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Enantiomeric resolution of pyrethroids by high-performance liquid chromatography with diode-laser polarimetric detection

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Abstract

Liquid chromatographic separation of the pyrethroid insecticides, bifenthrin and fenpropathrin, has been investigated with various achiral and chiral stationary phases. The identification of enantiomers of fenpropathrin and bifenthrin has been carried out by using two detectors in high-performance liquid chromatography: a UV spectrometer and a diode-laser polarimeter. Optimization of the mobile phase has been performed dealing regard to analyte resolution and polarimetric signal values.

Keywords: Enantiomer separation; Pyrethroids; Pesticides; Bifenthrin; Fenpropathrin

1. Introduction

High-performance liquid chromatographic (HPLC) coupled to spectrophotometric UV detection is widely used for the separation, identification and quantification of the diastereoisomers, such as those found in the series of pyrethroids [1]. However, this technique is not appropriate to determine the absolute configuration when the enantiomeric forms are present; the separation of the latter requires the use of a chiral chromatographic selector [2,3]. Another way to identify enantiomers is by using the combination of a photometric detector with a polarimetric detector, both coupled to the HPLC system. An optical activity based detector can give information on the elution order of the antipodes which could be very useful in understanding the recognition mechanism of the chiral stationary phase. For peak confirmation a diode array detector was used and for information on the chirality of the eluates, polarimetric or circular dichroism detectors would be ideal. Unfortunately, these detectors are costly and have limited sensitivity.

The development of laser-based polarimeters with optical rotation sensitivity of microdegrees [4–6] has provided chromatographers with a powerful technique for studying chiral molecules. As polarimetry is a specific detection technique for optically active species it is particularly suited for studies of chiral discrimination and the determination of enantiomeric purity [4,6–8]. A laser based polarimeter operating at a single wavelength is in fact a universal detector for chiral molecules, since optical rotation is a dispersive property which can be probed at wavelengths remote from an optically active absorption band. Lamp-based chiroptical detectors have been covered by Mannschreck [9], so the aim of this article is to discuss developments following previous reviews and to focus on applications of laser-based polarimetry in high-performance liquid chromatography, flow-injection analysis and enzyme kinetics.

Pyrethrins and their structural analogues, pyrethroids (e.g., cypermethrin, deltametrhrin, fenpropathrin, tetramethrin, permethrin, etc) are of growing

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importance as insecticides in agriculture, forestry, horticulture, public health and household usage. The toxicity and metabolic detoxification of the active compounds in insects and in non-target organisms strongly depends on molecular shape as do all biological processes involving membranes, receptors or enzymes [10–12].

Pyrethroids are synthesized, tested, marketed and either used as a single, most active isomer or as isomeric mixtures containing two, four or eight different stereoisomers, depending on the number of chiral centers in the molecules and the synthesis route [13,14]. Thus chromatographic separations of the diastereoisomers and enantiomers into individual stereoisomers provide either compounds (via micropreparation) or a means for thorough examination of biological activity and enzymatic metabolism. Furthermore biotic or abiotic transformation yield products with either the identical reduced or increased number of asymmetric C atoms depending on the kind and the alteration site [15–17].

Fenpropathrin [(*RS*)-α cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropane carboxilate] is a synthetic pyrethroid. It is an optical compound that contains an asymmetric carbon atom with the configuration *R*αCN+*S*αCN (Fig. 1).

Bifenthrin [2-methylbiphenyl-3-ylmethyl-(Z)-(1*RS*)-*cis*-3-(2 chloro- 3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcicloprapanecarboxilate], a synthetic pyrethroid possessing asymmetric centers at C-1 and C-3 of cyclopropane ring, is a mixture of four stereoisomers which come in two diastereoisomers containing two diastereomer forms (Fig. 1).

Several methods have been proposed for the determination of fenpropathrin, by liquid and gas chromatography in formulations, fruit and vegetables without separation of the isomers [18–21]. The separation and identification of the isomers of fenpropathrin by liquid chromatography was made using a chiral phase column with photometric detection [22,23].

The bibliographic search indicates that no quantitative methods by liquid chromatography for the determination of bifenthrin have been published until now. A gas chromatographic–mass spectrometry method for the determination of bifenthrin in pumpkins [24] has been described.

This work has been designed to carry out the resolution of enantiomers of pyrethroids (bifenthrin and fenpropathrin) through liquid chromatography of high resolution with photometric and diode laser polarimetric detection.

