Enantiomeric analysis of pyrethroids and organophosphorus insecticides

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ABSTRACT

The use of pesticides has increased sharply in the last decades, not only in agriculture, but also in industry, public health, and other areas. Pyrethroids and organophosphorus insecticides are among the most employed pesticides. These chemicals usually contain asymmetric chiral atoms; thus, they are characterized by stereoisomerism. Although most of these chiral pesticides are produced, used, and released as racemic mixtures, the different enantiomers of these compounds can present different insecticidal activity, different toxicity against vertebrates and invertebrates, and also different persistence in the environment. In fact, in some cases, only one enantiomer is active, while the other can be less active or even toxic to non-target organisms. Therefore, the development of enantioselective analytical methodologies enabling their determination presents a high interest. Different separation techniques, including high performance liquid chromatography, gas chromatography, supercritical fluid chromatography, and capillary electrophoresis, have been employed to achieve the chiral analysis of pyrethroids and organophosphorus insecticides. This review presents the characteristics of the stereoselective analytical methodologies developed with this aim from 2010 to April 2019 and their applications to the analysis of real samples as well as for toxicity and biodegradation studies.

Keywords: pyrethroids, organophosphorus, pesticides, enantioseparation, toxicity, biodegradation

1. Introduction

Pesticides can be defined as compounds which purpose is to prevent, destroy or control any pest. These substances or mixture of substances are used in agriculture, industry, green area servicing, public health, water reservoirs, etc., to control vectors and pests and to protect the production of harmful organisms and the quality of crops. Additionally, they also have a domestic and livestock use. Since the first pesticide (dichloro diphenyl trichloroethane) was synthesized in 1874, the application of these chemicals has become a common practice worldwide [1]. In particular, from 1950, the use of pesticides has increased approximately 50-fold [2].

Pesticides can be classified according to their median lifetime, toxicity and chemical structure. Regarding their chemical structure, pesticides can be divided into organophosphorus, carbamate, pyrethrin, and organochlorine groups [1]. Among them, organophosphorus pesticides (OPPs) have been the most used for protection against household and agricultural pests because of their ease use, rapid degradation under natural conditions and high activity [3]. Initially, most OPPs were achiral chemicals, but in the late 1960s chiral centers began to be introduced into OPPs. Nowadays, a 30% of the OPPs used have at least one chiral center. For the past three decades, OPPs have been the most widely employed insecticides because of their ability to block the enzyme acetylcholinesterase (AChE) in the target species [4]. However, due to their potentially toxic effects on humans, the US Environmental Protection Agency (EPA) started to ban some of their uses (e.g., non-agricultural applications). This fact has led to the gradual replacement of OPPs by pyrethroid pesticides [5]. Pyrethroid pesticides are synthesized derivatives of pyrethrins. They were first synthesized in 1949 to improve their biological activity and stability [6]. Unlike OPPs, their mode of action affects the transmission of electric impulses as they act on axonal membranes in the nervous system, interacting with sodium channels [6]. These pesticides are persistent

compounds with high hydrophobicity. Therefore, pyrethroids are not very soluble in water [5]. As OPPs, pyrethroids are also chiral compounds, as they can have from one to three chiral centers, which can arise from the alcohol moiety, the acid moiety or both [5, 6]. Thus, pyrethroids can have two, four or eight stereoisomers, and usually they contain one or two pairs of cis/trans diastereomers, or two or four pairs of enantiomers [5].

Although stereoisomers of chiral compounds present the same molecular formula, they deliver different three-dimensional arrangement. Among them, enantiomers have equal physicochemical properties, with the exception of being able to deflect the plane of polarized light to the right or to the left [7]. Moreover, when different enantiomers are exposed to an identical biological environment they can present different biological activity [2]. Additionally, enantiomers can show different toxicity against vertebrates and invertebrates, different biological activity and different persistence in the environment [6]. Likewise, sometimes only one of the enantiomers is active, while the other can be less active or even toxic to non-target organisms [6].

In 1996, around a quarter of the herbicides and insecticides sold were chiral [8]. Nowadays, approximately a 30% of the active principles of the pesticides registered to date possess asymmetry centers [8]. Nevertheless, despite in most of the racemic formulations insecticidal action is mainly attributed to one enantiomer, it is estimated that only a 7% of the currently registered pesticides are marketed as an enriched mixture of the active stereoisomer or as a pure stereoisomer [9]. This is probably due to the high costs involved in the purification and/or production processes [9].

Since stereochemistry affects insecticidal activity, toxicity and distribution in the environment, it is important and necessary to distinguish the enantiomeric and diastereomeric patterns of chiral pesticides. For this reason, chiral methodologies have been developed in the last decades to achieve the enantiomeric separation of pyrethroids and OPPs. For this purpose,

different techniques have been employed, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical fluid chromatography (SFC) and capillary electrophoresis (CE). This article aims to review the analytical chiral separation methodologies reported between 2010 and April 2019 enabling the enantioseparation of pyrethroids and OPPs, and their applications, which supposes an update of the bibliographic review carried out in 2010 by Pérez-Fernández et al. in the case of pyrethroids [6].

2. Enantiomeric separation of pyrethroids

2.1. High Performance Liquid Chromatography

HPLC is the most widely employed technique for the enantiomeric separation of pyrethroids due to its advantageous characteristics, such as its high efficiency and non-destructive property [6, 10]. Moreover, in comparison to GC, HPLC is suitable for the determination of non-volatile, polar, and thermally labile compounds, it also tolerates large injection volumes of sample and has little risk of enantiomerization during analysis [11]. Additionally, HPLC is very useful to obtain enantiopure compounds by small-scale preparation [6]. **Table 1** groups the characteristics of the enantioselective analytical methodologies developed for HPLC analysis of pyrethroids in the period reviewed in this article. UV detection was generally used, except in one case, in which triple quadrupole MS was employed [12]. Applications of some of the methodologies developed included the determination of pyrethroids in real samples [11, 13-18] although no chiral multicomponent separations were reported.

Permethrin (PM) is one of the most studied pyrethroids. This compound is an ester of the dichloro-analogue of chrysanthemic acid and 3-phenoxybenzyl alcohol. It has a cyclopropane ring with two asymmetric carbon atoms, resulting in four enantiomers (two pairs of enantiomers): (+)-cis-PM and (-)-cis-PM (cis-enantiomers), and (+)-trans-PM and (-)-trans-PM (trans-enantiomers) [11, 19]. PM strongly acts on the nervous system of insects;

therefore, it is mainly employed to control pests [11]. However, its use has several drawbacks, since its residues can affect other non-target organisms, such as humans and other living beings. For instance, the exposure to PM residues may affect reproduction in humans and mammals. In addition, PM presents immuno-suppressive potential, and it is considered to be carcinogenic and neurotoxic [19]. Therefore, the presence of this pesticide in the environment needs to be monitored. Shishovska & Trajkovska separated the four PM enantiomers in 42.3 min by RP-HPLC, using a β-cyclodextrin-based stationary phase (ChiraDex® column) with a combination of methanol (MeOH) and water in gradient mode as mobile phase [11]. Van't Hoff's curves for each enantiomer were constructed in the temperature range from 298 to 318 K. Results showed linearity and the influence of more than one process acting simultaneously in the separation of the enantiomers, such as hydrogen bonding and hydrophobic interactions. Limits of detection (LODs) were 0.07 and 0.19 μg for trans-enantiomers and cis-enantiomers, respectively. PM enantiomeric ratio values were determined in a veterinary powder formulation showing that trans-enantiomers/cis-enantiomers ratio in the sample was 74/23% (m/m). Jin et al. also achieved the separation of the four individual PM enantiomers in 11 min, in this case by semipreparative HPLC [19]. A cellulose column (Chiralcel® OJ-H) and a hexane/ethanol (EtOH)/acetic acid mobile phase were used. This method was faster than the one previously developed by Shishovska & Trajkovska. Moreover, cis-enantiomers eluted first, while in the method described by Shishovska & Trajkovska [11], the first-eluting were trans-enantiomers. Once the enantiomers were separated and isolated, they were orally administered at different doses to male mice in order to evaluate their enantioselective toxicity and endocrine disruption activity. Results revealed that (+)-cis-PM, (-)-cis-PM and (-)-trans-PM produced important testicular histopathological damage and endocrine disruption activity, while (+)-trans-PM was the less toxic. More recently, Chalányová and Petránová used an achiral stationary phase (Silasorb Phenyl) to preconcentrate PM, and after preconcentration,

they achieved its enantiomeric separation using a ChiraDex® column and MeOH/water as mobile phase [20]. When comparing this work with that reported by Shishovska & Trajkovska with the same column [11], the analysis time of the method developed by Chalányová and Petránová was reduced in more than a half, achieving the separation of enantiomers in less than 18.0 min.

Another popular pyrethroid used to control insect pests and acarids is fenpropathrin (FPT). Commercial samples of FPT have two enantiomers (R-FPT and S-FPT) at a 1:1 ratio [13]. Tian et al. performed an enantiomeric separation of 8 chiral pesticides, including FPT as the only pyrethroid, by RP-HPLC using cellulose-(tris-3,5-dimehtyl-phenylcarbamate) (CDMPC) and amylose-(tris-3,5-dimethylphenyl-carbamate) (ADMPC) polysaccharide-based stationary phases [21]. Under optimized conditions, the resolution values obtained for FPT enantiomers were 0.35 and 0.59 in the CDMPC and ADMPC columns, respectively. A circular dichroism detector was used to determine the elution order of the enantiomers which was different for both columns (the (+)-enantiomer eluted first in the CDMPC column while in the ADMPC column, the (-)-enantiomer was the first-eluting enantiomer). Also, analysis time was different (16.4 min for CDMPC column and 24.7 min for ADMPC column). More recently, Zhang et al. also developed a method for the determination of FPT enantiomers by RP-HPLC [13]. They tested different cellulose-based chiral stationary phases: Lux™ Cellulose-1, Lux™ Cellulose-3 and Chiralpak® IC, evaluating as well the effect of other parameters, such as the mobile phase composition and the temperature. The best results were achieved on the Lux™ Cellulose-3 column using MeOH/water (85/15, v/v) as mobile phase, with resolution values of 2.30 and eluting first the S-FPT enantiomer. LODs were 0.015 μg g^{-1} for both FPT enantiomers and the method was applied to evaluate the enantiomeric degradation of FPT in soil samples, observing that R-FPT degraded faster than S-FPT.

Bifenthrin (BF), also known as uranus [22], has one chiral center [23] and it is used worldwide in agriculture for pest control and in health care products [24]. It is commercialized in the racemic form of (Z)-cis-BF, which consists of 1R-cis-BF and 1S-cis-BF [12, 23]. Fan et al. developed a method enabling the enantiomeric separation of BF isomers with a polysaccharide derivative bonded chiral column based on amylose (Chiralpak® IF-3) [22]. Liu et al. developed a semipreparative method by HPLC with a cellulose-based chiral column (Chiralcel® OJ) [23] where 1S-cis-BF eluted at 13.9 min and 1R-cis-BF at 16.1 min. Once the enantiomers were separated and isolated, their enantioselective disrupting effects on progesterone and prostaglandin E2 (PGE2) synthesis via protein kinasa C (PKC) pathway in rat ovarian cells was evaluated. Jin et al. developed a semipreparative method employing the same cellulose column to isolate BF enantiomers [24], which were used to evaluate their individual effects on the locomotor behaviour of zebrafish, and whether the locomotor activity is associated with developmental toxicities. Results revealed that 1R-cis-BF is more toxic to invertebrates. Nevertheless, 1S-cis-BF was more harmful to mammals, as previously reported by Liu et al. [23]. Hence, it can be assumed that BF shows opposite enantioselective toxicity in mammalian cells compared to invertebrates. Finally, Yang et al. also developed a semipreparative method to isolate BF enantiomers to evaluate their toxicity and metabolism in zebrafish, in this case, in the presence of cadmium, copper and lead, observing that the toxicity of cis-BF racemate and R-cis-BF increased when metals were added [12]. First, the separation of cis-BF enantiomers was achieved using a Lux™ Cellulose-3 column and hexane/EtOH as mobile phase. The purified fractions of the enantiomers were individually collected and were used for exposure experiments. Zebrafish were exposed to cis-BF enantiomers at different doses under different conditions. 1R-cis-BF caused more mortality than 1S-cis-BF at the same concentration levels, what is in accordance to the results previously reported by Jin et al. [24]. It is worth highlighting that this is the only

article found in the period reviewed which combines HPLC with triple quadrupole MS detection for the enantiomeric determination of pyrethroids.

([(RS)-α-cyano-3-phenoxybenzyl(1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate]), commonly known as cypermethrin (CYM), is a pyrethroid with three chiral carbon atoms, thus, it has 8 isomers (1R-cis-αR, 1S-cis-αS, 1R-cis-αS, 1S-cis-αR, 1R-trans-αS, 1S-trans-αR, 1R-trans-αR, and 1S-trans-αS). This pyrethroid is a semivolatile nonpolar compound used to control different insect pests, especially Lepidoptera [14]. However, among all its isomers, only (+)-1R-cis-S-CYM and (+)-1R-trans-S-CYM have insecticidal activity [15]. Its use presents some drawbacks, since it causes neurotoxicity, genotoxicity, immunotoxicity, endocrine disruption effects and reproductive toxicity in humans. Moreover, the United States Environmental Protection Agency affirms that CYM can act as carcinogen, and it is regarded as toxic to fish and invertebrates [15]. CYM can be commercially found in single enantiomer enriched, diastereomers or racemic formulations [14]. Kuang et al. developed a HPLC method to effectively measure CYM enantiomer concentrations in pig muscle tissue samples [14]. They achieved the partial separation of the eight CYM isomers in 25 min using a phenylcarbamate beta-cyclodextrin chiral column (Chiral CD-ph) and hexane/isopropyl alcohol as mobile phase. Baseline separation could be achieved only for 1R-cis-αR-CYM, 1S-cis-αS-CYM and 1R-trans-αR-CYM. Each peak of CYM was recognized using different commercially available isomer-enriched racemates. The elution order was established as follows: 1R-cis-αR-CYM, 1S-cis-αS-CYM, 1S-cis-αR-CYM, 1R-cis-αS-CYM, 1R-trans-αR-CYM, 1S-trans-αS-CYM, 1S-trans-αR-CYM and 1R-trans-αS-CYM. The method was applied to the analysis of pig samples with LODs for each isomer of 17 µg/kg or higher and recovery values ranging between 67 and 113%. The separation of α-CYM enantiomers, [(+)-(1R-cis-αS)] and [(-)-(1S-cis-αR)], was also carried out in soil samples on a cellulose chiral column (Chiralcel® OD)

and applied to evaluate the enantioselective transformation of α-CYM in soils and its toxicity to earthworms [15]. Xu et al. achieved the enantioselective separation of β-CYM, which contains two pairs of enantiomers (1R-cis-αS/1S-cis-αR and 1R-trans-αS/1S-trans-αR), on two polysaccharide-based chiral columns (Chiralpak® AD and Chiralcel® OD) by HPLC [25]. Chiralcel® OD was selected since good baseline separation was obtained in less time. The elution order of the four enantiomers in this case was established as follows: 1R-cis-αS-CYM, 1R-trans-αS-CYM, 1S-cis-αR-CYM and 1S-trans-αR-CYM. The individual enantiomers were used to evaluate their toxicity in zebrafish embryos, showing significant enantioselective toxicity. Therefore, this highlights the importance of individually considering toxicity of chiral pyrethroids [25].

Another popular pyrethroid is cyphenothrin (CPN), which has 4 pairs of enantiomers (1R-cis-αR, 1S-cis-αS, 1R-cis-αS, 1S-cis-αR, 1R-trans-αS, 1S-trans-αR, 1R-trans-αR and 1S-trans-αS). As other pyrethroids, it exhibits high insecticidal activity against flies, mosquitoes and cockroaches being 1R-trans-αS-CPN the most biologically active [16]. Suzuki et al. separated CPN enantiomers in soil and water samples with the aim of determining the dissipation and degradation profiles of this pesticide through aerobic metabolism in a water-sediment system and its aqueous photolysis [16]. Also, soil adsorption studies to determine the partition profiles of CPN enantiomers were carried out. Soil and water samples were individually analyzed by normal-phase HPLC using three Sumichiral OA-2000 columns connected in a series and hexane/butanol (300/1, v/v) as mobile phase in isocratic mode. Cis isomers eluted before trans isomers, but the analysis time was quite long (the first isomer eluted at 100.6 min and the last one at 119.7 min).

Other common pyrethroid used to control insects such as mosquitoes, fleas, flies and cockroaches is λ-cyhalothrin (λ-CYH). It has two pairs of cis-isomers, but the commercial formula only contains one, corresponding to [Z]-1R-cis-αS-CYH and [Z]-1S-cis-αR-CYH (in

231 a 1:1 ratio) [10]. Li et al. reported the enantiomeric separation of λ-CYH by HPLC under
232 normal phase mode using a cellulose-based chiral column (Chiralcel® OD-H) [10]. Better
233 separation was achieved using hexane/isobutanol (98/2, v/v) as mobile phase with resolutions
234 values > 4.00, achieving the elution of the first enantiomer at 9.5 min and 13.1 min for the
235 second one [10].
236 Some pyrethroids are under development, such as terallethrin (TLL) ((2-methyl-4-oxo-3-
237 prop-2-enylcyclopent-2-en-1-yl) 2,2,3,3-tetramethylcyclopropane-1-carboxylate). This
238 pyrethroid can be used to repel sanitary insects and is still under study to become, in the near
239 future, a mosquito control agent [26]. Twenty chiral pesticides were analyzed by Tian et al.
240 including TLL, for which baseline separation was achieved on an ADMPC column [27]. The
241 use of an ACN/water (60/40, v/v) mobile phase was chosen as optimum with a resolution
242 value of 2.14 for TLL enantiomers being (+)-TLL which eluted first.
243 Regarding the published works reporting the chiral separation of more than one pyrethroid by
244 HPLC [17, 18, 28, 29], as mentioned before, they described different chromatographic
245 conditions for each pyrethroid analyzed. Three pyrethroids (PM, CYM and cyfluthrin (CYF))
246 were separated in all their stereoisomers by Li et al. [28]. As it has been previously described,
247 PM has four stereoisomers, while CYM and CYF have eight. First, all the isomers of the three
248 pyrethroids were separated in an achiral silica gel column and the fractions were collected.
249 Afterwards, for enantioselective separation of the fractions, two chiral columns were tested.
250 With Chiralcel® OJ-H column baseline separation of the four PM stereoisomers was achieved
251 in less than 20.0 min, using hexane/isopropanol as mobile phase. The cis form of PM eluted
252 first than the trans, and the elution order of the four enantiomers was 1S-cis-PM, 1R-cis-PM,
253 1S-trans-PM and 1R-trans-PM. However, baseline separation was not possible in this column
254 for CYM and CYF, so a Chiralcel® OD-H column was used. With this column, baseline
255 separation of the eight CYM and eight CYF enantiomers were achieved using

256 hexane/isopropanol mobile phases. The elution order was 1R-cis-αR-CYM, 1S-cis-αS-CYM,
257 1R-cis-αS-CYM, 1S-cis-αR-CYM, 1S-trans-αS-CYM, 1R-trans-αR-CYM, 1R-trans-αS-
258 CYM, 1S-trans-αR-CYM, for CYM, and 1S-cis-αS-CYF, 1R-cis-αR-CYF, 1R-cis-αS-CYF,
259 1S-cis-αR-CYF, 1S-trans-αS-CYF, 1R-trans-αR-CYF, 1R-trans-αS-CYF, 1S-trans-αR-CYF
260 for CYF. Although CYM and CYF structurally only differ in one atom, the elution order of
261 one of the diastereomers was reversed. Once the separation was performed, the fractions were
262 collected and solutions of three specific enantiomers (1R-trans-PM, 1R-trans-αS-CYM and
263 1R-trans- αS-CYF) were prepared and subjected to photolysis experiments. More recently, the
264 same research group used the same chiral HPLC method for the stereoselective separation of
265 the same 3 pyrethroids in soil samples [17]. The method was applied to determine the stereo-
266 and enantioselective degradation of this 3 pyrethroids in different soil samples, a Shijiazhuang
267 alkaline yellow soil and a Wuhan acidic red soil. Soil samples were first extracted by matrix
268 solid-phase dispersion (MSPD) using florisil as sorbent and hexane/ethyl acetate (7/1, v/v) as
269 elution solvent. Degradation was more favourable in the alkaline soil than in the acidic soil. In
270 addition, trans-diastereomers degraded faster and showed higher enantioselectivity than their
271 corresponding cis-diastereomers.
272 Zhang et al. studied the enantiomeric separation of two pyrethroids, BF and λ-CYH [18]. The
273 best separation for BF was achieved using Lux™ Cellulose-3 with MeOH/water (95/5, v/v).
274 For λ-CYH, when the mobile phase consisted of MeOH/water, baseline separation was
275 obtained on Lux™ Cellulose-1 and in Lux™ Cellulose-3 although this last column gave rise
276 to a higher resolution. A 90/10 MeOH/water mobile phase was selected for method validation
277 and quantitative analysis of BF and λ-CYH in soil and water samples (**Fig. 1**). LODs for BF
278 and λ-CYH were 0.01 and 0.015 mg L^{-1}, respectively. Recovery values of BF ranged between
279 91-100 and 91-101% and for λ-CYH between 91-100 and 93-98% in soil and water samples,
280 respectively. Wang et al. used chiral HPLC as a semipreparative method to separate the

enantiomers of cis-BF, PM and fenvalerate (FEN) [29]. To resolve the enantiomers, a Chiralcel® OJ column and different mobile phases were used for each pyrethroid: hexane/EtOH (95/5, v/v) for cis-BF, hexane/isopropanol (95/5, v/v) for PM and hexane/EtOH (90/10, v/v) for FEN. Under these conditions the elution order was: 1R-cis-BF, 1S-cis-BF; 1R-cis-PM, 1S-cis-PM, 1R-trans-PM; 1S-trans-PM, αR-2S-FEN, αR-2R-FEN, αS-2R-FEN, αS-2S-FEN. The individual enantiomeric fractions (EFs) were collected and subsequently subjected to bioassays to determine their endocrine disruption activity. PM and FEN did not experience estrogenic potential activities at the tested concentrations.

