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Enantiomeric separations of halogen-substituted 2-aryloxypropionic acids by high-performance liquid chromatography on a terguride-based chiral stationary phase

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Abstract

A number of racemic halogen-substituted 2-aryloxypropionic acids, most of which have not yet been resolved, were examined by HPLC on an ergot alkaloid-based chiral stationary phase (CSP). Baseline enantiomeric separations were obtained with a selectivity factor (α) within the range 1.06–1.7, thus showing the suitability of this CSP for resolving these compounds in optical purity control of formulations, in stereochemical studies as well as in transformation processes in soil and vegetable tissues. Furthermore, a semipreparative-scale separation of 2-(2,4,5-trichlorophenoxy)propionic acid (Fenoprop) enantiomers was carried out on a 250×7.8 mm I.D. column, yielding approximately 1.0 mg of each enantiomer in a single chromatographic run, with a recovery of 88% and optical purity greater than 99%.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Terguride; Aryloxypropionic acids; Halogenated compounds; Pesticides

1. Introduction

Chloro-2-phenoxypropionic (Cl-APAs) and halogen-substituted 2-aryloxyphenoxypropionic (APPAs) acids, as well as N-benzoyl-N-(3-chloro-4-fluorophenyl) aminopropionic acid (Flamprop) are herbicides or structurally related compounds which are produced in hundreds of thousands of tons annually [1]. Cl-APAs are generally used as free acids, whereas halogen-substituted APPAs are commercialized as herbicide esters. It has been shown that the ester derivatives undergo fast hydrolysis in the presence of vegetable tissues and soil bacteria, yielding the corresponding free acid, which is assumed to possess biological activity [2,3]. APAs and APPAs have a center of asymmetry at the carbon atom in position 2 of the substituted propionic acid, and it has been demonstrated that one of the enantiomers exhibits the strongest herbicidal activity: (−)-(*R*)-isomers represent the active forms of chlorophenoxy- and aryloxypropionic acids [1,4,5], whereas the active form of Flamprop is the (+)-(*R*)-isomer [4]. Fluazifop, (*R,S*)-butyl 2-(-5-trifluoromethyl-2-pyridyloxyphenoxy) acid butyl ester, undergoes stereoselective interconversion during the hydrolysis, giving a product in which the (*R*)-enantiomer is predominant [5].

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Different toxicities have been ascribed to the optical isomers of chlorophenoxy propionic acids [6]. Consequently, recent legislation in several European countries has led to the marketing of enantiomerically pure formulations, the (+)-(*R*)-isomers [7]. Flamprop isopropyl, Halossifop methyl and Quizalofop butyl esters are also produced in an optically pure form in the (*R*)-configuration [8].

The pressure to restrict the environmental diffusion of herbicides without compromising their application in agriculture and to obtain a deeper insight into their metabolic fate, has created demand for enantioselective analytical and preparative-scale methods suitable for optical purity control of formulations and for the study of metabolites and/or reaction intermediates.

Several chromatographic procedures, involving the separation of chlorophenoxy propionates by gas chromatography on a modified cyclodextrin column and of ester and amide derivatives by high-performance liquid chromatography (HPLC) on a Pirkle-type chiral stationary phase (CSP) [4,6,9], or by HPLC using a ODS column eluted with a chiral mobile phase containing L-prolyl-*n*-octylamide-Ni(II) [10], or the separation of free acid on an α-cid glycoprotein-based [1,9] and on a π–π electron acceptor–donor containing groups CSPs [11] have been reported. Chlorophenoxypropionic acids were recently separated by capillary zone electrophoresis using cyclodextrins in an electrolyte [7,12,13].

The resolution of APPA esters on a cellulose-type CSP has recently been described [14]. On the other hand, with the exception of Fluazifop, which was resolved by an indirect method involving the use of L-prolyl-N-octylamine as a chiral modifier in the eluent on a reversed-phase column [5,10], no other enantiomeric separation of aryloxyphenoxypropionic acids has been reported. This has led us to examine a series of racemic structurally different herbicide acids and free acids from herbicide esters using a novel CSP derived from the ergot alkaloid (+)-(5*R*,8*S*,10*R*)-terguride, which in previous studies showed high selectivity for several carboxylic group-containing organic acids [15]. Semipreparative-scale separations of Fenoprop enantiomers are also reported.

