HPLC-Method for Determination of Permethrin Enantiomers Using Chiral β-Cyclodextrin-based Stationary Phase

MAJA SHISHOVSKA[1]* AND VERA TRAJKOVSKA[2]

[1]*Department for Medicines Quality Control, Republic Institute for Health Protection, 1000 Skopje, Republic of Macedonia*
[2]*Faculty of Natural Sciences and Mathematics, Institute of Chemistry, St's Cyril and Methodius University, 1000 Skopje, Republic of Macedonia*

ABSTRACT The liquid chromatographic separation of permethrin enantiomers on chiral β-cyclodextrin-based stationary phase has been investigated. All four enantiomers are obtained by using simple methanol and water mobile phase, under gradient mode. The method was optimized and validated. The relationship between temperature and chromatographic parameters: k' (capacity factor), α (separation factor) and *Rs* (resolution factor) was studied. Van't Hoff's curves for each enantiomer were plotted for temperature range 288–318 K. It was noticed that the response factor ratio of permethrin isomers differ and calculated value is found to be 1.66 (*cis/trans*, for $n = 5$). This method has been used for determining permethrin enantiomer ratio for a few samples of working standards and one formulation. *Chirality 22:527–533, 2010.*

KEY WORDS: chiral separation; β-cyclodextrin-based stationary phase; HPLC; permethrin

INTRODUCTION

Great public concern is associated with the use of pesticides. The environmental significance of chirality is still poorly understood although every fourth currently used pesticide is chiral.[1] Now-a-days, the demand for stereoselective separation techniques and analytical assays to evaluate enantiomer purity has increased. Pyrethroids are synthetic compounds with 1–4 chiral centers,[2] which makes them the family of pesticides with the highest number of enantiomers. The insecticide permethrin is a member of this group.

This broad spectrum of pyrethroid is used in veterinary medicines, agriculture, forestry, home pest control, and public health programs. It is approved by the Food and Drug Administration for treatment of headlice[3] and lately are used for therapy of opportunistic infections in HIV infected patients.[4]

Permethrin is an ester of the dichloro-analogue of chrysanthemic acid and 3-phenoxybenzyl alcohol. The cyclopropane ring in its structure results in asymmetric carbon atoms at positions C-1 and C-3 giving four stereoisomers (Fig. 1). as two pairs of *cis*- and *trans*-configurations with respect to the plane of the cyclopropane ring.[5] The optical ratio of 1R:1S is racemic. The individual stereoisomers differ widely in toxicity,[6] metabolism, and biological activites.[7–9] High insecticide toxicity is generally associated with the 1R configuration of the chiral cyclopropane ring adjacent to the carbonyl group.[10] It was reported that *cis*-permethrin is 10-fold more toxic than the *trans*-isomer,[11] 1R-*cis*-enantiomer is 15–38 times more active than the 1S-*cis*-enantiomer, and the 1R-*trans*-isomer is substantially more toxic than the 1S-*trans*-enantiomer.[4] *Trans*-isomers of pyrethroids degrade faster in environment than the *cis*-isomers.[12,13] In soil media, under aerobic conditions, 1R-*cis*-permethrin degrades more slowly than 1S-*cis*-permethrin.[14]

Permethrin is a neurotoxin as are all synthetic pyrethroids, and it kills insects by strongly exciting the nervous system. Its mode of action is similar to that of the organochlorine DDT.[15] Permethrin's health effects include suppression of the immune system,[16] lymph node and spleen damage, and carcinogenesis.[17] Residues in foods are a public health problem because it is known that a major route of exposure to pesticides and other environmental contaminants is through the diet.[18–20] It is reported that permethrin residues are the 10th most frequently detected in food.[21] Thus, it was found in 44% of analyzed peach baby food products.[21] Also, there are data that permethrin may be five times more toxic to children than adults.[21] In spite of the many recent reports[2,22] about pesticide residues of pyrethroids, there are few data on their enantiomers in foodstuffs.

Chromatography is the most common method for enantiomeric analysis. Gas chromatography (GC) is the primary method used in analyses of pesticides.[23] Many of published GC-methods are not adequate for enantioseparation of compounds.[24] In comparison to GC, high-performance liquid chromatography (HPLC) has little risk of epimerization during analysis.[7] However, HPLC is more suitable for nonvolatile, polar, and thermally labile pesti-

*Correspondence to: Maja Shishovska, Department for Medicines Quality Control, Republic Institute for Health Protection, 50 Divizija No 6, Skopje 1000, Macedonia, F.Y.R. E-mail: mzivas@yahoo.com
Received for publication 17 November 2008; Accepted 13 July 2009
DOI: 10.1002/chir.20773
Published online 2 November 2009 in Wiley InterScience (www.interscience.wiley.com).

