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ON ENANTIOSELECTIVE SEPARATION OF PHENOXYPROPIONATES USING PERMETHYLATED ß-CYCLODEXTRIN HPLC AND GC COLUMNS

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dedicated to the 60th birthday of Prof. Dr. W. Klein

Abstract

Investigations on chiral phenoxypropionates using permethylated ß-cyclodextrin HPLC and GC columns showed a decrease in enantioselective separation efficiency from mecoprop-methyl and dichlorprop-methyl to the threefold chlorinated fenoprop-methyl. This corresponded to decreasing electron density in the aromatic system due to the increasing negative inductive effect of 3 chlorine substituents. Investigation on methyl-(RS)-2-(2,4-dichloro-3,6-dinitrophenoxy)-propionate confirmed the influence of electrophilic substituents while determination of ethyl-(RS)-2-methoxypropionate emphasized the necessity of an aromatic system for enantioselective separation on ß-cyclodextrin stationary phases. For fenoprop-methyl as well as for the aryloxyphenoxypropionates diclofop-methyl and fluazifop-butyl, reversed phase HPLC showed higher separation performance than high resolution capillary GC. © 1997 Elsevier Science Ltd

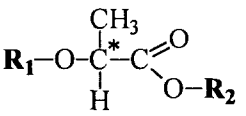
Key words: phenoxypropionates, enantioselective separation, ß-cyclodextrins, HPLC, GC

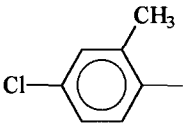
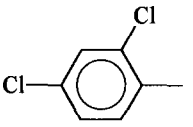
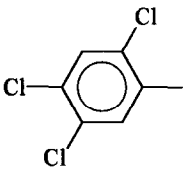
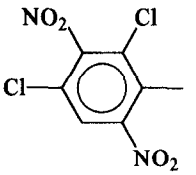
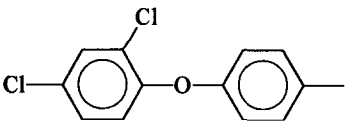
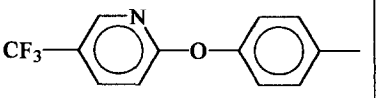
Introduction

Chiral phenoxypropionates are widely used herbicides and are rapidly hydrolyzed in soil to their corresponding acids which are the real active substances. Hydrolysis depending on soil moisture and supported by soil microbial activity occurs without changing the optical configuration of the enantiomers [1, 2]. In contrast, degradation of phenoxypropionic acids is combined with a preceding inversion of S- to R-enantiomer

followed by formation of further degradation products [3, 4]. Since it has been found that R-enantiomers are the more active forms several herbicides were formulated as optically pure agents [5].

Table 1: Structures of phenoxy-, alkoxy- and aryloxyphenoxypropionates.



compound	R_1	R_2
mecoprop-methyl methyl-(RS)-2-(2-methyl-4-chloro-phenoxy)-propionate		CH_3
dichlorprop-methyl methyl-(RS)-2-(2,4-dichlorophenoxy)-propionate		CH_3
fenoprop-methyl methyl-(RS)-2-(2,4,5-trichlorophenoxy)-propionate		CH_3
methyl-(RS)-2-(2,4-dichloro-3,6-dinitro-phenoxy)-propionate		CH_3
ethyl-(RS)-2-methoxypropionate	CH_3	C_2H_5
diclofop-methyl methyl-(RS)-2-[4-(2,4-dichlorophenoxy) phenoxy]propionate		CH_3
fluazifop-butyl butyl-(RS)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate		C_4H_9

Both aspects emphasized the importance of enantioselective separation by applying sophisticated chromatographic systems. So, determination of enantiomers in standard solutions and extracts of environmental samples was performed by employing GC [6], HPLC [7] and capillary electrophoresis [8] with different chiral stationary phases.

