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PROPOSED PRIMARY REFERENCE METHODS FOR THE DETERMINATION OF SOME COMMERCIALLY IMPORTANT CHIRAL ARYLOXYPROPIONATE HERBICIDES IN BOTH FREE ACID AND ESTER FORMS

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SUMMARY

The direct enantiomeric high-performance liquid chromatographic resolution of the free acid form of the herbicide 2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP) was demonstrated using a second-generation α_1-acid glycoprotein (Chiral-AGP) chiral stationary phase (CSP). Analysis times were short, separations and retention times were reproducible during intermittent use and the baseline resolution of the enantiomers of the herbicide 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP) could also be achieved. This system was found to be far superior to the earlier separation using EnantioPac α_1-AGP CSP.

Commercially important 2-butoxyethyl and 2-ethylhexyl esters and also model methyl and ethyl esters of racemic CMPP were resolved using an ionically bonded D-phenylglycine (Pirkle type 1-A) CSP. The same esters were chromatographed using a covalent D-phenylglycine CSP but the separations observed were inferior. These CSPs were capable of recognizing chirality at the phenoxypropionic moiety but no recognition of chirality associated with the 2-ethylhexyl ester was observed.

The reproducibility and reliability of these methods of chiral analysis makes them suitable for both reference purposes and also for the routine determination of the newly introduced, optically active phenoxypropionate herbicides.

INTRODUCTION

Aryloxypropionate herbicides are produced in hundreds of thousands of tons per annum. Several of these herbicides, such as 2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP) and 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP) have centres of asymmetry and have been used in racemic form for many years, even though marked differences in the activities of different enantiomers have been reported. Environmental pressure has inevitably lead to the production and marketing of the single *R*(+) active enantiomers[1] for both CMPP and 2,4-DP. Recent legislation in several EEC countries will ban the use of such herbicides in the racemic form[2].

Aryloxypropionate herbicides are formulated and supplied in both the free acid

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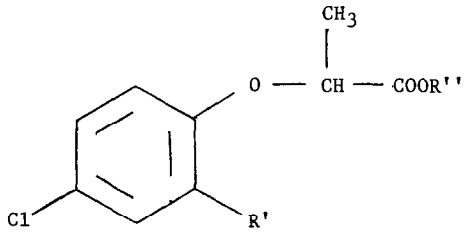

Compound	R'	R''
CMPP	CH_3	H
2,4-DP	Cl	H
CMPP-BOE ester	CH_3	$CH_2CH_2OCH_2CH_2CH_2CH_3$
CMPP-2-EH ester	CH_3	$CH_2CHCH_2CH_2CH_2CH_3$ (with C_2H_5 branch)

and ester forms as better penetration of the plant cuticle can be achieved by esters[3]. Commercially important esters of CMPP and 2,4-DP are the 2-ethylhexyl (2-EH) and the 2-butoxyethyl (BOE) esters. Robust analytical methods are therefore essential for the chiral analysis of CMPP and 2,4-DP in both free acid and ester forms.

In previous papers[4–6], several methods were presented for the determination of these herbicides in the acid form. Of these, the only direct method (*i.e.*, one involving no chemical modification of the analyte) was the separation of CMPP using a first-generation α_1-acid glycoprotein (EnantioPac; LKB, Stockholm, Sweden) high-performance liquid chromatographic (HPLC) chiral stationary phase (CSP)[4]. The analysis time was long (*ca.* 40 min) and 2,4-DP could not be separated into its enantiomers[5]. EnantioPac columns have also been found to be unreliable and non-reproducible by many workers[7]. Moreover, this direct approach could not be applied to mixtures of phenoxypropionate herbicides[5]. To our knowledge, only one method, showing limited detail and only partial resolution, for the direct determination of these free acid herbicides has been reported[8]. For these reasons, we developed indirect (derivatization) methods for chiral HPLC (using Pirkle columns)[5] and achiral gas chromatography (GC) of diastereomeric derivatives[6].

The need for direct primary reference methods is pressing. To our knowledge, the only published work concerning the usable chiral separation of herbicide esters is that of Muller and Bosshardt[9]. The separations reported were for the methyl and ethyl esters, which are not used commercially. Their method was in fact designed as an indirect method for the determination of the free acid herbicides.

We report here a much improved direct chiral separation of both CMPP and 2,4-DP free acids using the second-generation α_1-acid glycoprotein CSP (Chiral-AGP) together with a direct separation of BOE and 2-EH esters of CMPP using Pirkle-type CSPs.

