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One- and Two-Dimensional Direct Chiral Liquid Chromatographic Determination of Mixtures of Diclofop-Acid and Diclofop-Methyl Herbicides

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Simple one- and two-dimensional high-performance liquid chromatography (HPLC) methods for the simultaneous enantiomeric determination of alkyloxyphenoxypropionic acid herbicides is presented. Compounds studied were (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid (diclofop-acid) and (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)]methyl propionate (diclofop-methyl). Mobile phases necessary to separate their enantiomers on an α_1-acid glycoprotein chiral stationary phase are different; therefore, the simultaneous separation by an isocratic mode is not possible. The chiral separation method proposed involves a one-step gradient allowing for the simultaneous determination of both racemic enantiomers. Detection limits of the method were 0.03 mg/L for both diclofop-acid enantiomers and 0.14 and 0.15 mg/L for diclofop-methyl enantiomers, respectively. The two-dimensional method involves the use of two chromatographs in one achiral-chiral coupling. The LC−LC method is more suitable for complex samples because it involves an online cleanup effect. Detection limits were 1.25 and 1.87 mg/L for diclofop-acid and 2.70 and 3.02 mg/L for diclofop-methyl enantiomers, respectively. Accuracy, repeatability, and reproducibility have been studied in standard samples and a technical product.

KEYWORDS: Diclofop; chiral analysis; two-dimensional HPLC; column switching; chiral herbicides

1. INTRODUCTION

At present, about 25% of the agrochemicals used in the world are chiral compounds, which are usually applied as racemic mixtures, but in general, only one of the enantiomers is significantly more active than the other and produces the desired effect. That is because, although enantiomers have identical physical and chemical properties, their behavior in biological systems could be completely different, because biological transformation of chiral compounds in animals, plants, and microorganisms can be stereoselective (*1, 2*). Despite the importance of this enantiomer behavior, only a small fraction of pesticides are manufactured and used as pure enantiomers (*3, 4*). In particular, (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid (diclofop-acid) and (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)]methyl propionate (diclofop-methyl) (**Figure 1**) are organochlorine herbicides that present chirality; they are fatty acid synthesis inhibitors that destroy the cell membrane, prevent the translocation of assimilates to roots, reduce the chlorophyll content, inhibit photosynthesis, and have meristem activity. The diclofop-methyl (*R*) enantiomer shows significantly greater herbicidal activity than the (*S*) enantiomer (*5*); therefore, to reduce the amount of herbicides used and prevent unnecessary enantiomer use causing some adverse impact, several European countries have suggested that only the active enantiomer should be employed. Under alkaline conditions, diclofop-methyl rapidly hydrolyzes into diclofop-acid, which has higher solubility in water and lower acute toxicity than its parent compound. Consequently, there is an urgent need to develop analytical methods to determine the optical purity, stereoselective bioactivity, and environmental behavior of these chiral pesticides.

Thus, several analytical methods have been used to control the enantiomeric purity of herbicides formulations, including

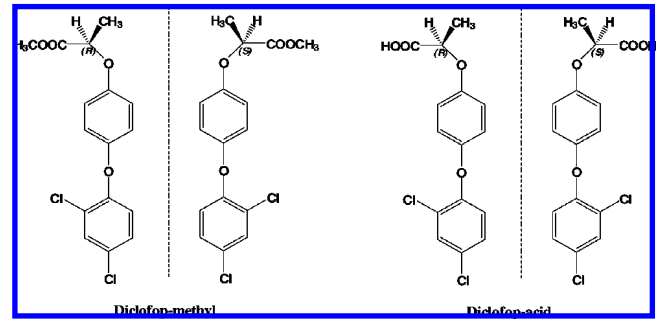

Figure 1. Chemical structures of the two diclofop herbicide forms.

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capillary electrophoresis, immunoassays, and biosensors (*6–9*). Nowadays, chromatographic and electromigration methods seem to be the most popular techniques applied in this field. Direct chiral high-performance liquid chromatography (HPLC) methods are generally preferred over gas chromatography (GC) ones (*10*) for nonvolatile compounds, because HPLC can be used without derivatization (*2, 11*). Several chiral stationary phases have been used to separate chiral pesticides; for example, the two enantiomers of malathion have been resolved by mixed-mode electrokinetic capillary chromatography (*12*) with a Chiralcel OJ chiral stationary phase (*13*); diclofop-methyl enantiomers have been separated on a chiral stationary phase based on cellulose tris(4-methylbenzoate) (*14*) or on permethylated β-cyclodextrin HPLC (*13*); chiral stationary phases based on terguride (*15*) and on Pirkle ionic or α_1-acid glycoprotein (*16, 17*) have been used for chiral HPLC of phenoxy acid herbicides mixtures but not for diclofop herbicides. The advantages of protein-based chiral stationary phases, such as α-acid glycoprotein (AGP), generally include the use of aqueous mobile phases, enantioselectivity for a wide range of compounds, and direct analysis (*18*), although in some cases, it can not discriminate between acid and ester forms simultaneously (*19*). However, some drawbacks still remain when the analyte is present in more complex samples containing other chiral or achiral compounds as well as endogenous matter that can coelute with the analyte peaks. In general, the chiral stationary phase shows selectivity for the separation of the enantiomer pair, but it is less selective for other achiral compounds present in the sample. These problems could be overcome by two-dimensional HPLC, where two columns are linked together via a switching valve in a manner such that any component flowing through the first column can be directed into a second column in which further resolution can be obtained. When all of the components from the first column (primary column) are transferred to the second column, the process is named comprehensive two-dimensional chromatography (also LC×LC); however, if the sample is fractionated and only a group of peaks is transferred to the second column, the process is known as "linear" or "heart cut" two-dimensional chromatography (LC−LC mode) (*20, 21*). The advantages of this technique include increasing selectivity because two different columns are used, as well as an online cleanup of the sample. This strategy is useful to resolve complex mixtures when enantiomers are involved (*22–24*). In particular, the online cleanup involved in LC−LC prevents the degradation of the chiral stationary phases, maintaining the column performance for longer times.