2.2. Gas Chromatography

Although HPLC is the most widely used technique for the enantiomeric analysis of pyrethroids, GC has also been extensively employed with this aim as shown in **Table 2**. GC is a very suitable technique because of its low injection volumes and its high sensitivity, which provide low LODs values able to determine pyrethroids residues at very low levels in accordance to their environmental occurrence [6, 30]. Nevertheless, despite its advantages, GC also presents some drawbacks, which may be the reason for its lower popularity compared to HPLC, such as the long analysis times required and the thermal instability of some pyrethroids [6]. In the period reviewed, BGB-172 column, which is based on β-cyclodextrin (20% tert-butyldimethylsilyl-β-cyclodextrin in 15% phenyl-, 85%-methylpolysiloxane), was used as chiral stationary phase. Moreover, unlike HPLC, GC methods developed were often based on the use of MS detection, using triple quadrupole mass analysers.

Khazri et al. achieved the enantioseparation of CYM using GC coupled to tandem MS [31]. Under the optimized conditions, only baseline separation of cis-CYM enantiomers was possible, according to the following elution order: 1R-3R-αR-CYM (1st peak), 1S-3S-αS-

306 CYM (2^{nd} peak), 1R-3R-αS-CYM (5^{th} peak) and 1S-3S-αR-CYM (6^{th} peak). Pairs of peaks
307 three and four (1S-3R-αS-CYM/1R-3S-αR-CYM) and seven and eight (1R-3S-αS-CYM/1S-
308 3R-αR-CYM) corresponded to trans-CYM diastereoisomers, but it was not possible to
309 discriminate between the enantiomers. CYM stereoselectivity in freshwater mussels was
310 evaluated. Yao et al. separated α-CYM enantiomers to study its enantioselective degradation
311 behaviour and metabolism in bullfrog through its oral administration and water exposure [32].
312 Multicomponent analysis methods enabling the simultaneous enantiomeric separation of
313 pyrethroids have been developed by GC. Kuang et al. separated the enantiomers of CYM and
314 cis-BF in order to obtain their EF in Chinese tea samples [33]. Helium as carrier gas and an
315 electron capture detector (ECD) were used. Baseline separation was achieved for cis-BF,
316 eluting first the (+)-enantiomer. In the case of CYM, from its eight stereoisomers, only six
317 were resolved on the column since two of them were not baseline separated, according to the
318 subsequent elution order: 1R-3R-αR-CYM (1^{st} enantiomer), 1S-3S-αS-CYM (2^{nd}
319 enantiomer), 1R-3S-αR-CYM/1S-3R-αS-CYM (3^{rd} and 4^{th} enantiomers not baseline
320 separated), 1R-3R-αS-CYM (5^{th} enantiomer), 1S-3S-αR-CYM (6^{th} enantiomer) and 1R-3S-
321 αS-CYM/1S-3R-αR-CYM (7^{th} and 8^{th} enantiomers not baseline separated). The analysis of 19
322 tea samples revealed the presence of cis-BF residues, ranging from 14.25 to 3071.29 μg kg^{-1},
323 while 17 teas presented CYM residues ranging between 20.13 and 187.65 μg kg^{-1}, what
324 highlights the exposure risk of consumers to these contaminants. Ulrich et al. also developed a
325 multicomponent method for the chiral separation of pyrethroids by GC [34]. First, the elution
326 order of BF, PM, CYF and CYM was studied in combination with helium as carrier gas and a
327 MS detector. BF was the first pyrethroid eluted (79.0 min), with the (+)-R-enantiomer
328 preceding the (-)-S-enantiomer. PM eluted later (95.0 min), with an elution order of (+)-1R-
329 cis, (-)-1S-cis and trans-(±) isomers, which eluted in a single peak since it was not possible to
330 enantiomerically separate them. At approximately 100.0 min, five of the eight CYF

stereoisomers eluted separately. Finally, CYM eluted at approximately 105.0 min, with five of the eight stereoisomer peaks separated. Since baseline separation was only achieved for BF and cis-PM, further studies were only related to the enantiomeric determination of these two pyrethroids in sediments, water and fish samples.

Corcellas and co-workers achieved the multicomponent determination of the enantiomers of 6 pyrethroids (cis-BF, CYH, CYF, CYM, PM and tetramethrin (TRM)) in less than 75.0 min with resolution values higher than 0.58 [35]. Triple quadrupole MS was used for detection and the temperature gradient program was optimized. EFs were determined in commercial insecticides and human breast milk samples. Results showed that insecticides samples contained the two pairs of TRM enantiomers in racemic proportion. Also, cis-isomers of CYM were found in racemic proportion in these samples, while cis-isomers of PM were in a non-racemic mixture (values for the EF of cis-PM ranged between 0.35 and 0.38, which showed an enrichement of 1S-3S-enantiomer). In the case of human breast milk samples, CYF was the only pyrethroid not detected. The EF value for cis-PM in these samples was 0.43±0.02 which indicated higher accumulation of the first eluting enantiomer 1R-3R-PM. For cis-BF the mean EF value was 0.47, which indicated racemic behaviour. Cis-TRM also showed racemic behaviour, however, the second eluting enantiomer of trans-TRM was more predominant in human breast milk samples. For CYH, the enantiomer 1S-3S-αR-CYH was more abundant in milk samples, while in the case of CYM no significant differences were detected among these samples and the racemic standard, nor among the insecticide samples. Thus, this study suggested selective bioaccumulation of CYH, CYM and TRM in humans [35]. The enantioselective bioaccumulation of the target analytes in edible river fish samples was also evaluated [36]. Different types of fish such as gudgeons, barbels, catfish and trouts were analyzed. As an example, **Fig. 2** shows the achiral and chiral separation of CYM (**Fig. 2a**) and its determination in barbel and catfish samples (**Fig. 2b**). Pyrethroids were detected in

all the samples analyzed, at levels ranging between 12-4938 ng g^{-1} lipid weight. Preferential bioaccumulation of cis-isomers was observed, except in the case of TRM. EF of PM showed very dissimilar values depending on the type of fish. These differences could be due to the different commercial mixture used. For CYH, the EFcis1 (1R-3R-αR-CYH and 1S-3S-αS-CYH) showed racemic enantiomeric behaviour in gudgeons, while the EFcis2 (1R-3R-αS-CYH and 1S-3S-αR-CYH) indicated enrichment of 1R-3R-αS-CYH. The opposite behaviour was observed in barbel and catfish, which could be attributed to different exposures, since gudgeons come from different rivers than barbels and catfishes. All samples analyzed, except in the case of catfishes, presented CYM EF values lower than 0.5, being the cis1-CYM enantiomeric pair enriched in the second eluting enantiomer 1S-3S-αS-CYM. However, the cis2-CYM EF presented enrichment of the second eluting enantiomer (1S-3S-αR-CYM) in all samples, including the catfish's samples. The cis1-CYF EF was always lower than 0.39, but the EFcis2 of CYF was only calculated in one sample, being 0.60. Therefore, authors concluded that there was no correlation between enantioselectivity of one enantiomeric pair and the other [36]. More recently, the same analytical methodology was applied to the characterization of the EFs of BF, CYH, CYF, CYM, PM and TRM and the evaluation of their enantioselective bioaccumulation in wild bird egg samples [37]. As in the two previous works, cis-isomers of PM and CYM were more accumulative than trans ones (cis/trans ratio values greater than 1). In the case of TRM, cis/trans ratio values were around 0.25, as in a previous work [36]. This is probably because commercial mixtures are usually enriched in trans-TRM as it is the enantiomer with more insecticidal efficacy. CYH and CYM cis1/cis2 ratio values were close to 1, which indicated that there was no preference between both cis isomers, except in the case of CYM in gadwalls, where enrichment of cis2 isomers was observed. In relation to enantiomeric factors, most samples showed racemic mixtures of cis-enantiomers for PM and TRM. However, for black kites and black-headed gulls, the cis-PM

EF values indicated selective accumulation of the second eluting enantiomer 1S-1S-cis-PM, while cis-PM EF values in glossy ibis showed the opposite behaviour. Trans-TRM EF values indicated selective accumulation of 1S-1R-trans-TRM, which was the second eluting enantiomer. The same result was obtained in the previous study described for human breast milk samples [35]. When white storks' eggs samples were analyzed, BF presented racemic behaviour. However, in black-headed gull and black kite, a preference for the first enantiomer 1S-1S-BF was observed. Regarding EF of CYH and CYM, results showed similar enantiomeric-selective accumulation to the ones observed in the previous studies reported for biotic samples and river fishes [36, 37]. To sum up, Corcellas et al. developed an effective, reproducible and sensitive chiral methodology for pyrethroids, which has been applied for the first time to the analysis of terrestrial biota tissues, river fish and human breast milk samples.

2.3. Supercritical Fluid Chromatography

In supercritical fluid chromatography (SFC), supercritical fluids with low viscosity and high diffusivity are used as mobile phase, being supercritical carbon dioxide the most widely used. This technique can be employed for the separation and purification of chiral and achiral molecules since stationary phases used are the same as in standard HPLC systems. However, SFC presents some advantages over HPLC. For instance, when the same columns are used in both techniques, SFC provides shorter analysis times than HPLC, due to the lower viscosity of the supercritical fluid compared to that of the liquid. Therefore, higher linear velocities can be expected in SFC. This increase in the flow rates reduces the analysis time and greatly improves productivity of the enantiomers separation. Moreover, this technique is very suitable for semipreparative purposes and presents green features, since the eluent used can be easily removed [30, 38, 39]. Nevertheless, despite the advantages, SFC has scarcely been used to perform chiral separation of pyrethroids in the period reviewed as shown in **Table 3**. Yan et

al. [39] separated β-CYM stereoisomers by SFC using polysaccharide-based chiral columns. First, a one-step direct method using an amylose column (EnantioPak® AD) with supercritical CO_2/isopropanol (95/5, v/v) was developed enabling the effective separation of the four stereoisomers of β-CYM. To improve separation efficiency and reduce solvent consumption, a two-step combined strategy using different polysaccharide-based chiral stationary phases was proposed. In the first step, β-CYM was separated in two stereoisomeric pairs, denoted P1 and P2, using a cellulose-derived chiral column (EnantioPak® OD) with supercritical CO_2/isopropanol (95/5, v/v) as mobile phase. Fraction P1 corresponded to 1R-cis-αS and 1R-trans-αS, while fraction P2 consisted of S-cis-αS and 1S-trans-αS. Both pairs were separated into four enantiopure isomers using an EnantioPak® AD column. EtOH was chosen instead of isopropanol to accelerate the elution of samples. P1 was separated using supercritical CO_2/EtOH (80/20, v/v) as mobile phase, while P2 was separated with supercritical CO_2/EtOH (85/15, v/v). According to the elution order, the absolute configurations of the four enantiopure stereoisomers were 1R-cis-αS, 1R-trans-αS, 1S-cis-αR and 1S-trans-αR. Circular dichroism spectra confirmed that the first and third eluted isomers were a pair of enantiomers, as well as the second and fourth. Jin et al. also used SFC for the enantiomeric separation of cis-BF [40]. A cellulose-based column (Chromegachiral™ CCJ) was used with supercrital CO_2/MeOH (85/15, v/v) as mobile phase in order to achieve a complete enantioseparation of the pyrethroid. Two separated peaks were obtained at 3.3 and 3.7 min, corresponding to 1R-cis-BF and 1S-cis-BF, respectively. SFC was used as a semipreparative method, so once the enantiomers were separated, their fractions were collected in order to evaluate their enantioselective toxicity and endocrine disruption activity in male mice, observing that both enantiomers showed endocrine disruption activities.

2.4. Capillary Electrophoresis

In capillary electrophoresis (CE), analytes migrate through electrolyte solutions inside a capillary tube under the influence of an electric field and their separation is achieved according to their ionic mobility and/or their non-covalent partitioning with alternative phases. CE can be considered a powerful analytical technique to achieve chiral separations, since it presents numerous advantages, including high efficiency, simplicity (since no chiral columns are needed) and low consumption of chiral selectors, reagents and samples [41]. In addition, shorter analysis times and higher resolutions can be achieved with CE compared with other techniques such as HPLC or GC, and it can be applied for the enantioseparation of a wide range of analytes in different research fields such as pharmaceutical analysis or environmental and food samples. However, despite all its advantages, few articles reported the chiral separation of pyrethroids by CE [6]. Pérez-Fernández et al. used micellar electrokinetic chromatography (MEKC) for the first time for the enantiomeric separation of cis-BF [42]. In MEKC, analytes are separated by differential partitioning between micelles (acting as a pseudo-stationary phase) and an aqueous solution [43]. The new chiral analytical methodology for cis-BF was developed using MEKC with cyclodextrins (CDs) as chiral selectors. Baseline separation of cis-BF enantiomers was achieved using 100 mM sodium cholate as surfactant in combination with 20 mM of heptakis-(2,3,6-tri-*O*-methyl)-β-CD in a 100 mM borate buffer (pH 8.0) with 2 M urea at 15 ºC and a separation voltage of 30 kV. 1S,3S-BF and 1R,3R-BF were separated in 9.2 min with a resolution of 2.8. The method was applied to the quantitation of cis-BF enantiomers in a polyvalent commercial insecticide formulation (**Fig. 3**). The LODs for the first and second migrating enantiomers were 4.8 and 3.9 mg L^{-1}, respectively. The quantitative determination of cis-BF in the commercial insecticide revealed a total concentration of 2077 ± 89 mg L^{-1} (labelled content 2000 mg L^{-1}), being 1060±39 and 1017±49 mg L^{-1} the concentration of the first and second migrating enantiomers, respectively [42].

3. Enantiomeric separation of organophosphorus pesticides

3.1. High Performance Liquid Chromatography

As in the case of pyrethroids, HPLC has been the most employed technique to achieve the enantiomeric separation of organophosphorus pesticides (OPPs). **Table 4** groups the characteristics of the chiral methods developed in the last years. UV or MS/MS have been selected as detection systems for the enantiomeric determination of OPPs. Most of these works focused on the separation of one single OPP [4, 21, 27, 44-68] and describe applications of the developed methods to the determination of OPPs in real samples [4, 46, 47, 49-51, 53-55, 62, 64-67]. In some of these works, the simultaneous enantiomeric analysis of OPPs was reported [69-75].

Chai et al. separated the enantiomers of crufomate (CRF). This insecticide is one of the most important OPPs, and it is mainly used to handle livestock and prevent torsaloes, parasites *in vitro* and intestinal worms [44]. It only has one asymmetric phosphorus center, which results into two enantiomers. Several polysaccharide-based chiral columns (Lux™ Cellulose-1, Lux™ Cellulose-2, Lux™ Amylose-2 and Lux™ Cellulose-3) were tested in NP-HPLC and RP-HPLC. Baseline separation was achieved by NP-HPLC when Lux™ Cellulose-1, Lux™ Cellulose-2 or Lux™ Amylose-2 were used. Also, separation was obtained in RP-HPLC with Lux™ Cellulose-1 or Lux™ Amylose-2. In NP-HPLC, on Lux™ Cellulose-2, (-)-CRF was firstly eluted, while on Lux™ Cellulose-1 and Lux™ Amylose-2 (+)-CRF was the first-eluting enantiomer. This was due to the different substituted groups of the chiral stationary phases, which affect their chiral discrimination power for CRF. In RP-HPLC the elution order of the enantiomers on Lux™ Cellulose-1 and Lux™ Amylose-2 was the same as in NP-HPLC. The best resolution was obtained using Lux™ Cellulose-2 on NP-HPLC [44].

O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN), is another OPP with acaracide activity, which is also an endocrine-disrupting chemical with estrogenic and antiandrogenic activity [76]. Due to its low cost and broad spectrum activity it has been widely used for agricultural purposes in many countries. Sun et al. investigated the enantiomeric separation of EPN in four different polysaccharide-based chiral columns (Chiralpak® AD, Chiralpak® AS, Chiralcel® OD and Chiralcel® OJ) using hexane as mobile phase in combination with a polar modifier (EtOH or isopropanol) [45]. Baseline separation of EPN enantiomers was successfully achieved using Chiralpak® AD and Chiralpak® AS columns with hexane/isopropanol and hexane/EtOH as mobile phases, respectively. Although good separation was achieved with both columns, better resolution was obtained when using Chiralpak® AD column. The two enantiomers of EPN were collected and used for aquatic toxicity assays using *Daphnia magna* and zebrafish embryos. Results in *Daphina magna* revealed that (+)-EPN was about 10 times more toxic than (-)-EPN. However, in zebrafish embryos, EPN presented an opposite enantioselective behavior [45].

