ENANTIOMERIC SEPARATION OF PROTHIOCONAZOLE AND PROTHIOCONAZOLE-DESTHIO BY CAPILLARY ELECTROPHORESIS. DEGRADATION STUDIES IN ENVIRONMENTAL SAMPLES

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Highlights

- The first enantiomeric separation of prothioconazole by CE is presented
- Enantiomeric analysis of prothioconazole-based commercial agrochemical formulations
- First simultaneous chiral analysis of prothioconazole and its main metabolite by CE
- Degradation study of prothioconazole and its metabolite in soil and sand samples
- First-order kinetic equations for degradation studies are provided

ABSTRACT

In this work, two analytical methodologies by Capillary Electrophoresis were developed. The first one enabled the rapid and cost-effective enantioseparation of prothioconazole and was applied to the analysis of prothioconazole-based commercial agrochemical formulations. The second methodology enabled the simultaneous enantioseparation of prothioconazole and its metabolite prothioconazole-desthio and was applied to degradation studies of both compounds in soil and sand samples. The influence of several experimental variables was investigated to develop both methodologies. The separation of prothioconazole enantiomers was achieved in 4.5 min with a resolution of 2.8 employing a neutral cyclodextrin (heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin). Given the nature of prothioconazole-desthio, a neutral cyclodextrin cannot be used for its chiral separation. For this reason, the simultaneous enantioseparation of prothioconazole and prothioconazole-desthio was achieved in 5.5 min with resolution values of 1.9 and 8.2, respectively, using a negatively charged cyclodextrin (sulfated-γ-cyclodextrin). The analytical characteristics of the developed methodologies were evaluated and both methods showed good performance to be applied to the quantitation of the enantiomers of prothioconazole in commercial agrochemical formulations (LOD 0.7 mg L^{-1}) and to carry out degradation studies for both compounds in environmental matrices (LODs lower than 0.9 and 1.3 mg L^{-1} for prothioconazole and prothioconazole-desthio enantiomers, respectively). The recovery values obtained were in the range between 94-104 % for the agrochemical formulations, between 96-99 % for the sand samples and between 97-100 % for the soil samples.

Keywords: capillary electrophoresis, chiral separation, prothioconazole, prothioconazole-desthio, agricultural formulations, degradation studies.

1. Introduction

The Food and Agriculture Organization of the United Nations (FAO) defines pesticide as any substance (pure or mixture) that can prevent, destroy, or control pests [1]. Since the first pesticide was synthesized in 1874, the Environmental Protection Agency (EPA) has registered more than 20000 products as pesticides [2]. The increase in the world population in recent decades has demanded greater agricultural productivity, as well as better quality of products. For this reason, the use of pesticides has increased significantly [1]. Specifically, since 1950 its use has increased 50 times [3]. Approximately, a 30 % of the active ingredients of the current registered pesticides contain asymmetric centers [4]. Frequently, pesticides have from one to three chiral centers giving rise to two, four or eight enantiomers which can present different biological activity, persistence in the environment and even, different toxicity. Despite this, most chiral pesticides are constituted by racemic mixtures [5]. In fact, possibly due to the high costs of purification and production of pure enantiomers, it is estimated that only a 7 % of the current registered pesticides are marketed as an enriched mixture of the active enantiomer or as a pure enantiomer [1]. In many cases, only one of the pesticide enantiomers is active, while the other can be less active or even toxic to non-target organisms [6]. The use of racemic mixtures in which only one of the enantiomers is active, implies the emission to the environment of a major amount of the pesticide to obtain the same results.

(R,S)-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione), commonly known as prothioconazole and introduced on the market in 2004 [7], is a widely used chiral triazole fungicide [8] for the control of rusts, leaf spot diseases, powdery mildew, *Pyricularia grisea, Sclerotinia sclerotiorum, Puccinia striiformis* and *Fusarium head blight* in economic crops such as soybean and cereals [9, 10]. It acts by inhibiting the C-14α-demethylase enzyme, which is involved in

the biosynthesis of fungal sterols [8, 10]. In soils, animals and plants, prothioconazole can be degraded by desulfurization to its main metabolite, prothioconazole-desthio [(R,S)-(2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol)], which exhibits greater mammalian toxicity than prothioconazole [7]. Both prothioconazole and prothioconazole-desthio have an asymmetrical carbon atom, so both consist of a pair of enantiomers: R-(-) and S-(+)-prothioconazole and R-(+) and S-(-)-prothioconazole-desthio [8]. R-enantiomers of both analytes have demonstrated to be more potent against pathogenic fungi and more effective inhibiting the biosynthesis of mycotoxins than the S-enantiomers [11]. Zhai *et al.* also found that R-(-)-prothioconazole was more active than S-(+)-prothioconazole towards different target organisms such as rice blast fungus, *Alternaria triticina*, exserohilum turcicum, wheat phytoalexin and *Fusarium avenaceum* [12].

The different behavior that enantiomers can exhibit makes relevant the development of chiral methodologies enabling the enantiomers of prothioconazole and its main metabolite to be distinguished. To date, the studies focused on the enantiomeric separation of prothioconazole and prothioconazole-desthio are scarce (see **Table 1**) [7-10, 12-18]. Among the analytical techniques used, the most employed has been HPLC [7, 9, 12-17]. As shown in **Table 1**, different polysaccharide chiral columns have been employed in combination with MS [7, 9, 13-15] and UV [12, 16, 17] detectors. Basically, the application of HPLC-based methodologies have enabled: i) to study their enantioselective degradation and transformation in soils, finding that under native conditions, R-(-)-prothioconazole was preferentially degraded and prothioconazole-desthio was rapidly formed during prothioconazole dissipation, while, under sterile conditions, prothioconazole and prothioconazole-desthio enantiomers were more slowly degraded without enantioselectivity [9]; ii) to study the accumulation of prothioconazole

113 **Table 1.** Articles previously published related to the chiral separation of prothioconazole and/or prothiconazole-desthio.
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Technique/Compound	Separation conditions	R_s and t_a	Application	Ref.
HPLC **Prothioconazole**	CSP: Lux™ Cellulose-1 Mobile phase: ACN/0.1 % formic acid (60/40, v/v) Detection: MS	R_s: 1.6 t_a: 6.9 min	Enantioseparation of prothioconazole standard solution.	[15]
HPLC **Prothioconazole**	CSP: Chiralcel® OD-RH Mobile phase: ACN/water (7/3, v/v) Detection: n.p.	R_s: n.p. t_a: n.p.	Enantioseparation of prothioconazole standard solution. Study the differences of inhibition of pathogens growth (wheat phytoalexin, rice blast fungus, exerohilum turcicum, *Alternaria triticina and Fusarium avenaceum*) and the toxicity to three nontarget aquatic organisms (*Daphnia magna, Chlorella pyrenoidosa and Lemna minor).*	[12]
HPLC **Prothioconazole**	CSP: Chiralcel® OD-RH Mobile phase: ACN/0.1 % formic acid (80/20, v/v) Detection: MS	R_s: n.p. t_a: 5.1 min	Enantioseparation and determination of prothioconazole in soil and earthworm samples. Study the enantioselective degradation in soils and its enantioselective accumulation and degradation in earthworms.	[13]
SFC **Prothioconazole**	CSP: EnantioPak® OD Mobile phase: CO_2/isopropanol (80/20, v/v) Detection: ECD 254 nm	R_s: 3.6 t_a: 3.5 min	Enantioselective determination and residual quantitative analysis of prothioconazole in food and environmental samples.	[10]
HPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Lux™ Cellulose-1 Mobile phase: ACN/water (80/20, v/v) Detection: UV 220 nm	$R_{s(Prot)}$: 4.7 $R_{s(Desthio\text{-}Prot)}$: 2.4 t_a: 8.5 min	Simultaneous enantioseparation of prothioconazole and prothioconazole-desthio in a standard solution.	[16]
HPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Lux™ Cellulose-1 Mobile phase: ACN/water (45/55, v/v) Detection: MS	$R_{s(Prot)}$: 3.4 $R_{s(Desthio\text{-}Prot)}$: 1.6 t_a: 14.5 min	Simultaneous determination of prothioconazole and prothioconazole-desthio enantiomers. Study of the stereoselective degradation and transformation of prothioconazole and its metabolite in soil.	[9]
HPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Chiralcel® OD-3R Mobile phase: water containing formic acid (0.05 %)/ACN (35/65, v/v) Detection: MS	R_s: n.p. t_a: 11.7 min	Simultaneous enantiomeric separation of prothioconazole and prothioconazole-desthio. Study the toxicokinetic process and enantioselective degradation of prothioconazole and desthio-protioconazole in *Eremias argus*.	[7]

HPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Lux^{TM} Cellulose-1 Mobile phase: water containing formic acid (0.1 %)/ACN Detection: MS	R_s: n.p. t_a: 13.7 min	Simultaneous enantiomeric separation of prothioconazole and prothioconazole-desthio. Study the enantioselective metabolism of prothioconazole and desthio-prothioconazole in rat liver microsomes.	[14]
HPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Daicel Chiralpak® OD-RH Mobile phase: ACN/water (85/15, v/v) Detection: UV 220 and 255 nm	R_s: n.p. t_a: 12.8 min	Simultaneous enantiomeric determination of prothioconazole and its metabolite residues in water, beer, Baijiu and vinegar samples.	[17]
UPLC **Prothioconazole** **Prothioconazole-desthio**	CSP: Lux^{TM} Cellulose-3 Mobile phase: ACN/water (40/60, v/v) Detection: UV 220 nm	$R_{s(Prot)}$: 1.9 $R_{s(Desthio\text{-}Prot)}$: 2.1 t_a: 19.3 min	Simultaneous enantiomeric determination of prothioconazole and prothioconazole-desthio enantiomers in agricultural products (cucumber and pear) and environment samples (water and soil). Study the enantioselective degradation and metabolism of prothioconazole in soil.	[8]
SFC **Prothioconazole** **Prothioconazole-desthio**	CSP: Chiralcel® OD-3 Mobile phase: CO_2/0.2 % HAc 5 mmol L^{-1} NH_4OAc IPA (85/15, v/v) Detection: MS	$R_{s(Prot)}$: 3.4 $R_{s(Desthio\text{-}Prot)}$: 3.1 t_a: 2.0 min	Simultaneous enantiomeric determination of prothioconazole and prothioconazole-desthio enantiomers in tomato, cucumber and pepper and study their enantioselective degradation.	[18]

ACN: acetonitrile; Chiralcel® OD-3: cellulose tris(3,5-dimethylphenylcarbamate); Chiralcel® OD-3R: cellulose tris(3,5-dimethylphenylcarbamate); Chiralcel® OD-RH: cellulose tris(3,5-dimethylphenylcarbamate) coated on 5 µm silica-gel; CSP: chiral stationary phase; Daicel Chiralpak® OD-RH: cellulose tris(3,5-dimethylphenylcarbamate) coated on 5 µm silica-gel; ECD: electron capture detector; Enantiopak® OD: cellulose tris(3,5-dimethylphenylcarbamate); LOD: limit of detection; Lux^{TM} Cellulose-1: cellulose tris(3,5-dimethylphenylcarbamate); Lux^{TM} Cellulose-3: cellulose tris(4-methylbenzoate); MS: mass spectrometry; n.p.: not provided; R_s: resolution; t_a: analysis time.

in earthworms and investigate its degradation in different soils (R-(-)-prothioconazole was preferentially degraded in soil and earthworms accelerate this degradation) [13]; iii) to investigate the biological activity of prothioconazole in different cereals and its toxicity towards non-target aquatic plants and algae [12]; iv) to evaluate the enantioselective metabolism of prothioconazole in rat liver microsomes [14] and, v) to assess the toxicokinetic properties of both analytes in Chinese lizards [7].

Ultraperformance liquid chromatography (UPLC) has been employed by Zhang and co-workers to achieve the simultaneous enantioseparation of prothioconazole and prothioconazole-desthio in less than 20 min with resolution values of 1.9 and 2.1, respectively (**Table 1**). The methodology was applied to determine both analytes in food and environmental samples and to study prothioconazole degradation and metabolism in soil [8]. Results showed that R-(-)-prothioconazole degraded preferentially and that prothioconazole-desthio enantiomers were formed during prothioconazole dissipation [8]. Finally, Jiang and co-workers developed a Supercritical Fluid Chromatography (SFC) methodology that enabled the determination of the four enantiomers of both compounds within 2 min (Rs > 3) and studied their enantioselective degradation in some vegetables [18]. Results showed that R-(-)-prothioconazole was preferentially degraded in cucumber, tomato and pepper and R-(+)-prothioconazole-desthio in pepper while S-(-)-prothioconazole-desthio was preferentially degraded in cucumber and tomato [18]. Moreover, Jiang et al. developed a SFC method enabling the enantioseparation of prothioconazole in 3.5 min (Rs 3.6) that was applied to the determination of prothioconazole enantiomers in tomato and soil samples [10].

Due to its high efficiency, simplicity (no chiral columns are needed), low consumption of chiral selectors, reagents and samples, and applicability to a wide range of compounds,

CE has proven to be an effective and powerful choice to carry out chiral separations [19]. However, to date, CE has never been applied to the separation of prothioconazole and its metabolite prothioconazole-desthio. For this reason, the aim of this work was to develop chiral analytical methodologies by CE, allowing the chiral separation of prothioconazole as well as its simultaneous enantiomeric separation from prothioconazole-desthio and to apply them to the determination of prothioconazole in commercial agrochemical formulations and to carry out degradation studies of these compounds in sand and soil samples.

2. Materials and methods

2.1. Reagents and samples

All chemicals and reagents used were of analytical grade. Sodium hydroxide and boric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was provided by Scharlau (Barcelona, Spain). The chiral selectors carboxymethyl-α-CD (CM-α-CD, DS ~ 3.5), succinyl-β-CD (Succ-β-CD, DS ~ 3.5), succinyl-γ-CD (Succ-γ-CD, DS ~ 3.5), (2-carboxyethyl)-β-CD (CE-β-CD, DS ~ 3.5), (2-carboxyethyl)-γ-CD (CE-γ-CD, DS ~ 3.5), phosphated β-CD (Ph-β-CD, DS ~ 4), sulfated α-CD (S-α-CD, DS ~ 12) and sulfated γ-CD (S-γ-CD, DS ~ 10) were purchased from Cyclolab (Budapest, Hungary). Sulfated β-CD (S-β-CD, DS ~ 18), heptakis(2,3,6-tri-O-methyl)-β-CD (TM-β-CD) and heptakis(2,6-di-O-methyl)-β-CD (DM-β-CD) were from Sigma-Aldrich. Finally, water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA).

Prothioconazole racemate and prothioconazole-desthio racemate (their structures are shown in **Fig. S1** in supplementary material) were from Sigma-Aldrich. The commercial

agrochemical formulations (AF1 and AF2) were acquired in an agricultural store from Valdepeñas (Ciudad Real, Spain). The composition of these commercial formulations was: 12,5 % (p/v) prothioconazole + 12,5 % (p/v) tebuconazole for AF1 and 10,0 % (p/v) prothioconazole + 10 % fluoxastrobin for AF2. Cleaned and dried sand was from Labkem (Barcelona, Spain). Soil used was collected from Alcalá de Henares (Madrid, Spain).

2.2. Apparatus

Electrophoretic experiments were carried out using an Agilent 7100 CE system from Agilent Technologies (Waldbronn, Germany) with a diode array detector (DAD) working at 205 nm with a bandwidth of 4 nm. The electrophoretic system was controlled with the HP ^{3D}CE ChemStation software that included data collection and analysis. Separations were carried out in an uncoated fused-silica capillary of 50 μm I.D. and a total length of 58.5 cm (50 cm effective length) provided by Polymicro Technologies (Phoenix, AZ, USA).

To weigh the different reagents and standards, an OHAUS Adventurer Analytical Balance (Nänikon, Switzerland) was used. pH measurements were carried out in a pH-meter model 744 from Metrohm (Herisau, Switzerland). Centrifuge 5424 R from Eppendorf was used to centrifuge soil and sand samples. All solutions were sonicated using an ultrasonic bath B200 from Branson Ultrasonic Corporation (Danbury, USA) using a power of 19 W and a frequency of 50 Hz.

Before its first use, the capillary was conditioned (applying 1 bar) with 1 M sodium hydroxide for 30 min, followed by 15 min with Milli-Q water and then with buffer solution for 60 min. At the beginning of each working day, the capillary was flushed (applying 1 bar) with 0.1 M sodium hydroxide for 10 min, Milli-Q water for 5 min and

buffer solution for 30 min. Between injections, in order to ensure the repeatability, the capillary was conditioned with methanol (3 min), 0.1 M sodium hydroxide (2 min), Milli-Q water (2 min) and background electrolyte (BGE) (4 min).