The purpose of the present work is to explore the ability of the AGP chiral stationary phase for the enantiomeric separation of the acid and ester forms of the diclofop herbicide, setting methods for their simultaneous quantitative and enantiomeric analysis. In this approach, the optimum chemical and experimental conditions to achieve the enantiomeric separation to determine enantiomeric ratios ($ER_{1/2}$) of the two diclofop forms were optimized both by one- and two-dimensional liquid chromatography using experimental design. The analysis of diclofop-acid and diclofop-methyl by the two-dimensional HPLC method has been carried out in a LC−LC system consisting of a reverse-phase ODS column switched to an α_1-acid glycoprotein protein chiral column. The main attention is paid to the compatibilization of the mobile phases of these two columns.

2. EXPERIMENTAL PROCEDURES

2.1. Reagents and Standards. All reagents and solvents were of analytical reagent grade. HPLC-grade methanol and 2-propanol were supplied by Scharlab (Barcelona, Spain), and purified water was obtained from a Milli-Q system from Millipore (Bedford, MA). Herbicide standards were of a purity between 96.5 and 99%; (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid (CAS number [40843-25-2]) (diclofop-acid) and (*R*,*S*)-2-[4-(2,4-dichlorophenoxy)]methyl propionate (diclofop-methyl) (CAS number [51338-27-3]) were supplied by Ehrensturfer Quality (Augsburg, Germany) as racemic mixtures. Stock solutions were prepared by dissolving 5 mg of each herbicide in 25 mL of methanol (200 mg/L). These solutions were stored at 4 °C in the dark for 3 months maximum. Working standard solutions were prepared in the respective mobile phase by diluting the stock solutions as required. To prevent the influence of the possible pesticide degradation on the results, the working solutions were prepared daily.

2.2. Commercial Sample. The commercial formulation SIROFOP [concentrated emulsifiable liquid that nominally contains 36% (w/v) of diclofop-methyl] was supplied by Proplant-Plant Protection Company S.L. (Madrid, Spain). SIROFOP is a commercial postemergence herbicide containing diclofop-methyl in a complex matrix formed by an organic solvent, which is not specified by the maker, and an emulsifiable liquid, which acts against wild oats, wild millets, and other annual grass weeds in wheat, barley, beet, and other crops.

2.3. Instrumentation. Two analytical HPLC systems were used to carry out experiments in one or two dimensions. The achiral chromatographic separation of both racemates was made using a 150 × 4.60 mm C_{18}-Luna column (5 μm) (Phenomenex, Torrance, CA) in a HPLC chromatograph (system A), consisting of an injection valve with a 20 μL sample loop (Rheodyne, Cotati, CA), a one-channel pump Varian Pro Star Solvent Module (Varian, Palo Alto, CA), and a Waters Variable Wavelength 481 detector (Waters Corporation, Milford, MA), both interfaced to a computer that contains Peak Simple Workstation Software (version 1.99) for Windows for chromatographic data processing. Chiral separation of the enantiomers of diclofop-acid and diclofop-methyl enantiomers were carried out in a HPLC chromatograph (system B) that includes a four-channel-gradient pump Jasco PV-2089 Plus Solvent Module (Jasco, Tokyo, Japan); one of the four channels is used to deliver the mobile phase suitable for the enantiomeric separation of the acid form of diclofop, and another one is used to supply the mobile phase to separate the methyl form. The injector employed was a manual Rheodyne valve, model 7010, with a 20 μL sample loop (Rheodyne, Cotati, CA). A Jasco programmable variable wavelength UV-2075 Plus detector (Jasco, Tokyo, Japan) was used, and all components were interfaced to a PC data station with the Borwin Workstation Software (version 1.50) for Windows (Jasco). A α_1-acid glycoprotein, Chiral-AGP column (100 × 30 mm, 5 μm), was used for the chiral separation (Chrom-Tech, Cheshire, U.K.). The two-dimensional LC−LC method was developed by switching system A to system B by means of a Laboratory Pro Rheodyne six-port valve (Rheodyne, Cotati, CA) (**Figure 2**).

