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Enantiomeric separation of organophosphorus pesticides by capillary electrophoresis Application to the determination of malathion in water samples after preconcentration by off-line solid-phase extraction

Carmen García-Ruiz [a], Gloria Álvarez-Llamas [b], Ángel Puerta [c], Elisa Blanco [b], Alfredo Sanz-Medel [b], Mª Luisa Marina [a,c,*]

[a] *Departamento de Química Analítica, Facultad de Química, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain*
[b] *Departamento de Química Física y Analítica, Facultad de Química, Universidad de Oviedo, C/Julián Clavería 8, 33006 Oviedo, Spain*
[c] *Centro de Tecnología de Alimentos y Servicios Biosanitarios, Facultad de Farmacia, Universidad de Alcalá, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain*

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Abstract

The separation of the enantiomers of a group of organophosphorus pesticides (OPs) has been investigated by electrokinetic chromatography (EKC) using different anionic cyclodextrins as chiral selectors. The use of a 25 mM Tris buffer (pH 7.0), 20 mM in CM-β-CD together with an applied voltage of 24 kV and a temperature of 25 °C enabled the individual enantiomeric separation of malathion and phenthoate each one into its two enantiomers, the partial separation of the enantiomers of phenamiphos and the separation of three of the four enantiomers of isomalathion. Since naled is very reactive in aqueous solutions, its enantiomeric separation achieved in about 8 min in 25 mM borate buffer at pH 9 with 10 mM CM-β-CD resulted in the observation of a broad peak due to degradation products. A preconcentration step by disk solid-phase extraction was studied and used together with the chiral EKC method developed for the enantiomeric separation of malathion in order to determine this pesticide in tap water samples spiked at the $\mu g\,mL^{-1}$ level. Finally, the precision and recovery of the method was established.

Keywords: Enantiomeric separation; organophosphorus pesticides; Capillary electrophoresis; Malathion; Solid-phase extraction

1. Introduction

Nowadays, organophosphorus pesticides (OPs) constitute one of the largest classes of agricultural insecticides used in the world and characterized by their high degradation rates. They act as pesticides because of their property of inhibiting acetilcholinesterase (AchE) in insects. Although these pesticides show preferential toxicity to insects, they are also toxic to mammals existing regulatory limits for human exposure based on inhibition of AchE either in experimental animals or in humans [1–3]. Human intake of OPs is through the remaining pesticides in food products due to their indiscriminate application by farmers [4]. Numerous OPs are chiral and their potential biological effects may be enantioselective. In addition, for some of these pesticides one enantiomer is more toxic that the other one. In these cases, it would be desirable to use single enantiomers as pesticides producing a reduction of the chemical used in the environment. However, the stereospecific synthesis and purification of individual enan-

* Corresponding author. Tel.: 34 91 8854915; fax: +34 91 8854971.
E-mail address: mluisa.marina@uah.es (M.L. Marina).

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tiomers is difficult and costly, which accounts for the fact that the worldwide production of single enantiomers of pesticides is very reduced [5–7]. In spite of this fact, the evaluation of the real toxicity of environmental samples and the understanding of the chiral discrimination in biological processes require the development of analytical methodologies to achieve the enantiomeric separation of these chiral compounds. These chiral methodologies will also be applicable to the control of products in enantioselective production plants since this practice is expected to increase in the future together with the appearance of legal regulations controlling the advantageous use of single enantiomers as agricultural. Among the different separation techniques enabling enantiomeric analysis, capillary electrophoresis (CE) has show an enormous potential in this field due to its interesting characteristics as high separation efficiency, versatility and low consumption of chiral selectors [8]. However, an enantiomeric separation in CE is not based on an electrophoretic mechanism because the electrophoretic mobilities of the enantiomers of a chiral compound are equal and nonselective. Actually, the enantioselective recognition of the enantiomers of a chiral compound is due to their different interaction with a chiral selector and therefore caused by a chromatographic mechanism. As a consequence, chiral separations by CE are performed in the electrokinetic chromatography (EKC) format [9].