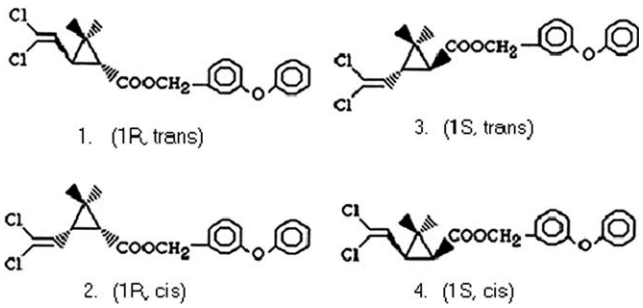

Fig. 1. Chemical structure of the four stereoisomers of permethrin.

cides,[25,26] and also, can tolerate large volume injections of samples.[23] Among chiral stationary phases used for HPLC columns, there are various types of sorbents based on cyclodextrins and their derivatives.[27] The sorbent Chira-Dex® consists of spherical silica particles with covalently bonded β-cyclodextrin[28] with seven glucose units, as chiral selector. Geometrically seen, cyclodextrins are described as truncated cones, were all the secondary hydroxy groups are directed toward the larger opening, and the smaller opening at the other end is formed by primary hydroxy groups. The enantiomers of racemic substance mixture, due to their opposite configurations, can be associated to different degrees with the cyclodextrin molecules. Thus, "inclusion complexes" are formed, based on hydrophilic interaction between cavity and guest molecule and stereoselective hydrogen bonds between glucose molecules and the guest molecule. All three usual cyclodextrin (CD) sorbents (α, β, and γ) have different selectivities and enantiorecognition capacities due to their different cavity diameters and lengths, and they are suitable for chiral resolution of small, medium, and large molecules, respectively. However, there are data that α-CD includes single phenyl and naphthyl groups (small molecules), β-CD accepts naphthyl and heavily substituted phenyl groups, and γ-CD attracts bulky steroid molecules.[29]

The separation and analysis of permethrin stereoisomers has been investigated.[7,30–36] Different HPLC columns were used in RP and NP modes of analysis. The separations were not very successful, especially for *trans*-permethrin enantiomers, and the reported analyses had very long run times. Yang et al.[7] investigated chiral chromatographic separation of permethrin on five different polysaccharide-based stationary phases and obtained only three separated peaks of permethrin. Lee and Salvador[30] presented chiral HPLC analysis methods and proposed them for various studies of pesticide enantiomer pairs in soil and water with two overlapped peaks of *cis*-enantiomers and not separated *trans*-enantiomers. Edwards and Ford[31] used irregular silica with cellulose based chiral HPLC-column and as they reported "slightly" improved resolution with a longer run time of 80 min. Liu et al.[35] investigated the enantioselectivity by HPLC and GC. By GC on a β-cyclodextrin-coated column they separated the *cis*-permethrin enantiomers with retention times of 84.0 and 85.4 min, but *trans*-permethrin was not separated. Novas et al.[36] with NP chromatography obtained four permethrin enantiomer peaks using Pirkle type columns. Anadon et al.[37] in their toxicokinetic studies with HPLC, and also Chalanyova et al.[38] and Tulve et al.[22] in their recent study separated and quantified only *cis*- from *trans*-permethrin.

The purpose of our study was to achieve better separation of permethrin enantiomers and to develop a faster, simpler, and more suitable HPLC method in comparison with earlier reported methods. Permethrin may be described as medium-large molecule, and, there are no published data for successful RP HPLC analysis of permethrin enantiomers. In this study, a chiral β-cyclodextrin-based stationary phase was employed because it contains 35 stereogenic centers. We assumed that this sorbent can be useful because of its many applications for various compounds. The proposed method was optimized and validated. Also, the influence of temperature on the chromatographic parameters: k' (retention factor), α (separation factor), Rs (resolution factor) was studied. From the obtained experimental data, Van't Hoff's curves were plotted over the temperature range: 288–318 K. The response factor ratio of the permethrin isomers was also calculated. The validated method was finally applied for enantiomer ratio analysis of a few samples of working standards and powder formulations for veterinary use.

MATERIALS AND METHODS

Chemicals and Materials

A racemic permethrin standard substance was used, which is a mixture of isomers: *cis-/trans-* 69/30%, m/m (Supelco, Bellefonte, PA). Another working standard of racemic permethrin standard mixture (94%, m/m) and racemic *trans*-permethrin (97%, m/m) was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Sample of "Tapilan"-veterinary powder (permethrin 2%, m/m), produced by Dovert Ltd. (Israel) was purchased.

HPLC grade solvents were used in this work: Chromosolv^R ethanol was from Riedel-de Haën^R (Sigma-Aldrich, Germany), and LiChrosolv® methanol was from Merck (Darmstadt, Germany). Purified water was obtained from TKA water purification system (Germany). All solvents and water were filtered by 0.45 μm white Nylon Millipore filters (Millipore Corporation, Billerica, MA), and all working standard solutions and working sample solutions were filtered by Econofilter nylon membrane syringe filters (0.45 μm) from Agilent Technologies (Germany), just before application.

Apparatus

The chromatography was performed with a Perkin Elmer HPLC-system equipped with: quaternary pump model Series 200, autosampler model SERIES 200, detector DiodeArray 235C, link Nelson 600 Series, and LC 101 Oven equipped with a L-7614 degasser from Merck (Germany). The software used was TotalChrom Navigator 6 (Perkin Elmer).

Chromatographic Conditions

A LiChroCART® HPLC-column with ChiraDex® stationary phase was used with dimensions: length 250 mm and i.d. 4.0 mm, particle size 5 μm and pore size 10 nm, from Merck (Darmstadt, Germany). Separation of permethrin enantiomers was achieved with mobile phase of methanol/water, at a constant flow rate 1.4 ml min^{-1}, UV-detection at 215 nm and with a column oven set at 298 K. The gradient elution program found as optimal was as follows: 0–16 min isocratically methanol/water 56/44 %, V/V, then to 30 min linear gradient from 56 to 70% methanol, and then methanol/water ratio returned directly to the starting conditions and re-equilibration of the system for 10 min.

