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Review

Characteristics and enantiomeric analysis of chiral pyrethroids

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ABSTRACT

Pyrethroids are synthetic pesticides that originated from the modification of natural pyrethrins to improve their biological activity and stability. They are a family of chiral pesticides with a large number of stereoisomers. Enantiomers of synthetic pyretroids present different insecticidal activity, toxicity against aquatic invertebrates and persistence in the environment so the development of rapid and sensitive chiral methods for the determination of different enantiomers is necessary. Several techniques have been employed for this purpose including gas chromatography, high performance liquid chromatography or more recently capillary electrophoresis and sub or supercritical fluid chromatography. A general view on the different chiral separation methods applied to the analysis of pyrethroids and the most important information about these pesticides is provided in this review.

Contents

1. Introduction

Separation of chiral compounds is an interesting and challenging topic of research in many analytical chemistry areas, especially in pharmaceutical, biomedical, and environmental fields where pure enantiomeric forms are widely required [1,2]. It is already well-known that enantiomers, in spite of their very similar structure, when exposed to an identical biological environment can show very different biological activity.

Pesticides in general and especially insecticides, are considered a class of important pollutants that are widespread over the environment. About 25% of the existing agrochemicals contain chiral centers and are produced and used as racemic mixtures [3]. In some cases, only one of the isomers of the pesticide is active, while the other may have less activity or even toxic effects against non-target organisms; unfortunately, no such specific studies are routinely required for active substances constituted by isomeric mixtures [4]. Another fact that has to be taken into account is that when racemic mixtures are used as pesticides, the enantiomers are often degraded at different rates [5]. Therefore, the chiral separation of pesticides is necessary in order to optimize enantioselective production processes, assessing the enantiopurity of formulations and monitoring their presence in the environment or into different types of matrices.

The use of insecticides in agriculture is growing at a high speed due to the rise in the productivity. There are different types of insec-

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ticides including organophosphorous, carbamates, organochloride, etc., but nowadays, pyrethroids are the most frequently used insecticides. Furthermore, it is expected to be even more important in the future given increasing restrictions on organophosphorous pesticides [6]. Pyrethroids are used worldwide as insecticides in agriculture, forestry, households, public health and stored products [7,8]. They act on the axons in the nervous systems, interact with the sodium channels and affect the electric impulse transmission. This stimulates the nervous cells and produces several electric shocks producing a total paralysis of the insect [9]. These chemicals are much less toxic for mammals than organophosphorous and carbamate compounds but they present an acute toxicity for a wide range of aquatic organisms and honeybees at low concentrations [10]. Pyrethroids are known to be strongly adsorbed to soil particles. However, this is not likely to render them immobile post-application, because they can be moved in runoff with soil particles to which they are attached, and end up in sediments [6]. Once in sediments, they can enter in aquatic ecosystems [11] and be bioavailable to the aquatic food web [12,13]. For this reason they have a devastating effect on aquatic invertebrates, with a median lethal concentration (LC_{50}) lower than 1 ppb [14].

One of the most significant differences of pyrethroids in comparison with many other pesticides is that most of them have one to three chiral centers [11]. Pyrethroids constitute the 20% of the insecticides used in the world [15], but only 6% of the market products are sold as single isomers [3]. Usually, pyrethroid insecticide formulations are mixtures of isomers [16]. These compounds show enantiomeric selectivity, with biological activity generally residing in only one of the enantiomers. This enantioselectivity phenomenon has important implications in the manufacture and use of chiral agrochemicals in general [17]. Thus, there is an increasing need for rapid separation methods for the determination of enantiomers of pyrethroids.

The most important information on chiral pyrethroids (chemical characteristics, classification, insecticide activity, toxicology and environmental behaviour) and the chiral separation techniques employed in their determination have been reviewed in this article.

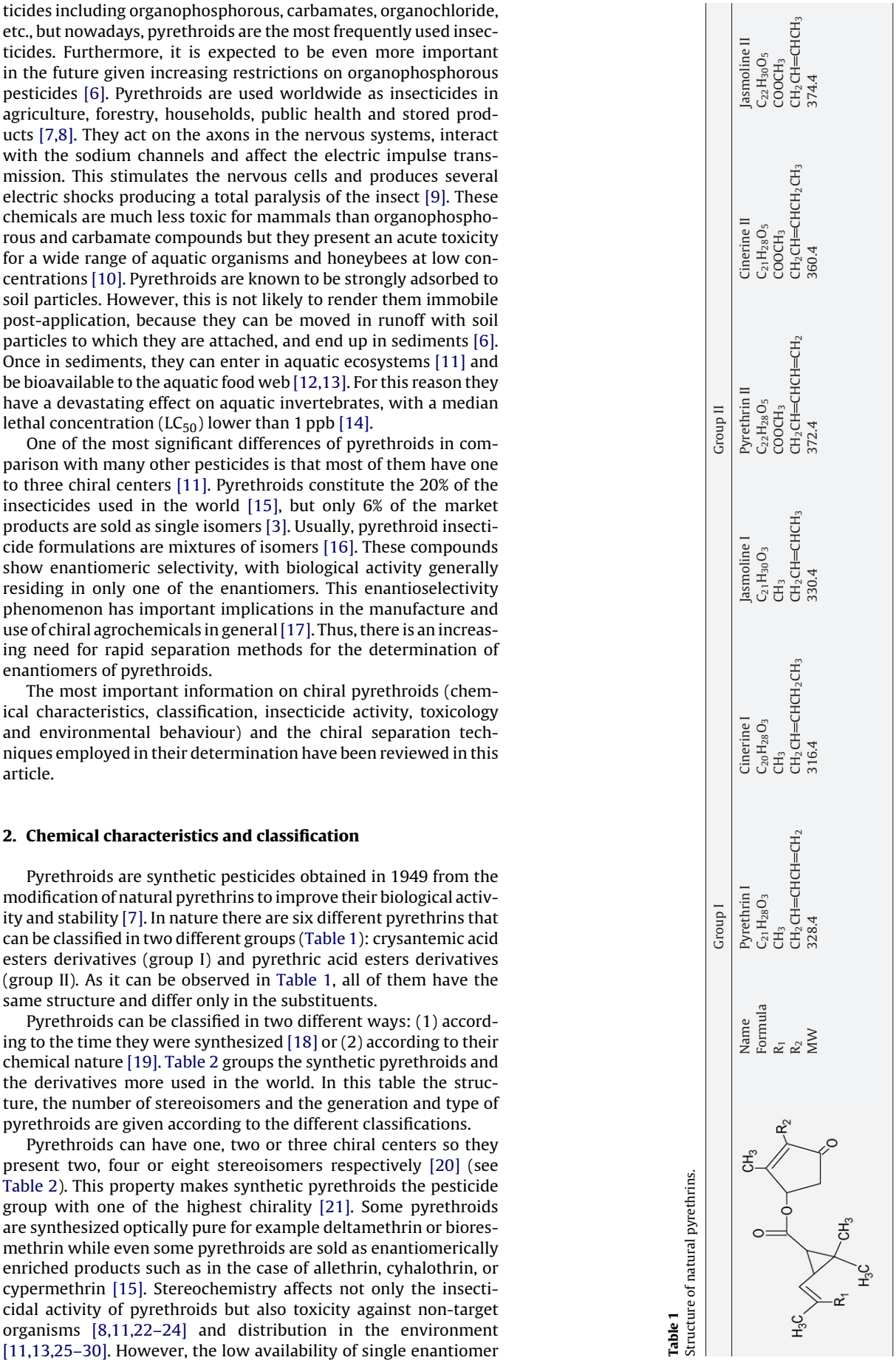
2. Chemical characteristics and classification

Pyrethroids are synthetic pesticides obtained in 1949 from the modification of natural pyrethrins to improve their biological activity and stability [7]. In nature there are six different pyrethrins that can be classified in two different groups (Table 1): crysantemic acid esters derivatives (group I) and pyrethric acid esters derivatives (group II). As it can be observed in Table 1, all of them have the same structure and differ only in the substituents.

Pyrethroids can be classified in two different ways: (1) according to the time they were synthesized [18] or (2) according to their chemical nature [19]. Table 2 groups the synthetic pyrethroids and the derivatives more used in the world. In this table the structure, the number of stereoisomers and the generation and type of pyrethroids are given according to the different classifications.

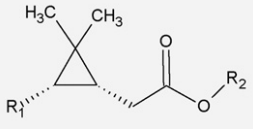
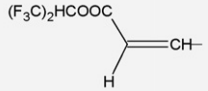
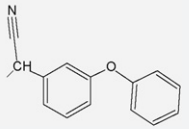
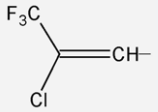
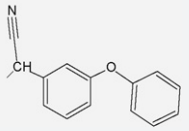
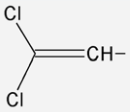
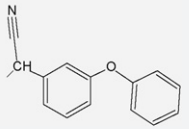
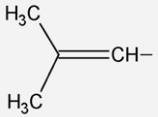
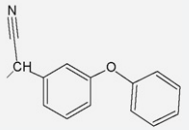
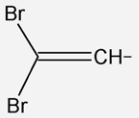
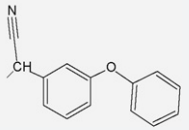
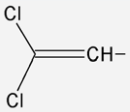
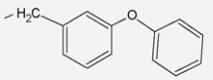
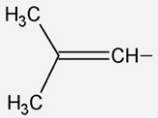
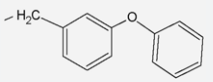
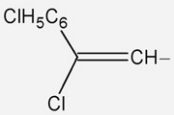
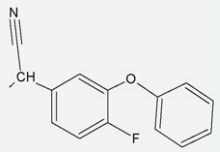
Pyrethroids can have one, two or three chiral centers so they present two, four or eight stereoisomers respectively [20] (see Table 2). This property makes synthetic pyrethroids the pesticide group with one of the highest chirality [21]. Some pyrethroids are synthesized optically pure for example deltamethrin or bioresmethrin while even some pyrethroids are sold as enantiomerically enriched products such as in the case of allethrin, cyhalothrin, or cypermethrin [15]. Stereochemistry affects not only the insecticidal activity of pyrethroids but also toxicity against non-target organisms [8,11,22–24] and distribution in the environment [11,13,25–30]. However, the low availability of single enantiomer

Table 1
Structure of natural pyrethrins.



	Group I			Group II	
Name	Pyrethrin I	Cinerine I	Jasmoline I	Pyrethrin II	Cinerine II	Jasmoline II
Formula	$C_{21}H_{28}O_3$	$C_{20}H_{28}O_3$	$C_{21}H_{30}O_3$	$C_{22}H_{28}O_5$	$C_{21}H_{28}O_5$	$C_{22}H_{30}O_5$
R_1	CH_3	CH_3	CH_3	$COOCH_3$	$COOCH_3$	$COOCH_3$
R_2	$CH_2CH{=}CHCH{=}CH_2$	$CH_2CH{=}CHCH_2CH_3$	$CH_2CH{=}CHCH_3$	$CH_2CH{=}CHCH{=}CH_2$	$CH_2CH{=}CHCH_2CH_3$	$CH_2CH{=}CHCH_3$
MW	328.4	316.4	330.4	372.4	360.4	374.4

Table 2
Structure and classification of pyrethroids.