Ethyl 4-methylthion-m-tolyl isopropylphosphoramidate, commonly known as fenamiphos (FAP), is a thioether insecticide OPP [4, 46, 47]. FAP has an asymmetric chiral center at the phosphorus atom, and therefore one pair of enantiomers [4]. It is a racemic chiral nematicide commonly used in the production of crops such as fruits, vegetables, tobacco and grains. In addition, it is considered potentially toxic to land and aquatic organisms [46]. Tian et al. studied the enantiomeric separation of twenty chiral pesticides, including 5 OPPs (FAP, profenofos (PFF), malathion (MA), isofenphos-methyl (IFM) and phenthoate (PTH)) [27]. Among these OPPs, only the separation of FAP enantiomers on an ADMPC column was possible. An ACN/water (60/40, v/v) mobile phase was chosen as optimum giving rise to a resolution of 1.89 in 7.5 min. The use of a circular dichroism detector showed that the (+)-FAP isomer eluted first than (-)-FAP. Wang et al. developed a semi-preparative method for

505 the separation of FAP enantiomers using an amylose-based column (Chiralpak® AD-H) with
506 hexane/EtOH as mobile phase [48]. Unlike in the previous study carried out by Tian et al.
507 [27], (-)-FAP enantiomer eluted first but better resolution was obtained. The separated and
508 isolated enantiomers were used to study their toxicity to arthropods and their inhibition
509 potential towards AChE in the rat pheochromocytoma 12 (PC12) cell line. In order to
510 evaluate the enantioselectivity in aquatic toxicity of FAP, *Daphnia magna* was used because
511 it is very sensitive to OPPs, showing that R-(+)-FAP enantiomer was about 2.4 times more
512 toxic than the S-(-)-FAP enantiomer. Cai et al. also separated FAP enantiomers employing a
513 Chiralpak® AD-H chiral stationary phase to investigate the stereochemistry of the successive
514 sulfoxidation of FAP in three different soils as well as their toxicity in zebrafish embryos
515 [47]. FAP sulfoxidation to the sulfoxide intermediate (FSO) was the primary transformation
516 process. Additionally, FSO was subsequently oxidated to the sulfone intermediate (FSO_2).
517 Both processes were biotic and stereoselective. Enantiomerization/diastereomerization of
518 FSO also took place. Hydrolysis of FAP, FSO and FSO_2 to phenols, which was biotically
519 favourable, but not stereoselective, took place at lower rates. More recently, Pérez de
520 Albuquerque and co-workers developed a new method in order to analyse FAP and its
521 metabolites [4]. Eleven chiral columns were evaluated obtaining the best separation with an
522 amylose-based column (Chiralpak® AS-H). It was necessary to couple an achiral silica
523 column to the Chiralpak® AS-H column, because coelution of FSO and FSO_2 enantiomers
524 was observed when performing simultaneous injection of FAP and its metabolites. As in the
525 previous investigation by Wang et al. [48], the (-)-FAP enantiomer eluted first. The method
526 was applied to the analysis of FAP in human liver microsomes, followed by characterization
527 of its metabolism and prediction of some toxicokinetic properties. FAP was stereoselectively
528 eliminated from the liver. Unlike the previous works, Damianys et al. separated FAP
529 enantiomers on a cellulose-based chiral stationary phase (Chiralcel® OJ column) instead of

530 an amylose-based column as in the previous studies described for FAP enantioseparation [46].
531 Hexane/EtOH (99/1, v/v) was used as mobile phase. As for Tian et al. [27], the first eluted
532 enantiomer was (+)-FAP. However, the resolution was lower and the analysis time was longer
533 than that obtained by Tian and co-workers [27]. The metabolic evaluation of FAP by the
534 alloforms of PON1 192 from human serum of children and adults was achieved. A low
535 hydrolysis for both FAP enantiomers by the three alloenzymes of PON1 Q192R from human
536 sera of children and adults was observed due to the different bonding modes of the insecticide
537 in the active site of PON1 and due to the differences in the reaction rate of limiting reaction
538 step. This lack of hydrolysis demonstrated that PON1 has limited role as a detoxifying agent
539 of FAP. Studies carried out in the period reviewed indicated that amylose-based columns are
540 more effective for separating FAP enantiomers than cellulose-based columns, achieving better
541 resolution values and lower analysis times.
542 Isocarbophos ((R,S)-O-2-isopropoxycarbonylphenyl O-methylphosphoramidothioate; ICP) is
543 one of the most employed OPPs and acaricides [49]. ICP is a potent AChE inhibitor and is
544 widely used to control sucking and chewing insects and spider mites on crops [50]. Due to its
545 high toxicity, China has banned its use on vegetables and fruits. However, it is still widely
546 used in rice and cotton cultivation [51]. ICP possesses a chiral center at the phosphorus atom
547 resulting in two enantiomers [52]. Works reporting the enantiomeric separation of ICP by
548 HPLC in the last years used polysaccharide-based chiral columns of cellulose and amylose.
549 Liu et al. developed a method enabling the enantiomeric separation of ICP [52] using
550 Chiralcel® OD column in normal phase mode. The resolved enantiomers were collected
551 manually and were used for bioassays. Liver hepatocellular (Hep G2) cells were used as *in*
552 *vitro* model to assay the cytotoxicity of ICP enantiomers showing that (-)-ICP enantiomer was
553 about two times more toxic than its antipode in Hep G2 cells. Zhao and co-workers also used
554 a cellulose-based chiral column (Chiralcel® OD-RH) to achieve the enantiomeric separation

555 of ICP, but in reverse phase mode [53]. The use of a 0.1% formic acid/ACN (60/40, v/v) as
556 mobile phase allowed the separation of (+)-ICP and (-)-ICP at 16.2 min and 17.4 min,
557 respectively. Before analysis, aqueous environmental samples were subjected to solid-phase
558 extraction (SPE) followed by dispersive liquid-liquid microextraction (DLLME) to extract
559 and purify the analytes. ICP was not detected in the river water and effluent samples and it
560 was detected in the influent sample at a concentration level lower than its limit of
561 quantification (LOQ). More recently, the same chromatographic method was employed by the
562 same research group for the simultaneous enantiomeric analysis of eight pesticides, being ICP
563 the only organophosphorus among them [54]. In the preliminary experiments, an amylose-
564 based chiral column (Chiralpak® IA) and four cellulose-based chiral columns (Chiralpak®
565 IC, Chiralpak® IB, Chiralcel® OJ-RH and Chiralcel® OD-RH) were tested, but best results
566 were achieved with Chiralcel® OD-RH column. Soils and river sediments (green belt soil,
567 farmland soil and river sediment) were analyzed after combined DLLME and MSPD. No
568 obvious stereoselectivity occurred during the biological degradation process. Additionally, in
569 the green belt soil, ICP was not detected, while in farmland soil and in river sediment, ICP
570 was not quantified, since it was detected at lower levels than its LOQ. Tian and co-workers
571 also achieved the enantiomeric separation of 8 chiral pesticides including ICP [21] by RP-
572 HPLC using cellulose CDMPC and amylose ADMPC polysaccharide based stationary phases
573 synthesized by them. Complete baseline separation of ICP enantiomers was achieved in the
574 ADMPC column (R_s=1.79) in 41.3 min, while near-besaline separation was obtained in the
575 CDMPC column (R_s=1.33) in 13.5 min. Additionally, both columns provided different elution
576 order. In the case of the CDMPC column, the (+)-enantiomer eluted first as in the previous
577 works reported by Zhao et al. [53, 54], while in the ADMPC column, the (-)-enantiomer was
578 the first-eluting enantiomer. Zhang et al. also isolated ICP enantiomers, using a Chiralpak®
579 AD-RH (amylose tris(3,5-dimethylphenylcarbamate)) column [49] and obtained the elution of

580 the two ICP enantiomers in 2.3 min, eluting first the R-(-)-ICP enantiomer, as reported by
581 Tian et al. for ADMPC colum [21]. The degradation of ICP was studied in three different
582 soils (Hangzhou, Zhengzhou and Changchun) under native or sterilized conditions (**Fig. 4**).
583 Under sterilized conditions, ICP enantiomers were stable, while under native conditions an
584 enantioselective degradation of ICP occurred. Yao et al. enantioselectively determined ICP
585 and its main metabolite ICP oxon in soil, as well as in rice and water samples [51]. They also
586 used an amylose-based chiral column (Chiralpak® AD-3R) and a gradient elution as mobile
587 phase. The elution order was the same as that reported by Zhang et al. [49] ((-)-ICP eluted
588 first than (+)-ICP). However, the analysis time achieved by Yao and co-workers was longer
589 (9.4 min) [51]. The simultaneous determination of ICP and ICP oxon was achieved in ten rice
590 samples, fifteen soil samples and five water samples. Among all the samples, just one soil
591 sample was positive. Qi et al. used the same chiral stationary phase as Yao et al. [51], but they
592 achieved the enantiomeric separation of ICP in 3.0 min with a resolution value of 2.46 [50].
593 ICP enantiomers were determined in one hundred samples of orange pulp, peel and kumquat.
594 Results showed that only orange pulp was free of ICP. As a conclusion, it can be affirmed that
595 the elution order of ICP enantiomers when cellulose-based columns are used is +/-. However,
596 when amylose-based columns are used, (-)-ICP enantiomer eluted first. In addition, to date,
597 the best separation conditions have been achieved with amylose-based chiral columns.
598 Another OPP very effective to control the presence of insects in soil and in a wide range of
599 fruits, vegetables and crops, such as maize, soybean, sweet potato, peanut, apple and wheat is
600 (R,S)-O-methyl-O-(2-isopropoxcarbonyl)-phenyl-N-isopropylphosphoramidothioate,
601 commonly known as isofenphos-methyl (IFM). IFM is a chiral OPP which acts through skin
602 penetration and stomach poisoning. Additionaly, this chiral insecticide can inhibit the activity
603 of AChE in the nervous system, avoiding its breakdown and resulting in different hazardous
604 effects. Gao et al. separated IFM in its two enantiomers in approximately 20.0 min using a

chiral Lux™ Cellulose-3 column in reversed mode [55]. Under the optimized conditions, (S)-(+)-IFM was the first eluted enantiomer. LODs for the two IFM enantiomers in different vegetables, fruits and soil matrices were in the range of 0.008 to 0.011 mg kg^{-1}.

Methamidophos (O,S-dimethyl phophoramidothioate; MTD) is a chiral OPP with an asymmetric center at the phosphorus atom. MTD is used in agriculture to control chewing and sucking insects and spider mites on different crops [56]. Nevertheless, its toxicity is not only limited to target insects, it also affects to human and animal causing them acute and delayed toxic effects [57]. For this reason, Emerick et al. developed a method to separate MTD enantiomers by HPLC to study their toxicity [56-58]. Four different analytical columns were tested [56] and finally, a Chiralcel® OD column was used with hexane/isopropanol (90/10, v/v) as mobile phase, eluting first the (+)-MTD enantiomer [56-58]. Once MTD enantiomers were separated, their *in vitro* inhibition activity of butyrylcolinesterase (BChE) in hens [56] and their delayed neuropathy effects in hens [57, 58] and humans [58] were evaluated. Enantioselective toxicity and significant differences between species were observed.

Because of the high toxicity of MTD, great efforts to synthesize new derived insecticides have been made in order to replace its use. This is the case of O,S-dimethyl-N-(2,2,2-trichloro-1-methoxyethyl)phosphoramidothioate, commonly known as MCP, which is a new chiral OPP that consists of four stereoisomers. MCP is highly active to insects and has low acute toxicity towards humans. However, it potentially induces delayed neuropathy when it is used as racemic mixture. Zhou et al. achieved its synthesis from MTD and separated MCP into its four stereoisomers [59]. Although various polysaccharide chiral stationary phases were tested, the enantiomeric separation, with resolution higher than 1.5, was achieved using a Chiralpak® AD column. The toxicity of the four enantiomers was evaluated in *Daphnia magna* studying the stereomeric selectivity of MCP in acute and delayed neurotoxicities. Among the enantiomers of MCP, the first eluted one was the most effective against insects

and produced less neurotoxic effects. For this reason, MCP should be formulated not as racemic, but as a pure enantiomer [59].

Another organophosphorothiolate insecticide employed in agriculture for pest control is PFF (O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate). PFF has a chiral center at the phosphorus atom, resulting in two enantiomers. Lu et al. obtained pure enantiomers of PFF using a Chiralcel® OJ column with hexane/isopropanol (99/1, v/v) as mobile phase [60]. They also evaluated their cytotoxicity and the DNA damage in PC12 cells showing that cell viability is enantioselectively reduced by PFF and that DNA damage in PC12 cells is induced by PFF.

Pyraclofos (PYR) is also an OPP with an optically active phosphorus atom [61]. This OPP belongs both to veterinary and pesticide categories [62]. Due to its high efficiency and capacity to manage multi-OPP-resistant pests, it is commonly used to control nematode, lepidoptera, coleoptera and acarina pests [63]. Also, it can be used in combination with the medication albendazole as an antihelmintic in sheep [62]. All works reporting the enantiomeric separation of PYR used a cellulose-based chiral stationary phase. Zhang et al. used a Chiralcel® OD column in normal phase mode from which the (-)-isomer eluted first [61]. The enantioselective toxicity of PYR enantiomers to human BChE and *Daphnia magna* was evaluated. IC_{50} values from anti-BChE tests demonstrated that (-)-PYR was more potent than its antipode, (+)-PYR. Nonetheless, aquatic assays showed that (+)-PYR was about six times more toxic than (-)-PYR.

Xu et al. separated PYR enantiomers in a cellulose-based chiral stationary phase (Lux™ Cellulose-4 column), but in this case, in reverse phase mode in just 10.4 min (S-(+)-PYR eluted first) [62]. The enantioselective degradation of PYR in three soils (Nanchang, Hangzhou and Zhengzhou) was investigated under native and sterilized conditions. Zhuang et al. used this same method to obtain the two enantiomers of PYR to evaluate their

enantioselective potential aquatic toxicity towards zebrafish [63]. R-enantiomer mainly contributed to the acute aquatic toxicity of PYR racemate, thus, R-PYR is more potent to promote malformations.

(O,O-dimethyl-(2,2,2-trichloro-1-hydroxyethyl)-phosphonate, commonly known as trichlorfon (TF), is a chiral OPP which has an asymmetric carbon center, resulting in two enantiomers [64]. It is water-soluble and can act as an antiparasitic agent in seawater aquaculture [65]. A Chiralpak® IC column was used in normal phase mode for the separation of TF enantiomers and under the optimized conditions, R-(-)-TF eluted first [64, 65]. TF enantiomers were determined and their enantioselective degradation was evaluated in mariculture pond water [64] and fish samples [65]. LODs obtained in the fish samples were 0.016 and 0.018 μg g^{-1} for S-(+)-TF and R-(-)-TF, respectively, which are lower than the maximum residue limits in animal muscle, established by the Food and Agricultural Organization/World Health Organization in 2000 [77].

Malathion (MA) is also a chiral OPP which contains an asymmetric α-carbon atom on the succinyl ligand, resulting in two enantiomers [66]. Like other OPPs, MA is mainly used in agriculture to protect crops froms pests such as wheat midge, weevils or cutworms, among others [67]. A cellulose-based chiral stationary phase in normal phase was used for the separation of MA enantiomers [66-68]. Enríquez-Núñez et al. employed a Chiralcel® OJ column [68]. The R-enantiomer, which is 65 times more toxic than the S-configuration eluted at 11.3 min, while the S-enantiomer eluted at 13.0 min. Sun et al. also achieved the enantiomeric separation of this chiral insecticide in different plant matrices using a CDMPC chiral stationary phase [67]. The two enantiomers were resolved with a resolution value of 1.88, being R-(+)-MA the first eluted enantiomer as in the previous work of Enríquez-Núñez et al. [68]. Sun et al. also evaluated the enantioselective dissipation behaviour of MA in vegetables and crops such as rape, cabbage or wheat. Additionally, the enantioselective

toxicity of the individual enantiomers of MA in earthworms and bees was evaluated showing that R-(+)-MA enantiomer is more toxic than S-(-)-MA enantiomer. The same chiral HPLC method was used by the research group for the stereoselective separation of MA in soil and water samples, as well as for evaluating its enantioselective degradation and chiral stability in these matrices [66]. Inactive S-enantiomer degraded faster than the active R-enantiomer. According to previous results, the first eluted enantiomer in these samples was R-(+)-MA [67, 68].

The enantiomeric separation of MA and its metabolite isomalathion (IMA), which is also considered an OPP, was also reported. Zhang et al. [69] separated simultaneously the two enantiomers of MA and the four enantiomers of IMA on a Chiralcel® OD column with hexane/isopropanol (80/20, v/v) as mobile phase. The aquatic toxicity of the enantiomers was evaluated using *Daphnia magna.* The stereoselective toxicity of IMA was evaluated on acid α-naphthyl acetate esterase (ANAE). (R)-MA was about 1.5-3 times more toxic than (S)-MA and in the case of IMA, (1R, 3R)-IMA was the most toxic enantiomer. The same research group also carried out the enantioseparation of MA and IMA using other chromatographic conditions [70]. However, the separation of both OPPs was not performed simultaneously. Authors assigned the absolute configurations of each peak employing binding energy computations. Results showed that (R)-MA eluted first, according to that reported previously [66-68]. For IMA, the first eluted enantiomer was the (1R, 3R) isomer, followed by (1S, 3R), (1S, 3S) and (1R, 3S) isomers. More recently, the same chromatographic method was used to study the enantioselective interaction of MA and IMA enantiomers with ANAE [71]. It was observed that inhibition of ANAE by IMA enantiomers followed the order (1R, 3R) > (1R, 3S) > (1S, 3R) > (1S, 3S). Finally, studies related to the enantioselective inhibition of ANAE by MA enantiomers suggested that (S)-MA enantiomer exhibited higher potencial to inhibit ANAE than (R)-MA enantiomer.

Zhao and co-workers separated simultaneously the enantiomers of FAP, PFF and ICP [72, 73]. Two different methods were developed using the same amylose-based chiral stationary phase (Chiralpak® IG) and different composition of the mobile phase (ACN/water containing 5 mM ammonium acetate and 0.05% formic acid (53/47, v/v) [72] and ACN/water containing 5 mM ammonium acetate and 0.1% formic acid (65/35, v/v) [73]). The resolution values differed from one method to another, being better for the three OPPs when ACN/water containing 5 mM ammonium acetate and 0.05% formic acid (53/47, v/v) was used as mobile phase [72], although good resolution and shorter analysis time was obtained when increasing the percentage of ACN. These methods were used for the determination of FAP, ICP and PFF enantiomers in water, soil, river sediments, fruits and vegetables [72, 73]. Water, soil and river sediment samples were extracted and purified by magnetic solid-phase extraction (MSPE) using amino modified multiwalled carbon nanotubes (MWCNTs-NH_2) [72]. LODs were in the range of 0.34-0.55 ng L^{-1}, 0.07-0.13 ng L^{-1} and 0.07-0.11 ng L^{-1} for water, soil and sediment samples respectively. Moreover, results suggested that adsorption played an important role because pesticides adsorbed to river sediments were at higher levels than those found in water samples. Fruit and vegetable samples were also extracted with MSPE using magnetic-graphene nanocomposite [73]. 42 samples were analyzed and the target pesticides were not detected in any of them.

The chiral separation of the three OPPs fensulfothion (FTN), MTD and PFF was achieved using Chiralcel® OD and Chiralcel® OJ columns in normal phase [74]. The enantioselective inhibition potential on AChE and toxicity in *Daphnia magna* were studied for their enantiomers. Studies showed that the activity of AChE is more supressed by the (+)-enantiomer of PFF and FTN than the (-)-enantiomer, unlike for MTD. Regarding the enantioselective toxicity in *Daphnia magna*, it was observed that MTD and FTN enantiomers had an additive effect. However, PFF enantiomers showed a synergistic effect.