Data Analysis

The chromatographic parameters were calculated from the following equations:

$$k' \text{ (retention factor)} : (t - t_o)/t_o;$$

$$\alpha\text{(separation factor)} : k'_2/k'_1;$$

$$\text{Rs(resolution factor)} : 2(t_2 - t_1)/(w_1 + w_2);$$

$$\text{Gibbs}-\text{Helmholtz equation} : \Delta G^\circ = -RT \ln (k'/\varphi) \quad (1)$$

(R is the gas constant, T is the absolute temperature, and φ is the column phase ratio (ratio of the stationary phase volume to the mobile phase volume)).

From the Gibbs energy equation, using the enthalpy, ΔH^o, and entropy, ΔS^o, changes, the retention factors are:

$$\ln k' = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \varphi \quad (2)$$

$$\ln \alpha = \ln (k'_2/k'_1) \quad (3)$$

$$\Delta(\Delta G^\circ) = -RT \ln \alpha = \Delta(\Delta H^\circ) - T\Delta(\Delta S^\circ) \quad (4)$$

RESULTS AND DISCUSSION

Method Development

The retention behavior of permethrin enantiomers was studied under different chromatographic conditions. During optimization of the method, at first the isocratic mode was used and then gradient elution with mobile phases consisting of methanol and water in different ratios, at various column temperatures and with varying flow rates to maintain adequate separation. Methanol was used as the most powerful organic modifier for the chosen stationary phase, as recommended by the column manufacturer.[39] The idea was to avoid buffer solutions using simpler mobile phases. The separation of *cis*-enantiomers was very simple, but this was not the case with the *trans*-enantiomers. The separation of *cis*-enantiomers can be achieved under isocratic conditions at lower flow. Separation of *trans*-enantiomers was more successful with a lower content of organic modifier, but this increased the separation time of *cis*-enantiomers and the run time of analysis as well as peak broadening. So, it was necessary to raise the flow rate and/or apply a gradient. The linear gradient was used after the 16th min of analysis to elute the *cis*-enantiomers faster and to give better peak shape.

The UV spectrum of the permethrin isomers standard dissolved in ethanol shows two absorption bands at about 215 and at 273 nm. To give higher sensitivity, the first band with higher absorption was selected as the detection wavelength.

The suitability and selectivity of the method in the presence of alkaline produced degradation products was investigated. Permethrin is stable in acid but unstable in alkaline solutions.[32] Alkali-induced degradation of permethrin was previously described[5] but it was not studied with respect to enantiomer separation. The degradation products are observed in the chromatogram of the permethrin standard solution with a few drops of 0.1M NaOH. They have retention times in the range 6–8 min, and they do not interfere with the permethrin enantiomer peaks. The major degradation products of permethrin isomers are the *cis*- and *trans*-isomers of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzyl alcohol.[40] Furthermore, 3-phenoxybenzoic acid, 3-(4′-hydroxyphenoxy) benzyl alcohol and 3-(4′-hydroxyphenoxy)-benzoic acid may also be present.[3,33,40] It was not the purpose of this work to identify these. The UV spectra of the dominant degradation products are very similar to all four *trans*- and *cis*-enantiomers. They all have bands with maxima at 200 nm, shoulder at 225 nm, minima at 255 nm, and bands with low intensity at 275 nm.

Influence of Temperature to Chromatographic Parameters

Temperature is one of the conditions which influence separation.[41,42] There are at least two different effects of temperature on resolution.[43,44] The first effect has a kinetic nature, and it is related to viscosity and diffusion coefficients in the mobile phase. At higher temperature, viscosity is lower and the mass transfer is enhanced in the mobile phase, and, thus, diffusion coefficient is higher at mobile phase and at stationary phase. The second effect has a thermodynamic nature, and it is in relationship with α and with the separation of the components. The degree to which temperature affects resolution is dependent on the analyte and the sorbent. The kinetics of inclusion complex formation between enantiomers and cyclodextrins vary with temperature, ΔG^o, the Gibbs free energy change of the solute phase transfer is expressed by the Gibbs–Helmholtz equation (eq. 1). The two enantiomers are similarly retained by achiral interactions and this may be expressed by the Gibbs energy using the enthalpy, ΔH^o, and entropy, ΔS^o, changes (eq. 2). The retention factor, k', is the sum of both achiral and chiral interactions. The enantioselectivity ratio, α, is the most important parameter measuring the relative chiral retention difference between

TABLE 1. The effect of temperature on the chromatographic parameters: retention factor (k), the separation factor (α) and resolution (Rs) obtained for *trans*- the first (1), the second (2), and for *cis*- the third (3) and the fourth (4) permethrin eluated peak

T (K)	k'_1	k'_2	α_{12}	Rs_{12}	k'_3	k'_4	α_{34}	Rs_{34}
288	14.16	15.67	1.11	1.18	23.91	26.34	1.10	2.02
295	12.01	14.60	1.21	1.26	23.96	26.47	1.10	2.20
296	11.01	12.46	1.13	1.34	20.57	22.76	1.11	2.21
298	9.67	11.19	1.16	1.32	19.51	21.75	1.11	2.27
303	7.64	8.88	1.16	1.12	17.78	20.27	1.14	2.25
308	6.64	7.64	1.15	1.02	16.83	19.68	1.17	2.31
313	4.36	4.99	1.14	0.93	11.38	14.14	1.24	2.00
318	3.27	3.74	1.14	0.78	7.82	10.26	1.31	1.87