Because application notes for gas chromatographic separation of chiral agrochemicals and related xenobiotics predominate in the literature [9-11], it was the main objective of the present study to investigate comparatively HPLC and GC separation performances. Columns used were permethylated ß-cyclodextrin phases and selected target compounds were mecoprop-methyl, dichlorprop-methyl and fenoprop-methyl as well as diclofop-methyl and fluazifop-butyl. In order to detect the influence of electrophilic substituents, dichlorprop was derivatized to a dinitro-dichloro-phenoxypropionate. Supplementary, ethyl-(RS)-2-methoxypropionate was synthesized to determine chromatographic behaviour of this alkoxypropionate. All structures are shown in Table 1. Additionally, batch experiments with mecoprop and diclofop were carried out to determine influences of coextractants from a complex soil matrix on the separation efficiency.

Material and Methods

Analytical standards and reagents

Reference substances dichlorprop, dichlorprop-methyl, mecoprop and mecoprop-methyl were supplied by Riedel-de Haën (Seelze, Germany) while diclofop, diclofop-methyl, fenoprop-methyl, fluazifop and fluazifop-butyl were purchased from Ehrenstorfer (Augsburg, Germany). The enantiomers of ethyl-2-hydroxypropionate were obtained from Fluka (Buchs, Switzerland). All chemicals used were of analytical grade. Solvents used were acetone, cyclohexane, dichloromethane, ethyl acetate, hexane, methanol and toluene (Baker, Griesheim, Germany). Stock standard solutions with 1 µg/µL of the target compounds were prepared in hexane and methanol for GC and HPLC analysis, respectively.

Enantioselective HPLC

HPLC analyses were performed using an HP Series 1050 System with quaternary pump, autosampler, HP 1040 diode array detector (DAD) and an HP Pascal ChemStation (Hewlett Packard, Waldbronn, Germany). For enantioselective separation, a Nucleodex ß-PM column (heptakis-(2,3,6-tri-O-methyl)-ß-cyclodextrin covalently bound on Nucleosil 100; Macherey-Nagel, Düren, Germany) with 200 mm column length, 4 mm ID and 5 µm particle size was applied. Isocratic elution with water/methanol mixtures (HPLC grade; Baker, Griesheim, Germany) was done at substance specific chromatographic conditions. 20 µL of standard solutions were injected and target compounds were detected at 233 nm.

In addition to enantioselective determination, mecoprop-methyl enantiomers were separated by micro-preparative HPLC using the Nucleodex ß-PM column. Standard solution of 0.1 µg/µL was injected 30 times and injection volume was 20 µL. Eluent mixture was water/methanol with a ratio of 40/60 and flow rate was 0.5 mL/min. Both enantiomers were detected with DAD at 233 nm and then isolated. After control of enantiomeric purity by HPLC, solutions were evaporated to dryness and dissolved in 1 mL methanol. In order to measure specific optical rotation of the enantiomers, the concentrated solutions were analyzed using a Perkin-Elmer 241 polarimeter (Überlingen, Germany). Rotation was measured at 589 nm and 20 °C.

Enantioselective GC

GC analysis was carried out using an HP 5890 Series II gaschromatograph equipped with HP 7673 auto-sampler, ^{63}Ni electron capture detector (ECD) and HP 3365 ChemStation for data analysis (Hewlett Packard, Waldbronn, Germany). For enantioselective separation, a Hydrodex ß-PM column (heptakis-(2,3,6-tri-O-methyl)-ß-cyclodextrin diluted in OV 1701; Macherey-Nagel, Düren, Germany) with 25 m length and 0.25 mm ID was applied. Temperature settings were 200 °C for injector and 230 °C for ECD. Carrier gas was helium with 1.4 mL/min and make up gas was nitrogen with 60 mL/min (Linde, Hannover, Germany). 1 µL of standard solutions was injected with the splitter closed for 0.75 min. Temperature programmes were optimized on target compounds.

Derivatization

In order to investigate substance specific impacts on enantioselective separation, dichlorprop was derivatized to a dinitro-dichloro-phenoxypropionate according to the DFG Method W4 [12]. 2 mL of nitrating acid (1.8 mL of concentrated sulfuric acid and 0.2 mL of fuming nitric acid) were added to 60 µL of the stock standard solution. After a reaction time of 4 min, the mixture was diluted with 20 mL deionized water. Liquid/liquid partition with 20 mL dichloromethane followed. Then, the organic phase was dried over sodium sulphate (Merck, Darmstadt, Germany) and rotary evaporated to dryness. Subsequently, the residue was methylated with 5 mL esterification mixture (10 % concentrated sulfuric acid in methanol). After 10 min reaction time, 15 mL deionized water were added and liquid/liquid partition with 10 mL toluene followed. After additional washing with 15 mL sodium hydrogencarbonate solution, the organic phase was separated, dried with sodium sulphate, evaporated approximately to dryness and dissolved in 1 mL hexane for control of the derivative by conventional GC/MS and for enantioselective GC. For HPLC analysis, the derivative was dissolved in methanol.

