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Journal of Chromatography A, 799 (1998) 355–360

JOURNAL OF CHROMATOGRAPHY A

Short communication

Simultaneous determination and enantiomeric resolution of mecoprop and dichlorprop in soil samples by high-performance liquid chromatography and gas chromatography–mass spectrometry

F. Sánchez-Rasero*, M.B. Matallo, G. Dios, E. Romero, A. Peña

Departamento de Ciencias de la Tierra y Química Ambiental, Estación Experimental del Zaidín, CSIC, Prof. Albareda 1, E-18008 Granada, Spain

Received 17 July 1997; received in revised form 3 October 1997; accepted 6 October 1997

Abstract

A rapid, precise and sensitive method was developed for the quantitative simultaneous determination of mecoprop and dichlorprop in soil samples by reversed-phase high-performance liquid chromatography (HPLC), thus making it very appropriate for degradation studies where many samples have to be analysed. This method was supplemented with a simple gas chromatography–mass spectrometry (GC–MS) ion trap method developed using a chiral permethylated-β-cyclodextrin stationary phase in order to determine the relative amounts of the *R* and *S* enantiomeric forms of these herbicides. Recovery rates for natural soils were around 80% at concentrations of 0.5 and 1.0 μg/g. Lower recoveries were achieved for the 10% peat-modified soil samples. The analytical sensitivity of the HPLC technique was 0.11 and 0.07 ng/μl, for 20 μl injections, for mecoprop and dichlorprop, respectively. © 1998 Elsevier Science B.V.

Keywords: Enantiomer separation; Environmental analysis; Soil; Pesticides; Mecoprop; Dichlorprop

1. Introduction

Even though some countries, such as Denmark and the Netherlands, have established programmes for cutting down the consumption of pesticides by 50% in the next few years and that there is a general concern about the environmental contamination by pesticides [1], the fact is that this contamination continues to exist. The European Union [2] strongly recommends that studies of the behaviour and fate [3] of priority pesticides, such as mecoprop and some other phenoxyacid herbicides [4], are carried out in the major types of aquifers. In order to carry out such studies, sensitive, precise, accurate and rapid analytical methods are needed.

The structures of the propionic acid derived herbicides [e.g., mecoprop (MCPP) and dichlorprop (DCPP)] contain an asymmetrically substituted carbon atom. Therefore, these products consist of two optical isomers and the herbicidal activity is concentrated almost exclusively in the *R*-form. Environmental pollution by both enantiomers is possible, when formulations containing the racemic forms are employed. This is another reason to encourage scientists to study the environmental behaviour of both enantiomeric forms [5], including adsorption, degradation (biotic and abiotic) and leaching processes in soils. These processes are affected by the different

*Corresponding author.

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PII S0021-9673(97)01024-8

soil constituents [6], and hence three soils from this region, presenting the most different physicochemical properties, have been chosen for this study. Because addition of organic matter to soil samples in the Mediterranean area is a common agricultural corrective practice, the influence on recovery of the addition of exogenous organic matter to the soil has been also considered.

Most of these studies are more conclusive when performed in an aqueous medium and that is the reason why, although different analytical methods are already available for determining phenoxyacid herbicides, either in their racemic forms [7–10] or with enantiomeric separation [11–15], the purpose of this paper is to develop a rapid high-performance liquid chromatography (HPLC) method for the quantitative determination of the racemic forms of these herbicides in soil, complemented with a gas chromatography–mass spectrometry (GC–MS) method for the determination of the relationship between their enantiomeric forms when it is considered appropriate.

2. Experimental

2.1. Reagents

n-Hexane, methanol HPLC-grade, 99.6% acetic acid, methylene chloride, orthophosphoric acid and hydrochloric acid were obtained from Panreac (Madrid, Spain). Boron trifluoride–methanol 14% solution was supplied by Sigma (Madrid, Spain). Mecoprop, mecoprop-P, dichlorprop and dichlorprop-P of known purity (99.9%) were a gift from BASF (Limburgerhof, Germany) and water was purified with a Milli-Q water purification system (Millipore).

2.2. Materials

Three soils from ''La Vega de Granada'' (southeast of Spain), classified as silty loam, sandy loam and clay loam, respectively, and characterized as indicated in Table 1, were adjusted to 80% of their field capacity moisture, and 10% of peat from Padul (Granada, Spain) was or was not added. Peat analysis showed a 78% of organic matter (OM) content and a cation-exchange capacity of 158 mequiv./100 g. An orbital shaker (Heidolph, Reax 2), filter paper (Schleicher-Schüll No. 604) and Millex filters (Millipore) type HV_4, pore size 0.45 μm, were employed.