2.4. General Procedures. *2.4.1. One-Dimensional Achiral and Direct Chiral HPLC Determination of Diclofop-Acid and Diclofop-Methyl.* Achiral separations of samples containing diclofop-acid and diclofop-methyl racemate mixtures were carried out in system A by injecting 20 μL of sample on the C_{18}-LUNA. An isocratic mobile phase containing 27% phosphate buffer (30 mM, pH 7) and 73% MeOH at a flow rate of 1.0 mL/min was used.

The two herbicide racemics were analyzed by chiral HPLC in system B using the AGP-Chiral column at a flow rate of 0.8 mL/min. A volume of 20 μL of diclofop-acid and diclofop-methyl standard solutions was injected into the HPLC system. For the isocratic elution of diclofop-acid enantiomers, the aqueous component of the mobile phase was phosphate buffer (70 mM, pH 7) and the organic modifier was 2-propanol (0.5%), whereas the chiral separation of the diclofop-methyl enantiomers was carried out using a phosphate buffer (30 mM, pH 7) modified with 2-propanol (9%) as the mobile phase. The pump program for the simultaneous enantiomeric determination of both herbicides started with the optimized mobile phase for the acid form (100%

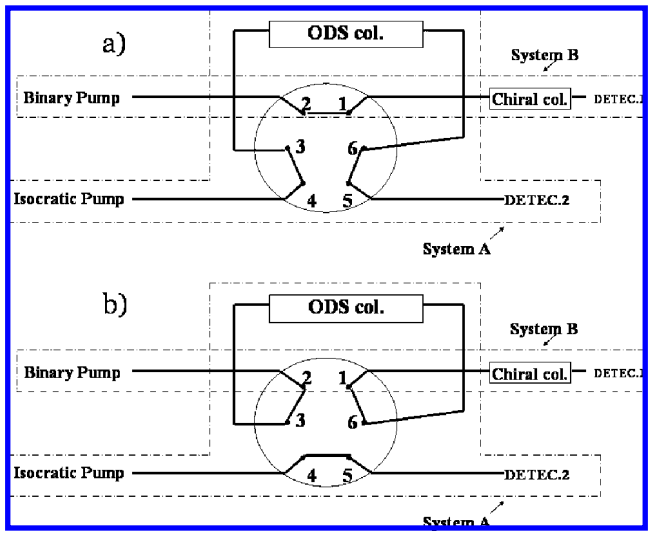

Figure 2. Switching scheme of the two-dimensional HPLC system: (a) position 1 and (b) position 2.

channel A) and changing to the mobile phase optimized for the methyl form at minute 14 (100% channel B). After 70 min from the injection, the pump changes again to channel A to equilibrate the system for a new injection (around 20 min).

The UV detection wavelength in both chiral and achiral analysis was made at 230 nm.

2.4.2. Chiral Separation of Diclofop-Acid and Diclofop-Methyl by "Heart Cut" LC−LC Chromatography. Once the chiral column on system B has been conditioned with the optimum mobile phase for the enantiomeric separation of the acid form, a volume of 20 μL of the sample mixture containing diclofop-acid and diclofop-methyl was injected into the primary column (C_{18}-LUNA) in system A, which has been previously conditioned with the optimum mobile phase for the achiral separation, keeping the switching valve in position 1. When diclofop-acid begins to be eluted from the primary column, a portion of this analyte is transferred to the chiral column (system B) by changing the switching valve to position 2 for 15 s. Afterward, the valve is switched back to its initial position, allowing the system to be ready for the subsequent diclofop-methyl transference. Meanwhile, after the diclofop-acid enantiomers have been eluted from the AGP-chiral column, this column is conditioned with the mobile phase used for the chiral separation of diclofop-methyl by changing the solvent module of system B to the appropriate channel. When this herbicide begins to elute from the primary column, the switching valve is changed again to position 2 for 38 s, transferring the diclofop-methyl portion to the secondary column, in which their enantiomers are separated. Finally, the valve is switched to position 1, allowing for the next sample injection. The primary chromatographic separation was carried out at a flow rate of 1.0 mL/min, and enantiomeric separation was carried out at a flow rate of 0.8 mL/min. **Table 1** summarizes the overall LC−LC procedure, showing the switch valve positions for each operation (**Figure 2**).

2.4.3. Sample Preparation of a Technical Product (SIROFOP). For racemic quantitation, 1 mL of SIROFOP was dissolved in 100 mL of methanol, obtaining an intermediate diluted sample of the technical product. Injection samples were prepared by diluting 0.25 mL of this intermediate solution into 10 mL of the respective mobile phase; these samples contain about 90 mg/L of diclofop-methyl. For determination of diclofop-acid and diclofop-methyl $ER_{1/2}$ values by simultaneous one-dimensional chiral chromatography, working samples were prepared by diluting as required the intermediate sample into 10 mL of the diclofop-acid mobile phase. For the same determination by two-dimensional chromatography, working samples were prepared by diluting 3 mL of the above intermediate sample solution into 10 mL of the achiral separation mobile phase. In all cases, volumes of 20 μL of these working solutions were injected.