Racemic and enantiomeric pure ethyl-2-methoxypropionate were synthesized by catalytic methylation in order to investigate the chromatographic behaviour of these alkoxypropionates [13, 14]. 20 µL tetrafluoroboric acid and diazomethane in 2.5 mL diethyl ether were added to 500 µL ethyl-2-hydoxy-propionate. After 2 h reaction time at -10 °C, 10 µL dodecane as keeper were added and the excess of solvent was removed in a gentle stream of nitrogen. Then, the residue was dissolved in 1 mL hexane. After additional washing with 15 mL sodium hydrogencarbonate solution, the organic phase was separated and dried with sodium sulphate. The concentrated hexane extract was analyzed by conventional GC/MS for control of the derivative and by enantioselective GC. For HPLC analysis, the solution was evaporated approximately to dryness and the residue was dissolved in methanol.

Batch experiments

50 g clayey silt soil samples sieved to < 2 mm were fortified with mecoprop and diclofop solutions in methanol to give 2 mg/kg dry soil which corresponded to the double application rate of common agricultural practice. Samples were incubated in flasks covered with cotton plugs at 20 ± 2 °C in the dark. Throughout the incubation period, soil moisture was maintained at approximately 40 % maximum water capacity by weighing each flask and adding deionized water to compensate losses. At 1, 3, 7, 14 and 21 days after application, flasks were closed and frozen at -20 °C until analysis.

Soil samples were analyzed according to the principles of the DFG S19 multi method [15] and the on-line extraction method reported by Steinwandter [16]. After extraction with water/acetone mixture, liquid/liquid

partition with cyclohexane and clean up by gel permeation chromatography, the phenoxypropionic acids were methylated with diazomethane in diethyl ether. HPLC analysis was performed with gradient elution to separate target compounds and coextractants from the soil matrix. The gradient started at a water/methanol ratio of 40/60. After 22 min of isocratic operation, methanol amount linearly increased to 70 % within 30 min. Flow rate was 0.4 mL/min and target compounds were detected at 233, 254 and 260 nm. Quantitation of residues was carried out by external calibration with diluted standard solutions in a concentration range of 5-85 ng/µL.

