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# The enantiomeric resolution of ciprofibrate and related compounds by HPLC using chiral stationary phases

N.H. ANDERSON,* D. JOHNSTON*† and P.R. VOJVODIC*‡

* *Sterling Winthrop Pharmaceuticals Research Division, Alnwick, Northumberland NE66 2JH, UK*
† *Present address: Sterling Winthrop Pharmaceuticals Research Division, Rensselaer, NY 12144, USA*

**Abstract**: The attempted chromatographic resolution of the drug ciprofibrate and a range of related compounds, containing the chiral moiety 2,2-dichlorocyclopropylbenzene, using five different chiral stationary phases is described. Aqueous mobile phases were used throughout and the successful separation of nine out of 12 pairs of enantiomers was achieved. Structures remote from the chiral centre were seen to affect chiral recognition.

**Keywords**: *Ciprofibrate; chiral HPLC; cellulose triacetate; cyclodextrin; alpha 1 acid glycoprotein.*

## Introduction

It has long been recognized that the individual enantiomers of chiral drugs are likely to possess different potencies because of differences in intrinsic activity, in pharmacokinetic behaviour or in metabolism [1]. According to Reidenberg [1], less frequently, enantiomers may exhibit different pharmacological properties. Although, in principle, it is desirable to develop drug substances as single enantiomers, the technology to enable this to be done in a cost-effective manner has only recently become available and therefore most drugs are marketed as racemates. The development of chiral stationary phases for GLC and HPLC has greatly facilitated the determination of enantiomeric purity [2] and the chiral analysis of a number of drug substances has been reported [3, 4].

The drug ciprofibrate, (2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methyl propanoic acid), (Fig. 1) is a hypolipidaemic agent currently marketed as a racemic mixture [5]. The objective of this work was to develop HPLC methods to resolve, at the analytical scale, enantiomers of the synthetic precursors, the drug and simple derivatives of the drug to allow synthetic procedures for preparative scale resolution to be developed. The approach is based on the use of a range of HPLC chiral stationary phases with reversed-phase (aqueous) mobile phases. As all the compounds tested contain the 2,2-dichlorocyclopropylphenyl moiety the effect on separation of structures remote from the chiral centre could be determined.

## Experimental

Five columns were chosen for the investigation. These were:

(1) Beta cyclodextrin–Cyclobond I (25 cm × 4.6 mm i.d.);

(2) gamma cyclodextrin–Cyclobond II (25 cm × 4.6 mm i.d.);

(3) alpha 1 acid glycoprotein–EnantioPac (10 cm × 4.0 mm, i.d.);

(4) cellulose triacetate–ChiralCel OA (25 cm × 4.6 mm i.d.); and

(5) cellulose triacetate–ChiralCel CA-1 (25 cm × 4.6 mm, i.d.).

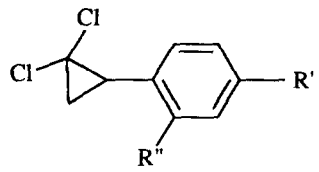
Columns 1 and 2 are manufactured by Advanced Separation Technologies Inc. (USA), column 3 by LKB-Produkter AB (Sweden) and columns 4 and 5 by Daicel Chemical Industries Ltd (Tokyo, Japan).

The ChiralCel OA column consists of cellulose triacetate bonded to a silica substrate while the CA-1 column is packed with pure microcrystalline cellulose triacetate.

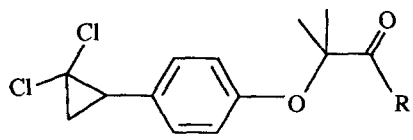
All columns were used with reversed-phase solvents as follows:

Cyclobond columns: methanol–water and acetonitrile–water mixtures.

‡ Author to whom correspondence should be addressed.




| | R' | R" |
|---|---|---|
| 1 | H | H |
| 2 | H | $NH_2$ |
| 3 | $NH_2$ | H |
| 4 | OH | H |
| 5 | OMe | H |
| 6 | OAc | H |
| 7 | $OSO_2Me$ | H |
| 8 | OCOPh | H |




| | R | |
|---|---|---|
| 9 | OH | Ciprofibrate |
| 10 | OMe | |
| 11 | OEt | |
| 12 | $NMe_2$ | |

**Figure 1**
Structures of ciprofibrate and related compounds.

EnantioPac column: propan-2-ol–10 mM, pH 6.0 phosphate buffer mixtures.

Cellulose triacetate columns: ethanol–water and methanol–water mixtures.

HPLC grade organic solvents were supplied by Rathburn Chemicals Ltd (UK).

The compounds of interest are shown in Fig. 1. All contain the 2,2-dichlorocyclopropylphenyl moiety which possesses a chiral carbon atom in the cyclopropyl ring adjacent to the phenyl ring.