730 Finally, Li et al. [75] enantioseparated eight chiral pesticides, including four OPPs (MA, PTH,
731 PFF and FAP) using different chromatographic conditions for each insecticide. Baseline
732 separation was achieved for all of them. FAP separation required 49.4 min with a resolution
733 value of 1.79, which is a worst separation than the one previously described by other authors
734 [27], while separation of MA and PFF were in general better than the ones previously
735 described. Racemization of the target OPPs enantiomers was evaluated in organic solvents
736 and buffer solutions. PFF and FAP did not experience racemization in any of the organic
737 solvents, nor in buffer solutions tested. However, results showed an opposite behaviour for
738 MA and PTH, which exhibited racemization when the second eluted enantiomer was
739 incubated in MeOH or EtOH, being faster in MeOH. Influence of temperature on
740 racemization of MA and PTH in organic solvents was also evaluated, showing that the extent
741 of conversion was smaller at lower temperatures. In addition, MA and PTH exhibited
742 racemization in buffer solutions, but it was lower than in MeOH and EtOH.
743
744 ***3.2. Gas Chromatography***
745 GC has also been used for the enantiomeric separation of OPPs although in a lesser extent
746 than for pyrethroids. As **Table 5** shows, three articles reported the GC enantiomeric
747 separation of OPPs in the period reviewed [78-80]. In all of them, the simultaneous separation
748 of acephate (APT) and MTD enantiomers was reported and the method was applied to the
749 analysis of real samples, such as vegetables, soil, and tea. Moreover, the QuEChERS (quick,
750 easy, cheap, effective, rugged and safe) procedure was used as sample preparation strategy
751 before GC analysis coupled to MS/MS [78, 79] or Flame Photometric Detector (FPD) [80].
752 Wang et al. tested different chiral columns for the enantioseparation of APT and MTD, being
753 the BGE column 176 selected to optimize the rest of the experimental conditions [78]. The
754 same group obtained the chiral separation of APT and MTD, but on a Cyclosil-B column

[79], using the same chromatographic conditions than in their previous study [78], but not significant differences were observed between both columns. Nonetheless, regardless of the column and the conditions used, in all cases the elution order was the same: R-(+)-MTD, S-(-)-MTD, R-(+)-APT, S-(+)-APT. One of the methods developed was applied to the determination of APT and MTD enantiomers in vegetables with the aim of studying their enantioselective metabolism [78]. Recovery values ranged from 72 to 81%, LODs between 5 and 8 μg kg^{-1}, and the results confirmed that the metabolism of both insecticides in vegetables is enantioselective. The other method was also validated in order to evaluate the transformation and degradation of APT, as well as its metabolite MTD, in these samples [79]. Recovery values achieved were higher than 72%. Pan et al. used a BGB-176 column under different chromatographic conditions to develop and validate a method to evaluate the enantioselective dissipation of APT and MTD during tea cultivation, manufacturing and infusion (**Fig. 5**) [80]. Despite using the QuEChERS procedure for sample preparation, recoveries achieved were not satisfactory (58-65% and 51-57% for MTD and APT enantiomers, respectively). Also, the LOQs were higher than the ones previously reported by Wang et al. [78].

3.3. Supercritical Fluid Chromatography

As shown in **Table 6**, some articles reported the chiral separation of OPPs by SFC [81, 82]. Chen et al. achieved the enantiomeric separation of IFM in less than two min with a resolution of 2.20 using SFC-MS/MS [81]. Four different polysaccharide-based chiral columns (Chiralpak® IA-3, Chiralpak® IA-5, Chiralpak® IB-3 and Chiralpak® IC-3) were evaluated and Chiralpak® IA-3 was chosen since it provided best resolution and a shorter retention time than the other columns. The composition of the mobile phase was also evaluated and four co-solvents (ACN, EtOH, MeOH and isopropanol) were tested. Baseline

780 separation was only achieved with isopropanol, so it was selected as modifier. The method
781 was applied to the determination of IFM enantiomers in wheat, corn, peanut and soil samples
782 (**Fig. 6**). As in GC, the QuEChERS procedure was used for sample preparation. Recovery
783 values ranged from 73 to 111% and LOQs for both enantiomers varied from 0.02 to 0.15 µg
784 Kg^{-1}. The analysis of rice, corn and peanut purchased in a local market showed the absence of
785 IFM enantiomers in the food samples analyzed. Nevertheless, IFM enantiomers were detected
786 in soil samples, at concentrations ranging between 1.19 and 1.36 mg Kg^{-1}. More recently,
787 Zhang et al. also employed SFC for the enantiomeric separation of IFM, in addition to ICP
788 and isofenphos (IFP) [82]. As in the previous study, different chiral columns were evaluated
789 (Chiralcel® OD-H, Chiralpak® AS-H, Chiralpak® AD-3, Chiralpak® IB, Lux™ 3u
790 Cellulose-1 and Sino-Chiral OJ). Although ICP could be well separated on Chiralpak® AD-3,
791 Chiralcel® OD-H and Lux™ 3u Cellulose-1, shorter retention times and better resolutions
792 were obtained using Lux™ 3u Cellulose-1 column. IFM and IFP were only separated on the
793 Chiralpak® AD-3 column. IFM was partially separated in the Chiralpak® AD-3 column in
794 5.0 min with a resolution of 2.03, which is a less effective separation than that previously
795 reported by Chen et al. [81] with Chiralpak® IA-3, which requires less analysis time and
796 provides better resolution.
797

798 ## 4. Conclusions

799 This article reviews the works dealing with the enantiomeric separation of pyrethroid and
800 OPPs insecticides and published between 2010 and April 2019. HPLC has been by far the
801 most widely used technique to achieve the chiral separation of pyrethroids and OPPS, while
802 SFC, GC and CE have been used in a lesser extent. Different polysaccharide chiral columns
803 have been evaluated, being the cellulose-based the most employed. Additionally, about a 70%
804 of the methods developed used UV detection. Nevertheless, there are some authors which

805 have used MS for detection (18 out of the 69 methods reviewed), due to its high sensitivity
806 and selectivity. In several studies, both for pyrethroids and OPPs, authors applied the methods
807 developed to the analysis of real samples, such as soil, water sediments, vegetables and fruits,
808 among others. Regarding multicomponent analysis, there are some works which
809 simultaneously analyse more than one OPP using general chiral separation conditions for all
810 of them. On the other hand, there are no multicomponent methods reported for pyrethroids,
811 since no general conditions are established for the simultaneous enantiomeric separation of
812 them. In addition, it should be noted that, although the EPA has banned the use of OPPs for
813 several applications, there are more articles published which described new methods to carry
814 out their chiral separation than for pyrethroids.
815 Many articles cited in this review evaluated the toxicity of pyrethroids and OPPs enantiomers
816 to non-target organisms, insecticide enantiomer activity and their degradation in the
817 environment, which reveal their risk and exposure to living beings. Thus, it is necessary to
818 continue investigating and developing chiral methodologies to control the presence of these
819 compounds, as well as raising awareness of the importance of formulating enantiomerically
820 pure pesticides instead of racemic ones, that is, having only the active and effective
821 enantiomer in their formulation in order to reduce the release of the other enantiomers that
822 may be toxic for living beings and the environment.
823

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827

828 ## References

[1] F.P. Garcia, S.Y.C. Ascencio, J.C.G. Oyarzun, A.C. Hernandez, P.V. Alavarado, Pesticides: Classification, uses and toxicity. Measures of exposure and genotoxic risks, J. Res. Environ. Sci. Toxicol. 1 (2012) 279-293.

[2] C. Wang, Q. Zhang, M. Zhao, W. Liu, Enantioselectivity in estrogenic potential of chiral pesticides, in A. Garrison et al. (Eds.), In chiral pesticides: Stereoselectivity and its consequences, American Chemical Society, Washington, 2011, pp.121-134.

[3] L. Li, S. Zhou, L. Jin, C. Zhang, W. Liu, Enantiomeric separation of organophosphorus pesticides by high-performance liquid chromatography, gas chromatography and capillary electrophoresis and their applications to environmental fate and toxicity assays, J. Chromatogr. B 878 (2010) 1264-1276.

[4] N.C. Perez de Albuquerque, J. Vicentin de Matos, A.R. Moraes de Oliveira, In-line coupling o fan achiral-chiral column to investigate the enantioselective in vitro metabolism of the pesticide fenamiphos by human liver microsomes, J. Chromatogr. A 1467 (2016) 326-334.

[5] M.L. Feo, E. Eljarrat, D. Barceló, Determination of pyrethroid insecticides in environmental samples, Trends in Anal. Chem. 29 (2010) 692-705.

[6] V. Pérez-Fernández, M.A. García, M.L. Marina, Characteristics and enantiomeric analysis of chiral pyrethroids, J. Chromatogr. A 1217 (2010) 968-989.

[7] C.E.T Parente, C.E. Azevedo-Silva, R.O. Meire, O. Malm, Pyrethroid stereoisomerism: Diastereomeric and enantiomeric selectivity in environmental matrices – A review, Orbital: Electron. J. Chem. 10 (2018) 337-345.

[8] J. Ye, M. Zhao, L. Niu, W. Liu, Enantioselective environmental toxicology of chiral pesticides, Chem. Res. Toxicol. 28 (2015) 325-338.

[9] N.C. Perez de Albuquerque, D.B. Carrão, M.D. Habenschus, A.R. Moraes de Oliveira, Metabolism studies of chiral pesticides: A critical review, J. Pharm. Biomed. Anal. 147 (2018) 89-109.

[10] X. Li, Y. Liu, C. Hu, L. Bai, Direct optical resolution of chiral pesticides by high performance liquid chromatography, Chin. J. Chem. Eng. 19 (2011) 603-609.

[11] M. Shishovska, V. Trajkovska, HPLC-method for determination of permethrin enantiomers using chiral β-cyclodextrin-based stationary phase, Chirality 22 (2010) 527-533.

[12] Y. Yang, D. Ji, X. Huang, J. Zhang, J. Liu, Effects of metals on enantioselective toxicity and biotransformation of cis-bifenthrin in zebrafish, Environ. Toxicol. Chem. 36 (2017) 2139-2146.

[13] P. Zhang, Q. Yu, Y. He, W. Zhu, Z. Zhou, L. He, Chiral pyrethroid insecticide fenpropathrin and its metabolite: enantiomeric separation and pharmacokinetic degradation in soils by reverse-phase high-performance liquid chromatography, Anal. Methods 9 (2017) 4439-4446.

[14] H. Kuang, H. Miao, Y. Wu, J. Shen, C. Xu, Enantioselective determination of cypermethrin in pig muscle tissue by immunoaffinity extraction and high performance liquid chromatography, Int. J. Food Sci. Technol. 45 (2010) 656-660.

[15] G. Yao, X. Jing, W. Peng, X. Liu, Z. Zhou, D. Liu, Chiral insecticide α-cypermethrin and its metabolites: stereoselective degradation behavior in soils and the toxicity to earthworm eisenia fetida, J. Agric. Food Chem. 63 (2015) 7714-7720.

[16] Y. Suzuki, M. Yoshida, T. Sugano, A. Shibata, R. Kodaka, T. Fujisawa, T. Katagi, Behavior of cyphenothrin in aquatic environment, J. Pestic. Sci. 42 (2017) 17-24.

[17] S. Li, Z. Li, Q. Li, J. Zhao, S. Li, Characterization of diastereo- and enantioselectivity in degradation of synthetic pyrethroids in soils, Chirality 28 (2016) 72-77.

[18] P. Zhang, Q. Yu, X. He, K. Qian, W. Xiao, Z. Xu, T. Li, L. He, Enantiomeric separation of type I and type II pyrethroid insecticides with different chiral stationary phases by reversed-phase high-performance liquid chromatography, Chirality 30 (2018) 420-431.

878 [19] Y. Jin, J. Liu, L. Wang, R. Chen, C. Zhou, Y. Yang, W. Liu, Z. Fu, Permethrin exposure
879 during puberty has the potential to enantioselectively induce reproductive toxicity in mice,
880 Environ. Int. 42 (2012) 144-151.
881 [20] M. Chalányová, I. Petránová, HPLC study of on-line transfer and chiral separation
882 options for selected pesticides using column switching techniques, Chem. Listy 110 (2016)
883 185-189.
884 [21] Q. Tian, Z. Zhou, C. Lv, J. Yang, Direct enantiomeric separation of chiral pesticides by
885 liquid chromatography on polysaccharide-based chiral stationary phases under reversed phase
886 conditions, Anal. Methods 4 (2012) 2307-2317.
887 [22] S. Fan, J. Shi, L. Zhou, Y. Hang, Analysis of bifenthrin and its enantiomer using high
888 performance liquid chromatography, Appl. Mech. Mater. 675-677 (2014) 275-279.
889 [23] J. Liu, Y. Yang, S. Zhuang, Y. Yang, F. Li, W. Liu, Enantioselective endocrine-
890 disrupting effects of bifenthrin on hormone synthesis in rat ovarian cells, Toxicology 290
891 (2011) 42-49.
892 [24] M. Jin, Y. Zhang, J. Ye, C. Huang, M. Zhao, W. Liu, Dual enantioselective effect of the
893 insecticide bifenthrin on locomotor behavior and development in embryonic-larval zebrafish,
894 Environ. Toxicol. Chem. 29 (2010) 1561-1567.
895 [25] C. Xu, W. Tu, C. Lou, Y. Hong, M. Zhao, Enantioselective separation and zebrafish
896 embryo toxicity of insecticide beta-cypermethrin, J. Environ. Sci. 22 (2010) 738-743.
897 [26] K. Mihara, H. Ohkawa, J. Miyamoto, Metabolism of terallethrin in rats, J. Pesticide Sci.
898 6 (1981) 211-222.
899 [27] Q. Tian, C. Lv, L. Ren, Z. Zhou, Direct enantiomeric separation of chiral pesticides by
900 LC on amylose tris(3,5-dimethylphenylcarbamate) stationary phase under reversed phase
901 conditions, Chromatographia 71 (2010) 855-865.

902 [28] Z. Y. Li, X. N. Luo, Q. L. Li, E. Q. Zhang, Stereo and enantioselective separation and
903 identification of synthetic pyrethroids, and photolytical isomerization analysis, Bull. Environ.
904 Contam. Toxicol. 94 (2015) 254-259.
905 [29] C. Wang, Q. Zhang, X. Zhang, J. Liu, W. Liu, Understanding the endocrine disruption of
906 chiral pesticides: The enantioselectivity in estrogenic activity of synthetic pyrethroids, Sci.
907 China Chem. 53 (2010) 1003-1009.
908 [30] J. Ye, M. Jin, W. Liu, Enantioselective separation and analysis of synthetic pyrethroids,
909 in: A. Garrison et al. (Eds.), In chiral pesticides: Stereoselectivity and its consequences,
910 American Chemical Society, Washington, 2011, pp. 81-94.
911 [31] A. Khazri, B. Sellami, M. Dellali, C. Corcellas, E. Eljarrat, D. Barceló, H. Beyrem, E.
912 Mahmoudi, Diastereomeric and enantiomeric selective accumulation of cypermethrin in the
913 freshwater mussel *Unio gibbus* and its effects on biochemical parameters, Pestic. Biochem.
914 Physiol. 129 (2016) 83-88.
915 [32] G. Yao, X. Jing, C. Liu, P. Wang, X. Liu, Y. Hou, Z. Zhou, Enantioselective degradation
916 of alpha-cypermethrin and detection of its metabolites in bullfrog (*rana catesbeiana*),
917 Ecotoxicol. Environ. Saf. 141 (2017) 93-97.
918 [33] H. Kuang, H. Miao, X. Hou, Y. Zhao, J. Shen, Y. Wu, Determination of enantiomeric
919 fractions of cypermethrin and cis-bifenthrin in Chinese teas by GC/ECD, J. Sci. Food Agric.
920 90 (2010) 1374-1379.
921 [34] E.M. Ulrich, P.L. TenBrook, L.M MacMillan, Q. Wang, W. Lao, Enantiomer-specific
922 measurements of current-use pesticides in aquatic systems, Environ. Toxicol. Chem. 37
923 (2018) 99-106.
924 [35] C. Corcellas, E. Eljarrat, D. Barceló, Enantiomeric-selective determination of
925 pyrethroids: Application to human samples, Anal. Bioanal. Chem. 407 (2015) 779-786.

926 [36] C. Corcellas, E. Eljarrat, D. Barceló, First report of pyrethroid bioaccumulation in wild
927 river fish: A case study in Iberian river basins (Spain), Environ. Int. 75 (2015) 110-116.
928 [37] C. Corcellas, A. Andreu, M. Máñez, F. Sergio, F. Hiraldo, E. Eljarrat, D. Barceló,
929 Pyrethroid insecticides in wild bird eggs from a world heritage listed park: A case study in
930 Doñana National Park (Spain), Environ. Pollut. 228 (2017) 321-330.
931 [38] Y. Nishikawa, Enantiomer separation of synthetic pyrethroids by subcritical and
932 supercritical fluid chromatography with chiral stationary phases, Anal. Sci. 9 (1993) 33-37.
933 [39] Y. Yan, J. Fan, Y. Lai, J. He, D. Guo, H. Zhang, W. Zhang, Efficient preparative
934 separation of β-cypermethrin stereoisomers by supercritical fluid chromatography with a two-
935 step combined strategy, J. Sep. Sci. 41 (2018) 1442-1449.
936 [40] Y. Jin, J. Wang, X. Pan, W. Miao, X. Lin, L. Wang, Z. Fu, Enantioselective disruption of
937 the endocrine system by cis-bifenthrin in the male mice, Environ. Toxicol. 30 (2015) 746-
938 754.
939 [41] E. Sánchez-López, M. Castro-Puyana, M.L. Marina, A.L. Crego, Chiral Separations by
940 capillary electrophoresis, in: J.L. Anderson, A. Berthod, V.P. Estévez, A.M. Stalcup (Eds.),
941 Analytical Separation Science, Wiley-VCH, USA, 2015, pp. 731-774.
942 [42] V. Pérez-Fernández, M.A. García, M.L. Marina, Enantiomeric separation of cis-
943 bifenthrin by CD-MEKC: Quantitative analysis in a commercial insecticide formulation,
944 Electrophoresis 31 (2010) 1533-1539.
945 [43] D.R. Baker, Capillary electrophoresis, Wiley, New York, 1995.
946 [44] T. Chai, X. Wang, M. Bie, Comparative enantioseparation of crufomate with cellulose-
947 and amylose-based chiral stationary phases on reverse-phase and normal-phase high-
948 performance liquid chromatography, Asian J. Chem. 2 (2013) 797-802.

949 [45] J. Sun, J. Liu, W. Tu, C. Xu, Separation and aquatic toxicity of enantiomers of the
950 organophosphorus insecticide O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN),
951 Chemosphere 81 (2010) 1308-1313.
952 [46] D. Almenares-López, M.F. Martínez-Salazar, M.L. Ortiz-Hernández, R. Vazquez-
953 Duhalt, A. Monroy-Noyola, Fenamiphos is recalcitrant to the hydrolysis by alloforms PON1
954 Q192R of human serum, Toxicol. In Vitro 27 (2013) 681-685.
955 [47] X. Cai, W. Xiong, T. Xia, J. Chen, Probing the stereochemistry of successive
956 sulfoxidation of the insecticide fenamiphos in soils, Environ. Sci. Technol. 48 (2014) 11277-
957 11285.
958 [48] C. Wang, N. Zhang, L. Li, Q. Zhang, M. Zhao, W. Liu, Enantioselective interaction with
959 acetylcholinesterase of an organophosphate insecticide fenamiphos, Chirality 22 (2010) 612-
960 617.
961 [49] H. Zhang, X. Wang, S. Zhuang, N. Jin, X. Wang, M. Qian, H. Xu, P. Qi, Q. Wang, M.
962 Wang, Enantioselective analysis and degradation studies of isocarbophos in soils by chiral
963 liquid chromatography-tandem mass spectrometry, J. Agric. Food Chem. 60 (2012) 10188-
964 10195.
965 [50] P. Qi, X. Wang, H. Zhang, X. Wang, H. Xu, Q. Wang, Rapid enantioseparation and
966 determination of isocarbophos enantiomers in orange pulp, peel, and kumquat by chiral
967 HPLC-MS/MS, Food Anal. Methods 8 (2015) 531-538.
968 [51] Z. Yao, M. Lin, M. Xu, T. Wang, X. Ping, S. Wu, Q. Wang, H. Zhang, Simultaneous
969 enantioselective determination of isocarbophos and its main metabolite isocarbophos oxon in
970 rice, soil, and water by chiral liquid chromatography and tándem mass spectrometry, J. Sep.
971 Sci. 38 (2015) 1663-1672.

