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Enantiomeric separation of acidic herbicides by capillary electrophoresis using vancomycin as chiral selector

C. Desiderio, C.M. Polcaro, P. Padiglioni, S. Fanali*

Istituto di Cromatografia del Consiglio Nazionale delle Ricerche, Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Scalo, Rome, Italy

Abstract

In this study vancomycin has been used as chiral selector for the enantiomeric separation of several free acid herbicides, namely aryloxypropionic (mecoprop, fenoprop and dichlorprop), *N*-benzoyl-*N*-(3-chloro-4-fluorophenyl)-2-aminopropionic acid (flamprop) and aryloxyphenoxypropionic (haloxyfop, fluazifop, diclofop and fenoxaprop). The partial filling method was used in order to increase the sensitivity of the electrophoretic method; vancomycin was not present at the detector path during the detection due to its positive charge and to the absence/reduction of the electroosmotic flow at the operating pH. The pH of the BGE, the capillary temperature and the vancomycin concentration influenced both effective mobility and resolution of the studied compounds. The increase of vancomycin concentration caused a general increase of migration time, resolution and selectivity, the best results have been achieved when a 6 m*M* concentration of chiral selector was used. Using such a concentration of vancomycin, baseline resolution was obtained for all the studied enantiomers in 4.5–8.4 min. The optimized CE method has been tested for the analysis of haloxyfop free acid metabolite in a soil sample spiked with racemic haloxyfop ethoxyethyl ester solution. The metabolite resulted to be a mixture of *R* and *S* enantiomers where the *R* form was about 72% (peak areas ratio). The method showed good precision for both migration time and corrected peak areas with a detection limit of 5×10^{-7} *M*. © 1997 Elsevier Science B.V.

Keywords: Enantiomer separation; Soil; Pesticides; Vancomycin

1. Introduction

The separation of enantiomers is an interesting topic of research applied to different fields, e.g., pharmaceutical, clinical, forensic, agrochemical, etc.

Analytical methods so far used for the separation of enantiomeric compounds include: gas chromatography (GC) [1–3], thin-layer chromatography (TLC) [4], high-performance liquid chromatography (HPLC) [5–7] and more recently capillary electrophoresis (CE) [8–17]. In the last decade capillary electrophoresis has been shown to be a powerful tool for chiral separations mainly using the direct separation method, where the chiral selector is added to the background electrolyte (BGE) [12]. In some instances the chiral selector has been bound to the capillary wall or included into a gel [9,18]; examples of enantiomeric resolutions using packed capillaries have also been shown [19].

The high resolution power and the high efficiency of CE, combined with the wide number of chiral selectors that can be employed in order to improve the stereoselectivity of the separations, and the low amount of both electrolyte and sample, make this technique complementary or even competitive with HPLC, where expensive chiral columns are requested.

*Corresponding auhtor. Tel.: +39 6 90672256; fax: +39 6 90625849; e-mail: fanali@nserv.icmat.mlib.cnr.it.

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It has been recently reported that about 25% of agrochemicals are chiral molecules even if a few of the new commercial formulations contain only one enantiomer as active compound [20,21]. Consequently, the different biological behaviour of the two agrochemical enantiomers and/or their metabolites as well as the stereoselective processes of synthesis are becoming an interesting topic of research in different fields [22], including the agrochemical industry, biochemistry, analytical chemistry, etc. Therefore analytical methods for enantiomeric separation of agrochemical compounds has been the subject of several publications [23–35].

Chlorophenoxy acid herbicide enantiomers or other herbicides have been separated by cyclodextrin-modified capillary electrophoresis [30,34]. Mechref and El Rassi used CE for the enantiomeric separation of herbicides after precolumn derivatization with a fluorescent tag in order to increase the sensitivity [36].

Recently we described the stereoselective separation of some aryloxyphenoxy propionate herbicides and flamprop isopropyl as well as several non-structurally related compounds by CZE using sulfobutyl(IV)–β-cyclodextrin (SBE–β-CD) [35].

In this study, several aryloxy-, aryloxyphenoxypropionic acids and an aminopropionic acid derivative (flamprop) have been selected for enantiomeric separations by CE. The selected compounds, with the exception of aryloxypropionic acids, are usually utilized as esters; they are rapidly hydrolyzed in soil or vegetable tissues to the corresponding acids that are the biologically active agrochemicals. Due to the presence of an asymmetric carbon in the chemical structure (see Fig. 1) all studied compounds are chiral, and the two enantiomers exhibit different herbicide activity (the *R* form being the biologically active one) and persistence in the environment. It has been reported that the hydrolysis of fluazifop butyl ester in soil gives rise to the enrichment of the *R*-(+)-enantiomer (acidic form) [23]. Furthermore, stereoselective degradation of aryloxypropionic acids has been exhibited by soil and aquatic microorganisms [26,37].

In this work we investigated the enantioselective properties of vancomycin towards the above-described compounds in order to develop a fast and cheap CE method suitable for the stereoselective analysis of samples of environmental interest, such as water or soil. Although 2-phenoxypropionic acid is not utilized as herbicide, it has been considered as reference compound in order to investigate the influence of chemical structure of aryloxypropionic acids on the stereoselectivity of vancomycin.