EXPERIMENTAL

Apparatus and materials

Several HPLC systems were used in interchangeable fashion during these studies. One consisted of an LKB Model 2150 pump, a Model 2151 variable-wavelength UV detector, a Rheodyne loop-valve injector (20-μl loop) a Hewlett-Packard HP 3396A integrator and a Kipp and Zonen BD 40 chart recorder. Another HPLC system (Pye Unicam, Cambridge, U.K.) consisted of a PU 4010 pump, PU 4020 UV detector, PU 4047 column module and a DP 88 computing integrator. Chiral columns used were Chiral-AGP, 10 cm × 4 mm I.D. (ChromTech, Stockholm, Sweden), ionically bonded N-(3,5-dinitrobenzoyl)-(*R*)(−)-phenylglycine (D-phenylglycine) (Pirkle type 1-A), 25 cm × 4.6 mm I.D. (Regis Chemical, Morton Grove, IL, U.S.A.) and covalently bonded D-phenylglycine, 25 cm × 4.9 mm I.D. (HiChrom, Reading, U.K.). All columns were subjected to the manufacturers' test conditions and found to comply.

All solvents used were of HPLC grade. Methyl and ethyl esters of racemic CMPP were synthesized by Mr. A. Beiraghi (University of Bradford). Details of eluent compositions, flow-rates, etc., are given in Tables I and II.

Linearity studies

Solutions of *R*(+)-CMPP in the mobile phase at concentrations of 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml were prepared and each was injected in triplicate onto a

TABLE I

DETERMINATION OF CMPP AND 2,4-DP FREE ACIDS USING THE SECOND-GENERATION CHIRAL-AGP α_1-ACID GLYCOPROTEIN CSP

Some of these data were previously reported in poster form at the *Perkin-Elmer Chromatography Symposium, London, April 1989*. Mobile phase, 10 m*M* phosphate buffer with various pH and concentrations of propan-2-ol; flow-rate, 0.9 ml/min; detection, 240 nm.

Propan-2-ol concentration (%, v/v)	*pH*	*Sample*	*Retention time (min)*		*Resolution (R_s)[a] or PSF[b]*
			1st	*2nd*	
4	6.9	(±)-CMPP	1.15	1.30	*PSF* = 0.65
4	5.2	(±)-CMPP	4.90	7.70	R_s = 2.84
		(±)-2,4-DP	4.55	5.85	R_s = 1.25
		(+)-2,4-DP	–	5.80	–
8	5.2	(±)-CMPP	3.00	4.20	R_s = 1.71
		(±)-2,4-DP	2.90	3.40	*PSF* = 0.91
4	6.0	(±)-CMPP	2.09	2.97	R_s = 1.28
		(±)-CMPP	–	2.93	–
		(−)-CMPP	2.03	–	–

[a] $R_s = \dfrac{2(\text{retention time of 2nd peak} - \text{retention time of 1st peak})}{\text{base peak width of 1st peak} + \text{base peak width of 2nd peak}}$

[b] For a pair of partially resolved peaks,

$$PSF = \frac{\text{mean of peak heights} - \text{trough to baseline height}}{\text{mean of peak heights}}$$

Chiral-AGP column. For each concentration the mean integrated peak area was calculated. The means were subjected to regression analysis using an in-house statistics package and linear regression coefficients (r) were ob tained. Solutions of $R(+)$-CMPP-BOE ester in hexane at concentrations of 0.05, 0.10, 0.15, 0.20 and 0.25 mg/ml were prepared and analysed by triplicate injection onto an ionically bonded D-phenylglycine column. The peak areas were processed using the same statistics package and the results are presented later.

RESULTS AND DISCUSSION

Racemic and optically pure samples of CMPP and 2,4-DP free acids were chromatographed on the Chiral-AGP column under various conditions as shown in Table I. Where non-baseline separation was obtained, the peak separation function (PSF), introduced by Kaiser[9,10], was used instead of the resolution value to express the separation obtained. Retention times for the acids predictably decreased with increasing pH and concentration of propan-2-ol. The Chiral-AGP column was found to be significantly more selective for CMPP than for 2,4-DP but baseline separation was achieved for both solutes after optimization.