Table 1. Operational Condition and Switch Valve Position in the LC−LC Determination of Diclofop-Acid and Diclofop-Methyl

time	pump channel (system B)	valve position	event
−30 to 0 min (approximate)	A	1	AGP-chiral column equilibration for diclofop-acid analysis
0	A	1	sample injection in system A
minute 6.90	A	2	diclofop-acid transference
after 15 s	A	1	end of the transference
minute 14.00	B	1	AGP-chiral column equilibration for diclofop-methyl analysis
minute 27.00	B	2	diclofop-ethyl transference
after 38 s	B	1	end of the transference
minute 70	A	1	AGP-chiral column equilibration for a new analysis

3. RESULTS AND DISCUSSION

3.1. Optimization of Chiral Separation Conditions of Racemic Standards. Resolution factor, Rs, was used to evaluate the separation quality in this study. Several preliminary experiments to establish best conditions for the enantiomeric analysis of diclofop-acid and diclofop-methyl on the AGP-chiral column were carried out, including the effect of the nature and concentration of the organic modifier, pH, and buffer concentration. These studies showed that using 2-propanol as organic modifier in aqueous solutions of phosphate buffer around pH 7 allows us to obtain peak resolutions close to 1; therefore, to achieve the best conditions for enantiomeric separation of both herbicides, a systematic study was carried out using this modifier. Optimal separation conditions were determined from the response surface plots after response modeling.

For diclofop-acid, an experimental design was used to optimize the factors that can affect the enantiomeric resolution, and 27 experiments on the basis of a factorial design for three levels and three factors were performed. The three parameters studied were pH, buffer concentration, and organic modifier (2-propanol) ratio. The ranges studied were 6–7 for pH, 40–100 mM for the concentration of the buffer solution, and 0.5–1.5% for the ratio of organic modifier. The Rs calculated from each of these experiments were then correlated with the above-mentioned factors by means of the general second-order equation, which takes into account the linear and quadratic factor effects on the resolution and the effect of the interactions between them

$$\mathrm{Rs} = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1^2 + a_8x_2^2 + a_9x_3^2 \quad (1)$$

where x_1 represents the 2-propanol ratio (%) in the mobile phase, x_2 is the millimolar concentration of the phosphate buffer, and x_3 is the pH. **Table 2** shows the parameter values obtained for each compound as well as the p values. As can be seen, a mathematical model with a correlation factor (R^2) of 0.992 and a standard error of estimation (SEE) of 0.04 for the diclofop-acid was obtained. The SEE value is a quality parameter of the mathematical model adjustment that shows the deviation of the residuals. This correlated model allows us to predict both the resolution of the enantiomeric pair under any selected experimental conditions and the experimental conditions necessary to get a predetermined resolution. Thus, an acceptable resolution of 1.4 is expected when the chiral separation is carried out on an AGP-chiral column with a 70 mM phosphate buffer at pH 7 containing 0.5% of 2-propanol as an organic modifier. Under these conditions, the first enantiomer elutes at a retention time of 8.50 min and the second one elutes at 11.16 min.

Table 2. Adjusted Parameters Obtained for the Two Diclofop Forms[a]

compound	a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9	R^2	SEE[b]
diclofop-acid	8.374	3.433	−0.019	−3.437	−0.0003	−0.570	0.0044	−0.018	−0.00005	0.342	0.992	0.04
	(0.008)	(0.001)	(0.004)	(0.001)	(0.670)	(0.001)	(0.001)	(0.784)	(0.018)	(0.010)		
diclofop-methyl	−10.48	0.067	0.023	3.346	−0.0002	−0.056	−0.0012	−0.0003	−0.00015	0.220	0.987	0.06
	(0.164)	(0.432)	(0.620)	(0.170)	(0.715)	(0.073)	(0.793)	(0.905)	(0.723)	(0.227)		

[a] *p* values (in parentheses). [b] SEE = standard error of estimation.

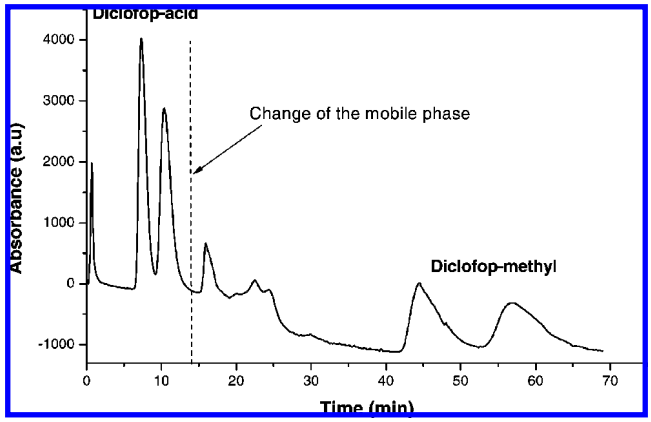

Figure 3. Simultaneous chiral separation of diclofop-acid and diclofop-methyl using a one-step-gradient program. Chromatographic conditions are mentioned in section 2.4.