2. Experimental

2.1. Instrumentation

Chromatography was performed using modular instrumentation consisting of a Series 400 Model (Perkin-Elmer, Norwalk, CT, USA) solvent delivery pump equipped with a Rheodyne 7125 Model injection valve provided with 10- or 500-μl injection loop, a 2550 Model (Varian, Walnut Creek, CA, USA) variable-wavelength detector, and an SPD-M6A Model (Shimadzu, Kyoto, Japan) photodiode array UV–Vis detector equipped with a computer data acquisition system. Chromatograms were recorded by a Chromatopac CR3A Model (Shimadzu, Kyoto, Japan) integrator.

2.2. Methods

Analytical and semipreparative-scale separations were carried out on columns 150×4.6 and 250×7.8 mm I.D. packed with CSPs derived from terguride (5 and 10 μm particle size silica-based), the synthesis of which is described in detail elsewhere [16] (Fig. 1).

Semipreparative-scale separations were carried out by injecting samples of a 4 mg/ml solution of Fenoprop in methanol. The parameter mass saturation capacity (w_s) was determined in order to check the loading performance of the CSP, following the method described by Snyder et al. [17]:

$$w_s = (2N)^{1/2}[(w_x)k''/(1 + k')]k_0''/(k_0'' - k'')$$

where N is the efficiency at high load, w_x is the injected amount of racemic mixture in mg, k' is the capacity factor at a load w_x and k'_0 is the capacity factor obtained under linear conditions (about 50 μg sample injected). The w_s values were calculated based on injections averaging of 0.6 and 2.0 mg of racemate. The mobile phase was 0.02 *M* potassium acetate buffer (pH 3.5)–acetonitrile (1:1, v/v) solution, which was delivered to the column at a flow-rate of 2.0 ml/min. The efficiency was determined by the half height equation.

The recovery yield related to the preparative separations was also considered. In order to de-

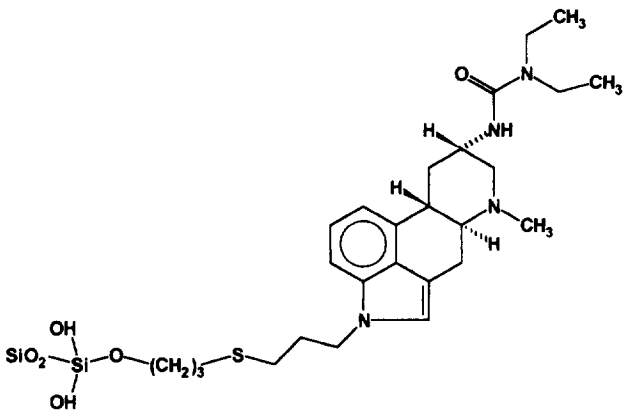

Fig. 1. Structure of terguride bonded to silica gel.

termine this parameter with accuracy, the elution profiles of the racemic mixtures were converted by direct calibration of the detector response to concentration profiles. Accordingly, fractions of the eluate from the column were collected at 30 s intervals; a measure of these fractions was then reinjected under linear conditions for quantitative analysis. At the wavelength used, i.e. 230 nm, the calibration curves were linear and the regressions were performed using a standard procedure. This method permitted an exact determination of the concentration of each enantiomer. The fractions obtained from replicate injections were acidified with aqueous 20% sulphuric acid, extracted with chloroform, and the organic phase was removed under vacuum. A sample of the first eluted pure enantiomer (1.2 mg) was dissolved in methanol and submitted to polarimetric analysis using a 541 Model (Perkin-Elmer) spectropolarimeter. The corresponding specific optical rotation was $[\alpha]^{21} = +83$ (Hg_{365}, $c =$ 0.12 g/100 ml, $T = 22°C$).

2.3. Materials

All solvents were of HPLC grade and were obtained from Merck (Darmstadt, Germany). Other chemicals, all of analytical grade, were purchased from Carlo Erba (Milan, Italy).

