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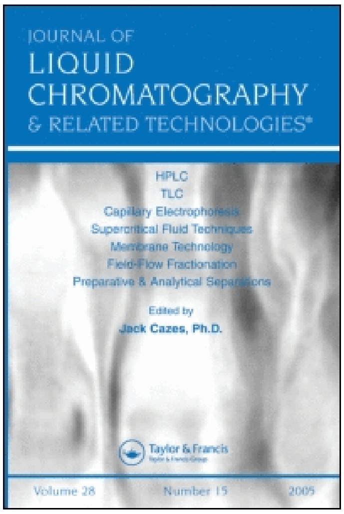

Journal of Liquid Chromatography & Related Technologies

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

Separation of Cypermethrin Diastereomers by Normal Phase Liquid Chromatography

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To cite this Article Hegyi, Cs. , Olah, E. , Fekete, J. and Szainik, G.(2006) 'Separation of Cypermethrin Diastereomers by Normal Phase Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 29: 19, 2835 — 2851
To link to this Article: DOI: 10.1080/10826070600959401
URL: http://dx.doi.org/10.1080/10826070600959401

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Journal of Liquid Chromatography & Related Technologies®, 29: 2835–2851, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600959401

Separation of Cypermethrin Diastereomers by Normal Phase Liquid Chromatography

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Abstract: Insecticides cause great environmental problems. We studied the separation of cypermethrin diastereomers. Cypermethrin has three asymmetric carbon atoms; it has four diastereomers. Research with reversed phase high performance liquid chromatography was unsuccessful. For the separation, we applied normal phase high performance liquid chromatography. In a systematic study, the best column was selected and the mobile phase was optimized. When we used acetonitrile as a polar modifier in the mobile phase, we found that the behaviour of acetonitrile is different in the separation of the four diastereomers from the other modifiers.

Keywords: Diastereomers, Cypermethrin, NPLC, Organic modifiers, Acetonitrile

INTRODUCTION

Pesticides are used routinely, both indoors and outdoors. The general trend is to ban the more dangerous ones. This is the reason why the use of pyrethroids[1] has increased worldwide. Toxicity for mammals is low, but excessive application will cause environmental problems. From this point of view, there is a general need to analyze them in water and in soil. In the past, the main focus was on determination in crops or in foodstuffs.[2–5]

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Either gas chromatography or liquid chromatography were used. In these cases, the total residue concentration was the main interest. All pyrethroids have asymmetric carbon atoms and are sold, in practice, mainly as diastereometric mixtures. Both for identification of products, and for study of fate in the environment, separation of diastereomers represents a basic need.

Pyrethroids are the most general compounds of available insecticides. American scientists found that they kill aquatic species and small species in water absorb them. Weston[6] detected high concentrations of pyrethroids in creeks passing through agricultural areas. In a new study, they examined samples from Roseville. In half of the samples, pyrethroids were at toxic levels.[7]

Cypermethrin is a synthetic pyrethroid with three asymmetric centers and, therefore, four diastereomers (Figure 1). In practice, the most toxic isomer is 1R-cis in the cyclopropane ring and the 1S configuration of the α-cyano group in the alcoholic part of permethrenic acid. Today, this product is used as a diastereomeric mixture.

Cypermethrin and other pyrethroids are now being used throughout the world in household applications and in agriculture, forestry and other fields (Figs. 2–4). They undergo rapid biotransformation and excretion by the mammalian catabolic system.[8–10] However, high toxicity to bees and fish has been recognized.[10] Due to the broad range of their application, appreciable levels of their residues may occur in food produced from crops, foods of animal origin, and in soils, sediments, and surface water. Most of the analytical efforts were focused on this field. GC, with electron capture detection,[2–4] and flame ionization detection were applied for residue analysis.[5]

Only a few publications deal with separation of diastereomers by HPLC[11,12] Edwards[13] baseline separated of the 4 diastereomers using a Partisil column and various organic modifiers, but only a little information was given about that work.

Rivas[14] examined microencapsulated pesticides such as cypermethrin. In their process, small drops of cypermethrin were surrounded with polyurea or polyamides. Toxic effects of pesticides on mammals and humans were reduced by this technology. A normal phase liquid chromatographic method is used as a reference to identify the cis and trans isomers from the peaks obtained by a reversed phase liquid chromatographic method. Spherisorb S5 CN 25 × 0.5 cm stationary phase was used, the mobile phase was 500 mL hexane mixed with 1.1 mL tetrahydrofuran. The scope of their study was the examination of recovery from microcapsules with reversed phase liquid chromatography. Rivas examined only the ratio of cis/trans isomers. Our aim is to study the possible use of both normal and reversed phase chromatography, to obtain detailed information of stationary effects in normal phase chromatography and optimization of the separation with respect to the mobile phase. Based on this study, the selection of a liquid chromatographic method will be easier for those who are working in the environmental field.