The separation of diclofop-methyl enantiomers was optimized in a similar way. In this case, the ranges of values studied were 6–7 for pH, 30–50 mM for the concentration of the phosphate buffer, and 9–17% for the 2-propanol ratio. As in the case of diclofop-acid, **Table 2** shows the parameter values that adjust experimental data to eq 1. Now, R^2 and SEE were 0.987 and 0.06, respectively. It was observed that a 30 mM phosphate buffer (pH 7) containing 9% of 2-propanol as an organic modifier was one of the most suitable mobile phases. Under these conditions, the first enantiomer elutes at a retention time of 21.37 min and the second one elutes at 23.08 min; the resolution obtained was 1.2.

3.2. Simultaneous One-Dimensional Analysis of Diclofop-Acid and Diclofop-Methyl for Racemic Standards. As has been mentioned in the previous section, enantiomeric separation of diclofop-acid and diclofop-methyl requires different mobile phases; therefore, chiral chromatographic separation of both herbicides cannot be performed simultaneously by the isocratic mode. In the optimized conditions for diclofop-methyl, diclofop-acid is eluted quickly and its enantiomers cannot be separated, while in the optimized conditions for diclofop-acid, diclofop-methyl is not eluted even after 60 min of analysis. Because the simultaneous enantiomeric determination of both racemics was not possible using a single mobile phase, different elution gradients were tried. Better results were found by a one-step gradient, changing the optimum conditions for diclofop-acid to the optimum conditions for diclofop-methyl. **Figure 3** shows the chromatogram obtained when the mobile phase used for chiral separation of diclofop-acid was changed at minute 14 to the suitable mobile phase for the separation of the enantiomers of the methyl form. As can be seen, it is possible to separate the enantiomers of both racemics. The analytical characteristics of the method were determined. Calibrations for diclofop-acid and diclofop-methyl showed a good linearity in the concentration range investigated, between 50 and 0.5 mg/L, and correlation coefficients for all of the peak areas were in the range of 0.9964–0.9996. **Table 3** shows the detection and quantitation limits as well as the reproducibility for the one-step-gradient chromatographic method. The enantiomeric ratio ($ER_{1/2}$), when the concentrations are unknown, can be expressed as the integrated area of the first enantiomer eluted divided by the integrated area of the second enantiomer peak (*4*). This term was calculated for 4 mg/L of each racemic standard and nine replicates, and as can be seen in **Table 3**, the method is quite reproducible, with standard deviations for the calculated $ER_{1/2}$ of 0.02 and 0.01 for diclofop-acid and diclofop-methyl, respectively.

Table 3. Analytical Characteristics for Standards Obtained by One-Dimensional Chiral Chromatography

			reproducibility	
herbicide	detection limits[a] (mg L^{-1})	quantitation limits[a] (mg L^{-1})	concentration[a,b] (mg L^{-1})	enantiomeric ratio[a,b] ($ER_{1/2}$)
diclofop-acid	0.03/0.03	0.10/0.12	1.87 (0.02)/1.82 (0.02)	1.03 (0.02)
diclofop-methyl	0.14/0.15	0.45/0.50	1.88 (0.02)/1.80 (0.02)	1.04 (0.01)

[a] First enantiomer eluted/second enantiomer eluted. [b] Calculated for 4.0 mg/L of each racemic standard and nine replicates. The standard deviations (SDs) are in parentheses.

3.3. Chiral Analysis of Diclofop-Acid and Diclofop-Methyl by "Heart Cut" LC−LC in Standards. As indicated in the Introduction, LC−LC can specially prevent deterioration of the chiral column. To overcome the low capacity of protein-based chiral stationary phases, two-dimensional HPLC should be recommended.

For this purpose, two chromatographs were interconnected by means of a switching valve, according to the scheme in **Figure 2** and descriptions given in section 2.3. To carry out chiral analysis of diclofop-methyl and diclofop-acid in the same sample, an optimization of the following chemical and operational parameters, as well as other aspects, was required.

(a) Mobile-phase compatibility between the columns: To connect two columns in a two-dimensional HPLC system, the most important requirement is mobile-phase compatibility. Because chiral separations of the diclofop-acid and diclofop-methyl enantiomers are carried out in aqueous mobile phases, the primary separation must be performed in a polar medium. Consequently, a C_{18} (ODS, primary column) column was selected for the separation under reversed-phase conditions, and several mobile phases were tested. The mobile phases studied contained methanol as an organic component and a phosphate buffer (30 mM/pH 7) as an aqueous component. This composition allowed for the adjustment of the 2-propanol ratio used as a modifier in the secondary column. It was concluded that there are no incompatibilities between mobile phases.