Phosphate and acetate buffers were prepared by adding potassium hydroxide to the acid, monitoring the pH by means of a Crison 2000 micro pH meter, then filtering the mixtures through Millipore (Bedford, MA, USA) type GS (0.22 μm) filter-disks.

Racemic herbicides (Fig. 2) were purchased as follows: Mecoprop (**1a**), 2-(4-chloro-2-methylphenoxy)propionic acid; Dichlorprop (**1b**), 2-(2,4-dichlorophenoxy)propionic acid; Fenoprop (**1c**), 2-(2,4,5-trichlorophenoxy)propionic acid; Fluazifop (**2a**), 2-(4-{[5-(trifluoromethyl)-2-pyridinyl]oxy}-phenoxy)propionic acid; Halossifop (**2b**), 2-(4-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy}phenoxy) propionic acid; Flamprop (**3a**), N-benzoyl-N-(3-chloro-4-fluorophenyl)-DL-alanine and Quizalofop-ethyl, ethyl 2-{4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy}-propionate were purchased from LabService (Milan, Italy). Diclofop (**2c**), 2-[4-(2,4-dichlorophenoxy)-phenoxy]propionic acid, and Fenoxaprop (**2d**), 2-[4-(6-chloro-2-benzoxazolyl)oxy]phenoxy-propionic acids were kindly provided by AgrEvo (Frankfurt am Main, Germany).

Quizalofop-ethyl ester was hydrolysed in 0.1 *M* aqueous sodium hydroxide at room temperature for 48 h, until the ester layer of the suspension disappeared. The alkaline mixture was acidified with aqueous 20% sulphuric acid (v/v), then the corresponding free acid (**2e**) was extracted with ethyl acetate and dried under vacuum.

Proton NMR spectra of the samples dissolved in $C^2H_3COC^2H_3$ ([$^2H_6$]acetone) were recorded by a Bruker AMX 600 spectrometer operating at 600.13 MHz.

(±)-(*R,S*)-Quizalofop-ethyl ester, ethyl 2-[4-(6-

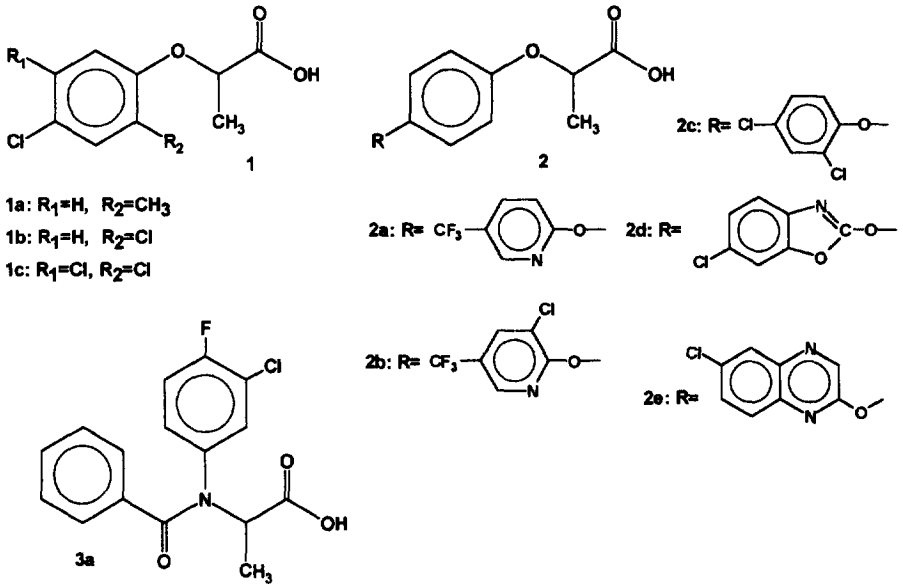

Fig. 2. Structures of the examined compounds.

chloro-2-quinoxalinyloxy)phenoxy]propionate: δ: 8.80 (s, 1H), 8.09 (d, 1H, J=2.16 Hz), 7.77 (d, 1H, J=8.94, 2.28 Hz), 7.72 (d, 1H, J=8.88 Hz), 7.30 (d, 1H, J=8.93 Hz), 7.30 (d, 1H, J=8.93 Hz), 7.05 (d, 1H, J=8.93 Hz), 7.05 (d, 1H, J=8.93 Hz), 4.93 (q, 1H, J=6.72 Hz), 4.25 (m, 2H), 1.63 (d, 3H, J=6.72 Hz), 1.28 (t, 3H, J=7.08 Hz).