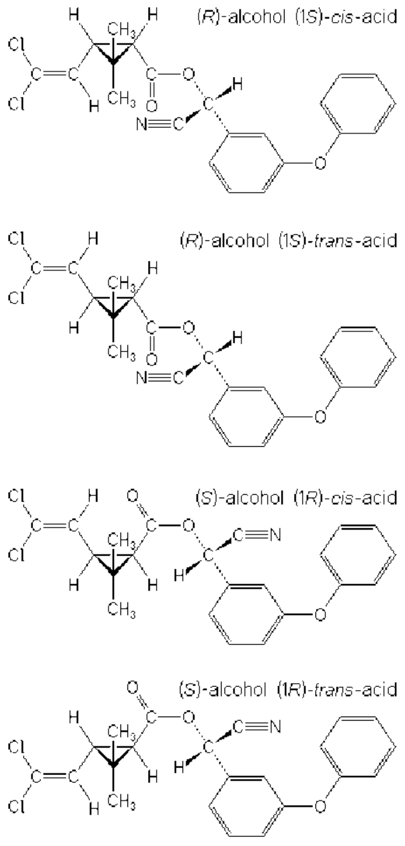

Figure 1. Structures of pyrethroid pesticides: cypermethrine. (R)-alcohol (1S)-cis-acid or (R)-α-cyano-3-phenoxybenzyl (1S, 3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, short form used in further Cis-I; (R)-alcohol (1S)-trans-acid or (R)-α-cyano-3-phenoxybenzyl (1S, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate,short form used in further Trans-I; (S)-alcohol (1R)-cis-acid or (S)-α-cyano-3-phenoxybenzyl (1R, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate short form used in further Cis-II; (S)-alcohol (1R)-trans-acid or (S)-α-cyano-3-phenoxybenzyl (1R, 3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, short form used in further Trans-II.

EXPERIMENTAL

Chromatographic Systems

Normal Phase Chromatography

Pump: 6100 Hitachi (Merck, Darmstadt, Germany); sampler: Rheodyne 7125, equipped with 20 μL loop (Rheodyne Inc., Cotati, USA); detector: PE 715A

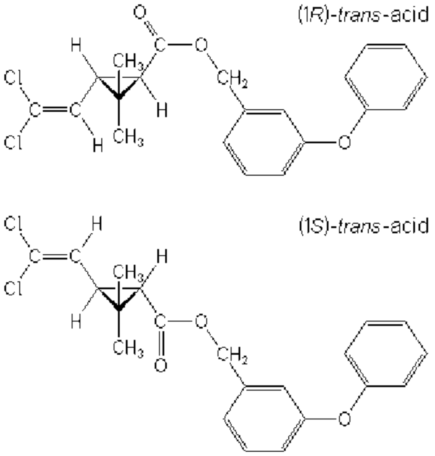

Figure 2. Structures of pyrethroid pesticides: permethrin.

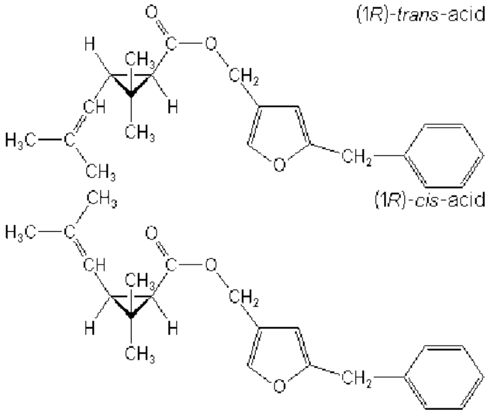

Figure 3. Structures of pyrethroid pesticides: resmethrin.

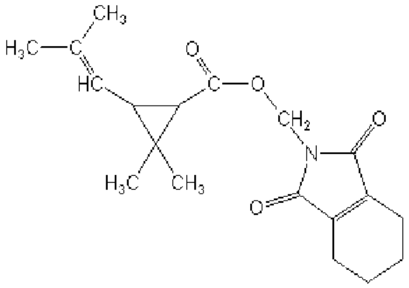

Figure 4. Structures of pyrethroid pesticides: tetramethrin.

UV/VIS detector (Perkin Elmer Corp., Norwalk, USA); data system: Turbo Chrom 4.1 (Perkin Elmer Corp., Norwalk, USA).

Reversed Phase Chromatography

Pump: Perkin Elmer 200 quaternary gradient pump; sampler: ISS 200 autosampler; detector: 235C diode array detector; data system: Turbo Chrom NT version (Perkin Elmer Corp., Norwalk, USA).

Stationary Phases used and Parameters (see Tables 1 and 2).