The chromatograms obtained for CMPP and 2,4-DP under optimum conditions are shown in Figs. 1 and 2, respectively. Preliminary validation exercises showed the detector response to $R(+)$-CMPP to be linear ($r = 0.9995$) over the range 0.02–0.1 mg/ml (corresponding to about 2–10 nmol on-column). The retention times were reproducible and independent of solute concentration. For both herbicides the inactive $S(-)$-enantiomer was eluted first, aiding its quantification as an impurity in samples of the active $R(+)$-enantiomer.

The rapid analysis times, good peak shapes and the fact that both herbicides could be resolved represent great improvements over the earlier EnantioPac method. The second-generation system also appears to be far more robust as the columns have been subjected to intermittent use and several hundred injections without observable changes in performances. These improvements lead us to propose this as a primary reference method for the determination of CMPP and 2,4-DP free acid enantiomers. As such, the method would be suitable for the routine analysis of production samples and could serve as a referee method for indirect separation systems, which may still be required to overcome interference from other components present in formulated mixtures or biological samples. The sensitivity and selectivity offered by electron-capture detectors would make an indirect GC method attractive for the determination of low levels of herbicides in biological matrices.

Herbicide esters of CMPP were examined using Pirkle-type CSPs. Prior to the examination of the relatively bulky BOE and 2-EH esters, simple methyl and ethyl esters were chromatographed as model compounds on the ionically bonded column. On CSPs of this type, dipole stacking appears to be the most important mechanism of retention and chiral recognition[11]. As esters have relatively weak dipole moments, initial experiments used hexane containing only 1% of propan-2-ol as the mobile phase (5–10% of propan-2-ol is required to achieve reasonable analysis times for amide derivatives of these herbicides[4,5]). Even at this low concentration, the methyl and ethyl esters were rapidly eluted but a promising separation was already evident. The results and conditions of analysis for all ester samples on the ionic column are

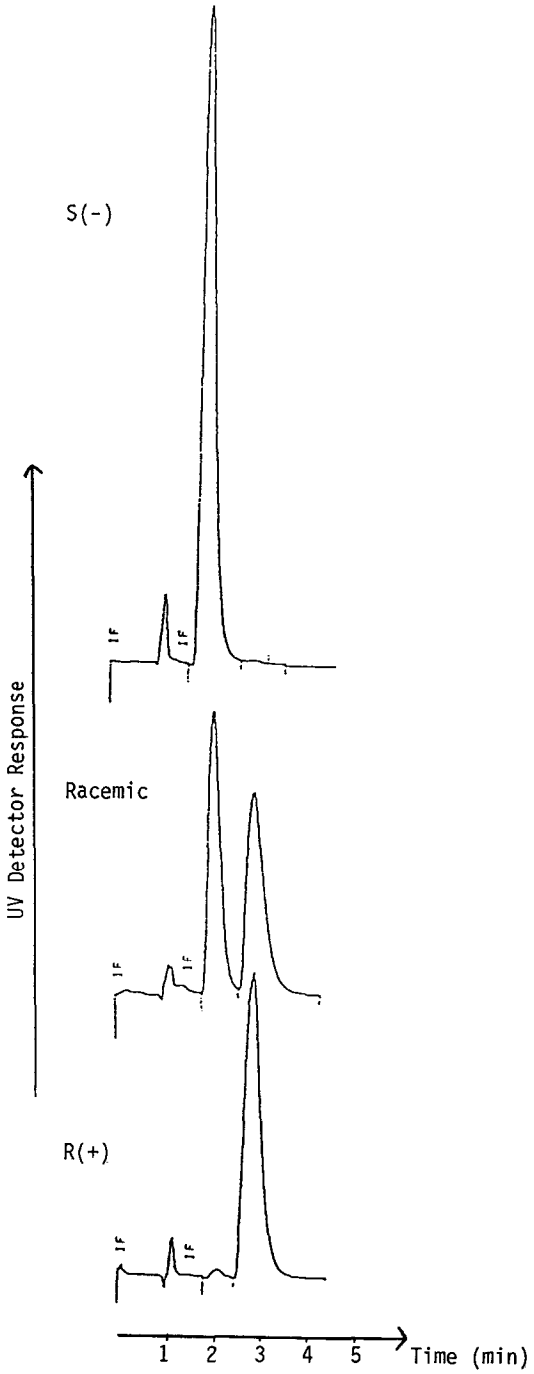

Fig. 1. Determination of CMPP free acids using the Chiral-AGP CSP. Mobile phase, 10 m*M* phosphate buffer (pH 6)–propan-2-ol (96:4, v/v); flow-rate, 0.9 ml/min; detection, 240 nm.

