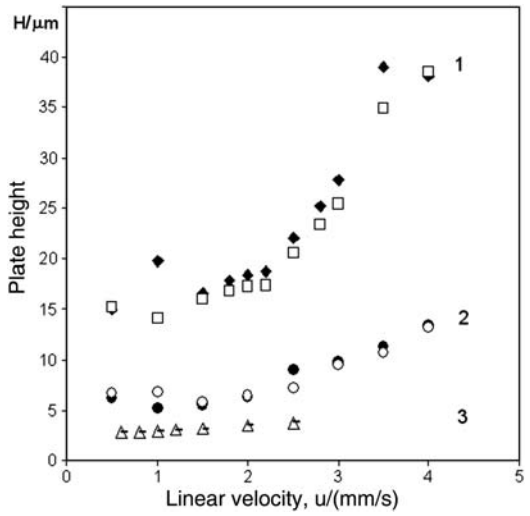

Figure 4. Efficiency of the HPLC columns presented as correlation between theoretical plate height H (μm) of the permethrin peaks versus linear velocity of the mobile phase u (mm/s) using: Rapid Resolution SB-C18 (5 cm x 4.6 i.d., 1.8 μm particle-size) (1); Chromolith Performance RP-18e (10 cm × 4.6, rod column) (2); and Zorbax SB-C18 (25 cm x 4.6 mm i.d., 5 μm particle-size) (3). Chromatographic conditions used are: mobile phase acetonitrile/water ratio 75/25 % (V/V), linear velocity in range: 0.5-2.5 or 4.0 mm/s, column temperature 45°C and UV-detection at 215 nm. Legend: *cis*-permethrin (□, ○, Δ); *trans*-permethrin (■, •, -).

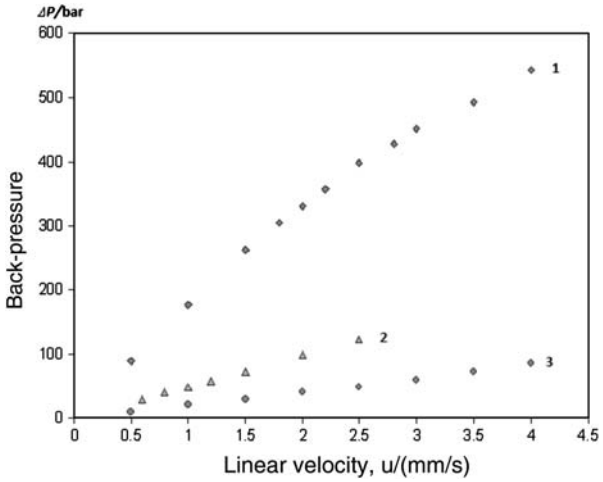

Figure 3. Comparison data of column back-pressure (ΔP) versus linear velocity of mobile phase [u/(mm/s)] using: Rapid Resolution Zorbax SB-C18 column (5 cm × 4.6 mm i.d., 1.8 μm particle-size) (1); analytical Zorbax column SB-C18 (25 cm × 4.6 mm i.d., 5 μm particle-size) (2); and Chromolith Performance RP-18e column (10 cm × 4.6 mm i.d., rod) (3). Chromatographic conditions used are: mobile phase acetonitrile–water ratio 75:25 % (V/V), linear velocity in range: 0.5–2.5 or 4.0 mm/s, column temperature 45°C and UV-detection at 215 nm.

conventional HPLC systems. It was found, also, that optimal efficiency is reached in u range 0.5–2.5 mm/s, where the curve shows near or flat minimum (Figure 4).

Performance comparison of different type columns

Evaluation of the performance of the 1.8-μm particle packed column compared with the other types of frequently used HPLC columns has been done by testing resolution (R_S), back-pressure (ΔP, bar), and efficiency (H, μm) versus linear velocity (u, mm/s), using the proposed method for obtaining comparable data. Chromolith Performance RP-18e (10 cm × 4.6 mm) monolith rod and Zorbax SB-C18 (25 cm × 4.6 mm) packed with 5 μm particles were chosen as columns for comparison because these types and shape are often used in laboratories. The obtained experimental data are given in Table I, and comparable plotted graphs are presented in Figures 2–4.

