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Enantiomeric separation by packed column chiral supercritical fluid chromatography

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Abstract

Supercritical fluid chromatography (SFC), is now a well established chromatographic technique for the separation of a range of compounds both analytically and preparatively. The advantages of SFC over HPLC are lower fluid viscosity and higher efficiencies per unit time. Furthermore, SFC is more convenient than HPLC in operation, because solvent power of the supercritical fluids, an important operational factor, is easily controlled by pressure and temperature. Cellulose and amylose derivatives coated onto a functionalised silica backbone have been used extensively for chiral resolution by HPLC. This article illustrates the preparative separation of several racemic glibenclamide analogues using both OD (tris-3,5-dimethylphenyl carbamate supported on cellulose) and AD (tris-3,5-dimethylphenyl carbamate supported on amylose) chiral phases packed into preparative columns (250 mm × 10 mm I.D.). The effect of loading, throughput and recovery of material are discussed together with the optical purity obtained from preparative chiral SFC. Comparison with chiral HPLC for some of these compounds is shown highlighting several important advantages of SFC over HPLC.

1. Introduction

Glibenclamide is known to be a potent potassium channel blocker active in the heart and pancreas. A series of glibenclamide analogues have been synthesised for assessing their potency and selectivity in the heart versus the pancreas. A preparative chiral method was required to separate the enantiomers of a series of racemic compounds for in vitro testing.

Supercritical fluid chromatography (SFC) is now a well established technique for the separation of a range of compounds both analytically and preparatively [1]. The advantages of SFC over HPLC are lower fluid viscosity and higher efficiencies per unit time. Furthermore, SFC is more convenient than HPLC in operation, because solvent power of the supercritical fluids, an important operational factor, is easily controlled by pressure and temperature [2]. The ability to programme the density or pressure of the mobile phase is unique to SFC. These considerations led to a study of the conditions required to optimise the separation of seven glibenclamide analogues.

Cellulose and amylose derivatives coated onto a functionalised silica backbone have been used extensively for chiral resolution by HPLC [3,4]. This paper describes the preparative separation of seven racemic glibenclamide analogues using Daicel OD (tris-3,5-dimethylphenyl carbamate supported on cellulose) and AD (tris-3,5-dimethylphenyl carbamate supported on amylose) chiral phases packed into both analytical (250 × 4.6 mm I.D.) and preparative columns (250 × 10 mm I.D.).

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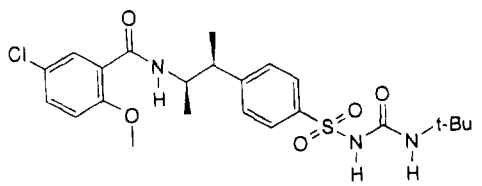

Fig. 1. Structure of a potassium channel blocker, Ro 32-1964.

2. Experimental

Industrial grade carbon dioxide was obtained from British Oxygen Company (London, UK). HPLC grade solvents, ethanol, hexane and isopropanol were purchased from Rathburn Chemicals (Walkerburn, UK). Daicel chiral columns were purchased from J.T. Baker.

Preparative chiral SFC was carried out on a Gilson SFC 3 system (Anachem, UK) equipped with a variable-wavelength UV detector fitted with a flow cell rated to 41.3 MPa. Gradient elution using ethanol–carbon dioxide was used to achieve the desired separation of the enantiomers. The Nupro valve (2 ml volume) supplied with the instrument was replaced with a Rheodyne valve Model 7037 fitted with a 3-μl volume internal loop. This valve situated downstream of the column allowed fractions to be collected as seen from the detector response avoiding any time lag.