Structure	Name (number of stereoisomers)	Derivatives (number of stereoisomers)	R_1	R_2	Generation according to developing time
	Acrinathrin (8)[a]				4
	Cyhalothrin (8)[a]	γ-Cihalothrin (1) λ-Cihalothrin (2)			4
	Cypermethrin (8)[a]	α-Cypermethrin (2) β-Cypermethrin (4) θ-Cypermethrin (2) ζ-Cypermethrin (4)			4
	Cyphenothrin (8)[a]				4
	Deltamethrin (1)[a]				4
	Permethrin (4)[a]	Biopermethrin (1) Transpermethrin (2)			3
	Phenothrin (4)[a]				2
	Flumethrin (8)[a]				4

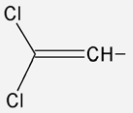
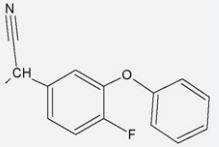
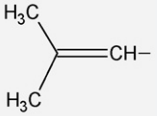
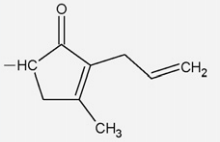
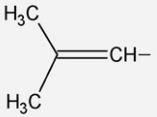
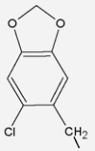
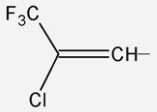
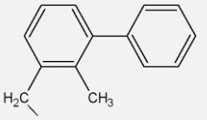
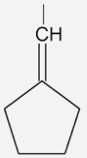
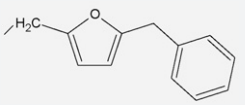
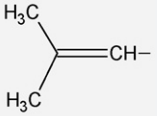
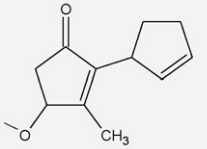
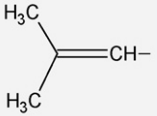
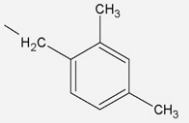
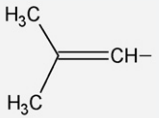
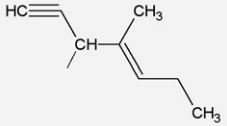
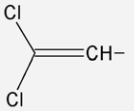
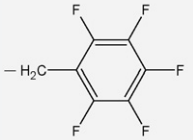
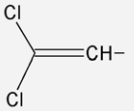
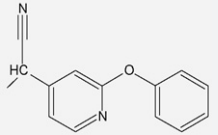
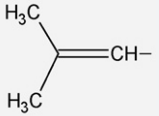
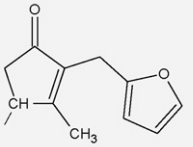
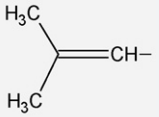
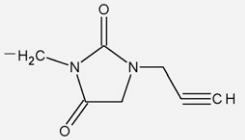
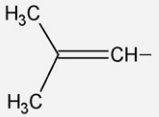
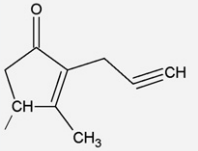
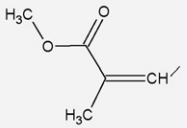
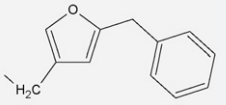
Cyfluthrin (8)[a]	β-Cyfluthrin (4)			4
Allethrin (8)[a]	Bioallethrin (2)			1
Barthrin (4)[a]				4
Bifenthrin (4)[a]				4
Bioetanomethrin (4)[a]				4
Cyclethrin (8)[a]				4
Dimethrin (4)[a]				4

Table 2 (*Continued*)

Structure	Name (number of stereoisomers)	Derivatives (number of stereoisomers)	R_1	R_2	Generation according to developing time
	Empenthrin (8)[a]				4
	Fenfluthrin (4)[a]				4
	Fenpirithrin (8)[a]				4
	Furethrin (8)[a]				4
	Imiprothrin (4)[a]				4
	Prallethrin (8)[a]				4
	Pyresmethrin (4)[a]				4

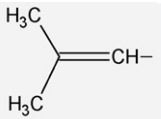
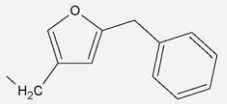
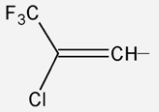
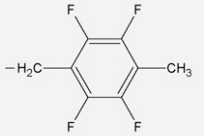
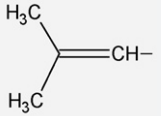
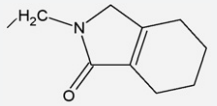
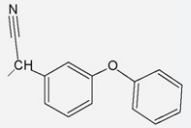
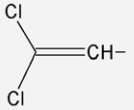
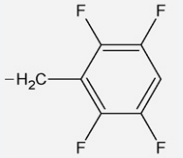
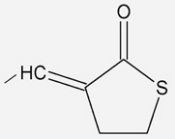
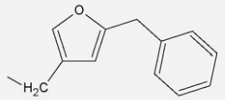
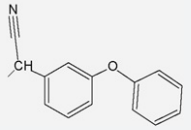
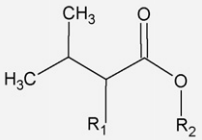
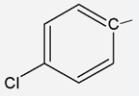
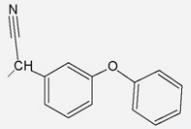
	Resmethrin (4)[a]	Bioresmethrin (1) Cismethrin (2)			2
	Tefluthrin (4)[a]				4
	Tetramethrin (4)[a]				2
	Tralomethrin (1)[a]	–CHBr-C(Br)$_3$			4
	Transfluthrin (4)[a]				4
	Kadethrin (2)[a]				4
	Fenpropathrin (2)[a]	–(CH$_3$)$_2$			4
	Fenvalerate (4)[a]	Esfenvalerate (1)			3

Table 2 (*Continued*)

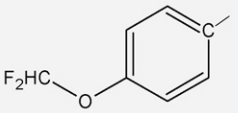
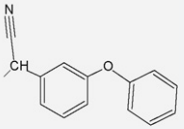
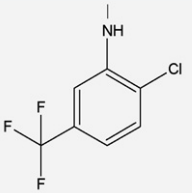
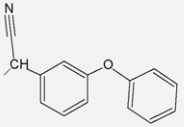
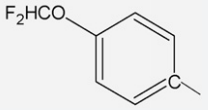
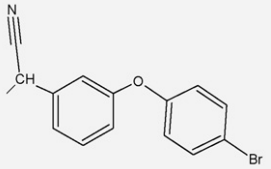
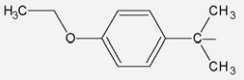
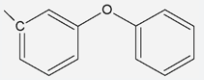
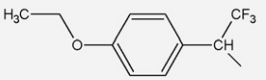
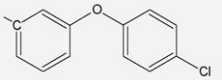
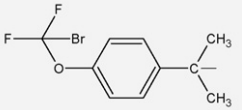
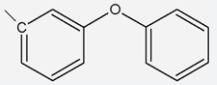
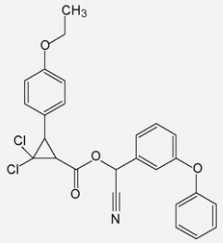
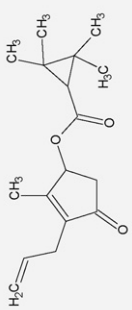
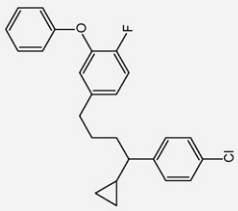
Structure	Name (number of stereoisomers)	Derivatives (number of stereoisomers)	R_1	R_2	Generation according to developing time
	Flucythrinate (4)[a]				4
	Fluvalinate (4)[a]	τ-Fluvalinate (2)			4
	Brofluthrinate (4)[a]				4
R_1-CH_2-O-CH_2-R_2	Etofenprox (1)[b]				4
	Flufenprox (2)[b]				4
	Halfenprox (1)[b]				4
	Cycloprothrin (4)[a]				4

Table 2 (*Continued*)

Structure	Name (number of stereoisomers)	Derivatives (number of stereoisomers)	R_1	R_2	Generation according to developing time
	Terallethrin (2)[a]				4
	Protifenbute (2)[c]				4

[a] Ester pyrethroids.
[b] Ether pyrethroids.
[c] Non-ester pyrethroids.

standards limits the ability to conduct enantiomer specific toxicity studies [15].

3. Insecticidal activity and toxicology

Pyrethroid toxicity is highly dependent on stereochemistry and each isomer has its own toxicity [8]. It has been reported that the toxicity of pyrethroids is dependent on the configuration of the chiral carbon adjacent to the carboxylic group [31]. For example, permethrin enantiomers having the *R* configuration at this carbon are about 25 times more toxic to houseflies than those with the *S* configuration [32]. Fenvalerate enantiomers having the *S* configuration at this carbon (spatially equivalent to the *R* configuration in the dimethylcyclopropanecarboxylic acids) are 10–100 times more toxic to houseflies than those with the *R* configuration [31] and the same has been observed for fluvalinate [17]. Also, the toxicity of the insecticides having a chiral cyano-substituted benzylic carbon present in the 3 phenoxybenzyl alcohol portion of the molecule (e.g., fenvalerate or cypermethrin) are affected by the configuration of this chiral carbon, with the *S* configuration being more toxic to houseflies by a factor of 20–100 [33]. Furthermore, in general the *cis* isomers are more toxic than the *trans* ones. Acute toxicity of a mixture of two isomers depends on the ratio of amounts of the two isomers in the formulation. For example, the female rat acute oral LD_{50} of permethrin increases from 224 mg/kg of bodyweight to 6000 mg/kg as the proportion of the *trans* isomer increases from 20 to 80% [8].

In many cases there is only one active enantiomer in the formulation. This is the case of cypermethrin whose insecticidal activity is only associated to 1*R*,*cis*,α*S* and 1*R*,*trans*,α*S* and the other six isomers have been demonstrated to be inactive [34]. Furthermore, from the two active isomers the *cis* one is much more persistent in soil. In conclusion, the employment of the racemic mixture is illogical because useless isomers are applied to the environment and bigger amounts of product are needed compared to the use of optically pure product [6]. In another sense, formulations made of a single isomer (for example, deltamethrin) are likely to be much more effective than those with four to eight isomers [8].

Pyrethroids are not toxic for mammals but their lethal doses for aquatic invertebrates are very low. For instance, the LC_{50} of *cis*-bifenthrin against *Ceriodaphnia dubia*, a commonly used indicator for invertebrate toxicity, is only 0.078 mg/L and that of permethrin is 0.550 mg/L [35].

The toxicity against aquatic invertebrates may also be enantioselective. Significant differences were observed in LC_{50} for the eight isomers of cypermethrin, with two enantiomers at least 10 times more toxic [22]. The commercial formulation of bifenthrin is made with the *cis* enantiomers: 1*R*-*cis*-bifenthrin and 1*S*-*cis*-bifenthrin. In this case (*cis*-bifenthrin) the toxicity of (1*R*)-bifenthrin is greater than that of (1*S*)-bifenthrin for *Daphnia* and fish [23]. Regression of the survival rate of *Ceriodaphnia dubia* neonates with the initial pesticide concentration gave LC_{50} values of 0.079 ± 0.018 and 0.144 ± 0.026 mg/L for 1*R*-*cis*-bifenthrin and the racemic *cis*-bifenthrin, respectively. The difference in the measured toxicity suggests that 1*R*-*cis*-bifenthrin was probably the only active isomer in *cis*-bifenthrin for causing toxicity to *Ceriodaphnia dubia* [11,22].

The same pattern has been observed for permethrin [11]. Formulations of permethrin are made of equal ratios of *cis*-(1*R*,*cis* and 1*S*,*cis*) and *trans*-permethrin (1*R*,*trans* and 1*S*,*trans*). Toxicity assays yielded similar LC_{50} values for *cis*-permethrin (0.540 ± 0.055 mg/L) and *trans*-permethrin (0.519 ± 0.058 mg/L) diastereomers which was consistent with the previously reported value of 0.550 mg/L for the permethrin mixture. Because of the lack of standards for permethrin enantiomers, the measured biological activity was not related experimentally to the individual stereoisomers. However,

given the great similarities in the chemical structure between bifenthrin and permethrin and the fact that chirality for both compounds originates from C1 and C3 on the cyclopropane ring, it is reasonable to assume that as with *cis*-bifenthrin, the aquatically active enantiomer in *cis*-permethrin was probably 1*R*,*cis*-permethrin.

Xu et al. [24] reported the aquatic toxicity of enantiomers of λ-cyhalothrin. λ-Cyhalothrin is the form of cyhalothrin mainly used in the commercial formulations to control mosquitoes, flies, etc. It contains only two of the eight isomers of the pyrethroid: 1*R*,*cis*,α*S* and 1*S*,*cis*,α*R*, that are considered the most active. Although the activity against target organisms is the same, the toxicity of the two enantiomers differs a lot. Enantioselectivity in acute aquatic toxicity was evaluated via 96 h toxicity tests using *zebrafish*. Significant differences in LC_{50} were observed for the two isomers being the (−)-enantiomer 60 times more toxic after 24 h test and 162 times more toxic after 96-h exposure than the (+)-λ-cyhalothrin. Finally, for cycloprothrin, the insecticidal activity of 1*R*,α*R* towards *larvae* of *Mythimaseparata* and *Aphismedicagini* is respectively six times and four times higher than that of the racemate [36].

4. Environmental behaviour

In the environment, pyrethroids are usually degraded by one or more biotic and abiotic processes: metabolic degradation by plants, animals, and microorganisms and degradation by light [8]. Degradation may be enantioselective, resulting in different distribution patterns and bioaccumulation potentials between enantiomers [21].