2.3. Calibration solutions

For the HPLC method a MCPP/DCPP standard aqueous solution was prepared at concentrations of ca. 12.0 ng/μl each. Four additional solutions were prepared by dilution in water at ca. 9.0, 6.0, 3.0 and 1.5 ng/μl, respectively.

2.4. Chromatography

A 1090 Hewlett-Packard liquid chromatograph was used, equipped with a 4.5 μl spectrometer cell, a diode-array detector and a DPU multichannel integrator [16]. A 250×4 mm stainless steel analytical column and a 4×4 mm precolumn were used, both packed with LiChrospher 100 RP-18 endcapped, 5 μm, as stationary phase. The different parameters for the method were, flow-rate 1.0 ml/min, temperature 40°C, stop-time 7.5 min, injected volume 20 μl, detection wavelengths 229 and 234 nm (bandwith 4 nm), reference wavelength 450 nm (bandwith 100 nm), spectra setting in apex, base and slope from 200 to 300 nm, chart speed 2 cm/min and range 25 mAU (milli-absorbance units). The mobile phase consisted of methanol–0.05 *M* phosphoric acid pH 2.5 (70:30).

Table 1
Physicochemical characteristics and textural composition of the three soils

Textural class	Organic matter (%)	pH	Cation-exchange capacity (mequiv./100 g)	Sand (%)	Silt (%)	Clay (%)
Silty loam	2.10	8.2	10.44	30.7	61.4	7.9
Sandy loam	1.50	7.5	6.38	67.6	32.9	–
Clay loam	1.42	8.1	22.41	22.0	45.3	32.7

A Varian Star 3400 CX gas chromatograph was used, fitted with a Varian 8200 CX automatic injector and connected to a Varian Saturn 3 ion-trap mass spectrometer. A 1-μl volume of the sample was injected splitless on a deactivated fused-silica column 2 m×0.32 mm I.D. connected to an analytical chiral column of 20 m×0.25 mm I.D., containing 2.3% OV-1701 and 15% permethylated-β-cyclodextrin (PM-β-CD) (a gift from Dr. M. Müller, Wädenswil, Switzerland). The injector, transfer line and detector manifold temperatures were 260, 190 and 190°C, respectively. Helium was the carrier gas at a column flow-rate of 1 ml/min and the temperature program was as follows: 60°C for 1 min, ramp to 100°C at 20°C/min, then to 145°C at 2°C/min. Each second a spectrum was recorded from m/z 40–300 in electron impact mode, starting 5 min after injection.

3. Methods

3.1. Extraction and analysis

Two 20-g aliquots of conditioned soil (moisture or moisture/peat) were each mixed with 20 and 10 μg of mecoprop and dichlorprop in aqueous solution and homogenized at room temperature for at least 1 h. For the isolation of the herbicides each treated soil was mechanically shaken for 1 h, twice, with 50 and 25 ml of methanol–water–acetic acid (49:49:2). After every extraction the suspensions were centrifuged for 20 min at 5000 rpm and filtered through paper. The mixtures of the two filtrates were acidified with conc. HCl to pH 1–2 and extracted twice with 50 and 25 ml of methylene chloride. The organic phases were mixed together and evaporated by means of a rotary evaporation under vacuum up to approximately 2 ml and later on to dryness by means of a stream of dry nitrogen. The evaporated extracts were dissolved in 2.0 ml water. A 1-ml volume of each aqueous solution was diluted with 1 ml of methanol, the mixture filtered through a Millex HV_4 filter and the total amount of the racemic herbicides contents determined by HPLC. The dilution with methanol of the aqueous soil solutions was carried out in order to avoid precipitations inside the liquid chromatographic column of water-soluble soil substances which are not soluble in methanol.

The remaining 1-ml volumes of the aqueous solutions were acidified with conc. HCl to pH 1–2 and manually extracted with 10 ml of methylene chloride. The organic phase was evaporated to dryness with a stream of dry nitrogen. A 2-ml volume of BF_3–methanol was added, the mixture kept at 70°C for 30 min, cooled in an ice bath, and 1 ml of water and 8 ml of *n*-hexane were added. After 2 min of energetic shaking, the solution was centrifuged for 1 min at 1000 rpm, the organic phase transferred to another tube, and concentrated under a stream of dry nitrogen to a final volume of 2 ml. A 1-μl volume of the solution was analysed by GC–MS for the determination of the percentage of each enantiomeric form in both herbicides.

4. Results and discussion

For the HPLC method, calibration lines were obtained from triplicate injections of the calibration solutions, by plotting absorbance versus herbicide concentrations. The curve was linear for MCPP over the range 1.07–11.89 ng/μl and for DCPP over the range 0.70–8.85 ng/μl. The straight lines obtained correspond to the equations $y=27.30x-0.78$ for MCPP, and $y=26.38x+0.53$ for DCPP. Other statistical parameters corresponding to the calibration curves calculated in accordance with Cuadros et al. [17] are shown in Table 2.

