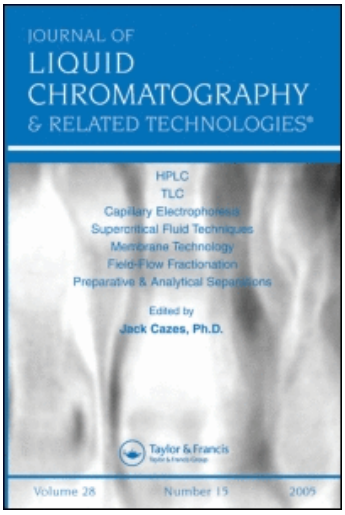

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Speed Determination of β-Cyfluthrin in EC and SC Pesticide Formulations by Reverse Phase and Normal Phase Liquid Chromatography

Lila Vodeb[a]; Biljana Petanovska-Ilievska[a]

[a] Laboratory for Pesticide Formulation Control, Department of Chemistry, Faculty of Agricultural Sciences and Food, Skopje, Republic of Macedonia

To cite this Article Vodeb, Lila and Petanovska-Ilievska, Biljana(2006) 'High Speed Determination of β-Cyfluthrin in EC and SC Pesticide Formulations by Reverse Phase and Normal Phase Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 29: 5, 649 — 660

To link to this Article: DOI: 10.1080/10826070500509207

URL: http://dx.doi.org/10.1080/10826070500509207

Journal of Liquid Chromatography & Related Technologies®, 29: 649–660, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500509207

High Speed Determination of β-Cyfluthrin in EC and SC Pesticide Formulations by Reverse Phase and Normal Phase Liquid Chromatography

Lila Vodeb and Biljana Petanovska-Ilievska
Laboratory for Pesticide Formulation Control, Department of Chemistry, Faculty of Agricultural Sciences and Food, Skopje, Republic of Macedonia

Abstract: The isocratic HPLC procedures for determination of β-cyfluthrin in two different (SC-suspension concentrate and EC-emulsifiable concentrate) pesticide formulations are described. High speed analytical columns for normal phase (HS Pecospher 3×3 Silica (3 μm, 3.3 × 0.46 cm)) and reversed phase liquid chromatography (HS Pecospher 3 × 3 C_{18}, (3 μm, 3.3 × 0.46 cm)) were used. Normal phase liquid chromatography was conducted using a mobile phase of n-hexane/dichloromethane (60/40, v/v) at UV detection of 265 nm; and acetonitrile/water (70/30, v/v), with UV detection at 220 nm for reversed phase liquid chromatography, respectively. The statistical evaluation for intra-day (n = 8) repeatability and inter-day (n = 3) precision assays was found to be satisfactory with high accuracy and precision results as indicated by the ANOVA test. The average percent of recovered analyte, at three concentration levels, was 100.8% with RSD = 1.68% for the HS Pecospher 3 × 3 Silica column and 101.69% with RSD = 3.25% for the HS Pecospher 3 × 3 C_{18} column. The sensitivity of the NP and RP HPLC procedures for investigated pesticides were evaluated by calculating the limit of detection (LOD) and limit of quantification (LOQ). The separation of two dominant isomers of β-cyfluthrin were obtained with a selectivity factor (α) of 1.41 for HS Pecospher Silica column, and 1.06 for HS Pecospher C_{18} column.

Keywords: β-Cyfluthrin, NP-HPLC, RP-HPLC, High speed columns

Address correspondence to Lila Vodeb, Laboratory for Pesticide Formulation Control, Department of Chemistry, Faculty of Agricultural Sciences and Food, Bul. Aleksandar Makedonski bb, 1000 Skopje, Republic of Macedonia. E-mail: lvodeb@zf.ukim.edu.mk

INTRODUCTION

3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid cyano (4-fluoro-3-phenoxyphenyl)-methyl ester (IUPAC), with the common name β-cyfluthrin (ISO), is a synthetic pyrethroid insecticide of the parent compound cyfluthrin. β-Cyfluthrin and cyfluthrin have the same toxicological profile, but β-cyfluthrin has an approximately 2 to 5 times higher acute toxicity than cyfluthrin.[1] β-cyfluthrin is a mixture of four diastereomers (diastereomer I, max. 2.0%; diastereomer II, 30.0–40.0%; diastereomer III, max. 3.0% and diastereomer IV, 57.0–67.0%). Percentage of the sum of the four diastereomers is 98.7%. It is assumed that only two of these diastereomers (II and IV) contribute directly to the biological performance[2] against most species of pest organisms. This photostable synthetic pyrethroid has an ester and an ether linkage in addition to a dichlorovinyl group attached to a cyclopropane moiety (Figure 1).