2.3. Preparation of solutions and samples

Borate buffer solutions (100 mM or 75 mM, pH 9.0) were prepared by dissolving the appropriate amount of boric acid to reach the desired concentration and adjusting the pH with sodium hydroxide 1M before completing the volume with Milli-Q water. BGEs were obtained by dissolving the appropriate amount of chiral selectors in the borate buffer solution.

Stock standard solutions of prothioconazole (1000 mg L^{-1}) and prothioconazole-desthio (1000 mg L^{-1}) were prepared by dissolving the appropriate amount of the pesticides in methanol and stored at 4 °C. Standard working solutions containing the racemic analytes at different concentration levels (between 2 and 400 mg L^{-1}) were prepared by appropriate dilution of the stock standard solutions in methanol (for the individual enantiomeric separation of prothioconazole) and water (for the simultaneous enantiomeric separation of prothioconazole and prothioconazole-desthio). Commercial agrochemical formulations AF1 and AF2 were prepared by diluting the appropriate amount in methanol up to a final concentration of 1000 mg L^{-1}.

Soil was collected, air-dried, and stored in the dark. Sand was already bought dried and cleaned. To confirm that the soil and sand samples did not contain prothioconazole or prothioconazole-desthio, the samples were submitted to extraction with water, followed by a centrifugation at 25 ºC for 10 min at 5000 rpm. Supernatants were collected and injected in triplicate in the CE system. The incubation experiments of sand and soil with

prothioconazole, prothioconazole-desthio and the mixture of both analytes were conducted in polypropylene centrifuge tubes. Accurately measured 1.0 g quantities of the soil or sand were treated with the appropriate amount of each compound or their mixture in methanol to achieve the desired final concentrations (50 mg L^{-1} for racemic prothioconazole-desthio, 200 mg L^{-1} for racemic prothioconazole and 75 mg L^{-1} for each racemic analyte when added together). The mixtures were vortexed at high speed for 1 min. The samples were then incubated for 0 and 18 h and 3 and 7 days. Once the incubation time has elapsed, samples were extracted with water and centrifuged at 25 ºC for 10 min at 5000 rpm. Supernatants were collected and injected in triplicate. This procedure was carried out for triplicate.

All solutions were filtered before use through disposable nylon 0.45 μm pore size filters purchased from Scharlau (Barcelona, Spain).

2.4. Data treatment

Migration times, resolution values (Rs) and area values were obtained using the Chemstation software from Agilent Technologies. Experimental data analysis, composition of graphs with different electrophoregrams and calculation of different parameters were carried out using Excel Microsoft, Origin Pro 8 and Statgraphics Centurion XVII software.

The degradation kinetic equation for prothioconazole and prothioconazole-desthio enantiomers were described using the first-order kinetic equation [9]:

$$C_t = C_0 e^{-kt}$$

where C_t and C_0 are the concentration at t time and the initial concentration in soil, respectively, and k is the degradation rate constant.

3. Results and discussion

3.1. Development of an analytical methodology for the enantiomeric separation of prothioconazole by CE

In order to achieve the first enantioselective separation of prothioconazole by CE, different cyclodextrins (CDs) were used as chiral selectors. A pH value of 9.0 was chosen at which prothioconazole is partially ionized (pk_a (NH group) = 9.6) [20] so neutral and anionic CDs can be assayed. Nine anionic (CM-α-CD, Succ-β-CD, Succ-γ-CD, CE-β-CD, CE-γ-CD, Ph-β-CD, S-α-CD, S-γ-CD and S-β-CD) and two neutral (TM-β-CD and DM-β-CD) CDs were selected to evaluate their discrimination power for prothioconazole. All CDs were tested at a concentration of 10 mM (except S-β-CD, which was prepared at a concentration of 2 % w/v) in 100 mM borate buffer. These experiments were carried out using a voltage of +20 kV, a temperature of 20 ˚C and an injection of 50 mbar x 10 s. Among the 11 CDs studied, only one anionic CD (S-γ-CD) and one neutral CD (TM-β-CD) enabled the partial enantiomeric separation of prothioconazole. Resolution values were similar for both CDs (1.2 for S-γ-CD and 1.4 for TM-β-CD). However, TM-β-CD gave rise to a shorter analysis time (6 min versus 10.6 min). For this reason, TM-β-CD was chosen as chiral selector.

It is known that the concentration of the chiral selector directly affects the affinity of the enantiomers for it [21]. However, there is not an optimum concentration since, depending on the analyte, the interaction with the CD is different. The influence of the concentration of TM-β-CD on the enantiomeric separation of prothioconazole was evaluated in the range from 2.5 to 20 mM (2.5, 5, 10, 15, and 20 mM) (**Fig. S2** in supplementary material). It was observed that, as the concentration of the chiral selector increased, the analysis time slightly decreased (approximately from 6.3 to 6.0 min). However, the resolution increased when the CD concentration raised from 2.5 mM to 5 mM and then, it decreased

as the concentration of CD continued to increase. In this way, no enantiomeric resolution was observed for prothioconazole when the concentration of TM-β-CD was 20 mM. Thus, the best enantiomeric resolution (Rs 2.3) was obtained at a concentration of TM-β-CD of 5 mM in a relatively short analysis time (6.1 min). As a compromise between resolution and analysis time, this CD concentration was chosen as the optimum.

Since a variation of the temperature can affect the formation constant of the analyte-selector chiral complex, the viscosity of the separation medium and the EOF, among other variables, the effect of the temperature (15, 20 and 25 ˚C) was studied. A decrease in the temperature gave rise to higher resolution values with only slightly higher analysis times. Thus, 15 ˚C was chosen (Rs 3.2 in 6.9 min). Finally, the influence of the voltage was evaluated in the range of +20-+30 kV. As the voltage increased, shorter analysis times were obtained but resolution values did not vary appreciably. As a consequence, +30 kV was chosen as the optimum applied voltage value, allowing to obtain for the first time the separation of prothioconazole enantiomers by CE. As **Fig. 1A** shows, a resolution of 2.8 was obtained in 4.5 min. The identification of each prothioconazole enantiomer was not possible because their pure enantiomer standards were not available.

In order to apply the developed chiral methodology to the quantitative analysis of prothioconazole in commercial agrochemical formulations, the analytical characteristics of the CE method were evaluated (**Table 2**).

The linearity of the method was established from twelve standard solutions at different concentration levels, ranging from 2 to 400 mg L^{-1} (1 to 200 mg L^{-1} for each enantiomer), by plotting corrected peak areas as a function of the enantiomer concentration. Linearity was proved to be adequate as correlation coefficient were higher than 0.99 for both enantiomers and confidence intervals for the intercept included de zero value while

confidence intervals for the slope did not include the zero value (in both cases for a 95 % confidence level).

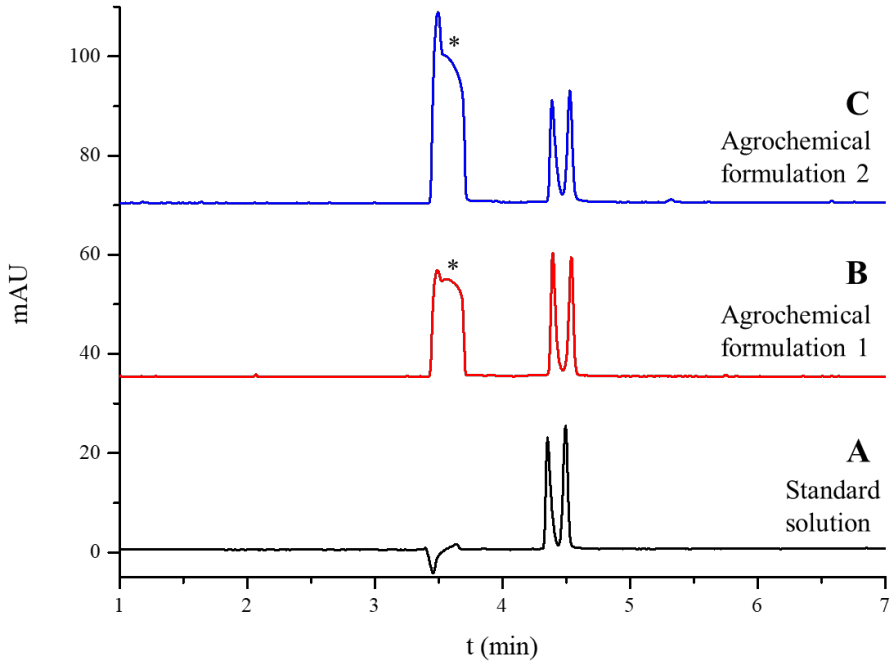

Fig. 1. Electrophoregrams obtained under optimized conditions for (A) a prothioconazole standard solution (150 mg L^{-1}), (B) agrochemical formulation 1 and (C) agrochemical formulation 2 containing each the fungicide at a concentration of 150 mg L^{-1} (according to the label of the commercial formulations). Asterisks could correspond to tebuconazole for agrochemical formulation 1 and fluoxastrobin for agrochemical formulation 2. Experimental conditions: BGE, 5 mM TM-β-CD in 100 mM borate buffer (pH 9.0); uncoated fused-silica capillary 50 µm id × 50 cm (58.5 cm to the detector); injection by pressure 50 mbar × 10 s; applied voltage +30 kV; temperature 15 ºC and UV detection 205 ± 4 nm.