(b) Separation time between peaks in the primary column: The elution time interval between the two racemics in the primary column must be long enough to allow for the complete elution of the enantiomer pair of diclofop acid and the stabilization of the chiral column for the chiral separation of diclofop-methyl. To achieve this, different ratios of methanol/phosphate buffer (30 mM/pH 7) as the mobile phase in the primary column were tested; the range of values studied was

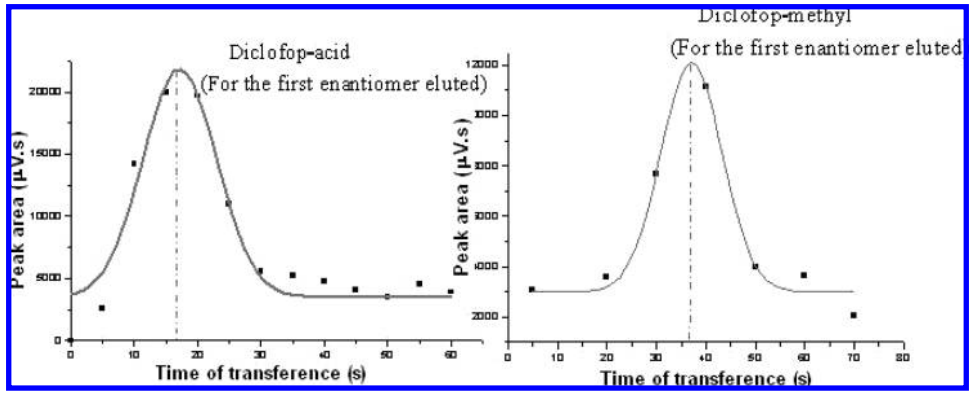

Figure 4. Plots of enantiomer peak area versus transference time. The flow rate was 0.8 mL/min, and detection was at 230 nm.

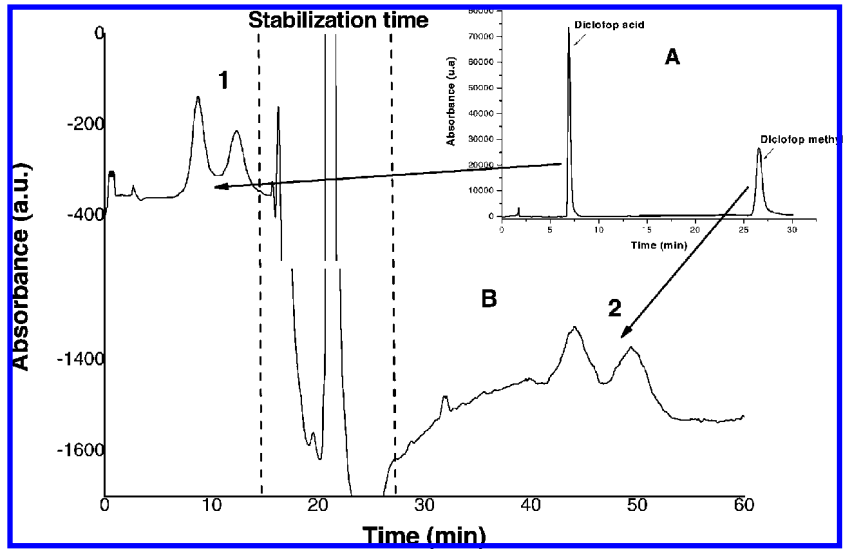

Figure 5. LC−LC chromatograms from achiral−chiral coupling: (1) enantiomeric separation of 20 mg/L diclofop-acid and (2) enantiomeric separation of 20 mg/L diclofop-methyl. Chromatographic conditions were specified in section 2.4.

70–80% for the organic component. From this study, it was concluded that the best conditions must be 73% methanol/27% phosphate buffer (30 mM/pH 7). Under these conditions, diclofop-acid is eluted at a retention time of 6.90 min and diclofop-methyl is eluted at 29.60 min.

(c) Transference time from the primary column to the secondary column: This time obviously controls the amount of analyte transferred from the primary column to the chiral column. To achieve the highest sensitivity, it is necessary to establish the optimum transference time. Different elution volumes from the primary column were transferred to the chiral column by switching the valve to apply different transference times starting from 5 s and ending with times similar to peak width. The time transference was optimized by plotting the integrated enantiomer peak areas versus transference time and fitting the curve to a Gaussian function (**Figure 4**). The maximum of this Gaussian was taken as the optimum transference time, which was 15 s for diclofop-acid and 38 s for diclofop-methyl. When these operational and chemical aspects were taken into account, the two-dimensional HPLC method was optimized. **Figure 5** shows the two-dimensional separation of a mixture containing the two diclofop herbicides. As can be seen, a good enantiomeric separation is obtained (**Figure 5**B) in both cases, similar to the one predicted by the mathematical model; therefore, it can be concluded that the change from one- to two-dimensional modes does not affect the enantiomeric separation of diclofop-acid and diclofop-methyl herbicides.

Table 4. Analytical Characteristics for Standards Obtained by LC−LC

herbicide	detection limits[a] (mg L^{-1})	quantification limits[a] (mg L^{-1})	enantiomeric ratio[b] ($ER_{1/2}$)	linearity range (mg L^{-1})
diclofop-acid	1.25/1.87	4.16/6.25	1.1 (SD = 0.1)	0.25–5 (r = 0.996) 5–150 (r = 0.998)
diclofop-methyl	2.70/3.02	9.12/10.1	1.09 (SD = 0.09)	5–150 (r = 0.999)

[a] First enantiomer eluted/second enantiomer eluted. [b] Calculated for 20 mg/L of each racemic standard and five replicates.