Micropak Si-10, (30 cm × 4 mm, Varian), Spherisorb Alumina (5 μm, 250 × 4 mm, Waters), Aluspher Al-100, (5 μm, 250 × 4 mm, Merck), NovaPak Silica (3.9 × 150 mm, Waters), LiChrosorb CN (5 μm, 250 × 4 mm, Merck), Purospher Star Si (5 μm, 250 × 4 mm, Merck), NovaPak C_{18} (5 μm, 3.9 × 150 mm, Waters), Purospher RP18e (5 μm, 125 × 3 mm, Merck), LiChrospher 100 RP-18 (5 μm, 125 × 4 mm, Merck),

Table 1. List and parameters of used RP-stationary phases

	NovaPak C18 (Waters)	Purospher RP18e (Merck)	LiChrospher 100 RP18 (Merck)	LiChrosorb RP Select B (Merck)	Chromolith RP18e (Merck)
Particle size [μm]	4	5	5	5	n.a.
Pore size [A^0]	60	120	100	60	n.a.
Particle shape	Spherical	Spherical	Spherical	Irregular	Monolithic
Pore volume [ml/g]	0.3	1	1,25	0,75	n.a.
Surface area [m^2/g]	120	350	350	300	n.a.
Coating [μmol/m^2]	n.a.	n.a.	3.61	4.21	n.a.
Theorethical plates [N/m]	n.a.	>80.000	55.000	55.000	>50.000
Carbon load [%]	7.3	18.0	21.0	11.4	n.a.
pH stability	n.a.	2-7.5	2-7.5	2-7.5	n.a.
Metal content [ppm]	n.a.	<5	<350	>350	<5
Endcapped	Yes	Yes	No	Yes	Yes

n.a.: not available.

Table 2. List and parameters of used NP-stationary phases

	NovaPak silica (Waters)	Purospher Star Si (Merck)	Micropak Si-10 (Varian)	Spherisorb alumina (Waters)	Aluspher Al-100 (Merck)	LiChrosorb CN (Merck)
Particle size [μm]	4	5	5	5	5	5
Pore size [A^0]	90	120	100	80	100	100
Particle shape	Spherical	Spherical	n.a.	Spherical	Spherical	irregular
Pore volume [ml/g]	0.3	1.1	n.a	n.a	n.a	1
Surface area [m^2/g]	120	330	300	200	n.a	300
Theorethical plates [N/m]	50000–60000	>50000	n.a	45000–50000	n.a	>25000
Metal contant [ppm]	120–150 ppm	Na, Ca, Mg, Al: 1 ppm Fe: 3 ppm	n.a	n.a	n.a	n.a

n.a. not available.

Table 3. Polarity of used solvents

Solvent	ε^{o}
Diisopropyl ether	0.28
Dichloromethane	0.39
1,4-Dioxane	0.56
Ethyl-acetate	0.62
Tert-buthyl-methyl-ether	0.62
Acetonitril	0.65
Isopropyl-alcohol	0.82

LiChrosorb RP Select B (5 μm, 250 × 4 mm, Merck), Chromolith RP18e (100 × 4.6 mm, Merck), C_{30}-as oszlop (6 μm, 250 × 4.6 mm)

For a list of solvents used and polarities according to Snyder,[15] see Table 3.

RESULTS AND DISCUSSION

In spite of using different types of alkyl modified silicas, baseline separation of four diastereomers of cypermethrin on the reversed phase columns was not achieved. The best results were achieved with Chromsil C_{30} (home-made stationary phase donated by Z. Szabó from the University of Pécs) (see Figure 5), but only the cis and trans isomers could be separated. The mobile phases did not contain buffers because there are no ionizable groups in the

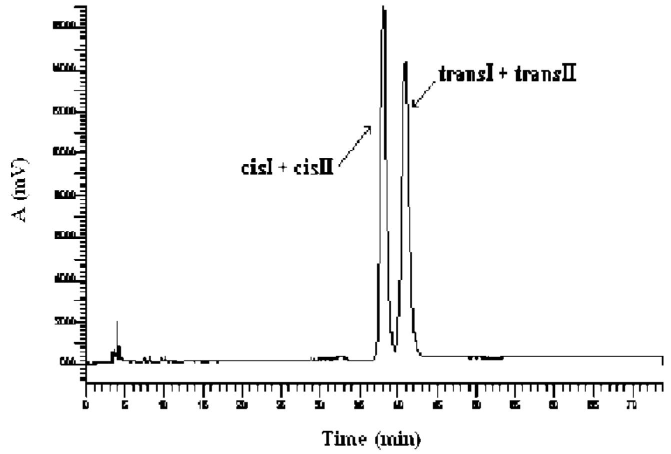

Figure 5. Separation of cypermethrine diastereomers on Chromsil C_{30} column with acetonitrile/water (70/30) mobile phase.

molecules. The change of the organic solvent to methanol did not affect the diastereomer selectivity. The final conclusion concerning the use of the reversed phase column was that the diastereomer selectivity for cypermethrin is not enough for the determination of either diastereomer composition or diastereomer purity.

The normal phase columns used can be divided into three different categories: Silica (LiChrosorb Si 60, Micropak Si-10, NovaPak Silica, Purospher Star Si), Alumina (Spherisorb Alumina, Aluspher Al-100) and polar bonded phase (LiChrosorb CN). Silica columns can be divided further according to their metal content. The high metal containing silicas (Silica A) are LiChrosorb Si 60, Micropak Si-10, NovaPak Silica; and low metal containing one (Silica B): Purospher Star Si. The silanol activity and polar character of silica depends on its metal content.