The longest column (5 μm) showed the best resolution characteristics, but the other two tested gave, also, quite satisfactory resolution in u range 0.5–3.0 mm/s (Figure 2). This is the column of choice in cases where the resolution is the main issue, in testing multi-component samples.

Using the 1.8-μm particle packed column, the back-pressure increased very rapidly with linear velocity, in comparison with the two other columns (Figure 3). At 2 mm/s for this column, the ΔP is 331 bar-s and it is 8.1 times higher than for the monolithic column (41 bar), or 2.4 times higher than at 5 μm column (98 bar). Thus, it makes this column incompatible with HPLC systems with lower limited pressure characteristics of the pumps. However, this also means that the monolithic column has excellent opportunities at higher flows because of its bimodal pore structure. The macropores reduce the column back-pressure and allow the use of higher flow-rates and the mesopores form the fine porous structure and provide the very large active surface area for high efficiency separations (21).

Figure 4 presents the comparison of efficiency of the tested columns. As H is lower, the column is more efficient. In other words, if the minimum of the graph is flat and wide then the column range is wide for different linear velocity values, which may be used for reaching the maximum efficiency of the column. The calculated values of H for both isomers for three tested columns are presented in Table I. These H values for the

Table I
Chromatographic Data for the Three Different Columns Obtained Using the Same Chromatographic Method for Assay Determination of Permethrin*

HPLC-column type	Rapid Resolution Zorbax SB-C18 (50–4.6 mm, 1.8 mm)		Analytical Zorbax SB-C18 (250–4.6 mm, 5mm)		Rod Chromolith Performance RP-18e (100–4.6 mm, rod)	
Parameter	cis-isomer	trans-isomer	cis-isomer	trans-isomer	cis-isomer	trans-isomer
Retention time (tr /min)	4.15	3.54	19.4	16.8	6.02	5.31
Run-time (t/min)	4.5		21		7	
Retention factor (k)	7.57	6.36	11.52	9.81	4.23	3.62
Separation factor (a)	1.19		1.17		1.17	
Resolution (Rs)	2.19		4.84		2.98	
Plate number (N)	3543	2527	17336	17177	7421	9683
Plate height (H/μm)	14.111	19.786	2.884	2.911	6.737	5.164
Tailing (T)	1.15	1.16	1.25	1.25	1.19	1.21
Peak area (A)	225.6	321.5	226.0	324.7	230.7	314.6
Back-pressure (bar)	176		49		20	
Organic solvent /per run (V, mL)	3.56		15.75		5.25	

* Chromatographic conditions with the given HPLC-columns are: mobile phase composed with acetonitrile and water in ratio 75:25% (V/V), flow-rate 1 mL/min, column at 45°C, and UV-detection at 215 nm. The solution used was with following concentrations of isomers: 8.27 μg/mL and 12.41 μg/mL for the cis- and trans- isomer, respectively.

all three columns and two isomers can be thus compared by calculating the factors (i.e., ratios between each other). It is found that the 1.8-μm particle packed column are not the most efficient. It shows lower efficiency in comparison with other columns, presented here as theoretical plate height (*H*). Thus, it is lower efficient for factors 6.8 and 4.9 in comparison with 5-μm particle columns and for factors 3.8 and 2.0 in comparison with the monolithic column, calculated for both permethrin isomers, separately.

The benefit with using a 1.8-μm particle packed column is in the shorter analysis run-time (by factor of 4.7 or 1.5) and the corresponding significant reduction in organic solvent consumption (by factor of 4.4 and 1.5). The weakness of this column type is in the very high back-pressure, which is higher for a factor of 3.8 or even 8.5 in case of the monolithic column. The columns were tested employing variable linear velocities (Figure 4) showing that all columns were most efficient in the range 0.5–2.5 mm/s, but with a minimum near 1 mm/s. Considering the height of the theoretical plates, the most efficient column was found to be the one with 5 μm particles.