A comparison of the confidence intervals for the slopes obtained by the external standard calibration method and the standard additions calibrations method (eight known amounts of prothioconazole standard solution were added to two different agrochemical

Table 2. Analytical characteristics of the developed CE methodology for the enantiomeric determination of prothioconazole in commercial agricultural formulations using TM-β-CD as chiral selector.

	First-migrating enantiomer				Second-migrating enantiomer			
External standard calibration method[a]								
Range	1-200 mg L^{-1}				1-200 mg L^{-1}			
Slope ± t · S_{slope}	0.234 ± 0.004				0.226 ± 0.004			
Intercept ± t · $S_{intercept}$	-0.4 ± 0.4				-0.4 ± 0.4			
r	0.9995				0.9996			
Standard additions calibration method[b]								
Range	0-125 mg L^{-1}				0-125 mg L^{-1}			
Slope ± t · S_{slope}	AF 1		AF 2		AF 1		AF 2	
	0.24 ± 0.02		0.24 ± 0.01		0.237 ± 0.008		0.228 ± 0.008	
r	0.9966		0.9989		0.9963		0.9986	
p-value of ANOVA	0.1341		0.6442		0.0510		0.6701	
Accuracy								
Recovery (%)[c]	5 mg L^{-1}		75 mg L^{-1}		5 mg L^{-1}		75 mg L^{-1}	
	AF 1	AF 2	AF 1	AF 2	AF 1	AF 2	AF 1	AF 2
	101 ± 7	94 ± 8	104 ± 7	97 ± 5	94 ± 7	103 ± 7	104 ± 4	101 ± 5
Precision								
Instrumental repeatability[d]								
	25 mg L^{-1}		100 mg L^{-1}		25 mg L^{-1}		100 mg L^{-1}	
t, RSD (%)	0.3		0.2		0.3		0.2	
A_c, RSD (%)	1.5		1.1		1.8		0.6	
Method repeatability[e]								
	25 mg L^{-1}		100 mg L^{-1}		25 mg L^{-1}		100 mg L^{-1}	
t, RSD (%)	0.6		0.2		0.6		0.2	
A_c, RSD (%)	1.8		2.0		2.2		2.0	
Intermediate precision[f]								
	25 mg L^{-1}		100 mg L^{-1}		25 mg L^{-1}		100 mg L^{-1}	
t, RSD (%)	0.7		0.9		0.7		0.8	
A_c, RSD (%)	4.3		2.1		4.6		2.5	
LOD[g]	0.7 mg L^{-1}				0.7 mg L^{-1}			
LOQ[h]	2.3 mg L^{-1}				2.3 mg L^{-1}			

A_c: corrected area. AF: agrochemical formulation

[a] Twelve standard solutions at different concentration levels injected in triplicate. [b] Addition of eight known amounts of prothioconazole standard solution to two different agrochemical formulations samples (AF1 and AF2) containing a constant concentration of prothioconazole. [c] Accuracy was evaluated as the recovery obtained from six agrochemical samples solutions (n=6) containing 150 mg L^{-1} of prothioconazole (as labeled amount) spiked with 10 and 150 mg L^{-1} of prothioconazole. [d] Instrumental repeatability was calculated from six consecutive injections of prothioconazole standard solution (n=6) at two levels of concentration (50 and 200 mg L^{-1}). [e] Method repeatability was determined by using the value obtained for three replicates of prothioconazole standards solutions injected in triplicate on the same day (n=9) at two levels of concentration (50 and 200 mg L^{-1}). [f] Intermediate precision was calculated by using the value obtained for three replicates (injected in triplicate during three consecutive days) of prothioconazole standard solution (n=9) at two levels of concentration (50 and 200 mg L^{-1}). [g] LOD obtained experimentally for a S/N = 3. [h] LOQ obtained experimentally for a S/N = 10.

formulations samples containing a constant concentration of prothioconazole of 75 mg L^{-1} for each enantiomer) showed that there were no statistically significant differences between the slopes of each calibration straight line (for a 95 % confidence level).

323 Therefore, there are not matrix interferences and the external calibration method can be
324 used to quantify the content of prothioconazole in the agrochemical formulations.

325 The trueness of the analytical method was evaluated as the recovery values (%) obtained
326 for prothioconazole enantiomers when spiking agrochemical formulations solutions with
327 known concentrations of racemic prothioconazole standard solution (10 and 150 mg L^{-1}).
328 **Table 2** shows that the recovery percentage values obtained were acceptable as they
329 included the 100 %.

330 Precision of the method was evaluated as instrumental repeatability, method repeatability
331 and intermediate precision using in all cases standard solutions of racemic
332 prothioconazole at two concentration levels (50 and 200 mg L^{-1}) (**Table 2**). Instrumental
333 repeatability was determined from six repeated injections of these standard solutions on
334 the same day. RSD values (%) obtained were lower than 0.3 % for migration times and
335 lower than 1.8 % for corrected peak areas. The method repeatability was assessed using
336 three replicates of the standard solutions injected in triplicate and on the same day
337 obtaining RSDs lower than 0.6 % and 2.2 % for migration times and corrected peak areas,
338 respectively. Finally, intermediate precision was evaluated injecting, in triplicate, three
339 replicates of the standard solutions of racemic prothioconazole at two concentration levels
340 during three consecutive days giving rise to RSD values lower than 0.9 % for migration
341 times and lower than 4.6 % for corrected peak areas.

342 Finally, LOD and LOQ values (calculated as the minimum concentration yielding an S/N
343 ratio of 3 and 10 times, respectively) were 0.7 and 2.3 mg L^{-1}, respectively, for both
344 enantiomers.

345 The developed methodology was applied to the quantitation of prothioconazole
346 enantiomers in two agrochemical formulations. With this aim, three independent diluted

347 samples of the two agrochemical formulations were injected in triplicate, containing each
348 of them prothioconazole at a concentration of approximately 150 mg L^{-1} (75 mg L^{-1} for
349 each enantiomer).

350 **Fig. 1B and 1C** show the electrophoregrams corresponding to the two agrochemical
351 formulations analysed. As it can be seen, the developed method shows an adequate
352 selectivity, since no interfering signals are observed. For both agrochemical formulations,
353 a signal next to the EOF can be seen due to the neutral nature of the other components of
354 the samples (tebuconazole in agrochemical formulation 1 and fluoxastrobin in
355 agrochemical formulation 2). This originated wider and more deformed peaks than the
356 peak corresponding to the EOF in the standard solution.

357 Contents of prothioconazole of 122 ± 6 and 95 ± 5 g L^{-1} were obtained for agrochemical
358 formulation 1 and agrochemical formulation 2, respectively. These values correspond to
359 percentages of 98 ± 5 and 95 ± 5 % of the labeled amounts, which demonstrates the correct
360 labeling of the two commercial formulations analyzed and the suitability of the method
361 to determine the content of prothioconazole in these samples.

362

363 ***3.2. Development of a chiral analytical methodology for the simultaneous***
364 ***enantioseparation of prothioconazole and prothioconazole-desthio by CE***

365 Since the fast methodology developed for the enantioseparation of prothioconazole did
366 not allow the simultaneous enantioselective separation of prothioconazole and its main
367 metabolite, prothioconazole-desthio, a chiral methodology was developed by CE
368 enabling the separation of the four enantiomers simultaneously in order to perform
369 degradation studies in environmental samples.

