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High-performance chiral displacement chromatographic separations in the normal-phase mode III. Separation of the enantiomers of 5-vinylpyrrolidin-2-one using the Chiralcel-OD stationary phase

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Abstract

A normal-phase displacement chromatographic method has been developed for the preparative-scale separation of the enantiomers of 5-vinylpyrrolidin-2-one using Chiralcel OD as chiral stationary phase, a solvent mixture of isopropanol–*n*-hexane (10:90, v/v) as carrier solution and 2-pyrrolidinone as displacer. Sample loads as high as 28 mg could be applied onto the 4.6 mm I.D. columns. Effluent fractions of 100 μl were collected during the displacement chromatographic runs and analyzed for enantiomeric purity in order to calculate the percent recoveries and the production rates.

1. Introduction

Earlier, we demonstrated [1] that successful normal-phase displacement chromatographic separations of enantiomers could be achieved using a family of 3,5-dinitrobenzoyl ester derivatives as displacers [2] on the N-(2)-naphthylalaninate silica π-electron donor chiral stationary phase (CSP) developed by Pirkle et al. [3–7]. This paper describes the first successful use of the chiral stationary phase Chiralcel OD, a silica-based material physically coated with cellulose tris(3,5-dimethylphenyl carbamate), in the normal-phase displacement chromatographic mode of preparative separation.

Both enantiomers of the potent GABA-T inhibitor, 4-amino-5-hexenoic acid, have been prepared by the potassium hydroxide hydrolysis [8] of the corresponding enantiomer of 5-vinylpyrrolidin-2-one (VP) [9,10]. A preparative separation method was sought to augment the enantioselective synthetic efforts to produce laboratory-scale quantities of enantiomerically pure *S*-VP. The enantiomers could be well separated on the Chiralcel OD column on the analytical scale, with the interesting *S*-enantiomer eluting last [11]. Therefore, VP was selected to demonstrate that effective displacement chromatographic separations can be developed on the Chiralcel OD stationary phase when operated in the normal-phase mode.

2. Experimental

Three 4.6 mm I.D. × 250 mm Chiralcel OD

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columns, containing cellulose tris(3,5-dimethylphenyl carbamate) physically coated onto silica, were obtained from Chiral Technologies (Exton, PA, USA). One of the columns was used for the linear elution mode retention studies and the fraction analysis work. The other two columns, connected in series, were used for the displacement chromatographic separations. The columns were thermostatted at 30°C by a water jacket and a Model UF-3 recirculating water bath (Science/Electronics, Dayton, OH, USA).

The displacement chromatographic separations were carried out with a displacement chromatograph, built in our laboratory, as described in Ref. [1]. The system consisted of two Type 2020 pumps, a Type 2050 variable-wavelength UV detector (set at 210 nm) and a Type RI-3 differential refractive index detector (all from Varian, Walnut Creek, CA, USA). The samples were injected by a pneumatically activated, computer-controlled Type 7125 injection valve, equipped with a 1-ml sample loop (Rheodyne, Cotati, CA, USA). The carrier and displacer solutions, delivered by the two Type 2020 pumps, were switched by a Type 7010 switching valve (Rheodyne). System control, data collection and analysis was achieved with the aid of a Model 4270 integrator (Varian), connected to a NEC Powermate I AT-compatible computer (Computer Access, College Station, TX, USA), and the Chrom1 program developed in our laboratory [12]. Both the UV detector signals and the RI detector signals were recorded simultaneously. During the displacement chromatographic separations, 100 μl effluent fractions were collected with a Cygnet fraction collector (ISCO, Lincoln, NE, USA) as soon as the detector signals began to rise. Both data and fraction collection were halted when the RI detector signal reached its upper plateau. After the displacement chromatographic run, the displacer was removed from the columns by pumping an isopropanol–*n*-hexane (10:90, v/v) solvent mixture through them until the absorbance of the column effluent matched that of the fresh wash solvent.

A liquid chromatograph consisting of a Type 2020 pump, a Type 2050 variable-wavelength UV detector (Varian), a pneumatically activated, computer-controlled Type 7125 injection valve (Rheodyne) equipped with a 10-μl sample loop, and a Maxima 820 Chromatographic Work Station (Millipore, Bedford, MA, USA), was used to complete the elution mode retention studies and the fraction analysis work.

The carrier solution, used at a flow-rate of 1 ml/min, was an isopropanol–n-hexane (10:90, v/v) solvent mixture prepared from HPLC grade solvents (Baxter, Muskegon, MI, USA). The displacer, reagent grade 2-pyrrolidinone (PD), was obtained from Aldrich (Milwaukee, WI, USA). The sample, VP, was obtained from Dow Chemical (Midland, MI, USA), and used without further purification. Both the sample and the displacer were dissolved in the carrier solution.