There are numerous papers dedicated to the separation and also quantification of OPs by gas chromatography (GC) [10,11] or high performance liquid chromatography (HPLC) [12,13]. However, only few of them are dedicated to the enantiomeric separation of some of these compounds by HPLC [14–16], or CE [7,17,18]. Using CE, dialifos was enantiomerically separated in less than 8 min in a sodium dodecyl sulfate (SDS) micellar EKC (MEKC) system using γ-cyclodextrin (γ-CD) (40 mM) as chiral selector and methanol (10%) in the separation buffer. Nevertheless, malathion and ruelene were only partially resolved in a similar MEKC system using hydroxypropyl-β-CD (60 mM) as chiral selector without and with methanol (20%) [17]. Ruelene enantiomers were also separated by a similar MEKC system containing hydroxypropyl-β-CD (40 mM) with acetonitrile (20%) [7]. Finally, the enantiomeric resolution of organophosphorus triesters using the anionic cyclodextrin derivative octakis(2,3-diacetyl-6-sulfo)-γ-cyclodextrin (ODAS-γ-CD, 20 mM) as chiral selector with methanol (10%) as organic modifier has been achieved [18]. Then, although there are many OPs having an asymmetric center (phosphorus or carbon) some of them have not been enantiomerically resolved yet by CE. At this respect, the use of cyclodextrin derivatives has shown enormous possibilities for the enantiomeric separation of compounds of pharmaceutical and environmental interest by CE [6,8,9]. Some possibilities of the use of cyclodextrin derivatives on the enantiomeric separation of compounds of environmental interest by CE have also been shown previously in different works of our research team [19–21].

Therefore, the aims of this work were two:

(i) To select the experimental conditions enabling the enantiomeric separation by CE of a group of OPs: malathion, malaoxon, isomalathion, phenthoate, naled, isofenphos, ruelene and phenamiphos due to most of them have not been separated enantiomerically by CE. In fact, as far as we know this holds for malaoxon and isomalathion (which are the main metabolites of malathion), phenthoate, naled, isofenphos and phenamiphos have not been enantiomerically separated previously by CE. In addition, although malathion has been partially enantiomerically separated by a MEKC system, its baseline separation is needed in order to apply the chiral method to the quantitative analysis of its enantiomers in different samples.
(ii) The determination of malathion, which is one of the most used OPs in agriculture, in water samples spiked at the $\mu g\,mL^{-1}$ concentration level, concentration levels reported for malathion in drinking waters [2], by chiral CE after preconcentration of this pesticide by disk solid-phase extraction.

2. Experimental

2.1. Chemicals and samples

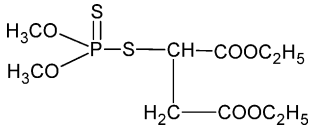
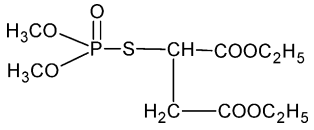
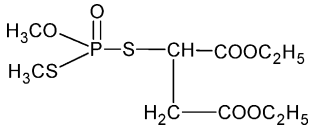
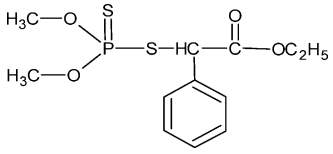
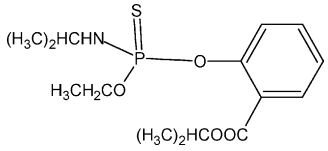
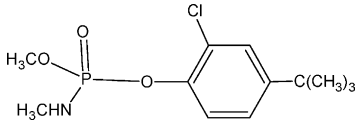
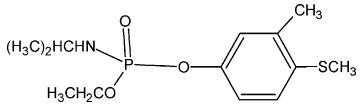
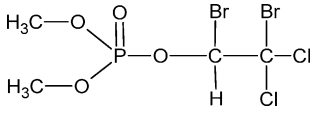
All reagents were of analytical grade. 3-[*N*-Morpholine] propanesulfonic acid (MOPS) was purchased from Sigma (St. Louis, MO, USA); sodium hydroxide, hydrochloric acid, methanol, hexane, ammonium acetate, and Tris (*N*-tris (hydroxymethyl) aminomethane) were supplied from Merck (Darmstadt, Germany); ethyl acetate and diethyl ether were from Panreac (Barcelona, Spain); tetrahydrofuran was from Scharlau (Barcelona, Spain); boric acid was from Fluka (Buchs, Switzerland); carboxymethylated γ-cyclodextrin (CM-γ-CD, degree of substitution (DS) ∼3), carboxymethylated β-cyclodextrin (CM-β-CD, DS ∼3), (Succ-β-CD, DS ∼3.5), β-cyclodextrin sulfated (β-CD sulfated, DS ∼12) were obtained from Cyclolab (Budapest, Hungary). Water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA). All solutions were filtered through 45 μm pore size disposable nylon filters from Waters (Milford, MA, USA).

Malathion, malaoxon, isomalathion, phenthoate, isofenphos, ruelene, phenamiphos, and naled were purchased from Chemservices (West Chester, PA, USA). The chemical structures of these OPs are shown in Table 1.

