Journal of Chromatography A, 652 (1993) 259–262
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 089

Separation of positional isomers and enantiomers using capillary zone electrophoresis with neutral and charged cyclodextrins

N.W. Smith

Chemical Analysis Department, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 0HE (UK)

ABSTRACT

Capillary zone electrophoresis is a highly efficient analytical technique that has been shown to be particularly useful for the analysis of isomers. The neutral chiral host compound β-cyclodextrin is capable of resolving analytes that possess charged functional groups. The enantiomers of two cyclic amines are resolved in a single run using a background electrolyte containing β-cyclodextrin at pH 2.7, conditions under which the compounds are positively charged. In order to resolve the enantiomers of neutral analytes, it is necessary to use a chiral selector capable of being ionised, and examples are given of the separation of neutral positional isomers and enantiomers using a carboxymethylethyl-β-cyclodextrin at high pH.

INTRODUCTION

Cyclodextrins are well established compounds capable of forming inclusion complexes in aqueous solutions. These cyclodextrins are cyclic oligosaccharides consisting of either 6, 7 or 8 glucose units and are termed α, β or γ, respectively. They consist of a truncated cone containing secondary hydroxyl groups at the entrance of the cavity, with a relatively hydrophobic interior. Therefore, molecules of certain size and stereochemistry can form inclusion complexes by hydrophobic interaction in the cyclodextrin cavity and from hydrogen bonding by the secondary hydroxyl groups at the entrance of the cavity. There have been numerous papers published on the use of cyclodextrins to separate positional and geometric isomers and also enantiomers [1–3]. In addition to the use of neutral cyclodextrins, Terabe *et al.* [1,4] advocated the use of cyclodextrins with ionic groups to separate enantiomers and geometrical isomers. Terabe *et al.* pointed out that carboxylated cyclodextrins behave in an analogous way to micelles in conventional micellar electrokinetic chromatography and will electrophorese towards the anode, but will be transported towards the cathode by the electroosmotic flow since this is much stronger than the electrophoretic mobility of the cyclodextrin. During this process, solutes will be separated by inclusion into the charged cyclodextrin as it travels towards the cathode, in a way that is analogous to micellar solubilisation.

EXPERIMENTAL

The data contained in this paper were produced using an Applied Biosystems (Foster City, CA, USA) Model 270HT instrument. β-Cyclodextrin was supplied by Sigma (Dorset, UK). The Resolvosil-7 column was supplied by Technicol (Cheshire, UK). All buffers and urea were supplied by BDH (Poole, UK). Capillaries were supplied by Polymicro Technologies (Phoenix, AZ, USA).

RESULTS AND DISCUSSION

o-Nitrobenzyl alcohol (Fig. 1) is used as a starting material for a new drug entity currently

0021-9673/93/$06.00

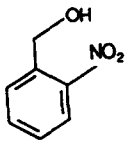


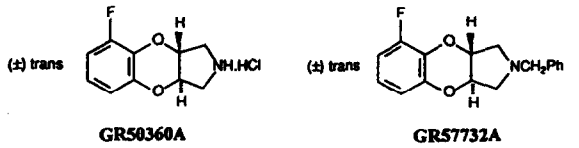
Fig. 1. *o*-Nitrobenzyl alcohol.

being progressed within Glaxo Group Research (GGR).

Attempts by HPLC to resolve *o*-nitrobenzyl alcohol from the *meta* and *para* analogues, as well as from *o*-nitrobenzoic acid have been unsuccessful. Although Terabe *et al.* [4] have shown that it was possible to resolve the *ortho*, *meta* and *para* isomers of nitrophenol using 0.1 *M* Na_2HPO_4 at pH 7.0 with and without α-cyclodextrin, we were unable to repeat these separations using the nitrobenzyl alcohol analogues. However, using carboxymethylethyl-β-cyclodextrin, we were able to achieve the desired separations (Fig. 2).

GR50360A and its N-benzyl analogue GR57732A (Fig. 3) are compounds passing through exploratory development within Glaxo Group Research.

The enantiomers of GR50360A have proved particularly difficult to resolve by HPLC, although some success was achieved using a Resolvosil column and 0.5 *M* K_2HPO_4–NaH_2PO_4 as mobile phase (Fig. 4).



GR50360A GR57732A

Fig. 3. GR50360A and its N-benzyl analogue GR57732A.