To interpret the enantioselective degradation of environmental pollutants the enantiomeric ratio is usually defined as (ER = (+)-enantiomer/(−)-enantiomer), the enantiomeric fraction ($EF = Area_1/(Area_1 + Area_2)$) or the enantiomer ratio doubling time ($T_{ER} = 2$) [13]. If a chiral contaminant is applied as racemate its EF is 0.5, and any deviation of this value in environmental samples is a direct indicator of bioactivity of the given chiral compound. The comparison of concentration of individual enantiomers or the change in the stereoisomeric profiles can also be used for this purpose [13].

After pyrethroids based insecticide application, the *trans* isomer is generally degraded faster than the *cis* isomer [15]. Moreover, in some cases it has been demonstrated that the less active enantiomer persists in the environment for much more time than the enantiomer with more insecticidal activity. The direction of the enantioselective degradation can be influenced by the sampling location and environmental conditions [13]. This may be because there are variations in the microbial population as a result of pH, soil oxidation state, etc. [26].

Morgan et al. [37] determined the EF of *cis*-permethrin after application to prove the enantioselective degradation of the pesticide. The samples analyzed were dust, floor surface wipes and food preparation wipes. The EF values for *cis*-permethrin obtained ranged from 0.412 to 0.535 in all samples so it proves that there is enantioselective degradation. The same degradation pattern for permethrin was observed by Qin and Gan [27] in soil and sediments. In this case, the degradation products for *cis*-permethrin were more persistent than those for *trans*-permethrin.

Due to the similarities in the structure of permethrin and bifenthrin, similar behaviour was observed in their degradation in sediments as shown in Fig. 1. A preferential degradation of the (−)-enantiomer of *cis*-bifenthrin resulted in a relative enrichment of the aquatically active (+)-enantiomer [29]. The same pattern was observed by Liu and Gan in water [38]. A preferential degradation of the 1*S*,*cis* enantiomer over 1*R*,*cis* enantiomer for (Z)-*cis*-bifenthrin and *cis*-permethrin in water was shown.

Liu et al. demonstrated the enantioselective degradation of bifenthrin, permethrin, cypermethrin and cyfluthrin in soil and sediments [26,30]. Both, soil and sediment were collected in the southern California region from the 0–10 cm surface layer. By comparing the chromatograms of the standards and soil and sediment samples, clear enantioselective degradation could be observed. However, the environmental conditions such as the season were important parameters in the degradation of synthetic pyrethroids.

Studies using 14C-labeled isomers showed that significantly faster degradation occurred with the *trans* diastereomers than with the corresponding *cis* diastereomers, and in the same diastereomeric pair, with the *R*,*S*-enantiomer than with the corresponding *R*,*R*-enantiomer of cypermethrin [39]. Under laboratory controlled conditions several studies demonstrated the same degradation trends [13]. Cypermethrin was spiked in sediment from Irvine, Canada with no cypermethrin residue in origin. The samples were stored at room temperature under aerobic conditions and changes in isomer composition were determined. The results showed that the *cis* pair of enantiomers was considerably more persistent than the *trans* one [40].

Isomer conversion or enantiomerization is another important process that may occur with pyrethroids [13]. There are several factors (e.g., heat, water, light, microorganisms.) that can induce the enantioselective degradation or isomer conversion of pyrethroids. Liu et al. [28] reported the chiral stability of *cis*-bifenthrin, permethrin, cypermethrin and cyfluthrin during gas chromatography analysis, where high temperatures are needed and different solvents are used for sample preparation. In nature, the most important solvent is water so the study of the chiral stability in this medium is also important. Only temperatures above 180 °C were enough to show isomer conversion in some cases. Pyrethroids with chirality only in the cyclopropyl ring (bifenthrin and permethrin) were found to be stable, but those with αC (cypermethrin and cyfluthrin) were unstable and gave an epimer. In organic solvents such as hexane, ethyl acetate and dichloromethane, and in sterile water, stereoisomers of *cis*-bifenthrin and permethrin did not show any isomer conversion. However for cypermethrin and cyfluthrin, when exposed to water a slow enantiomerization was observed.

Compared with natural pyrethrins, pyrethroids have an enhanced stability under light. However, photochemical reactions are very common. Holmstead et al. [41] observed that when permethrin was irradiated with UV light (λ > 290 nm) or sunlight the isomers underwent an extensive isomerization of the cyclo-

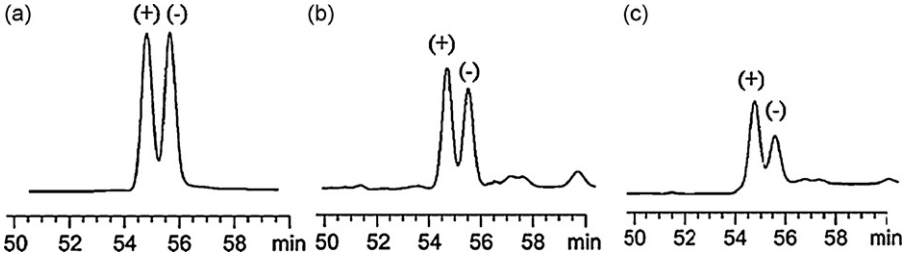


Fig. 1. Chiral GC chromatograms of *cis*-bifenthrin in sediments from different depths. (a) 0–15 cm, (b) 15–30 cm, and (c) 30–45 cm. Reprinted with permission from Ref. [29].

propane ring. Maguire [13,42] performed the evaluation of the isomerization of deltamethrin (1*R*,*cis*,α*R*) in hexane under bright summer sunshine for 5 days. The treatment resulted in the formation of 1*S*-*cis*-α*S*, 1*R*-*trans*-α*S* and 1*S*-*trans*-α*R*. No isomerization was observed when samples were stored in hexane in the dark so this effect was probably due to the light. However, this situation does not occur always, sometimes solvents can induce this epimerization [12,34].

In other studies the isomerization of some pyrethroids in water was observed even without light. Maguire [42] in a study on deltamethrin stability, observed the isomerization of 1*R*,*cis*,α*S* to 1*S*,*cis*,α*S*. However, no isomer conversion occurred for *cis*-bifenthrin and permethrin [13,28]. In the case of cyfluthrin interconversion from *trans* to *cis* isomer was observed after incubation in soil. This leads to a time dependent variation of the isomeric ratio of permethrinic acids in the soil extracts [43].

5. Enantiomeric analysis

5.1. Sample preparation

Sample treatment can be the most laborious part of every analytical methodology. Frequently, when real samples are analyzed, the components of interest are present at levels too low for their detection. Therefore, previous preconcentration of the analytes is required in order to make their concentration adequate to the separation technique employed. In the case of pyrethroids, their low levels in environmental samples make necessary the use of this type of procedures. Different extraction techniques have been employed for this purpose including solid-phase microextraction (SPME), solid-phase extraction (SPE), liquid–liquid extraction (LLE), supercritical fluid extraction (SFE), and micro solid-phase dispersion extraction (MSPD) enabling the chiral determination of pyrethroids in several matrices such as soil, water, sediments or biological samples.

The extraction techniques mainly employed previous enantiomeric determination of pyrethroids in soil, water, sediments and biological samples (*Ceriodaphnia dubia*) by GC were mainly SPME and LLE. Liu and Gan [38] developed a SPME method for the extraction of *cis*-bifenthrin and *cis*-permethrin from water samples. The SPME methodology was carefully optimized to obtain as good recuperation as possible for the studied analytes. Factors like sampling time, temperature and organic solvents used were the most important parameters to be optimized. The comparison of SPME with LLE for the two above-mentioned pyrethroids indicated a clear higher selectivity of the first one in the extraction of these compounds. However, when cypermethrin and cyfluthrin were determined by the same authors in water [21] and sediments [29] samples, LLE with ethyl acetate or acetone:hexane (1:1, v/v) was the extraction technique chosen. A very similar procedure was followed for the extraction of bifenthrin, permethrin, cypermethrin and cyfluthrin from soils [26]. In the case of biological matrices like *Ceriodaphnia dubia* [11] a more laborious extraction method was required for the determination of bifenthrin and permethrin. Several consecutive LLE steps were performed with different solvents before GC analysis.

HPLC has been the most employed technique to achieve the chiral separation of synthetic pyrethroids. Several extraction techniques have been applied previous to HPLC analysis of pyrethroids in environmental (soil and water) samples. Although the extraction from soil has been mainly achieved by LLE, MSPD was also employed for this purpose [30]. When using LLE for soil samples different solvents were tested: hexane, acetone, methanol and mixtures of them. Each author has his own methodology for the extraction. Chapman [31] performed the extraction of cypermethrin by adding acetone to the soil that after evaporation was redissolved in hexane. The extract was transferred to a silica Sep-Pak cartridge to preconcentrate the analyte. Qin and Gan [25] performed the extraction of permethrin by LLE with a mixture of acetone:hexane (1:1, v/v). When water samples were analyzed, SPE with C-18 cartridges enabled the determination of τ-fluvalinate and permethrin [44]. The initial volume of 500 mL of water was passed through the cartridge and eluted three times with a total volume of 50 mL of n-hexane. The extract was then dried and evaporated to dryness and the residue was redissolved in 1 mL of n-hexane. Finally, a MSPD methodology was used by Li et al. [30] for the extraction of β-cypermethrin and β-cyfluthrin from soils. In this case, soil samples were placed into a mortar and Florisil adsorbent and distilled water were added. Once the mixture was homogenized, it was introduced into a glass chromatographic column and a mixture of n-hexane/ethyl acetate was added and allowed to elute dropwise by gravity. The initial 15 mL eluent was collected and blown to dryness with nitrogen. The final residue was dissolved in the HPLC mobile phase (n-hexane:propan-2-ol, 100:0.1, v/v) and subjected to HPLC analysis.

The limited LODs achieved with the CE methodologies require good preconcentration methodologies. Water samples containing bioallethrin, fenpropathrin and phenothrin were extracted by SPE with Oasis HLB cartridges (Waters Corp. Milford, MA) [17]. Moreover, the extraction of fenpropathrin, fenvalerate and fluvalinate from a cellulose matrix by SFE with supercritical CO_2 was also reported [7].

5.2. Separation and determination

5.2.1. Gas chromatography

GC has been widely used for the enantioseparation of several insecticides and environmental pollutants mainly due to the low LODs achieved in general by GC compared with other techniques [2,45]. The direct separation involving the use of chiral stationary phases (CSP) usually based on cyclodextrins (CD) is the most commonly approach used. However, the main problems are the high cost of this type of columns, large time of analysis and the thermal instability of some pesticides [16].

There are several works in which GC is employed for the chiral separation of pyrethroid pesticides (see Table 3). Kutter and Class [46] carried out the separation of the enantiomers of allethrin and cypermethrin. For allethrin resolution, a CDX-B chiral column (permethylated-β-CD) (J&W, Folson, CA, USA) coupled to an achiral DB 1701 (14% cyanopropyl phenyl methyl polysiloxane) (J&W, Folson, CA, USA) column was employed. The enantioresolution of *trans* isomers was achieved but *cis* isomers were not separated. Diastereomeric but not enantiomeric selectivity by GC was achieved for cypermethrin with an apolar DB 5 column (5% phenyl)-methylpolysiloxane) (J&W, Folson, CA, USA). The separation was applied to study the composition of cypermethrin in insecticidal formulations (Ripcord) and to compare with the extracts obtained after forestry application.

Nie et al. [47] performed the enantiomeric separation of some ester pyretroids with different chiral stationary phases: permethylated-β-CD (PM-β-CD), heptakis (2,6-di-O-butyl-3-O-butyryl)-β-CD (DBB-β-CD), heptakis (2,6-di-O-nonyl-3-O-trifluoroacetyl)-β-CD (DNT-β-CD), the mixture of PM-β-CD and DBB-β-CD and the mixture of PM-β-CD and DNT-β-CD. With CSPs containing the mixtures of derivatized cyclodextrins, the enantiomeric separation was improved significantly for some compounds when compared with the single cyclodextrin CSPs derivatives. Synergistic effects were observed for some racemate compounds on the mixed cyclodextrin derivative CSPs. In general, the best resolution was obtained with PM-β-CD + DBB-β-CD

Table 3
Chiral separation of pyrethroids by GC.