Polar bonded silica was chosen to investigate the effect of modification of silica surface. In our case, the bonded group was Si-(CH_2)-CN; the nitrile group has a high dipole moment and undergoes dipole-dipole interactions. In spite of this, the bonded nitrile phase couldn't separate any of the four isomers, even when using the weakest polar modifiers (diisopropyl ether and dichloromethane). The cypermethrin Cis-II and Trans-I cannot be resolved, or even partly resolved, with polar modifiers, except for 2.7 v/v% acetonitrile-n-hexane mobile phase (Figure 8). This acetonitrile concentration is near the solubility and probably the pores of the stationary phase are filled partly with it and a liquid-liquid distribution might occurring. This theory is backed up by adsorption isotherm of acetonitrile in n-hexane (Figure 12).[16] The solubility of acetonitrile in hexane is about 3.5 v/v% at 25°C (Table 4).[17] According to a pore filling model, the sorbed amount was about the same on two different silicas. The unique selectivity of acetonitrile near the solubility was demonstrated in other separations as well, for example, for separation of cannaboids.[18]

We have summarized the results in Table 5. From the table, it can be concluded that the separation of cypermethrin Cis-II and Trans-I diastereomers were the lowest; for silicas and aluminas close to baseline separation, and for nitrile phase was 0.75. The best separation can be achieved with the

Table 4. Acetonitrile content of n-hexane saturated with acetonitrile on different temperatures

Temperature (°C)	Concentration (mol %)	Concentration (v/v %)
20	8.99	3.82
25	9.20	3.92
30	9.41	4.01
35	9.63	4.10
40	9.84	4.20

Table 5. Resolution values for cypermethrin diastereomers in different conditions (stationary phases and polar modificators)

	Spherisorb alumina		Aluspher Al-100		LiChrosorb CN		NovaPak Silica		Micropak Si-10		Purospher Star Si	
Acetonitril	%	3.0	%	3.0	%	2.7	%	2.7	%	2.7	%	2.7
	Rs(1–2)	2.20	Rs(1–2)	2.89	Rs(1–2)	2.02	Rs(1–2)	3.47	Rs(1–2)	2.45	Rs(1–2)	3.21
	Rs(2–3)	1.39	Rs(2–3)	1.83	Rs(2–3)	0.75	Rs(2–3)	1.56	Rs(2–3)	1.31	Rs(2–3)	1.48
	Rs(3–4)	1.53	Rs(3–4)	2.35	Rs(3–4)	1.49	Rs(3–4)	2.70	Rs(3–4)	1.71	Rs(3–4)	2.43
Dichloromethane	%	20	%	20	%	10	%	20	%	20	%	10
	Rs(1–2)	2.42	Rs(1–2)	2.91	Rs(1–2)	1.94	Rs(1–2)	1.56	Rs(1–2)	0.54	Rs(1–2)	1.99
	Rs(2–3)	2.55	Rs(2–3)	2.64	Rs(2–3)	<1.5	Rs(2–3)	4.35	Rs(2–3)	4.97	Rs(2–3)	7.25
	Rs(3–4)	1.74	Rs(3–4)	2.78	Rs(3–4)	1.33	Rs(3–4)	1.83	Rs(3–4)	1.20	Rs(3–4)	2.66
Diisoprophyl-ether	%	4.0	%	5.0	%	1.0	%	4.0	%	2.5	%	8.0
	Rs(1–2)	4.92	Rs(1–2)	5.09	Rs(1–2)	2.41	Rs(1–2)	2.49	Rs(1–2)	1.75	Rs(1–2)	2.67
	Rs(2–3)	3.68	Rs(2–3)	5.57	Rs(2–3)	<1.5	Rs(2–3)	2.23	Rs(2–3)	2.59	Rs(2–3)	5.00
	Rs(3–4)	3.24	Rs(3–4)	3.82	Rs(3–4)	2.46	Rs(3–4)	2.40	Rs(3–4)	1.85	Rs(3–4)	3.11
1.4-Dioxane	%	1.25	%	5.0	%	0.625	%	0.625	%	0.625	%	1.25
	Rs(1–2)	2.75	Rs(1–2)	3.43	Rs(1–2)	3.01	Rs(1–2)	2.67	Rs(1–2)	2.09	Rs(1–2)	2.84
	Rs(2–3)	2.45	Rs(2–3)	1.57	Rs(2–3)	0.71	Rs(2–3)	1.31	Rs(2–3)	1.66	Rs(2–3)	1.88
	Rs(3–4)	3.17	Rs(3–4)	4.13	Rs(3–4)	2.80	Rs(3–4)	2.44	Rs(3–4)	2.01	Rs(3–4)	2.70
Ethyl-acetate	%	1.0	%	2.50	%	0.50	%	0.50	%	0.50	%	0.50
	Rs(1–2)	4.11	Rs(1–2)	3.39	Rs(1–2)	2.70	Rs(1–2)	3.31	Rs(1–2)	2.13	Rs(1–2)	3.19
	Rs(2–3)	3.15	Rs(2–3)	2.71	Rs(2–3)	<1.5	Rs(2–3)	3.43	Rs(2–3)	3.54	Rs(2–3)	6.60
	Rs(3–4)	2.95	Rs(3–4)	2.90	Rs(3–4)	2.55	Rs(3–4)	2.84	Rs(3–4)	2.08	Rs(3–4)	3.18

