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Capillary electrophoretic separation of herbicidal enantiomers applying ergot alkaloids

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Abstract

The capillary electrophoretic separation of some herbicidal enantiomers is shown applying 1-allylterguride as chiral selector. Baseline separation is shown for the enantiomers of fluazifop, halossifop and fenoxaprop, whereas the optical isomers of flamprop could be partially resolved. Separation times are short compared to similar analyses, applying HPLC and a terguride chiral stationary phase. The degree of dissociation of the acidic analytes, as well as the amount of methanol present in the background electrolyte, are shown to have a major influence on enantioresolution, as expected form earlier studies. © 1997 Elsevier Science B.V.

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1. Introduction

The introduction of automated equipment for capillary zone electrophoresis (CZE), applying fused-silica capillaries, in the early 1980s has strongly increased the number of applications of CZE. Chiral analysis has become one of the main areas of interest, resulting in some detailed reviews, listing many applications and hundreds of references [1–3]

Chloro-2-phenoxypropionic (Cl-APA) and halogen substituted 2-aryloxyphenoxy-propionic (APPA) acids, as well as N-benzoyl-N-(3-chloro-4-fluro-phenyl)amino-propionic acid (flamprop) are (structurally related to) herbicides. These compounds have a stereocenter in position 2 of the propionic acid functional group. It has been shown that the (*R*)-(−)-isomers of Cl-APAs and APPAs, and the (*R*)-(+)-isomer of flamprop exhibit the strongest herbicidal activity [4–6]. However, both optical isomers of these compounds are toxic [7], and their use should therefore be minimized. Consequently, recent legislation in several European countries has resulted in the marketing of pure enantiomers. Analytical methods are needed in order to determine the optical purity of these formulations. Liquid chromatography, using a Pirkle-type chiral stationary phase (CSP) can be applied for the above purpose [5,7]. The capillary electrophoretic separation of phenoxy acid herbicide enantiomers, applying α-CD and DIME-β as chiral selector, was shown by Nielen [8]. Recently, Padiglioni et al. showed the enantioseparation of some herbicides applying a CSP derived from terguride [9].

Earlier, this terguride CSP showed high selec-

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tivities for the enantiomers of several organic acids [10]. In recent studies [11,12] we have shown the applicability of terguride and structurally related compounds, as chiral selectors in CE. In this study, CE using the 1-allyl derivative of (5*R*,8*S*,10*R*)-terguride (allyl-TER) as chiral selector was applied for the chiral separation of some herbicidal compounds. The same analytes as those used in the HPLC study [9] were chosen in order to make a fair comparison between both separation techniques.

2. Experimental

A P/ACE 2200 (Beckman, Fullerton, CA, USA) was used for all electrophoretic experiments. The instrument used uncoated and polyacrylamide coated capillaries [13] of 37 cm, with an effective length of 30 cm and an I.D. of 50 μm. The UV detector was operated at 230 nm. The applied voltage was 20 kV or 30 kV. The capillary cartridge was thermostated at 25°C.

β-Alanine and acetic acid were purchased from Merck (Darmstadt, Germany). Allyl-TER was synthesized by a previously published method [14]. Fluazifop (2-(4-{[5-(trifluoromethyl)-2-pyridinyl]oxy}-phenoxy)propionic acid), halossifop (2-(4-{[3-chloro- 5 -(trifluoromethyl)- 2 -pyridinyl]oxy}phenoxy)propionic acid), fenoxaprop (2-[4-(6-chloro-2-benzoxazolyl)oxy]phenoxypropionic acid) and flamprop (N-benzoyl-N-(3-chloro-4 fluorophenyl)-DL-alanine were donated by the Istituto di Cromatografia (CNR, Rome, Italy). The structure of these compounds is shown in Fig. 1.