Pyrethroid	Matrix	Separation conditions	Sample preparation/comments	References
Allethrin Cypermethrin	Ripcord (CP) formulation in extracts of bark	**Allethrin** CSP: CDX-B + DB1701 columns 170 °C isothermal program **Cypermethrin** CSP: DB 5 T program: 70 °C (1 min), 30 °C/min to 220 °C, 1 °C/min to 240 °C Detection: ECD, 310 °C	Higher photostability was observed for *trans* isomers of cypermethrin after forestry applications. Only partial resolution Analysis time Cypermethrin ≈ 13 min Allethrin ≈ 75 min	[46]
- Chrysanthemic methyl ester - Chrysantemic l-methyl ester - Permethrinic methyl ester - 2,2-dimethylcyclopropane carboxylic methyl ester - 3-(2,2,2-trichloroethyl)-2,2-dimethylcyclopropane carboxylic ethyl ester	–	CSP PM-β-CD DBB-β-CD DNT-β-CD PM-β-CD + DBB-β-CD PM-β-CD + DNT-β-CD Detection: FID, 250 °C	Different CDs and CD combinations CSPs were tested	[47]
cis-Bifenthrin *cis*-Permethrin	Water	CSP: BGB 172 T program: Initial hold at 180 °C for 2 min, ramped at 1 °C/min to 230 °C until elution Detection: ECD, 310 °C	On-line SPME with 100 μm layer PDMS fiber. Fiber was introduced in the sample 2.0 cm from the surface and it was desorbed in the GC inlet during 3.0 min Analysis time *cis*-Bifenthrin ≈ 78 min *cis*-Permethrin ≈ 51 min	[38]
cis-Bifenthrin *cis*-Permethrin	Field sediments	CSP: BGB 172 column T program: 180 °C for 2 min, ramp at 5 °C/min to 230 °C and held at this T until complete elution. Detection: ECD, 310 °C	LLE with acetone:hexane (1:1, v/v) No interconversion of enantiomers was observed	[29]
Cypermethrin Cyfluthrin	Water and sediments	CSP: BGB 172 column T program: 160 °C for 2 min, ramp at 1 °C/min to 220 °C, held at this T for 60 min, ramped at 5 °C/min to 230 °C, held at 230 °C till complete elution Detection: ECD, 310 °C	LLE with ethyl acetate and after evaporation the extract was redissolved in 4.0 mL acetone:hexane (1:1, v/v) Only partial resolution of six peaks Analysis time Cypermethrin ≈ 145 min Cyfluthrin ≈ 134 min	[21]
Bifenthrin Permethrin	*Ceriodaphnia dubia*	CSP: BGB 172 column T program: 180 °C (2 min), ramp at 5 °C/min to 230 °C, followed by an isothermal hold at 230 °C until elution Detection: ECD, 310 °C	LLE with ethyl acetate and after evaporation the extract was redissolved in 4.0 mL acetone:hexane (1:1, v/v) No resolution for *trans*-permethrin Extraction of the samples with ethyl acetate Analysis time *cis*-Bifenthrin ≈ 55.5 min *cis*-Permethrin ≈ 88.5 min	[11]
cis-Bifenthrin Permethrin Cypermethrin Cyfluthrin	–	CSP: BGB 172 column T program: 160 °C for 2 min, ramped at 1 °C/min to 220 °C, held at 220 °C for 60 min, ramped at 5 °C/min to 230 °C and held at this T till complete elution Detection: ECD, 310 °C	Conversion at the αC	[28]

Table 3 (*Continued*)

Pyrethroid	Matrix	Separation conditions	Sample preparation/comments	References
cis-Bifenthrin Permethrin Cyfluthrin Cypermethrin	Soil and sediments	CSP: BGB 172 column T program: 160 °C for 1 min and then ramped to 230 °C at 1 °C/min Detection: ECD, 310 °C	Centrifugation of the sample during 25 min at 10,000 rpm. The supernatant was decanted. The residue was extracted with acetone:methylene chloride (1:1, v/v) that after evaporation was reconstituted in 1 mL hexane In lab incubation experiments, an enantioselective degradation was observed for *cis*-bifenthrin, permethrin and cifluthrin	[26]
Permethrin Cypermethrin	–	CSP: BGB 172 column T program: 160 °C for 1 min and then ramped to 230 °C at 1 °C/min. Hold at 230 °C till complete elution Detection: ECD, 310 °C	Enantiomerization was observed: (1) cypermethrin was stable in propan-2-ol and methanol but not in n-hexane, acetone and methylenechloride (2) Permethrin was stable under all conditions	[27]

stationary phase. All separations were performed isothermally and flame-ionization detector (FID) was used.

Several methods were developed by Liu and Gan for determining optical isomers of (Z)-*cis*-bifenthrin, *cis*-permethrin, cypermethrin and cyfluthrin [11,20,26,28,29,38] and the aquatic toxicity of their enantiomers against *Ceriodaphnia dubia* [11] using a BGB 172 column (20% tert-butyldimethylsilyl-β-CD dissolved in 15% diphenyl and 85% dimethylpolysiloxane) (BGB Analytik, Adliswil, Switzerland) after probing different CSPs. It was the first time that coupled solid-phase microextraction and enantioselective GC with electron capture detector (ECD) was used for the analysis of the two first compounds [38] and the first time GC was applied to a biological matrix for the chiral analysis of pyrethroids [11]. Moreover, the thermal stability of these four commonly used pyrethroids during GC analysis and sample preparation was also studied [28] showing that chiral stability of pyrethroids depends on the origin of chirality. In the first work, the enantiomers of (Z)-*cis*-bifenthrin and *cis*-permethrin were baseline separated [38] with LODs of 0.05 μg/L and 0.10 μg/L for each pyrethroid. The integrated SPME-enantioselective GC method was used to analyze surface runoff water samples. The analysis showed preferential degradation of the 1*S*,3*S* enantiomer over 1*R*,3*R* enantiomer for both compounds studied. The concentrations detected were substantially smaller than those determined following solvent extraction, suggesting that SPME-enantioselective GC analysis selectively measured the dissolved fraction. Once the method was optimized it was applied to sediment samples containing residues of these pyrethroids, extracted with acetone:hexane 1:1 (v/v) for the evaluation of changes in the ER [29]. The same method was applied to the analysis of cyfluthrin and cypermethrin, giving six peaks that could be identified using enantiomerically enriched products [20]. Complete separation of enantiomers occurred for both *cis* diastereomers and between *cis* and *trans* diastereomers, but not for enantiomers from the same *trans* diastereomers. The method was also applied to quantify the enantiomers in water and sediment samples. The detection limits (LODs) obtained ranged from 7.5 to 15.0 μg/L for cypermethrin and from 3.5 to 7.5 μg/L for cyfluthrin. Finally, in the last paper the enantioselective degradation of the cited pyrethroids in soil and sediments was evaluated by comparing changes of stereoisomers profiles from the original values [26]. Good resolution was obtained for *cis* diastereomers but not for the *trans* diastereomers, as it can be observed in Fig. 2 that shows the GC chromatograms of permethrin under enantioselective analytical conditions in standard (Fig. 2a) and a sediment sample (Fig. 2b). Clear enantioselective degradation can be observed.

As said, some pyrethroids can show thermal instability and may be degraded during GC analysis resulting in enantiomer conversion. Sometimes pyrethroids can show this effect in organic solvents. In this sense, Qin and Gan [27] demonstrated by using GC analysis, that permethrin is stable in all the organic solvents used (n-hexane, methylene chloride, propan-2-ol, acetone and methanol) but cypermethrin was unstable in acetone and methylene chloride. The extent of enantiomerization was affected by temperature dependence and was also influenced by water as a cosolvent. Results from this study suggest that the exposure to certain solvents and water may cause artefacts in chiral analysis. Also, they showed that for isomer-enriched pyrethroids products such abiotic enantiomerization may render the products less effective because the conversion leads to the formation of inactive stereoisomers.

5.2.2. High performance liquid chromatography

HPLC is a very useful technique for the separation of enantiomers because it is quite rapid, non-destructive and there is little possibility of epimerization during the analysis as seen in GC [48,49]. Apart from analysis, HPLC is also the best technique for small-scale preparation of enantiopure chemicals.

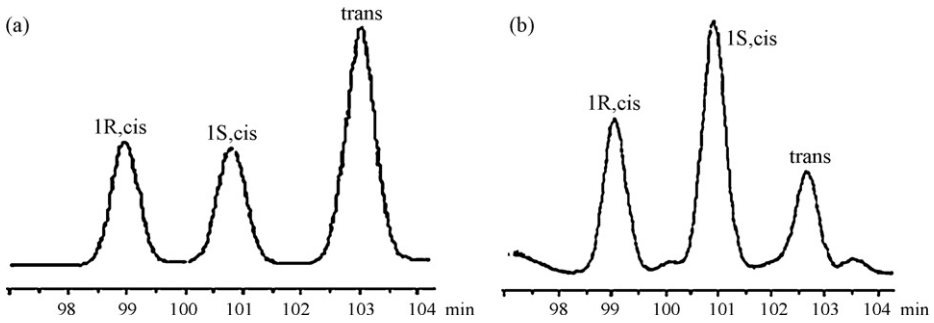


Fig. 2. Chiral GC chromatograms of permethrin pyrethroid. (a) The standard reference racemic mixture and (b) a sediment sample from San Diego Creek in California. Experimental conditions in the text. Reprinted with permission from Ref. [26].

In chiral HPLC the selectors used in the chiral stationary phases include cyclodextrins, proteins, crown ethers, polysaccharides, polyacrylamides, polymeric chiral surfactants, macrocyclic antibiotics and some low-molecular-weight molecules such as Pirkle type compounds [50].

Table 4 groups all the chiral separations of pyrethroids performed by HPLC including the compounds separated and the most important separation conditions. It can be seen that several chiral columns have been employed for the enantiomeric resolution of pyrethroid insecticides. While for no α-cyano pyrethroids polymeric CSPs based on cellulose derivatives seemed to be the most suitable for their resolution, multiple interaction Pirkle type CSPs showed advantages in the enantiomeric separation of α-cyano pyrethroids [51]. UV detection is used in all the articles and sometimes other detection modes are used as complementary detection systems (laser polarimeter detector, NMR, etc). Only in seven of the publications the developed method has been applied to a real matrix [25,30,31,44,46,52,53] to confirm the existence of these compounds and in two of the total articles the degradation products of pyrethroids were analyzed [43,54]. One of the most popular column is Pirkle type 1-A (NH_2 bonded Spherisorb column containing (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine) (Regis, Morton, Grove, IL, USA) and (Technicol, Stockport, UK) [31,52,55]. This column enabled the chiral separation of fenproanate, fenvalerate, cypermethrin and cyfluthrin using 0.1% propan-2-ol in hexane as mobile phase [31]. The method was applied to the analysis of cypermethrin residues in soil samples in different periods after application of the pesticide as shown in Fig. 3.

A systematic study of the separation of thirteen pyrethroids grouped in four different categories, was carried out by Cayley and Simpson [55]. The separation was achieved with a mobile phase composition ranging from 0.025 to 0.1% propan-2-ol in hexane. Useful resolution was obtained in analysis times between 8 and 60 min. These results were confirmed by Lisseter and Hambling and demonstrated that the bonding of the column (ionically or covalently bonded) can influence the separation of the pyrethroids [52]. In effect, while for allethrin, α-cypermethrin, cypermethrin, fenpropathrin, fenvalerate, permethrin, phenothrin, resmethrin and tetramethrin a better separation was obtained with the ionically bonded column, for cyfluthrin, λ-cyhalothrin, cyhalothrin, flucythrinate and flumethrin better separations were obtained with the covalently bonded column. The authors applied the method to the analysis of a formulated product of cypermethrin, fenvalerate and permethrin. It was noticed that the commercial ionic column rapidly lost selectivity when exposed to the formulation.

Sumichiral OA (Sumika Chemical Analysis Service, Osaka, Japan) type columns were for the first time used for the chiral analysis of pyrethroids by Oi et al. [48] for the resolution of ten pyretroids with three different columns: Sumichiral OA-4000 ((*S*)-valine and (*S*)-1-(α-naphthyl)-ethylamine), Sumichiral OA-4600 ((*S*)-tert-leucine and (*S*)-1-(α-naphthyl)-ethylamine) and Sumichiral OA-2500 ((*R*)-1-naphthylglycine and 3,5-dinitrobenzoic acid). Each CSP was capable to manage the enantioseparation of a number of pyrethroids using hexane–dichloroethane–ethanol in different proportions as mobile phases. The resolutions obtained were higher than 1.03 for all compounds. Sumichiral OA-2500 CSP was also employed for the enantioseparation of *cis*-bifenthrin with hexane–1,2-dichloroethane [22,29] or n-hexane–propan-2-ol–ethanol (99.8:0.06:0.14) as mobile phases with reasonable retention times (14.8–16.3 min) and high resolution ($R_s = 3$) [23]. A laser polarimeter detector was used in the detection of pyrethroids [23]. This detector was employed earlier for fenpropathrin detection [56].