Vancomycin is a macrocyclic antibiotic containing in its structure 18 asymmetric centers with several functional groups (hydroxyl, amino, amide, carboxylic, aromatic, etc.) responsible for stereoselective interactions such as hydrogen bonds, and hydrophobic and electrostatic interactions. Due to the strong absorption of vancomycin at low wavelengths, the partial filling method was used in order to increase the sensitivity of the CE method. The capillary was firstly filled with the BGE-chiral selector free and then with BGE-vancomycin until the detector; a polyacrylamide-coated capillary was used in order to eliminate/minimize electroosmotic flow and adsorption on the capillary wall. The effect of pH of the BGE, capillary temperature and vancomycin concentration on effective mobility and enantiomeric resolution of the selected samples and selectivity of the separation have been investigated. Furthermore, a soil sample was fortified with a commercial formulation containing racemic haloxyfop ethoxyethyl herbicide and the extract was analyzed using the optimized CE method.

2. Experimental

2.1. Chemicals

All the solvents used were of HPLC or analytical grade. Methanol and acetonitrile (ACN) were purchased from BDH (Poole, UK) and acetone was from J.T. Baker (Deventer, The Netherlands). Sodium hydroxide and phosphoric acid were from Fluka (Buchs, Switzerland). Boric acid, glacial acetic acid, hydrochloric acid and potassium acetate were from Carlo Erba (Milan, Italy). Vancomycin pure substance was purchased from Sigma (St. Louis, MO, USA). Double-distilled water was used for solution preparation. Haloxyfop, fluazifop, fenoxaprop and flamprop free acids, diclofop, mecoprop, dichlorprop, fenoprop, 2-phenoxypropionic acid racemic standard compounds were purchased from Lab-Service Analytica (Anzola Emilia, Bologna, Italy).

Haloxyfop–ethoxyethyl commercial herbicide, marketed as Gallant, and declared to contain 125 g/l of the herbicide, was purchased from Siapa, DowElanco Italy (Milan, Italy).

2.2. Apparatus and procedures

A Biofocus 3000 automated capillary electrophoresis apparatus (Bio-Rad Labs., Hercules, CA, USA) equipped with a multiwavelength UV detector has been used for experiments. A polyacrylamide-coated capillary, 37.5 cm total length (effective length, 33 cm)×0.05 mm I.D. was introduced into the user assembler cartridge (Bio-Rad) after removing 0.5 cm of the polyimide layer (detector cell) (for the coating procedure see Ref. [38]). The negative vs. positive electric polarity was used for the runs at a 20-kV applied voltage.

Buffers (75 m*M*, pH 5–7) were prepared from a 150 m*M* Britton–Robinson buffer system (150 m*M* B.R.) composed of equal concentrations (50 m*M*) of boric acid, acetic acid and phosphoric acid after titration to the desired pH value with sodium hydroxide and dilution.

Between runs the capillary was rinsed, at high pressure, with water (70 s) and buffer (70 s) and then the vancomycin-containing buffer was purged at low pressure (at a value of 5 p.s.i. (1 p.s.i. corresponds to 6.895 kPa) for a variable time ranging from 29 to 32 s, depending on vancomycin concentration) in order to fill only part of the capillary leaving the detector path chiral agent free. Vancomycin solutions in buffer were prepared daily.

Stock solutions of samples (10^{-3} *M*) were prepared in methanol with the exception of dichlorprop, mecoprop and 2-phenoxypropionic acid which were dissolved in acetone. The concentrated sample solutions were diluted to 10^{-4} *M* with 10 m*M* Britton–Robinson buffer for injection.

Samples were injected by pressure, applying a set low pressure value of 34.475 kPa×4 s at the cathodic end of the capillary.

2.3. Preparation of a haloxyfop ethoxyethyl ester-contaminated soil sample

One hundred grams of soil, collected in a CNR Research Area, were sieved to 2 mm and spiked with 1 ml of a methanolic solution (8 μl/ml, corresponding to 1 mg of active principle) of Gallant (Down Elanco) a commercial herbicide formulation containing 125 g/l of racemic haloxyfop ethoxyethyl ester.

The fortified soil sample was carefully mixed and allowed to stay for 72 h at room temperature: literature data show that, after this period of incubation in soil, aryloxyphenoxy propionates are almost completely hydrolyzed to the corresponding acids [23,39].

2.4. Extraction of haloxyfop free acid from soil

The extraction of haloxyfop free acid followed the procedure obtained from Ref. [39]. A methanol–1 *M* hydrochloric acid (9:1, v/v) solution was added to the soil sample (B): the mixture was stirred at room temperature for 1 h, then filtered under reduced pressure. Twenty ml of water were added and the hydroalcoholic solution was extracted three times with 100 ml of methylene chloride. The less polar phase was dried on anhydrous sodium sulphate, then evaporated under vacuum up to about 2 ml of volume.

2.5. HPLC separation of haloxyfop free acid enantiomers

Haloxyfop free acid enantiomers were separated by HPLC using an analytical column terguride-based CSP (150×46 mm) and 0.02 *M* potassium acetate buffer (pH 3.5)–ACN (45:55, v/v) [29] as the mobile phase. Two fractions, corresponding to the two enantiomers of haloxyfop, were separately collected, evaporated under vacuum and reconstituted in methanol. An aliquot of the above-described solutions were diluted 10 times using 10 m*M* of BGE-free vancomycin and injected for the electrophoretic analysis in order to characterize the soil extract.

