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Preparative isolation of the eutomer of cyclothiazide by high-performance liquid chromatography on a cellulose-derived chiral stationary phase with toluene–acetone as the mobile phase

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Abstract

The enantiomeric pair containing the eutomer of cyclothiazide can be resolved by HPLC on commercially available cellulose-derived chiral stationary phases (CSPs). However, the poor solubility of cyclothiazide in solvents compatible with this kind of CSP makes this separation difficult on a preparative scale. This operation was achieved with a CSP constituted by a 3,5-dimethylphenylcarbamate/10-undecenoate derivative of cellulose fixed on allylsilica gel. This CSP is resistant to all the polar solvents usually applied in HPLC.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Preparative chromatography; Diastereomer separation; Cyclothiazide

1. Introduction

Polysaccharide derivatives, in beads or coated on silica gel, are among the most widely used chromatographic sorbents for the resolution of racemic compounds by liquid chromatography, on both analytical and preparative scales [1,2]. These chiral stationary phases (CSPs) have a broad range of applications. However, their lack of stability in a number of organic solvents limits the choice of eluent, under normal-phase conditions, to heptane or hexane with a variable amount of ethanol or 2-propanol added. This is not an impediment on the analytical scale when dilute solutions are used. However, this is not the case on the preparative scale. The productivity of a preparative liquid chromatographic separation is affected not only by the selectivity factor shown by the racemic compound on the CSP but also by the solubility of the sample in the mobile phase [3].

In this study, the separation of the eutomer of

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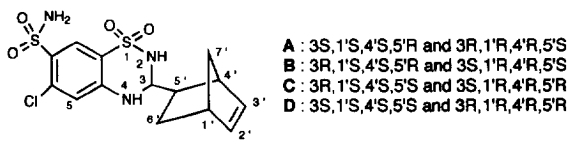

Fig. 1. Structure of cyclothiazide.

cyclothiazide was achieved using a CSP constituted of a mixed 3,5-dimethylphenylcarbamate/10-undecenoate cellulose derivative fixed on allylsilica gel [4,5]. This CSP allows the use of the solvent that is best adapted to the solution of the racemic compound in the mobile phase, without loss of column performance.

2. Experimental

The chromatographic system used consisted of a Model 590 pump (Waters, Milford, MA, USA), equipped with a Knauer (Berlin, Germany) variable-wavelength UV detector and an injector with a 30-ml loop.

2.1. Products

The racemic compound **D** (Fig. 1) was isolated from commercially available cyclothiazide by preparative chromatography [6] using a Nucleoprep 100-20 column (Macherey–Nagel, Düren, Germany) (50 × 4 cm I.D.) and toluene–ethyl acetate (75:25) as the mobile phase. The load was 900 mg of the racemic mixture. The diastereomeric purity of the product obtained, measured using a Nucleosil 10-5 column (25 × 0.46 cm I.D.) and heptane–ethanol (87:13) as the mobile phase, was 97.7%.

2.2. Chiral stationary phase

The chiral stationary phase used was prepared as indicated in Fig. 2 from Avicel cellulose (Merck, Darmstadt, Germany) and Nucleosil 100-5 silica gel (Macherey–Nagel) [4]. The elemental analysis of the resulting CSP was C 10.07, H 1.70 and N 0.78%. This analysis corresponds to a CSP with 11.0% of polysaccharide derivative (based on the percentage of nitrogen). The chiral support was packed by the slurry method into a 50 × 2 cm I.D. stainless-steel column.