(*continued*)

Table 5. Continued

	Spherisorb alumina		Aluspher Al-100		LiChrosorb CN		NovaPak Silica		Micropak Si-10		Purospher Star Si	
Tert-buthyl methyl ether	%	5.0	%	5.0	%	1.25	%	2.50	%	2.50	%	2.50
	Rs(1–2)	2.98	Rs(1–2)	4.22	Rs(1–2)	2.44	Rs(1–2)	2.45	Rs(1–2)	1.95	Rs(1–2)	2.79
	Rs(2–3)	2.54	Rs(2–3)	4.74	Rs(2–3)	<1.5	Rs(2–3)	1.61	Rs(2–3)	1.82	Rs(2–3)	3.24
	Rs(3–4)	2.70	Rs(3–4)	3.81	Rs(3–4)	2.44	Rs(3–4)	2.24	Rs(3–4)	1.93	Rs(3–4)	3.09
Isopropyl-alcool	%	0.05	%	0.05	%	0.05	%	0.025	%	0.05	%	0.05
	Rs(1–2)	3.55	Rs(1–2)	<1.5	Rs(1–2)	2.64	Rs(1–2)	2.42	Rs(1–2)	<1.5	Rs(1–2)	<1.5
	Rs(2–3)	3.54	Rs(2–3)	<1.5	Rs(2–3)	<1.5	Rs(2–3)	3.69	Rs(2–3)	<1.5	Rs(2–3)	<1.5
	Rs(3–4)	2.68	Rs(3–4)	<1.5	Rs(3–4)	2.39	Rs(3–4)	2.49	Rs(3–4)	<1.5	Rs(3–4)	<1.5

Aluspher Alumina phase. There were other indications for changing the retention mechanism. The pyrethroid based pesticides have different polarities. The more polar tetramethrine diastereomers' retentions were reduced dramatically and retention order of diastereomers was reversed (Figure 9c–d). The retention order followed the n-octanol-water distribution values. The two logP values are: 6.09 for cypermethrin and 4.53 for tetramethrin. The retention order and selectivity achieved by acetonitrile as polar modifier compared with results with other polar modificators, the difference showed that the mechanism of separation is now not adsorption onto the surface of the stationary phases, but might be a distribution between the mobile phase and acetonitrile within the pores and retention order follows the polarities of the pyrethroids. The separation can be seen in Figure 9c and tetramethrine can be eluted without use of a gradient.

Using dichloromethane as a modifier, the cis and trans isomers elute close to each other and selectivity between cypermethrin cis-II and trans I is relatively high. Dichloromethane is a non-localized solvent.[19] The data can be seen in Table 5.

In the Aluspher Alumina column, the four isomers were distributed evenly in the chromatogram and the separation time was acceptable at 20 v/v% dichloromethane, and also, other pyrethroids can be separated. Tetramethrine was eluted by gradient elution (Figure 9d).

Using diisopropylether as a polar modifier, retention of cypermethrin on alumina columns was higher, compared to the Silica A column, but on Silica B (PurospherStar) it was higher and selectivity was also better. It was possible to reduce the analysis time by increasing the amount of diisopropyl ether in n-hexane (8 v/v%). The four peaks were distributed evenly in the chromatogram (Figure 11b).

In the dioxane-n-hexane mobile phase, the separation of cis-I and cis-II and trans-I and trans-II was high but cis-II and trans-I were eluted close to each other. Only the Aluspher column gave acceptable retention and resolution at 1.25 v/v% dioxane.

The PurosherStar column is a Silica B; the metal content is low and silanol group activity is lower, compared to Silica A stationary phases (LiChrosorb Si 60, Micropak Si-10, NovaPak Silica). Separation between Cis-II and Trans I is lower than between Cis–I and Cis-II, and Trans-I and Trans-II, indicating partly similar retention as if using dichloromethane as modifier.

According to the elutropic series (Snyder[19]), the next tested eluent was ethyl acetate ($\varepsilon^o \sim 0.62$)-n-hexane mixture. At 1 v/v% ethyl acetate/n-hexane mixture, the peaks were evenly distributed. For the NovaPak Si column, the ethyl acetate concentration must be reduced to 0.5 v/v% to obtain a resolution higher than 2.