All samples were dissolved in MeOH–H_2O (1:5) to a concentration of 10^{-4} *M* and injected hydrodynamically (5 s, $3 \cdot 10^3$ Pa). The BGE was prepared by adjusting a 200 m*M* β-alanine solution with acetic acid to pH 4.0. Subsequently, 1 part of this electrolyte solution was diluted with 1 part of MeOH. This resulted in a BGE consisting of 100 m*M* β-alanine–acetate, 50% MeOH (pH 5.3).

3. Results and discussion

According to the literature, a phenoxy substituent at the α-position of propionic acid decreases the pK_a value of the analyte from 4.9 to 3.1 [15]. Therefore, it can be assumed that fluazifop, halossifop, and fenoxaprop have a high degree of dissociation, whereas flamprop is assumed to have a relatively low degree of dissociation, at the selected pH value. This is confirmed by the migration behavior, where no chiral selector was added to the BGE. The phenoxy substituted analytes pass the detection window well within 9 min in the order of their molecular masses (M_r) (1st fluazifop; M_r=327, 2nd fenoxaprop; M_r= 333.5, 3rd halossifop; M_r=361.5), whereas flamprop (M_r=321.5) passes the detection window after approximately 12 min.

In order to separate the optical isomers of the

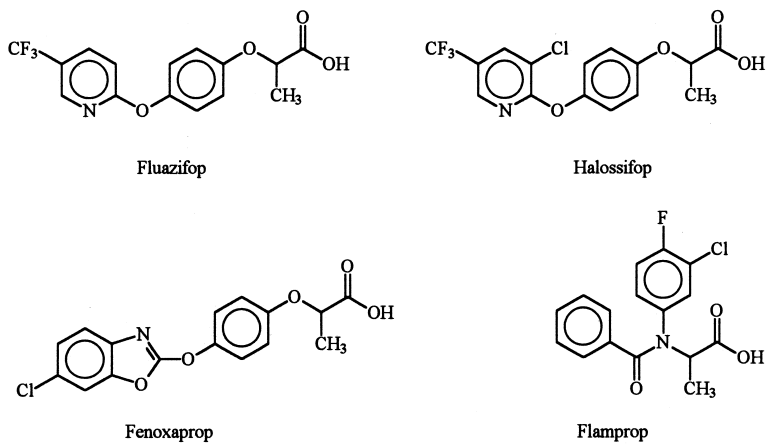

Fig. 1. Chemical structures of the examined herbicides.

herbicidal analytes, the buffer was supported with 25 m*M* allyl-TER, which resulted in a slight increase of the pH of the BGE. Before injecting the racemic analytes, the capillary was rinsed with BGE containing allyl-TER. The in- and outlet consisted of pure BGE, without chiral selector. The boundary between the zones with and without allyl-TER has self-sharpening properties, following the Kohlrausch regulation function. The properties of this boundary have been extensively discussed elsewhere [12,16]. In the electropherogram shown in Fig. 2, the boundary, which is migrating in the direction of the anode, passes the detection window after approximately 2.5 min.

An electropherogram of the separation of the optical isomers of the herbicidal compounds is shown in Fig. 2. The enantiomers of the phenoxy substituted propionic acids are well separated in approximately 13 min. No resolution was observed for the flamprop enantiomers. A possible explanation of the limited enantioselectivity of allyl-TER towards flamprop is the lower degree of dissociation of this compound. In recent studies, it is shown that only the dissociated acids interact stereoselective with the chiral selector [12,17].

An impurity, originating from the fenoxaprop sample, is visible as two small peaks after approximately 8 min. The impurity seems to be a racemate since only one small peak is visible without the addition of the chiral selector to the BGE. It is possibly a degradation product of fenoxaprop: 2-(4′-hydroxyphenoxy)propanoic acid.