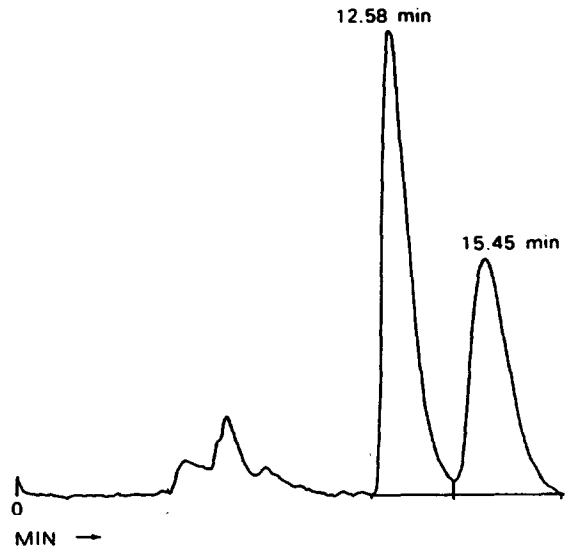


Fig. 4. Chiral HPLC separation of the enantiomers of GR50360A. Column: 150 mm × 4.6 mm I.D. Resolvosil-7; flow 0.3 ml/min; temperature 35°C; detection at 220 nm, 0.5 AUFS; injection volume 10 μl; mobile phase: 125 ml 0.5 *M* K_2HPO_4–250 ml 0.5 *M* NaH_2PO_4–250 ml water adjusted to pH 6.2.

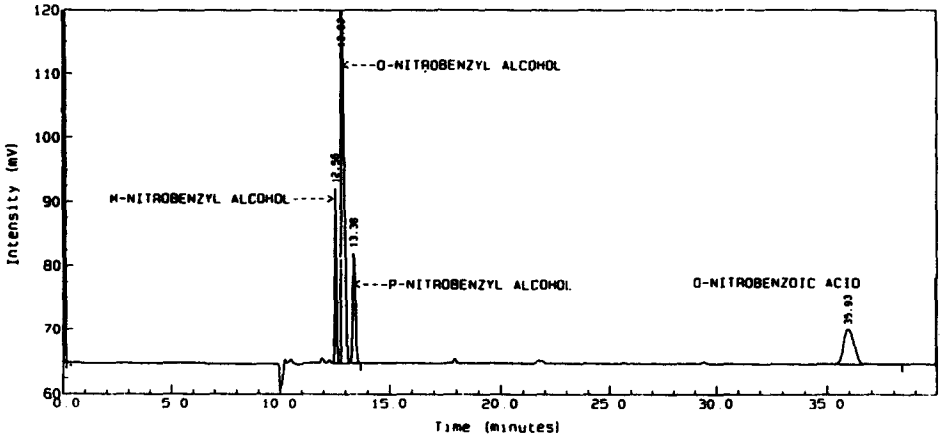


Fig. 2. Chiral separation of nitrobenzyl alcohols. Conditions: capillary 72 cm × 50 μm I.D.; applied voltage 15 kV; detection at 220 nm, range 0.03 AUFS; vacuum injection for 0.4 s; temperature 40°C; carrier: 0.025 *M* carboxymethylethyl-β-cyclodextrin in 0.05 *M* PO_4–0.03 *M* borate overall pH 8.4.