The actual CIPAC Handbook[3] referee method for analysis of β-cyfluthrin is by normal phase HPLC, using UV detection at 235 nm with external standardization. Harbin,[4] in his collaborative study, has developed an isocratic reversed phase HPLC method (250 × 4.6 mm analytical column packed with 5 μm cyanopropylsilane modified silica gel) with 55% (v/v) acetonitrile in water as mobile phase, UV detection at 230 nm, and decanophenone as an internal standard, to determine total cyfluthrin levels in liquid and solid formulations. Liu and Gan[5] applied the GLC system with an HP-5 column, with electron capture detector (ECD) and mass selective detector (MSD) for separation and analysis of diastereomers and enantiomers of cypermethrin and cyfluthrin. The quantitative estimation of β-cyfluthrin was carried out from Saikia and Gopal[6] by using a gas chromatography instrument equipped with a Ni^{63} electron capture detector.

The stability and biological activity of cyfluthrin and β-cyfluthrin isomers were examined from Leicht et al.[7] In their assays they used several strains of lepidopteran larvae and found that the isomers I and III were much less biologically active than the isomers II and IV. Therefore, they applied a GC method equipped with a HP-1 capillary column and a mass selective detector, capable of completely separating the four pairs of diastereomers of cyfluthrin. Isomers of four commonly used pyrethroids, including cyfluthrin were separated at the enantiomeric level by enantioselective HPLC on two

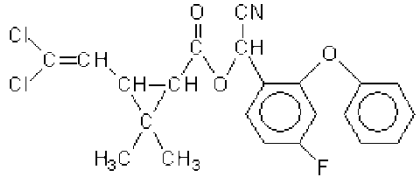

Figure 1. β-Cyfluthrin.

chained Chirex columns, using hexane/1,2-dichloromethane/ethanol as the mobile phase, and UV wavelength for detection was set at 230 nm. The polarity of each resolved enantiomer was determined by an in-line laser polarimeter detector.[8] Some other authors using chiral phase HPLC to the separation of pyrethroid enantiomers have proven that several investigated peaks were not completely separated.[9,10] Most of these methods require either expensive speciality columns or relatively long retention times. The difficulty is manifested by a general lack of standards that are hard to synthesize or purify.[5] Therefore, the aim of this paper is to investigate the possibility of developing a relatively fast, accurate, and economically high speed HPLC method for determination of two dominated and biologically active diastereomers (II and IV) of β-cyfluthrin in its EC and SC pesticide formulations.

EXPERIMENTAL

Equipment and Materials

A Perkin Elmer HPLC, equipped with Binary LC Pump (model 250, Perkin Elmer) and UV Diode Array Detector (model 235, Model Perkin Elmer) were used. Constant column temperature was maintained with a column thermostat Spark Holland "Mistral" (type 880). The investigations were performed with a high speed Pecospher 3×3 Silica column, with particle size of 3 μm, and column dimensions 3.3×0.46 cm (Perkin Elmer), and high speed Pecospher 3×3 C_{18} column, with particle size of 3 μm, and column dimensions 3.3×0.46 cm (Perkin Elmer). HPLC grade acetonitrile, n-hexane, dichloromethane, and methanol were supplied from Sigma-Aldrich (Deisenhofen, Germany). Water was deionized and after that distilled from a glass apparatus. All solvents and solutions for HPLC analysis were degassed in an ultrasonic bath before use. Beta-cyfluthrin analytical standard (98.7%), the pesticide formulations Responsar SC 025 and Bulldock 025 EC, were procured free of charge from Bayer (Germany).