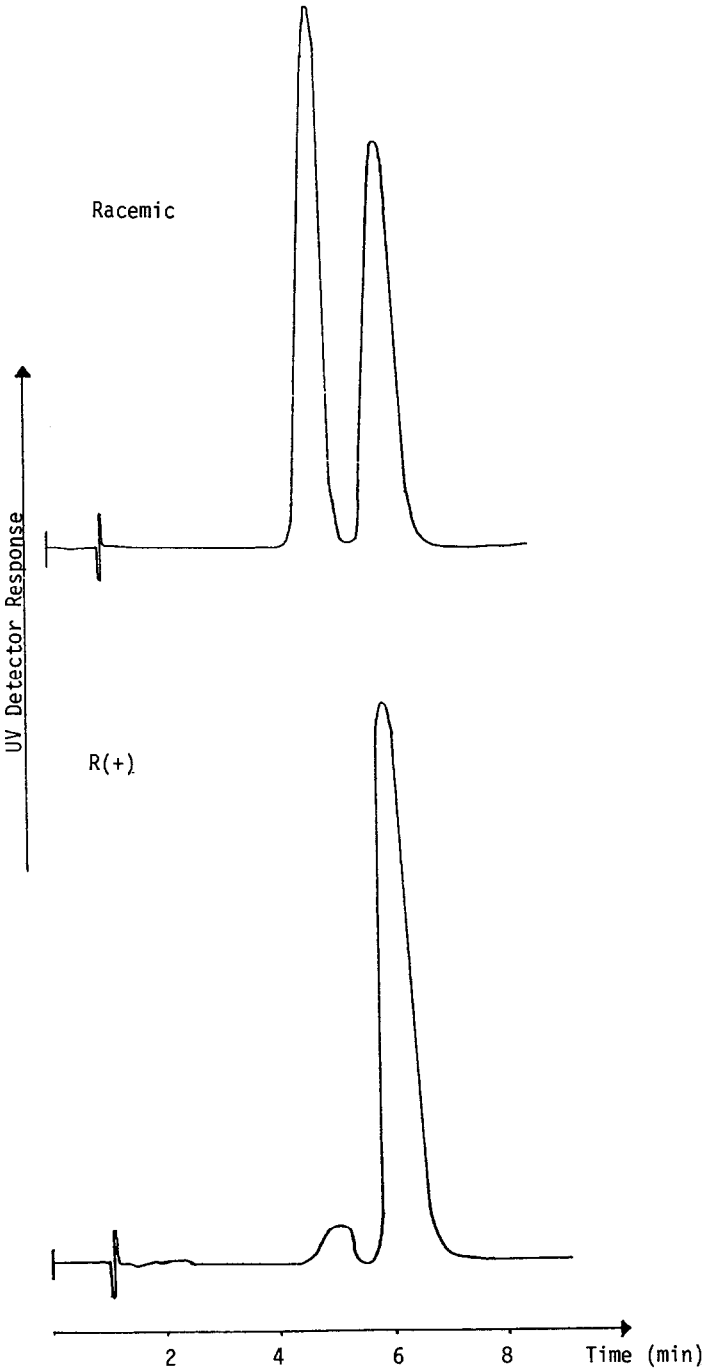

Fig. 2. Determination of 2,4-DP free acids using the Chiral-AGP CSP. Mobile phase, 10 m*M* phosphate buffer (pH 5.2)–propan-2-ol (96:4, v/v); flow-rate, 0.9 ml/min; detection, 240 nm.

TABLE II

THE ANALYSIS OF CMPP ESTERS USING D-PHENYLGLYCINE CSPs (BOTH IONICALLY AND COVALENTLY BONDED)

Mobile phase, hexane with various concentrations of propan-2-ol; flow-rate, 1.0 ml/min; detection, 240 nm.