Once all chemical and operational conditions have been optimized, detection limits and reproducibility of method were determined. Detection limits were calculated by injecting solutions containing progressively smaller amounts of the two racemics (50–2 mg/L for diclofop-acid and 50–5 mg/L for diclofop-methyl) until the response obtained in the secondary chromatograph had a peak height only 3 times larger than the average height of noise around the respective enantiomer. The accuracy and reproducibility of the method were evaluated in terms of the enantiomeric ratio ($ER_{1/2}$). It was calculated from 20 mg/L of each racemic standard and five replicates. The results are shown in **Table 4**, where it can be seen that the method is quite reproducible, with standard deviations for the calculated $ER_{1/2}$ of 0.1 and 0.09 for diclofop-acid and diclofop-methyl, respectively (**Table 4**), similar to those obtained by the one-dimensional LC method. As could be expected, the LC−LC method is less sensitive than the one-dimensional one, because

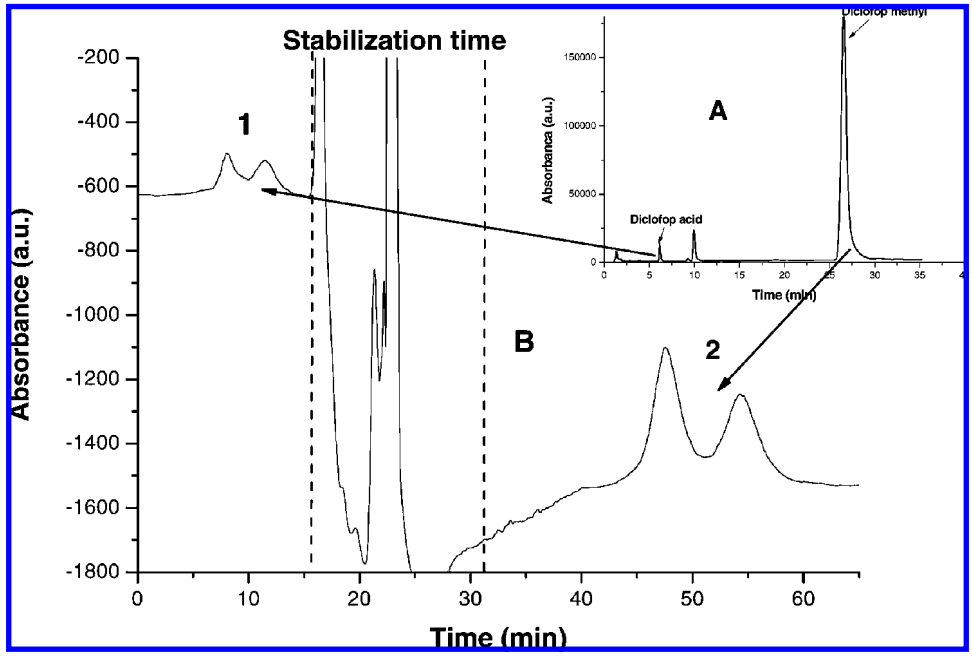

Figure 6. Chromatograms from LC−LC separation of SIROFOP components by achiral−chiral coupling. The chromatographic conditions are the same as mentioned in section 2.4.

Table 5. Analysis of SIROFOP by LC−LC

	diclofop-acid (%)[a]	diclofop-methyl (%)[b]
	Racemic Analysis (w/v)[a,c]	
	5.9 ± 0.6	41 ± 7
	Enantiomeric Analysis ($ER_{1/2}$)[b]	
first day	1.2 ± 0.4	1.2 ± 0.2
second day	0.9 ± 0.2	1.1 ± 0.3
third day	1.1 ± 0.2	0.9 ± 0.1
intraday	1.0 ± 0.4	1.0 ± 0.4

[a] A total of 0.25 mL of the intermediate solution [36% (w/v) diclofop-methyl as an active content]. [b] $n = 3$ replicates per day. [c] $n = 4$.

only a part of the peak is transferred each time and an additional dilution effect has to be taken into account. On the other hand, cleaner chromatograms and therefore more increasing precision are expected.

3.4. Determination of Chiral Herbicides in the Technical Product. The technical product SIROFOP is a complex matrix as indicated in section 2.2. The achiral chromatographic analysis using an ODS reverse-phase column of this product showed that, in fact, the main active component is the diclofop-methyl but that it also contains a small amount of diclofop-acid and other nonidentified compounds (**Figure 6A**). Using the primary chromatographic system (system A described in section 2.3), the quantitative composition of the two racemics herbicides was determined (**Table 5**). Moreover, previous attempts to analyze this product by means of the one-dimensional chiral chromatographic method showed the presence of an intense peak at 7 min, overlapping the diclofop-acid first enatiomer eluted, making it difficult to analyze this component of the sample. The chiral analysis showed $ER_{1/2}$ between 0.72 and 0.90, depending upon the aliquot sample taken, far from the 1.0 expected. However, the LC−LC method provides a cleaner chiral chromatogram than the one-dimensional method, allowing a more precise chiral analysis of the diclofop herbicides. Thus, enantiomeric ratios ($ER_{1/2}$) for diclofop-acid and diclofop-methyl were established using the two-dimensional system; also, the repeatability and reproducibility of the method, in day and intraday terms, were studied. The results obtained are listed in **Table 5**, and first- and second-dimension chromatograms are shown in **Figure 6**.