(±)(*R,S*)-Quizalofop, 2-[4-(6-chloro-2-quinoxalinyloxy)phenoxy]propionic acid (±**2e**) δ: 8.67 (m, 1H), 8.02 (d, 1H, J=2.6 Hz), 7.67 (d, 1H, J=8.0 Hz), 7.67 (d, 1H, J=8.0 Hz), 7.14 (d, 1H, J=8.2 Hz), 7.14 (d, 1H, J=8.2 Hz), 7.00 (d, 1H, J=8.2 Hz), 7.00 (d, 1H, J=8.2 Hz), 4.53 (q, 1H, J=6.3 Hz), 1.51 (d, 3H, J=6.3 Hz), COOH (not detected).

3. Results and discussion

The organic modifier content (%), the capacity factor of the more retained enantiomer (k'_2) and the selectivity factor (α) of a number of halogen-substituted 2-aryloxy-(**1a–1c**), 2-aryloxyphenoxy-(**2a–2d**) propionic acids, and Flamprop (**3a**) (Fig. 2) are summarized in Table 1. The terguride-based CSP was found to provide baseline resolution for all the examined compounds and, to a lesser extent, for Flamprop.

Chromatographic data show that the selectivity strongly depends on the polarity of the chiral radical in α position to the carboxylic group; the higher the hydrophobicity of the moiety, the better the resolution of the racemate. These findings are in good agreement with the assumptions made on the chiral recognition operating by ergot alkaloid-based CSPs [18]. In fact, studies of diastereoisomeric complexes formed between terguride and 2-arylpropionic acid

Table 1
Chromatographic data of herbicide acids (Fig. 1)

Compound	k'_2	α[a]	Mobile phase (%AcN)
1a	10.1	1.20	60
1b	11.9	1.32	60
1c[b]	9.0	1.35	70
2a	24.2	1.11	50
2b	8.30	1.65	55
2c	21.6	1.06	50
2d	15.2	1.15	50
2e	9.55	1.21	60
3a	10.0	1.09	55

Chromatographic conditions: packing, terguride-based CSP; column, 150×4.6 mm I.D.; eluent, 0.02 *M* potassium acetate buffer (pH 3.5)–acetonitrile (AcN); flow-rate, 0.8 ml/min; UV detector, 254 nm; room temperature.

[a] α, Selectivity factor (k'_2/k'_1).

[b] R_s=6.45, determined using the equation: $t_2-t_1/(w^1_{0.5}+w^2_{0.5})$.

enantiomers showed that the enantiodiscrimination by the selector is partially governed by π–π interactions, implying high selectivity toward strong π electron donators.

This effect is shown in Fig. 3, which gives the resolutions of three Cl-APAs. A 30% increase of the selectivity factor is observed by comparing the resolution of mono- (**1a**, $\alpha = 1.20$) up to trichloro- (**1c**, $\alpha = 1.35$) derivatives. An opposing trend, i.e. a decreasing selectivity corresponding to an increasing number of substituted chlorine atoms in the phenoxy moiety, has been observed on packings derived from α-glycoproteins [9].

It should be noted that groups in the *ortho* position of solutes **1a–1c** seem to be the determinants of resolution, since they resemble a molecular structure which favours stabilizing interactions with the ergoline skeleton. On the other hand, the ether bond linking the phenoxy-substituent to the aryloxypropionic acids determines an opposite effect of "opening" the molecular structure with the result of decreasing the selectivity. This effect is evidenced by comparing the behaviour of solutes **1b** and **2c**.