[52] H. Liu, J. Liu, L. Xu, S. Zhou, L. Li, W. Liu, Enantioselective cytotoxicity of isocarbophos is mediated by oxidative stress-induced JNK activation in human hepatocytes, Toxicology 276 (2010) 115-121.

[53] P. Zhao, S. Lei, M. Xing, S. Xiong, X. Guo, Simultaneous enantioselective determination of six pesticides in aqueous environmental samples by chiral liquid chromatography with tandem mass spectrometry, J. Sep. Sci. 41 (2018) 1287-1297.

[54] P. Zhao, J. Zhao, S. Lei, X. Guo, L. Zhao, Simultaneous enantiomeric analysis of eight pesticides in soils and river sediments by chiral liquid chromatography-tandem mass spectrometry, Chemosphere 204 (2018) 210-219.

[55] B. Gao, Q. Zhang, M. Tian, Z. Zhang, M. Wang, Enantioselective determination of the chiral pesticide isofenphos-methyl in vegetables, fruits, and soil and its enantioselective degradation in pak choi usin HPLC with UV detection, Anal. Bioanal. Chem. 408 (2016) 6719-6727.

[56] G.L. Emerick, R.V. Oliveira, K.R.A. Belaz, M. Gonçalves, G.H. DeOliveira, Semipreparative enantioseparation of methamidophos by HPLC-UV and preliminary in vitro study of butyrylcholinesterase inhibition, Environ. Chem. 31 (2011) 239-245.

[57] G.L. Emerick, M. Ehrich, B.S. Jortner, R.V. Oliveira, G.H. DeOliveira, Biochemical, histopathological and clinical evaluation of delayed effects caused by methamidophos isoforms and TOCP in hens: Ameliorative effects using control of calcium homeostasis, Toxicology 302 (2012) 88-95.

[58] G.L. Emerick, G.H. DeOliveira, R.V. Oliveira, M. Ehrich, Comparative *in vitro* study of the inhibition of human and hen esterases by methamidophos enantiomers, Toxicology 292 (2012) 145-150.

[59] S. Zhou, W. Liu, Application of stereoselective bioassays for improvement in pesticide design: An example from China using methamidophos and its derivatives, in: J.J. Gan, A.W.

997 Garrison, W. Liu (Eds.), Chiral pesticides: Stereoselectivity and its consequences, ACS
998 Symposium Series, American Chemical Society, Washington, DC, 2011, pp. 201-212.
999 [60] X. Lu, C. Yu, Enantiomer-specific profenofos-induced cytotoxicity and DNA damage
1000 mediated by oxidative stress in rat adrenal pheochromocytoma (PC12) cells, J. Appl. Toxicol.
1001 34 (2014) 166-175.
1002 [61] H. Zhang, S. Chen, S. Zhou, Enantiomeric separation and toxicity of an organophosporus
1003 insecticide, pyraclofos, J. Agric. Food Chem. 60 (2012) 6953-6959.
1004 [62] Y. Xu, H. Zhang, S. Zhuang, M. Yu, H. Xiao, M. Qian, Different enantioselective
1005 degradation of pyraclofos in soils, J. Agric. Food Chem. 60 (2012) 4173-4178.
1006 [63] S. Zhuang, Z. Zhang, W. Zhang, L. Bao, C.Xu, H. Zhang, Enantioselective
1007 developmental toxicity and immunotoxicity of pyraclofos toward zebrafish (Danio rerio),
1008 Aquat. Toxicol. 159 (2015) 119-126.
1009 [64] J. Nie, Y.G. Wang, L.Y. Yang, W.J. Wu, D. Yu, X.K. OuYang, Chiral separation and
1010 enantioselective degradation of trichlorfon enantiomers in mariculture pond water, Anal.
1011 Methods 8 (2016) 3196-3203.
1012 [65] J. Nie, L.Y. Yang, X.K. OuYang, W.J. Wu, Y.G. Wang, D. Yu, Investigation into the
1013 enantiospecific behavior of trichlorfon enantiomers during microorganism degradation, R.
1014 Soc. Chem. 6 (2016) 3934-3941.
1015 [66] M. Sun, D. Liu, G. Zhou, J. Li, X. Qiu, Z. Zhou, P. Wang, Enantioselective degradation
1016 and chiral stability of malathion in environmental samples, J. Agric. Food Chem. 60 (2012)
1017 372-379.
1018 [67] M. Sun, D. Liu, Z. Dang, R. Li, Z. Zhou, P. Wang, Enantioselective behavior of
1019 malathion enantiomers in toxicity to beneficial organisms and their dissipation in vegetables
1020 and crops, J. Hazard. Mater. 237-238 (2012) 140-146.

[68] C.A. Enríquez-Núñez, A.A. Camacho-Dávila, V.H. Ramos-Sánchez, G. Zaragoza-Galán, L. Ballinas-Casarrubias, D. Chávez-Flores, Chemoenzymatic kinetic resolution of (R)-malathion in aqueous media, Chem. Cent. J. 9 (2015) 46-55.

[69] A. Zhang, X. Xie, J. Ye, C. Lin, X. Hu, Stereoselective toxicity of malathion and its metabolites, malaoxon and isomalathion, Environ. Chem. Lett. 9 (2011) 369-373.

[70] A. Zhang, W. Lai, J. Sun, G. Hu, W. Liu, Probing the chiral separation mechanism and the absolute configuration of malathion, malaoxon and isomalathion enantiomers by chiral high performance liquid chromatography coupled with chiral detector-binding energy computations, J. Chromatogr. A 1281 (2013) 26-31.

[71] A. Zhang, J. Sun, C. Lin, X. Hu, W. Liu, Enantioselective interaction of acid α-naphthyl acetate esterase with chiral organophosphorus insecticides, J. Agric. Food Chem. 62 (2014) 1477-1481.

[72] P. Zhao, Z. Wang, K. Li, X. Guo, L. Zhao, Multi-residue enantiomeric analysis of 18 chiral pesticides in water, soil and river sediment using MSPE base on amino modified multiwalled carbon nanotubes and chiral liquid chromatography coupled with tándem mass spectrometry, J. Chromatogr. A 1568 (2018) 8-21.

[73] P. Zhao, Z. Wang, X. Gao, X. Guo, L. Zhao, Simultaneous enantioselective determination of 22 chiral pesticides in fruits and vegetables using chiral liquid chromatography coupled with tandem mass spectrometry, Food Chem. 277 (2019) 298-306.

[74] Q. Zhang, C. Wang, Toxicity of binary mixtures of enantiomers in chiral organophosphorus insecticides: The significance of joint effects between enantiomers, Chirality 25 (2013) 787-792.

[75] Z. Li, T. Wu, Q. Li, B. Zhang, W. Wang, J. Li, Characterization of racemization of chiral pesticides in organic solvents and water, J. Chromatogr. A 1217 (2010) 5718-5723.

1045 [76] J.Y Shim, Y.A. Kim, Y.T. Lee, B.D. Hammock, H.S. Lee, Monoclonal antibody-based
1046 enzyme-linked immunosorbent assays for the organophosphorus insecticide *O*-ethyl *O*-4-
1047 nitrophenyl phenylphosphonothioate (EPN), J. Agric. Food Chem. 58 (2010) 5241-5247.
1048 [77] G.M. Wang, H. Dai, Y.G. Li, X.L. Li, J.Z. Zhang, L. Zhang, Y.Y. Fu, Z.G. Li,
1049 Simultaneous determination of residues of trichlorfon and dichlorvos in animal tissues by LC-
1050 MS/MS, Food Addit. Contam. 27 (2010) 983-988.
1051 [78] X. Wang, H. Zhang, H. Xu, P. Qi, X. Ji, Q. Wang, X. Wang, Direct chiral determination
1052 of acephate and its metabolite methamidophos in vegetables using QuEChERS by gas
1053 chromatography-tandem mass spectrometry, Food Anal. Methods 6 (2013) 133-140.
1054 [79] X. Wang, Z. Li, H. Zhang, J. Xu, P. Qi, H. Xu, Q. Wang, X. Wang, Environmental
1055 behavior of the chiral organophosphorus insecticide acephate and its chiral metabolite
1056 methamidophos: Enantioselective transformation and degradation in soils, Environ. Sci.
1057 Technol. 47 (2013) 9233-9240.
1058 [80] R. Pan, H. Chen, C. Wang, Q. Wang, Y. Jiang, X. Liu, Enantioselective dissipation of
1059 acephate and its metabolite, methamidophos, during tea cultivation, manufacturing, and
1060 infusion, J. Agric. Food Chem. 63 (2015) 1300-1308.
1061 [81] X. Chen, F. Dong, J. Xu, X. Liu, Z. Chen, N. Liu, Y. Zheng, Enantioseparation and
1062 determination of isofenphos-methyl enantiomers in wheat, corn, peanut and soil with
1063 Supercritical fluid chromatography/tandem mass spectrometric method, J. Chromatogr. B
1064 1015-1016 (2016) 13-21.
1065 [82] L. Zhang, Y. Miao, C. Lin, Enantiomeric separation of six chiral pesticides that contain
1066 chiral sulfur/phosphorus atoms by supercritical fluid chromatography, J. Sep. Sci. 41 (2018)
1067 1460-1470.

FIGURE CAPTIONS

Fig. 1 HPLC representative chromatograms of bifenthrin (BF; Lux Cellulose-3, MeOH/H_2O = 95/5) and λ-cyhalothrin (Lux Cellulose-3, MeOH/H_2O = 90/10) enantiomers extracted from soil and water. (A) Bifenthrin standard solution; (B) bifenthrin extracted from water at 5 mg L^{-1} spiked level; (C) bifenthrin extracted from soil at 5 mg kg^{-1} spiked level; (D) λ-cyhalothrin standard solution; (E) λ-cyhalothrin extracted from water at 5 mg L^{-1} spiked level; (F) λ-cyhalothrin extracted from soil at 5 mg kg^{-1} spiked level. Readapted and reproduced with permission [18].

Fig. 2 a) Peak assignation for the GC chromatograms obtained in diastereomeric and enantiomeric analyses of cypermethrin (BGB-172 column, helium as carrier gas). **b)** GC chromatograms obtained for the chiral determination of cypermethrin in barbel and catfish samples. Readapted and reproduced with permission [36].

Fig. 3 Electropherograms corresponding to the separation of cis-bifenthrin (A) in a standard solution of 200 mg/L and (B) in a polyvalent comercial insecticide formulation solution with a concentration of approximately 200 mg/L (according to the label of the product) prepared in methanol using 100 mM SC with 20 mM TM-β-CD in 100 mM borate buffer (pH 8.0) with 2 M urea. Experimental conditions: uncoated fused-silica capillary 50 μm id × 50 cm (58.5 cm to the detector), injection by pressure 50 mbar × 2s, applied voltaje 30 kV, temperature 15ºC and UV detection 210±2 nm. Readapted and reproduced with permission [42].

Fig. 4 HPLC chromatograms of the R-(-)- and S-(+)-isocarbophos enantiomers in a standard solution and in different soils after seven days of incubation. Experimental conditions: 30 ˚C, Chiralpak AD-RH column and a mobile phase of ACN/2 mM ammonium acetate aqueous solution containing 0.1% formic acid (60/40, v/v). Readapted and reproduced with permission [49].

Fig. 5 GC chromatograms of acephate and methamidophos enantiomers in matrix standard solution (fresh tea leaves, 0.8 mg/kg) (A), fresh tea leaves on day 3 (B), fresh tea leaves on day 14 (C), green tea on day 3 (D), spent leaves of green tea on day 3 (E), black tea on day 3 (F), and spent leaves of black tea on day 3 (G). Peaks: 1, (+)-methamidophos; 2, (-)-methamidophos; 3, (+)-acephate; 4, (-)-acephate. Experimental conditions: BGB-176 column, carrier gas nitrogen and temperature program: 80 ºC for 1 min, ramped at 10 ºC/min to 220 ºC and held for 5 min. Readapted and reproduced with permission [80].

Fig. 6 SFC-MS/MS (MRM) chromatograms of the racemate of isofenphos-methyl in a standard solution (A), wheat blank (B), wheat spiked (C), corn blank (D), corn spiked (E), peanut blank (F), peanut spiked (G), soil blank (H), soil spiked (I). Experimental conditions: Chiralpak IA-3 column, mobile phase CO_2/MeOH (90/10, v/v), flow rate 2.2 mL/min, temperature 30˚C. Readapted and reproduced with permission [81].