Column: ChiraDex® 25 cm × 4.0 mm id, mobile phase: gradient methanol/water, flow rate: 1.4 ml/min, detection: UV 215 nm.

the two enantiomers (eq. 3), and it may be affected by temperature changes especially in the entropy-dominated situation often encountered in chiral separation.[45] In general, low temperature improves in chiral resolution and usually, the α-values are lower at higher temperature. The chromatographic parameters in correlated with temperature are presented in Table 1. At higher temperature k'-values for all enantiomers are lower, as expected. The α-values for *trans*-enantiomers are similar within the range tested, but the *cis*-enantiomers tend to be higher α-values at higher temperatures. This difference is probably due to change of the mobile phase ratio. Changes in column temperature in the range tested have an effect on resolution, The results show (Table 1), the Rs-values under the ambient temperature can be taken as optimal.

Van't Hoff plots (ln k versus $1/T$) give the thermodynamic parameters of the solute phase transfer from the mobile phase to the stationary phase. These curves were plotted for each enantiomer over the temperature range 288–318 K. The correlations obtained are presented in

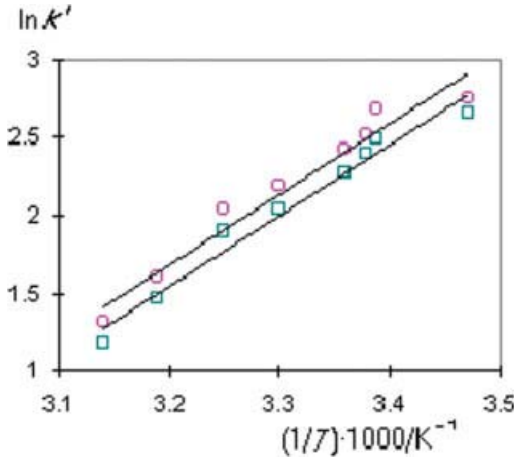

Fig. 2. Van't Hoff's curves obtained for *trans*-permethrin first eluted peak (□), with regression equation $y = 4.5401x - 12.979$ and $R^2 = 0.9748$ and for the second eluted peak (o), with equation $y = 4.5267x - 12.794$ and $R^2 = 0.9620$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

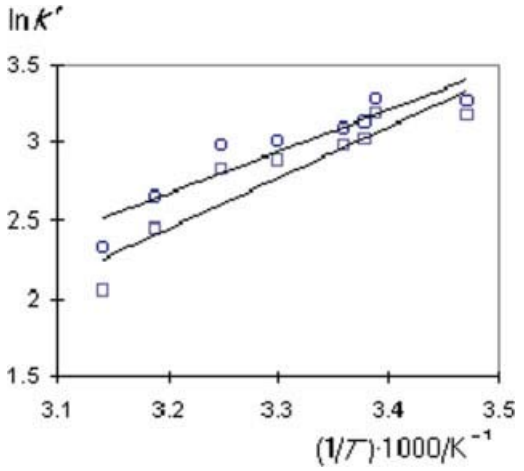

Fig. 3. Van't Hoff's curves obtained for the *cis*-permethrin first eluted peak (□), with regression equation $y = 3.2706x - 8.0089$ and $R^2 = 0.8773$ and for second eluted peak (o) with $y = 2.7273x - 6.0625$ and $R^2 = 0.8724$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figures 2 and 3, and show linearity in observed temperature range. The correlation coefficient is closer to linearity for the *trans*-enantiomers. Van't Hoff plots for all enantiomers have high slopes and large intercepts is probably due to interactions with high enthalpy change and high entropy change.[46] This is an indication that there is more than one process influencing the separation of permethrin enantiomers. Solute inclusions in β-cyclodextrin cavities involves stereogenic centers of the sorbent and various interactions are acting simultaneously (hydrogen bonding, hydrophobic interactions, Van der Waals forces, dipole-induced dipole interactions, ionic interactions, π–π forces, steric effects, solvation effects).[29] From eqs. 1–3 and Van't Hoff's plots, ΔG, ΔH, and ΔS values were calculated for all enantiomers. Comparison with data obtained at 298 K is shown in Table 2. It can be noticed that the Gibbs free energy values for the *cis*-pair are higher than for the *trans*-pair which means better chiral recognition. Also, higher values for ΔH mean that the separation process is enthalpy-driven. For the *trans*-enantiomer pair, ΔH-values are similar, but for the *cis*-enantiomer pair, they differ more as there is better chiral resolution.

Furthermore, the chiral part of the phase transfer of enantiomers is reflected in the difference between the ΔG^o

TABLE 2. Thermodynamic data of enantioselectivity between permethrin enantiomers. Effect of temperature on *trans*- and *cis*-permethrin enantiomers at 298 K

Enantiomer	$\Delta G_{298\ K}$ (kJ/mol)	$\Delta H_{298\ K}$ (kJ/mol)	ΔS (J/Kmol)
trans- first eluted	−10.846	−11.248	104
trans- second eluted	−11.208	−11.215	102
cis- first eluted	−12.585	−8.103	62
cis- second eluted	−12.854	−6.757	45

Column: ChiraDex® 25 cm × 4.0 mm id, mobile phase gradient: methanol/water, flow rate: 1.4 ml/min, detection: UV 215 nm.