Taking into account all the obtained data, it was concluded that for the method to be applicable to most HPLC systems, it would be appropriate to use a flow-rate of 1 mL/min, which corresponds in this case to linear velocity (*u*) of 1 mm/s; which on the other hand corresponds to resolution (R_s) of 2.19; back-pressure (ΔP) of 176 bar-s, and plate height (*H*) of around 14.111 μm for *cis* and 19.786 μm for *trans*-isomer.

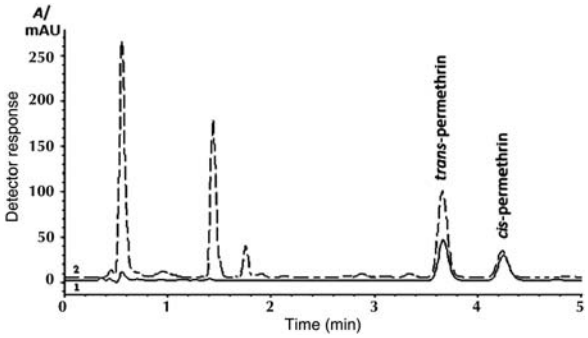

Figure 5. Typical chromatograms of: permethrin standard solution (1); and permethrin standard solution with degradation products (2). Chromatographic conditions used are: Rapid Resolution column Zorbax SB-C18 (5 cm × 4.6 mm i.d., 1.8 μm particle-size), mobile phase acetonitrile–water ratio 75:25 % (*V/V*), flow-rate 1 mL/min, column temperature at 45°C and UV-detection at 215 nm.

Optimal chromatographic conditions

The most suitable chromatographic conditions applicable with convenient HPLC systems and using the rapid resolution (RR) column Zorbax SB-C18 (5 cm × 4.6 mm, 1.8 μm particle-size) are: mobile phase acetonitrile–water in ratio 75:25%, V/V; flow-rate, 1.0 mL/min; column temperature, 45°C; and UV-detection, 215 nm. Employing these conditions, the *trans*-isomer elute at 3.54 min and the *cis*-isomer at 4.15 min. A typical chromatogram obtained by using these conditions is presented in Figure 5, and the important chromatographic data are given in Table I. Comparative chromatograms obtained analysing the permethrin standard solution on different columns were as follows: Rapid Resolution column Zorbax SB-C18 (5 cm × 4.6 mm i.d., 1.8 μm particle-size) (A); Chromolith Performance RP-18e (10 cm × 4.6, rod column) (B); and Zorbax SB-C18 (25 cm × 4.6 mm i.d., 5 μm particle-size) (C) are presented on Figure 6.

The resolution factor obtained for permethrin's isomers of 2.19 and back-pressure of 176 bars make this method applicable with conventional HPLC systems.

Validation results

The selectivity of the method was tested by comparing the freshly prepared permethrin standard solution (Figure 5) with standard solution with derived degradation products (Figure 5). The degradation products are clearly observed in the chromatogram of the solution kept at room temperature for a long period of time. They elute in time range from 0.4 to 1.8 min and appear before the *trans*-permethrin peak. It is noticeable that permethrin degradation products do not overlap or interfere with permethrin isomers peaks. UV spectra were also compared and the peak purity test showed that the permethrin peaks are due to only one component each.