3. Results and discussion

Due to the widely demonstrated resolution power of vancomycin towards anionic compounds with carboxylic function, this antibiotic was chosen as chiral selector to separate the enantiomers of the aryloxypropionic acid and the free acids form of aryloxyphenoxypropionic herbicides, as well as flam-

prop, an aminopropionic acid derivative. The chemical structures of the studied compounds are reported in Fig. 1.

Improvement of sensitivity can be obtained in CE, e.g., using a low wavelength (190–210 nm), however when a strong absorbing chiral selector is employed for stereoselective analysis, such wavelengths are not useful. Thus, in order to avoid the decrease of sensitivity due to the absorption of vancomycin we applied the 'partial filling method' where the BGE-

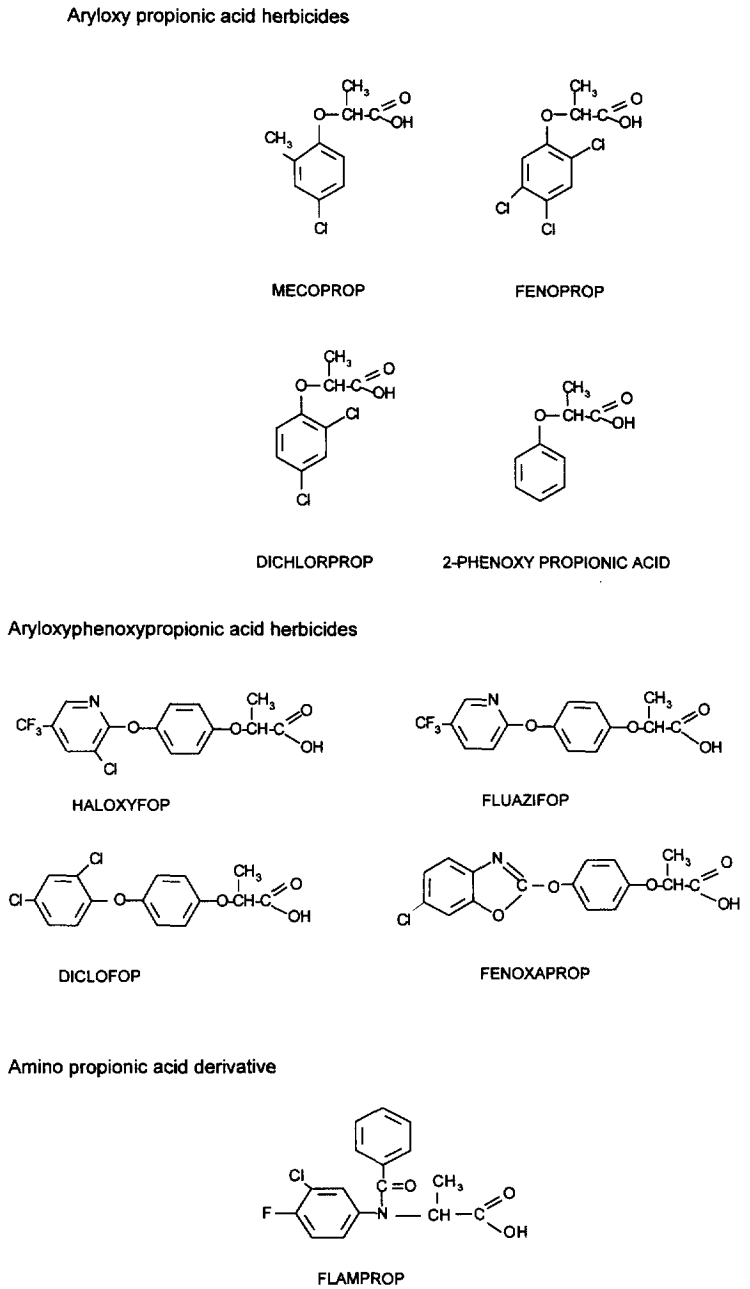

Fig. 1. Chemical structures of the studied compounds.

chiral selector filled only part of the capillary, keeping the detector cell free of vancomycin during the electrophoretic run and allowing good sensitivity. This method could be applied because, at the selected pH range (pH 5–7), vancomycin exhibited a positive charge and thus moved in the opposite direction to the analytes. Furthermore, the use of a coated capillary allowed to perform the electrophoretic runs in absence/very low electroosmotic flow as well as to reduce the adsorption of vancomycin on the capillary wall. In addition the counter-current process, produced by the opposite migration of the chiral selector and analytes, allowed the increase of enantioselective discrimination [14,40].

In order to test this chiral selector for our purposes and to find the optimum experimental conditions we studied the effect of several parameters, namely vancomycin concentration, buffer pH and capillary temperature, on the enantiomeric separation.

3.1. Effect of vancomycin concentration

Enantiomeric resolution (R) and selectivity (S) were calculated using Eq. (1) and Eq. (2), respectively:

$$R = 2\frac{t_2 - t_1}{w_2 + w_1} \quad (1)$$

$$S = 2\frac{\mu_2 - \mu_1}{\mu_2 + \mu_1} \quad (2)$$

where t, w and μ are the migration time, the width at the base-line and the apparent mobility of the two enantiomers (2 moving slower than 1).

To study the influence of vancomycin concentration on the enantiomeric resolution of the investigated herbicides, a BGE at pH 5 was selected for experiments according to the published data and our own experience [40–42]. At the experimental operating conditions (absence of the chiral selector) all analytes were moving negatively charged and reached the detector in less than 6.5 min.