## Results and Discussion

In most reported cases of resolution of racemates by HPLC, analytes have contained hydrogen bonding groups close to the chiral centre and this appears to have played a major role in the chiral recognition process. In ciprofibrate the hydrogen bonding groups are remote from the chiral centre and there appeared to be no good literature precedents for the separation of this type of molecule.

### *Cyclodextrin columns*

The cyclodextrins used in these columns consist of a ring of six, seven or eight glucose molecules bonded to a silica support [6]. The rings take the form of hollow truncated cones. Solute molecules of the correct size can fit inside the cyclodextrin molecule and, if correctly positioned, chiral functions on the solute can interact with the open edge of the cyclodextrin ring giving rise to a chiral interaction and ultimately separation. The recognition mechanism in most cases appears to include steric and hydrogen bonding interactions involving groups adjacent to the chiral centre of the solute molecule. The compounds under investigation do not possess any hydrogen bonding ability close to the chiral centre but it was felt that the interaction of the C—Cl dipole of the solute with dipoles on the cyclodextrin molecule might permit interactions capable of allowing chiral recognition.

To achieve the correct orientation of ciprofibrate in the cyclodextrin ring, allowing interaction of the chiral centre with the open edge of the ring, the methyl propanoic acid moiety must sit in or protrude through the base of the ring. This is unlikely to occur with alpha cyclodextrin as the diameter of the cavity is only 4.5–6.0 Å [6], so attempted separations were restricted to the beta and gamma cyclodextrins (6–8 and 8–10 Å diameter, respectively).

The mobile phases used consisted of simple methanol–water or acetonitrile–water mixtures. Flow rates of 0.5 or 1 ml $min^{-1}$ were employed at ambient temperature (20–25°C).

Initially, only the two anilines, ciprofibrate and the methyl and ethyl esters of ciprofibrate were available for investigation on these columns (compounds 2, 3, 9, 10 and 11). No resolution of any of these compounds was observed.

### *The alpha 1 acid glycoprotein column*

Numerous separations of pharmaceutical compounds, in a wide range of different structural classes have been reported for the alpha 1 acid glycoprotein column [4, 7].

Of the 12 compounds used in this study six were successfully separated (Table 1). Inspection of these structures (compounds 1, 2, 3, 6, 7 and 12) did not show any common

**Table 1**
Chromatographic conditions and results

| Compound | EnantioPac column | | | | ChiralCel OA column | | | | ChiralCel CA-1 column | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mobile phase $^{i}$PrOH:buffer* | $k'$† | α | $R$ | Mobile phase MeOH:water | $k'$† | α | $R$ | Mobile phase EtOH:water | $k'$† | α | $R$ |
| 1 | 3:97 | 11.5 | 1.22 | <0.6 | Not tested | | | | 90:10 | 3.8 | 1.46 | 1.83 |
| 2 | 7:93 | 3.1 | 1.20 | 1.01 | 40:60 | 9.0 | 1.31 | 1.18 | 90:10 | 5.0 | 1.35 | 1.30 |
| 3 | 5:95 | 6.3 | 1.64 | 3.23 | 40:60 | 6.3 | 1.40 | 1.39 | 90:10 | 4.3 | 1.29 | 0.8 |
| 4 | Not resolved | | | | Not resolved | | | | Not resolved | | | |
| 5 | Not resolved | | | | 50:50 | 8.0 | <1.1 | <0.6 | 90:10 | 3.0 | 1.33 | 1.38 |
| 6 | 3:97 | 5.5 | 1.18 | <0.6 | Not resolved | | | | Not resolved | | | |
| 7 | 3:97 | 7.5 | 1.10 | <0.6 | 50:50 | 8.0 | 1.13 | <0.6 | 85:15 | 6.3 | 1.16 | <0.6 |
| 8 | Not resolved | | | | Not resolved | | | | Not resolved | | | |
| 9 | Not resolved | | | | Not resolved | | | | Not resolved | | | |
| 10 | Not resolved | | | | 50:50 | 4.0 | 1.63 | 1.7 | 70:30 | 5.4 | 2.66 | 5.23 |
| 11 | Not resolved | | | | 60:40 | 5.6 | 1.13 | 0.88 | Not tested | | | |
| 12 | 15:85 | 3.6 | 1.76 | 3.33 | Not tested | | | | 75:25 | 1.1 | 1.56 | 0.9 |

*Buffer is 10 mM pH 6.0 phosphate buffer.
†$k'$ is the capacity factor of the first enantiomer eluted.

structural features. What is clear is that structures remote from the chiral centre have the ability to influence the separations. An example of this is the amide derivative of ciprofibrate which is separated and the methyl and ethyl esters which cannot be resolved. The differences in the structures of these molecules are well removed from the chiral centre. This behaviour suggests that the interaction of the solute with the protein substrate is more complex than the three-point interaction proposed by Dalgleish [8]. The protein surface possesses many different potential binding sites capable of chiral recognition so the effect of solute structure remote from the chiral centre could in principle alter the site of binding. Both these factors make any prediction or rationalization of separation on alpha 1 acid glycoprotein difficult.