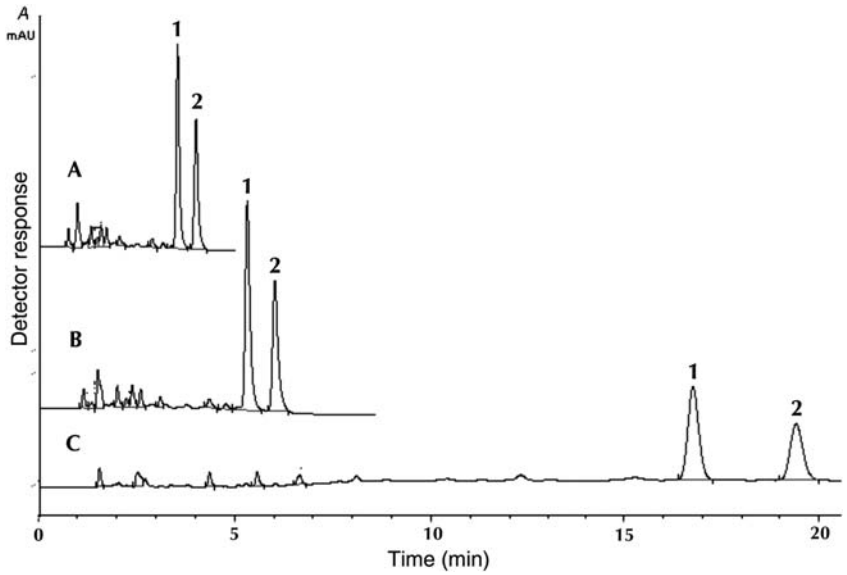

Figure 6. Comparative chromatograms obtained analysing the permethrin standard solution on different columns: Rapid Resolution column Zorbax SB-C18 (5 cm × 4.6 mm i.d., 1.8 μm particle-size) (A); Chromolith™ Performance RP-18e (10 cm × 4.6, rod column) (B); Zorbax SB-C18 (25 cm × 4.6 mm i.d., 5 μm particle-size) (C). *Trans*-permethrin (1), *cis*-permethrin (2). Chromatographic conditions used are:, mobile phase acetonitrile–water ratio 75:25 % (V/V), flow-rate 1 mL/min, column temperature at 45°C and UV-detection at 215 nm.

Table II
Regression Equations, Correlation Coefficients (R2), and Standard Deviations (SD) Found During Linearity, LOQ, and LOD Testing*

Isomer	Amounts interval	Regression equations	R2	SD	Validation test
cis	8.27–165.44 ng	y = 5.3085x − 4.8621	0.9999	3.0964	Linearity
trans	12.41–248.16 ng	y = 4.9954x − 4.6971	0.9999	3.6079	Linearity
cis	2.48–16.54 ng	y = 4.4920x + 3.1543	0.9951	2.4276	LOQ, LOD
trans	3.72–24.82 ng	y = 4.4595x + 3.5840	0.9973	2.6709	LOQ, LOD

* Chromatographic conditions used: HPLC-column Zorbax SB-C18 (5 cm × 4.6 mm i.d.; 1.8 μm particle-size), mobile phase acetonitrile/water in ratio 75:25 % (V/V), flow-rate 1 mL/min, column temperature 45°C, UV-detection at 215 nm.

Further, by testing three different randomly chosen formulations of different composition, it has been demonstrated that the proposed chromatographic conditions of this method are suitable for analyzing various permethrin formulations. It was found that none of the formulations contained any component that interferes with the analysis, although they are for different purposes, from different origin, in different form, and with different permethrin concentration.

Linearity of the method was tested in a concentration range of 8.27–165.44 ng for *cis*-isomer and 12.41–248.16 ng for *trans*-isomer. The obtained results showed a linear relationship between peak area and quantity of permethrin isomers applied on column, which is supported by the regression coefficient values for both isomers ($R^2 = 0.9999$). The obtained regression equations are presented in Table II.

The precision of the method was tested by evaluating the repeatability of the retention times (t_r) and peak areas (A) for both isomers. Results are given as RSD (%) in Table III. The obtained instrumental (intra-day) precision data for retention times are in the range of 0.02–0.45% and for peak areas and in the range of 0.05–0.56%. The obtained intermediate (inter-day) precision data for retention times are in the range of 0.09–

Table III
Precision: Repeatability Data (intra-day, instrumental precision, n = 6) and Method Repeatability Data, MRD (Inter-day, Intermediate Precision, n = 4) Tested for Three Concentration Levels for Retention Time (tr) and Peak Area (A)*

Permethrin concentration (μg/mL)		RSD of tr (%)		RSD of A (%)	
	Day No.	trans-isomer	cis-isomer	trans-isomer	cis-isomer
12.41	1	0.02	0.02	0.35	0.32
	2	0.05	0.05	0.05	0.13
	3	0.14	0.14	0.24	0.26
	4	0.09	0.09	0.39	0.41
	MRD	0.90	0.89	0.28	0.40
20.68	1	0.10	0.11	0.43	0.36
	2	0.06	0.06	0.33	0.24
	3	0.07	0.07	0.20	0.17
	4	0.08	0.09	0.12	0.09
	MRD	0.09	0.09	0.58	0.58
41.36	1	0.41	0.46	0.31	0.56
	2	0.19	0.20	0.11	0.18
	3	0.20	0.23	0.42	0.29
	4	0.31	0.31	0.23	0.29
	MRD	0.35	0.38	0.25	0.26