Column	*Propan-2-ol concentration (% v/v)*	*Ester of CMPP*	*Retention times (min)*		*Resolution (R_s)[a] or PSF[b]*
			1st	*2nd*	
Ionic	1.0	(±)-Methyl	2.7	2.9	*PSF* = 0.71
		(±)-Ethyl	2.7	3.0	*PSF* = 0.77
	0.2	(±)-Methyl	7.43	8.25	R_s = 1.50
		(±)-Ethyl	6.61	7.30	R_s = 1.18
		(±)-2-EH	5.36	5.66	*PSF* = 0.60
		(±)-BOE	9.51	10.63	R_s = 1.50
		(+)-BOE	9.50	–	–
	0.1	(±)-2-EH	6.91	7.41	*PSF* = 0.78
	0.05	(±)-2-EH	9.12	9.88	*PSF* = 0.85
		(+)-2-EH	9.10	–	–
	0.00	(±)-2-EH	23.15	25.10	*PSF* = 0.79
Covalent	0.2	(±)-Methyl	8.79	9.54	*PSF* = 0.76
		(±)-Ethyl	7.97	8.53	*PSF* = 0.71
		(±)-BOE	12.63	13.67	*PSF* = 0.79
		(±)-2-EH	6.06	6.29	*PSF* = 0.33

[a,b] See Table I.

shown in Table II. At a concentration of 0.2% propan-2-ol, baseline separation was obtained for the model CMPP methyl and ethyl esters and for the commercially important BOE ester. A further decrease in propan-2-ol concentration to 0.05% was necessary to obtain a reasonable separation of the 2-EH ester. Figs. 3 and 4 show the chromatograms obtained under optimum conditions for the BOE and 2-EH esters of CMPP.

Linearity studies using the *R*(+)-BOE ester showed the detector response to be linear over the range 0.05–0.25 mg/ml (r = 0.9994); the retention times were reproducible and independent of solute concentration. It should be noted that for the 2-EH ester a second chiral centre is associated with the 2-EH group. Racemic 2-ethylhexanol is used commercially in their production, so four isomers (*RR, RS, SR* and *SS*) are possible for the racemic ester. Only two peaks were observed when racemic CMPP 2-EH ester was chromatographed. Only one major peak was observed for the 2-EH ester of *R*(+)-CMPP. Chiral recognition was therefore limited to the asymmetric centre associated with the CMPP component of the ester. This is understandable as the 2-EH chiral centre is not adjacent to groups capable of dipole stacking or participating in π–π interactions.

The retentions of the BOE and 2-EH esters on the covalent column (Table II) were similar to those on the ionic column and the elution order was the same. The separation obtained, however, was poorer in the former instance. This may be due to the ionic CSP having an additional interactions involving the ionic linkage.