TABLE 3. Calculated Δ(Δ*G*)-data for *trans*- and *cis*-permethrin enantiomers, for temperature range 288–318 K

T (K)	$\Delta(\Delta G)_{trans}$ (kJ/mol)	$\Delta(\Delta G)_{cis}$ (kJ/mol)
288	−0.243	−0.718
295	−0.479	−0.565
296	−0.304	−0.401
298	−0.362	−0.330
303	−0.379	−0.269
308	−0.359	−0.249
313	−0.351	−0.244
318	−0.355	−0.232

Column: ChiraDex® 25 cm × 4.0 mm id, mobile phase: gradient methanol/water, flow rate: 1.4 ml/min, detection: UV 215 nm.

of the two pairs of enantiomers, and the term $\Delta(\Delta G^{o})$, is responsible for the higher retention of the second enantiomer pair. The ΔH° and ΔS° represent the enthalpy and entropy terms for each enantiomer, and $\Delta(\Delta H^{\circ})$ and $\Delta(\Delta S^{\circ})$ represent their differences. According to eqs. 1–4, both the retention and the separation factors are influenced by an enthalpic contribution, which decreases with increasing temperature, and an entropic contribution, which is not temperature-dependent. The selectivity is a compromise between differences in the enantiomeric binding enthalpy and disruptive entropic effects.[29] Also, enantioselectivity may be expressed as $-\Delta(\Delta G)$, resembling the different interaction of the individual permethrin enantiomers with the cyclodextrin selector. The $\Delta(\Delta G)$-values, for both enantiomer pairs, calculated using eqs. 1–4 and experimental data from Table 1, are given in Table 3. Since the highest enantiomeric selectivity is achieved by maximizing the energy difference in the diastereoisomeric complexes formed between the solutes and the sorbent, these energy differences become smaller with increasing temperature. It is noticeable that the *cis*-enantiomeric pair is more stable especially at lower temperatures, but this is not the case with the *trans*-pair.

Correlations between ln α and $1/T$ for *cis*- and *trans*-enantiomeric pair are also presented (Fig. 4). Van't Hoff plots obtained for all enantiomers are only linear in a very narrow temperature range 318–303 K (for *trans:* $y = 0.104x - 0.1949$, with $R^2 = 0.8754$ and for *cis*: $y = -0.9273x + 3.1815$, $R^2 = 0.9710$). The *cis*-enantiomers are more stable at higher temperatures. At lower temperatures, both pairs act similarly. The results show that the enthalpic and the entropic contribution to enantioselectivity are important in the enantioseparation of permethrin enantiomers. Thus, in the temperature range investigated, enantioselectivity is governed by the enthalpic contribution and the separation factor α is increased by lowering the temperature for *cis*-enantiomers, while for *trans*-enantiomers it is onlyslightly changed, with a maximum at 298–303 K. The chiral recognition of these enantiomers is mainly enthalpy-driven because the main contribution (eq. 4) for Gibbs free energy comes from this effect (enantiometric binding enthalpy).

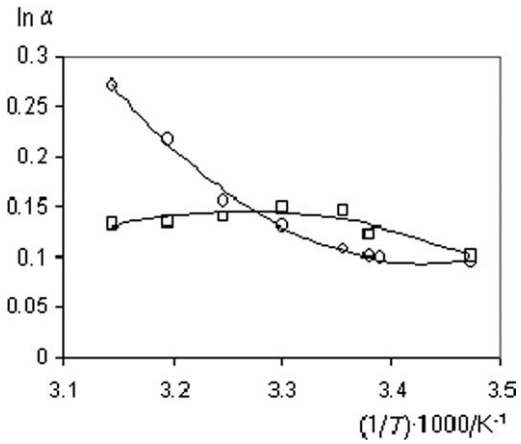

Fig. 4. Van't Hoff's plots of ln α vs. $1/T$ for *cis*-permethrin pair of enantiomers (o) ($y = 2.212x^2 - 15.159x + 26.067$, $R^2 = 0.9955$), and for **trans*-permethrin pair of enantiomers (□) ($y = -1.0416x^2 + 16.8037x - 10.965$, $R^2 = 0.8402$). *It is not taken account the point for 295 K because of its deviation; it is one of eight points; and the next point comes from very close 296 K.

Method Validation

To test linearity of response, 0.4257–2.1285 μg *trans*-permethrin and 0.1419–0.7095 μg *cis*-permethrin were applied on column. Regression analysis revealed a linear relationship between response and quantity of permethrin loaded for both *trans*- and *cis*-permethrin enantiomers, with correlation coefficients of 0.9964 for the *cis*-isomer and 0.9953 for the *trans*-isomer. The obtained regression equations are: $y = 1E + 06x - 39565$ for sum of *cis*-, and $y = 2E + 06x - 152706$ for sum of *trans*-forms. Results for reproducibility and precision are calculated and expressed as RSD (%). The values for reproducibility for the *trans*-forms are: 2.59 and 2.07% and for *cis*-forms: 1.45 and 1.38%. For precision, the obtained RSD values are: 2.90 and 2.60% for *trans*- and 3.30 and 3.32% for *cis*-enantiomers. The obtained values for accuracy are in range 95.6–98.0% with RSD < 3.0%. The LOD and LOQ values were found to be 0.19 and 0.57 μg for *trans*- and 0.07 and 0.21 μg for *cis*-enantiomers.