3. Results and discussion

A recent study involved the separation of the two enantiomers of the most active racemic pair of cyclothiazide, which is slightly soluble in mobile phases used with cellulose derivative-coated CSPs. Cyclothiazide (Fig. 1), a diuretic of long duration of action used in the control of hypertension, has four asymmetric carbon atoms but only eight stereoisomers are possible. The separation on the preparative scale of the four pairs of enantiomers **A–D** (Fig. 3) can be performed on a silica gel column. Owing to its low solubility in heptane–2-propanol mixtures [6], the resolution of enantiomers in the D fraction, which contains the most active isomer of cyclothiazide, was laborious on a commercially available CSP based on the 3,5-dimethylphenylcarbamate of cellulose coated on silica gel. However, acetone is a good solvent for cyclothiazide, and thus mixtures of this solvent with toluene have been used to dissolve the sample and also

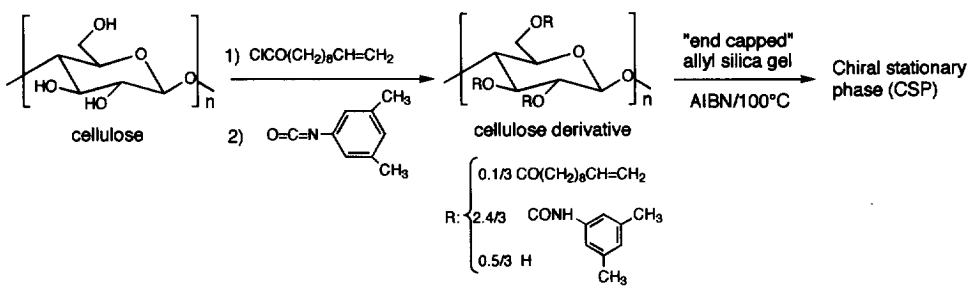

Fig. 2. Preparation of the chiral stationary phase.

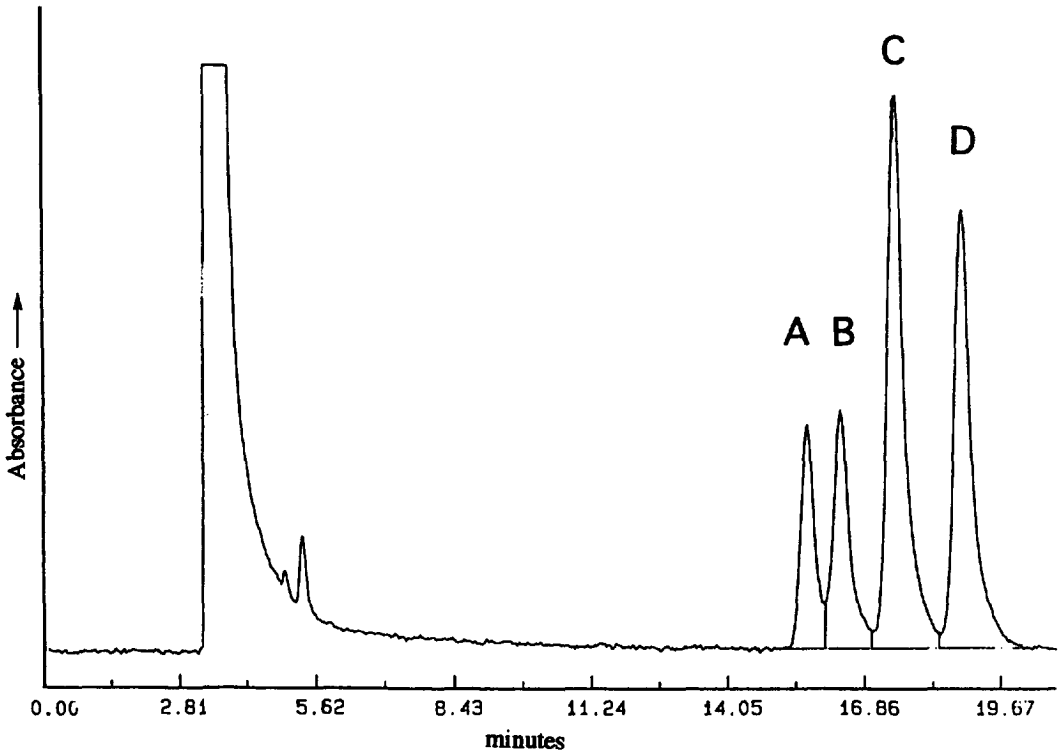

Fig. 3. Analytical separation of the four diastereomers (**A–D**) of cyclothiazide. Column, Nucleosil 100-5 (25 × 0.46 cm I.D.); mobile phase, heptane–ethanol (87:13); flow-rate, 1 ml/min; detection, UV at 210 nm.