Figure 1

Fig. 1

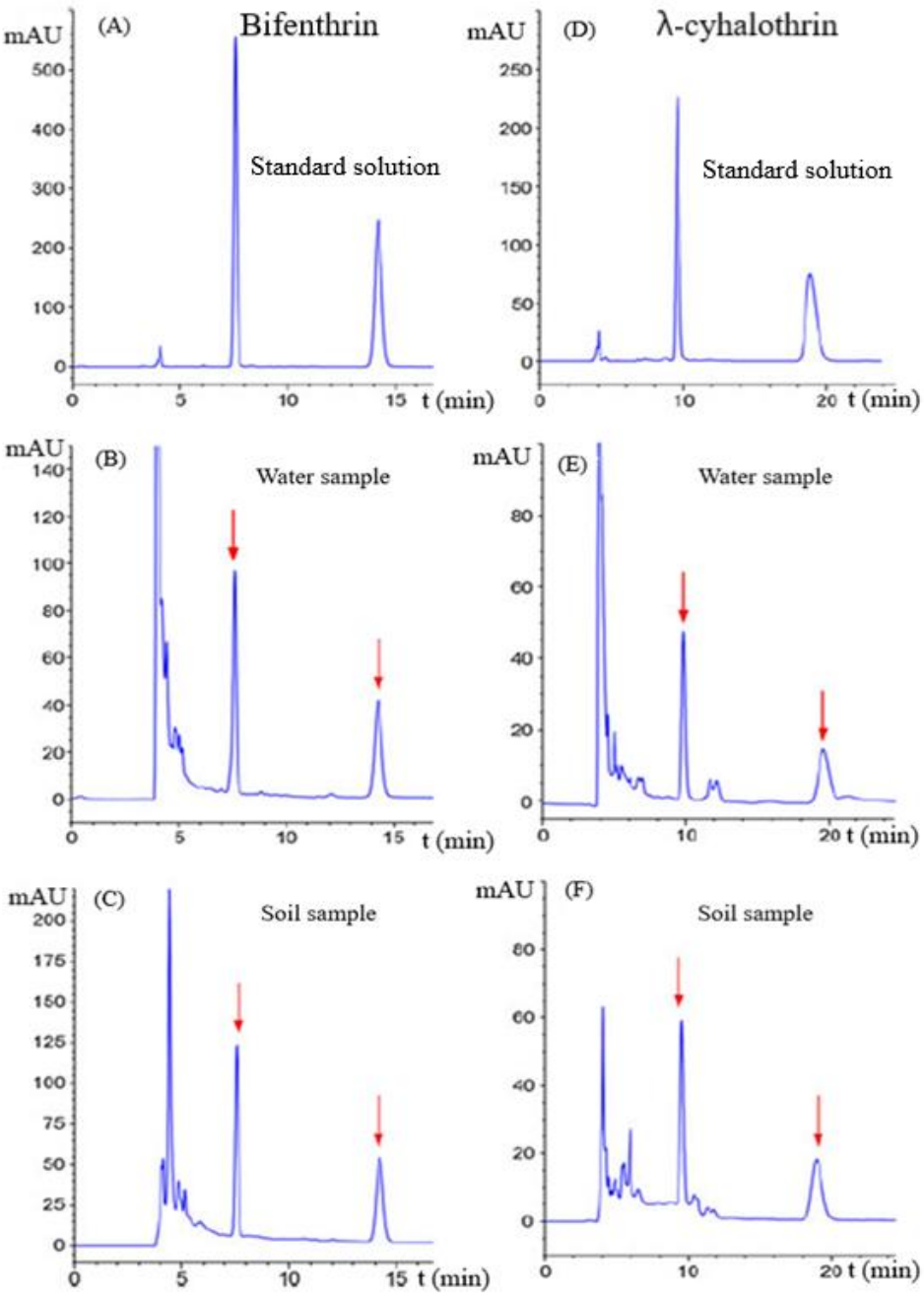

Figure 2

Fig. 2

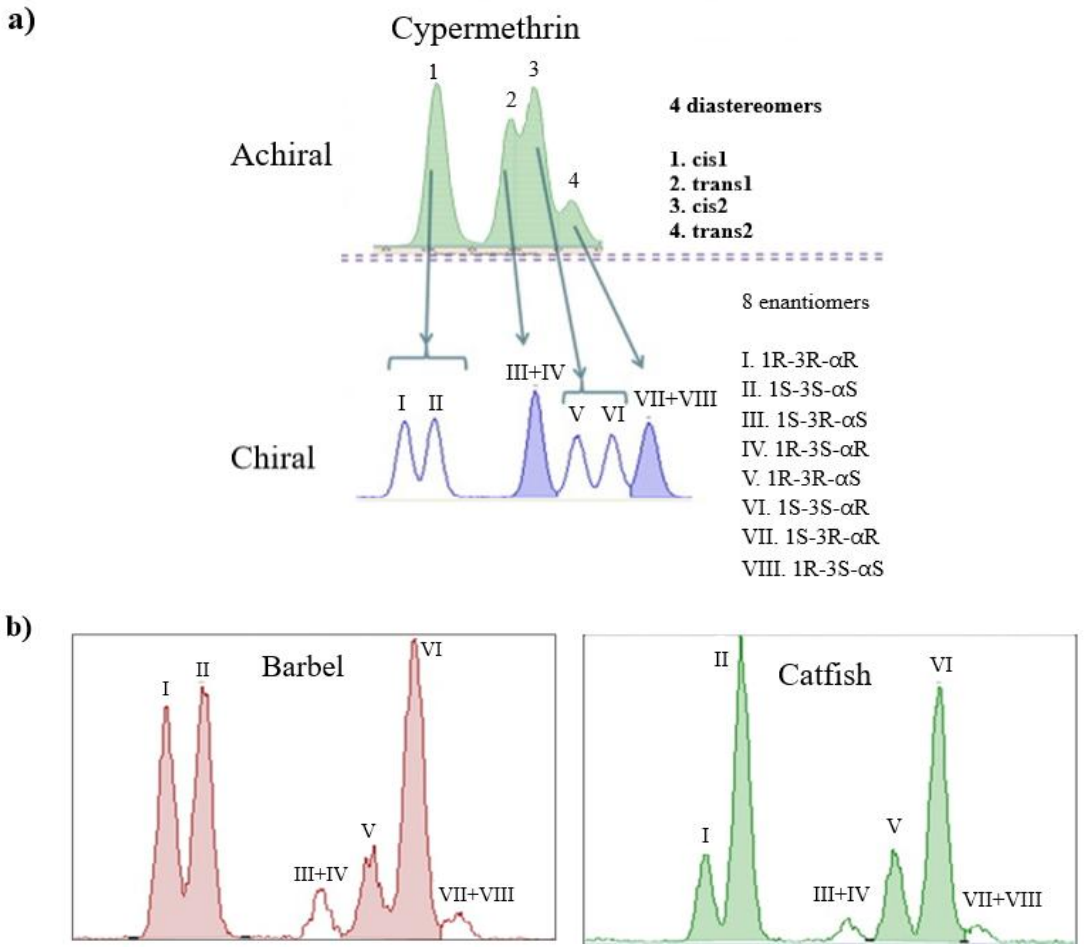

Figure 3

Fig. 3

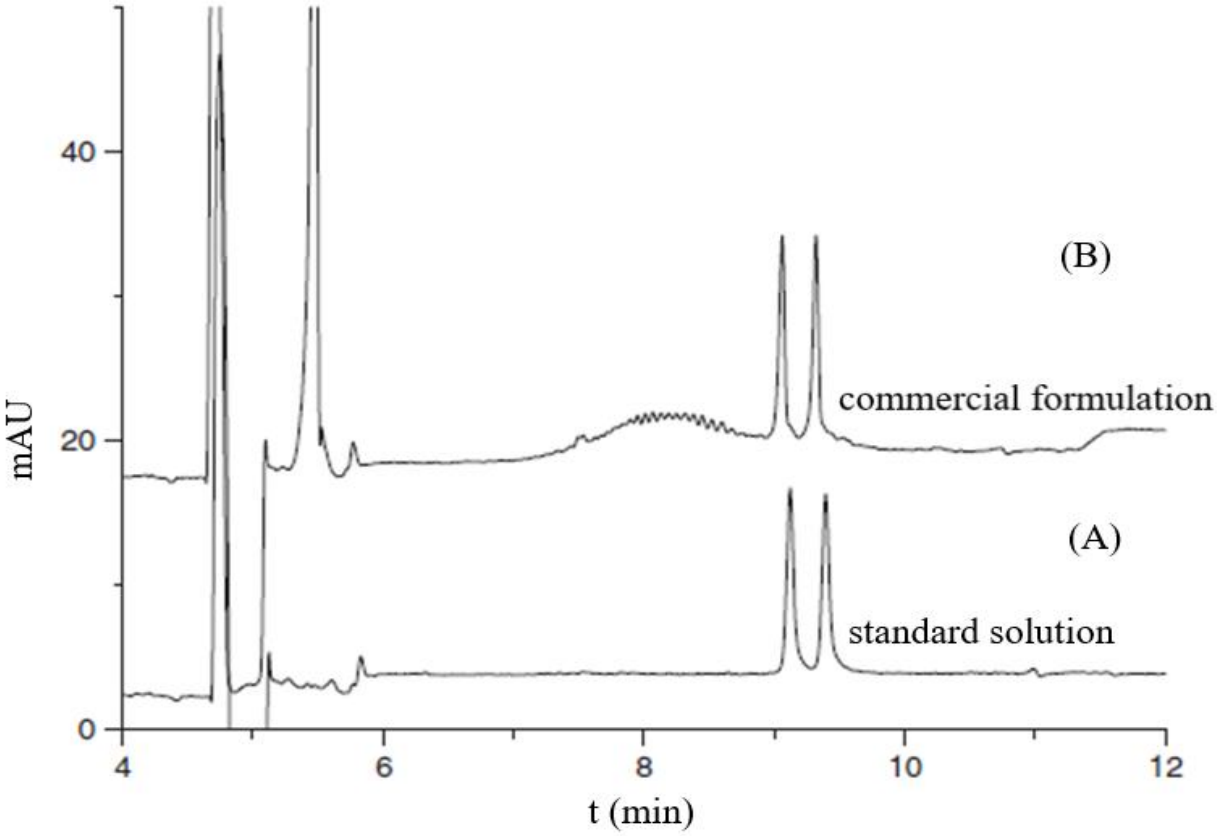

Figure 4

Fig. 4

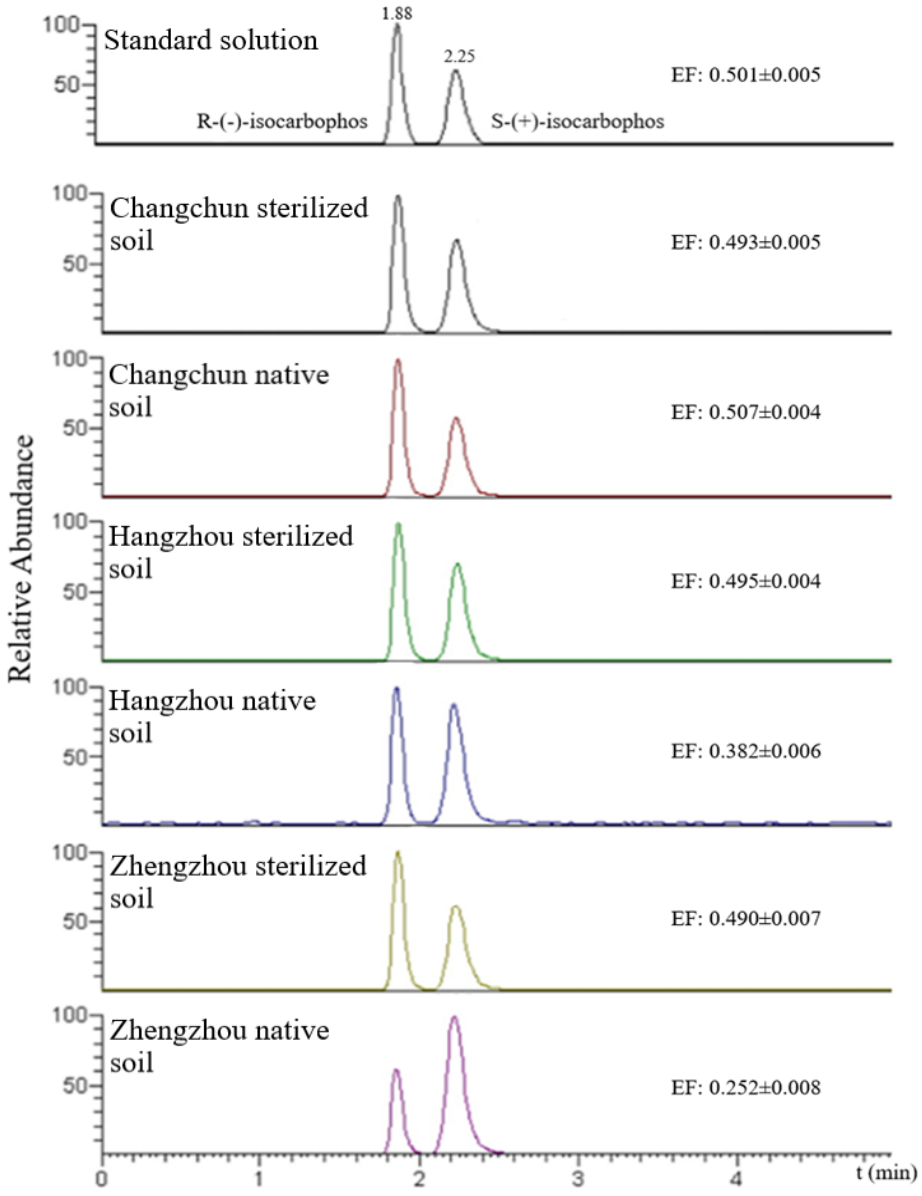

Figure 5

Fig. 5

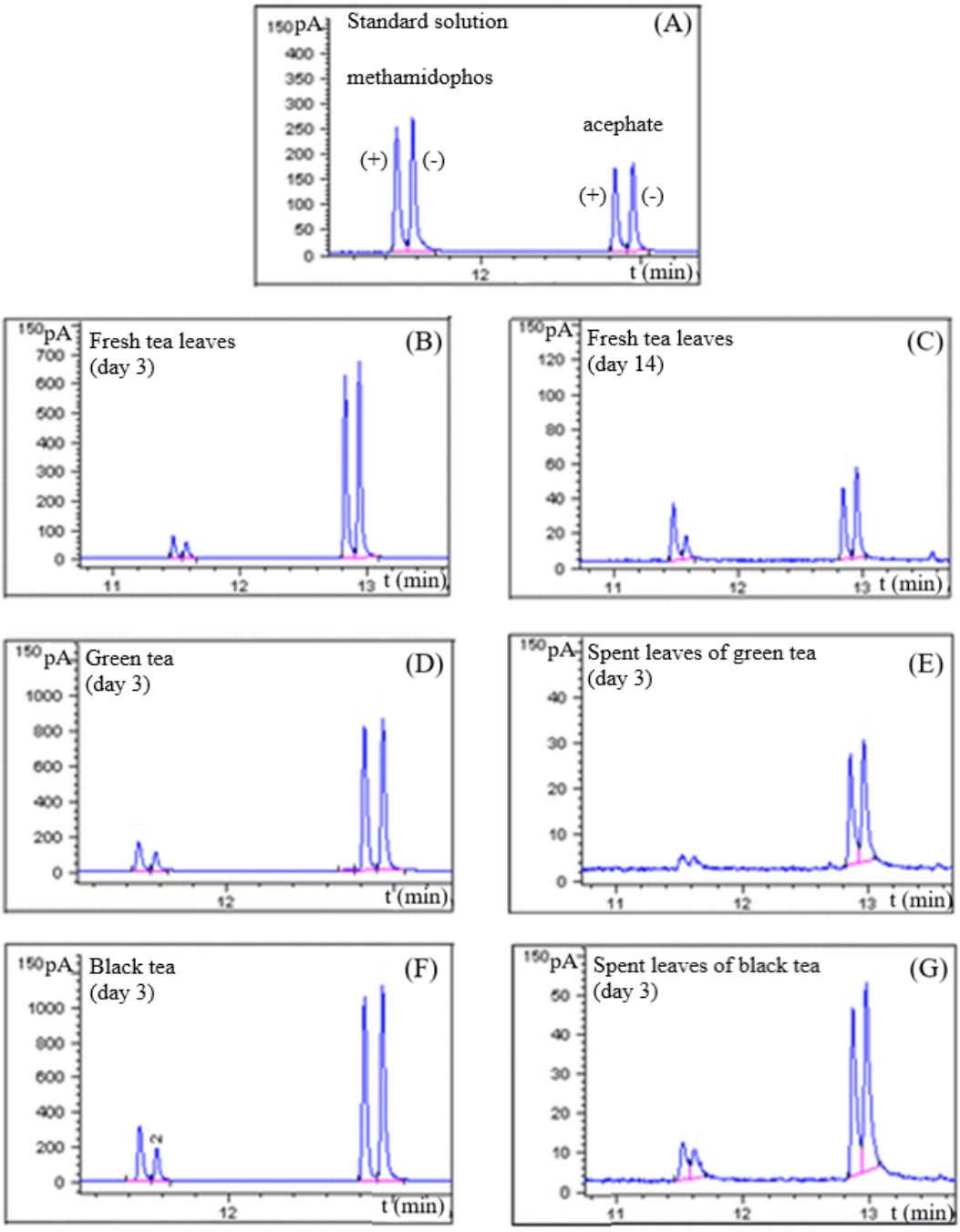

Figure 6

Fig. 6

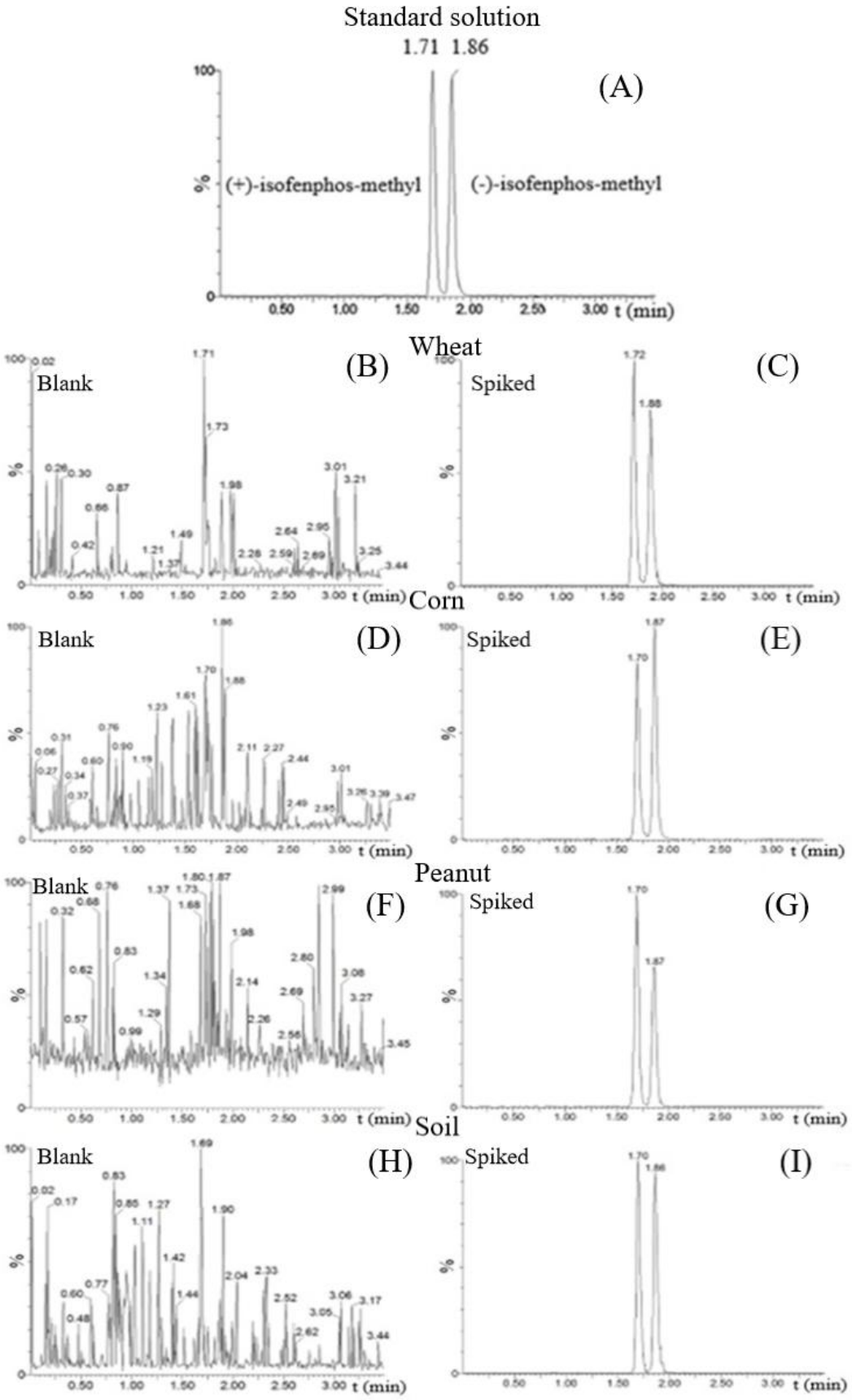

Table 1. Chiral separation of pyrethroids by HPLC

Pyrethroid	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
Permethrin (PM)	Veterinary powder formulation	n.p.	CSP: ChiraDex® column Mobile phase: MeOH (solvent A) and water (solvent B): 56% A (0 min), 56% A (16 min), 70% A (30 min), 56% A (40 min). Dectection: UV-DAD 215 nm	$R_{s1\text{-}2(trans)}$: 1.32 $R_{s3\text{-}4(cis)}$: 2.27 t_a: 42.3 min	0.07-0.19 µg	Determination of permethrin enantiomer ratio in veterinary formulation.	[11]
	Standard solution	-	CSP: Chiralcel® OJ-H column Mobile phase: hexane/EtOH/acetic acid (95/5/0.1, v/v/v) Detection: UV 220 nm	R_s: n.p. t_a: 11.0 min	n.p.	Semipreparative method to separate permethrin enantiomers. Evaluation of their enantioselective toxicity and endocrine disruption activity in male mice.	[19]
	Standard solution	-	CSP: ChiraDex® column Mobile phase: MeOH/water (70/30, v/v) Detection: UV 230 nm	$R_{s1\text{-}2(trans)}$: 1.20 $R_{s3\text{-}4(cis)}$: 2.20 t_a: 16.5 min	n.p.	-	[20]
Fenpropathrin (FPT)	Standard solution	-	(a) CSP: CDMPC column Mobile phase: MeOH/water (85/15, v/v) (b) CSP: ADMPC column Mobile phase: MeOH/water (80/20, v/v) Detection: UV-DAD 230 nm	(a) R_s: 0.35 t_a: 16.4 min (b) R_s: 0.59 t_a: 24.7 min	n.p.	-	[21]
	Soil	Extraction with anhydrous sodium sulfate, ethyl acetate, sodium chloride and acetic acid, filtration, evaporation to dryness and reconstitution in ACN.	CSP: Lux™ Cellulose-3 column Mobile phase: MeOH/water (85/15, v/v) Detection: UV-DAD 230 nm	R_s: 2.30 t_a: n.p.	0.015 µg g^{-1}	Evaluation of the enantioselective degradation of fenpropathrin in soil samples.	[13]

Bifenthrin (BF)	Standard solution	-	CSP: Chiralpak® IF-3 column Mobile phase: MeOH/ammonium acetate (80/20, v/v) Detection: UV 220 nm	R_s: 3.95 t_a: n.p.	n.p.	-	[22]
	Standard solution	-	CSP: Chiralcel® OJ column Mobile phase: hexane/EtOH (ratio n.p.) Detection: UV 230 nm	R_s: n.p. t_a: 16.1 min	n.p.	Semipreparative method to separate bifenthrin enantiomers. Evaluation of their enantioselective disrupting effects on progesterone and prostaglandin E2 synthesis via protein kinase C pathway in rat ovarian cells.	[23]
	Standard solution	-	CSP: Chiralcel® OJ column Mobile phase: hexane/1,2-dichloroethane (500/1, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate bifenthrin enantiomers. Evaluation of their effect on the acute locomotor activity and development of embryonic-larval zebrafish.	[24]
	Standard solution	Water samples: SPE with Oasis HLB cartridges as sorbent, elution with ethyl acetate, evaporation to dryness and reconstitution in MeOH. Zebrafish samples: extraction with ACN, evaporation to dryness and reconstitution in hexane. Clean-up in a glass column packed with sodium sulfate, neutral aluminium oxide, silica gel and florisil. Elution with hexane/dichloromethane (70/30, v/v), evaporation to dryness and reconstitution in MeOH.	CSP: Lux™ Cellulose-3 column Mobile phase: 0.1% ammonium formate in MeOH Detection: triple quadrupole MS	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate the enantiomers of cis-bifenthrin. Evaluation of their toxicity and metabolism in zebrafish in the presence of cadmium, copper and lead.	[12]

Cypermethrin (CYM)	Pig muscle	Extraction and clean-up with immunoaffinity columns.	CSP: Chiral CD-ph column Mobile phase: hexane/isopropyl alcohol (99.3/0.7, v/v) Detection: UV 230 nm	R_s: n.p. t_a: 19.4 min	17 µg Kg^{-1}	Determination of cypermethrin enantiomer concentrations in pig muscle tissue samples.	[14]
	Soil	Extraction with anhydrous sodium sulfate and ethyl acetate, filtration, evaporation to dryness and reconstitution in hexane.	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (98/2, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Evaluation of the enantioselective transformation of α-cypermethrin in soils and its toxicity to earthworms.	[15]
	Standard solution	-	CSP: Chiralcel® OD and Chiralpak® AD columns Mobile phase: hexane/isopropanol (97/3, v/v) Detection: UV 236 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate the enantiomers of cypermethrin. Evaluation of their toxicity in zebrafish embryos.	[25]
Cyphenothrin (CPN)	Soil and water sediments	Soil samples: extraction with 0.01 M $CaCl_2$, ethyl acetate and acetone. Water sediments: extraction with acetone and acetone/0.5 M HCl (8/2, v/v), evaporation to dryness and reconstitution in ethyl alcohol.	CSP: Sumichiral OA-2000 column Mobile phase: hexane/butanol (300/1, v/v) Detection: UV 254 and/or 278 nm	R_s: n.p. t_a: 119.7 min	n.p.	Determination of the dissipation and degradation profiles of cyphenothrin through aerobic metabolism in a water-sediment system and its aqueous photolysis. Soil adsorption studies to determine the partition profiles of cyphenothrin enantiomers.	[16]
λ-Cyhalothrin (λ-CYH)	Standard solution	-	CSP: Chiralcel® OD-H column Mobile phase: hexane/isobutanol (98/2, v/v) Detection: UV 254 nm	R_s > 4.00 t_a: 13.1 min	n.p.	-	[10]
Terallethrin (TLL)	Standard solution	-	CSP: ADMPC column Mobile phase: ACN/water (60/40, v/v) Detection: UV 230 nm	R_s: 2.14 t_a: 12.6 min	n.p.	-	[27]

