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CHROMATOGRAPHIC APPROACHES TO THE QUALITY CONTROL OF CHIRAL PROPRIONATE ANTI-INFLAMMATORY DRUGS AND HERBICIDES

B. BLESSINGTON*, N. CRABB and S. KARKEE
Department of Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP (U.K.)
and
A. NORTHAGE
Perkin Elmer Demonstration Unit, Phoenix Building, University of Bradford, Bradford, West Yorkshire BD7 1DP (U.K.)
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SUMMARY

The chiral analysis of a range of proprionate anti-inflammatory drugs and herbicides was accomplished by gas chromatographic separation of their *R*-α-phenylethylamide diastereomeric derivatives. Using a packed column (3% OV-1 on Chromosorb G) good separations were obtained but analysis times were rather long. Examination of the same crystalline derivatives using a capillary column (bonded methylsilicone) resulted in good separations together with much reduced total analysis times. The technique was validated through the analysis of several derivatives prepared on a micro scale and examined directly by gas chromatography without any crystallisation step. Racemic materials all gave virtually 50:50 response ratios while the analysis of optically pure samples produced no evidence of significant racemisation. Derivatives of phenoxyacetic acid and myristic acid were also prepared and chromatographed as potential internal standards.

INTRODUCTION

Marked differences in the activities of different enantiomers have been reported for chiral proprionate anti-inflammatory drugs[1] and phenoxyproprionate herbicides[2]. In some cases this has lead to the commercial production and marketing of single active enantiomers[2]. Such developments have stimulated the search for analytical methods which will form the basis of legally enforcable statutes. We have already reported on the role of the two chiral stationary phases (CSPs), the α_1- acid glycoprotein and "Ionic Pirkle" phases for the high-performance liquid chromatographic (HPLC) analysis of such materials[3,4]. The translation of such methods to routine quality control, statutory evaluation laboratories and laboratories analyzing biological matrices produces some problems. The sheer volume of samples for analysis and their chemical complexity means that methods must not only work but they should be

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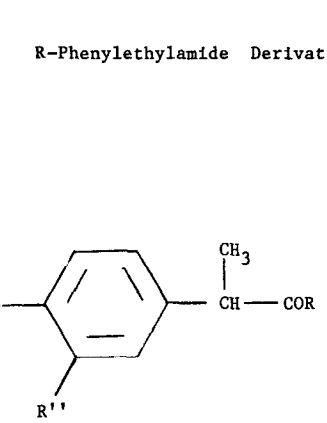
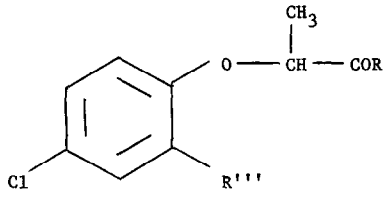
robust. Their speed and reproducibility are as important as their capacity to separate. Using these criteria CSPs have serious limitations since they are all rather "delicate". In this paper we wish to report a method for the chiral analysis of such compounds which is based on the gas chromatographic (GC) method of Vangiessen and Kaiser[5], involving the preparation of diastereomeric *R*-phenylethylamide derivatives with subsequent separation by GC (both packed and capillary columns). The samples examined were the (±), (+) and (−) forms of ibuprofen (A), flurbiprofen (B), ketoprofen (C), 2-(2,4-dichlorophenoxy)-propanoic acid (2,4-DP) (D) and 2-(4-chloro-2-methylphenoxy)-propanoic acid (CMPP) (E). Phenoxyacetic acid (F) and myristic acid (G) were also examined as potential internal standards.

Free Acids: R = OH

R-Phenylethylamide Derivatives : R = NH ► C ◄ H





A: R'= $(CH_3)_2$- CH- CH_2 R''= H

B: R'= C_6H_5 R'' = F

C: R'= C_6H_5- CO R'' = H

D: R'''= Cl

E: R'''= CH_3

F: = C_6H_5-O- CH_2- COR

G: = CH_3- $(CH_2)_{12}$- COR

EXPERIMENTAL

Materials

All CMPP samples and (*R*)-(+)-phenylethylamine were obtained as gifts from A.H. Marks & Co. (Bradford, U.K.) or May & Baker (Dagenham, U.K.). Ibuprofen and flurbiprofen samples were obtained as gifts from Boots plc. (Nottingham, U.K.) or Approved Prescription Services (Bradford, U.K.). Ketoprofen samples were gifts from May & Baker. All solvents used were HPLC grade, other materials were of laboratory grade.