Preparation of Standard Solutions

A primary standard solution of beta-cyluthrin for NP-HPLC was prepared by dissolving 0.0311 g of pure analytical standard with n-hexane in a 25 mL volumetric flask and stored in a refrigerator at 4°C. Working solutions were prepared daily from 0.15, 0.3, 0.6, 1.2, 2.4, 4.8, 7.2, and 9.6 mL of stock solution in 10 mL volumetric flasks and dissolved with n-hexane. The β-cyfluthrin stock solution for reversed phase HPLC was prepared by dissolving 0.0142 g of analytical standard β-cyfluthrin with methanol in a 25 mL volumetric flask and stored in a refrigerator at 4°C. Working solutions were

prepared daily from 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution in 10 mL volumetric flasks and dissolved with acetonitrile. The calibration curve of β-cyfluthrin was obtained with triplicate injections (20 μL each) of working solutions. The area and height counts of individual peaks and the corresponding amount of β-cyfluthrin were used to construct the standard curve using the least-squares method by the OMEGA statistical program[11] with external standard multilevel calibration by linear fit.

Preparation of Sample Solutions

For NP liquid chromatography the sample solutions of pesticide formulations Responsar SC 025 (1.2639 g) and Bulldock 025 EC (0.908 g) were prepared in 25 mL volumetric flasks by dissolving the weighed amounts with n-hexane, and for RP liquid chromatography samples were prepared in a 25 mL volumetric flask by dissolving 0.5695 g (Responsar SC 025) and 0.5532 g (Bulldock 025 EC) in methanol. The samples were degassed for 20 min in an ultrasonic bath and from each sample solution, 5 mL (for NP HPLC) and 1 mL (for RP HPLC), were transferred in a 10 mL volumetric flask and dissolved with the adequate solvents. All the sample solutions were filtered through 0.45 μm Spartan-T syringe filters. From these solutions, three injections were performed with 20 μL each.

RESULTS AND DISCUSSION

An isocratic HPLC method for determination of two dominated and biologically active diastereomers (II and IV) of β-cyfluthrin in its EC and SC pesticide formulations was accomplished on HS Pecospher 3 × 3 Silica columns (Method I) with a mobile phase of n-hexane/dichloromethane (60/40, v/v). The flow rate of 1.3 mL/min at a constant column temperature of 25°C, resulted in two completely separated peaks. The retention time (t_r) of the first peak was 2.65 min (RSD = 0.902%), and for the second peak was 3.60 min (RSD = 1.133%) (Figure 2). The obtained peaks of interest were sharp and symmetrical. Beta-cyfluthrin consists, predominantly, of two diastereomeric pairs (diastereomer II, 30.0–40.0% and diastereomer IV, 57.0–67.0%) of enantiomers;[12] therefore, it was possible to tentatively identify the resolved peaks like diastereomer II and diastereomer IV.

In addition, to confirm the specificity of the developed method, UV diode array detection was used to check the peak purity and analyte peak identity.[13] Figure 3 shows the overlay spectra with a purity index of 1.1, obtained by comparing the absorption spectra of a pure analytical standard and absorption spectra of an active ingredient in both formulations.

The absorption spectra of β-cyfluthrin has a band with maximum absorption approximately at 267 nm. The chromatographic peak, obtained at a

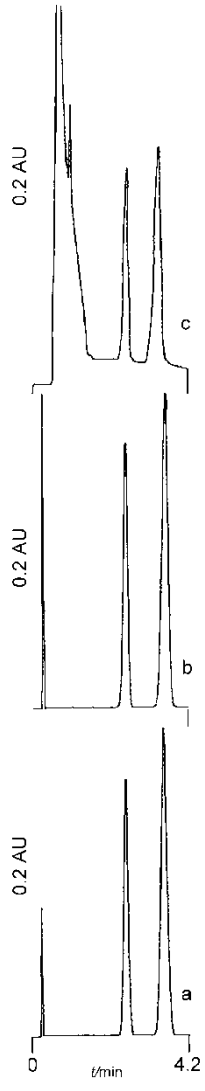

Figure 2. Chromatograms of β-cyfluthrin from: standard solution (a), pesticide formulation Responsar 025 SC (b) and Bulldock 025 EC (c), eluted on a HS Pecospher 3×3 Silica, mobile phase n-hexane/dichloromethane (60/40, v/v), flow rate 1.3 mL/min, column temperature 25°C, UV detection 265 nm (Method I).

