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Note

Chiral high-performance liquid chromatographic analysis of phenoxy herbicide mixtures

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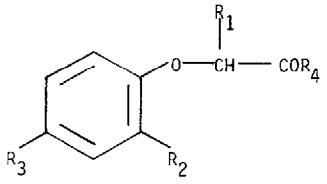
2-(4-Chloro-2-methylphenoxy)propanoic acid (CMPP) is a member of the phenoxy herbicide group; its annual U.K. and world production have been estimated at 3500 and 20 000 tons, respectively[1]. It has been recognised that its herbicidal activity is stereoselective, since only the (+)-enantiomer has appreciable activity[2]. Recent developments have led to the marketing of this agent in optically pure form by BASF (trade name Duplosan). Within the next two years The Netherlands and Switzerland will restrict the scale of CMPP to the active enantiomer only. Other countries are considering similar legislation. Such developments require assay methods for quality control and regulatory purposes.

We have previously reported the direct chiral separation of CMPP using an α_1-acid glycoprotein (Enantiopac) chiral stationary phase (CSP)[3]. The same enantiomers could also be separated, after conversion to their diphenylamides, using a Pirkle ionic CSP [with N-(3,5-dinitrobenzoyl)-(*R*)-(−)phenylglycine as chiral ligand]. We now wish to report preliminary work on the analysis of mixed-herbicide formulations. Such formulations arise because different phenoxy herbicides have different specificities, so much broader herbicidal action will result from the use of mixed formulations. Typical blends include CMPP, the related chiral 2-(2,4-dichlorophenoxy)-propanoic acid (2,4-DP) and the non-chiral 4-chloro-2-methylphenoxyacetic acid (MCPA).



	R_1	R_2	R_3
CMPP	CH_3	CH_3	Cl
2,4-DP	CH_3	Cl	Cl
MCPA	H	CH_3	Cl

R_4 = OH (free acids) or $N(C_6H_5)_2$ (diphenylamide derivatives)

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EXPERIMENTAL

Materials

Herbicide samples were gifts from A. H. Marks Co. (Bradford, U.K.) and May & Baker (Dagenham, U.K.), γ-Aminopropyl silica (5 μm) was purchased as Spherisorb NH_2 from Jones Chromatography and packed into a 30 cm × 4.6 mm I.D. column. N-(3,5-Dinitrobenzoyl)-(*R*)-(−)-phenylglycine was purchased from Sigma. The 10-cm Enantiopac column was purchased from LKB (Bromma, Sweden). All solvents used were HPLC grade, other materials were of laboratory grade and used as purchased.

Equipment

The Pye Unicam HPLC system used consisted of a PU 4010 pump, PU 4020 UV detector, PU 4047 column module and a DP 88 computing integrator.

Storage and testing of Enantiopac column

The Enantiopac column was stored in propan-2-ol–water (50:50, v/v) at a temperature of 10°C. Prior to each use the column was equilibrated in a mobile phase of propan-2-ol–0.1 *M* sodium chloride in phosphate buffer (10 m*M*, pH 6) (8:92, v/v) and tested by the injection of 20 μl disopyramide (0.1 mg/ml) in the mobile phase. Baseline resolution for this reference racemate was always obtained during our study.

Preparation, testing and storage of Pirkle column

Preparation of the Pirkle CSP, from a prepacked 30-cm γ-aminopropyl silica column and a solution of N-(3,5-dinitrobenzoyl)-(*R*)-(−)-phenylglycine was in accordance with the *in situ* method described by Pirkle *et al.*[4]. The final stage of preparation involved equilibration in a mobile phase of propan-2-ol–hexane (10:90, v/v). On achieving a stable baseline the column was tested using phensuximide as the test racemate. On injection of 20 μl of a 1-mg/ml solution, clear separation (but not to baseline) of the enantiomes was considered satisfactory.