For safety reasons a stainless-steel pressure

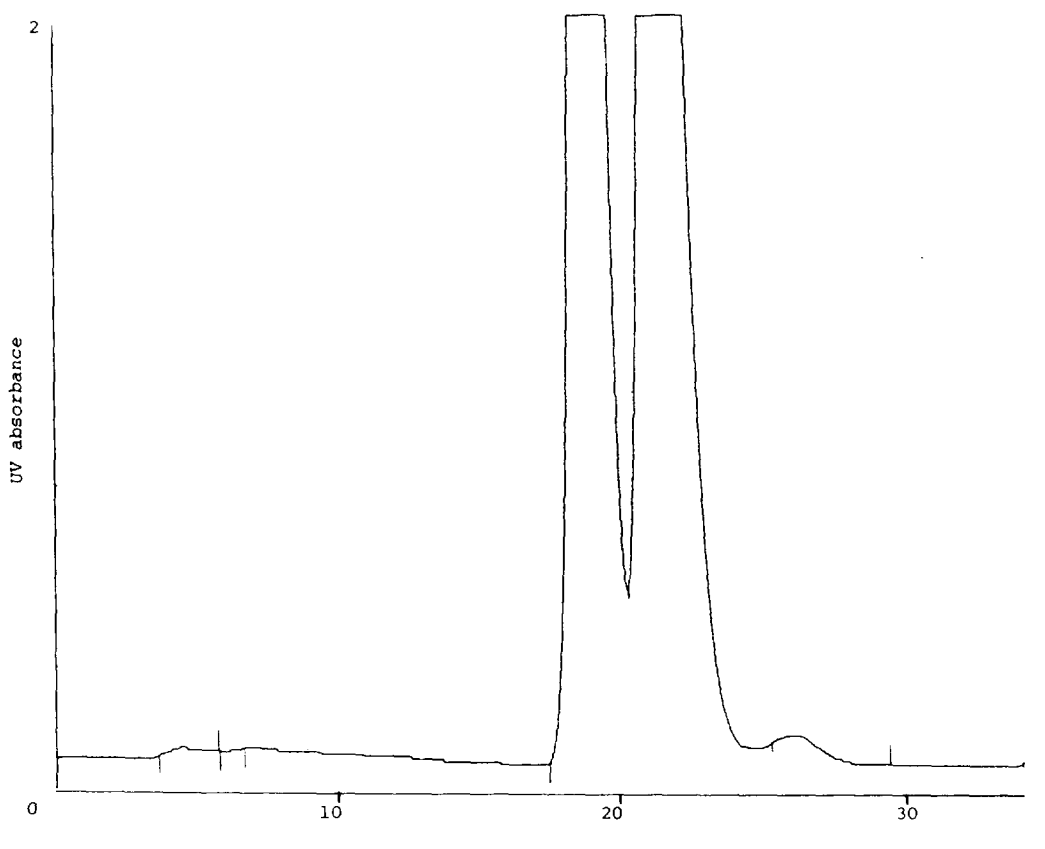

Fig. 2. Chiral SFC separation of Ro 32-1964. SFC conditions: Chiralpak AD column (250 × 10 mm I.D.). Gradient program: 2 ml 20% ethanol–carbon dioxide to 3 ml 60% ethanol–carbon dioxide over 30 min. Pressure program: 13.8 MPa to 20.6 MPa. Temperature: 45°C. UV Detection: λ_{265}, 2.0 a.u.f.s. Loading: 5 mg/0.5 ml ethanol.

guard column was purchased from Lancashire Fittings (Harrogate, UK). It was considered important that due safety precautions be taken to contain any burst that might occur when using high pressures. The guard column is certificated to 55 MPa and will accommodate a column of dimensions 250 × 20 mm I.D.

3. Results and discussion

Fig. 1 represents the typical structure of the racemic glibenclamide analogues separated by packed-column chiral SFC. Fig. 2 is the chiral SFC separation of Ro 32-1964 on a Chiralpak AD column using gradient elution. This separation is representative of the chiral separations achieved for the series of compounds under test.

Separated enantiomers of the glibenclamide analogues were checked for optical purity by chiral HPLC using either a Chiralcel OD column or a Chiralpak AD column. The first eluting enantiomer from each separation had 100% e.e. The second eluting enantiomer varied in optical purity, ranging from 99% e.e. down to 90% e.e.

Previous attempts to separate one of the glibenclamide analogues by preparative HPLC on a Chiralcel OD column using ethanol–hexane had met with limited success, Fig. 3. Low loadings and poor resolution meant that extensive chromatography was required to achieve the desired quantity and purity of enantiomers for biological testing. By contrast, SFC on a Chiralcel OD column allowed higher loadings with better peak symmetry and consequently faster throughput to make preparative SFC the method of choice, Fig. 4.