2.2. Apparatus

Two capillary electrophoresis instruments have been used: (i) a UNICAM CRYSTAL 300 CE system (Unicam Emmen, The Netherlands) equipped with a UV detector (UNICAM 4225). Electropherograms were recorded using a Philips

Table 1
Structure and name of the OPs pesticides studied in this work

Pesticide	Structure
Malathion	
Malaoxon	
Isomalathion	
Phenthoate	
Isofenphos	
Ruelene	
Phenamiphos	
Naled	

PU1815 Integrator (Cambridge, GB) and (ii) a HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) and a HP 3D-CE Chemstation software.

Uncoated fused-silica capillaries from Teknokroma (Barcelona, Spain) with 75 μm inner diameter (i.d.) and 363 μm outer diameter (o.d.) with an effective length of 61.5 cm (70 cm total length) when the HP^{3D} CE system was used or with 50 μm i.d. and 363 μm o.d. with an effective length of 65 cm (77 cm total length) when the UNICAM CRYSTAL 300 CE system was used, were employed.

Capillary temperature was set to 25 °C and UV detection was performed at 210, 230, and 254 nm.

A microp H 2000 (Crison, Barcelona, Spain) was employed to adjust the pH of the separation buffers.

Extraction of malathion was performed in a vacuum filtration system into a ISOLUTE SPE column (C8/ENV+) from International Sorbent Technology (Mid Glamorgan, UK).

2.3. Procedure

2.3.1. Conditioning of the capillary and EKC separation

Electrolytic solutions were prepared weighing and dissolving the appropriate amount of buffer and chiral selector in Milli-Q water to obtain the required concentration and adjusting the pH to the desired value with 1 M sodium hydroxide or diluted hydrochloric acid solutions prior to adjust the final volume.

Between injections, the capillary was washed with NaOH 0.1 M (5 bar for 2 min) followed by Milli-Q water (5 bar for 2 min) and separation buffer (5 bar for 2 min) in the HP^{3D} CE system whereas in the UNICAM CRYSTAL 300 CE system the capillary was washed with NaOH 0.1 M (2 bar for 2 min) followed by Milli-Q water (2 bar for 2 min) and separation buffer (2 bar for 2 min). The injections were made by pressure: 50 mbar for 3–6 s (50 mbar for 3 s for the determination of malathion in water samples). The applied voltage employed was from 15 to 30 kV. A range of voltage of 24 kV in 0.6 min was employed for the selected conditions and for the determination of malathion in water samples.

2.3.2. Standards and sample preparation

Standard solutions were prepared by dissolving each compound in methanol (malathion, malaoxon, isomalathion, phenthoate, isofenphos, and ruelene) or hexane (phenamiphos and naled) up to a final concentration of 10.00 mg mL^{-1} approximately. Dilutions of these solutions were made in order to obtain a concentration of approximately 0.10 mg mL^{-1} for all the pesticides studied except for naled for which a concentration of about 1.00 mg mL^{-1} was employed. In the application of the chiral EKC method developed to the determination of malathion in water samples, different standard solutions were prepared by diluting the stock solution of 10.00 mg mL^{-1} in methanol in order to obtain increasing concentrations ranging from 0.10 to 1.40 mg mL^{-1}.

Malathion was determined in tap water samples (from Alcalá de Henares, Madrid) spiked at the μg mL^{-1} concentration level (0.8, 1.0, and 1.2 μg mL^{-1}).

2.3.3. Solid-phase extraction

ISOLUTE SPE disks (C8/ENV+) were placed in the vacuum filtration system and activated and conditioned with 2× 5 mL methanol and 2 × 5 mL of Milli-Q water. After percolating the spiked water sample (100 mL), the adsorbent was washed with 5 mL Milli-Q water and the malathion was eluted with 2× 2 mL of elution solvent (ethyl ether, ethyl acetate, ethyl acetate–tetrahydrofurane, or ethyl acetate–ethyl ether). Prior to the injection in the CE system the solvent was evaporated by a nitrogen flow and the pesticide was redissolved in 0.4 mL methanol.