* RSD of tr: Relative Standard Deviation calculated for retention time; RSD of A: Relative Standard Deviation calculated for peak area. Chromatographic conditions: HPLC-column Zorbax SB-C18 (5 cm × 4.6 mm i.d.; 1.8 μm particle-size), mobile phase acetonitrile–water in ratio 75/25 % (V/V), flow-rate 1 mL/min, column at 45°C, and UV-detection at 215 nm.

Table IV
Recovery Results for Testing Accuracy of the Method*

Permethrin added (μg/mL)	Found (n = 3, mg/L)	SD	RSD (%)	Recovery (%)
0.549	0.516	0.015	2.96	93.98
1.099	1.056	0.040	3.76	96.13
2.198	2.096	0.038	1.80	95.34

* SD: Standard Deviation; RSD: Relative Standard Deviation. Chromatographic conditions: HPLC-column Zorbax SB-C18 (5 cm × 4.6 mm i.d.; 1.8 μm particle-size), mobile phase acetonitrile–water in ratio 75:25 % (V/V), flow-rate 1 mL/min, column at 45°C, and UV-detection at 215 nm.

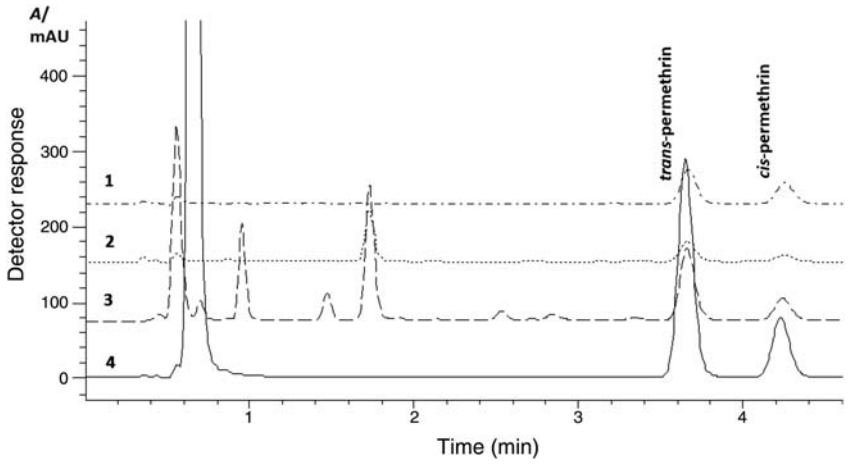

Figure 7. Comparative chromatograms of: permethrin standard solution (1); mattresses cleaner solution (2); shampoo solution (3); and veterinary powder solution (4). Chromatographic conditions used are: Rapid Resolution column Zorbax SB-C18 (5 cm × 4.6 mm i.d., 1.8 μm particle-size), mobile phase acetonitrile–water ratio 75:25 % (V/V), flow-rate 1 mL/min, column temperature at 45°C and UV-detection at 215 nm.

0.90% and 0.25–0.58% for peak areas. There was no significant difference for the isomers area and for the retention times within day and between days, which demonstrates the high precision of the method. These results are approximately two times higher than previous reported results (16, 33) obtained using these type of columns, but they are still fully satisfactory because they all exhibit RSD lower than 1%.

To confirm the accuracy, recovery experiments were carried out by the standard addition technique to one of the tested samples (veterinary powder). Three different portions of permethrin standard solution were added to the prepared sample solution. The results calculated as recovery of found/added in % are given in Table IV. The obtained values are in range of 94.0–96.1%.