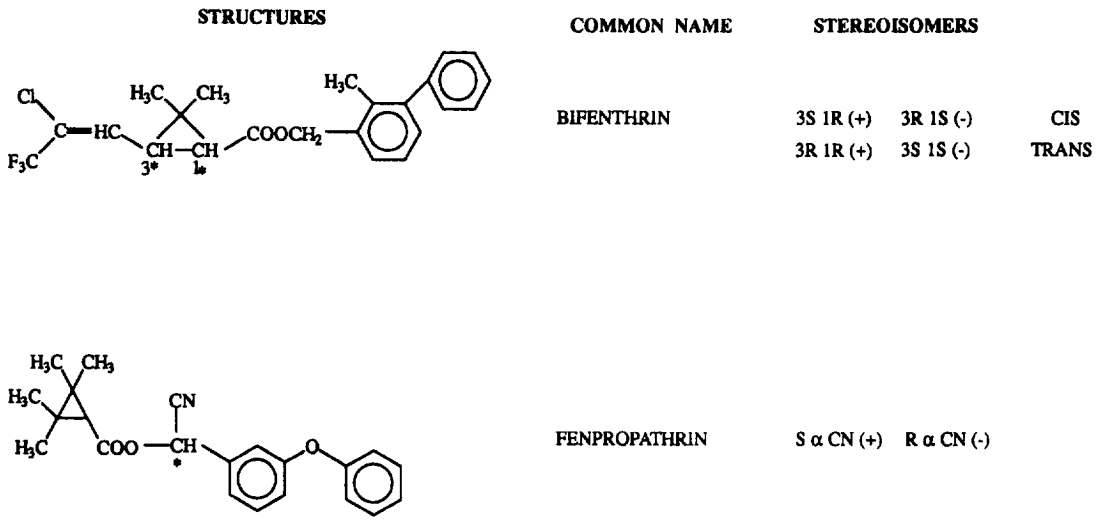

Fig. 1. Structures of the pyrethroids.

2. Experimental

2.1. Chemicals

Fenpropathrin was obtained from Dr. S. Ehrenstorfer (Augsburg, Germany) and bifenthrin from Riedel-de Haën (Hannover, Germany). Methanol, water, acetonitrile, 2-propanol, tetrahydrofuran, ethanol and hexane were gradient grade Lichrosolv Merck (Darmstadt, Germany).

Stock standard solutions of fenpropathrin and bifenthrin (5 g l^{-1}) were prepared by dissolving the compounds in ethanol and protecting them from light. Working standard solutions were prepared by dilution with ethanol. The solvents used as mobile phase were filtered through 0.2 μm nylon membrane filters and degassed.

2.2. Liquid chromatography

The measurements were performed with a Merck–Hitachi (Darmstadt, Germany) Liquid Chromatograph consisting of a L-6200 pump, an AS-4000 autosampler, a L-4250 UV-visible detector and a D-6000 interface. Integration was carried out using a PC/AT computer and instrumental parameters were controlled by Hitachi–Merck HM software.

A ChiralMonitor 2000 optical rotation detector (Applied Chromatography System Limited, Macclesfield, UK) placed in series with and after the UV-visible detector, was equipped with a collimated laser diode and provided up to 30 mW of light at 830 nm, a flow cell of 0.48 dm path length, 73 μl volume. The polarimetric detection system has been described in detail elsewhere [4]. As analogic output of ChiralMonitor 2000 was not associated with software managing data acquisition and processing, we implemented this complementary instrumentation. Data acquisition and transformation were accomplished by the Pico ADC-100 (Picotechnology, Cambridge, UK) which is an analog to digital converter with two input ranges. The instrumental parameters were controlled by Picolog Software (Picotechnology) and the calculation of the area (negative and positive peaks), the peak height and retention time were performed with Lab-Cal LC software (Galactic, Salem, NH, USA). The picolog program has been described in detail elsewhere [25].

The columns utilized are a Lichrospher RP18 (25 cm×4 mm I.D.; 10 μm particle size), a Lichrospher Si 60 (25 cm×4 mm I.D.; 10 μm particle size) and a Chriraspher (25 cm×4 mm I.D.; 5 μm particle size).