370 Prothioconazole-desthio is a neutral compound in a wide range of pH values (pk_a (OH
371 group) = 13.0) [20]. Then, in order to achieve its enantioselective separation together with
372 prothioconazole, a pH 9.0 and a charged CD was chosen. Among the 9 different anionic
373 CDs tested at this pH (CM-α-CD, Succ-β-CD, Succ-γ-CD, CE-β-CD, CE-γ-CD, Ph-β-
374 CD, S-α-CD, S-γ-CD and S-β-CD) to achieve the separation of the enantiomers of
375 prothioconazole, only S-γ-CD enabled its partial enantiomeric separation, as previously
376 mentioned. Thus, this CD was tested at a concentration of 10 mM in 100 mM borate
377 buffer (pH 9.0) using a voltage of +20 kV, a temperature of 20 ˚C and an injection of 50
378 mbar x 10 s. Under these conditions, the enantiomers of prothioconazole (Rs 1.9) and
379 prothioconazole-desthio (Rs 9.8) were simultaneously separated in 11.5 min, with
380 prothioconazole-desthio enantiomers eluting first. Since the peaks obtained for
381 prothioconazole-desthio were very small, standard working solutions of prothioconazole
382 and prothioconazole-desthio were prepared from the stock standard solution by dilution
383 in water instead of methanol. This change allowed us to obtain much larger areas and
384 therefore, the following experiments were carried out by diluting the stock standard
385 solutions in water.

386 The influence of the concentration of the CD was evaluated in the range from 5 to 15
387 mM. Results are shown in **Fig. S3** (supplementary material). As it can be observed, an
388 increase in the S-γ-CD concentration led to higher analysis times and, moreover, the worst
389 separation was obtained for a CD concentration of 15 mM. Taking into account that with
390 a 5 mM concentration of S-γ-CD, prothioconazole enantiomers were only partially
391 separated, a concentration of S-γ-CD of 10 mM was selected as a compromise between
392 analysis time and resolution.

393 In order to improve the shape of the peaks of prothioconazole-desthio, the effect of the
394 injection time was studied. Values of 2, 3, 4, 5, 6 and 10 s were tested at 50 mbar. An

395 injection time of 6 s was chosen since it resulted in a better peak shape and, therefore, a
396 higher resolution (10.6 for prothioconazole-desthio and 2.0 for prothioconazole). With
397 the aim of reducing the analysis time, the effect of the buffer concentration was studied
398 (50, 75 and 100). As expected, as the buffer concentration increased, higher analysis time
399 was obtained. However, with the lowest concentration, the second-migrating enantiomer
400 of prothioconazole-desthio eluted with the first-migrating enantiomer of prothioconazole.
401 A concentration of borate buffer of 75 mM was chosen since it gave rise to shorter
402 analysis time (10.5 min) without losing resolution. Then, three different temperatures
403 were tested (15, 20 and 25 ˚C). As a compromise between resolution and analysis time, a
404 temperature of 20 ˚C was selected. Finally, the effect of the voltage was evaluated in the
405 range of +20-+30 kV. As the voltage increased, resolution values decreased. However,
406 shorter analysis times were obtained, so +30 kV was chosen as the optimum applied
407 voltage value, enabling the simultaneous enantioseparation of prothioconazole-desthio
408 and prothioconazole in 5.5 min with resolution values of 8.2 and 1.9, respectively, as can
409 be seen in **Fig. 2A**. Again, it was not possible to establish the order of elution of each
410 prothioconazole-desthio enantiomers due to the lack of commercial pure enantiomer
411 standards.

412 The analytical characteristics of the methodology were evaluated to demonstrate the
413 method suitability to carry out degradation studies. The results obtained are grouped in
414 **Table 3**.

415 Linearity was determined with twelve standard solutions containing racemic
416 prothioconazole and racemic prothioconazole-desthio from 2 to 400 mg L^{-1} obtaining
417 satisfactory results in terms of linearity as can be observed in **Table 3.**
418

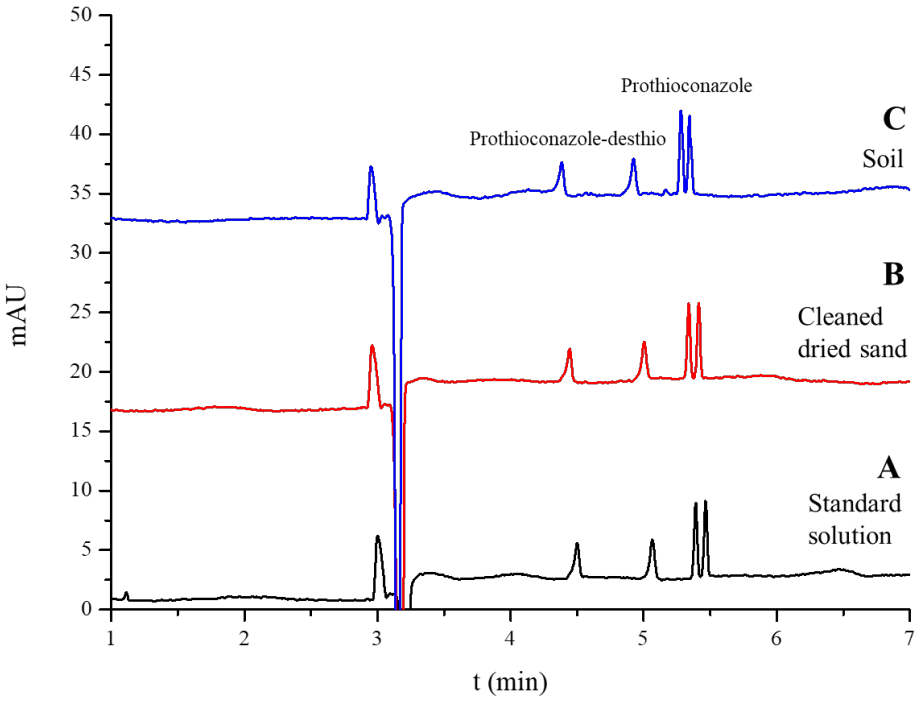

Fig. 2. Electrophoregrams obtained under the optimized conditions for (A) a standard solution containing prothioconazole and prothioconazole-desthio (50 mg L^{-1} each), (B) an extract from a cleaned dried sand sample spiked with a mixture of prothioconazole and prothioconazole-desthio standard solution (50 mg L^{-1} each), and (C) an extract from a soil sample spiked with a mixture of prothioconazole and prothioconazole-desthio standard solution (50 mg L^{-1} each). Experimental conditions: BGE, 10 mM S-γ-CD in 75 mM borate buffer (pH 9.0); uncoated fused-silica capillary 50 µm id × 50 cm (58.5 cm to the detector); injection by pressure 50 mbar × 6 s; applied voltage +30 kV; temperature 20 ºC and UV detection 205 ± 4 nm.

As for the previous methodology, the existence of matrix interferences was studied for sand and soil samples. No statistically significant differences were obtained for the slopes obtained by the external and the standard additions calibration methods for both, sand and soil samples. Thus, there were no matrix interferences and therefore, the external calibration methods can be employed to quantify the content of the analytes in both samples. **Fig. 2B and 2C** show that no interfering peaks were observed when cleaned

Table 3. Analytical characteristics of the developed CE methodology for the simultaneous enantiomeric determination of prothioconazole and prothioconazole-desthio in sand and soil samples using S-γ-CD as chiral selector.

	Prothioconazole		Prothioconazole-desthio	
	E1	**E2**	**E1**	**E2**
External standard calibration method[a]				
Range	1-200 mg L^{-1}	1-200 mg L^{-1}	2.5-50 mg L^{-1}	2.5-50 mg L^{-1}
Slope ± t · S_{slope}	0.101 ± 0.001	0.0990 ± 0.0009	0.085 ± 0.005	0.085 ± 0.004
Intercept ± t · $S_{intercept}$	-0.1 ± 0.1	-0.08 ± 0.08	-0.1 ± 0.1	-0.1 ± 0.1
r	0.9997	0.9998	0.9980	0.9983