As with 2-aryloxypropionic acids, halogen-substituted 2-phenoxy-aryloxypropionic acids that possess strong electron acceptor–donor functions are also well resolved by terguride. In this case the indole ring of the selector produces again strong stabilizing

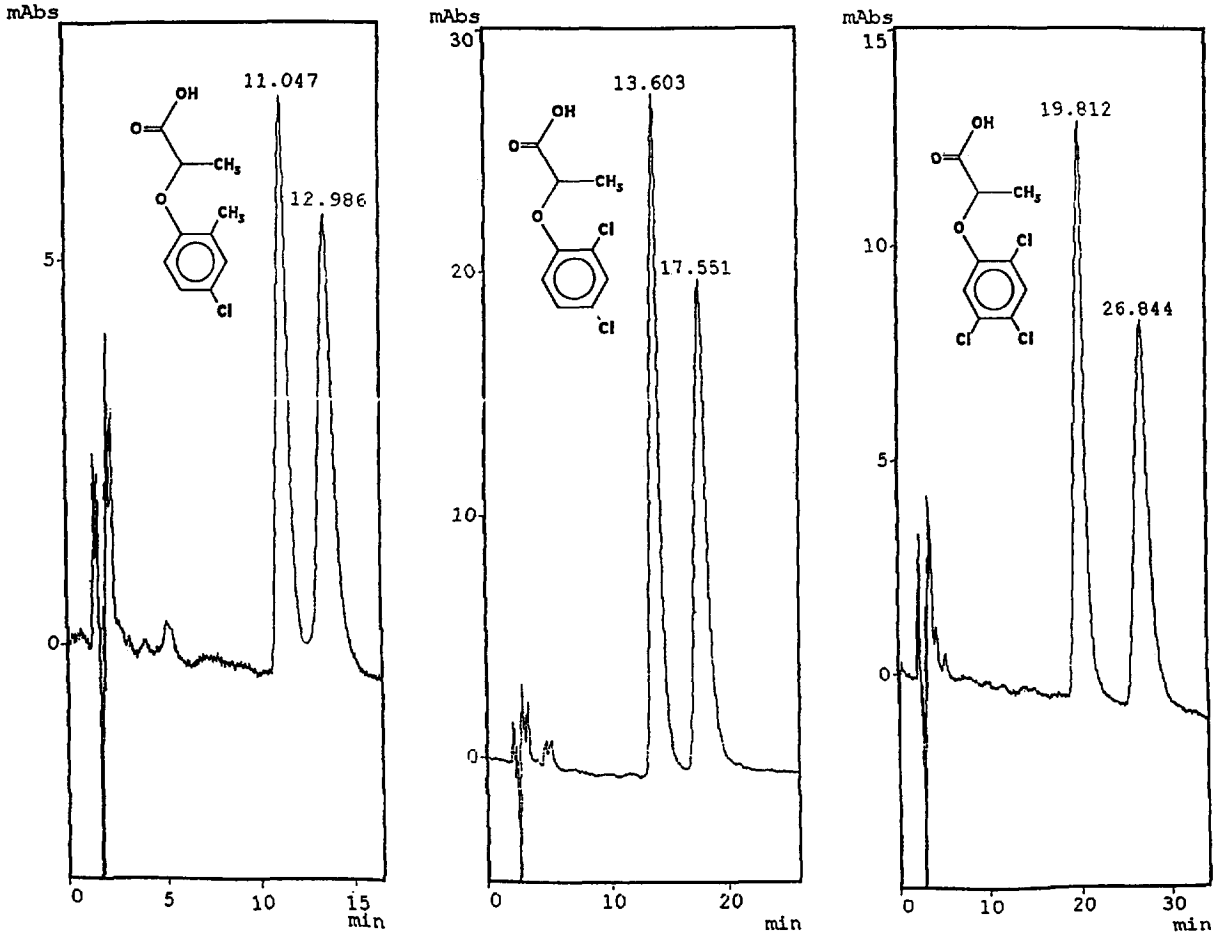

Fig. 3. Enantiomeric separation of Mecoprop (**1a**), Dichlorprop (**1b**) and Fenoprop (**1c**) on terguride-based CSP. Chromatographic conditions: column size, 150×4.2 mm I.D.; eluent, 0.02 *M* potassium acetate buffer (pH 3.5)–acetonitrile (1:1, v/v); flow-rate, 0.8 ml/min; detector UV, 254 nm. Room temperature.