2.3. LC operating conditions

Fenpropathrin and bifenthrin are analyzed using a Chiraspher column (25 cm×4 mm I.D.; 5 μm particle size) from Merck (Darmstadt, Germany). The mobile phase composition was hexane–ethanol (99.5:0.5 v/v) at 1 ml min^{-1} flow-rate and photometric ($\lambda_{absorbance}$=230 nm) and polarimetric detection.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

Preliminary experiences show that neither reversed-phase (Lichrospher RP18) nor normal-phase (Lichrospher Si 60) as stationary phases gave good separation of the pyrethroids studied. The best results were obtained using a chiral stationary phase (Chiraspher).

In the optimization of the mobile phase, we need to take into account that in the polarimetric detector, the mobile phase composition is a key parameter because of its influence on the signal (rotatory power).

Thus, the mobile phase must achieve two objectives: first, promote the separation of the analytes, and second give the adequate medium (polarity) to obtain great polarimetric signals.

In Table 1 we ordered the results obtained in the optimization of the stationary and mobile phases, including the range of the polarimetric signal obtained.

As can be seen in Table 1, the resolution (R_s) values obtained are within 0.07–0.90 in the case of the reversed- and normal-phases, although polarimetric signals are high when using as a mobile phase hexane–ethanol–2-propanol.

Table 1
Effect of stationary/mobile phases on the resolution and polarimetric signal

Stationary phase	Mobile phase composition	Resolution (Bifenthrin-Fenpropathrin)	Polarimetric signal (voltage, mV)
Lichrospher RP18 (250×4 mm I.D.) 10 μm	Methanol–water (80:20)	0.07	–
	Acetonitrile–water (80:20)	0.18	+50 to 0
	Ethanol–water (80:20)	0.23	+40 to −40
	2-Propanol–water (70:30)	0.12	+60 to −60
Lichrospher Si60 (250×4 mm I.D.) 10 μm	Hexane–ethanol–2-propanol (90:5:5)	0.15	+200 to ≥−200
	Hexane–ethanol–2-propanol (95:2.5:2.5)	0.26	+200 to ≥−200
	Hexane–ethanol–2-propanol (99:0.5:0.5)	0.83	+50 to ≥−100
	Hexane–ethanol (99.9:0.1)	0.90	+50 to −50
ChiraSpher (250×4 mm I.D.) 5 μm	Hexane	0.66	+10 to −10
	Hexane–ethanol (99:1)	0.51	+40 to −40
	Hexane–ethanol (99.5:0.5)	1.04	+80 to −80
	Hexane–ethanol–2-propanol (99.9:0.05:0.05)	0.85	+25 to −25