CONCLUSIONS

Although the α_1-acid glycoprotein chiral stationary phase requires different mobile phases for the enantiomeric separation of the two diclofop racemics, the simultaneous enantiomeric determination of both alkyloxyphenoxypropionic acid racemic herbicides by chiral HPLC is possible by means of one-step-gradient elution. When the sample is complex, the LC−LC method is more convenient for this determination. Besides the online cleanup effect, the method is practical and easy to perform and it allows a more accurate determination of the enantiomeric ratio ($ER_{1/2}$) for these herbicides.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; LC, liquid chromatography; ODS, octadecyl silane; AGP, α-acid glycoprotein; SEE, standard error of the estimation; SD, standard deviation; ER, enantiomeric ratio.

LITERATURE CITED

(1) Rosales-Conrado, N.; León-González, M. E.; D'Orazio, G.; Fanali, S. *J. Sep. Sci.* **2004**, *27* (15–16), 1303–1308.
(2) Wang, P.; Jiang, S.; Liu, D.; Zhang, H.; Zhou, Z. *J. Agric. Food. Chem.* **2006**, *54* (5), 1577–1583.
(3) Spencer, E. Y. *Guide to Chemicals Used in Crop Protection*, 7th ed.; Canadian Government Publishing Centre: Ottawa, Canada, 1981.
(4) Ali, I.; Aboul-Enein, H. Y. *Chiral Pollutants: Distribution, Toxicity and Analysis by Chromatography and Capillary Electrophoresis*; John Wiley and Sons, Ltd.: Chichester, U.K., 2004.
(5) *The Pesticide Manual*, 11th ed.; Tomlin, C. D. S., Ed.; British Crop Protection Council: Surrey, U.K., 1997.
(6) Menzinger, F.; Schmitt-Kopplin, Ph.; Freitag, D.; Kettrup, A. *J. Chromatogr., A* **2000**, *981* (1), 45–67.
(7) Desiderio, C.; Polcaro, C. M.; Padiglioni, P.; Fanali, S. *J. Chromatogr., A* **1997**, *781* (1–2), 503–513.

(8) Morimune, K.; Yamaguchi, Y.; Beppu, Y.; Miyake, S.; Takewaki, S.; Kawata, M.; Yuasa, Y. *Anal. Chim. Acta* **1998**, *376* (1), 37–40.
(9) Trajkovska, S.; Tosheska, K.; Aaron, J. J.; Spirovski, F.; Zdravkovski, Z. *Luminiscence* **2005**, *20* (3), 192–196.
(10) Liu, W.; Chem, Z.; Xu, H.; Shi, Y.; Chen, Y. *J. Chromatogr., A* **1991**, *547*, 509–515.
(11) Kallenborn, R.; Hühnerfuss, H. *Chiral Environmental Pollutants. Trace Analysis and Ecotoxicology*; Springer: Berlin, Germany, 2001.
(12) Anigbogu, V. C.; Woldeab, H.; Garrison, A. W.; Avants, J. K. *Int. J. Environ. Anal. Chem.* **2003**, *83* (2), 89–100.
(13) Weber, K.; Kreuzing, R.; Bahadir, M. *Chemosphere* **1997**, *35*, 13–20.
(14) Chen, P.; Na, P. J.; Han, X. Q.; Pan, C. P.; Hou, J. G.; Du, X. Z.; Gao, J. Z. *J. Instrum. Anal.* **2003**, *22*, 39–41.
(15) Padigliorni, P.; Polcado, C. M.; Marchese, S.; Sinibaldi, M.; Flieger, M. *J. Chromatog., A* **1996**, *756* (1–2), 119–127.
(16) Blessington, B. *J. Chromatogr.* **1987**, *396*, 177–182.
(17) Blessington, B.; Crabb, B. *J. Chromatogr.* **1988**, *454*, 450–454.
(18) Haginaka, J. *J. Chromatog., A* **2001**, *906* (1–2), 253–273.
(19) Félix, G. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *27* (2), 237–273.
(20) Mondello, L.; Lewis, A. C.; Bartle, K. D. *Multidimensional Chromatography*. John Wiley and Sons, Ltd.: New York, 2002.
(21) Schoenmakers, P.; Marriot, P.; Beens, J. *LC−GC Europe* **2003**, *16*, 335–339.
(22) Faraoni, M.; Messina, A.; Polcaro, C. M.; Aturki, Z.; Sinibaldi, M. *J. Liq. Chromatogr. Relat. Technol.* **2004**, *27* (6), 995–1012.
(23) Hutta, M.; Rybár, I.; Chalányová, M. *J Chromatogr., A* **2002**, *959* (1–2), 143–152.
(24) Cass, Q. B.; Oliveira, R. V.; De Pietro, A. C. *J. Agric. Food Chem.* **2004**, *52* (19), 5822–5827.

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