Vancomycin at different concentrations, in the range 1–6 mM, was added to the BGE at pH 5 and the electrophoretic runs were performed using the partial filling method.

Table 1 shows migration times, resolution (R) and selectivity (S) values of the studied herbicides at increasing concentrations of vancomycin.

Complete enantiomeric resolution was achieved for all the studied herbicides with the exception of flamprop free acid which showed an R value of 0.69 at the highest vancomycin concentration used (6 mM). A relatively low vancomycin concentration of 2 mM caused sufficient differences in electrophoretic mobilities to start the enantiomeric separation of almost all the analyzed substances.

Fig. 2a,b depict the electropherograms of the enantiomeric separation of two different mixtures of the studied herbicides obtained in less than 9 min using the pH 5 buffer containing 6 mM of vancomycin.

Considering the group of aryloxyphenoxypropionic acid herbicides it is interesting to remark that, for haloxyfop and diclofop acids, a relatively high enantiomeric resolution value was achieved, whereas for fluazifop and fenoxaprop acids R reached a significantly lower value. By observing the chemical structures in Fig. 1 it can be seen that the analytes exhibiting the highest resolution have chlorine atoms in the molecule.

The influence of chlorine atoms in enhancing the stereoselectivity was particularly evident for haloxyfop ($R=3.69$) and fluazifop ($R=1.37$), which differ in their chemical structure only for the presence of a chlorine in the haloxyfop molecule. Thus, it can be supposed that the chlorine atoms play a key role in modulating the chiral selector/analyte interaction increasing the stereoselective discriminating power. This effect is also confirmed by the behaviour of the aryloxypropionic acid herbicides. In fact, the compound showing the lowest R value was 2-phenoxypropionic acid that does not contain any chlorine atom in the molecule. The fenoprop with the highest number of chlorine atoms exhibited the maximum enantiomeric separation. In fact, using 6 mM of vancomycin the resolution order was as follows: fenoprop>dichlorprop>2-phenoxypropionic acid.

Mecoprop, with only one chlorine in its structure, exhibited an unexpected high enantiomeric resolution (R value between fenoprop and dichlorprop), showing that the stereoselective interaction, analyte–vancomycin, is also influenced by the substituent group type on the aromatic ring of analyte. In fact, in mecoprop the presence of one methyl substituent is

Table 1
Effect of vancomycin concentration on migration time (t_m), enantiomeric resolution (R) and selectivity (S) of aryloxypropionic acids, aryloxyphenoxypropionic acid and an aminopropionic acid derivative (flamprop)

Sample (free acid form)	0 m*M*, t_m	1 m*M*			2 m*M*			4 m*M*			6 m*M*		
		t_{m1} t_{m2}	R	S	t_{m1} t_{m2}	R	S	t_{m1} t_{m2}	R	S	t_{m1} t_{m2}	R	S
Haloxyfop	6.26	6.55	—	—	5.82	<0.50	0.013	6.59	1.31	0.046	7.21 7.95	3.69	0.098
					6.91			6.90					
Fluazifop	5.85	5.94	—	—	5.98	—	—	6.13	<0.50	0.017	6.64	1.37	0.033
								6.24			6.86		
Diclofop	5.79	6.03	—	—	6.10	<0.50	0.013	6.96	1.71	0.048	7.67	3.64	0.089
					6.18			7.30			8.38		
Fenoxaprop	6.15	6.23	—	—	6.60	—	—	6.70	<0.50	0.022	7.64	1.97	0.042
								6.85			7.97		
Flamprop	5.68	5.82	—	—	5.84	—	—	5.97	<0.50	0.010	6.43	0.69	0.020
								6.03			6.56		
Mecoprop	4.56	4.60	—	—	4.62	<0.50	0.010	5.00	1.51	0.049	5.47	4.32	0.096
					4.67			5.25			6.02		
Fenoprop	4.68	4.77	—	—	4.91	0.92	0.018	5.08	3.47	0.046	5.46	4.47	0.092
					5.00			5.32			5.99		
Dichlorprop	4.46	4.50	—	—	4.55	<0.50	0.011	4.80	0.91	0.035	4.98	3.22	0.064
					4.60			4.97			5.31		
2-Phenoxy-proprionic acid	4.03	4.04	—	—	4.07	<0.50	0.007	4.10	<0.50	0.024	4.31	2.41	0.050
					4.10			4.20			4.53		

Apparatus, Biofocus 3000; capillary, polyacrylamide-coated 37.5×0.05 mm I.D. (effective length 33 cm); background electrolyte, 75 m*M* Britton–Robinson (B.R.), pH 5. The BGE-chiral selector rinsed the capillary at 34.475 kPa×30–32 s. Injection was done by pressure at the cathodic end at 34.475 kPa×2 s of 10^{-4} *M* of racemic herbicide solutions. Applied voltage, 20 kV, 43–47 A; capillary temperature, 25°C.

responsible for the strong interaction with vancomycin, and thus the relatively high enantiomeric resolution.

It is also interesting to observe that, when the vancomycin concentration increased, the increase in migration time was very low (in the range of 1–2 min) in comparison with the high resolution values obtained, particularly for the aryloxypropionic acids. In fact as previously discussed [43], with respect to the most widely used cyclodextrins, the vancomycin–analyte interaction does not usually greatly affect the mobility of the analyzed compounds, indicating a very high stereoselective discriminating power for this chiral selector, producing high enantiomeric resolutions in short analysis time.