Tert-butyl methyl ether (TBME) has the same ε^o value as ethyl acetate. The separation factors and peak symmetry were good for Aluspher Alumina (5 v/v% TBME-n-hexane) and Purospher Star Si column (2.5 v/v% TBME-n-hexane). The retention times of cis-II were 8.9 and 6.29 min

Aluspher and Purospher columns, respectively. The retentivity of Spherisorb Alumina column at this condition is higher when compared to silica.

Isopropanol is a strong hydrogen bonding polar modifier. To realize reasonable retention, the concentration in the eluent must be decreased to 0.05 v/v% or below. Even at this low polar organic concentration, baseline separation was not achieved on PurospherStar Si, Aluspher Alumina, LiChrosorb Si 60, Micropak Si − 1, and LiChrosorb CN. Baseline separation was achieved on NovaPak Si (Figure 6c) and Spherisorb Alumina (Figure 7c), but the retention factors (k > 10) and analysis times were high.

In Figures 6, 7, 8, 9, 10, and 11, separation of the four diastereomers in various stationary phases can be seen.

CONCLUSIONS

Separation of cypermethin isomers can be performed by normal phase chromatography with various types of stationary phases. The separation of four diasteremers of cypermethrin on the tested reversed phase columns was unsuccessful. The RP columns can be divided into separate categories. The LiChrospher RP-18, so called Silica A, is not end-capped; LiChrosorb RP Select B is a specially treated material for the separation of basic solutes, Purospher RP-18e is prepared from low metal content silica (Silica B) and end-capped. Chromolith RP-18e is a monolithic column. Using the above listed columns, in all cases, the separation time was about 90 minutes. This is unacceptable; we can state that reversed phase chromatography is not a choice for separation of cypermetrin diastereomers.

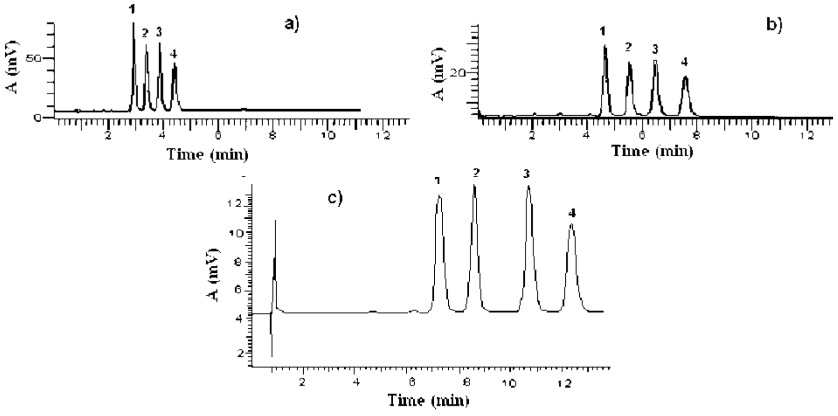

Figure 6. Separation of cypermethrine diastereomers (1: cis-I; 2: cis-II; 3: trans-I; 4: trans-II) on NovaPak Silica column with various mobile phases. a) 4 v/v % diisopropylether; b) 2.5 v/v % tertbuthylmethylether; c) 0.025 v/v % isopropanol.

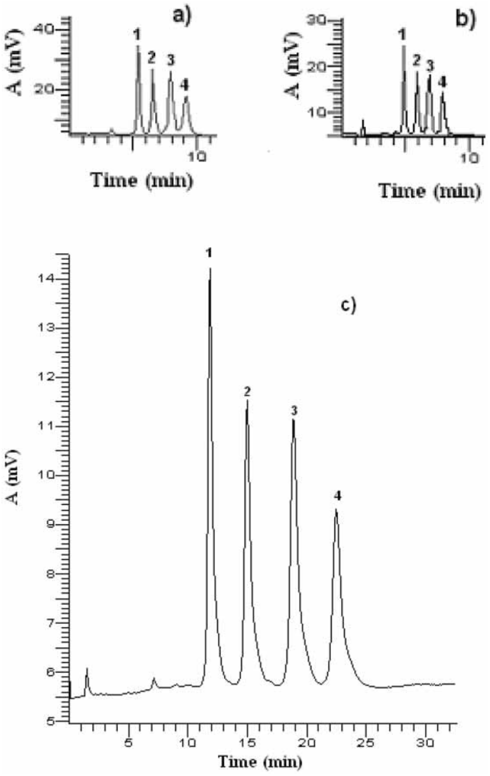

Figure 7. Separation of cypermethrine diastereomers (1: cis-I; 2: cis-II; 3: trans-I; 4: trans-II) on Spherisorb Alumina column with various mobile phases. a) 20 v/v % dichloromethane; b) 5 v/v % tertbuthylmethylether; c) 0.05 v/v % isopropanol.