One disadvantage of the method presented is the elution order, the minor com-

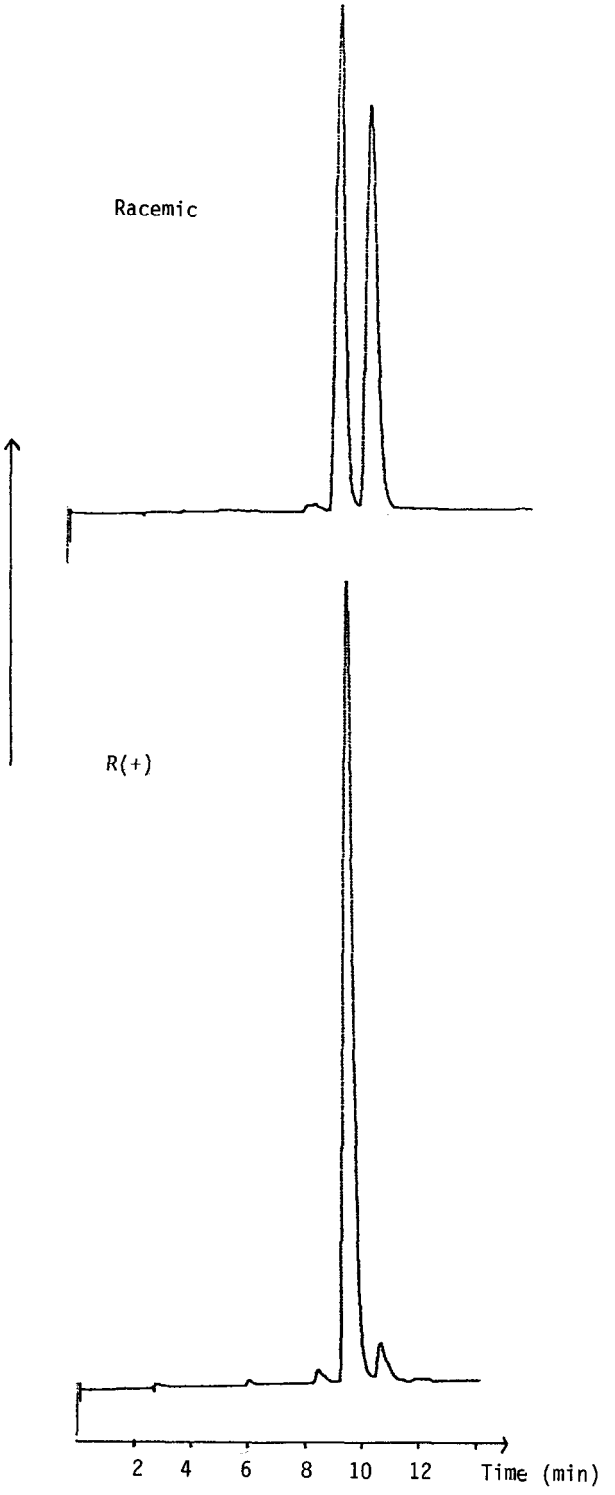

Fig. 3. Determination of CMPP-BOE esters using the ionic D-Phenylglycine CSP. Mobile phase, hexane propan-2-ol (99.8:0.2, v/v); flow-rate, 1 ml/min; detection, 240 nm.

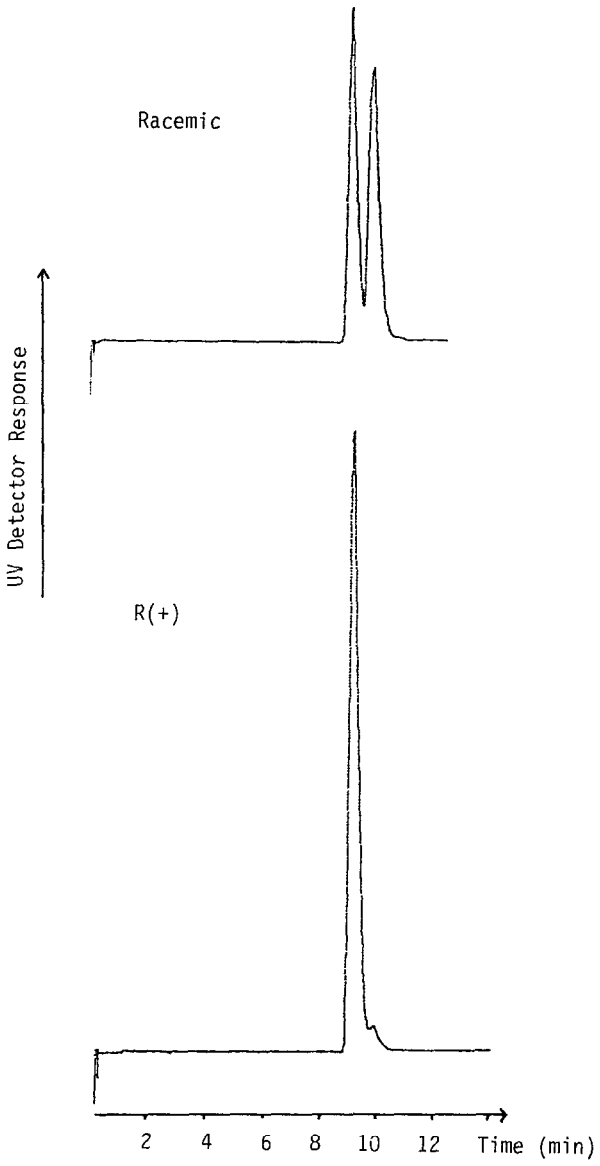

Fig. 4. Determination of CMPP-2EH esters using the ionic D-phenylglycine CSP. Mobile phase, hexane–propan-2-ol (99.95:0.05, v/v); flow-rate, 1 ml/min; detection, 240 nm.

ponent eluting after the active *R*(+)-enantiomer. This should be easily overcome by using a Pirkle phase of opposite configuration [*i.e.* N-(3,5-dinitrobenzoyl)-(*S*)(+)-phenylglycine]; such phases are commercially available in both the ionic and covalent forms.

CONCLUSIONS

CMPP and 2,4-DP free acids can both be determined directly using the second-generation Chiral-AGP α_1-acid glycoprotein CSP. BOE and 2-EH esters of CMPP can be determined directly using an ionically bonded D-phenyglycine (Pirkle type 1-A) CSP. Both methods are robust and the analysis times are short, making them suitable for adoption as primary reference methods.

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