Table 2
Experimental conditions tested for the enantiomeric separation of the OPs pesticides studied

Buffer	Chiral selector	Voltage, kV (current intensity, μA)	Pesticide enantiomerically separated (enantiomeric resolution)
25 mM Borate (pH 9)[a]	10 mM CM-β-CD	20 (20)	Naled (>5), phenthoate (1.4)
25 mM Borate (pH 9)[a]	10 mM CM-β-CD	20 (27)	Naled (>5)
25 mM Borate (pH 9)[a]	10 mM Succ-β-CD	20 (10.5)	None
25 mM Borate (pH 9)[a]	10 mM β-CD sulfated	20 (40)	None
25 mM Tris (pH 7.0)[b]	5 mM CM-β-CD	24 (59)	Isomalathion (1.7, 0.3)[c]
25 mM Tris (pH 7.0)[b]	10 mM CM-β-CD	24 (102)	Naled (>5), phenthoate (1.5), isomalathion (2.4, 0.6)[c]
25 mM Tris (pH 7.0)[b]	15 mM CM-β-CD	24 (155)	Naled (>5), phenthoate (1.7), phenamiphos (0.4), malathion (0.5), isomalathion (2.5, 0.9)[c]
25 mM Tris (pH 7.0)[b]	20 mM CM-β-CD	24 (162)	Naled (>5), phenthoate (2.0), phenamiphos (0.6), malathion (1.4), isomalathion (2.5, 1.1)[c]
25 mM MOPS (pH 7.0)[b]	20 mM CM-β-CD	24 (117)	Naled (>5), phenthoate (1.7), isomalathion (2.5, 1.2)[c]
25 mM Ammonium acetate (pH 5.0)[b]	20 mM CM-β-CD	24 (178)	Isomalathion (2.7, 1.1)[c]

[a] Instrumental conditions: uncoated fused sillica capillary 78.5 cm (70 cm to the detector) × 50 μm i.d.; UV-detection at 214 nm; 25 °C and 30 kV.
[b] Instrumental conditions: uncoated fused sillica capillary 70 cm (61.5 cm to the detector) × 75 μm i.d.; UV-detection at 230 nm and 254 nm; 25 °C and 24 kV.
[c] Enantiomeric resolution between peaks 1 and 2 and peaks 3 and 4.

2.4. Data treatment

In order to increase the precision of the data, corrected migration times and corrected peak areas were used [22]. Corrected migration times were calculated as the migration time of the peak divided by the migration time corresponding to the electroosmotic flow (methanol). Corrected peak areas were calculated dividing the peak area by the migration time of the peak. Corrected total peak areas, used for the calibration, were calculated as the addition of the area corresponding to the first migrating enantiomer divided by its migration time and the area corresponding to the second migrating enantiomer divided by its migration time.

All the experimental data were manipulated using Excel 7.0 from Microsoft Office software [23].

3. Results and discussion

3.1. Enantiomeric separation of OPs: selection of the experimental conditions

Eight chiral OPs (malathion, malaoxon, isomalathion, phenthoate, isofenphos, ruelene, phenamiphos, and naled) have been injected in a CE system using different anionic cyclodextrins as chiral selectors in the separation buffer. All conditions used in this work to try the enantiomeric separation of these compounds are summarized at Table 2.

First, different chiral selectors were used under different experimental conditions in order to achieve the enantiomeric separation of the chiral OPs studied. When a fixed concentration (10 mM) of different anionic CD derivatives (CM-γ-CD, CM-β-CD, Succ-β-CD, β-CD sulfated) was used in a 25 mM borate buffer (pH 9) it was observed that CM-β-CD enabled the enantiomeric resolution of naled and phenthoate and CM-γ-CD enabled the enantiomeric resolution of naled whereas the use of Succ-β-CD or β-CD sulfated as chiral selectors did not enable the enantiomeric separation of any compound. Fig. 1 shows the enantiomeric separation of naled in less than 8 min when 25 mM borate buffer at pH 9 with 10 mM CM-β-CD and UV-detection at 214 nm were employed. It can be observed that the first enantiomer is partially overlapped with a peak, which was due to the degradation products of this pesticide, which is very reactive in aqueous solution. Then,