*Cellulose triacetate*

The cellulose triacetate column, ChiralCel OA was selected for this work on the basis of a number of successful separations reported in the literature [9]. Two of the separations were of compounds having chiral carbon atoms adjacent to phenyl rings and no hydrogen bonding capability (trans-1,2-diphenylcyclopropane and 2-phenylpropionitrile), features common to ciprofibrate and the related compounds.

Successful separations were achieved for six of the 10 compounds tested using the ChiralCel OA column (Table 1). The alternative CA-1 column uses pure cellulose triacetate without any support material. It was argued that the CA-1 column contained more cellulose triacetate than the OA column and would therefore give better separations (in terms of resolution) and would have a higher loading capacity. Successful separations of seven of the 11 compounds of interest were then achieved using this column. As expected resolution was increased in most cases (Fig. 2). All the compounds tested possess the same chiral group but the degree of separation differed widely and was influenced by features remote from the chiral centre. This was demonstrated by the four derivatives of the phenol: the methyl ether and mesylate were successfully resolved but the acetate and benzoate esters remained unresolved.

Review of the literature reveals that the precise mechanism for chiral recognition on cellulose triacetate is not clear. It does not

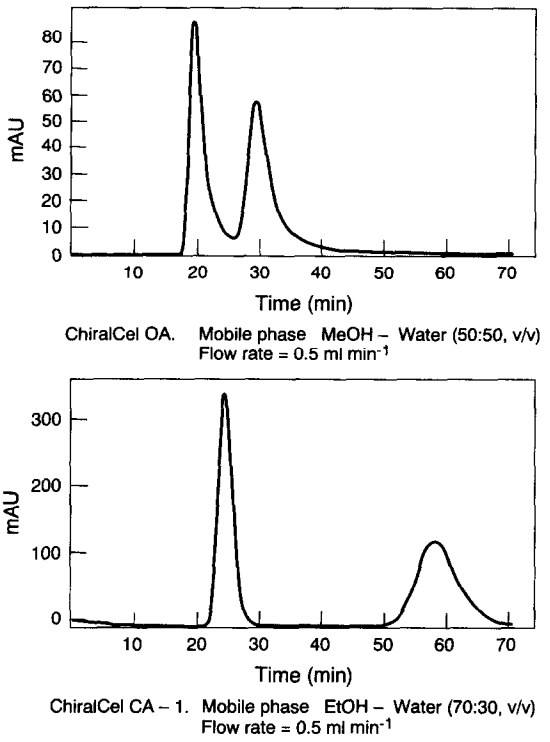



**Figure 2**
Resolution of ciprofibrate methyl ester.

appear to be a direct diastereomeric interaction between individual glucose units and solute molecules. It has been proposed that inclusion of the solute molecule into chiral cavities is the predominant interaction [10, 11]. The tertiary structure of the derivatized cellulose is therefore a key factor and several papers studying the effect of the crystallinity of the cellulose on chiral resolution have been published [12, 13].

Several cellulose derivatives are now commercially available but selection of the correct phase for a particular solute is still an art rather than a science. Rationalization of the behaviour of a set of related compounds on a particular phase is also difficult.

## Conclusions

Although one of the prime objectives, separation of the enantiomers of ciprofibrate, was not achieved, successful resolution of most of the enantiomers of the related compounds was achieved. Although the substances tested all contained the same chiral moiety, enantiomeric resolution was unpredictable as substituents remote from the chiral centre were shown to influence resolution.

Initially, the limited functionality adjacent to the chiral centre was expected to limit the potential for strong chiral interactions capable of resolving ciprofibrate enantiomers. The results show that this is not the case for either the protein or cellulose triacetate columns.

Despite the lack of success with the cyclodextrins, separations using cellulose triacetate as a stationary phase were achieved. Both the cyclodextrins and cellulose are constructed from glucose units so it is feasible that resolution can be achieved on the commercially available acetylated cyclodextrin columns which are chemically similar to cellulose triacetate.

Since completion of this work Armstrong has reported the separation of ciprofibrate enantiomers using a derivatized cyclodextrin column (beta cyclodextrin derivatized with *S*-(+)-1-naphthylethylisocyanate) using normal-phase conditions [14]. This separation does not appear to be dependent on the inclusion complexation mechanism that operates in the reversed-phase mode.

The successful separation of many of the related compounds now allows the optical purity of these materials to be measured. This ability is essential if the preparation of the individual enantiomers of a target molecule is to be achieved by conversion of a single enantiomer of a synthetic intermediate or derivative.

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