Separation of diastereomers of cypermethrin can be achieved in normal phase chromatography with various stationary phases and mobile phases. Based on our experiments, the alumina columns give good resolution and acceptable analysis times. The separation efficiency of Aluspher was good,

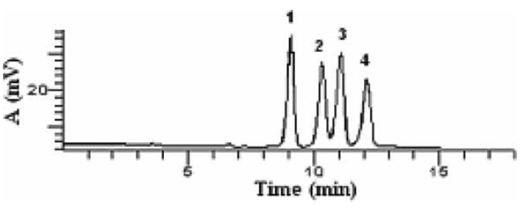

Figure 8. Separation of cypermethrine diastereomers (1: cis-I; 2: cis-II; 3: trans-I; 4: trans-II) on LiChrosorb CN column with 2.7 v/v % ACN.

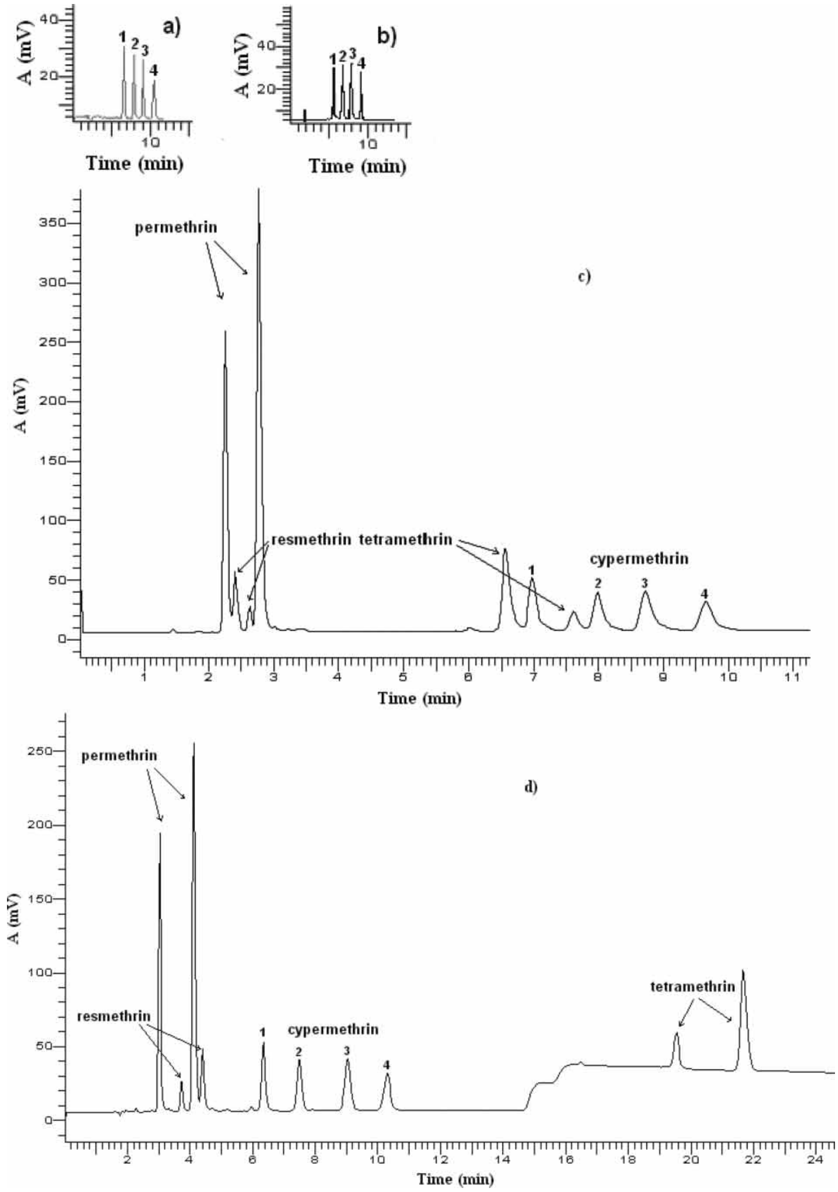

Figure 9. Separation of pyrethroid diastereomers (cypermethrine: 1 = cis-I; 2 = cis-II; 3 = trans-I; 4 = trans-II) on Aluspher alumina column with various mobile phases. a) 2.5 v/v % ethylacetate; b) 5 v/v % tertbuthylmethylether; c) 3 v/v % acetonitrile; d) 20 v/v % dichloromethane (from 11 min: 40 v/v % dichloromethane).

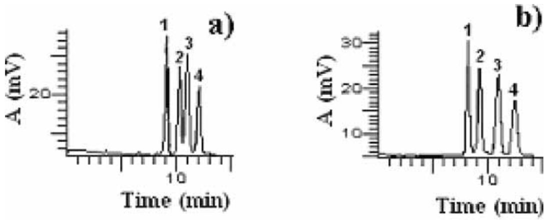

Figure 10. Separation of cypermethrine diastereomers (1: cis-I; 2: cis-II; 3: trans-I; 4: trans-II) on silica column with various mobile phases. a) 2.7 v/v % acetonitrile; b) 4 v/v % diisopropilether.