It is interesting to note that under HPLC conditions one of the racemic compounds could not be resolved on the Chiralpak AD column. However, when a Chiralpak AD column (250 × 4.6 mm I.D.) was installed in the Gilson SFC3 and run under similar SFC conditions to those already used, an acceptable separation was achieved. On scale-up to a preparative Chiralpak AD column (250 × 10 mm I.D.) this separation was maintained to afford milligram quantities of each enantiomer.

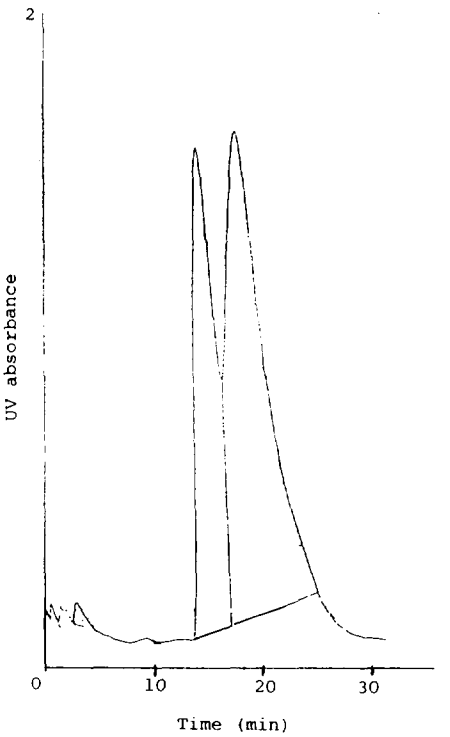

Fig. 3. HPLC separation of glibenclamide analogue. HPLC conditions: Chiralcel OD column (250 × 10 mm I.D.). Mobile Phase: 15% ethanol–hexane. Flow-rate: 20 ml/min. UV Detection: λ_{254}, 2.0 a.u.f.s. Loading: 2 mg/0.5 ml ethanol–hexane.

It is thought that this unexpected resolution under gradient SFC conditions is attributable to the low fluid viscosity and high solute diffusivity of the supercritical carbon dioxide into the pores of the silica. The result is greater permeability and therefore better chiral interaction with the stationary phase.

4. Enantiomeric recovery

It is well known that efficient containment of column eluates under SFC conditions can be difficult since the column effluent is emitted as a fine atomised mist. Before chiral separation of these compounds was undertaken an experiment was set up to investigate the recovery possible by trapping the column eluates in a suitable solvent surrounded by solid carbon dioxide.