The selectivity (S) of the enantiomeric separation of the studied compounds was strongly influenced by the vancomycin concentration. S increased by increasing the chiral selector concentration, and the highest value of S was recorded at 6 m*M* of vancomycin for all analytes. Very high selectivity was achieved for those analytes which exhibited the highest resolution.

As for the migration times, the increase in vancomycin concentration resulted in a decrease of the mobility value for all the studied compounds, mainly due to the complexing effect of the chiral selector. This effect was particularly evident for diclofop and mecoprop enantiomers in the groups of aryloxy-phenoxy- and aryloxy-propionated herbicides, respectively (data not shown).

3.2. Effect of the pH of the background electrolyte on enantiomeric separations

Since the BGE pH value can influence the charge, and thus the electrophoretic mobility of both vancomycin and analytes, we investigated the effect of this parameter on the enantiomeric resolution and selectivity of the studied compounds. The effect of the pH on resolution and selectivity was studied using BGEs at pH 5, 6 and 7 supplemented with 6

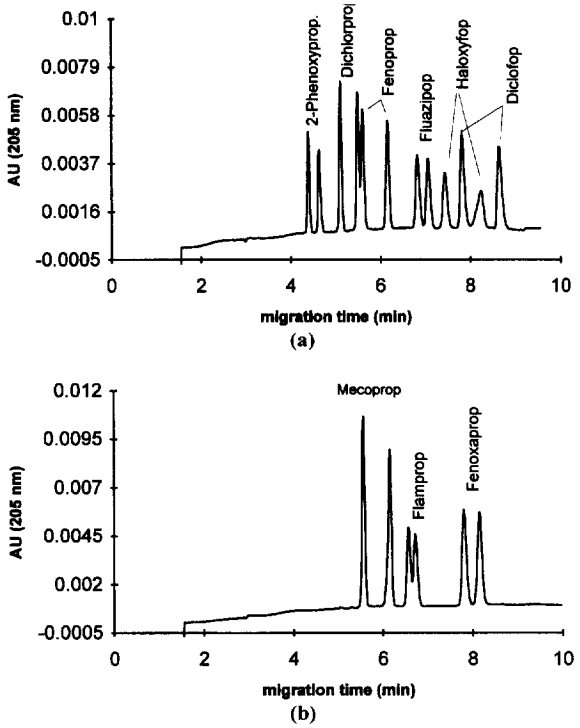

Fig. 2. Electropherograms of the enantiomeric separation of herbicides. (a) 2-Phenoxypropionic acid, dichlorprop, fenoprop, fluazifop, haloxyfop and diclofop; (b) mecoprop, flamprop and fenoxaprop. Six m*M* of vancomycin partial filling at 34.475 kPa×30 s; applied voltage, 20 kV, 47 μA; analyte concentration was 10^{-4} *M*, with exception of fenoprop and diclofop (5×10^{-5} *M*) and 2-phenoxypropionic acid (2×10^{-4} *M*). For other experimental conditions see Table 1.

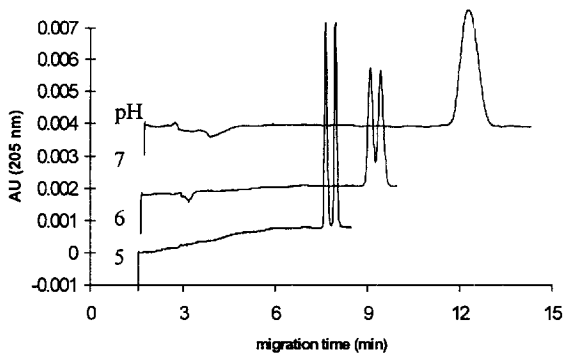

Fig. 4. Electropherograms of fenoxaprop enantiomeric separation at pH 5, 6 and 7. For experimental conditions see Fig. 3.

m*M* of vancomycin. The selected concentration of chiral agent allowed to obtain the best results for the enantiomeric resolution of all analytes.

As can be seen in Fig. 3a the increase of the pH of the BGE caused a general remarkable decrease of enantiomeric resolution and, in some cases, the resolution was completely lost.

The increase of the pH of the BGE also caused a decrease of the selectivity of the enantiomeric separation for all studied herbicides. This effect was remarkable at pH 7 for diclofop, mecoprop, fenoxaprop and fluazifop, while at pH 6 flamprop exhibited $S=0$ (see Fig. 3b).