wavelength of 220 nm, had a high intensity, compared to the chromatographic peak intensity obtained at 267 nm. Accordingly, the wavelength of 220 nm was chosen as the monitoring wavelength for HS Pecospher 3×3 C_{18} (Method II), but in this UV region, n-hexane and dichloromethane gave a

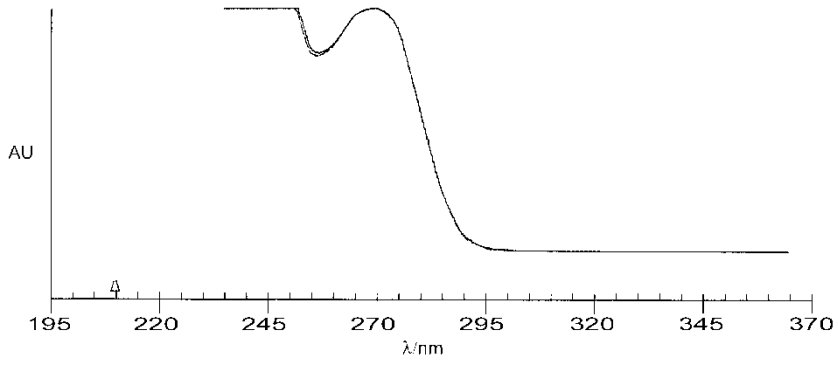

Figure 3. Overlay spectra of β-cyfluthrin from a pure analytical standard and β-cyfluthrin from pesticide formulations Responsar 025 SC and Bulldock 025 EC (Index purity = 1.1) (Method I).

significant absorption, which has a negative effect at the base line; therefore, measurements on HS Pecospher 3 × 3 Silica column (Method I) were performed at 265 nm.

When the high speed Pecospher 3 × 3 C_{18} column (Method II) was used, the mobile phase consisted of acetonitrile/water in a volume ratio of 70/30. The flow rate of 1.0 mL/min and column temperature of 30°C gave the retention time of β-cyfluthrin diastereomers around at 3.56 min and at 3.77 min (Figure 4).

In the chromatogram obtained with Method II, it is interesting to note the appearance of two peaks approximately at 1.30 min and 1.52 min, with a maximum absorption at around 211 nm. From day to day, the area of these peaks (1.30 min and 1.52 min) increased compared to the area of both dominant peaks that decreased (Figure 5). It is evident that isomerisation of β-cyfluthrin isomers in methanol is possible. A similar fairly rapid isomerization was observed by Leicht et al.[7] when cyfluthrin isomer-I was incubated in methanol or methanol + water, at room temperature and in darkness. It's possible that using the chromatographic condition described, the inactive diastereoisomers I and III eluted ahead of the biologically active diastereoisomers II and IV, although, no standards were used to validate the peak assignment. The overlay spectra of the four obtained chromatographic peaks showed the same spectral characteristics with index purity of 1.1 (Figure 6).

The values for the capacity factors are 5.76 for the diastereomer II, and 8.17 for the diastereomer IV. The column dead-time (t_o) on the HS Pecospher 3 × 3 Silica column (Method I) for β-cyfluthrin was about 0.39 min. The evaluated value for the separation factor (α) measured from the adjacent peaks was 1.41, which implies that these chromatographic conditions gave a high efficiency of separation to both diastereomers of