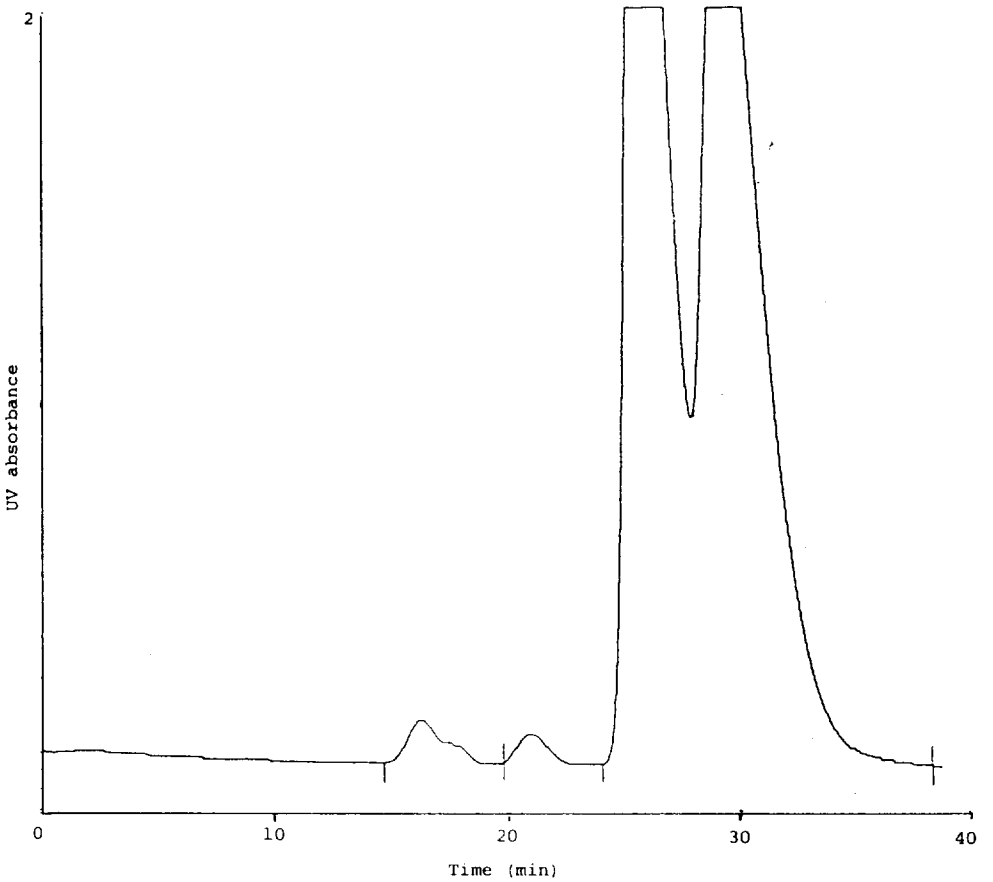

Fig. 4. Chiral SFC separation of glibenclamide analogue. SFC conditions: Chiralcel OD column (250 × 10 mm I.D.). Gradient program: 3 ml 15% ethanol–carbon dioxide to 3 ml 30% ethanol–carbon dioxide over 30 min. Pressure program: 17.3 MPa to 24.2 MPa. Temperature: 45°C. UV Detection: λ_{265}, 2.0 a.u.f.s. Loading: 3.5 mg/0.5 ml ethanol.

The compound Ro 32-2059 was chosen as a model for this exercise. A known amount (6.0 mg) was dissolved in ethanol (0.3 ml) and injected onto the Chiralcel OD column. Both eluted enantiomers were trapped by dipping the column outlet tube into 20 ml ethanol contained in a sealed vial with a small vent hole pierced in the plastic top. The vial was chilled by surrounding it with solid carbon dioxide. After evaporation of the ethanol 5.6 mg was recorded for the total enantiomers. This represented a very favourable recovery of 93%. This procedure of bubbling the column effluent into chilled ethanol contained in semi-sealed vials was adopted for all the compounds chromatographed. Recoveries from processing the other analogues varied, the worse case being 70% yield, which was considered to be quite acceptable. Ideally, the racemic compound being chromatographed should have good solubility in the trapping solvent. The better this solubility, the greater, in general, the recovery of separated isomers.

5. Loading and throughput

Typical loadings of 4 mg per injection were possible on the preparative column and 0.5 mg per injection on the analytical column. It was possible to process up to 8 mg of racemate per hour.

At the start of this work manual injection of

the glibenclamide compounds was made onto the preparative column. Very reproducible chromatography was observed with only small changes in retention time over a 30 min run time. Towards the end of this project the Gilson SFC3 system was upgraded to automatic sample injection and fraction collection controlled by the 232-401 processor.

6. Conclusions

Packed-column chiral SFC using both Chiralcel OD and Chiralpak AD chiral phases has proved to be a quick and convenient method for separating mg quantities of racemic glibenclamide analogues. The high resolving power and better efficiency resulted in superior chromatography compared to chiral HPLC. This was exemplified by the successful separation of a glibenclamide analogue under SFC conditions using the Chiralpak AD column compared to HPLC methodology where the racemate could not be resolved. One distinct advantage over HPLC is the small volume of solvent needed to be removed to recover the enantiomers. The bulk of the mobile phase comprises carbon dioxide which volatilises immediately at atmospheric pressure. Additionally there is no problem with concentration of residues in solvents, as in HPLC, where often several litres of solvent have to be evaporated to recover the isomers.

Environmentally SFC has much to offer since the volume of solvent to be removed is minimal compared to preparative HPLC, where litres of organic solvents have to be disposed of at high cost.

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