As a representative example, chromatograms of extracts from the silty loam sample with and without added peat, are shown in Fig. 1. It is clear that in both cases there is an excellent resolution of the MCPP and DCPP peaks. Their separation from impurities seems to be adequate and no peak was observed at the retention times of these herbicides when blank samples were chromatographed under the same conditions. Typical retention times for both

Table 2
Statistical parameters of the calibration curves for both herbicides ($y=a+bx$)

	n	a	s_a	b	s_b	$s_{R,c}$	r^2
MCPP	15	−0.78	1.51	27.30	0.21	3.07	0.9996
DCPP	15	0.53	1.10	26.38	0.20	1.94	0.9994

s_x=Standard deviation of each parameter.

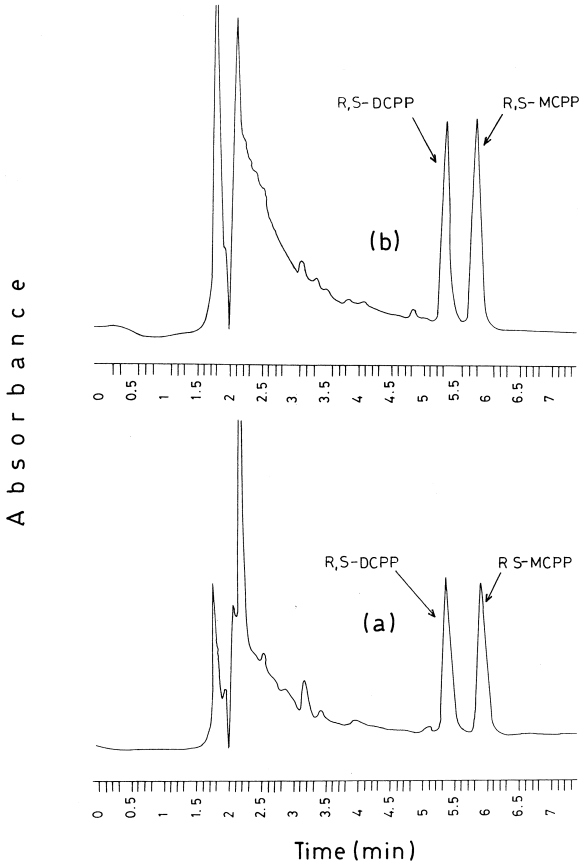

Fig. 1. HPLC chromatograms of *R*,*S*-MCPP and *R*,*S*-DCPP, in the silty loam sample, (a) without and (b) with added peat.

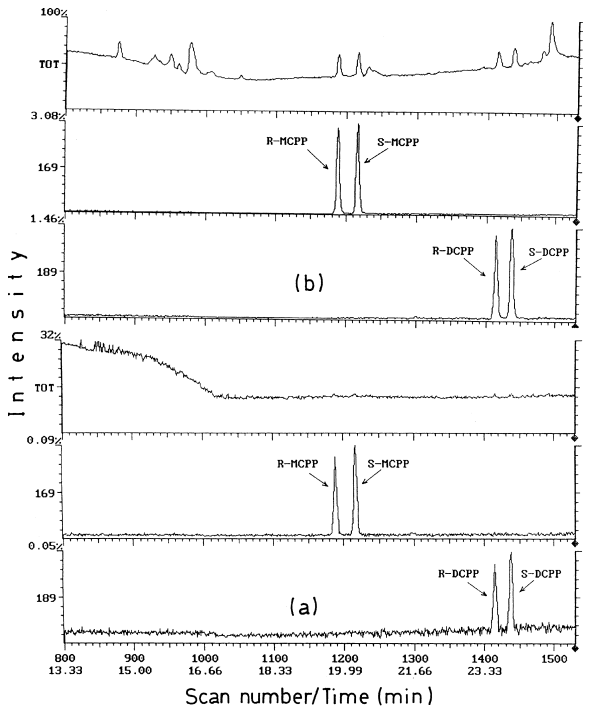

Fig. 2. GC–MS chromatograms of methylated *R*- and *S*-MCPP plus *R*- and *S*-DCPP, in the silty loam sample, (a) without and (b) with added peat. In both cases, the total-ion chromatogram and the single-ion chromatograms corresponding to m/z values of 169 (specific for the enantiomers of MCPP) and 189 (for those of DCPP) are presented.

herbicides are 6.0 min for MCPP and 5.5 min for DCPP and their peak areas are about 120 mAU.