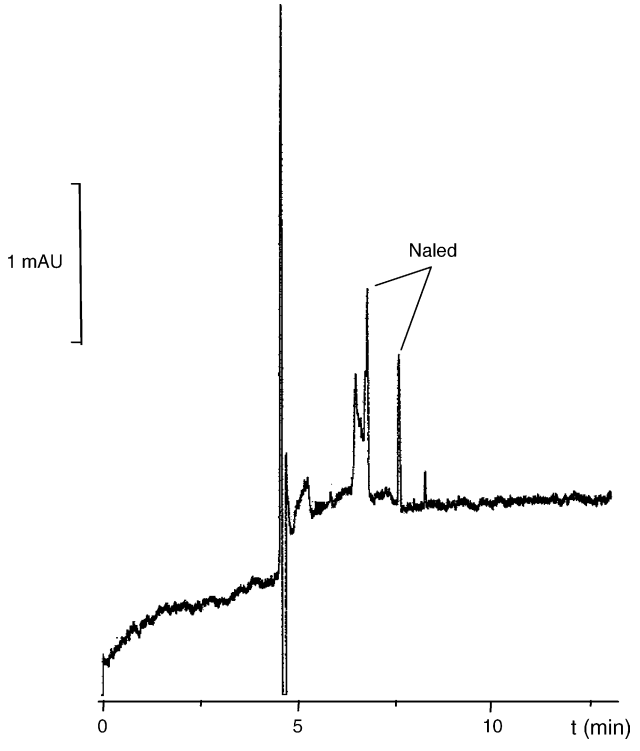


Fig. 1. Enantiomeric separation of naled (~1.00 mg mL^{-1}) in a 25 mM borate buffer (pH 9) with 10 mM CM-β-CD. Experimental conditions: ambient temperature; applied voltage 30 kV; UV detection at 214 nm; injection by pressure, 50 mbar for 12 s; 50 μm i.d., 363 μm o.d. capillary of 77 cm length (65 cm to the detector), UNICAM CRYSTAL 300 CE system.

CM-β-CD seems to be the best chiral selector among those checked. The use of CM-β-CD (10 mM) with 25 mM Tris buffer at neutral pH enabled the enantiomeric resolution of naled and phenthoate as in the case of borate buffer at pH 9, but in addition, isomalathion was enantiomerically discriminated in this case. When 25 mM Tris buffer (pH 7) with different concentrations (5, 10, 15, and 20 mM) of CM-β-CD was used, an increase in the enantiomeric resolution was observed with increasing concentrations of CD (see Table 2). Therefore, a 20 mM concentration of CM-β-CD was selected as the most appropriate to achieve the chiral separation of the compounds studied and higher concentrations were not employed due to the high current intensity (162 μA) obtained with this concentration of the anionic CD. Fig. 2a shows the separation of the two enantiomers of malathion, and the separation of three of the four enantiomers of isomalathion in 25 mM Tris buffer at pH 7 with 20 mM CM-β-CD (detection at 230 nm). On the other hand, Fig. 2b shows the enantiomeric separation of phenthoate and the partial separation obtained for the enantiomers of phenamiphos when 25 mM Tris buffer at pH 7 with 20 mM CM-β-CD (detection at 254 nm) was employed. The selected concentration of CM-β-CD (20 mM) was also employed with 25 mM MOPS (pH 7) and 25 mM ammonium acetate (pH 5). A decrease in the number of pesticides enantiomerically separated when Tris was changed by MOPS and when the pH decreased from 7 to 5 was observed (see Table 2).

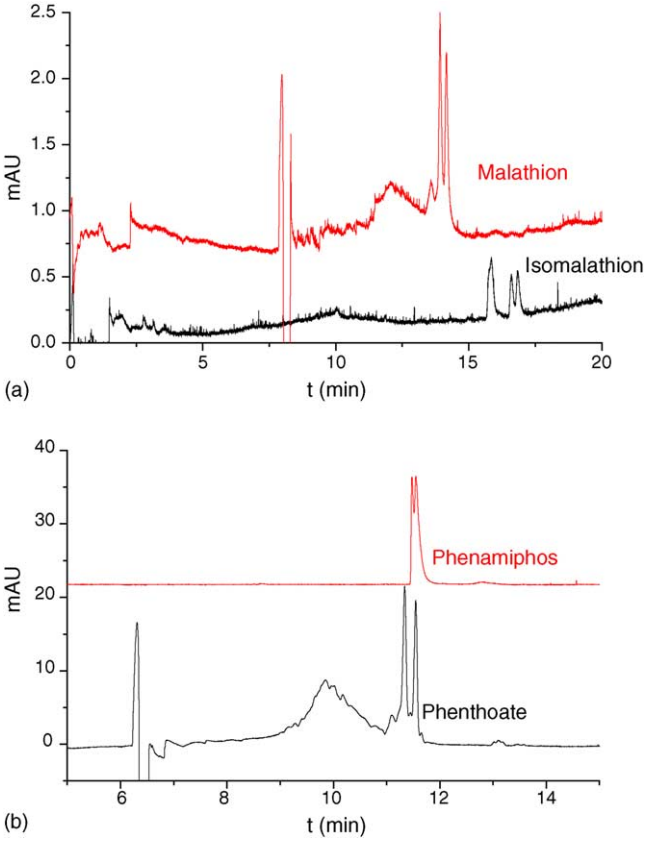


Fig. 2. (a) Separation of the two enantiomers of malathion (∼0.10 mg mL^{-1}) and of three of the four enantiomers of isomalathion (∼0.10 mg mL^{-1}) in a 25 mM Tris buffer (pH 7) with 20 mM CM-β-CD and UV-detection at 230 nm. (b) Enantiomeric separation of phenthoate (∼0.10 mg mL^{-1}) and phenamiphos (∼0.10 mg mL^{-1}) in a 25 mM Tris buffer (pH 7) with 20 mM CM-β-CD and UV-detection at 254 nm. Other experimental conditions: 25 °C; applied voltage 24 kV; injection by pressure, 50 mbar for 3 s; 75 μm i.d., 363 μm o.d. capillary of 70 cm length (61.5 cm to the detector), HP^{3D} CE system.