	Sand				Soil			
	Prothioconazole		**Prothioconazole-desthio**		**Prothioconazole**		**Prothioconazole-desthio**	
	E1	**E2**	**E1**	**E2**	**E1**	**E2**	**E1**	**E2**
Standard additions calibration method[b]								
Range	0-200 mg L^{-1}	0-200 mg L^{-1}	0-50 mg L^{-1}	0-50 mg L^{-1}	0-200 mg L^{-1}	0-200 mg L^{-1}	0-50 mg L^{-1}	0-50 mg L^{-1}
Slope ± t · S_{slope}	0.102 ± 0.002	0.101 ± 0.002	0.083 ± 0.002	0.084 ± 0.004	0.100 ± 0.001	0.099 ± 0.001	0.082 ± 0.004	0.083 ± 0.002
r	0.9983	0.9987	0.9987	0.9963	0.9994	0.9993	0.9961	0.9989
p-value of ANOVA	0.2075	0.1738	0.2324	0.6807	0.4678	0.7395	0.2346	0.3619
Accuracy								
Recovery (%)[c]	99 ± 6	96 ± 5	96 ± 4	96 ± 4	100 ± 5	100 ± 5	99 ± 3	97 ± 5
Precision								
Instrumental repeatability[d]								
t, RSD (%)	1.1	1.1	1.2	1.4	0.7	0.7	0.5	0.5
A_c, RSD (%)	0.7	0.2	0.2	0.7	1.3	1.7	2.5	2.0
Method repeatability[e]								
t, RSD (%)	3.7	3.7	3.1	3.6	0.8	0.8	1.2	1.3
A_c, RSD (%)	2.8	2.9	2.5	3.0	3.1	2.9	3.3	3.4
Intermediate precision[f]								
t, RSD (%)	5.8	5.6	3.9	4.5	0.9	0.9	1.3	1.4
A_c, RSD (%)	4.9	4.7	4.2	3.5	4.7	5.3	4.6	4.8
LOD[g]	0.9 mg L^{-1}	0.8 mg L^{-1}	1.3 mg L^{-1}	1.1 mg L^{-1}	0.9 mg L^{-1}	0.8 mg L^{-1}	1.3 mg L^{-1}	1.1 mg L^{-1}
LOQ[h]	3.1 mg L^{-1}	2.8 mg L^{-1}	4.3 mg L^{-1}	3.7 mg L^{-1}	3.1 mg L^{-1}	2.8 mg L^{-1}	4.3 mg L^{-1}	3.7 mg L^{-1}

A_c: corrected area. E1: first-migrating enantiomer. E2: second-migrating enantiomer.

[a] Twelve standard solutions at different concentration levels injected in triplicate. [b] Addition of thirteen known amounts of prothioconazole and thirteen known amounts of prothioconazole-desthio to cleaned dried sand and soil samples. [c] Accuracy was evaluated for prothioconazole as the recovery obtained when cleaned dried sand and soil samples were spiked, each, with 200 mg L^{-1} prothioconazole (n=6) and for prothioconazole-desthio as the recovery obtained when cleaned dried sand and soil samples were spiked, each, with 50 mg L^{-1} prothioconazole-desthio (n=6). [d] Instrumental repeatability was calculated for sand and soil samples from six consecutive injections of prothioconazole and prothioconazole-desthio sample extracts at a concentration of 200 and 50 mg L^{-1}, respectively. [e] Method repeatability was determined for sand and soil samples by using the value obtained for three replicates of prothioconazole and prothioconazole-desthio sample extracts injected in triplicate on the same day at a concentration of 200 and 50 mg L^{-1}, respectively. [f] Intermediate precision was calculated for sand and soil samples by using the value obtained for three replicates (injected in triplicate during three consecutive days) of prothioconazole and prothioconazole-desthio sample extracts at a concentration of 200 and 50 mg L^{-1}, respectively. [g] LOD obtained experimentally for a S/N = 3. [h] LOQ obtained experimentally for a S/N = 10.

447 dried sand and soil samples spiked with a mixture of prothioconazole and
448 prothioconazole-desthio were analysed.

449 Trueness of the method was evaluated as the recovery values (%) obtained for
450 prothioconazole-desthio and prothioconazole enantiomers when cleaned dried sand and
451 soil were, each, spiked with known concentrations of prothioconazole-desthio and
452 prothioconazole (50 mg L^{-1} of prothioconazole-desthio and 200 mg L^{-1} prothioconazole).
453 For sand samples, recoveries obtained were between 96 and 99 % and between 97 and
454 100 % for soil samples (**Table 3**).

455 Precision was evaluated considering the instrumental and method repeatability, and
456 intermediate precision for analysis time and corrected peak areas, for cleaned dried sand
457 as well as for soil samples (**Table 3**), obtaining adequate values in all cases (lower than
458 5.8 %).

459 Finally, LOD and LOQ values, experimentally determined as for the previous
460 methodology, were identical for both samples, sand and soil (**Table 3**). For
461 prothioconazole enantiomers, LODs were 0.9 and 0.8 mg L^{-1} and LOQs 3.1 and 2.8 mg
462 L^{-1}. In the case of prothioconazole-desthio, LODs were 1.3 and 1.1 mg L^{-1} and LOQs
463 were 4.3 and 3.7 mg L^{-1}.

464

465 ***3.3. Degradation studies in sand and soil samples***

466 The suitability and effectiveness of the developed methodology was used to carry out
467 degradation studies in cleaned dried sand and soil samples established through recovery
468 values in %. For both matrices, these studies were performed by spiking them only with
469 prothioconazole (racemate concentration 200 mg L^{-1}), only with prothioconazole-desthio

(racemate concentration 50 mg L^{-1}) and with both analytes (75 mg L^{-1} concentration for each racemate). Recovery values obtained after incubation of the analytes with the samples are shown in **Fig. 3** for sand and in **Fig. 4** for soil. As can be seen in both figures, no enantioselective degradation was observed under sterile conditions, in agreement to the results reported by Zhang and co-workers [9]. Moreover, results for sand showed a similar degradation trend when spiking each analyte separately, achieving a maximum reduction in the recovery values after 3 days (approximately a reduction of a 40 %) and then remaining constant. These results are in accordance with the degradation kinetic equations, which followed the first-order (**Table 4**) until the third day (from which degradation remained constant). In addition, the slopes obtained for the enantiomers of both analytes, were of the same order. However, when spiking both compounds (prothioconazole and prothioconazole-desthio) together, a major degradation for prothioconazole after 7 days of incubation was observed. In the case of prothioconazole-desthio, only a slight decrease in the recovery values was observed (approximately 15 %) after 7 days, which could indicate that prothioconazole is being transformed in prothioconazole-desthio. In this case, the degradation of the enantiomers of prothioconazole and prothioconazole-desthio followed the first-order kinetic equation until day 7 (**Table 4**), being the slopes much higher for prothioconazole enantiomers, which can again indicate that prothioconazole is being transformed in prothioconazole-desthio.

Regarding soil samples, when spiking each analyte separately, a major degradation of prothioconazole after 7 days of incubation was obtained (approximately a reduction of a 40 % for prothioconazole-desthio and of a 50 % for prothioconazole). In addition, unlike what happened in the sand samples, the recovery values do not remain constant after 3 days but continue to decrease for both compounds. All these results are in agreement with

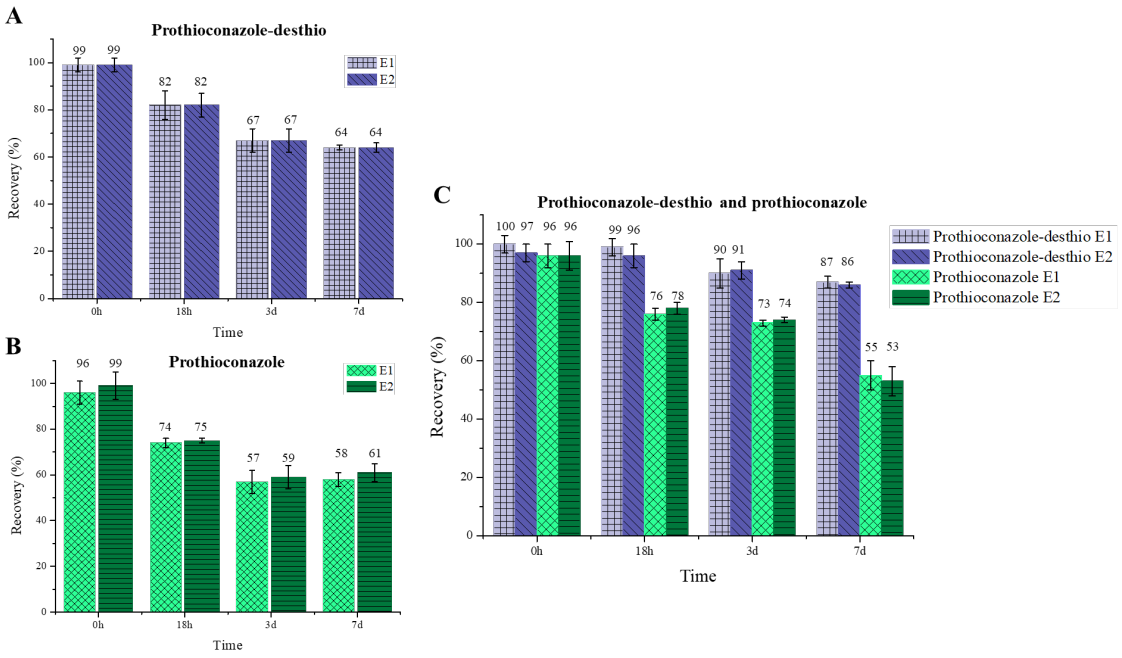

Fig. 3. Recovery values (%) obtained in cleaned dried sand (after 0 h, 18 h, 3 days and 7 days of incubation) for (A) prothioconazole-desthio, (B) prothioconazole and (C) prothioconazole-desthio and prothioconazole. Experimental conditions as in **Fig. 2**.