LOD and LOQ were evaluated by injecting 5 μL of each permethrin low-concentration working solutions, which corresponds to 2.48–16.54 ng of *cis*-isomer and 3.72–24.82 ng of *trans*-isomer onto column. From the obtained experimental data, LOQ was calculated to be 5.40 ng for *cis*-isomer and 5.99 ng for *trans*-isomer, and the LOD was found to be 1.78 ng for *cis*-isomer and 1.98 ng for *trans*-isomer. The regression parameters are presented in Table II.

An estimation of the LOD/LOQ for the two other columns can be made using the data in Table I, where the values for peaks areas of both isomers obtained with all three columns are almost identical. So, for the 1.8 μm and for the monolith column, similar LOD/LOQ are expected because of the similar areas and also retention times, whereas for the 5 μm column, these values will be higher due to the wider and lower peaks for low concentrations because of higher retention times.

Analysis of various formulation samples

The proposed method has been employed for permethrin analysis in three various randomly chosen formulations. They are for different uses (homes, humans, animals), in different forms (solution, emulsion, powder), with different labelled concentration of permethrin (0.1; 0.3 and 2.0%, m/V), and from different origins (producer). Chromatograms of permethrin standard solution and the ones obtained for the analyzed formulations are given in Figure 7. Peaks of the analytes were identified with retention times as compared with permethrin standard chromatogram (Figure 7) and confirmed with characteristic absorption spectra. These formulations contain different constituents besides permethrin. In all three formulations, the present components don't interfere with permethrin peaks. The purity of the peaks has been checked. One of the formulations contains piperonybutoxide, a compound often present with permethrin in different formulations. In chromatogram 3 of Figure 7, it can be seen that its presence also does not interfere with permethrin isomers. As demonstrated, the method works well in all three cases, so the obtained results for permethrin assay are accurate. The obtained data are separated for the *cis*- and for the *trans*-isomer and the isomer ratio can be calculated from the chromatographic data. It is noticeable that the method is fast with a runtime of 4.5 min, and economic with consumption of 3.5 mL organic solvent per run. The results from the analyzed samples of formulations are presented in Table V.

Table V
Results from Permethrin Determination in Various Formulation Samples

Sample	Label claim (%, m/m)	Mean result, n = 3 (%, m/m)	RSD (%)	Recovery (%)	Isomer's ratio (cis/trans, m/m)
Mattress cleaner	0.10	0.101	0.80	101.17	33.3/66.7
Shampoo	0.30	0.295	1.96	98.34	39.2/60.8
Veterinary powder	2.00	2.013	1.11	100.65	29.0/71.0

* RSD: Relative Standard Deviation. Chromatographic conditions: HPLC-column Zorbax SB-C18 (5 cm × 4.6 mm i.d.; 1.8 μm particle-size), mobile phase acetonitrile–water in ratio 75:25 % (V/V), flow-rate 1 mL/min, column at 45°C, and UV-detection at 215 nm.

Conclusion

The optimal chromatographic conditions for permethrin analysis using C18 Rapid Resolution column packed with 1.8 μm particles size (5 cm × 4.6 mm i.d.) are: mobile phase, acetonitrile–water in ratio 75:25 %, V/V; flow-rate, 1.0 mL/min; column temperature, at 45°C; and UV-detection, 215 nm. The proposed method is fast, simple, accurate, and precise for analysis of both

permethrin isomers in various types of formulations and applicable with convenient HPLC systems. It should be useful for analytical and quality control assays of permethrin isomers and their ratio in various products. There are benefits that come with 1.8 μm particles-packed column use. It has been shown that using it allows for saving time (for factor 4.7 or 1.5) and organic solvents (for factor 4.4 or 1.5); for example, in this case it results in a factor 4.4 or 2.0. At lower linear velocities of the mobile phase, this type of column is applicable with conventional HPLC systems, because at higher linear velocities it exhibits very high back-pressure, which limits its use. Further, the monolithic column showed excellent characteristics at higher linear velocities because of the extremely low back-pressure and good resolution characteristics. And, the 25 cm long, 5 μm particle-packed column showed that it is the column of choice in cases of analysis of multi-component samples where separation of the complex mixture needs higher column efficiency, but on behalf of longer analysis time and higher solvent consumption.

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