interactions with the CF_3-groups or quinoxalin rings of the solutes. This explains the high selectivity found for Quizalofop (**2e**, $\alpha = 1.21$), Halossifop (**2b**, $\alpha = 1.65$) and, to a lesser extent, for Fluazifop (**2a**, $\alpha = 1.11$). Fig. 4 shows the chromatographic resolution of Halossifop enantiomers. In order to identify the resolved peaks of Halossifop as the corresponding optical isomers, the column was connected to a photodiode array detector and the eluate monitored between 320–200 nm.

Solutes **2a** and **2b** contain a pirydil ring, the protonation of which, at the pH of the buffer used (3.5), increases the molecule polarity, reflecting a decrease in retention. By comparing the selectivity data of these compounds, the presence of a chlorine atom in **2b** increases the α value by a factor of 1.5.

However, the higher resolution generally found for 2-substituted aryloxypropionic acids as compared to the previously studied profens [19] cannot be explained only in terms of $\pi-\pi$ interactions. An additional interaction, possibly hydrogen bonding, probably occurs to stabilize the overlapping of nonpolar moieties between the solutes and the selector. In this respect, structural investigations of the diastereoisomeric adducts are currently in progress.

In order to obtain milligram amounts of both pure enantiomers suitable for stereochemical studies, we developed a semipreparative scale separation of racemic Fenoprop (**1c**) using a column of 250×7.8 mm I.D. The first eluted enantiomer exhibited a positive specific rotation of +88° (see Section 2). On the basis of structural investigations of the chiral