Kutter and Class [46] performed the enantioselective liquid and gas chromatography of allethrin and cypermethrin. Enantiomeric selectivity was observed for cypermetrin in normal phase-HPLC (NP-HPLC) with a Pirkle type chiral stationary phase made of L-tartaric acid and L-dinitrobenzylphenylethylamine (Marchery-Nagel, PA, USA), but very strong interactions and therefore long retention times prevented the separation of allethrin in these phases. *trans*-Allethrin isomers were separated on a chiral β-cyclodextrin RP-HPLC column but no selectivity was obtained for the *cis* isomers of allethrin and some difficulties due to isomerization of cypermethrin were also reported. The enantiomeric selectivity observed for cypermetrin in NP-HPLC with a Pirkle type chiral stationary phase was used by Class in other work [53] for

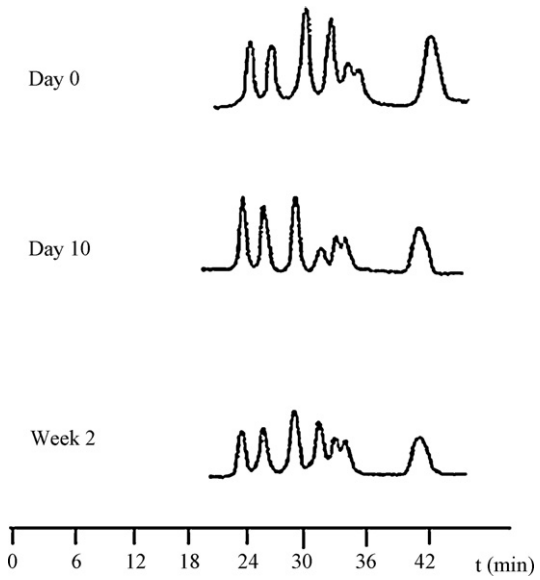


Fig. 3. Separation of cypermethrin soil extracts after forestry application by HPLC using 0.1% propan 2-ol in hexane. Column: Pirckle 1-A, flow 1 mL/min. Reprinted with permission from Ref. [31].

Table 4
Chiral separation of pyrethroids by HPLC.

Pyrethroid	Matrix	Separation conditions	Sample preparation/comments	References
Fenproanate Fenvalerate Cypermethrin Cyfluthrin	Soil	CSP: Pirkle type 1A column Mobile phase: propan-2-ol:hexane 0.1:99.9 Detection: UV 200 nm	LLE with acetone and after evaporation the extract was transferred to a silica cartridge and eluted with 20 mL propan-2-ol:hexane (1:99, v/v). The extract was evaporated to 5 mL. Analysis time Fenproanate ≈ 15 min Cypermethrin ≈ 31 min Cyfluthrin ≈ 35 min Fenvalerate ≈ 32 min	[31]
Type I: Fenpropathrin Type II: Resmethrin Bioresmethrin Permethrin Type III: Phenothrin Fenvalerate Fluvalinate Type IV: Allethrin Bioallethrin Cypermethrin Fastac® Karate® Deltamethrin	–	CSP: Pirkle type 1-A column Mobile phase Type I: 0.1% propan-2-ol in hexane as mobile phase Type II: 0.025% propan-2-ol in hexane Type III: 0.1% propan-2-ol in hexane Type IV: 0.1% propan-2-ol in hexane Detection: UV 240/280 nm	A cyano-bonded column was also tested	[55]
d-Allethrin (a) d-Allethrin (b) Cyfluthrin (c) λ-Cyhalothrin (d) Cyhalothrin (e) α-Cypermethirn (f) Cypermethrin (g) Fenpropathrin (h) Fenvalerate (i) Flucythrinate (j) Flumethrin (k) Permethrin (l) d-Phenothrin (m) Resmethrin (n) Tetramethrin (o)	Formulated products of permethrin	CSP: Pirkle type 1-A column (a), (b), (g), (h), (o) ionic column/0.15 (% propan-2-ol in hexane) (c), (j), (k) covalent column/0.05 (% propan-2-ol in hexane) (d), (e) covalent column/0.15 (% propan-2-ol in hexane) (f), (i) covalent, ionic column/0.15% propan-2-ol in hexane (l), (m), (n) ionic column/0.05 (% propan-2-ol in hexane) Detection: UV 230 nm	Analysis time Cypermethrin ≈ 36 min Cyfluthrin ≈ 80 min Permethrin ≈ 27 min	[52]
Terallethrin (a) Fenpropathrin (b) Resmethrin (c) Pemethrin (d) Phenothrin (e) Tetramethrin (f) Fenvalerate (g) Cypermethrin (h) Allethrin (i) Bioallethrin (j)	–	(a) CSP: Sumichiral OA-4000 column Mobile phase: hexane–1,2 dichloroethane–ethanol 500:30:0.15 (b), (h), (i) CSP: Sumichiral OA-4600 Mobile phase: hexane–1,2 dichloroethane–ethanol 500:10:0.05 (c), (d), (e), (f), (g) CSP: Sumichiral OA-2500I Mobile phase: hexane–1,2 dichloroethane–ethanol 500:1 Detection: UV 230 nm	The first time these columns were tested in the chiral separation of pyrethroids Analysis time: <60 min for all compounds	[48]
Type I: *cis*-bifenthrin Permethrin Type II: Cypermethrin Cyfluthrin	–	Type I: CSP: Sumichiral OA-2500-I column Mobile phase: hexane:1,2-dichloroethane 500:1 Type II: CSP: two chained Chirex 00G-3019-DO columns Mobile phase: hexane:1,2-dichloroethane:ethanol 500:10:0.05 Detection: laser polarimeter detector 675 nm and UV	Toxicity studies demonstrated the different contribution of each enantiomer in the toxicity against *Ceriodaphnia dubia* and *Daphnia magna* Analysis time *cis*-Bifenthrin ≈ 12 min Permethrin ≈ 38 min Cypermethrin ≈ 90 min	[22]
cis-Bifenthrin *cis*-Permethrin *trans*-Permethrin	–	CSP: Sumichiral OA-2500I column Mobile phase: hexane:dichloroethane 99.5:0.05 Detection: UV 230 nm	–	[29]

Table 4 (*Continued*)

Pyrethroid	Matrix	Separation conditions	Sample preparation/comments	References
Permethrin	Soil and sediment	CSP: Sumichiral OA-2500I column Mobile phase: hexane:dichloroethane 99.5:0.05 Detection: UV 230 nm	LLE with acetone:hexane (1:1, v/v) Enantioselective degradation was observed	[25]
Bifenthrin	–	CSP: Sumichiral OA-2500I column Mobile phase: propan-2-ol:ethanol:n-hexane 0.06:0.14:99.8 Detection: UV 230 nm, MS and polarimeter	Toxicity studies showed that (−)-Bifenthrin was 10 times more toxic than its enantiomer	[23]
Allethrin (a) Cypermethrin (b)	Allethrin indoor formulation	(a) CSP: silica-gel modified with β-CD. (Cyclobond I) column Mobile phase: acetonitrile:water 22:78 (b) CSP: silica-gel modified with L-tartaric acid and L-dinitrobenzylphenylethylamine (Pirkle type) Mobile phase: 0.05% trifluoruro acetic acid and 0.5% propan-2-ol in hexane Detection: UV 220 nm	Photoselective degradation was observed through the study of enantiomeric profiles Analysis time Cypermethrin ≈ 42 min Allethrin ≈ 16 min (*trans*)	[46]
Cypermethrin	Soil	CSP: silica-gel modified with L-tartaric acid and L-dinitrobenzylphenylethylamine (Pirkle type column) Mobile phase: 0.05% trifluoroacetic acid and 0.05% propan-2-ol in hexane Detection: UV 220 nm	Investigation of environmental degradation after forestry application	[53]
Fenpropathrin (a) Bifenthrin	–	CSP: Chirasper column Mobile phase: hexane:ethanol 99.5:0.5 Detection: diode laser polarimetric detector and UV detection 230 nm	Good resolution Quantification of enantiomers	[56]
Cypermethrin Permethrin	–	CSP: Daicel Chiralcel OD column material (cellulose tris-3,5-dimethylphenylcarbamate derivate bonded to silica) Detection: UV 280 nm and NMR	NMR was employed for the confirmation of the elution order Analysis time Cypermethrin ≈ 50 min Permethrin (incomplete R_s)	[57]
Permethrin Cyfluthrin *cis*-Bifenthrin Phenothrin	Soil	CSP: (+) (5*R*,8*S*,10*R*) 1-(3-aminopropyl)-terguride based column Mobile phase: 20 mM acetate (pH 4):acetonitrile 6:4 Detection: UV and Polar monitor detector	Monitorization of the hydrolytic degradation products of cyfluthrin in soil	[43]
Alphamethrin (a) Cypermethrin (b)	–	(a) CSP: Cyclobond I 2000 column Mobile phase: 150 mM triethylamine in water with H_3PO_4 (pH 3.5):methanol 50:50 (b) CSP: ChiraDex Mobile phase: 500 mM triethylamine in water with H_2SO_4 (pH 3.5):methanol 45:55 Detection: UV 210 nm	Two same CSPs columns were tested observing that the precedence of them was crucial in the separation Analysis times Cypermethrin ≈ 30 min Alphamethrin ≈ 25 min	[58]
Cyfluthrin (a) Resmethrin (b) *cis*-Bifenthrin (c) (1*R*)-Phenothrin (d)	–	(a) CSP: Chiralcel OD-H column coupled with urea CSP Mobile phase: hexane:1,4-dichlorobutane:ethylacetate 500:5:0.15 (b), (c), (d) CSP: Chiralcel OD-H column Mobile phases: n-hexane:propan-2-ol Detection: UV 220 nm	When α-cyano pyrethroids were separated multiple-interaction columns seem to be better while for no-α-cyano compounds cellulose based OD-H columns were most suitable	[51]
Chrysantemic acid (A) Permethrinic acid (B) Decamethrinic acid (C) And their esters	–	CSP: Chiralpak AS column (CSP amylose tris (*S*)-α-methylbenzyl carbamate) Mobile phase: hexane:propan-2-ol:trifluoroacetic acid 98.9:1:0.1 Detection: UV 215 nm	–	[54]
Acrinathrin (a) β-Cyfluthrin (b) λ-Cyhalothrin (c) Deltamethrin (d) Esfenvalerate (e) Fenpropathrin (f) fenvalerate (g) τ-Fluvalinate (h) Permethrin (i)	–	(a), (g) CSP: Chiralpak AD and Chiralcel OJ columns Mobile phase: n-hexane:propan-2-ol 90:10 and n-Hexane:ethanol 95:5 respectively (b), (i) CSP: Chiralcel OJ column; mobile phase: ethanol:n-hexane 90:10 (c) CSP: Chiralcel OD-R column; mobile phase: Acetonitrile:water 70:30 (d) CSP: RP-Chiralpak AD column; mobile phase: ethanol:water 85:15 (e), (h) CSP: Chiralcel OJ column; mobile phase: n-hexane:ethanol 95:5 (f) CSP: Chiralcel OD-R column; mobile phase: ethanol:water 85:15 Detection: PDAD 210 nm	Analysis time: λ-Cyhalothrin ≈ 37 min Fenpropathrin ≈ 36 min Deltamethrin ≈ 52 min β-Cyfluthrin ≈ 18 min Esfenvalerate ≈ 16 min Fenvalerate ≈ 30 min τ-Fluvalinate ≈ 16 min Permethrin ≈ 8 min	[59]