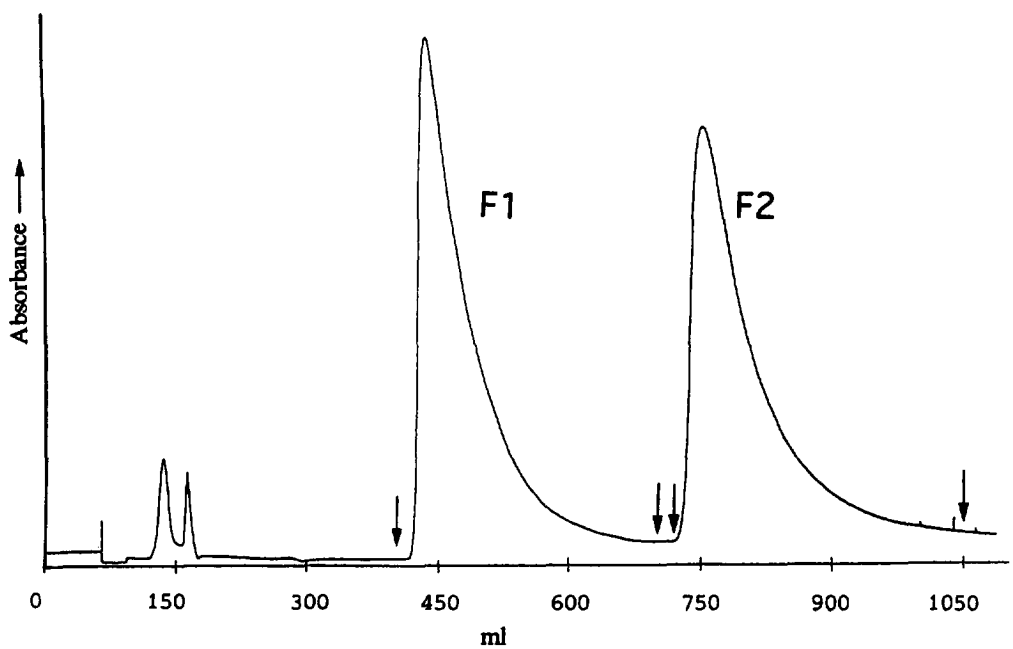

Fig. 4. Chiral preparative HPLC separation of racemic **D**. Column, 50 × 2 cm I.D.; mobile phase, toluene–acetone (90:10); flow-rate, 2.5 ml/min; detection, UV at 325 nm; sample, 30 mg of racemic **D** in 4.5 ml of toluene–acetone (2:1).

Table 1
Characteristics of the resolved enantiomeric compounds

Parameter	Fraction	
	F1	F2 (eutomer)
Volume eluted (ml)	From 400 to 700	From 725 to 1050
Diastereomeric purity (%)	97.4	97.7
Enantiomeric excess (%)	98.5	>99
$[\alpha]_D^{20}$ ($c = 1$, acetone) (°)	−78.5	+77.8

as eluents for the bonded cellulose-derived CSP. Cyclothiazide showed a UV absorption at 325 nm, which allows UV detection without interference with the mobile phase absorption.

In Fig. 4 a chromatogram is shown corresponding to the resolution of 30 mg of racemic **D** using a column filled with the CSP described. From fractions F1 and F2 15 mg of each enantiomer was recovered. The diastereomeric purity and enantiomeric excess of the two fractions were measured using analytical columns, with the same stationary and mobile phase as used in preparative separations. The results are given, together with the optical rotation values for both enantiomers, in Table 1. Fraction F2 contained the eutomer of cyclothiazide.

4. Conclusion

It is possible to resolve the most active racemic isomer of cyclothiazide on the preparative scale by HPLC using a CSP based on a cellulose derivative bonded on allylsilica gel. Thus the eutomer of cyclothiazide can be separated from its optical antipode.

This new kind of stationary phase allows the use of the best adapted eluent for the separation proposed. This is not always possible when using the commercially available polysaccharide-derived CSPs.

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