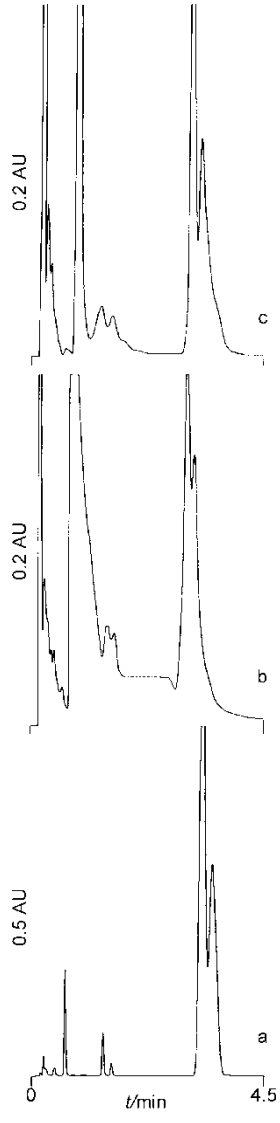

Figure 4. Chromatograms of β-cyfluthrin from: standard solution (a), pesticide formulation Responsar 025 SC (b), and Bulldock 025 EC (c), eluted on a high speed Pecospher 3×3 C_{18} column, mobile phase acetonitrile/water (70/30, v/v), flow rate 1.0 mL/min, column temperature 30°C, and UV detection at 220 nm (Method II).

beta-cyfluthrin, in contrast to the HS Pecospher 3×3 C_{18} column (Method II) where the obtained values for the capacity factors (k') were 10.14 and 10.80. The dead time for this column was 0.32 min. The calculated value for the separation factor between adjacent peaks (diastereomer II and IV) was 1.06. Thus,

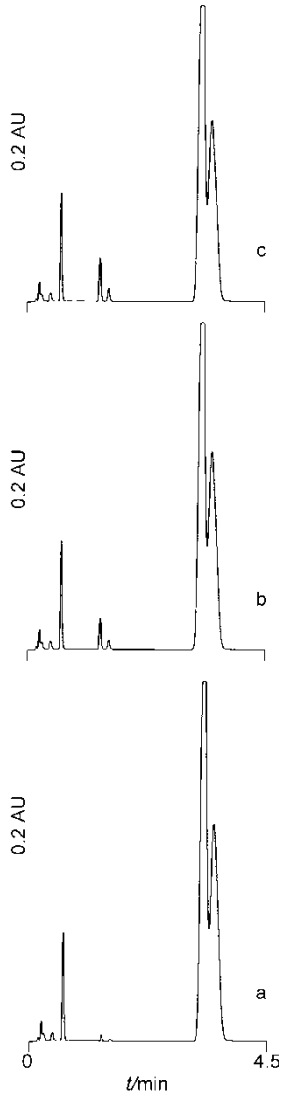

Figure 5. Chromatograms of β-cyfluthrin standard solution obtained during the first day of preparation (a), after seven days (b), and after fourteen days (c), on high speed Pecospher 3 × 3 C_{18} column, mobile phase acetonitrile/water (70/30, v/v), flow rate 1.0 mL/min, column temperature 30°C, and UV detection at 220 nm (Method II).

the high efficiency of separation and reduced time of analysis consumption[13] made the HS Pecospher 3 × 3 Silica column (Method I) for HPLC analysis of β-cyfluthrin more suitable than the HS Pecospher 3 × 3 C_{18} column (Method II).

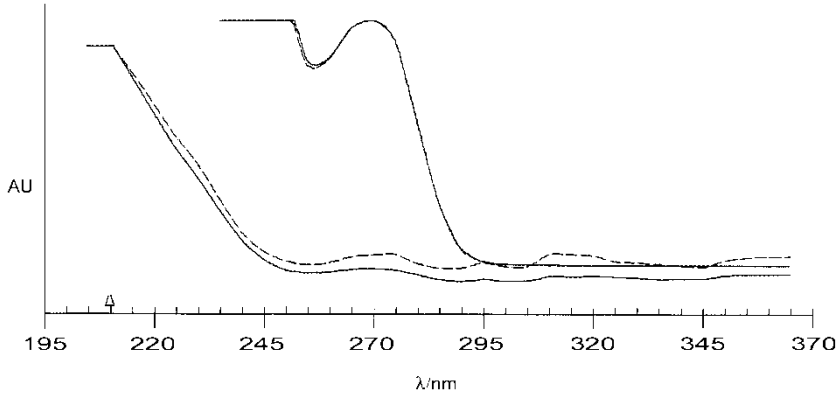

Figure 6. Overlay spectra of the four diastereomers of β-cyfluthrin (Index purity = 1.1).