τ-Fluvalinate Permethrin	Drinking water	CSP: Chiralcel OJ column; mobile phase: n-hexane:ethanol 90:10 Detection: PDAD 210 nm	SPE with C-18 cartridges (500 mL of sample). The sample was eluted three times with a total volume of 50 mL of n-hexane (20 + 20 + 10) Analysis times: Permethrin ≈ 9 min τ-Fluvalinate ≈ 19 min	[44]
Fenpropathrin (a) Fenvalerate (b) Brofluthrinate (c) Cypermethrin (d) Cyfluthrin (e)	–	CSP: A novel chiral stationary phase connecting (*R*)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivate of (*S*)-isoleucine to aminopropyl silica-gel through 2-amino-3,5-dinitro-1-carboxamido-benzene unit Mobile phase (a) n-Hexane–1,2-dichloromethane–propan-2-ol 94:9.5:0.5 (b) n-Hexane–1,2-dichloroethane–propan-2-ol 97.45:2.5:0.05 (c) n-Hexane–1,2-dichloromethane–propan-2-ol 94:9.5:0.5 (d) and (e) n-hexane–1,2-dichloromethane–propan-2-ol 96.8:3:0.2 Detection: UV/circular dichroism 230 nm	Elution orders were assigned by using different stereoisomer-enriched products R_s > 0.71 for all pyrethroids Analysis time: Fenpropathrin ≈ 20 min Brofuthrinate ≈ 30 min Fenvalerate ≈ 40 min Cypermethrin ≈ 38 min Cyfluthrin ≈ 50 min	[60]
Fenpropathrin (a) Fenvalerate (b) Brofluthrinate (c) Cypermethrin (d) Cyfluthrin (e)	–	CSP: new chiral stationary phase based on the (*R*)-1-phenil-2-(4-methylphenyl)ethylamine Amide derivate of (*S*)-valine and 2-chloro-3,5-dinitrobenzoic acid Mobile phase (a) Hexane–dichloromethane 94:6 (b) Hexane–1,2 dichloromethane–ethanol (98.45:1.2:0.35) (c) Hexane–1,2 dichloromethane–ethanol (93.8:6:0.2) (d) Incomplete (e) Incomplete Detection: UV 230 nm	Analysis time: Fenpropathrin ≈ 16 min Brofuthrinate ≈ 38 min Fenvalerate ≈ 32 min Cypermethrin ≈30 min Cyfluthrin ≈ 38 min	[61]
β-Cypermethrin β-Cyfluthrin	Soil	CSP: Chiralcel OD column Mobile phase: n-hexane–propan-2-ol 100:6 for cypermethrin/100:2 for cyfluthrin Detection: UV 230 nm	MSPD extraction, the final residue was redissolved in n-hexane:propan-2-ol (100:0.1, v/v) Quantification to prove enantioselective degradation Analysis time: <15 min previous separation of diastereoisomeric pairs	[30]
Cycloplothrin	–	1*S*,α* = CSP: Chiralcel OD-H column Mobile phase: hexane:propan-2-ol 9:1 1*R*,α* = CSP: Chiralcel OJ-H column Mobile phase: hexane:propan-2-ol 7:3 Detection: UV 254 nm	Enantioselective insecticidal activity against larvae of *Mythimaseparata* Analysis time 1*S*,α* 18.8-63.1 min 1*R*,α* 23.9-41.0 min	[36]
λ-Cyhalothrin	–	CSP: Chiralcel OD column Mobile phase: n-hexane:propan-2-ol 95:5 Detection: circular dichroism/UV 236 nm	Enantioselective toxicity of this pyrethroid and malformations induced mainly by one enantiomer Analysis time <20 min	[24]

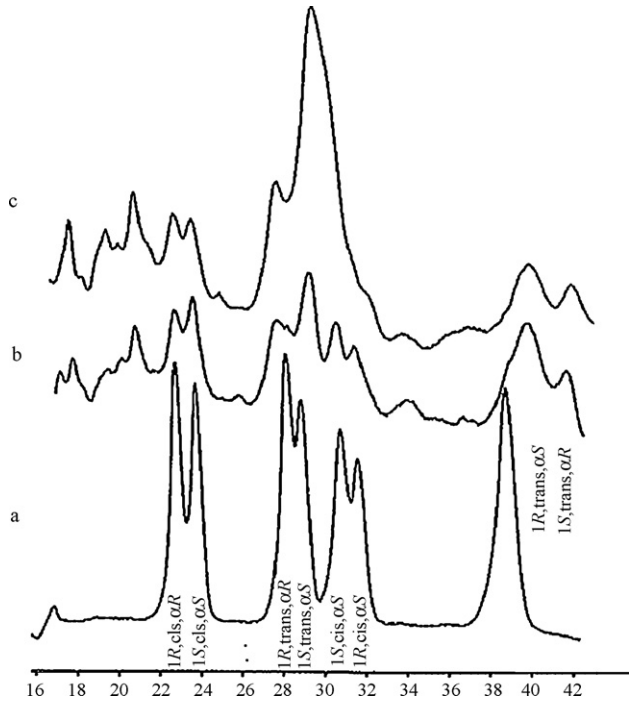


Fig. 4. NP-HPLC chromatograms of cypermethrin (a) standard (b) residual cypermethrin soil extract form the Rübhau (c) from the Tranke site. Column: Pirkle, mobile phase: 0.05% trifluoroacetic acid–0.05% 2-propanol in hexane. Reprinted with permission from Ref. [53].

the analysis of cypermethrin enantiomers and their degradation products in soil extract after forestry application of this compound. Fig. 4 shows chiral NP-HPLC with the Pirkle type phase and 0.05% trifluoroacetic acid (TFA)-0.05% propan-2-ol in hexane as mobile phase [53]. Eight cypermethrin stereoisomers were separated into seven peaks (Fig. 4a) and two soil extracts were also injected (Fig. 4b and c).

In some cases a previous separation of the diastereoisomers and the injection of the fractions in the chiral column were necessary to achieve a good enantioresolution [30,57]. Edwards and Ford [57] resolved the four diastereoisomeric pairs of cypermethrin and the two diastereoisomeric pairs of permethrin on an irregular silica column using hexane–chloroform–diethyl ether (200:1:6). All diastereoisomeric pairs were injected individually in cellulose based chiral HPLC column with hexane–propan-2-ol (250:1) as mobile phase. In the case of cypermethrin the separation of the four diastereoisomeric pairs was achieved with reasonable retention times (<50 min). For permethrin only two of the four enantiomers were baseline separated. Li et al. [30] resolved the four enantiomers of β-cypermethrin and β-cyfluthrin in a Chiralcel OD column (cellulose tris-(3,5-dimethylphenylcarbamate coated on 10 μm silica-gel) (Daicel Chemical Industries, Japan). First of all an achiral separation of the two diastereoisomeric pairs of each pyrethroid was carried out and only two peaks were observed that corresponded to 1*R*,*cis*,1*S* + 1*S*,*cis*,1*R* and 1*R*,*trans*,1*S* + 1*S*,*trans*,1*R*. To identify each peak a standard of α-cypermethrin and α-cyfluthrin (1*R*,*cis*,1*S* + 1*S*,*cis*,1*R*) was injected. This method was used for the determination of enantioselective degradation of β-cypermethrin and β-cyfluthrin in soil.

Sometimes there are significant differences among the results obtained with the same CSPs. These results can be due to the influence of the chiral selector bonding to the silica support and/or other possible variation in properties of stationary phases (different surface concentration of chiral selector, accessibility of silanol groups, etc.) [58]. Lemr et al. [58] proved the influence of two β-CD

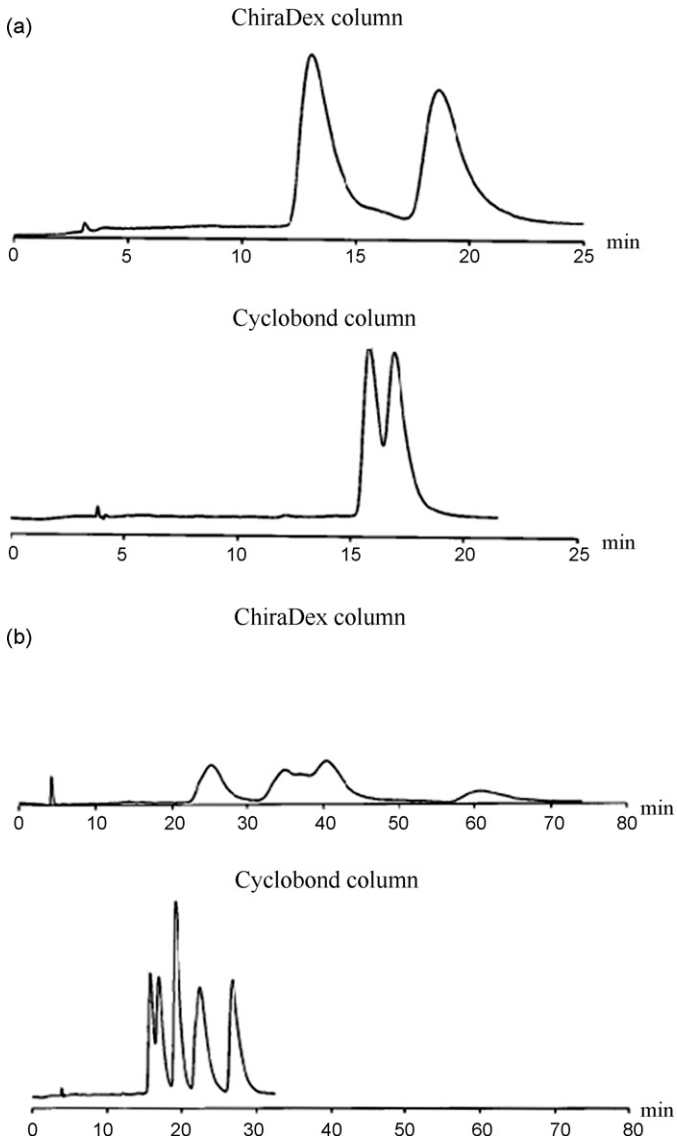


Fig. 5. Chiral separation by HPLC of (a) alphamethrin (b) cypermethrin in two different columns with the same CSP: ChiraDex (mobile phase: 150 mmol/L triethylamine with H_2SO_4 (pH 3.5): methanol 45:55 (v/v); detection: UV absorption at 210 nm; flow-rate: 0.8 mL/min for alphamethrin and 0.6 mL/min for cypermethrin; temperature: 20 °C for alphamethrin and 10 °C for cypermethrin) and Cyclobond I 2000 (mobile phase: 150 mmol/L triethylamine with H_3PO_4 (pH 3.5): methanol 50:50 (v/v); flow-rate: 0.8 mL/min; detection: UV absorption at 210 nm; flow-rate: 1.0 mL/min; temperature: 20 °C). (Reprinted with permission from Ref. [58]).

columns: ChiraDex (Merck, Darmstadt, Germany) and Cyclobond I 2000 (Astec, Whippany, NJ, USA) in the separation of alphamethrin and cypermethrin. As Fig. 5 shows better results were obtained for alphamethrin with ChiraDex-β-CD (Fig. 5a) while for cypermethrin Cyclobond I 2000 column was better (Fig. 5b) under the same other experimental conditions [58].

Yang et al. [59] separated the enantiomers of nine pyrethroids with three polysaccharide-based CSPs: Chiralpak AD (Amylose tris (3,5-dimethylphenylcarbamate)), Chiralcel OD-R (cellulose tris (3,5-dimethylphenylcarbamate)) and Chiralcel OJ (Cellulose tris (4-methylbenzoate)) all of them from Daicel Chemical Industries (Tokyo, Japan) both in reversed and normal HPLC modes. The composition of mobile phase played an important role in the resolution of pyrethroids enantiomers in both modes. Enantiomers of τ-fluvalinate and permethrin were also separated and simultaneously determined by Yang et al. [44] with a Chiralcel

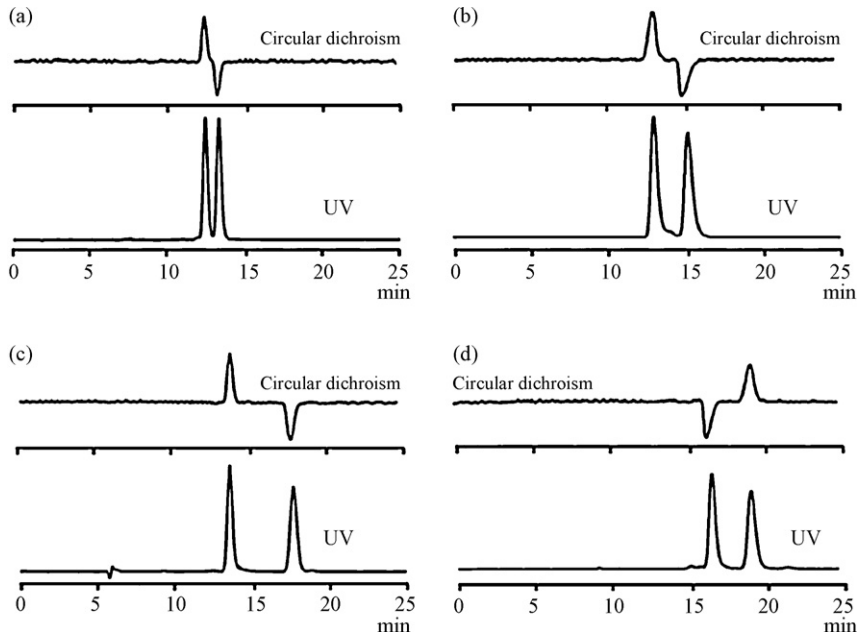


Fig. 6. Chromatograms of λ-cyhalothrin obtained by HPLC on chiral columns detected by circular dichroism and ultraviolet (UV) detection. (a) Chiralpak amylose tris (3,5-dimethylphenyl-carbamate), n-hexane/ethanol (98/2, v/v), 25 °C, 0.40 mL/min; (b) Chiralpak amylase tris-([*S*]-α-methylbenzyl-carbamate), n-hexane/ethanol (95/5, v/v), 25 °C, 0.60 mL/min; (c) Chiralcel cellulose tris (3,5-dimethylphenyl-carbamate), n-hexane/propan-2-ol (95/5, v/v), 25 °C, 0.50 mL/min; (d) Chiralcel cellulose tris-(4-methylbenzoate), n-hexane/ethanol (95/5, v/v), 25 °C, 0.60 mL min^{-1}. Reprinted with permission from Ref. [24].