UV spectra for each chromatographic peak, prior to, at and after the MCPP and DCPP maxima are very similar, demonstrating the purity of both peaks. In addition, the linear relationships between the signals obtained at 229 and 234 nm also confirm their purities.

Other analytical parameters for this HPLC method [17] are analytical sensitivity 0.11 and 0.07 ng/μl, limit of detection 0.32 and 0.21 ng/μl, limit of determination 1.07 and 0.70 ng/μl, and precision 1.60% and 1.09% at 5.90 ng/μl, for MCPP and DCPP, respectively.

Fig. 2 illustrates the total-ion current (TIC) profiles together with the single-ion chromatograms obtained from addition of the herbicides to and extraction from the same silty loam sample with and without added peat, derivatization and determination by GC–MS under the previously described conditions. Fig. 2 shows the complete separation of the enantiomeric forms with typical retention times of 19.8 and 20.3 min for *R*- and *S*-MCPP and 23.6 and 23.9 min for *R*- and *S*-DCPP, respectively. Quantitation was performed at m/z values of 169 and 189 for MCPP and DCPP respectively, corresponding, for both herbicides, to the loss of the $-COOCH_3$ fragment. Good correlation (>97%) between mass spectrum (background subtracted) and library spectrum was obtained for methylated MCPP.

The use of single ions to quantify the enantiomeric forms of the herbicides has clear benefits. The signal-to-noise ratio is increased and the number of interfering substances responding to the selected m/z values is diminished, with an improvement in analytical sensitivity, which is especially observed in

Fig. 2a. The average enantiomeric ratios calculated from peak areas, were 1.00:1.00 for MCPP (relative standard deviation of 4.2%) and 0.98:1.02 for DCPP (3.1%) for the *R* and *S* forms of the phenoxyacid herbicides, as expected for a racemate.

This can potentially provide a very useful procedure for studies on the degradation of the two enantiomeric forms. Determinations of the racemic forms should be performed in all the samples throughout the whole procedure by the simple HPLC method and the relative amounts of the enantiomers determined as appropriate with the more time-consuming GC–MS method.

Recovery rates and standard deviations for the three soils in their natural form as well as samples with 10% added peat, fortified at two levels with MCPP and DCPP, are given in Table 3. Recovery rates in natural soils are greater than 80% with the exception of one result (78.8%), both for the 0.5 and the 1.0 μg/g concentrations. When the soils are modified with 10% of added peat, the recovery rates are close to 80%. The values of the standard deviations are in the range 0.1–11%, but in most cases below 7%. After applying the *t*-test for comparison of averages, the following conclusions are drawn. Firstly, no statistical difference is found in the recovery rate among the three natural soils; this is also the case for the three modified soils. Secondly, a higher recovery rate is obtained for natural soils, in comparison with modified soils ($P<0.001$); this agrees with previous results for several other types of pesticides [18,19] and shows a dependence of recovery on the organic matter content of the soil.

Consequently, this method gives good recoveries and reproducibility for the determination of these phenoxy herbicides in soil without a clean-up step, which makes it very useful for degradation studies where lots of samples have to be analysed. The results obtained are comparable with those reported for the same family of chemicals in soil [9,20], but at the same time this method is easier and quicker.

Acknowledgements

We gratefully acknowledge support for this work from grant AMB94-0776 from the ''Comisión Interministerial de Ciencia y Tecnología (CICYT)''. The gift of MCPP and DCPP from BASF (Limburgerhof, Germany) as well as the valuable technical assistance of M.D. Maroto are gratefully acknowledged. M.B.M. is a Ph.D. student sponsored by ''Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)'', and a researcher at the Instituto Biológico, São Paulo, Brazil.

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Table 3
Recoveries of MCPP and DCPP from fortified natural (N) and 10% peat-modified (P) soils

Herbicide	Amount added (μg/g)	Recoveries (%)[a]					
		Silty loam		Sandy loam		Clay loam	
		N	P	N	P	N	P
MCPP	0.5	86.3(±4.5)	78.2(±1.2)	84.5(±5.0)	80.7(±4.5)	82.9(±4.2)	83.0(±3.5)
	1.0	80.7(±2.5)	70.7(±3.1)	82.8(±1.8)	73.7(±4.7)	82.7(±2.0)	74.1(±3.3)
DCPP	0.5	85.8(±0.7)	70.3(±0.5)	85.4(±6.1)	73.0(±8.8)	78.8(±0.5)	84.1(±11.1)
	1.0	93.2(±3.5)	77.4(±6.5)	89.1(±6.0)	82.5(±0.1)	84.8(±0.5)	81.4(±5.3)

[a] Average of three replicates.

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