Packed-column GC

The instrument used was a dual-column Pye Series 104 chromatograph, equipped with a flame ionization detector. A 5 ft. × 1/4 in. glass column packed with 3% OV-1 on Chromosorb G was used. The carrier gas was nitrogen at a flow-rate of 30 ml/min. The detector temperature was 336°C. A Hewlett-Packard 3388A integrator/printer terminal was used for recording and data processing.

Capillary-column GC

A Perkin-Elmer 8420 capillary chromatograph, connected to a Perkin-Elmer GP-100 graphics printer was used. A 12 m × 0.22 mm I.D. bonded methylsilicone (SGE BP-1) column was used. The carrier gas (flow-rate *ca.* 0,5 ml/min) was either nitrogen or helium used at an inlet pressure of 8.0 p.s.i. The line was fitted with a Supelco high-capacity carrier gas purifier. The oven temperature was 250°C for 1 min, then rising at 5°C/min to 270°C. The sample was introduced via split injection with split ratio 200:1. The injector temperature was 200°C. A high-sensitivity flame ionization detector was used at 350°C.

Identification of compounds

All samples were examined as KBr discs on a Perkin-Elmer Model 297 infrared spectrophotometer. UV spectra were recorded on a Pye Unicam SP 800 UV spectrophotometer. Ethanol (95%) was used as solvent and reference sample. Mass spectra were run, using a direct-insertion probe, on an AEI MS-902 instrument (source 250°C; 70 eV ionising energy; 100 μA emission) equipped with a Mass Spectrometry Services data system. Melting points were determined in capillary tubes on a Gallenkamp melting point apparatus.

Preparation and characterisation of R-phenylethylamide derivatives

Macro reaction. The required acid (1 g) was weighed into a 250-ml quickfit round bottomed flask. Thionyl chloride (0.4 ml) was added and the mixture was refluxed on a steam bath for 1 h. Excess thionyl chloride was removed under vacuum. The viscous residue was dissolved in dichloromethane (10 ml) and *R*-phenylethylamine (0.7 ml) was added dropwise, shaking the flask after each addition. The solution was frequently shaken for 20 min before being washed twice with hydrochloric acid (4 *M*; 5 ml) and finally with distilled water (5 ml). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. The residue was dissolved in aqueous ethanol (65%, v/v; 5 ml) by warming on a steam bath. The hot solution was filtered and then allowed to cool slowly. Crystals were collected after 1 h. A further crop of crystals were collected after keeping the mother liquor overnight at room temperature.

The crystalline derivatives were all characterised by their melting points, mass, IR and UV spectra. The principal spectroscopic peaks and melting points are shown for each derivative in Table I.

*Micro reaction.*The required acid (2 mg) was weighed into a quickfit centrifuge tube. Thionyl chloride (1 drop) was added and the tube was stoppered and heated on a steam bath for 10 min. The contents were then evaporated to dryness under reduced pressure and chloroform (0.5 ml) was added. *R*-phenylethylamide (1 drop) was added and the tube was shaken regularly over a period of 10 min. The solution was examined directly by GC.

TABLE I

CHARACTERISATION OF *R*-PHENYLETHYLAMIDE DERIVATIVES OF SOME CHIRAL PROPRIONATE ANTI-INFLAMMATORY DRUGS AND HERBICIDES, AND POTENTIAL INTERNAL STANDARDS

R-Phenylethylamide of	*Melting point (°C)*	*Principal spectroscopic data*			
		MS		*IR (cm^{-1}) (functional group region)*	*UV_{max}(nm)*
		Molecular peak(s)	*Base peak*		
(±)-CMPP	115–118	319, 317	105	3260, 1650	218, 320, 282
(−)-CMPP	124–126	319, 317	105	3250, 1650	213, 229, 280
(+)-Ibuprofen	94–96	309	161	3300, 1640	212, 258, 264
(−)-Ibuprofen	109–111	309	161	3300, 1640	212, 258, 265
(±)-Ibuprofen	73–75	309	161	3300, 1640	213, 259, 264
(+)-Flurbiprofen	144–146	347	199	3310, 1640	210, 249
(−)-Flurbiprofen	139–141	347	199	3375, 1640	210, 249
(±)-Flurbiprofen	124–130	347	199	3325, 1640	210, 249
(±)-Ketoprofen	84–86	357	105	3290, 1640	217, 255
Phenoxyacetic acid	73–74	256	105	3350, 1660	213, 270
Myristic acid	68–70	331	105	3320, 1640	213, 258