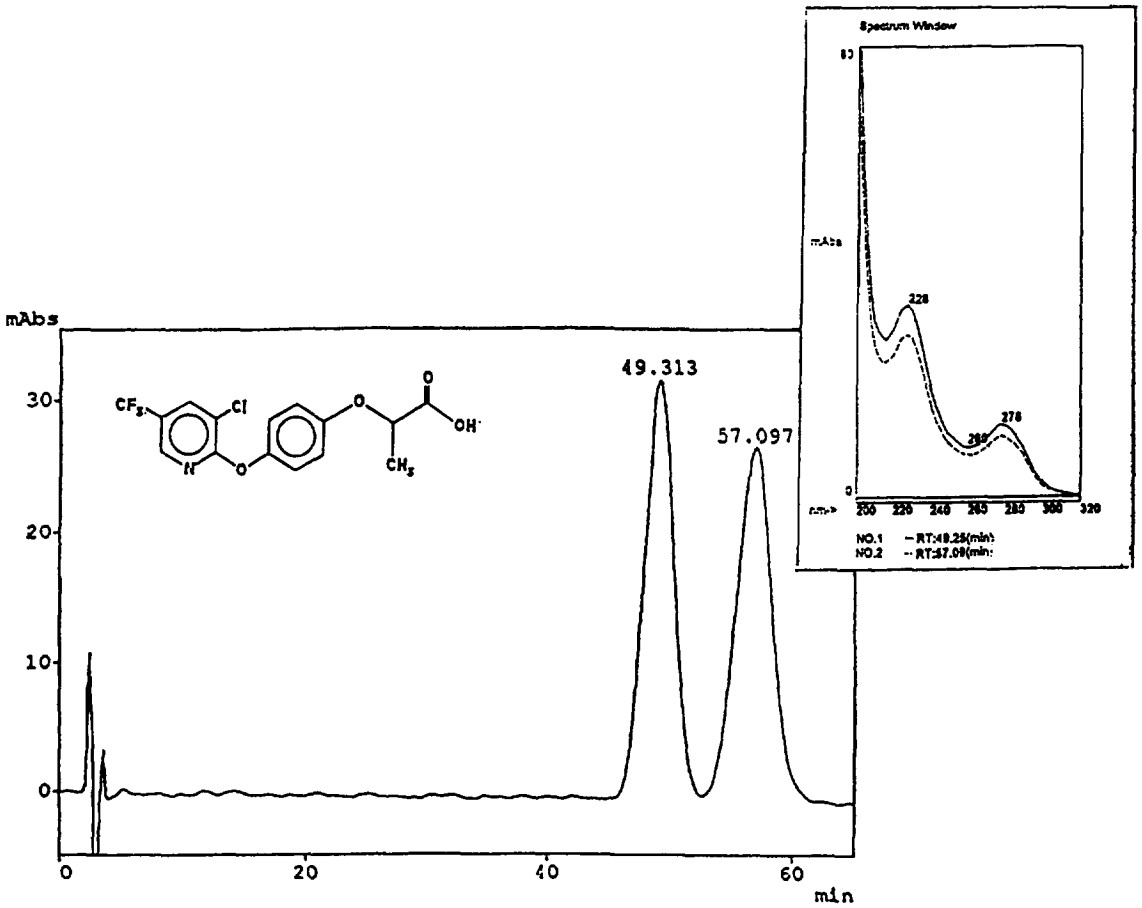

Fig. 4. Enantiomeric separation of Halossifop (**2b**): eluent, 0.02 *M* potassium acetate buffer (pH 3.5)–acetonitrile (45:55, v/v); other conditions as in Fig. 2.

discrimination mechanism [20] and by analogy with the elution sequence previously observed for profen enantiomers, e.g. (−)-(*S*) more retained than (+)-(*R*), (+)**1c** was assigned the (*R*)-configuration. This assignment is also in accordance with data in the literature [1].

Fig. 5 shows that the packing derived from 10 μm particle size silica gel provides a nearly baseline separation of about 2 mg of racemate (**1c**) injected in a single run, whereas at greater amounts a loss of resolution and column overload were observed as a consequence of the non-linear effects and competition for adsorption between molecules of the two enantiomers. As expected, owing to the displacement effect, the front of the first eluted isomer and the rear of the more retained one elute earlier than the times observed under linear conditions [21]. An enrichment of the peak maxima due to the displacement conditions of the chromatographic run was found by Jacobson et al. [22] to be useful, in terms of recovery yield, for the preparative separation of a racemic mixture.

The pure enantiomer production (in mg), the corresponding optical purity and the recovery yield (%) are reported in Table 2. The amount of each enantiomer was calculated by discarding the mixed