Preparation of herbicide derivatives

Diphenylamide derivatives of the herbicides were prepared in the following manner. A 2-mg amount of the herbicide was weighed into a quickfit centrifuge tube. Thionyl chloride (1 drop) was added and the tube was stoppered and heated over a steam bath for 10 min. The contents were then evaporated to dryness under reduced pressure and reconstituted in 1 ml of a solution of 2 mg/ml diphenylamine in chloroform. The tube was shaken regularly over a period of 10 min prior to reducing to dryness. The residue was dissolved in 10 ml of propan-2-ol–hexane (10:90, v/v) and this solution was diluted 10-fold prior to HPLC analysis on the Pirkle column.

Herbicidal agents were extracted from commercial blends by first acidifying the solution and then extracting the acidic herbicides into chloroform. After evaporating the chloroform layer to dryness, under reduced pressure, derivatisation was accomplished as described above.

RESULTS AND DISCUSSION

Examination of individual compounds on the Enantiopac column revealed good separation for CMPP, as previously reported, but only poor separation for 2,4-DP. Systematically varying the pH and propan-2-ol content of the mobile phase failed to yield useful separation of 2,4-DP (see Table I). This must reflect the strict specificity of the binding sites of the α_1-acid glycoprotein and means that Enantiopac separations cannot be considered as group separation systems. A further disadvantage with the Enantiopac column became apparent when MCPA was run under conditions optimised for CMPP because 2,4-DP gave poor, but evident separation. However, the retention time of MCPA corresponded to that of the (−)-enantiomer of CMPP.

These are serious limitations of the Enantiopac method. Taken in conjunction with the cost, erratic behaviour and short lifetime of these columns[5,6] they render the Enantiopac system unreliable for routine chiral analysis of phenoxy herbicides.

In contrast the ionic Pirkle column performed well when used to assay mixed phenoxy herbicides as their diphenylamide derivatives. Clear separation of the CMPP amide enantiomers (as previously reported[3]), 2,4-DP amide enantiomers and the non-interfering elution of MCPA amide was obtained (see Table II). For both chiral herbicides the derivatisation step was validated. Racemic materials gave virtually 50:50 response ratios for the enantiomeric amides whilst the purest samples of (+)-CMPP and (+)-2,4-DP available to us gave 0% and 3% of the (−)-enantiomers respectively (as calculated from integrated peak areas), so excluding significant racemisation. Further work is in hand to judge the exact extent of racemisation during these reactions and to formalise and validate conditions in a detailed assay method.

TABLE I

THE CHROMATOGRAPHIC EXAMINATION OF (±)-CMPP AND (±)-2,4-DP ON AN α_1-ACID GLYCOPROTEIN (ENANTIOPAC) CSP

Mobile phase: 0.1 *M* sodium chloride in phosphate buffer (10 m*M*); variable pH and Propan-2-ol concentration. Flow-rate: 0.2 ml/min. Detection: 240 nm. t_R = Retention time of first eluted enantiomer; k' = capacity factor; α = separation factor; R_s = resolution.

Mobile phase variable		*Solute*	t_R *(min)*	k'	α	R_s
Propan-2-ol in mobile phase (%)	*pH*					
8	6	(±)-CMPP	31	6.4	1.16	1.06
		(±)-2,4-DP	25	4.8	1.00	0.00
6	6	(±)-CMPP	36	6.9	1.21	1.35
		(±)-2,4-DP	30	5.6	1.00	0.00
4	6	(±)-CMPP	40	7.5	1.25	1.60
		(±)-2,4-DP	30	5.4	1.09	0.33
6	9	(±)-CMPP	34	6.8	1.21	1.34
		(±)-2,4-DP	27	5.2	1.00	0.00
6	4	(±)-CMPP	36	6.9	1.22	1.33
		(±)-2,4-DP	28	5.1	1.00	0.00

TABLE II

THE CHROMATOGRAPHIC BEHAVIOUR OF PHENOXY HERBICIDES AS DIPHENYLAMIDE DERIVATIVES ON A PIRKLE IONIC TYPE CSP

Mobile phase: propan-2-ol–hexane (10:90, v/v); flow-rate: 1.0 ml/min; detection: 240 nm.