RESULTS AND DISCUSSION

A good analytical method should be applicable to the analysis of a group of structurally related compounds. We have found that a wide range of racemic organic herbicides (CMPP, 2,4-DP) and anti-inflammatory drugs (ibuprofen, flurbiprofen and ketoprofen) can all be separated by GC as their *R*-phenylethylamide diastereomeric derivatives. This contrasted with our experiences with ODS reversed-phase HPLC analysis of the same compounds.

Initially these separations were carried out on packed GC columns (3% OV-1). Table II shows the results of these separations. Where the individual enantiomers of the organic acids were available, these were derivatised as their *R*-phenylethylamides and chromatographed. Their retention times, along with those for the *R*-phenylethylamides of phenoxyacetic acid and myristic acid (potential internal standards) are also shown in Table II. Usable separations were obtained but rather high temperatures were required for the higher-molecular-mass compounds. Inevitably retention times were long.

Vast improvements, however, were found when the same compounds were analysed using a bonded methylsilicone (BP-1) capillary GC column. Initially helium was used as the carrier gas since its low viscosity allows the use of high flow-rates with only modest reductions in column efficiency. In contrast, where nitrogen is used as the carrier gas, sharp reductions in efficiency are observed when flow-rates are altered from the optimum. In these investigations, however, good results were obtainable using helium or nitrogen; nitrogen was therefore selected on the grounds of economy. The results of the separations obtained using nitrogen as the carrier gas are also shown in Table II. Comparative chromatograms for the diastereomeric amides of (±) CMPP and (±) flurbiprofen run on the packed-column and capillary GC sys-

TABLE II

GC EXAMINATION OF R-PHENYLETHYLAMIDE DERIVATIVES OF SOME CHIRAL PROPRIONATE ANTI-INFLAMMATORY DRUGS AND HERBICIDES, AND POTENTIAL INTERNAL STANDARDS

t_R = Retention time (min) from injection. R_s = Resolution = $(t_{R2} - t_{R1})[2/(W_1 + W_2)]$, where W = peak width at the base.

R-Phenylethylamide of	*Packed column (3% OV-1)*[a]				*Capillary column (BP-1)*		
	Column temperature (°C)	t_R *1st Isomer*	t_R *2nd Isomer*	R_S	t_R *1st Isomer*	t_R *2nd Isomer*	R_S
(±)-CMPP	210	21.2	24.5	1.33	2.82	3.00	1.78
(+)-CMPP	210	21.1	–	–	2.83	–	–
(−)-CMPP	210	–	24.6	–	–	3.02	–
(±)-2,4-DP	210	24.2	28.1	1.10	3.17	3.40	2.30
(+)-2,4-DP	210	24.4	–	–	3.21	–	–
(±)-Ibuprofen	200	28.7	31.7	1.20	2.89	3.03	1.42
(+)-Ibuprofen	200	–	31.7	–	–	3.04	–
(−)-Ibuprofen	200	28.5	–	–	2.91	–	–
(±)-Flurbiprofen	220	52.8	60.5	1.60	5.54	5.88	2.30
(+)-Flurbiprofen	220	–	60.7	–	–	5.86	–
(−)-Flurbiprofen	220	53.1	–	–	5.56	–	–
(±)-Ketoprofen	234	51.5	58.0	1.30	7.60	8.10	2.00
(+)-Ketoprofen	234	–	58.2	–	–	8.21	–
(−)-Ketoprofen	234	51.3	–	–	7.56	–	–
Phenoxyacetic acid	200	16.0	–	–	1.93	–	–
Phenoxyacetic acid	210	11.5	–	–	–	–	–
Mysteric acid	220	43.5	–	–	4.39	–	–

[a] Some of the packed-column data were previously published as a poster at the *Chromatographic Society International Symposium on Chiral Separations, Guilford, September, 1987.*

tems are shown in Figs. 1 and 2. Phenoxyacetic acid was found to be a suitable internal standard for CMPP, 2,4-DP and ibuprofen on both the packed and capillary systems. Myristic acid was found to be a more suitable GC internal standard for ketoprofen and flurbiprofen.