The day to day and within day repeatability[14,15] of results was evaluated from obtained values for retention times and peak areas of β-cyfluthrin diastereomers (II and IV), after eight successive injections of analytical standard of β-cyfluthrin, with concentrations of 1.244 μg/μL for HS Pecospher Silica (Method I) and 0.580 μg/μL for HS Pecospher C_{18} column (Method II). The results were tested with an ANOVA test. Excellent repeatability of all tested parameters was achieved using the conditions described under Method I (Tables 1 and 2). The attempts of determination of β-cyfluthrin using HS Pecospher 3 × 3 C_{18} column (Method II) were not successful enough for both diastereomers, due to the problems with irreproducibility of the retention times (diastereomer II), and peaks area (diastereomer II and diastereomer IV).

Calibration graphs were constructed by plotting the injected amount of the standard of active ingredient as a function of the peak area and height. The peak areas and heights were used as dependent variables and their values were treated with the OMEGA statistical program[12] using external standard multilevel calibrations by linear fit. The results for statistical estimation for

Table 1. Statistical data for intra and inter day precisions of retention times/min for HS Pecospher 3 × 3 Silica column (Method I)

				Within day ($P = 0.05$)		Between day ($P = 0.05$)	
Diastereomer	Mean	SD	RSD (%)	$F_{2,14}$	$F_{2,14\ (crit.)}$	$F_{7,14}$	$F_{7,14\ (crit.)}$
II	2.65	0.024	0.90	0.652	3.739	1.464	2.764
IV	3.60	0.041	1.13	0.108		2.746	

Table 2. Statistical data for intra and inter day precisions of areas for HS Pecospher 3 × 3 Silica column (Method I)

Diastereomer	Mean	SD	RSD (%)	Within day ($P = 0.05$)		Between day ($P = 0.05$)	
				$F_{2,14}$	$F_{2,14\ (crit.)}$	$F_{7,14}$	$F_{7.14\ (crit.)}$
II	11555248	99781.6	0.863	2.647	3.739	0.918	2.764
IV	18802115	166084.1	0.883	2.222		1.061	

Method I are presented in Table 3. It is evident that the obtained results for multiple correlation coefficients indicated, preferably, the use of peak area as a variable. Method I with the HS Pecospher 3 × 3 Silica column showed good linearity, because the obtained values for multiple correlation coefficient were 0.9998 and 0.9995 for the diastereomer II and IV, respectively.

The sensitivity of the method for both diastereomers of β-cyfluthrin was determined by construction of a calibration curve in the low concentration region (0.018–0.149 μg/μL for Method I, and 0.011–0.045 μg/μL for Method II). The limit of detection (LOD) was calculated as three times the ratio between the SD and the slope of the low concentration curve ($LOD = 3 \cdot SD/Slope$), and the limit of quantification (LOQ) as ten times the same ratio ($LOQ = 10 \cdot SD/Slope$).[16] When the HS Pecospher Silica column was used (Method I), the obtained results showed that LOD for diastereomers II and IV is 10.03 μg or concentration of 0.501 μg/μL, and LOQ for both diastereomers is 3.00 μg (0.15 μg/μL), while in the case of the HS C_{18} column (Method II), the LOD is 3.22 μg (0.161 μg/μL) and the LOQ is 0.966 μg (0.048 μg/μL) for both diastereomers.

The accuracy was determined by the method of standard addition.[17] According to this method, known amounts of analyte are spiked at three different levels (20, 40, and 80%) into a sample matrix that already contains some quantity of the analyte. The percentage of recovered analyte at three

Table 3. Statistical evaluations for calibration curves for HS Pecospher 3 × 3 Silica column (Method I)

Diastereomer	Regression equation	Multiple correlation coefficient
Area		
II	$y = 478561x + 23824$	0.9958
IV	$y = 775305x + 4820.8$	0.9822
Height		
II	$y = 51.164x - 12.471$	0.9810
IV	$y = 56.058x + 1.0482$	0.9885

concentration levels with the method of HS Pecospher 3 × 3 Silica column (Method I), were 99.61, 98.7, and 100.86% for the diastereomer II and 99.72, 102.23, and 103.68% for the diastereomer IV, with relative standard deviation (RSD) of 1.68% (n = 9). The values of β-cyfluthrin diastereomers recoveries for the Method II with HS Pecospher 3 × 3 C_{18} column, varied from 94.95 to 104.69% with RSD = 3.25%.