Partial resolution of flamprop enantiomers can be obtained by increasing the concentration of the chiral selector or by increasing the degree of dissociation of the analyte. Therefore, a BGE was applied consisting of pure MeOH containing 100 m*M* acetic acid and 50 m*M* triethanolamine (TEA), supported with 100 m*M* allyl-TER. Approximately the first 28 cm of an uncoated capillary were filled with BGE containing the chiral selector. The rest of the capillary, including the in- and the outlet vial contained 100 m*M* acetate and 50 m*M* TEA in 100% MeOH. No migration of the boundary between the zones with and without allyl-TER was observed under these experimental conditions. Apparently, the electrophoretic mobility of the ergot alkaloid is largely compensated by the residual electroosmotic flow. The latter was reversed due to the presence of TEA in the BGE. The resulting electropherogram, applying 20 kV, is shown in Fig. 3. Partial resolution is obtained for the enantiomers of flamprop (R_s=0.7), whereas high resolutions were obtained for the optical isomers of the other compounds.

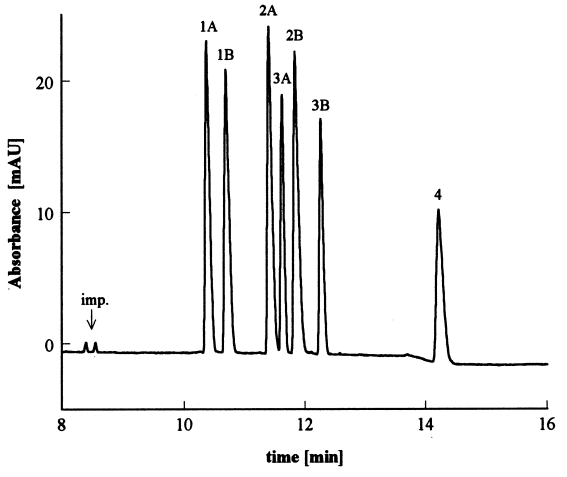

Fig. 2. Electropherogram of the chiral separation of some herbicidal compounds. 1A, 1B=fluazifop; 2A, 2B=halossifop; 3A, 3B=fenoxaprop; 4=flamprop; imp.=impurity. BGE: 100 m*M* β-alanine–acetate, 50% MeOH (pH 5.3) supported with 25 m*M* allyl-TER. Separation voltage 30 kV. Coated capillary: 30–37 cm×50 μm I.D.

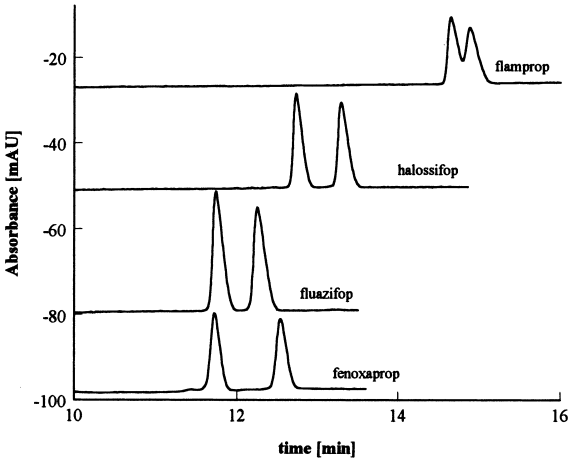

Fig. 3. Electropherogram of the chiral separation of some herbicidal compounds. BGE: 100 m*M* acetate, 50 m*M* TEA in 100% MeOH supported with 100 m*M* allyl-TER. Separation voltage 20 kV. Uncoated capillary: 30–37 cm×50 μm I.D.

Similar resolutions as shown in Fig. 3 could be obtained applying HPLC [9]. The selectivities obtained in the HPLC experiments (as defined by k_1'/k_2') however, were much higher than those obtained in the CE experiments (as defined by the ratio of the effective mobilities of the optical isomers). Equal resolutions must be explained by the much higher efficiencies, usually obtained in CE. The separation time in CE is shorter than in HPLC; e.g., separation of the phenoxy substituted enantiomers takes approximately 90 min, using the terguride packing in HPLC whereas only 15 min are needed applying CE with allyl-TER as chiral buffer additive (see Fig. 3).

In this study it is shown that CE can be successfully applied for the separation of the herbicidal optical isomers. The method can be useful for the analysis of real production samples and the determination of their enantiopurity.

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