The analysis of crystalline diastereomeric derivatives (*i.e.*, those produced by the macro reactions) is inappropriate for determining the optical constitution of a sample since the crystallisation process leads to the preferential crystallisation of one diastereomer. It is therefore essential that all diastereomeric product formed remains in solution. To ensure that this occured we developed the micro derivatisation procedure.

Strict validation is required because of potential problems associated with chiral analysis based on the separation of diastereomeric derivatives. Theoretically the enantiomers of a pair may react at different rates with the chiral derivatising agent and/or the chiral reagent may be of insufficient optical purity and/or the two diastereomers may give different detector responses and/or the derivatisation reaction could result in some racemisation (although the possibility of racemisation applies equally to the chiral separation of enantiomeric derivatives).

R-phenylethylamide derivatives of several of the herbicide and anti-inflammatory samples were prepared using the micro method. Using the capillary GC system,

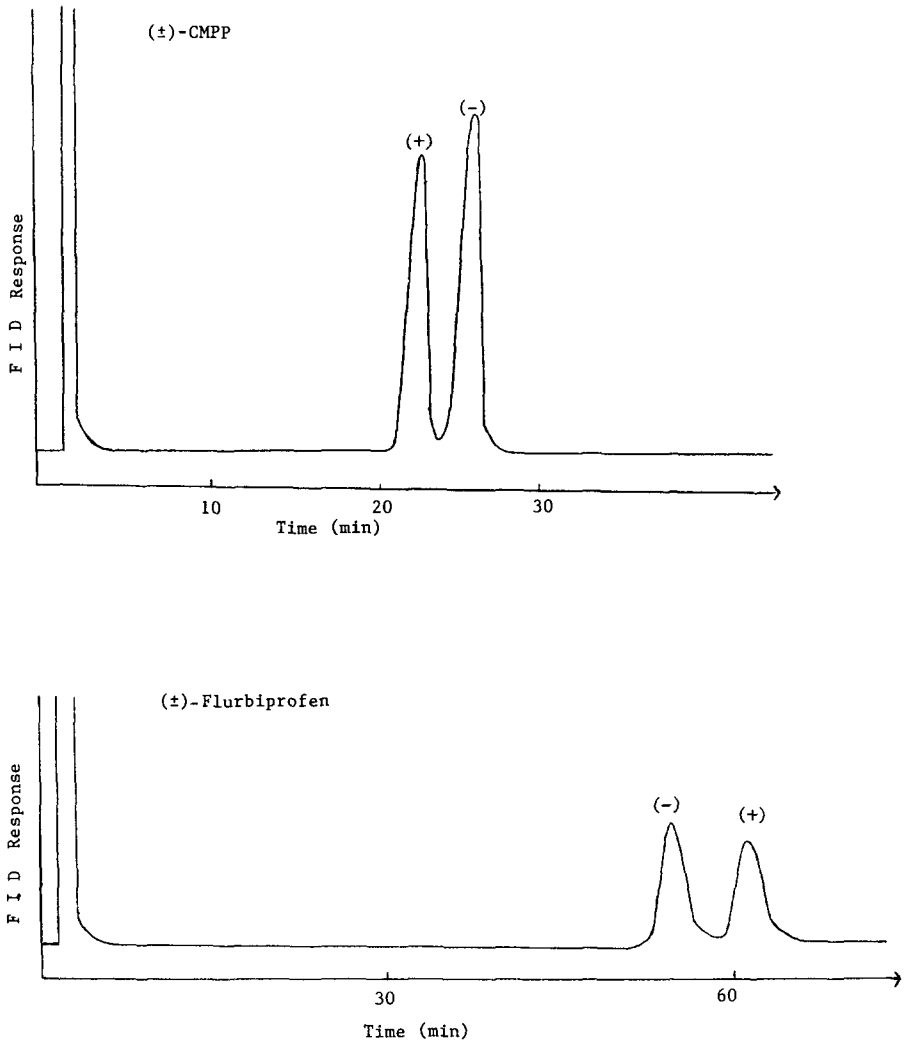


Fig. 1. Packed-column-analysis of (±)-CMPP (210°C) and (±)-flurbiprofen (220°C) as their diastereomeric *R*-phenylethylamide derivatives. FID = flame ionization detection.

each sample was chromatographed five times and the diastereomeric ratios were calculated from the integrated peak areas. For each sample the mean and standard deviation (S.D.) are shown in Table III. The racemic acids all gave virtually 50:50 response ratios for the diastereomeric amides while the optically pure samples gave between 2.9 and 5.3% of the unwanted isomer. These minor discrepancies may arise from reaction influences as discussed above. However we believe they are more likely to arise from small amounts of optical impurities present in the starting materials. We base this claim on our earlier CMPP studies, using a non-derivatising HPLC method[3]. It was therefore concluded that the method could be used to assess chiral purity.