The active substance quantity in pesticide formulations Responsar 025 SC and Bulldock 025 EC has the mean value equal to 2.52% (sum of both diastereomers) with RSD = 1.68 % (n = 24) (Method I). The determined content corresponds to the value (2.5%) declared by the manufacturer.

CONCLUSIONS

This study shows the possibility for determination of β-cyfluthrin in pesticide formulation Responsar 025 SC and Bulldock 025 EC, by the normal phase HPLC using Pecospher 3 × 3 Silica column (Method I) and with reversed phase HPLC with HS Pecosphere 3 × 3 C_{18} column (Method II). Validation of the methods in selected conditions shows that the method with HS Pecospher 3×3 Silica column (Method I) is more selective and precise with linear response of the detector for both pairs (II and IV) of enantiomers, than the method with HS Pecospher 3 × 3 C_{18} column (Method II). The tested NP HPLC method, showed a high value of multiple correlation coefficient and reproducibility of retention time and peak area. The high speed method with HS Pecospher 3×3 Silica column (Method I) is simple, fast, economical, and precise enough for routine analysis of β-cyfluthrin in the pesticide formulations Responsar 025 SC and Bulldock 025 EC.

REFERENCES

1. Europian Commission, Health & Consumer Protection Directorate-General, Beta-cyfluthrin. 2002.
2. Naumann, K. *Synthetic Pyrethroid Insecticides: Structure and Properties*; Springer—Verlag: Berlin, 1990.
3. CIPAC method, Handbook H. 1998; 43–51.
4. Harbin, N.D. Quantitation of cyfluthrin in liquid and solid formulations by reversed phase liquid chromatography: Collaborative study. J. AOAC Int'l. **1995**, *78* (6), 1335–1338.
5. Liu, W.; Gan, J.J. Separation and analysis of diastereomers and enantiomers of cypermethrin and cyfluthrin by gas chromatography. J. Agric. Food Chem. **2004**, *52*, 755–761.
6. Saikia, N.; Gopal, M. Biodegradation of β-cyfluthrin by Fungi. J. Agric. Food Chem. **2004**, *52*, 1220–1223.
7. Leicht, W.; Fuchs, R.; Londershausen, M. Stability and biological activity of cyfluthrin isomers. Pestic. Sci. **1996**, *48*, 325–332.

8. Liu, W.; Gan, J.J.; Qin, S. Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides. Chirality **2005**, *17*, S127–S133.
9. Lisseter, S.G.; Hambling, S.G. Chiral high-performance liquid chromatography of synthetic pyrethroids insecticides. J. Chromatogr. **1991**, *539*, 207–210.
10. Edwards, D.P.; Ford, M.G. Separation and analysis of the diastereomers and enantiomers of cypermethrin and related compounds. J. Chromatogr. **1997**, *777*, 363–369.
11. OMEGA, Perkin-Elmer Corp. Norwalk, USA, 1989.
12. FAO specifications and evaluations for plant protection products. Beta-cyfluthrin. Evaluation report 482, 1999.
13. Jenke, D.R. Chromatographic method validation: A review of current practices and procedures. I. general concepts and guidelines. J. Liq. Chromatogr. & Rel. Technol. **1996**, *19* (5), 737–757.
14. Meyer, V.R. *Practical High-Performance Liquid Chromatography*; 2nd Ed.; John Wiley & Sons, Ltd.: Chichester, 1994; 259–260.
15. Lough, W.J.; Wainer, I.W. *High Performance Liquid Chromatography Fundamental Principles and Practice*; Blackie Academic & Professional, Chapman & Hall: Glasgow, UK, 1990; 156–163.
16. Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*; 2nd Ed.; Ellis Horwood Ptr Prentice Hall, 1993; 101–141.
17. Snyder, R.L.; Kirkland, J.J.; Glajch, L.J. *Practical HPLC Method Development*, 2nd Ed.; John Wiley & Sons, Inc.: New York, 1997; 688–689.

Received August 16, 2005
Accepted November 8, 2005
Manuscript 6706