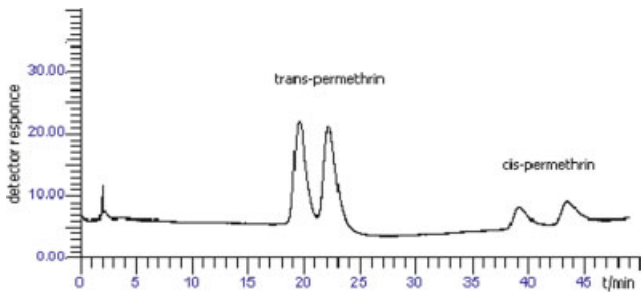

Fig. 5. HPLC chromatogram of racemic permethrin obtained under optimal chromatographic conditions. Retention times of *trans*-enantiomers are 19.8 and 22.7 min; and for *cis*-enantiomers are 38.2 and 42.3 min, respectively. Column: ChraDex® (250 × 4.6 mm, 5 μm particle size); temperature 298 K; injection volume 20 μl; mobile phase: methanol (A) water (B); elution: isocratic 56% A 16 min, gradient of 56–70% A in 30 min, additional 10 min kept at 56% A; flow rate: 1.4 ml/min; detection: UV at 215 nm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Response Factor Ratio

There are no data reported for response factor ratio for permethrin isomers, as authors usually assume they are equivalent. However, our results show that the response factors of *trans*- and *cis*-permethrin differ. With this HPLC method it was obtained that the *cis*-isomer has a higher response, 1.66 times greater than the *trans*-isomer. RSD% = 1.45 (n = 5). Permethrin is a very stable pyrethroid,[35] and the possibility of isomerisation during analysis is very low. This experimental data show that the different response factors of isomers have to be considered during analysis of permethrin.

Analytical Characteristics of the Method

Figure 5 shows a typical chromatogram obtained using the optimal chromatographic conditions. The retention times are 19.8 and 22.7 min for *trans*- and 38.2 and 42.3 min for *cis*-permethrin enantiomers. Identification of *trans*-enantiomers was confirmed with standards. There is little baseline rise because of the mobile phase change. The obtained retention times of the enantiomers are much shorter than in earlier work[30] By using RP-mode with methanol, ethanol, and water mixtures as mobile phase Yang et al.[7] obtained broad peaks with Rs $<$ 0.91. Worse separation was achieved with acetonitrile/water mixtures. Better resolution, Rs $>$ 1.5, was obtained only with one column, using NP mode with isopropanol/*n*-hexane mobile phase, but only three peaks instead of four were detected, and they were not identified.

Here, we have separated all four permethrin enantiomers, using the same type of column in the RP-mode. Edwards and Ford[31] separated the enantiomers, but with a run time of 80 min, which is much longer than our run time of 45 min. Other authors[30] have used five types of Pirkle-brush columns for separation of six pesticide stereoisomer mixtures, but have not separated *trans*-enantiomers, and they were only partly successful in separating the *cis*-forms. In that article,[30] it was concluded that normal-phase was more effective than reverse-phase separation. Liu et al.[35] used a β-cyclodextrin-coated column, and determined the polarity of the resolved enantiomers by using an inline laser polarimeter detector. Using both GC and HPLC, for all investigated insecticides including permethrin, the (+) enantiomer elutes before the (−) enantiomer. It is reported in the literature[47] as a disadvantage of cyclodextrins that such sorbents cannot invert elution order of enantiomers.

The resolution of the *cis*-enantiomer pair is better than that of the *trans*-enantiomer pair. The key difference of these two pairs is in geometry of the molecules. Even during usual RP separation of *cis*- and *trans*- permethrin, the *trans*-form elutes before the *cis*-form because of steric effects. The *cis*-permethrin molecules enter the cavity more easily and are attracted there, compared to the *trans*-permetrhin enantiomers. As result, the *cis*-enatiomers have higher retention times and because of the stronger interactions with the cavity, the *cis*-enantiomers are better resolved.

The stability constant of the complexes decreases with the addition of organic solvents and hence organic modifiers are used to optimize the chiral resolution. Gradient elution shortens the run time and improves the shape of the *cis*-enantiomer peaks. In this method, the first enantiomers elutes at 57.8%, the second at 59.1%, the third at 66.4% and the fourth at 68.3% methanol in the mobile phase.

Determination of Permethrin Enantiomers in Samples

This method was applied for determination of permethrin and enantiomers ratio values (ER). In the analysis of the formulation "Tapilan", excipients gave peaks in the range from 1 to 5 min and no interfering peaks were found in the analyte region of chromatograms due to sample excipients. Moreover, peak purity tests showed that analyte chromatographic peak is not attributable to more than one component with the diode array. For "Tapilan" formulation it was found that *trans*-/*cis*- ratio is 74/23%, m/m. The results for the analyzed samples of working standards and formulations showed that their ER of *cis*- and *trans*-permethrin is very close to racemic.