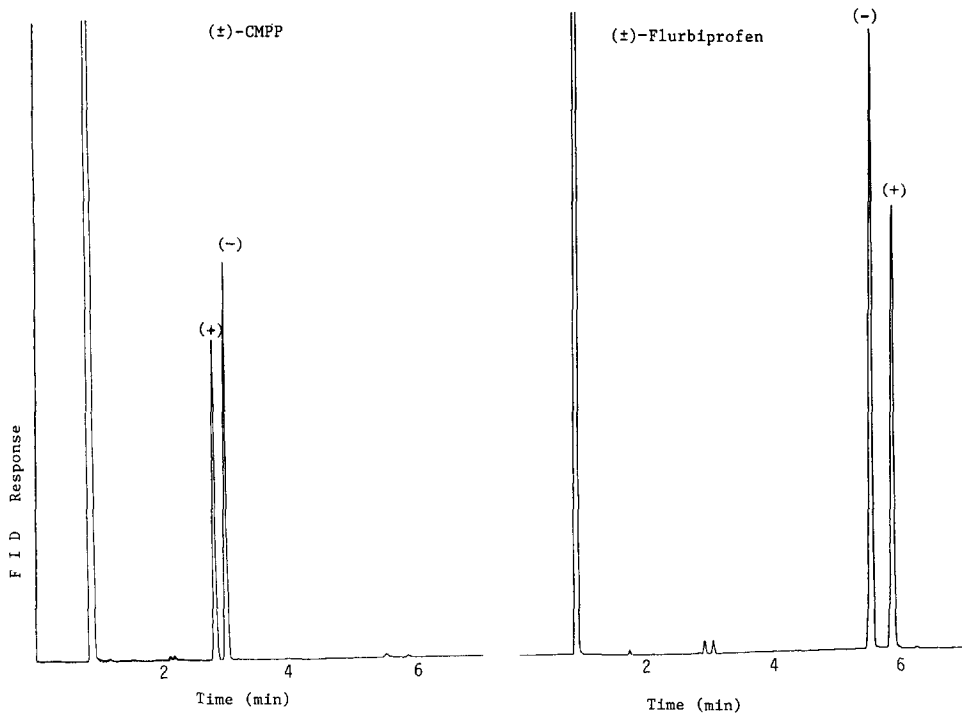


Fig. 2. Analysis of (±)-CMPP and (±)-flurbiprofen as their diastereomeric *R*-phenylethylamide derivatives using the capillary-column (BP-1) system.

TABLE III

ANALYSIS OF SAMPLES DERIVATISED USING THE MICRO METHOD

(+) and (−) designations refer to the starting enantiomeric acids, not their diastereomeric amides.

R-Phenylethylamide of	*% (+)- and (−)-isomers (Mean of 5 injections)*		*S.D.*
	(+)	*(−)*	
(±)-Ibuprofen	50.8	49.2	0.083
(+)-Ibuprofen	96.1	3.9	0.089
(−)-Ibuprofen	4.2	95.8	0.25
(±)-Flurbiprofen	48.8	51.2	0.24
(+)-Flurbiprofen	94.7	5.3	0.27
(−)-Flurbiprofen	4.5	95.5	0.32
(±)-Ketoprofen	48.4	51.6	0.31
(±)-CMPP	49.4	50.6	0.24
(+)-CMPP	97.1	2.9	0.65
(−)-CMPP	3.8	96.2	0.08
(±)-2,4-DP	51.0	49.0	0.16

Further work is in hand to optimize the reaction conditions and to determine and minimize the extent of racemisation. Comparative studies using chiral HPLC are also in hand.

To our knowledge no chromatographic methods have been reported for the direct chiral analysis (*i.e.*, without any form of derivatisation) of such a range of acids. If derivatisation is required for indirect methods then distereomer formation followed by separation on conventional GC phases offers a rapid and robust analysis suitable for quality control and statutory purposes.

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