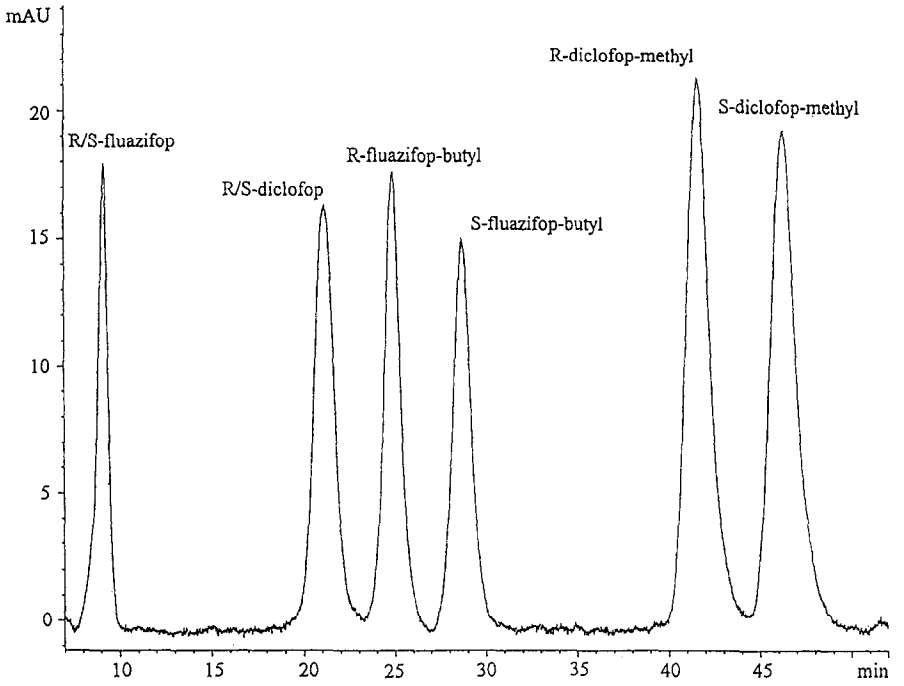


Figure 1: Separation of fluazifop, diclofop and the enantiomers of corresponding esters using chiral permethylated ß-cyclodextrin HPLC column (eluent: 70 % methanol buffered at pH 4 with 0.4 % triethyl ammonium acetate, flow rate: 0.4 mL/min, injection volume: 20 µL, detection: 233 nm).

Results and discussion

For enantioselective separation of differently substituted phenoxypropionates (Table 1), heptakis-(2,3,6-tri-O-methyl)-ß-cyclodextrin was used in this study as stationary phase of the chiral HPLC and GC column. HPLC analysis was applied with isocratic water/methanol eluents. Methanol amounts were optimized for each compound and varied from 60-75 % with flow rates from 0.3-0.6 mL/min. According to this, optimized GC temperature programmes were 115 °C (70 min) → 4 °C/min → 185 °C (1 min) for mecoprop-methyl and dichlorprop-methyl as well as 170 °C (30 min) → 1 °C/min → 250 °C (1 min) for diclofop-methyl and fluazifop-butyl. Application of both chromatographic systems showed a decrease in

enantioselective separation efficiency from mecoprop-methyl and dichlorprop-methyl to the threefold chlorinated fenoprop-methyl. While methyl esters of mecoprop and dichlorprop were baseline separated, fenoprop-methyl was detected with slight peak splitting by HPLC and only as single peak by GC. Similar effects were described by König et al. [6] and Garrison et al. [7]. In order to control the influence of electrophilic substituents on enantioselective separation 2 nitro groups were incorporated into dichlorprop by derivatization. By analogy with fenoprop-methyl, the dinitro-dichloro-propionate formed was only detected as a single peak although HPLC and GC conditions were varied in a wide range. Because of increased number of electrophilic substituents, electron density of the aromatic system decreased and intercalation complexes between lipophilic hollow spaces of cyclodextrins and nonpolar phenyl groups of phenoxypropionates were not formed sufficiently for enantioselectice separation. The significance of an aromatic functionality is additionally emphasized by analyzing ethyl-2-methoxypropionate. Thus, ethyl-2-hydroxypropionate was methylated under catalysis of tetrafluoroboric acid. According to the theory of enantioselective complexation [17], this racemic alkoxypropionate could not be separated.

Analysis of fenoprop-methyl enantiomers using ß-cyclodextrins already revealed higher separation performance for HPLC than for GC. This advantage of HPLC is shown more clearly by enantioselective separation of diclofop-methyl and fluazifop-butyl. Although high resolution capillary GC is well known to have generally a higher number of separation stages, separation of these aryloxyphenoxypropionates was not possible. Furthermore, HPLC permitted simultaneous detection of phenoxypropionates and their corresponding acids when the water/methanol eluent was buffered at pH 4 by adding 0.4 % triethyl ammonium acetate to prevent dissociation of the acids. The chromatogramme is shown in Figure 1. Even if the acids were not separated enantioselectively, the formation of diclofop and fluazifop by hydrolysis of the soil applied esters could be determined. Additionally, it could be shown that hydrolysis of the esters occurred without change in optical configuration of the enantiomers. This simultaneous differentiation is not possible by application of GC which generally requires derivatization of phenoxypropionic acids. Finally, micro-preparative isolation of the enantiomers by employing GC is more difficult since selected fractions have to be trapped from the gas phase using a special sampling device [18]. Contrary to GC, fractionation by HPLC was carried out with less expenditure of technical equipment [19]. After control of retention times by DAD, selected fractions were sampled and enantiomers of mecoprop-methyl were characterized by polarimetry in order to identify the absolute configuration by comparison with data in the literature [1, 3].