In the case of malathion, it was only partially enantiomerically separated by CE in a previous work [17] whereas under the conditions proposed in this work it was baseline separated although in a longer analysis time (∼15 min versus ∼5 min reported in Ref. [17]). On the other hand, the baseline separation of malathion enantiomers by HPLC has been reported using a polysaccharide chiral stationary phase using a hexane:ethanol (90:10) mobile phase [16]. Limits of detection for this pesticide by HPLC were not given in this work because its goal was the comparison of different stationary phases to achieve the chiral separation of a group of OPs. In addition, from an environmental point of view, CE may be considered a clean analytical technique compared to HPLC due to its low consumption of solvents and samples.

3.2. Determination of malathion in water samples

Malathion is one of the most widely used OPs insecticides and it is chiral due to an asymmetric carbon center on the succinyl ligand (see Table 1). This is why in this work, the chiral EKC method developed for the enantiomeric separation of malathion based on the use of 25 mM Tris buffer at pH 7 with 20 mM CM-β-CD (25 °C and 24 kV) was applied to the determination of malathion in water samples spiked at the μg mL^{-1} level.

First, solutions of malathion standard with concentrations ranging from 1.02 to 1.40 mg mL^{-1} were injected in order to determine the linear concentration range. A linear relationship between the total corrected area and the concentration of malathion was observed in the concentration range from 0.10 to 0.99 mg mL^{-1}. The equation obtained in this concentration range when the corrected total peak area against the concentration was plotted was $y = -0.03 + 0.77x$ being the correlation coefficient equal to 0.993, which indicated a relatively strong relationship between the variables. The sensitivity of this chiral method, corresponding to the slope of the calibration line, was about 0.8 mL mg^{-1}. The limit of detection (calculated as the concentration corresponding to a signal equal to the intercept plus three times the standard error of the calibration plot) was ∼0.10 mg mL^{-1} for malathion, that is, ∼0.05 mg mL^{-1} for each malathion enantiomer. As a consequence, we considered a preconcentration step to apply the chiral EKC method to the determination of malathion in spiked water samples.

Therefore, the preconcentration of malathion using ISOLUTE SPE disks (C8/ENV+) was studied due to the applicability of these disks to the extraction of OPs from water samples [24]. First, different elution solvents were used in order to obtain the highest recovery in the extraction process of

Table 3
Recovery of the extraction procedure of malathion from water samples when different elution solvents were employed with the ISOLUTE SPE disks (C8/ENV+)

Elution solvent	Recovery (%)
Ethyl acetate	30 ± 4[a]
Diethyl ether	63 ± 7[a]
Ethyl acetate–tetrahydrofuran (50:50)	47 ± 6[a]
Ethyl acetate–diethyl ether (50:50)	81 ± 5[b]

[a] Average values obtained from two determinations.
[b] Average value obtained from seven determinations.

malathion from spiked tap water samples. Table 3 shows the recovery obtained in the extraction procedure when different elution solvents were used. This recovery was measured as the ratio between the corrected total peak area measured for malathion in the tap spiked water samples after SPE extraction and the corrected total peak area of a standard solution with a concentration of malathion equal to the concentration that the final preconcentrated solution would have if the recovery was equal to 100%. The results obtained show that the best recovery for the extraction procedure was about 81% when ethyl acetate–diethyl ether (50:50) was used as elution solvent. Therefore, this elution solvent was used to apply the analytical method combining disk solid-phase extraction with EKC to the determination of malathion in water samples.