Solute	t_R *(min)*	k'	α	R_s	*(+)-Enantiomer (%)*	*(−)-Enantiomer (%)*
(±)-CMPP	8.2	2.00	1.10	1.20	50.8	49.2
(±)-2,4-DP	9.2	2.37	1.11	1.14	49.3	50.7
(+)-CMPP	8.2	2.00	–	–	100.0	0.0
(+)-2,4-DP	9.2	2.37	–	–	97.0	3.0
MCPA	12.1	3.41	–	–	–	–

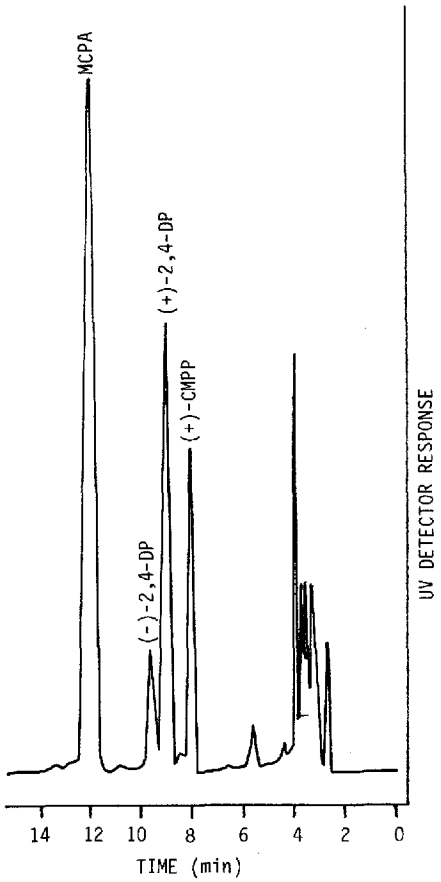

Fig. 1. The analysis of (+)-CMPP, (+)-2,4-DP and MCPA in a commercial mixed formulation using a Pirkle ionic CSP. The 2,4-DP in this non-compliant sample contains 20% of the (−)-enantiomer. Less than 5% of the (−)-CMPP isomer was found. Mobile phase: propan-2-ol–hexane (10:90, v/v); flow-rate: 1 ml/min; detection: 240 nm.

The technique was used to determine the optical purity of (+)-CMPP and (+)-2,4-DP in commercial mixed-herbicide formulations. Fig. 1 shows the chromatogram obtained for a non-compliant sample. Here the CMPP assay was acceptable but the 2,4-DP contained an unacceptably high level (20%) of the (−)-enantiomer. Peaks were identified by comparison with the retention times of authentic (+)-CMPP, (+)-2,4-DP and MCPA as well as the actual materials used in the manufacture of the commercial sample. It is important to note that the analysis of such formulations cannot be accomplished by polarimetric methods alone, chromatographic resolution is required. The low cost of Pirkle columns compared to Enantiopac columns is a notable feature. Moreover the Pirkle system seems to be considerably more robust. Both columns, however, are more temperamental than typical GC and reversed-phase HPLC systems. The requirement to derivatise the herbicides in order to obtain separations on Pirkle columns is a major limitation. The method must be compared with derivatisation methods to yield diastereomers which can then be separated by non-chiral GC and HPLC methods (details of such separations are in press).

CONCLUSIONS

The Pirkle ionic CSP can be used for the analysis of commercial blends of CMPP, 2,4-DP and MCPA herbicides. The method requires conversion of the free acids to diphenylamides prior to HPLC.

In contrast, the separation of such herbicides by direct analysis on an α_1-acid glycoprotein column (Enantiopac) cannot, in our hands, provide an effective analysis. Good separation of CMPP alone can be obtained. The chiral specificity of the Enantiopac column is such that minor substrate structural modification markedly alters the separation obtainable. Interference with the non-chiral MCPA was also found.

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