(a) Permethrin (PM) **(b) Cypermethrin (CYM)** **(c) Cyfluthrin (CYF)**	Standard solution	-	(a) CSP: Chiralcel® OJ-H column Mobile phase: hexane/isopropanol (100/2, v/v) Detection: UV 225 nm (b and c) CSP: Chiralcel® OD-H column Mobile phase: (b) hexane/isopropanol (100/1, v/v) (c) hexane/isopropanol (100/2 v/v) Detection: UV 225 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate the enantiomers of the target pyrethroids. Evaluation of their photolysis and chiral stability.	[28]
	Soil	Extraction by MSPD, elution with hexane/ethyl acetate (7/1, v/v), evaporation to dryness and reconstitution in hexane.	(a) CSP: Chiralcel® OJ-H column Mobile phase: hexane/isopropanol (100/2, v/v) Detection: UV 230 nm (b and c) CSP: Chiralcel® OD-H column Mobile phase: (b) hexane/isopropanol (100/1, v/v) (c) hexane/isopropanol (100/2 v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	< 0.03 µg g^{-1}	Measurement and comparison of the stereo- and enantioselective degradation of the target pyrethroids in soil samples.	[17]
(a) Bifenthrin (BF) **(b) λ- Cyhalothrin (λ-CYH)**	Soil and water	Soil samples: extraction with ACN, anhydrous sodium sulfate and sodium chloride. Water samples: extraction with ethyl acetate and sodium chloride. Both sample extracts were filtered, evaporated to dryness and reconstituted with ACN.	CSP: Lux™ Cellulose-3 column Mobile phase: (a) MeOH/water (95/5, v/v) (b) MeOH/water (90/10, v/v) Detection: UV-DAD 220 nm	(a) R_s: 8.88 (b) R_s: 6.47 t_a: n.p.	0.01-0.015 mg L^{-1}	Determination of bifenthrin and λ-cyhalothrin enantiomers in soil and water samples.	[18]

(a) Cis-bifenthrin (Cis-BF) **(b) Permethrin (PM)** **(c) Fenvalerate (FEN)**	Standard solution	-	CSP: Chiralcel® OJ column Mobile phase: (a) hexane/EtOH (95/5, v/v) (b) hexane/isopropanol (95/5, v/v) (c) hexane/EtOH (90/10, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate the enantiomers of the target pyrethroids. Subjected them to bioassays to determine their endocrine disruption activity.	[29]

ACN: acetonitrile; ADMPC: amylose tris(3,5-dimethylphenylcarbamate); CDMPC: cellulose tris(3,5-dimethylphenylcarbamate); ChiraDex®: β-cyclodextrin-based stationary phase; Chiral CD-ph column: phenylcarbamate beta-cyclodextrin; Chiralcel® OD: cellulose tris(3,5-dimethylphenylcarbamate) coated on 10 µm silica-gel; Chiralcel® OD-H: cellulose tris(3,5-dimethylphenylcarbamate) coated on 5 µm silica-gel; Chiralcel® OJ: cellulose tris(4-methylbenzoate) coated on 10 µm silica-gel; Chiralcel® OJ-H: cellulose tris(4-methylbenzoate) coated on 5 µm silica-gel; Chiralpak® AD: amylose tris(3,5-dimethylphenylcarbamate) coated on 10 µm silica-gel; Chiralpak® IF-3: amylose tris(3-chloro-4-methylphenylcarbamate); CSP: chiral stationary phase; DAD: diode array detector; EtOH: ethanol; HLB: hydrophilic-lipophilic balance; LOD: limit of detection; Lux™ Cellulose-3: cellulose tris(4-methylbenzoate); MeOH: methanol; MSPD: matrix solid-phase dispersion; n.p.: not provided; SPE: solid-phase extraction; Sumichiral OA-2000: (R)-phenylglycine as chiral selector coated on 5 µm silica-gel; t_a: analysis time (elution time for the last eluting enantiomer).

Table 2. Chiral separation of pyrethroids by GC

Pyrethroid	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
Cypermethrin (CYM)	Water and mussel (*Uniogibbus*)	Water samples: LLE with chloroform, evaporation to dryness and reconstitution in ethyl acetate. Mussel samples: extraction with hexane/dichloromethane (2/1, v/v), SPE using alumina and C_{18} cartridges as sorbents and ACN as elution solvent, evaporation to dryness and reconstitution in ethyl acetate.	CSP: BGB-172 column Carrier gas: Helium T program: 180 ºC for 2 min, ramped at 5 ºC/min to 220 ºC and held for 30 min, ramped at 5 ºC/min to 230 ºC and held for 25 min, finally ramped at 5 ºC/min to 240 ºC and held for 5 min. Detection: Triple quadrupole MS	R_s: n.p. t_a: n.p.	n.p.	Evaluation of the enantiomeric selective accumulation of cypermethrin and its toxicity in mussel samples.	[31]
α-Cypermethrin (α-CYM)	Bullfrog	Extraction with ethyl acetate, evaporation to dryness and reconstitution in ACN.	CSP: BGB-172 column Carrier gas: Nitrogen T program: 160 ºC for 2 min, ramped at 1 ºC/min to 220 ºC and held for 40 min, and finally ramped at 5 ºC/min to 230 ºC and held for 60 min. Detection: ECD at 300 ºC	R_s: n.p. t_a: n.p.	n.p.	Evaluation of the enantioselective degradation behavior and metabolism of α-cypermethrin in bullfrog organs.	[32]
(a) Cypermethrin (CYM) **(b) Cis-bifenthrin (Cis-BF)**	Tea	Extraction of samples with hot water (90-100 ºC), acetone and hexane, evaporation to dryness of the upper phase of the extract and reconstitution in petroleum ether. Clean-up of the extracts in a glass cartridge packed with anhydrous sodium and florisil, elution with petroleum ether/diethyl ether (85/15, v/v) and condensation of the cleaned extract to 200 µL for analysis.	CSP: BGB-172 column Carrier gas: Helium T program: 160 ºC for 2 min, ramped at 1 ºC/min to 220 ºC and held for 60 min, and finally ramped at 5 ºC/min to 230 ºC and held for 40 min. Detection: ECD at 270 ºC	R_s: n.p. (a) t_a: 134.4 min (b) t_a: 69.6 min	n.p.	Determination of the enantiomeric fractions of the target analytes in commercial tea samples.	[33]
(a) Bifenthrin (BF) **(b) Cis-permethrin (Cis-PM)**	Sediment, water and fish	n.p.	CSP: BGB-172 column Carrier gas: Helium T program: 50 ºC for 1 min, ramped at 5 ºC/min to 160 ºC, ramped at 1 ºC/min to 230 ºC and held for 20 min. Detection: Electron impact MS	R_s: n.p. (a) t_a: 79.0 min (b) t_a: 95.0 min	n.p.	Characterization of the enantiomer fractions of the target analytes in environmental sample extracts (sediment and water) and laboratory-dosed fish.	[34]

(a) Cis-bifenthrin (Cis-BF) **(b) Cyhalothrin (CYH)** **(c) Cyfluthrin (CYF)** **(d) Cypermethrin (CYM)** **(e) Permethrin (PM)** **(f) Tetramethrin (TRM)**	Commercial insecticides and human breast milk	Domestic insecticides samples: evaporation to dryness and reconstitution in ethyl acetate. Insecticide human skin cream sample: LLE with ethyl acetate. Human breast milk samples: extraction with hexane/dichloromethane (2/1, v/v), SPE using alumina and C_{18} cartridges as sorbents and ACN as elution solvent, evaporation to dryness and reconstitution in ethyl acetate.	CSP: BGB-172 column Carrier gas: Helium T program: 180 ºC for 2 min, ramped at 5 ºC/min to 220 ºC and held for 30 min, ramped at 5 ºC/min to 230 ºC and held for 25 min, finally ramped at 5 ºC/min to 240 ºC and held for 5 min. Detection: Triple quadrupole MS	(a) R_s: 0.74 (b) $R_{s1\text{-}2(cis)}$: 0.58 $R_{s5\text{-}6(cis)}$: 0.90 (c) $R_{s1\text{-}2(cis)}$: 0.77 $R_{s5\text{-}6(cis)}$: 0.98 (d) $R_{s1\text{-}2(cis)}$: 0.85 $R_{s5\text{-}6(cis)}$: 0.90 (e) $R_{s(cis)}$: 0.89 (f) $R_{s1\text{-}2(cis)}$: 1.21 $R_{s3\text{-}4(trans)}$: 0.87 t_a: n.p.	4-49 fg	Determination of the enantiomeric fractions in commercial insecticides and human breast milk samples.	[35]
	Wild river fish	Extraction of samples with hexane/dichloromethane (2/1, v/v), SPE using alumina and C_{18} cartridges as sorbents and ACN as elution solvent, evaporation to dryness and reconstitution in ethyl acetate.	CSP: BGB-172 column Carrier gas: Helium T program: 180 ºC for 2 min, ramped at 5 ºC/min to 220 ºC and held for 30 min, ramped at 5 ºC/min to 230 ºC and held for 25 min, finally ramped at 5 ºC/min to 240 ºC and held for 5 min. Detection: Triple quadrupole MS	R_s: n.p. t_a: n.p.	0.03-0.46 ng g^{-1} (lw)	Evaluation of the enantioselective bioaccumulation of the target analytes in edible river fish samples.	[36]
	Wild bird eggs	Extraction of samples with hexane/dichloromethane (2/1, v/v), SPE using alumina and C_{18} cartridges as sorbents and ACN as elution solvent, evaporation to dryness and reconstitution in ethyl acetate.	CSP: BGB-172 column Carrier gas: Helium T program: 180 ºC for 2 min, ramped at 5 ºC/min to 220 ºC and held for 30 min, ramped at 5 ºC/min to 230 ºC and held for 25 min, finally ramped at 5 ºC/min to 240 ºC and held for 5 min. Detection: Triple quadrupole MS	R_s: n.p. t_a: n.p.	0.03-0.46 ng g^{-1} (lw)	Characterization of the enantiomer fractions of the target analytes. Evaluation of their enantioselective bioaccumulation in wild bird egg samples.	[37]

ACN: acetonitrile; BGB-172: 20% tert-butyldimethylsilyl-β-cyclodextrin in 15% phenyl-, 85%-methylpolysiloxane; C_{18}: octadecyl; CSP: chiral stationary phase; ECD: electron capture detector; LLE: liquid-liquid extraction; LOD: limit of detection; lw: lipid weight; n.p.: not provided; SPE: solid-phase extraction; t_a: analysis time (elution time for the last eluting enantiomer).

Table 3. Chiral separation of pyrethroids by SFC

Pyrethroid	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
β-Cypermethrin (β-CYM)	Standard solution	-	CSP: EnantioPak® OD column Mobile phase: CO_2/isopropanol (95/5, v/v) Detection: UV 230 nm CSP: EnantioPak® AD column Mobile phase: (a) CO_2/EtOH (80/20, v/v) (b) CO_2/EtOH (85/15, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	-	[39]
Cis-bifenthrin (Cis-BF)	Standard solution	-	CSP: Chromegachiral™ CCJ column Mobile phase: CO_2/MeOH (85/15, v/v) Detection: UV 254 nm	R_s: n.p. t_a: 3.7 min	n.p.	Semipreparative method to separate cis-bifenthrin enantiomers. Evaluation of their enantioselective toxicity and endocrine disruption activity in male mice.	[40]

Chromegachiral™ CCJ: cellulose 4-methylbenzoate; CSP: chiral stationary phase; EnantioPak® AD: silica gel coated with amylose tris(3,5-dimethylphenylcarbamate); EnantioPak® OD: silica gel coated with cellulose tris(3,5-dimethylphenylcarbamate); EtOH: ethanol; LOD: limit of detection; MeOH: methanol; n.p.: not provided; t_a: analysis time (elution time for the last eluting enantiomer).

Table 4. Chiral separation of organophosphorus pesticides by HPLC

Organophosphorus pesticide	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
Crufomate (CRF)	Standard solution	-	**Normal Phase HPLC:** (a) CSP: Lux™ Cellulose-1 column Mobile phase: isopropanol/hexane (2/98, v/v) (b) CSP: Lux™ Amylose-2 column Mobile phase: isopropanol/hexane (2/98, v/v) (c) CSP: Lux™ Cellulose-2 column Mobile phase: isopropanol/hexane (5/95, v/v) **Reverse Phase HPLC:** (d) CSP: Lux™ Cellulose-1 column Mobile phase: ACN/water (40/60, v/v) (e) CSP: Lux™ Amylose-2 column Mobile phase: ACN/water (30/70, v/v) Detection: UV 210 nm	(a) R_s: 2.78 (b) R_s: 3.55 (c) R_s: 4.08 (d) R_s: 2.75 (e) R_s: 3.56 t_a: n.p.	n.p.	-	[44]
O-ethyl O-4-nitrophenyl phenylphosphonothioate (EPN)	Standard solution	-	(a) CSP: Chiralpak® AD column Mobile phase: hexane/isopropanol (99/1, v/v) (b) CSP: Chiralpak® AS column Mobile phase: hexane/EtOH (99/1, v/v) Detection: UV 236 nm	(a) R_s: 5.39 (b) R_s: 2.50 t_a: n.p.	n.p.	Semipreparative method to separate EPN enantiomers. Evaluation of their toxicity in *Daphnia magna* and zebrafish embryos.	[45]

Fenamiphos (FAP)	Standard solution	-	CSP: ADMPC column Mobile phase: ACN/water (60/40, v/v) Detection: UV 230 nm	R_s: 1.89 t_a: 7.5 min	n.p.	-	[27]
	Standard solution	-	CSP: Chiralpak® AD-H column Mobile phase: hexane/EtOH (98/2, v/v) Detection: UV 254 nm	R_s: 3.42 t_a: n.p.	n.p.	Semipreparative method to separate fenamiphos enantiomers. Evaluation of their toxicity in *Daphnia magna* and their inhibition potential towards AChE in rat PC12 cells.	[48]
	Soils	QuEChERS extraction with deionized water, ACN, NaCl and anhydrous $MgSO_4$ clean-up with $MgSO_4$ and PSA, evaporation to dryness and reconstitution in hexane.	CSP: Chiralpak® AD-H column Mobile phase: hexane/isopropanol (87/13, v/v) Detection: UV 225 nm	R_s: n.p. t_a: n.p.	10.0 µg kg^{-1}	Study stereochemistry of the successive sulfoxidation of fenamiphos in soils. Evaluation of its stereoselective toxicity in zebrafish embryos.	[47]
	Human liver microsomes	LLE with ethyl acetate and sodium metabisulfite solution, evaporation to dryness and reconstitution in mobile phase.	CSP: Chiralpak® AS-H column Mobile phase: hexane/EtOH/MeOH (85/12/3, v/v/v) Detection: UV-DAD 250 nm	R_s > 1.30 t_a: n.p.	n.p.	Study the in vitro metabolism of fenamiphos by human liver microsomes and predict some of its toxicokinetic properties.	[4]
	Human serum	Incubation of sera with racemic fenamiphos, tris-HCl and $CaCl_2$ or EDTA. Reaction stopped with HCl. LLE with hexane.	CSP: Chiralcel® OJ column Mobile phase: hexane/EtOH (99/1, v/v) Detection: UV 235 nm	R_s: 1.20 t_a: 23.0 min	(+)-fenamiphos: 0.6 µM (-)-fenamiphos: 0.7 µM	Study the stereoselective hydrolysis of fenamiphos by PON1 Q192 alloenzyme from human serum of children and adults.	[46]

Isocarbophos (ICP)	Standard solution	-	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (90/10, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate isocarbophos enantiomers and assay their cytotoxicity using Hep G2 cells.	[52]
	Aqueous environmental samples	SPE with C_{18} cartridges, elution with MeOH, evaporation to near dryness, addition of water, dichloromethane and ACN for DLLME, evaporation to dryness of the sedimented phase and reconstitution in the mobile phase.	CSP: Chiralcel® OD-RH column Mobile phase: 0.1% formic acid/ACN (60/40, v/v) Detection: MS/MS	R_s > 1.45 t_a: 17.4 min	0.82-1.54 ng g^{-1}	Determination of isocarbophos enantiomers in aqueous environmental samples.	[53]
	Soils and river sediments	MSPD with C_{18} sorbent, elution with MeOH, evaporation to dryness, addition of water, CAN and dichloromethane for DLLME, evaporation to dryness of the sedimented phase and reconstitution in the mobile phase.	CSP: Chiralcel® OD-RH column Mobile phase: 0.1% formic acid /ACN (60/40, v/v) Detection: MS/MS	R_s: 1.49 t_a: 17.4 min	0.40 µg L^{-1}	Determination of isocarbophos enantiomers in soils and river sediments.	[54]
	Standard solution	-	(a) CSP: CDMPC column Mobile phase: MeOH/water (65/35, v/v) (b) CSP: ADMPC column Mobile phase: ACN/water (30/70, v/v) Detection: UV-DAD 230 nm	(a) R_s: 1.33 t_a: 13.5 min (b) R_s: 1.79 t_a: 41.3 min	n.p.	-	[21]
	Soils	Extraction with water, ACN, anhydrous $MgSO_4$ and NaCl, evaporation to dryness and reconstitution in water.	CSP: Chiralpak® AD-RH column Mobile phase: ACN/2 mM ammonium acetate aqueous solution containing 0.1% formic acid (60/40, v/v) Detection: MS/MS	R_s: n.p. t_a: 2.3 min	0.005 µg g^{-1}	Evaluation of the enantioselective degradation of isocarbophos in soil samples.	[49]

	Rice, soil and water	Water samples: SPE with C_{18} cartridges and elution with MeOH. Soil and rice samples: extraction with water, 1% acetic acid in ACN, anhydrous magnesium sulfate and anhydrous sodium acetate, evaporation to dryness and reconstitution in water.	CSP: Chiralpak® AD-3R column Mobile phase: ACN with 0.1% formic acid solution (phase A) and 0.1% formic acid solution (phase B). Gradient elution: 0-4.0 min 30% A, 4.0-9.5 min 60% A, 9.5-11.0 min 30% A, 11.0-14.0 min 30% A. Detection: MS/MS	R_s: n.p. t_a: 9.4 min	Water samples: 0.1 µg kg^{-1} Rice and soil samples: 0.5 µg kg^{-1}	Determination of isocarbophos enantiomers in water, rice and soil samples.	[51]
	Orange pulp, peel and kumquat	QuEChERS extraction with ACN containing 1% of acetic acid, $MgSO_4$ and CH_3COONa, clean-up with $MgSO_4$ and PSA. Upper layer mixed with water and filtered.	CSP: Chiralpak® AD-3R column Mobile phase: ACN/water containing 2 mmol L^{-1} ammonium formate and 0.1% formic acid (60/40, v/v) Detection: MS/MS	R_s: 2.46 t_a: 3.0 min	0.2-0.5 µg kg^{-1}	Determination of isocarbophos enantiomers in orange pulp, peel and kumquat.	[50]
Isofenphos-methyl (IFM)	Vegetables, fruits and soils	Extraction with ACN (ultrapure water was also added in the case of soil samples), NaCl, $MgSO_4$ and anhydrous sodium sulfate, evaporation to dryness, reconstitution in hexane, SPE with Florisil cartridges (for fruits and soils sample extracts) and Alumina-A cartridges (for vegetables sample extracts), elution with hexane, evaporation to dryness and reconstitution in the mobile phase.	CSP: Lux™ Cellulose-3 column Mobile phase: ACN/water/MeOH (31/57/12, v/v/v) Detection: UV 228 nm	R_s: 1.52 t_a: n.p.	0.008–0.011 mg kg^{-1}	Determination of isofenphos-methyl enantiomers in vegetables, fruits and soils. Evaluation of the enantioselective degradation of isofenphos-methyl in pak choi.	[55]