OJ column under normal phase mode and a mobile phase of n-hexane–ethanol (90:10, v/v) with a baseline resolution ($R_s > 1.5$). LODs for τ-fluvalinate and permethrin were 0.12 and 0.14 μg/L. The developed method was applied to the determination of these compounds in drinking water samples at ppb levels before solid-phase extraction (SPE) with C-18 cartridges. Recoveries ranged between 103 and 113% and precision values were better than 10%.

The asymetrically synthesized isomers of cycloprothrin were resolved on a chiral column and the evaluation of the enantioselectivity of their insecticidal activity was studied by Jiang et al. [36]. Complete separation was achieved on a Chiralcel OD-H column (cellulose tris-(3,5-dimethylphenylcarbamate) coated on 5 μm silica-gel) (Daicel Chemical Industries, Japan) for (1*S*,α*)-cycloprothrin isomers with retention times of 18.8 min and 63.1 min. For (1*R*,α*)-cycloprothrin isomers, a Chiralcel OJ-H column (cellulose tris-(4-methylbenzoate) coated on 5 μm silica-gel) (Daicel Chemical Industries, Japan) gave two peaks with retention times of 23.9 min and 41.0 min. The method allowed individual isomers to be recovered for use in bioassays.

Xu et al. [24] performed the separation of λ-cyhalothrin by HPLC using four different chiral columns: Chiralpak AD, Chiralpak AS (amylose tris-[(*S*)-α-methylbenzylcarbamate] coated on 10 μm silica-gel) (Daicel Chemical Industries, Japan), Chiralcel OD and Chiralcel OJ. The enantiomers of λ-cyhalothrin were separated completely on all columns tested, but the best resolutions were obtained with Chiracel OD as stationary phase and n-hexane:propan-2-ol 95:5 as mobile phase. To identify each enantiomer a circular dicroism detector was used. Although lower temperatures and less amounts of modifiers resulted in better separations, the retention times of enantiomers and their peak tailing must also be taken into account when selecting chromatographic conditions. Fig. 6 shows chromatograms of λ-cyhalothrin in different experimental conditions, using circular dichroism and ultraviolet (UV) detection systems [24]. The chromatographic method developed in this work may be suitable for obtaining optically pure enantiomers of λ-cyhalothrin which would be a prerequisite for toxicity assessment.

Finally, Tan et al. [60,61] reported two novel CSPs by bonding (*R*)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (*S*)-isoleucine to aminopropyl silica-gel through 2-amino-3,5-dinitro-1-carboxamido-benzene unit in the first one and (*R*)-1-phenyl-2-(4-methylphenyl)ethylamine amide derivative of (*S*)-valine in the second column. The new CSPs were applied to the enantioresolution of five pyrethroid insecticides by NP-HPLC. Two enantiomers of fenpropathrin and four stereoisomers of fenvalerate were baseline separated using n-hexane:1,2-dichloroethane:propan-2-ol as mobile phase for the first CSP and n-hexane:1,2-dichloroethane:ethanol in the valine derivate CSP, showing that enantioselectivity of this stationary phase is better than that obtained with a Pirkle type 1-A column for the compounds. Only partial separations were observed for brofluthrinate, cypermethrin and cyfluthrin.

5.2.3. Capillary electrophoresis

Capillary electrophoresis is a quite new technique in the chiral separation of pyrethroids. Its main limitation is the low concentration sensitivity obtained with optical detection due to the small sample volumes injected and the limited path length employed for on-capillary detection [62]. To avoid these problems different options have been reported such as sample preconcentration procedures and alternative detection systems [62–65]. In on-line UV detection, which is the most common detection mode used in CE, a small enhancement of the sensitivity can be observed by using special designs of the detection window (bubble and Z-cells). Off-line sample preconcentration procedures have been developed to improve the LODs usually achieved by CE. Thus liquid–liquid extraction, solid-phase extraction (SPE), solid-phase microextraction, supercritical fluid extraction and cloud point extraction have demonstrated their usefulness prior to CE analysis of insecticides [62]. On-line preconcentration techniques based on electrophoretic principles such as: sample stacking [66], transient isotachophoresis [67], sweeping [68], and dynamic pH junction [69] have also been employed. However, the use of alternative detection systems, such as laser induced fluorescence (LIF), phosphorescence, MS and electrochemical detection has enabled to achieve the most promising

Table 5
Chiral separation of pyrethroids by CE.

Pyrethroid	Matrix	CE mode	Separation conditions	Sample preparation/comments	References
Boallethrin Fenpropathrin Pehnothrin	Water	CD-MEKC	Chiral selector: DM-β-CD BGE: 50 mM NaH_2PO_4 + sodium cholate + 15 mM DM-β-CD Capillary: 50 μm (i.d.) × 40 cm (47 cm t.l.) Voltage: 20 kV Temperature: 20 °C Detection: UV 214 nm	SPE preconcentration with Oasis HLB cartridges. The analytes were eluted with 6 mL methyl t-butyl ether (90:10, v/v) Analysis time - Bioallethrin ≈ 13 min - Fenpropathrin and Phenothrin < 6 min R_s > 1.5 for all compounds	[17]
DCA (Permethrin), CA (Phenotrin), DCA (Cypermethrin), CMBA (Sanmarton), TCCA (Fenpropatrhrin)	–	MECK	Chiral selector: OG (chiral surfactant) BGE: 100 mM sodium phosphate pH 6.5 + 10% (v/v) acetonitrile + OM 25 mM Capillary: 50 μm (i.d.) × 50 cm (57 cm t.l.) Voltage: 19 kV Temperature: 20 °C Detection: UV 255 nm and LIF: λex: 360 nm, λem: 380 ± 2 nm	Previous derivatization with ANDSA	[16]
CA (Phenothrin), DCA (Cypermethrin), CMBA (Sanmarton)	–	MEKC	Chiral selector: OG (chiral surfactant) BGE: 175 mM phosphate pH 6.5 containing 30 mM OM Capillary: 50 μm (i.d.) × 50 cm (57 cm t.l.) Voltage: 18 kV; Temperature: 18 °C Detection: LIF: λex: 325 nm, λem: 380 ± 2 nm and 420 ± 2 nm	Previous derivatization with ANDSA	[70]
Cypermethrin, Alphamethrin, Permethrin, Fenpropathrin	–	CD-MEKC	Chiral selector: γ-CD BGE: 50 mM sodium phosphate pH 2.5 + 150 mM SDS + 150 mg/mL γ-CD Capillary: 50 μm (i.d.) × 30 cm (37 cm t.l.) Voltage: −250 V/cm Temperature: 20 °C Detection: UV 214 nm	Analysis time <15 min for all pyrethroids R_s fenpropathrin: 10 R_s alphamethrin: 1.4	[49]
Fenpropathrin, fenvalerate Fluvalinate	Cellulose	CEC	Chiral selector: Me-β-CD BGE: methanol 25 mM Tris pH 8.3 + Me-β-CD 70 mM Capillary: 75 μm (i.d.) × 21 cm (30 cm t.l.) Voltage: 25 kV Temperature: 20 °C Detection: UV 214 nm	SFE from a cellulose matrix using CO_2 Analysis time - Fenpropathrin > 25 min Fenvalerate and fluvalinate were not completely resolved	[7]
Chrysanthemic acid Permethrinic acid Deltamethrinic acid	–	CD-EKC	Chiral selector: PMMA-β-CD BGE: 40 mM boric + 40 mM acetic + 40 mM phosphoric acids (1:2:2) Capillary: 50 μm (i.d.) × 50 cm (58.5 cm t.l.) Voltage: 30 kV Detection: UV 202 and 220 nm	–	[71]

sensitivity enhancements. The combination of preconcentration techniques with alternative detectors makes it possible to achieve the highest sensitivity enhancements [63–65].

A few articles have been published on the separation of pyrethroids (see Table 5) and only in some of them the developed method has been applied to the analysis of real matrices [17]. The most extended CE mode used for enantioseparations of pyrethroids is Micellar Electrokinetic Chromatography (MEKC) both with chiral and achiral surfactants and MEKC in presence of cyclodextrins (CD-MEKC), although Capillary Electrochromatography (CEC) and Electrokinetic Chromatography with cyclodextrin (CD-EKC) have also been employed.

Karcher and El Rassi [16] developed the separation of permethrin, phenothrin, cypermethrin, fenpropathrin and Sanmarton (fenvalerate) hydrolysis products by MEKC with n-octyl-β-D-glucoside (OG) and n-octyl-β-O-maltopyranoside (OM) as chiral surfactants. Hydrolysis gave as product the corresponding carboxylic acid that contains less chiral centers so this way the number of enantiomers was reduced, making the separation easier. The detection system used was LIF previous derivatization of the carboxylated hydrolytic products with 7-aminonaphtalene-1,3-disulfonic acid (ANDSA), which apart from making possible the LIF detection, increased the solubility of the analytes rendering them more amenable to analysis by aqueous CE. LIF detection is a good choice but it involves the introduction of an additional step in the analysis that has to be carefully optimized [1]. The same authors [70] intended to extend this method for the chiral separation of phenothrin, cypermethrin and Sanmarton (fenvalerate) and their respective hydrolysis products. The detection system was also LIF but in this case different fluorescent labels were tested: 5-aminonaphthalene-1-sulfonic acid (ANSA), ANDSA and 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), with different number of sulfonic acid groups (1, 2 or 3 respectively), and the same two chiral surfactants: OM and OG to improve the separations. The magnitude of enantioseparation was shown to vary greatly among the fluorescence labels, with ANDSA providing the best overall enantioresolution. Furthermore, a change in enantioresolution was observed when changing the ionic strength as well as the nature and concentration of surfactant. The best resolutions were obtained with ANDSA and OG.

Another electrophoresis mode that has been applied to the chiral separation of pyrethroids is CD-MEKC in which the enantiomers are separated based on their different interactions with the micelles and the cyclodextrin. Ševčkík et al. [49] compared CD-MEKC and HPLC for the separation of the lipophilic uncharged pyrethroids cypermethrin, alphamethrin, permethrin and fenpropathrin. Different kinds of cyclodextrins (β-CD, HP-β-CD, DM-β-CD, TM-β-CD, γ-CD), surfactants (SDS and CTAB), and some background electrolytes at pH 2.5 were tested. Optimized conditions (background electrolyte: 50 mmol/L sodium phosphate, pH 2.5, 150 mmol/L SDS, 150 mmol/L γ-cyclodextrin) allowed the separation of alphamethrin, the eight cypermethrin stereoisomers being eluted in seven peaks and the separation of two enantiomers of fenpropathrin with resolution $R_s = 10$. Under the tested experimental conditions, complete separation of four permethrin stereoisomers was not obtained. In comparison to HPLC, alphamethrin enantiomers were separated by both methods, CD-MEKC allowed enantioseparation of fenpronpathrin with very high resolution and better separation of cypermethrin stereoisomers, and HPLC offered better possibilities in the analysis of permethrin.