Separation performance of HPLC is often influenced by coextractants from the matrix investigated. Therefore, batch experiments were carried out with mecoprop and diclofop which were fortified to a clayey silt soil. After extraction, clean up procedure and methylation of the phenoxypropionic acids, the soil extracts were analyzed by HPLC with sufficient separation of the enantiomeric esters. So, it was possible to investigate the enantioselective degradation of the parent compounds applied. For diclofop enantiomers, concentrations differed especially during the first days of incubation period. While concentration of the S-enantiomer decreased continuously, concentration of the R-enantiomer increased till 3 days after application. Then, this compound continuously disappeared, too (Figure 2). Considering the complex soil matrix investigated having 18 % clay and 80 % silt, the results achieved agreed well with the studies of Wink and Luley [4]. These effects are explained by Bewick [3] with an inversion of S- to R-enantiomer which is followed by formation of further degradation products. In contrast, a change in optical

configuration of the enantiomers of mecoprop could not be derived from Figure 3. Here, the R-enantiomer rapidly disappeared while the S-enantiomer concentration decreased continuously. Similar results were reported for the enantioselective degradation of fenoxaprop-ethyl in soil [4]. They were explained with a slower inversion of S-diclofop than S-fenoxaprop. Additionally, little inversion of R-fenoxaprop was observed. Finally, it was described that the extent of inversion was largely dependent on soil type.

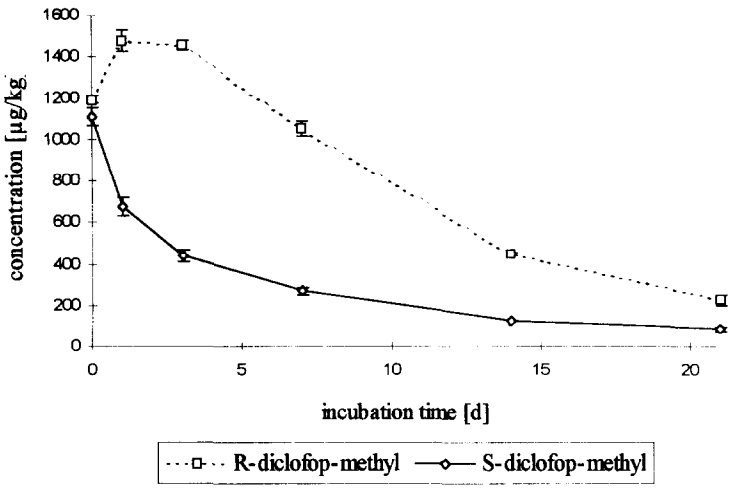


Figure 2: Enantioselective degradation of diclofop in soil. Samples were analyzed in duplicate.

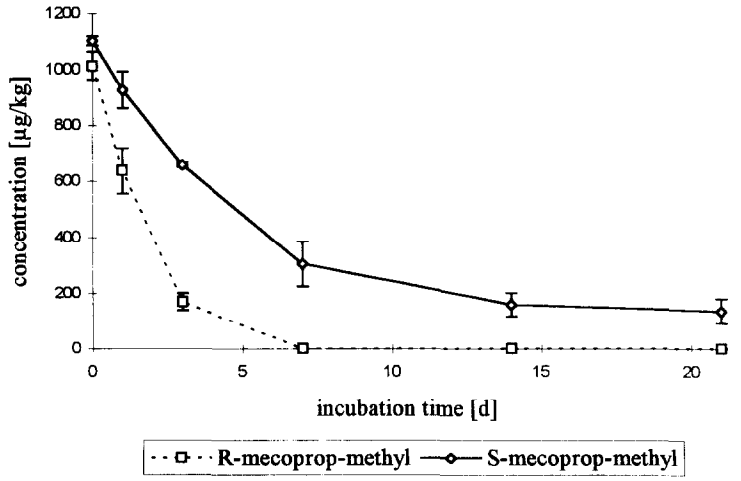


Figure 3: Enantioselective degradation of mecoprop in soil. Samples were analyzed in duplicate.

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