the kinetic degradation study, which demonstrated that the enantiomers of both analytes followed the first-order kinetic equation until day 7. However, slopes obtained for prothioconazole enantiomers were higher, revealing a higher degradation (see **Table 4**). When spiking both compounds (prothioconazole and prothioconazole-desthio) together, a similar trend than for sand was observed. Indeed, after 7 days of incubation only a slight decrease in the recovery values of prothioconazole-desthio (approximately 15 %) was observed, while a remarkable decrease for prothioconazole was obtained (approximately a reduction in the recoveries of 75 %). This could indicate again that prothioconazole was being transformed in prothioconazole-desthio, being this transformation more notable in soil than in sand. Indeed, in this case, kinetic equations for prothioconazole enantiomers followed the first-order until day 7 (**Table 4**). However, this did not occur for

prothioconazole-desthio enantiomers, effect that may be due to the major transformation of prothioconazole into prothioconazole-desthio in soil.

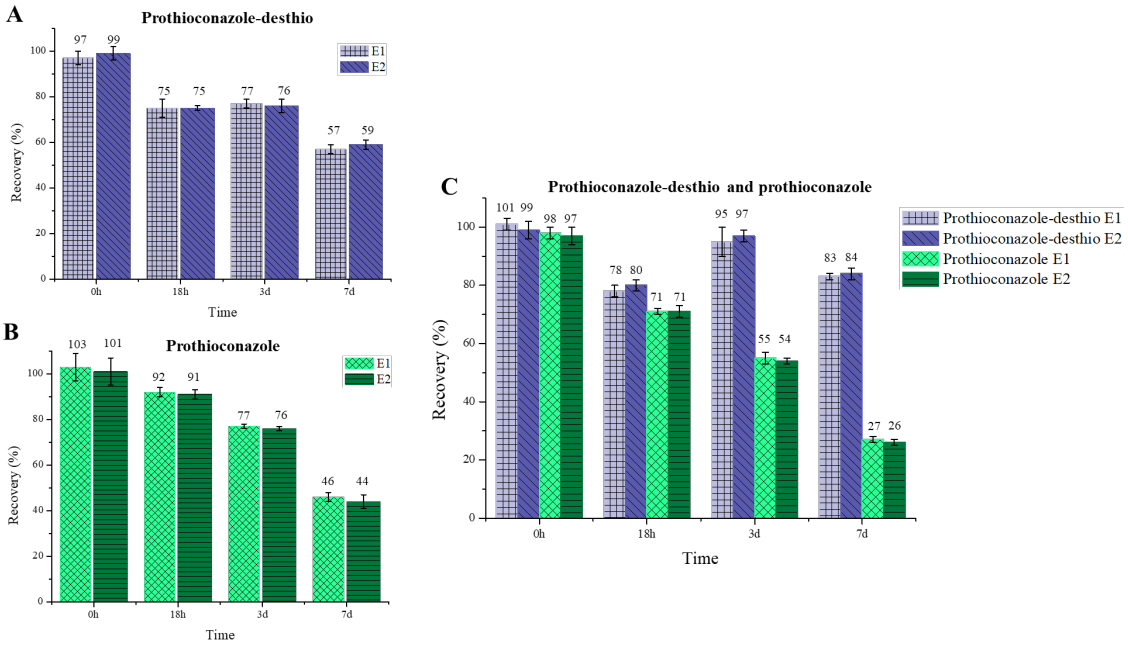

Fig. 4. Recovery values (%) obtained in soil (after 0 h, 18 h, 3 days and 7 days of incubation) for (A) prothioconazole-desthio, (B) prothioconazole and (C) prothioconazole-desthio and prothioconazole. Experimental conditions as in **Fig. 2**.

3.4. Comparison of the developed CE methods with other reported methodologies

Table 1 groups the methodologies previously reported for the chiral separation of prothioconazole and for the simultaneous enantiomeric separation of prothioconazole and prothiconazole-desthio. The technique and experimental conditions employed in each work as well as the enantiomeric resolution and analysis time obtained are included in this Table together with the applications achieved.

Regarding the chiral separation of prothiconazole, the CE methodology developed in this work enabled to reach a higher resolution value in a lower analysis time than those

obtained by HPLC [12, 13, 15]. Only the separation carried out by SFC [10] resulted in slightly better resolution and analysis time values.

Table 4. First-order kinetic equation and correlation coefficients of prothioconazole and prothioconazole-desthio enantiomers in sand and soil.

	Enantiomers	Kinetic equation	r
SAND			
Spiked only with prothioconazole-desthio (50 mg L^{-1})	First-migrating enantiomer	$y = 23.7e^{-0.005x}$	0.9095
	Second-migrating enantiomer	$y = 23.6e^{-0.005x}$	0.9252
Spiked only with prothioconazole (200 mg L^{-1})	First-migrating enantiomer	$y = 91.0e^{-0.007x}$	0.9443
	Second-migrating enantiomer	$y = 91.0e^{-0.006x}$	0.9137
Spiked with both analytes (75 mg L^{-1} for each racemate)	Desthio-prothioconazole first-migrating enantiomer	$y = 37.6e^{-0.0009x}$	0.9297
	Desthio-prothioconazole second-migrating enantiomer	$y = 36.5e^{-0.0008x}$	0.9001
	Prothioconazole first-migrating enantiomer	$y = 33.2e^{-0.003x}$	0.9188
	Prothioconazole second-migrating enantiomer	$y = 33.6e^{-0.003x}$	0.9436
SOIL			
Spiked only with prothioconazole-desthio (50 mg L^{-1})	First-migrating enantiomer	$y = 22.2e^{-0.003x}$	0.9337
	Second-migrating enantiomer	$y = 24.3e^{-0.003x}$	0.9872
Spiked only with prothioconazole (200 mg L^{-1})	First-migrating enantiomer	$y = 103.4e^{-0.005x}$	0.9909
	Second-migrating enantiomer	$y = 102.3e^{-0.005x}$	0.9877
Spiked with both analytes (75 mg L^{-1} for each racemate)	Desthio-prothioconazole first-migrating enantiomer	-	-
	Desthio-prothioconazole second-migrating enantiomer	-	-
	Prothioconazole first-migrating enantiomer	$y = 33.8e^{-0.007x}$	0.9884
	Prothioconazole second-migrating enantiomer	$y = 33.5e^{-0.007x}$	0.9899

With respect to the simultaneous enantiomeric separation of prothioconazole and prothioconazole-desthio, as shown in **Table 1**, the CE methodology developed in this work led to shorter analysis times and higher resolution values for prothioconazole-desthio than those obtained using other separation techniques such as HPLC [7, 9, 14, 16, 17] or UPLC [8] (see **Table 1**). The use of SFC [18] enabled to achieve better results in terms of analysis time but not for the enantiomeric resolution of prothioconazole-desthio.