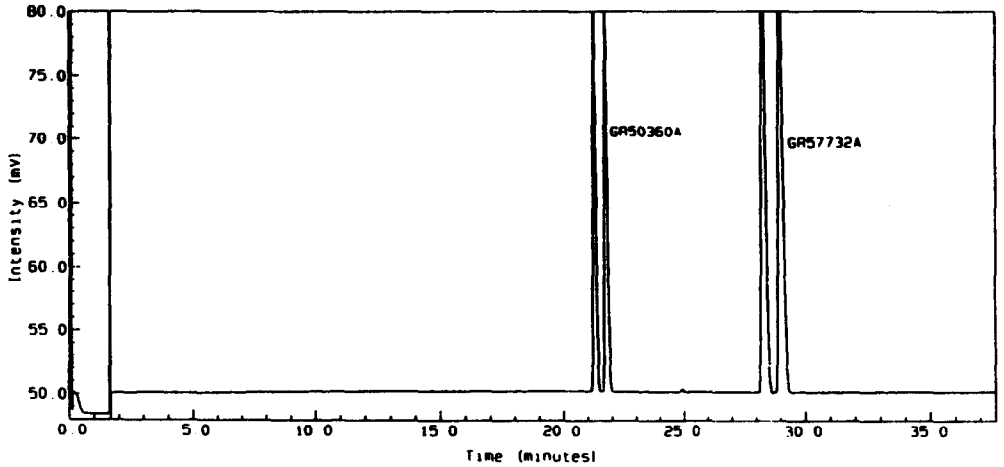


Fig. 5. Chiral separation of the enantiomers of GR50360A and the N-benzyl analogue GR57732A. Conditions: capillary 72 cm × 50 μm I.D.; applied voltage 30 kV; detection at 220 nm, range 0.03 AUFS; vacuum injection for 0.5 s; temperature 40°C; carrier: 0.1 *M* β-CD–5 *M* urea dissolved in 20% isopropanol in 0.01 *M* Tris–borate adjusted to pH 2.7 with dilute H_3PO_4.

Fig. 5 shows the baseline resolution of both pairs of enantiomers of GR50360A and the N-benzyl analogue in a single run, using β-CD as the chiral receptor.

GR50360A and its N-benzyl analogue are both

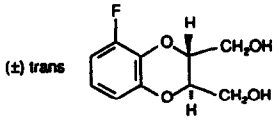


Fig. 6. GR57888X.

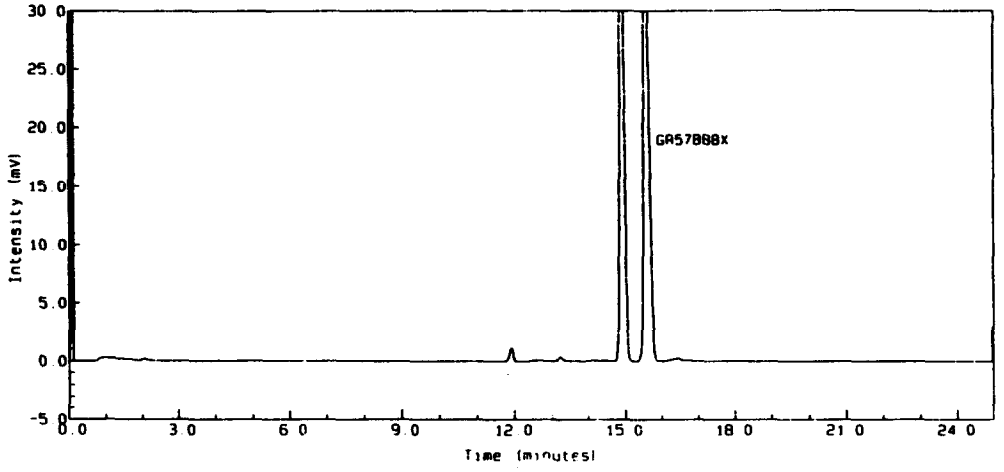


Fig. 7. Chiral separation of GR57888X. Conditions: capillary 72 cm × 50 μm I.D.; applied voltage 15 kV; detection at 220 nm, range 0.04 AUFS; vacuum injection for 1.5 s; temperature 30°C; carrier: 0.025 *M* carboxymethylethyl-β-cyclodextrin in 0.01 *M* Tris overall pH 12.4.

charged molecules at low pH and can therefore be separated using neutral cyclodextrins, however the related compound GR57888X, (Fig. 6) is neutral and can only be resolved by the use of a charged host compound.

Fig. 7 shows the baseline separation of enantiomers of GR57888X using a carboxylated methylethyl-β-cyclodextrin at pH 12.4.

DISCUSSION

There is an abundance of applications in the literature showing the use of cyclodextrins, particularly β-cyclodextrin, for the analysis of positional and geometrical isomers, as well as for enantiomers. However in order to achieve recognition for neutral species (without the use of a micelle) it is necessary to use charged cyclodextrins and we have been able to demonstrate in our laboratory several applications showing the resolution of enantiomers and positional isomers that would not be possible using neutral cyclodextrins, and that have also proved very difficult by HPLC.

REFERENCES

1 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129–134.
2 H. Nishi, T. Fukuyama and S. Terabe, *J. Chromatogr.*, 553 (1991) 503–516.
3 S. Fanali, *J. Chromatogr.*, 545 (1991) 437–444.
4 S. Terabe, H. Ozaki, K. Otsuka and T. Ando, *J. Chromatogr.*, 332 (1985) 211–217.