As an example, Figs. 4 and 5 show the electropherograms of fenoxaprop and mecoprop enantio-

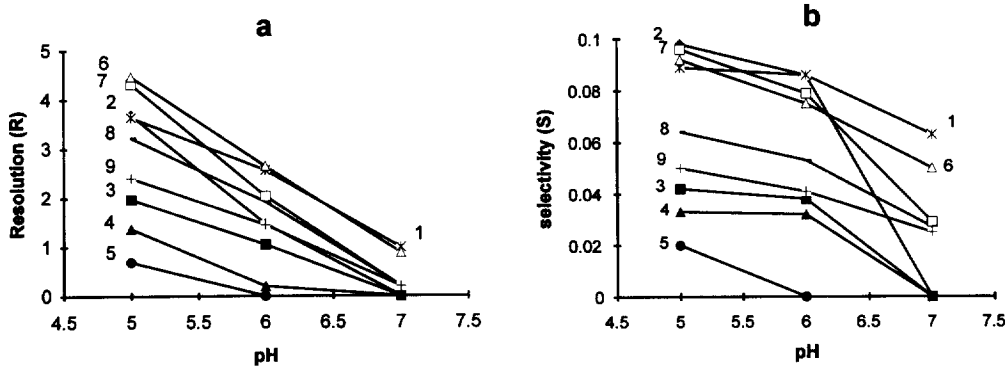

Fig. 3. Effect of pH of the background electrolyte on (a) resolution and (b) selectivity of the studied compounds. (1) Diclofop, (2) haloxyfop, (3) fenoxaprop, (4) fluazifop, (5) flamprop, (6) fenoprop, (7) mecoprop, (8) dichloprop and (9) 2-phenoxypropionic acid. Background electrolyte, 75 m*M* Britton–Robinson buffer containing 6 m*M* of vancomycin; analyte concentration, 10^{-4} *M*; applied voltage, 20 kV, 47–72 μA and 25°C. For other experimental conditions see Table 1.

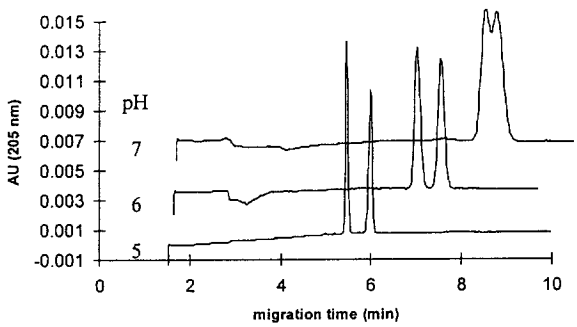

Fig. 5. Electropherograms of mecoprop enantiomeric separation at pH 5, 6 and 7. For experimental conditions see Fig. 3.

mers as representative of the studied aryloxyphenoxypropionic and aryloxypropionic groups, respectively.

In order to explain the above-reported results we have to consider that the increase of the pH caused a decrease of the positive charge of vancomycin (uncharged at pH 7.2–7.5) and thus a reduction of the counter current effect of the separation, as well as of the electrostatic interactions with analytes.

The increase of the pH of the BGE caused a general decrease of electrophoretic mobilities, and consequently longer migration times were recorded. The effect was remarkable for haloxyfop and fluazifop enantiomers; the two racemic compounds were not resolved at all into their enantiomers at pH 7. It was expected that, at this pH value, the analytes should migrate faster than at lower pHs accordingly to their negative charge and to the reduced positive charge of vancomycin, resulting in a negligible counter-current effect. It can be supposed that the hydrophobic interaction, chiral selector–analyte, was predominant in the complexation process that was, however, not stereoselective. Furthermore, we should take into account that the stability of vancomycin solutions can be strongly influenced by the BGE composition, its pH and the Joule heating generated during the electrophoretic process. Also, pH 7 is a critical value for the stability of the used chiral selector, even if semi-deteriorated solutions are not necessarily accomplished with complete loss of stereoselective properties and analyte interactions, however longer migrations times and reduced enantiomeric resolutions are expected [44]. Thus the BGE at pH 5 containing 6 m*M* of vancomycin seems to be the optimum electrolyte: (i) because it imparted the analytes and the vancomycin with the necessary charge, negative and positive, respectively; (ii) because of the stability of vancomycin and analytes; and (iii) because it allowed to perform enantiomeric separation of herbicides with high stereoselectivity in the shortest analysis time.

3.3. Effect of capillary temperature

Electrophoretic experiments were performed at 20, 25 and 30°C, using the BGE at pH 5 containing 6 m*M* of vancomycin, in order to study the effect of capillary temperature on enantiomeric resolution, selectivity, migration time and mobility. Temperatures higher than 30°C are not recommended due to the instability of vancomycin solutions, while values lower than 20°C were not trustworthy due to the room temperature in our laboratory during the experiments (summer time) which caused long equilibration times for the capillary system.

As expected, the increase of capillary temperature caused a general decrease of migration time for all the studied herbicides; this effect was more remarkable changing the temperature from 20 to 25°C than from 25 to 30°C.

The higher the capillary temperature the lower was the enantiomeric resolution (see Fig. 6). The effect was remarkable when the experiments were performed at 30°C, especially when analyzing chlorophenoxy herbicides. The above-reported results are in accordance with previous findings [45,46], and can be explained considering that changes in the

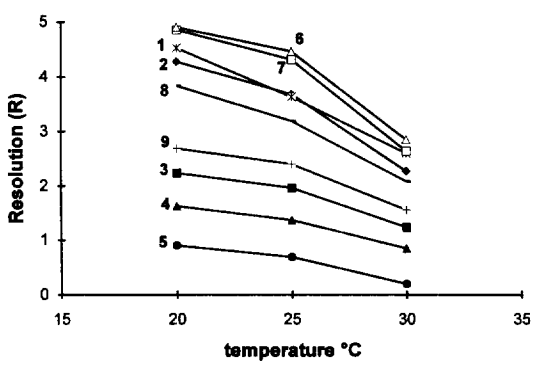

Fig. 6. Stereoselective analysis of haloxyfop free acid metabolite in a soil sample. Experimental conditions as in Fig. 2. For soil extract preparation see text.

capillary temperature affect both buffer viscosity and the thermodynamics of the complex formed between analytes and vancomycin.