4. Conclusions

Two chiral CE methodologies, one enabling the fast and cost-effective enantiomeric separation of prothioconazole and its determination in prothioconazole-based commercial agrochemical formulations, and other enabling the simultaneous enantioseparation of prothioconazole and prothioconazole-desthio to achieve degradation studies, have been developed for the first time in this work. The use of 5 mM TM-β-CD in a 100 mM borate buffer solution (pH 9.0), with a separation voltage of +30 kV and a temperature of 15 ˚C enabled the separation of prothioconazole enantiomers in an analysis time of 4.5 min with a resolution value of 2.8. Analytical characteristics of the method were evaluated, showing a good performance for the quantitation of prothioconazole in commercial agrochemical formulations. No statistically significant differences were found between the total concentration determined for each formulation and their labelled contents. In addition, the use of 10 mM S-γ-CD in a 75 mM borate buffer solution (pH 9.0), with a separation voltage of +30 kV and a temperature of 20 ˚C, enabled the simultaneous enantioseparation of prothioconazole-desthio and prothioconazole in 5.5 min with resolution values of 8.2 and 1.9, respectively. Again, the analytical characteristics of the method were evaluated and considered adequate to study the degradation of these compounds in soil and sand samples. No enantioselective degradation was observed for none of the analytes (prothioconazole and prothioconazole-desthio) neither in sand nor in soil. When spiking each analyte separately, a major degradation of prothioconazole was observed in soil (approximately 50 %) than in sand (approximately 40 %), while the degradation of prothioconazole-desthio was similar for both samples (approximately 40 %). When both compounds were spiked together, a slight decrease in the recovery values for prothioconazole-desthio (approximately 15 %) was observed, while a remarkable decrease for prothioconazole was obtained in both cases, being even higher in soil

562 (approximately 75 %) than in sand (approximately 45 %). This could indicate that
563 prothioconazole is being transformed in prothioconazole-desthio in both matrices. Results
564 obtained in this work demonstrate the potential of the chiral methodologies developed to
565 achieve the analysis of commercial agrochemical formulations and environmental
566 samples. In the first case, the use of a neutral CD four times less expensive that the anionic
567 one employed in the second methodology, enabled to have a more cost-effective
568 methodology when formulations containing only prothioconazole have to be analyzed.

569

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578

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ENANTIOMERIC SEPARATION OF PROTHIOCONAZOLE AND PROTHIOCONAZOLE-DESTHIO BY CAPILLARY ELECTROPHORESIS. DEGRADATION STUDIES IN ENVIRONMENTAL SAMPLES

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Prothioconazole **Prothioconazole-desthio**

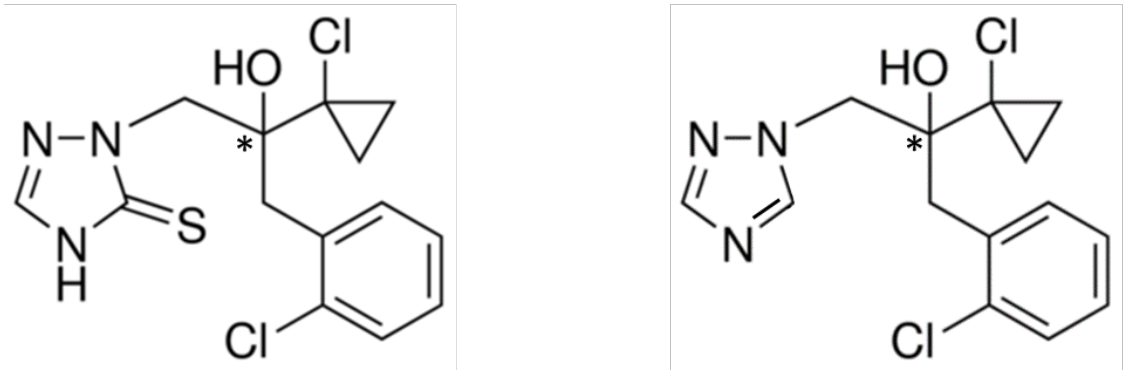

Fig. S1. Chemical structures of prothioconazole and prothioconazole-desthio. The asterisk represents an asymmetric carbon.

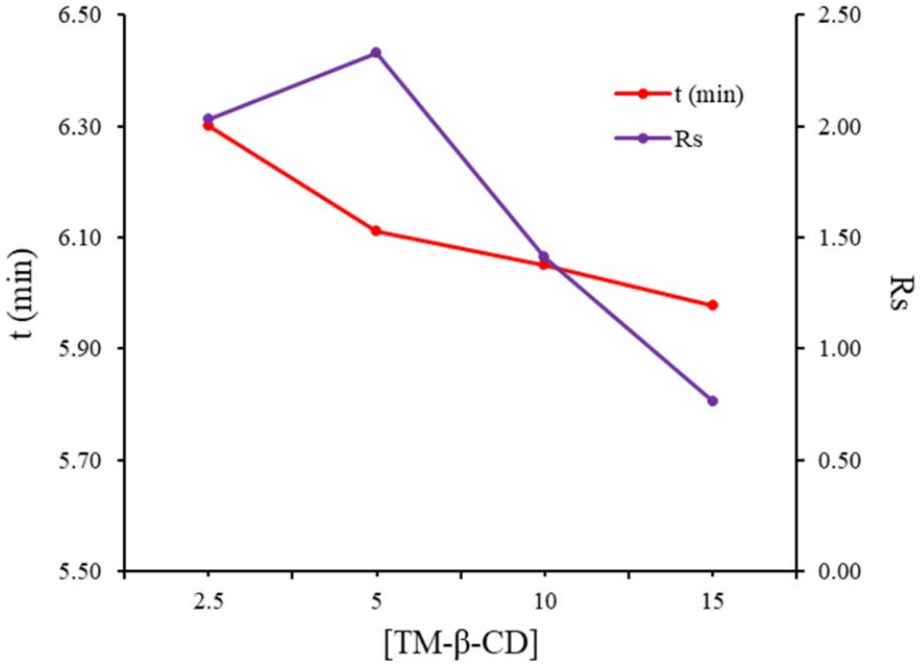

Fig. S2. Variation of the analysis time and the enantiomeric resolution for prothioconazole (racemate concentration 200 mg L^{-1}) as a function of the concentration of TM-β-CD. Experimental conditions: BGE, TM-β-CD in 100 mM borate buffer (pH 9.0); uncoated fused-silica capillary 50 µm id × 50 cm (58.5 cm to the detector); injection by pressure 50 mbar × 10 s; applied voltage +20 kV; temperature 20 ºC and UV detection 205 ± 4 nm.

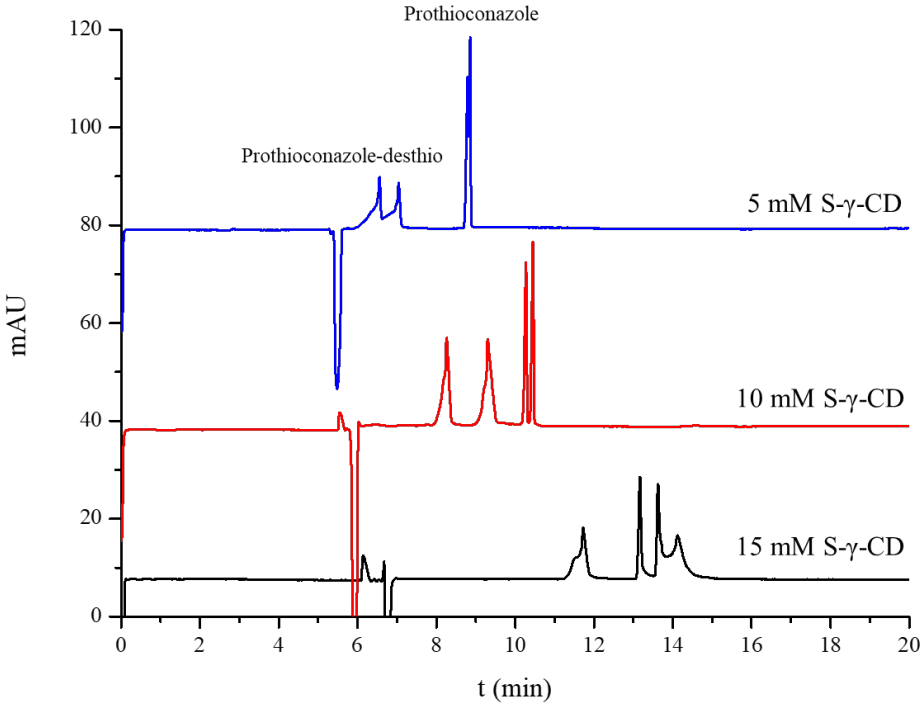

Fig. S3. Electrophoregrams obtained for a standard solution containing prothioconazole and prothioconazole-desthio (at a concentration of 200 mg L^{-1} each) when using S-γ-CD at different concentrations. Experimental conditions: BGE, S-γ-CD in 100 mM borate buffer (pH 9.0); uncoated fused-silica capillary 50 µm id × 50 cm (58.5 cm to the detector); injection by pressure 50 mbar × 10 s; applied voltage +20 kV; temperature 20 ºC and UV detection 205 ± 4 nm.