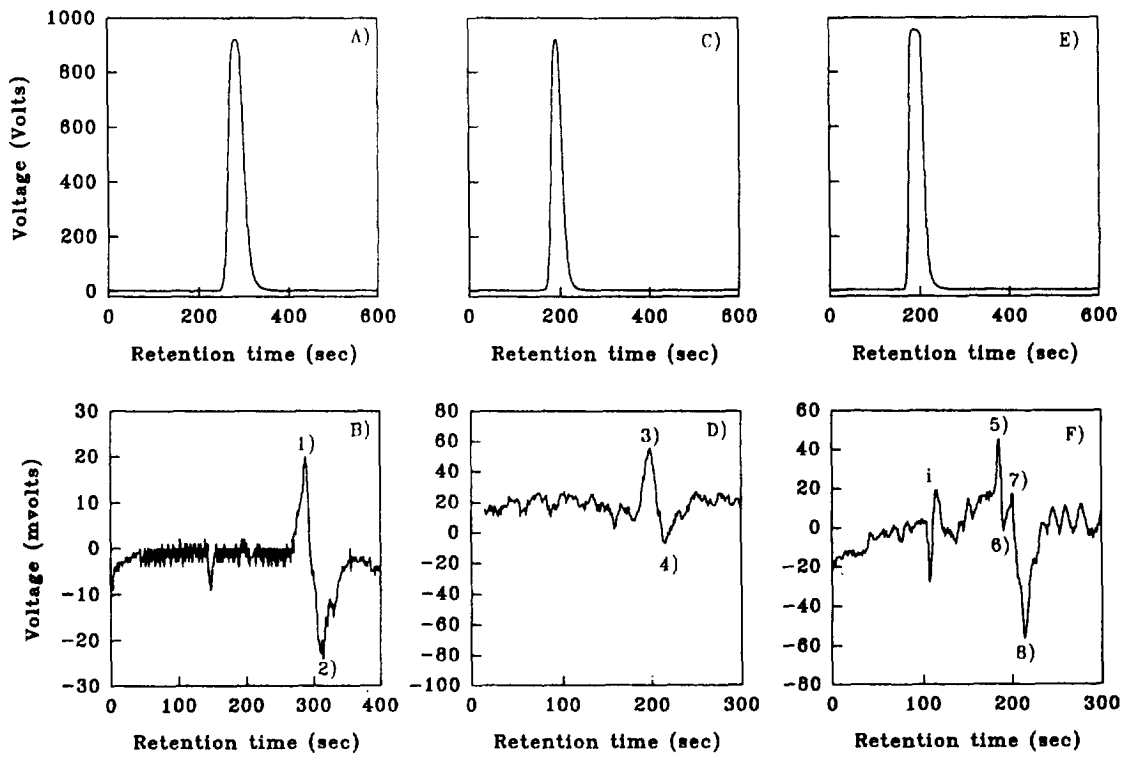

Fig. 2. Chromatograms of the pyrethroids, bifenthrin and fenpropathrin. (A–C–E) UV detection; (B–D–F) polarimetric detection; (A,B) fenpropathrin (150 μg); (C,D) bifenthrin (80 μg); (E,F) bifenthrin (220 μg). Peaks (3,5,7) 3*S*, 1*R*(+) or 3*R*, 1*R*(+); Peaks (4,6,8) 3*R*, 1*S*(−) or 3*S*, 1*S*(−); peak (i) injection peak. Chromatographic conditions are described in Section 2.3 (LC Operating Conditions).

The chiral stationary phase gives good separation of bifenthrin and fenpropathrin (R_s=0.66–1.04), and the best polarimetric signal is obtained when using the mixtures hexane–ethanol (99.5:0.5, v/v).

The general trend of polarimetric signal associated to the mobile phase composition is a reduction in the polarimetric signal with increasing water percentage. On the other hand, increasing ethanol and 2-propanol percentages increase the polarimetric signal.

In the optimum conditions for the resolution of fenpropathrin and bifenthrin using a column chiral, using as a mobile phase hexane–ethanol (99.5:0.5, v/v) at a flow-rate of 1 ml/min, the enantiomers of fenpropathrin and bifenthrin are identified by using two detectors, UV spectrometer and a diode-laser polarimeter.

In the UV spectrometer, the retention times of the pyrethroids together with relative standard deviation (n=9) are 3.10 min (1.63%) and 4.64 min (3.31%) for bifenthrin and fenpropathrin, respectively.

In the diode-laser polarimeter, the retention times of two enantiomers of fenpropathrin together with relative standard deviation (n=9) are 4.73 min (3.41%) and 5.15 min (2.94%) for the enantiomeric form *S*αCN(+) and *R*αCN(−), respectively.

For bifenthrin, we may distinguish four enantiomers which present retention times and relative standard deviation (n=9) of 3.06 min (0.94%) and 3.27 min (1.46%) for diastereomer form (Z)3*S*1*R*(+) or (Z)3*R*1*R*(+), and 3.16 min (1.15%) and 3.50 min (1.54%) for diastereomer form (Z)3*R*1*S*(−) or (Z)3*S*1*S*(−).

In the present conditions the enantiomers of the fenpropathrin are solved (Fig. 2B) while in the case of bifenthrin one can distinguish two situations: at low concentration in which two isomers of the four of the bifenthrin can be separated (isomer *cis* or *trans*, corresponding to one of the stereoisomers) (Fig. 2D); at high concentrations the four isomers of the bifenthrin are distinguished (Fig. 2F). It is deduced that the commercial bifenthrin constitutes a mixture of the two diastereoisomers which are not in equal amounts and the rotatory power is very different, or perhaps some dimerization may occur at high concentrations.