CONCLUSIONS

In this study, the successful HPLC separation and determination of all permethrin enantiomers is demonstrated. The optimal analytical conditions are achieved using β-cyclodextrin-based sorbent, methanol/water gradient, 1.4 ml/min flow, 298 K working temperature, and 215 nm detection. The method has been established and validated. The temperature influence on the chromatographic parameters has been studied and discussed. The Van't Hoff curves of permethrin enantiomers constructed for the temperature range from 288 to 318 K showed linearity and indicated that there is more than one process influencing the separation. The proposed HPLC method is simple, accurate, precise, reproducible, and stable. The method has been found to be improved when compared with the previously reported methods with respect to its linearity, simple sample preparation, availability and simple mobile phase, UV detection, faster (much lower retention times of the enantiomers), better *R*s-values, and acceptable validation data. It was calculated that response factor of *cis*-permethrin is higher for 1.66 times than the value for *trans*-permethrin. The method proposed in this study enables practical and sensitive RP HPLC analysis of permethrin enantiomers and should be useful for analytical and quality control in various kinds of studies related to permethrin enantiomers.

ACKNOWLEDGMENT

The authors thank Dr Marina Stefova for valuable suggestions.

LITERATURE CITED

1. Williams A. Opportunities of chiral agrochemicals. Pestic Sci 1996; 46:3–9.
2. Tomlin CDS. The Pesticide Manual 12th ed, British Crop Protection Concil, Farnham, UK, 2000.
3. Ochiai N, Sasamoto K, Kanda H, Yamagami T, David F, Sandra P. Multi-Residue Method for the Determination of Five Groups of Pesticides in Non-Fatty Food Samples by Dual Stil Bar Sorptive Extraction (Dual SBSE) and Thermal Desorption GC-MS. Gerstel Global Analytical Solutions, AppNote 3/2005, Germany.