The reduction of selectivity by increasing the capillary temperature was not noticeable for 2-phenoxypropionic acid, fenoxaprop, fluazifop and flamprop, while for dichlofop, haloxyfop, fenoprop and mecoprop a decrease of S was recorded (see Fig. 7).

3.4. Analysis from soil of haloxyfop free acid and data precision of the method

To show the applicability of the optimized analytical method we spiked a soil sample with the haloxyfop ethoxyethyl ester herbicide, marketed as Gallant commercial product, and after a period of incubation we extracted the sample and analysed it for the presence of the haloxyfop free acid metabolite.

Fig. 8 depicts the electropherogram of the stereoselective analysis of haloxyfop free acid metabolite in the extracted soil sample. The analysis of the soil sample after 72 h at room temperature revealed the stereoselectivity of the metabolism, as the production of enantiomeric haloxyfop free acids was not racemic. It can be observed that the separation is obtained in less than 9 min and the electropherogram profile is completely interference free.

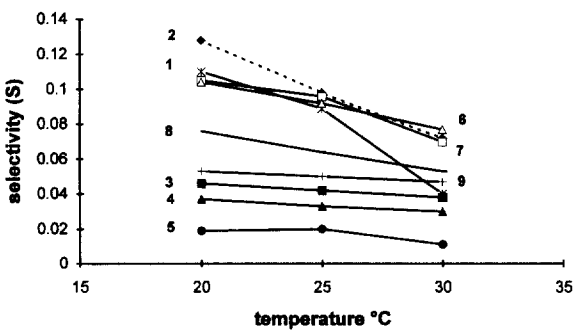

Fig. 7. Effect of capillary temperature on selectivity (S). (1) Diclofop, (2) haloxyfop, (3) fenoxaprop, (4) fluazifop, (5) flamprop, (6) fenoprop, (7) mecoprop, (8) dichloprop and (9) 2-phenoxypropionic acid. Experimental conditions: 75 mM Britton–Robinson buffer, pH 5, containing 6 mM of vancomycin; applied voltage 20 kV, 43–50 μA. For other experimental conditions see Fig. 2.

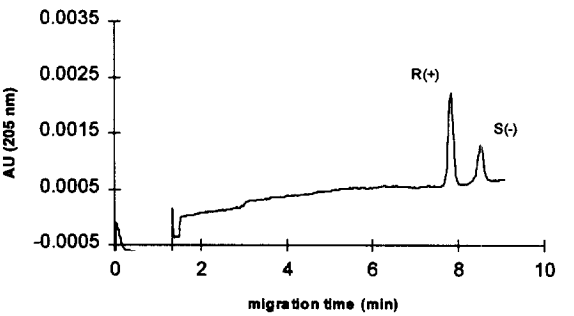

Fig. 8. Stereoselective analysis of haloxyfop free acid metabolite in a soil sample. Experimental conditions as in Fig. 2. For soil extract preparation see text.

The qualitative analysis of haloxyfop free acid in a real environmental sample was supported by the precision data of the method, as shown in Table 2. Very good repeatability for migration times, peak areas and corrected peak areas, were obtained for both intra- and inter-day data.

The method showed a detection limit (LOD) (signal-to-noise ratio=3) of 5×10^{-7} M for each enantiomer.

4. Conclusions

The results achieved in this study show that capillary electrophoresis can be used for the enantiomeric separation of herbicides, such as aryloxypropionic acids, aryloxyphenoxypropionic acids and an aminopropionic acid derivative (flamprop), by employing vancomycin as the chiral selector. Due to the strong UV absorption of the chiral additive at low wavelength, we used the partial filling method that allowed to keep the detector path free of absorbing vancomycin. The above method was very effective because the chiral selector moved in the opposite direction of the detector, being positively charged at the pH values used (pH 5–7), and with the electroosmotic flow absent or very low.

The concentration of chiral selector, the capillary temperature and the pH of the BGE strongly influenced the enantiomeric resolution and the selectivity of the separation of chiral herbicides. The increase of pH and capillary temperature caused a reduction of both selectivity and resolution which, on

Table 2
Intra- and inter-day precision data for haloxyfop free acid enantiomeric separation

Haloxyfop free acid	t_{m1} (min)	t_{m2} (min)	R (3.7)	A_1	A_2	A_{T1}	A_{T2}	A_{T1}/A_{T2}
Intra-day STD % data, $n=5$	0.50	0.59	1.46	1.32	1.67	0.95	1.18	0.55
Inter-day STD % data, $n=5$ (4 days)	0.67	0.70	4.09	3.06	2.93	2.58	3.01	0.41

Six mM of vancomycin, partial filling method, 34.475 kPa×30 s. Other experimental conditions as in Table 1.

the contrary, increased by increasing the vancomycin concentration. The best results were achieved with 6 mM vancomycin, pH 5, at 25°C.

The optimized method was tested for the qualitative analysis of a soil extract treated with haloxyfop ethoxyethyl ester where the herbicide was hydrolyzed to the acidic metabolite haloxyfop. Very good precision of the method was measured for both migration time and corrected peak areas. The detection limit (LOD) were as low as 5×10^{-7} M for each enantiomer.