In order to evaluate the precision of the solid-phase extraction-EKC method, repeatability and reproducibility were studied. The repeatability for corrected migration times, corrected total peak area, and concentration was determined (as relative standard deviation, R.S.D.) for eight consecutive injections of a solution in methanol obtained after solid-phase extraction of a water sample spiked with 0.8 μg mL^{-1} of malathion. As it can be observed in Table 4, R.S.D. values were less than 0.9% for the corrected migration times of the enantiomers, 9.2% for corrected total peak area, and 8.7% for the calculated concentration (value obtained after interpolation of the total peak area in the calibration line and applying the corresponding dilution factor). On the other hand, reproducibility in corrected migration times, in corrected total peak area, and in concentration was measured as the R.S.D. obtained in two different days when solutions in methanol were obtained after solid-phase extraction of two individual water samples spiked with 0.8 μg mL^{-1} of malathion. R.S.D. values obtained were less than 0.7% for the corrected migration times of the enantiomers, 11.0% for corrected total peak area, and 9.8% for the calculated concentration (see Table 4). Although R.S.D. values for corrected migration times are good, they are only acceptable for corrected peak areas (∼10%) and it may be attributed to the use of volatile solvents (the sample solvent is methanol) at room temperature.

Table 4
Precision measured as repeatability and reproducibility obtained in the application of the disk solid-phase extraction-EKC method to the analysis of malathion in tap water samples

Repeatability for corrected migration times[a]	0.9
Repeatability for corrected total peak area[a]	9.2
Repeatability for concentration[a]	8.7
Reproducibility for corrected migration times[b]	0.7
Reproducibility for corrected total peak area[b]	11.0
Reproducibility for concentration[b]	9.8

[a] R.S.D. (%) values determined for eight consecutive injections.
[b] R.S.D. (%) values determined for two different water samples.

The recovery of the method was studied by using water samples containing increasing amounts of malathion. For this purpose, water samples spiked with three different and known quantities of malathion standard (0.8, 1.0, and 1.2 μg mL^{-1}) were preconcentrated by the solid-phase extraction procedure and injected in the CE system. The recovery was obtained by dividing the corresponding concentration obtained by the EKC method (calculated by interpolating on the calibration line and applying the dilution factor) and corrected with the average value of the recovery of the extraction procedure (81%) [25] by the theoretical concentration. The mean recovery obtained was 107%.

Finally, Fig. 3 shows the electropherogram corresponding to the separation of the enantiomers of malathion in a tap water sample spiked with 0.8 μg mL^{-1} after preconcentration by disk solid-phase extraction (0.4 μg mL^{-1} of each enantiomer). It can be observed the enantiomeric separation of malathion and one unknown peak probably due to the sample. The instability observed for the baseline was also observed for the standard solutions being more appreciated in this case due to the scale used to see the small peaks corresponding to the pesticide. Finally, it is important to consider that the limit of detection (calculated from Fig. 3 as the concentration corresponding to a signal three times the noise background) of the SPE-EKC method developed is ∼200 ng mL^{-1} of each

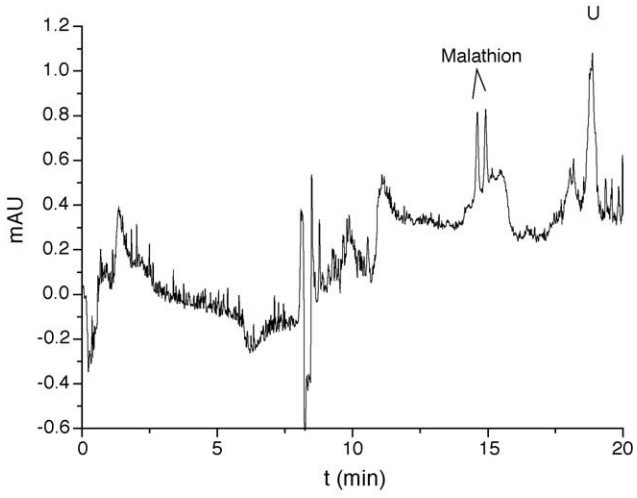


Fig. 3. Electropherogram corresponding to the separation of the enantiomers of malathion after preconcentration by disk solid-phase extraction of a tap water sample spiked with 0.8 μg mL^{-1} of malathion (0.4 μg mL^{-1} of each enantiomer). Electrophoretic conditions: 25 mM Tris buffer (pH 7) containing 20 mM CM-β-CD at 25 °C and 24 kV. Injection by pressure, 50 mbar for 3 s; UV-detection at 230 nm; HP^{3D} CE system. Preconcentration conditions: ISOLUTE SPE disk (C8/ENV+) using ethyl acetate–diethyl ether (50:50) as elution solvent; U, unknown peak.

malathion enantiomer in spite of the low absorption of this OP.

4. Conclusions

A screening of various anionic cyclodextrin derivatives under different experimental conditions (different buffer nature and pH), has shown that carboxymethylated β-cyclodextrin (CM-β-CD) enabled the enantiomeric separation of malathion and phenthoate, the partial enantiomeric separation of phenamiphos, and the separation of three of the four enantiomers of isomalathion in 25 mM Tris buffer (pH 7) containing 20 mM CM-β-CD. The separation of the enantiomers of naled was also possible in about 8 min in a 25 mM borate buffer (pH 9) containing 10 mM CM-β-CD as chiral selector, but in this case a signal corresponding to degradation products of this compound was also observed because it is rapidly degraded in aqueous solution.