4. Liu W, Gan JJ, Qin S. Separations and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. Chirality 2005;17:127–133.
5. International Programme on Chemical Safety, Environmental Health Criteria 94, Permethrin. 1. Pyrethrins. I. Series. WA 240: NLM Classification, p 1–84.
6. Qin S, Gan J. Difference of pyrethroid enantiomers in their degradation in soils. SETAC 2005;337–338. Available at: abstracts.co.allenpress.com/pweb/setac 2005/document 56232. Accessed August 2008.
7. Yang G-S, Vazquez PP, Frenich AG, Vidal JLM, Aboul-Enein HY. Separation and simultaneous determination of enantiomers of taufluvalinate and permethrin in drinking water. Chromatographia 2004; 60: 523–526.
8. Ray DE, Fry JR. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacol Ther 2006;111:174–193.
9. Leicht W, Fuchs R, Londershausen M. Stability and biological activity of cyfluthrin isomers. Pestic Sci 1996;48:325–332.
10. Burt PE, Elliott M, Farnham AW, Jones NF, Needham PH, Pulman DA. The pyrethroids and related compounds. XIX geometrical and optical isomers of 2,2-dimethyl-3-(2,2 dichlorovinyl) cyclopropane carboxylic acid and insecticidal esters with 5-benzyl-3-furymethyl and 3-phenoxybenzyl alcohols. Pestic Sci 2006;5:791–799.
11. World Health Organization Permethrin. Environmental Health Criteria 94. Geneva, Switzerland: WHO, United Nations Environment Programme, and International Labor Organization; 1990. p 1–26.
12. Liu W, Gan JJ. Separation and analysis of diastereomers and enantiomers of cypermethrin and cyfluthrin by gas chromatography. J Agric Food Chem 2004;52:755–761.
13. Qin S, Budd R, Bondarenko S, Liu W, Gan J. Enantioselective degradation and chiral stability of pyrethroids in soil and sediment. J Agric Food Chem 2006;54:5040–5045.
14. Qin S, Gan J. Enantioselective differences in permethrin degradation pathways in soil and sediment. J Agric Food Chem 2006;54:9145–9151.
15. Vijverberg HPM, Van Der Bercken J. Neurotoxicological effect and the mode of action of synthetic pyrethroids. Crit Rev Toxicol 1990; 21:105–126.
16. Repetto RC, Baligia SS. Pesticides and the immune system. The Public health risks. Washington, DC: WRI (World Resources Institute), National Center for Food and Agricultural Policy; 1996.
17. Park E-K, Kim J-H, Gee SJ, Watanabe T, Ahn KC, Hammock BD. Determination of pyrethroid residues in agricultural products by an enzyme-linked immunosorbent assay. J Agric Food Chem 2004;52:5572–5576.
18. Dougherty CP, Henricks HS, Reinert JC, Panyacosit L, Axelrad DA, Woodruff TJ. Dietary exposures to food contaminants across the United States. Environ Res 2000;84:170–185.
19. Muntean N, Jermini M, Small I, Falzon D, Furst P, Migliorati G, Scortichini G, Forti AF, Anklam E, Von Holst C, Niyazmatov B, Bahkridinov S, Aertgeerts R, Bertollini R, Tirado C, Kolb A. Environ Health Perspect. Assessment of dietary exposure to some persistent organic pollutants in the Republic of Karakalpakstan of Kzbekistan. 2003; 111:1306–1311.
20. CDC. Third national report on human exposure to environmental chemicals. Centers for disease control and prevention, Atlanta, GA 30341. Available at: http://www.cdc.gov/exposurereport. 2005.
21. Cox C. Permethrin insectiside fact sheet. J Pest Ref 1998;18:1–26.
22. Tulve NS, Jones PA, Nishioka MG, Fortmann RC, Croghan CW, Zhou JY, Fraser A, Cave C, Friedman W. Pesticide measurements from the first national environmental health survey of child care centers using multi-residue GC/MS analysis method. Environ Sci Technol 2006;40: 6269–6274.
23. Grosser AA, Ryan JF, Dong MW. Environmental chromatographic methods and regulations in the United States of America. J Chromatogr 1993;642:75–87.
24. Pang GF, Chao YZ, Fan CL, Zhang JJ, Li XM, Zhao TS. Modification of AOAC multiresidue method for determination of synthetic pyrethroid residues in fruits, vegetables, and grains. I. acetonitrile extraction system and optimization of florisil cleanup and gas chromatography. JAOAC Int 1995;78:1481–1488.
25. Buser H, Muller MD. Environmental behavior of acetamide pesticide stereoisomers. I. stereo and enantioselective determination using chiral high-resolution gas chromatography and chiral high-performance liquid chromatography. Environ Sci Tehnol 1995;29:2023–2030.
26. Farran A, Ruix S, Serra C, Aguilar M. Comparative study of high-performance liquid chromatography and micellar electrokinetic capillary chromatography applied to the analysis of different mixtures of pesticides. J Chromatogr A 1996;737:109–116.
27. Guidebook To Chiral HPLC. Column selection and method development techniques, Phenomenex. Available at: http://www.instrument.com.cn./Quotation/Manual/104005.pdf. Accessed November 2008.
28. ChromBook 2008/09, Chromatography at Merck-experience drives innovation, Merck; 2008. p 139. Available at: www.chromatography.merck.de.ChiraDex® DataSheet. Accessed November 2008.
29. Ali I, Aboul-Enein HY. Chiral polutants: distribution, toxicity and analysis by chromatography and capillary electrophoresis. Chichester: John Wiley & Sons; 2004. p 186.
30. Lee W-Y, Salvador JM. Environmental applications of chiral high-performance liquid chromatography. Proceedings of the 2000 conference on hazardous waste research, 2000. p 36–45. Available at: www.engg.ksu.edu/HSRC/Proceed/. Accessed November 2008.
31. Edvards DP, Ford MG. Separation and analysis of diastereoisomers and enantiomers of cypermethrin and related compounds. J Chromatogr A 1997;777:363–369.
32. Oi N, Kitahara H, Kira R. Enantiomer separation of pyrethroid insections by high-performance liquid chromatography by chiral stacionary phases. J Chromatogr 1990;515:441–450.
33. Cayley GR, Simpson BW. Separation of pyrethroid enantiomers by chiral high-performance liquid chromatography. J Chromatogr 1986; 356:123–134.
34. Enayati AA, Vontas JG, Small GJ, Mccarroll L, Hemingway J. Quantification of pyrethroid insecticides from treated bednets using a mosquito recombinant glutathione S-transferase. Med Vet Entomol 2001;15:58–63.
35. Liu W, Gan J, Schlenk D, Jury WA. Enantioselectivity in environmental safety of current chiral insecticides. PNAS USA 2005;102:701–706.
36. Novas Dias A, Garcia Sanchez F, Garcia Paveja A. Resolution of deltamethrin, permethrin, and cypermetrin enantiomers by high-performance liquid chromatography with diode-laser polarimetric detection. J Chromatogr Sci 1998;36:211–216.
37. Anadon A, Martinez-Larranage MR, Diaz MJ, Bringas P. Toxicokinetics of permethrin in the rat. Toxicol Appl Pharmacol 1990; 110:1–8.
38. Chalanyova M, Paulechova M, Hutta M. Method of a selected group of pyrethroids in soil samples off-line flow-trough extraction and on column direct large-volume inj in reversed phase high performance liquid chromatography. J Sep Sci 2006;29:2149–2157.
39. ChiraDex data sheet, Merck. Available at: http://www.uni-leipzig.de/bbz/dateien/abstract 2005.pdf 200803[1].023.pdf. Accessed November 2008.
40. Ariens EJ, Van Rensen JS, Welling W. Stereoselectivity of pesticides. biological and chemical problems. New York, NY: Elsevier Science Publishing; 1988. Chapter 4–10.
41. Cole LA, Dorsey JG. Temperature dependence of retention in reversed-phase liquid chromatography. I. Stationary-phase considerations. Anal Chem 1992;64:1317–1323.
42. Dolan JW, Snyder LR, Blanc T. Selectivity differences for C18 and C8 reversed-phase columns as a function of temperature and gradient steepness. II. Minimizing column reproducibility problems. J Chromatogr A 2000;897:51–63.
43. Pirkle WH. Universal effect of temperature on the retention of enantiomers on a chiral column. J Chromatogr A 1991;558:1–6.
44. Chen H, Horvath Cs. High-speed high-performance liquid chromatography of peptides and proteins. J Chromatogr A 1995;705:3–20.
45. Berthod A. Chiral recognition mechanisms with macrocyclic glycopeptide selectors. Chirality 2009;21:167–175.
46. Scott RPW. The Thermodynamics of chromatography, Chrom ed. series. Free online chromatography books. Available at: http://www.library4science.com. 2002–2005.
47. Review of Stereochemistry, Regis Technologies; 2000. p 19. Available at: www.educhimica.it/Organica/Chiralreview.pdf. Accessed August 2008.