Shea et al. [17] carried out the separation of the enantiomers of seven pesticides, three of them were pyrethroids (bioallethrin, fenpropathrin and phenothrin) by MEKC or CD-MEKC, and they applied the method to the analysis of fortified water samples. In this case, they investigated the use of two surfactants, sodium dodecyl sulfate (SDS) and sodium cholate (SC (chiral surfactant)) alone or in combination with different cyclodextrins (β-CD, DM-β-CD, TM-β-CD, HP-β-CD, γ-CD, HP-γ-CD) in a phosphate buffer (pH 7.0). With SDS, bioallethrin and fenpropathrin enantiomers were separated in presence of DM-β-CD ($R_s = 0.8$) and γ-CD ($R_s = 7.1$), respectively. With SC, bioallethrin enantiomers were resolved with β-CD ($R_s = 1.3$), DM-β-CD ($R_s = 1.5$), and HP-β-CD ($R_s = 2.4$); fenpropathrin enantiomers were resolved only in presence of DM-β-CD ($R_s = 1.5$); and phenothrin enantiomers were resolved when using DM-β-CD ($R_s = 1.5$) and HP-β-CD ($R_s = 0.7$). Fig. 7 shows the separation of bioallethrin enantiomers (Fig. 7a) with SDS and DM-

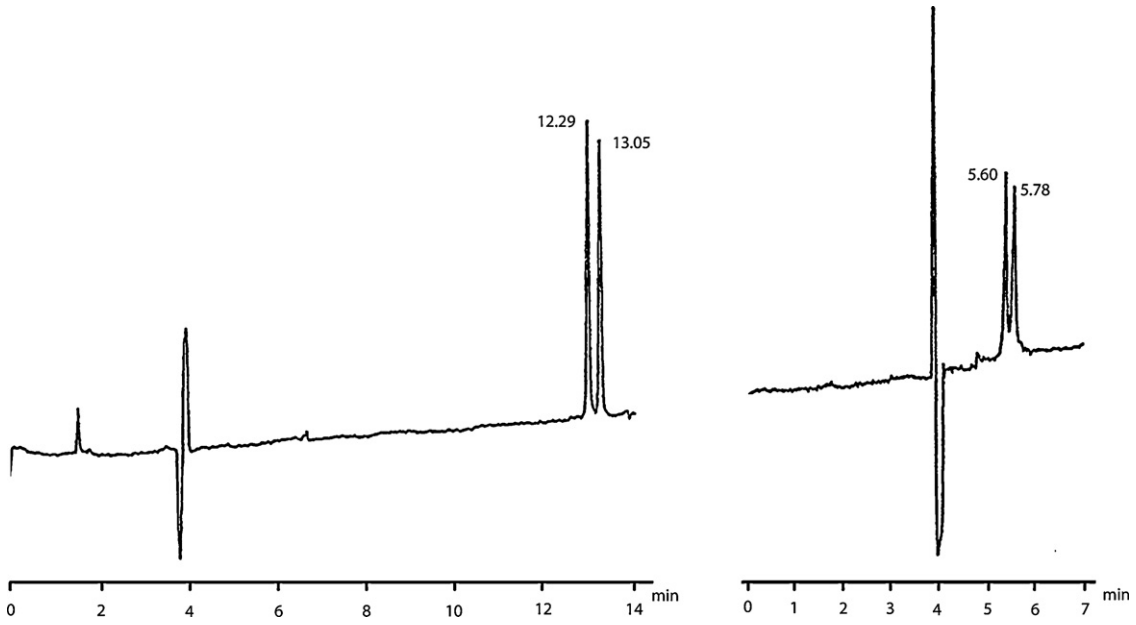


Fig. 7. Chiral separation by CD-MEKC for (a) bioallethrin with 50 mM NaH_2PO_4 + 50 mM SDS + 15 mM DM-β-CD buffer, pH 7 and (b) fenpropathrin enantiomers with 50 mM NaH_2PO_4 + 50 mM SC + 15 mM DM-β-CD buffer, pH 7. Other experimental condition (capillary: 50 μm i.d. × 47 cm L_{ef} (40 cm L_t); pressure injection 2 s; 20 kV; λ = 214 nm). Reprinted with permission from Ref. [17].

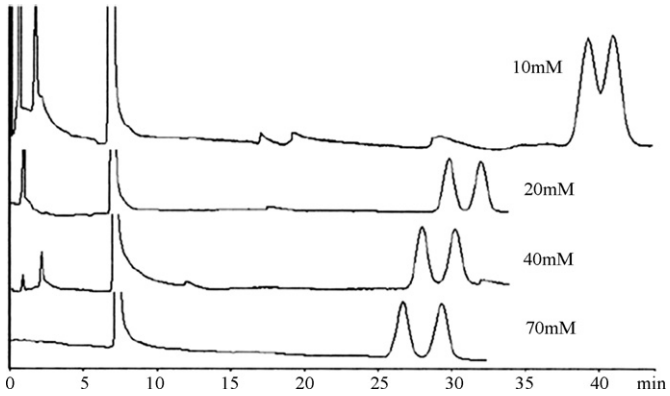


Fig. 8. Electropherograms corresponding to the separation of fenpropathrin with increasing Me-β-CD concentration. Experimental conditions: mobile phase: methanol–25 mM Tris pH 8.3 (75:25) (10, 20, 40, and 70 mM Me-β-CD); column: 30 cm × 75 μm i.d. (21 cm packed length) 5 μm packed Hypersil ODS; voltage: 25 kV; temperature: 20 °C; λ: 214 nm; electrokinetic injection: 5 kV × 3 s. Sample: thiourea and fenpropathrin in 100% methanol. Reprinted with permission from Ref. [7].

β-CD and fenpropathrin (Fig. 7b) with SC bile salt and DM-β-CD [17]. Analysis of fortified water samples under the experimental conditions suitable for baseline resolution of enantiomers of the three pyrethoids studied in this work, yielded recoveries (for the total concentration of each compound, not for the concentration of each enantiomer) ranging from 49 to 84% and detection limits ranging from 0.27 to 2.1 ppb.

There is only one publication in which CEC is used for the enantioseparation of three pyrethroids (fenpropathrin, fenvalerate and fluvalinate) [7]. The chiral separations were investigated by using Hypersil 5 μm ODS stationary phase (Shandon, Runcon, UK), Me-β-CD and HP-β-CD as chiral additives at different concentrations and methanol as organic modifier in a mobile phase 25 mM Tris at pH 8.3. No chiral discrimination was achieved for fluvalinate in any experimental condition. Fenvalerate only showed slight resolution (R_s = 0.42) with Me-β-CD (80 mM). However, the enantiomers of fenpropathrin were partially resolved with HP-β-CD (20 mM, R_s = 0.53) and baseline separation was obtained with Me-β-CD. Fig. 8 shows the separation of the enantiomers of fenpropathrin in a methanol–25 mM Tris pH 8.3 (75:25) mobile phase with different concentrations of Me-β-CD [7]. Optimum resolution (R_s = 1.4) was obtained at 70 mM cyclodextrin concentration. This work also reported the use of CEC for monitoring the extraction of three pyrethroids insecticides from a cellulose matrix by SFE using as supercritical fluid, CO_2. LODs achieved for fenpropathrin, fenvalerate and fluvalinate were 4.7, 3.8 and 2.1 μg/mL respectively.

Finally, Iványi et al. using positively ionizable permethyl monoamino β-cyclodextrin (PMMA-β-CD) separated all enantiomers and diastereomers of chrysanthemic, permethrinic and deltamethrinic pyrethroic acids in one run with the exception of *trans*-chrysanthemic acid, which was only partially separated [71]. PMMA-β-CD seems to be a much better chiral selector than other CDs used in this work.

It can be emphasized that only in one article dealing with the separation of pyrethroids by CE the quantification of the enantiomers was achieved [7].

5.2.4. Subcritical and supercritical fluid chromatography

Only one article has reported the use of sub or supercritical fluid chromatography for the separation of enantiomers of pyrethroids. Although the use of supercritical fluid chromatography is not very extended it can present some advantages when compared to HPLC. For example, when the same columns are used in HPLC and SFC, shorter analysis times can be expected in SFC since the viscosity of supercritical fluid is lower than that for a liquid [72]. Other advantages are easy elimination of the eluent and the use for preparative purposes [72]. Although SFC has been applied to the chiral separation of some compounds, it has rarely been applied to agrochemical compounds [72]. Nishikawa [72] performed the enantioseparation of fenpropathrin and fenvalerate by subcritical (SubFC) and SFC fluid chromatography using the Pirkle type chiral stationary phases developed for HPLC and CO_2 with ethanol, dichloromethane and hexane as modifier to the mobile phase. The Pirkle columns used were Sumichiral OA-2000 ((*R*)-phenylglycine and 3,5-dinitrobenzoic acid) for fenvalerate and Sumichiral OA-4000 for fenpropathrin. Fig. 9 shows the subcritical fluid chromatogram corresponding to the separation of the four enantiomers of fenvalerate (Fig. 9a) and of the two enantiomers of fenpropathrin (Fig. 9b) [72]. The modifiers were 10% ethanol in hexane and hexane respectively. Enantioselectivity mainly depended on the temperature, and not as strongly on other parameters, such

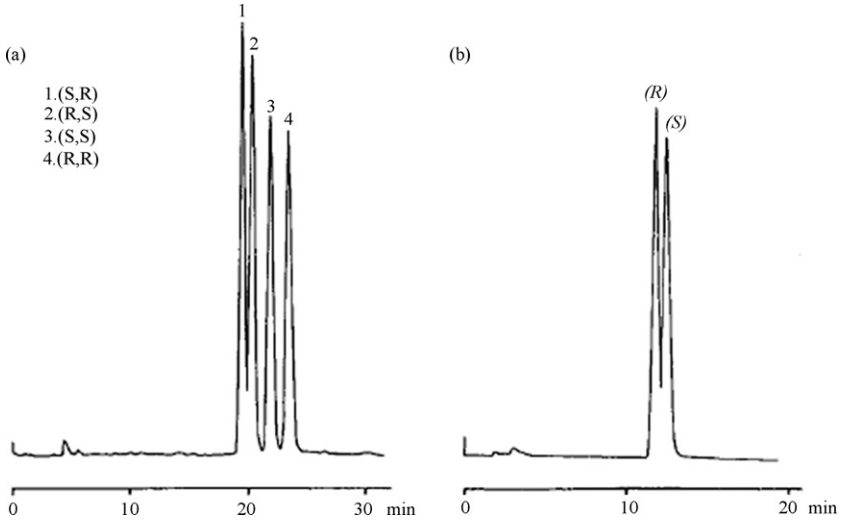


Fig. 9. Subcritical fluid chromatogram of (a) fenvalerate enantiomers on a Sumichiral OA-2000 chiral column (experimental conditions: mobile phase carbon dioxide at 300 μL/min and 10% ethanol in hexane at 10 μL/min; pressure 200 kg/cm^2 (backpressure); column temperature held at 20 °C; detection, UV at 210 nm) and (b) fenpropathrin enantiomers on a Sumichiral OA-4000 chiral column experimental conditions: mobile phase carbon dioxide at 300 μL/min and hexane at 2 μL/min; pressure, 170 kg/cm^2 (backpressure); column temperature held at 25 °C; detection, UV at 210 nm). Reprinted with permission from Ref. [72].

as the nature of the polar modifier. Operation below 20 °C improved the separation while with higher temperatures the resolution was scarce.

6. Concluding remarks and future trends

The chiral separation of synthetic pyrethroids has been mainly achieved by HPLC or GC techniques. Different types of chiral columns based on cyclodextrins have been used for this purpose. The developed methods were applied to different matrices like water, soil, sediments and biological samples. A search of literature cited here indicates few reports about the chiral resolution of these environmental pollutants by CE. MEKC is the mode of CE mainly used in all the publications both with achiral and chiral surfactants necessary due to the poor solubility of pyrethroids in aqueous media. The major concern in CE is the lack of sensitivity. Several approaches may be useful to overcome this problem. They include on-line preconcentration techniques or alternative detection systems. This seems to be a promising area in the study of pyrethroids in real samples.

About the different pyrethroids studied it is necessary to bring out that only few of them have been studied in the field of chiral separations perhaps due to the difficulties in the obtention of pure standards and single isomers. There is a wide range of synthetic pyrethroids yet to be studied for separating their enantiomers.

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