Methamidophos (MTD)	Standard solution	-	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (90/10, v/v) Detection: UV 230 nm	R_s: 1.25 t_a: n.p.	n.p.	Semipreparative method to isolate methamidophos enantiomers. Evaluation of their in vitro inhibition of plasma BChE of hens.	[56]
	Standard solution	-	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (90/10, v/v) Detection: UV 230 nm	R_s: 1.25 t_a: n.p.	n.p.	Semipreparative method to isolate methamidophos enantiomers. Evaluation of their delayed neuropathy effects in hens.	[57]
	Standard solution	-	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (90/10, v/v) Detection: UV 230 nm	R_s: 1.25 t_a: n.p.	n.p.	Semipreparative method to isolate methamidophos enantiomers. Evaluation of their potential to induce AChE inhibition and/or delayed neurotoxicity in human and hen cells.	[58]
O,S-dimethyl-N-(2,2,2-trichloro-1-methoxyethyl)phosphoramidothioate (MCP)	Standard solution	-	CSP: Chiralpak® AD column Mobile phase: hexane/EtOH (85/15, v/v) Detection: n.p.	$R_s > 1.5$ t_a: n.p.	n.p.	Semipreparative method to separate MCP enantiomers. Evaluation of their toxicities in *Daphnia magna*.	[59]
Profenofos (PFF)	Standard solution	-	CSP: Chiralcel® OJ column Mobile phase: hexane/isopropanol (99/1, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate profenofos enantiomers. Evaluation of their induced cytotoxicity and DNA damage in PC12 cells.	[60]

Pyraclofos (PYR)	Standard solution	-	CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (85/15, v/v) Detection: UV 254 nm	R_s: 8.10 t_a: n.p.	n.p.	Semipreparative method to separate pyraclofos enantiomers. Evaluation of their enantioselective toxicity to human BChE and *Daphnia magna*.	[61]
	Soil	Extraction with water, ACN, anhydrous $MgSO_4$ and NaCl, clean-up with PSA, C_{18} and $MgSO_4$ and dilution with water for analysis.	CSP: Phenomenex Lux™ Cellulose-4 column Mobile phase: MeOH/0.1% formic acid (55/45, v/v) Detection: MS/MS	R_s: n.p. t_a: 10.4 min	0.6 ng g^{-1}	Evaluation of the enantioselective degradation of pyraclofos in soil samples.	[62]
	Standard solution	-	CSP: Phenomenex Lux™ Cellulose-4 column Mobile phase: MeOH/0.1% formic acid (55/45, v/v) Detection: MS/MS	R_s: n.p. t_a: 10.4 min	n.p.	Semipreparative method to separate pyraclofos enantiomers. Evaluation of their enantioselective potential aquatic toxicity towards zebrafish.	[63]
Trichlorfon (TF)	Mariculture pond water	SPE with Oasis® HLB cartridges, elution with ethyl acetate, evaporation to dryness and reconstitution in the mobile phase.	CSP: Chiralpak® IC column Mobile phase: hexane/isopropanol/EtOH (90/8.5/1.5, v/v/v) Detection: UV 207 nm	R_s: 8.77 t_a: n.p.	R-(-)-trichlorfon: 0.012 mg L^{-1} S-(+)-trichlorfon: 0.015 mg L^{-1}	Determination of trichlorfon enantiomers and evaluation of their enantioselective degradation in mariculture pond water.	[64]
	Fish	Extraction with ACN containing 0.1% acetic acid, SPE with ProElut™ PLS cartridges, elution with ethyl acetate, evaporation to dryness and reconstitution in the mobile phase.	CSP: Chiralpak® IC column Mobile phase: hexane/isopropanol (91/9, v/v) Detection: UV 207 nm	R_s: n.p. t_a: n.p.	R-(-)-trichlorfon: 0.016 µg g^{-1} S-(+)-trichlorfon: 0.018 µg g^{-1}	Determination of trichlorfon enantiomers in fish samples and evaluation of their enantioselective degradation during fish storage.	[65]

Malathion (MA)	Standard solution	-	CSP: Chiralcel® OJ column Mobile phase: hexane/isopropanol/trifluoroacetic acid (95/4.9/0.1, v/v/v) Detection: UV 254 nm	R_s: n.p. t_a: 13.0 min	n.p.	-	[68]
	Vegetables and crops	Extraction with ethyl acetate, petroleum ether and sodium chloride, filtration with anhydrous sodium sulfate, evaporation to dryness, reconstitution in petroleum ether, clean-up through a column with anhydrous Na_2SO_4/alumina neutral + activated carbon/anhydrous Na_2SO_4, elution with petroleum ether/ethyl acetate (1/2, v/v), evaporation to dryness and reconstitution in isopropanol.	CSP: CDMPC column coated on aminopropylated spherical gel Mobile phase: hexane/isopropanol (99/1, v/v) Detection: UV 230 nm	R_s: 1.88 t_a: n.p.	0.015 µg g^{-1}	Determination of malathion enantiomers. Evaluation of their enantioselective dissipation behaviour in vegetables and crops. Semipreparative method to isolate malathion enantiomers. Evaluation of their enantioselective toxicity in earthworms and bees.	[67]
	Soil and water	Soil samples: extraction with ethyl acetate, filtration through anhydrous sodium sulfate, evaporation to dryness and reconstitution in isopropanol. Water samples: SPE with ODS-C_{18} cartridges, elution with MeOH, evaporation to dryness and reconstitution in isopropanol.	CSP: CDMPC column Mobile phase: hexane/ispropanol (98/2, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	Soil samples: 0.03 µg g^{-1} Water samples: 0.015 µg L^{-1}	Determination of malathion enantiomers and evaluation of their enantioselective degradation and chiral stability in soil and water samples.	[66]

(a) Isomalathion (IMA) **(b) Malathion (MA)**	Standard solution	-	(a, b) CSP: Chiralcel® OD column Mobile phase: hexane/isopropanol (80/20, v/v) Detection: UV 230 nm	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate malathion and isomalathion enantiomers. Evaluation of their stereoselective toxicity on *Daphnia magna* and their interaction with acid α-naphthyl acetate esterase.	[69]
	Standard solution	-	(a) CSP: Chiralpak® AD column Mobile phase: hexane/isopropanol (91/9, v/v) (b) CSP: Chiralcel® OJ column Mobile phase: hexane/isopropanol (97/3, v/v) Detector: UV 220 nm	(a) R_{s1-2}: 1.85 R_{s2-3}: 3.04 R_{s3-4}: 2.97 t_a: n.p. (b) R_s: 3.35 t_a: n.p.	n.p.	-	[70]
	Standard solution	-	(a) CSP: Chiralpak® AD column Mobile phase: hexane/isopropanol (91/9, v/v) (b) CSP: Chiralcel® OJ column Mobile phase: hexane/isopropanol (97/3, v/v) Detector: UV 220 nm	(a) R_{s1-2}: 1.85 R_{s2-3}: 3.04 R_{s3-4}: 2.97 t_a: n.p. (b) R_s: 3.35 t_a: n.p.	n.p.	Enantioseparation of isomalathion and malathion to study the enantioselective interaction of acid α-naphthyl acetate esterase with each enantiomer.	[71]

(a) Fenamiphos (FAP) **(b) Isocarbophos (ICP)** **(c) Profenofos (PFF)**	Water, soil and river sediments	Water samples: MSPE with m-MWCNTs-NH_2, elution with ACN, evaporation to dryness and reconstitution in the mobile phase. Soils and river sediments: extraction with ACN and water, MSPE with m-MWCNTs-NH_2, elution with ACN, evaporation to dryness and reconstitution in the mobile phase.	CSP: Chiralpak® IG column Mobile phase: ACN/water containing 5 mM ammonium acetate and 0.05% formic acid (53/47, v/v) Detection: MS/MS	(a) R_s: 3.20 t_a: 20.6 min (b) R_s: 4.25 t_a: 12.2 min (c) R_s: 1.52 t_a: 47.5 min	Water samples: (a) 0.34-0.48 ng L^{-1} (b) 0.51-0.55 ng L^{-1} (c) 0.35-0.42 ng L^{-1} Soil samples: (a) 0.07-0.13 ng g^{-1} (b) 0.08-0.11 ng g^{-1} (c) 0.08-0.10 ng g^{-1} Sediment samples: (a) 0.07 ng g^{-1} (b) 0.11 ng g^{-1} (c) 0.09 ng g^{-1}	Determination of fenamiphos, isocarbophos and profenofos enantiomers in water, soil and river sediments.	[72]
	Fruits and vegetables	Extraction with ACN and water, MSPE with magnetic-graphene nanocomposite, elution with ACN, evaporation to dryness and reconstitution in the mobile phase.	CSP: Chiralpak® IG column Mobile phase: ACN/water containing 5 mM ammonium acetate and 0.1% formic acid (65/35, v/v) Detection: MS/MS	(a) R_s: 2.34 t_a: 17.5 min (b) R_s: 3.27 t_a: 10.6 min (c) R_s: 1.44 t_a: 38.1 min	(a) 0.10-0.25 ng g^{-1} (b) 0.15-0.20 ng g^{-1} (c) 0.12-0.15 ng g^{-1}	Determination of fenamiphos, isocarbophos and profenofos enantiomers in fruits and vegetables.	[73]
(a) Fensulfothion (FTN) **(b) Methamidophos (MTD)** **(c) Profenofos (PFF)**	Standard solution	-	CSP: Chiralcel® OD and Chiralcel® OJ columns Mobile phase: (a) hexane/EtOH (95/5, v/v) (b) hexane/isopropanol (80/20, v/v) (c) hexane/isopropanol (99/1, v/v) Detection: n.p.	R_s: n.p. t_a: n.p.	n.p.	Semipreparative method to separate fensulfothion, methamidophos and profenofos enantiomers. Evaluation of their enantioselective inhibition potential on AChE and their toxicity in *Daphnia magna*.	[74]

(a) Fenamiphos (FAP) **(b) Malathion (MA)** **(c) Phenthoate (PTH)** **(d) Profenofos (PFF)**	Standard solution	-	(a, d) CSP: Chiralcel® OJ-H column (b, c) CSP: Chiralcel® OD-H column (a, c) Mobile phase: hexane/isopropanol (100/1, v/v) (b, d) Mobile phase: hexane/isopropanol (100/3, v/v) Detection: UV (a) 254 (b, c, d) 230 nm	(a) R_s: 1.79 t_a: 49.4 min (b) R_s: 3.79 t_a: 18.5 min (c) R_s: 1.83 t_a: 12.8 min (d) R_s: 2.61 t_a: 10.5 min	n.p.	Semipreparative method to separate fenamiphos, malathion, phentoate and profenofos enantiomers. Evaluation of their racemization in organic solvents and buffer solutions.	[75]

AChE: acetylcholinesterase; ACN: acetonitrile; ADMPC: amylose tris(3,5-dimethylphenylcarbamate); BChE: butyrylcholinesterase; C_{18}: octadecyl; CDMPC: cellulose tris(3,5-dimethylphenylcarbamate); Chiralcel® OD: cellulose tris(3,5-dimethylphenylcarbamate) coated on 10 µm silica-gel; Chiralcel® OD-H: cellulose tris (3,5-dimethylphenylcarbamate) coated on 5 µm silica-gel; Chiralcel® OD-RH: cellulose tris(3,5-dimethylphenylcarbamate) coated on 5 µm silica-gel; Chiralcel® OJ: cellulose tris(4-methylbenzoate) coated on 5 µm silica-gel; Chiralcel® OJ-H: cellulose tris(4-methylbenzoate) coated on 5 µm silica-gel; Chiralpak® AD: amylase tris(3,5-dimethylphenylcarbamate); Chiralpak® AD-3R: amylose tris(3,5-dimethylphenylcarbamate); Chiralpak® AD-H: amylase tris-(S)-1-methylphenylcarbamate; Chiralpak® AD-RH: amylose tris(3,5-dimethylphenylcarbamate); Chiralpak® AS: amylase tris-(S)-1-methylphenylcarbamte; Chiralpak® AS-H: amylose tris-(S)-α-methylbenzylcarbamate coated on 5 µm silica-gel; Chiralpak® IC: cellulose tris-(3,5-dichlorophenylcarbamate) immobilised on 5 µm silica-gel; Chiralpak® IG: amylose tris(3-chloro-5-methylphenylcarbamate); CSP: chiral stationary phase; DAD: diode array detector; DLLME: dispersive liquid-liquid microextraction; EPN: O-ethyl O-4-nitrophenyl phenylphosphonothioate; EtOH: ethanol; Hep G2: liver hepatocellular cells; HLB: hydrophilic-lipophilic balance; LLE: liquid-liquid extraction; LOD: limit of detection; Lux™ Amylose-2: amylose tris(5-chloro-2-methylphenylcarbamate); Lux™ Cellulose-1: cellulose tris(3,5-dimethylphenylcarbamate); Lux™ Cellulose-2: cellulose tris(3-chloro-4-methylphenylcarbamate); Lux™ Cellulose-3: cellulose tris(4-methylbenzoate); MCP: O,S-dimethyl-N-(2,2,2-trichloro-1-methoxyethyl)phosphoramidothioate; MeOH: methanol; m-MWCNTs-NH_2: magnetic amino modified multiwalled carbon nanotubes; MSPE: magnetic solid-phase extraction; n.p.: not provided; PC12 cells: pheochromocytoma 12 cells; Phenomenex Lux™ Cellulose-4: cellulose tri-(4-chloro-3-methylphenylcarbamate); PON1: paraoxonase 1; PSA: primary-secondary amine; QuEChERS: quick, easy, cheap, effective, rugged and safe; SPE: solid-phase extraction; t_a: analysis time (elution time for the last eluting enantiomer).

Table 5

Table 5. Chiral separation of organophosphorus pesticides by GC

Organophosphorus pesticide	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
(a) Acephate (APT) (b) Methamidophos (MTD)	Vegetables	QuEChERS extraction with ACN, $MgSO_4$ and sodium acetate, clean-up by dSPE with $MgSO_4$, PSA and C_{18}.	CSP: BGB-176 SE column Carrier gas: Helium T program: 90 ºC for 1 min, ramped at 8 ºC/min to 220 ºC and held for 10 min. Detection: MS/MS	(a) R_s > 1.50 t_a: 14.9 min (b) R_s > 1.50 t_a: 12.9 min	(a) 8 µg kg^{-1} (b) 5 µg kg^{-1}	Determination of acephate and methamidophos enantiomers in vegetables.	[78]
	Soil	QuEChERS extraction with ACN, $MgSO_4$ and NaCl, clean-up by dSPE with $MgSO_4$ and PSA, evaporation to dryness and reconstitution in acetone.	CSP: Cyclosil-B column Carrier gas: Helium T program: 90 ºC for 1 min, ramped at 8 ºC/min to 220 ºC and held for 10 min. Detection: MS/MS	(a) R_s: n.p. t_a: 14.3 min (b) R_s: n.p. t_a: 10.9 min	n.p.	Evaluation of the enantioselective degradation and transformation of acephate and methamidophos in soils.	[79]
	Tea	Made tea: QuEChERS extraction with boiled water, ACN, $MgSO_4$ and NaCl, clean-up by dSPE with PSA, C_{18}, GCB and $MgSO_4$, evaporation to dryness and reconstitution in acetone. Fresh tea leaves: QuEChERS extraction with ACN, $MgSO_4$ and NaCl, clean-up by dSPE with PSA, C_{18}, GCB and $MgSO_4$, evaporation to dryness and reconstitution in acetone. Tea soup: LLE with dichloromethane, evaporation to dryness and reconstitution in acetone. Spent tea leaves: QuEChERS extraction with ACN, $MgSO_4$ and NaCl, clean-up by dSPE with PSA, C_{18}, GCB and $MgSO_4$, evaporation to dryness and reconstitution in acetone.	CSP: BGB-176 column Carrier gas: Nitrogen T program: 80 ºC for 1 min, ramped at 10 ºC/min to 220 ºC and held for 5 min. Detection: FPD	(a) R_s: 1.37 t_a: 13.6 min (b) R_s: 1.97 t_a: 12.0 min	(a) 5-100 µg kg^{-1} (b) 3-30 µg kg^{-1}	Evaluation of the enantioselective dissipation of acephate and methamidophos during tea cultivation, manufacturing and infusion.	[80]

ACN: acetonitrile; BGB-176: 20% 2,3-dimethyl-6-tert-butyldimethylsilyl-β-cyclodextrin dissolved in BGB-15 (15% phenyl-, 85%-methylpolysiloxane); BGB-176 SE: 20%

2,3-dimethyl-6-tert-butyldimethylsilyl-β-cyclodextrin dissolved in SE-52 (5% phenyl-, 95%-methylpolysiloxane); C_{18}: octadecyl; CSP: chiral stationary phase; Cyclosil-B: heptakis (2,3-di-O-methyl-6-O-t-butyldimethylsilyl)-β-cyclodextrin; dSPE: dispersive solid-phase extraction; FPD: flame photometric detector; GCB: graphitized carbon black; LLE: liquid-liquid extraction; LOD: limit of detection; n.p.: not provided; PSA: primary-secondary amine; QuEChERS: quick, easy, cheap, effective, rugged and safe; t_a: analysis time (elution time for the last eluting enantiomer).

Table 6. Chiral separation of organophosphorus pesticides by SFC

Organophosphorus pesticide	Matrix	Sample preparation	Optimal separation conditions	R_s and t_a	LOD	Application	Ref.
Isofenphos-methyl (IFM)	Wheat, corn, peanut and soil	QuEChERS extraction with water, ACN, NaCl and $MgSO_4$, clean-up by dSPE with $MgSO_4$ and C_{18} (wheat, corn and soil) or florisil (peanut).	CSP: Chiralpak® IA-3 column Mobile phase: CO_2/isopropanol (90/10, v/v) Detection: MS/MS	R_s: 2.20 t_a: 1.9 min	0.02-0.15 µg kg^{-1}	Determination of isofenphos-methyl enantiomers in wheat, corn, peanut and soil samples.	[81]
(a) Isocarbophos (ICP) **(b) Isofenphos (IFP)** **(c) Isofenphos-methyl (IFM)**	Standard solution	-	(a) CSP: Lux™ 3u Cellulose-1 column Mobile phase: CO_2/EtOH (91/9, v/v) (b, c) CSP: Chiralpak® AD-3 column Mobile phase: CO_2/isopropanol (91/9, v/v) Detection: UV 230 nm	(a) R_s: 3.93 t_a: 5.7 min (b) R_s: 1.02 t_a: n.p. (c) R_s: 2.03 t_a: 5.0 min	n.p.	-	[82]

ACN: acetonitrile; C_{18}: octadecyl; Chiralpak® AD-3: amylose tris(3,5-dimethylphenylcarbamate) coated on a silica gel support; Chiralpak® IA-3: amylose tris(3,5-dimethylphenylcarbamate); CSP: chiral stationary phase; dSPE: dispersive solid-phase extraction; EtOH: ethanol; QuEChERS: quick, easy, cheap, effective, rugged and safe; LOD: limit of detection; Lux™ 3u Cellulose-1: cellulose tris(3,5-dimethylphenylcarbamate) coated on a silica gel support; n.p.: not provided; t_a: analysis time (elution time for the last eluting enantiomer).