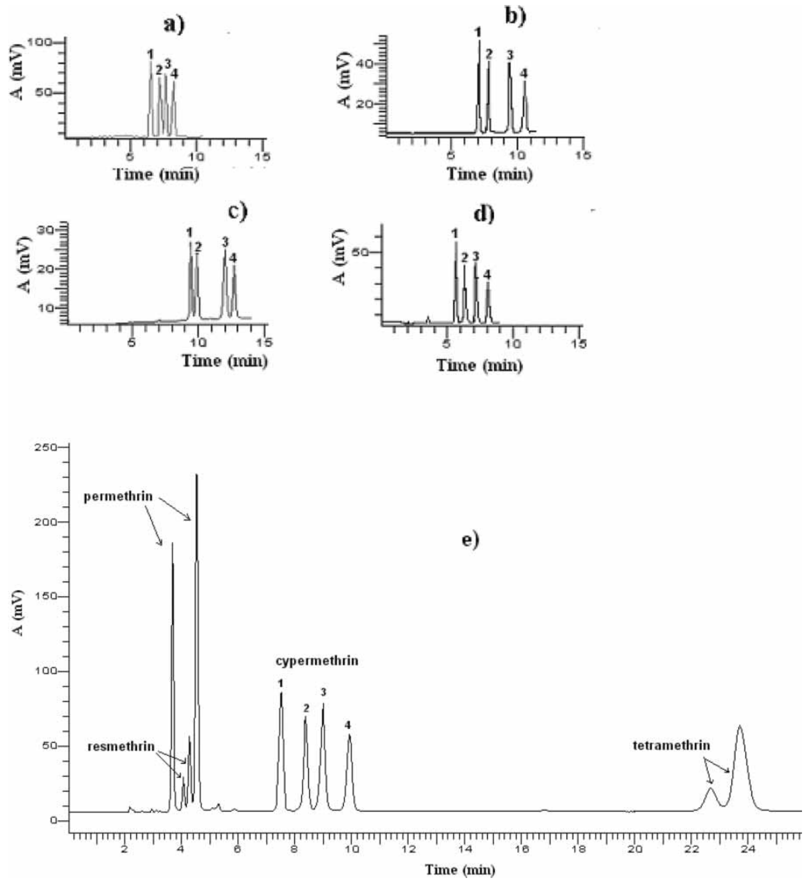

Figure 11. Separation of cypermethrine diastereomers (1: cis-I; 2: cis-II; 3: trans-I; 4: trans-II) on Purospher star column with various mobile phases. a) 2.7 v/v % acetonitrile; b) 8 v/v % diisopropilether; c) 20 v/v % dichloromethane; d) 2.5 v/v % tert-buthylmethylether; e) 1.25 v/v % dioxane.

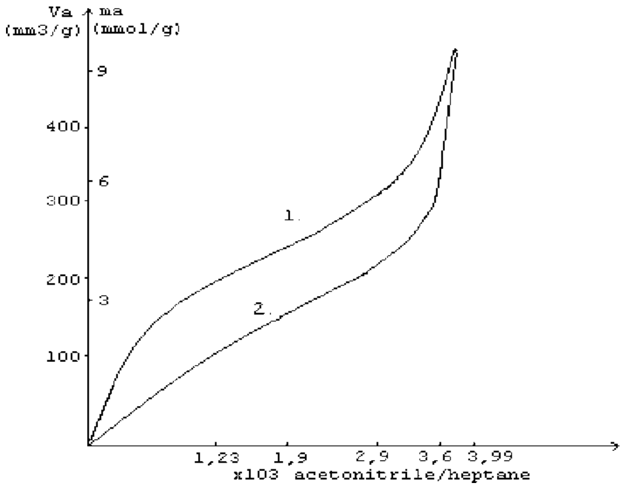

Figure 12. Adsorption isotherm of acetonitrile in n-hexane on silicas partisil 10 (1); and LiChrospher 100 Å (2).

except for isopropanol. In most cases, PurospherStar gave good selectivity and separation.

The polar bonded phase (LiChrosorb CN) can be used only with acetonitrile– n-hexane mobile phase to get acceptable resolution. The content of acetonitrile in the n-hexane is near the solubility limit and the pores of the nitrile phase are filled with acetonitrile and the separation mechanism is based on liquid-liquid distribution. An advantage of this chromatographic system is that the different pyrethroid pesticides can be separated without gradient elution. The liquid-liquid distribution was confirmed by different retention order to liquid solid or adsorption chromatography. The polar diastereomers of tethramethrine were eluted before the Cis-I and after it. These compounds, in other cases, could be eluted only with gradient elution and the retention times were higher than the late eluting trans-II isomer. Variations of the retention factors on different stationary phases are small caused by different pore volumes in the column. This points back to the hypothesis of liquid-liquid distribution. The peak shape on the nitrile column was better than on alumina and on silica. This indicates that the interaction with the surface of the stationary phase is weak or negligible.

ACKNOWLEDGMENT

This paper, in part, appeared in the Balaton Symposium 2005. Sept. 7–9, 2005.

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Received April 14, 2006
Accepted May 3, 2006
Manuscript 6886