With regard to the aforementioned results, the partial filling method with vancomycin can be advantageously used for the analysis of herbicides in environmental analysis. The method is rapid and cheap compared to other ones, e.g., HPLC, GC, where expensive chiral stationary phases or derivatizations are necessary.

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References

[1] F. Kobor, G. Schomburg, J. High Resolut. Chromatogr. 16 (1993) 693.
[2] C.P. Granville, B. Gehrcke, W.A. Konig, I.W. Wainer, J. Chromatogr. 622 (1993) 21.
[3] M. Jung, S. Mayer, V. Schurig, LC·GC-Mag. Sep. Sci. 12 (1994) 458.
[4] D.W. Armstrong, J.R.J. Faulkner, S.M. Han, J. Chromatogr. 452 (1988) 323.
[5] J. Debowski, D. Sybilska, J. Jurczak, J. Chromatogr. 282 (1983) 83.
[6] T.J. Ward, D.W. Armstrong, J. Liq. Chromatogr. 9 (1986) 407.
[7] G. Blaschke, J. Liq. Chromatogr. 9 (1986) 341.
[8] J. Snopek, I. Jelinek, E. Smolkova-Keulemansova, J. Chromatogr. 438 (1988) 211.
[9] A. Guttman, A. Paulus, A.S. Cohen, N. Grinberg, B.L. Karger, J. Chromatogr. 448 (1988) 41.
[10] S. Terabe, Trends Anal. Chem. 8 (1989) 129.
[11] S. Fanali, J. Chromatogr. 474 (1989) 441.
[12] S. Fanali, P. Bocek, Electrophoresis 11 (1990) 757.
[13] T. Schmitt, H. Engelhardt, Chromatographia 37 (1993) 475.
[14] B. Chankvetadze, G. Endresz, G. Blaschke, Electrophoresis 15 (1994) 804.
[15] S. Fanali, E. Camera, Chromatographia 43 (1996) 247.
[16] S. Terabe, K. Otsuka, H. Nishi, J. Chromatogr. A 666 (1994) 295.
[17] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[18] S. Mayer, V. Schurig, J. High Resolut. Chromatogr. 15 (1992) 129.
[19] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684.
[20] A. Wiliams, Pestic. Sci. 46 (1996) 3.
[21] J. Crosby, Pestic. Sci. 46 (1996) 11.
[22] G.M.R. Tombo, D. Bellus, Angew. Chem. 30 (1991) 1193.
[23] D.W. Bewick, Pestic. Sci. 17 (1986) 349.
[24] B. Blessington, N. Crabb, J. Chromatogr. 483 (1989) 349.
[25] W.A. Koning, D. Ichien, T. Runge, B. Pfaffenberg, P. Luduring, H. Huhnerfuss, J. High Resolut. Chromatogr. 14 (1991) 530.
[26] P. Ludwig, W. Gunkel, H. Hunerfuss, Chemosphere 24 (1992) 1423.
[27] M.M. Kirkland, J. Chromatogr. A 718 (1996) 9.
[28] M. Muller, H.R. Buser, Environ. Sci. Technol. 29 (1995) 2031.
[29] P. Padiglioni, C.M. Polcaro, S. Marchese, M. Sinibaldi, M. Flieger, J. Chromatogr. A 756 (1996) 119.
[30] M.W.F. Nielen, J. Chromatogr. 637 (1993) 81.
[31] M.W.F. Nielen, Trends Anal. Chem. 12 (1993) 345.
[32] D.W. Armstrong, K.L. Rundlett, J. Liq. Chromatogr. 18 (1995) 3659.
[33] A.W. Garrison, P. Schmitt, D. Martens, A. Kettrup, Environ. Sci. Technol. 30 (1996) 2449.
[34] Y.Z. Hsieh, H.Y. Huang, J. Chromatogr. A 745 (1996) 217.
[35] C. Desiderio, C.M. Polcaro, S. Fanali, Electrophoresis 18 (1997) 227.
[36] Y. Mechref, Z. El Rassi, Anal. Chem. 68 (1996) 1771.
[37] V.A. Tett, A.J. Willietts, H.M. Lappin-Scott, FEMS Microbiol. Ecol. 14 (1994) 191.

[38] W. Schutzner, G. Caponecchi, S. Fanali, A. Rizzi, E. Kenndler, Electrophoresis 15 (1994) 769.
[39] S.R. Day, K. Schelle, M.J. Mirbach, E. Zietz, Brighton Crop Protection Conference—Weeds, 1993, pp. 803–808.
[40] S. Fanali, C. Desiderio, J. High Resolut. Chromatogr. 19 (1996) 322.
[41] D.W. Armstrong, K.L. Rundlett, J.R. Chen, Chirality 6 (1994) 496.
[42] R. Vespalec, H.A.H. Billiet, J. Frank, P. Bocek, Electrophoresis 17 (1996) 1214.
[43] R. Vespalec, H. Corstjens, H.A.H. Billiet, J. Frank, K.C.A.M. Luyben, Anal. Chem. 67 (1995) 3223.
[44] M.P. Gasper, A. Berthod, U.B. Nair, D.W. Armstrong, Anal. Chem. 68 (1996) 2501.
[45] K.D. Altria, D.M. Goodall, M.M. Rogan, Chromatographia 34 (1992) 19.
[46] W. Schutzner, S. Fanali, Electrophoresis 13 (1992) 687.