In the optimum conditions the obtained calibration curves (Fig. 3) for each one of the isomers of fenpropathrin and bifenthrin in the range of 50–280 μg for fenpropathrin and of 40–120 μg for bifenthrin are the following:

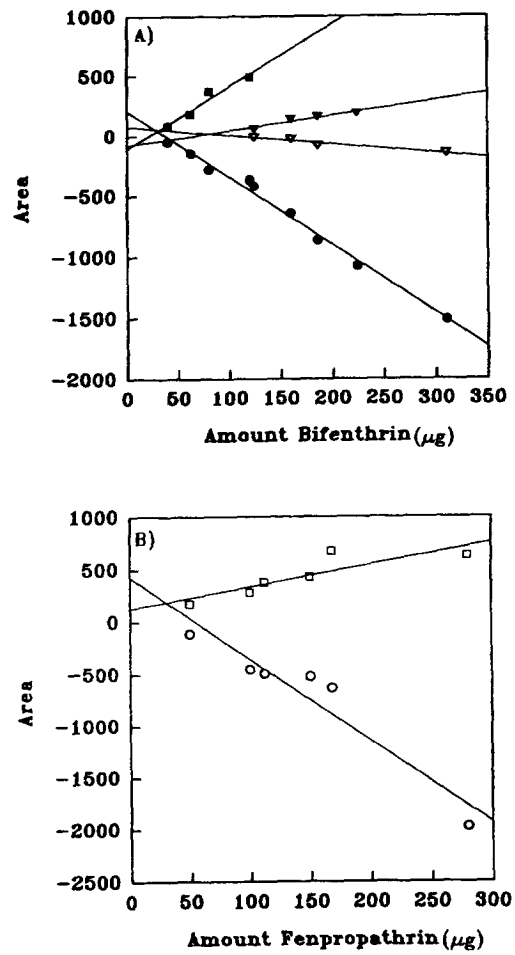

Fig. 3. Calibration graph of the isomers. (A) Bifenthrin; (B) fenpropathrin. (■,▼): 3*S*, 1*R*(+) or 3*R*, 1*R*(+); (●,△): 3*R*, 1*S*(−) or 3*S*, 1*S*(−); (□): *S*αCN(+); (○): *R*αCN(−).

Bifenthrin: area(+)=−111+5.23 (quantity of bifenthrin (μg)); area(+)=68+2.13 (μg); area(−)=79−0.80 (μg); area(−)=211−5.56 (μg). Fenpropathrin: area(+)=−2.05+2.80 (quantity of fenpropathrin (μg); area(−)=431−7.9 (μg). In each case the correlation coefficient is 0.99). The detection limits (signal-to-noise ratio n=3) of fenpropathrin and bifenthrin are 30 μg and 35 μg, respectively.

The linearity between detectors (UV and polarimeter) has been investigated. For this we have represented the area of polarimetric signal vs. the area of

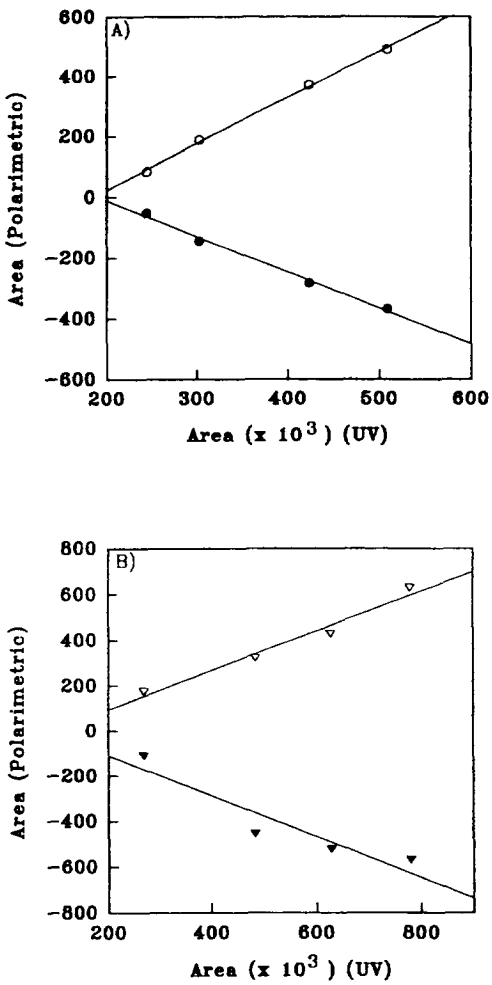

Fig. 4. Linearity series showing UV response vs. polarimetric response. (A) Bifenthrin (40–120 μg); (B) fenpropathrin (50–280 μg).

the UV for fenpropathrin (Fig. 4A) and bifenthrin (Fig. 4B). Areas of polarimetric signals were graphed against areas of UV response, eliminating any contribution to uncertainty from prepared or injected solutions. Good correlations were obtained in both cases. The magnitude of the slope of linearity plots of pure enantiomers should be identical while the signs should be opposite.

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