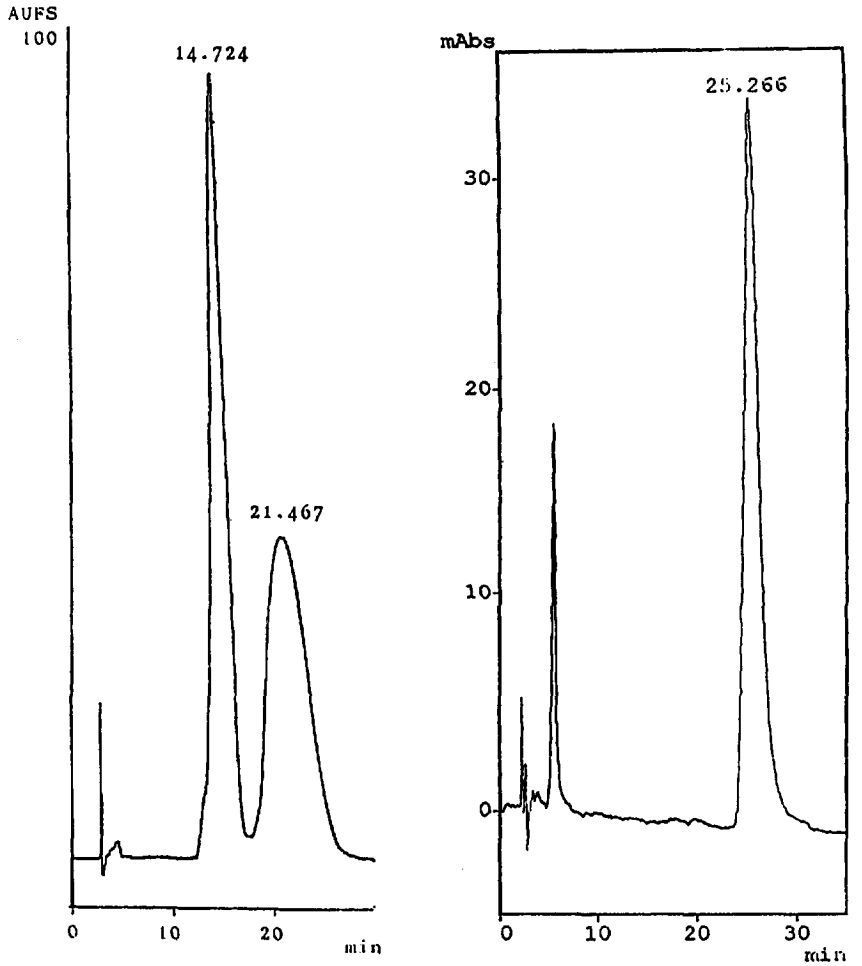

Fig. 5. (left) Semipreparative-scale separation of Fenoprop (**1c**) enantiomers. Sample size, 1.0 mg in 200 μl methanol. Chromatographic conditions: column, 250×7.8 mm I.D.; flowrate, 2.0 ml/min; eluent, 0.05 *M* potassium phosphate buffer (pH 3.5)–acetonitrile (4:6, v/v); detector UV, 254 nm; room temperature. (right) Optical purity control of the collected fraction corresponding to the more retained enantiomer. Column size, 150×4.6 mm I.D.; flow-rate, 0.8 ml/min; other conditions, as in the semi-preparative separation.

Table 2
Chromatographic data of Fenoprop (±**1c**) semi-preparative separations on a terguride-based CSP

Resolved enantiomer	Cut time (min)	Amount of pure enantiomer (mg)	Recovery yield (%)	Enantiomeric purity (%)
(*R*)*		0.48	97.8	>99
	1.7			
(*S*)		0.49	97.6	>99
(*R*)**		0.88	88.1	>99
	2.3			
(*S*)		0.87	88.0	>99

Chromatographic conditions: 10 μm particle size silica gel; column size, 250×7.8 mm I.D.; eluent, 0.05 *M* potassium phosphate (pH 3.5)–acetonitrile (4:6, v/v); flow-rate, 2.0 ml/min; UV detector, 230 nm. *1-mg and **2-mg sample size injections.

fractions, i.e. the fractions collected between cut times, defined as the stop point for collection of fractions of the first purified isomer (*R*) and the starting point for collection of the second isomer (*S*). The differences between these cut times are reported in Table 2. The yields corresponding to the injections of 1 and 2 mg of racemate were more than 98 and 88%, respectively. In both cases the optical purity was greater than 99%.

The higher efficiency of terguride-based packings in preparative scale separations, as compared to the α-glycoprotein-based CSPs [7] that have been extensively used for the resolution for these acidic compounds, relates to the grafting type of their active surface. Accordingly, the loadability of the CSP was also examined. This parameter was determined on the basis of the mass saturation capacity (w_s), following the two-injection method described by Snyder [17]. This procedure requires the knowledge of the capacity factor of an analytical and preparative load and the efficiency of the high load peak; these data and the actual mass load were then entered into the w_s equation (see Section 2). The resulting w_s value for the packing is reported in Table 3. As a reference, a typical C_{18}–100 Å packing has a w_s of approximately 500–700 mg/column. It has to be taken into account that this approach does not consider the influence of physical and chemical (for example ligand density) properties of the packing on the loading capacity.

Table 3
Capacity factor, efficiency and mass saturation capacity (w_s) data related to 1 mg sample size

Packing	k'_0	k'	Load (mg) efficiency	w_s (mg)
Terguride-CSP	5.05	4.40	474	208

Solute, (±)**1c**. Other chromatographic conditions as in Table 2.

In conclusion, the suitability of CSPs derived from terguride for the direct resolution of free acids of herbicides and for predicting the conformation of the resolved enantiomers has been demonstrated. Semi-preparative-scale resolution showed that appropriate packed columns could be conveniently used for the production of enantiomers in amounts suitable for standards (1–3 mg) or for biological and toxicological testing (20–30 mg).

Acknowledgments

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