Due to malathion is one of the most used OPs in the agriculture it has been monitored in tap water samples spiked with this pesticide in the $\mu g\,mL^{-1}$ concentration level. Nevertheless, due to the low sensitivity of the chiral EKC method developed, a off-line preconcentration step by disk solid-phase extraction was included and studied obtaining recoveries of about 81% when ethyl acetate–diethyl ether (50:50, v/v) was used for the elution of malathion in ISOLUTE SPE disks. This analytical method showed good performance in terms of precision and recovery (close to 100%). In this way, the potential of the developed method for the determination of malathion in water samples has been shown. In addition, it is important to take into account that when a racemic mixture of this widely used pesticide enters in the environment, it may be degraded at different rates exhibiting these degradation processes a high degree of stereoselectivity as has already been demonstrated for other agrochemicals [26,27]. As a consequence, the developed enantioselective method may be useful for this type of studies in future works.

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References

[1] www.pesticides.gov.uk (checked on January, 2005).
[2] www.epa.gov/pesticides (checked on January, 2005).
[3] J.R. Plimmer, Chemistry of Pesticides, Handbook of Pesticide Toxicology, vol. 1, Pesticide Risk Characterization, 2002, pp. 95–107 (Chapter 2).
[4] B.I.A. Kaipper, L.A.S. Madureira, H.X. Corseuil, J. Braz. Chem. Soc 12 (2001) 514.
[5] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[6] Z. El Rassi, Electrophoresis 18 (1997) 2465.
[7] D.L. Lewis, A.W. Garrison, K.E. Wommack, A. Whittemore, P. Steudler, J. Melillo, Nature 401 (1999) 898.
[8] B. Chankvetadze, Capillary Electrophoresis in Chiral Analysis, John Wiley & Sons, Chichester, 1997.
[9] B. Chankvetadze, G. Blaschke, J. Chromatogr. A 906 (2001) 309.
[10] A. Bouaid, L. Ramos, M.J. González, P. Fernández, C. Cámara, J. Chromatogr. A 939 (2001) 13.
[11] E.M. Kristenson, E.G.J. Haverkate, C.J. Slooten, L. Ramos, R.J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 917 (2001) 277.
[12] M.D. Beeson, W.J. Driskell, D.B. Barr, Anal. Chem. 71 (1999) 3526.
[13] A.W. Abu-Qare, M.B. Abou-Donia, J. Pharm. Biomed. Anal. 26 (2001) 291.
[14] C.E. Kientz, J.P. Langenberg, G.J. de Jong, U.A.Th. Brinkman, J. High Resolut. Chromatogr. 14 (1991) 460.
[15] C.J. Welch, T. Szczerba, Enantiomer 3 (1998) 37.
[16] J.J. Ellington, J.J. Evans, K.B. Prickett Jr., W.L. Champion, J. Chromatogr. A 928 (2001) 145.
[17] Ph. Schmitt, A.W. Garrison, D. Freitag, A. Kettrup, J. Chromatogr. A 792 (1997) 419.
[18] W. Zhu, F. Wu, F.M. Raushel, G. Vigh, J. Chromatogr. A 895 (2000) 247.
[19] Y. Martín-Biosca, C. García-Ruiz, M.L. Marina, Electrophoresis 22 (2001) 3216.
[20] Y. Martín-Biosca, C. García-Ruiz, M.L. Marina, Electrophoresis 21 (2000) 3240.
[21] C. García-Ruiz, Y. Martín-Biosca, A.L. Crego, M.L. Marina, J. Chromatogr. A 910 (2001) 157.
[22] J.P. Schaeper, M.J. Sepaniak, Electrophoresis 21 (2000) 1421.
[23] Microsoft Excel 7.0, Microsoft Corporation, 1985–1997.
[24] SPE Method Development Recommendations for Extraction of organophosphorus Pesticides from Water Samples, International Sorbent Technology (IST0092).
[25] C. Cámara (Ed.), Toma y Tratamiento de Muestras, Editorial Síntesis, Spain, 2002.
[26] C. Desiderio, C.M. Polcaro, P. Padiglioni, S. Fanali, J. Chromatogr. A 781 (1997) 503.
[27] S. Kodama, A. Yamamato, A. Matsunaga, K. Okamura, R. Kizu, K. Hayakawa, J. Sep. Sci. 25 (2002) 1055.