For fraction analysis, the solvent was evaporated from each collected 100 μl fraction, the residues were redissolved in 500 μl volumes of the eluent [isopropanol–*n*-hexane (10:90, v/v) solvent mixture] that contained 2-phenylethanol (Aldrich) as achiral internal standard, and analyzed for chiral purity using peak areas and individual calibration curves. The reconstructed displacement chromatograms were obtained from these data and used to calculate product purities, recoveries and production rates.

3. Results and discussion

The chromatogram of a dilute VP sample, obtained with the isopropanol–*n*-hexane (10:90, v/v) solvent mixture as eluent, is shown in Fig. 1. The capacity factors for the less retained and the more retained enantiomers are 1.38 and 1.85, and the separation selectivity, α, is 1.34. Because VP is lightly retained on the Chiralcel OD column, it was expected that its starting material, PD, which is somewhat more polar than VP itself, could act as a displacer candidate. Indeed, when analyzed at infinite dilution, the capacity factor of PD was found to be higher (2.66) than that of VP; its adsorption isotherm proved to be sufficiently competitive and there

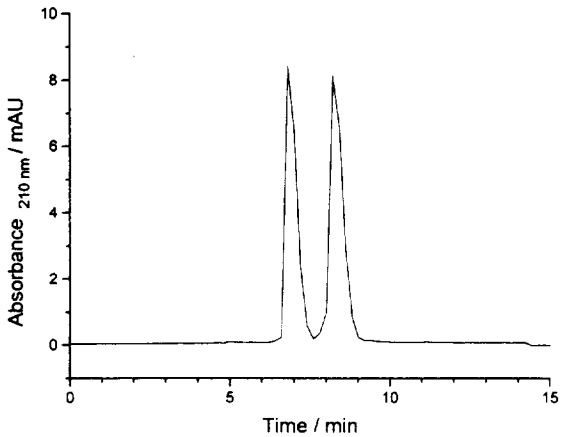

Fig. 1. Recorded elution mode chromatogram of a dilute sample of VP on a 4.6 mm I.D. × 250 mm Chiralcel OD column. Eluent: isopropanol–*n*-hexane (10:90, v/v) solvent mixture. Flow-rate: 1 ml/min. Temperature: 30°C.

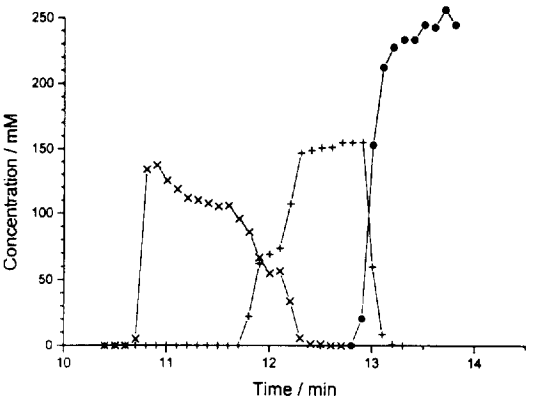

Fig. 3. Reconstructed displacement chromatogram of a 28-mg sample of VP. Conditions as in Fig. 2.

were no solubility or column stability problems up to a displacer concentration of 250 m*M* [13]. Thus, 2-pyrrolidinone, was deemed a suitable displacer for the preparative experiments.

A displacement chromatographic separation of a 28-mg VP sample, obtained with a 250-m*M* PD displacer solution is shown in Fig. 2. Analysis of the collected fractions (100 μl each) and the plotting of the results in the reconstructed displacement chromatogram (Fig. 3) revealed that at this load the displacement train is not quite developed yet (a portion of the less retained enantiomer leaves the column by elution), and that there is a small mixed zone between the two pure enantiomers. However, the more retained *S*-enantiomer and the displacer are separated by a well-defined, sharp zone.

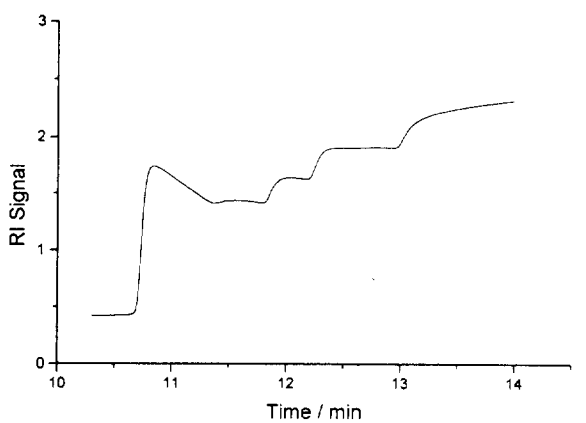

Fig. 2. Recorded displacement chromatogram of a 28-mg sample of VP obtained on two 4.6 mm I.D. × 250 mm Chiralcel OD columns connected in series. Displacer solution: 250 m*M* PD dissolved in a solvent mixture of isopropanol–*n*-hexane (10:90, v/v) used as carrier solution. Flow-rate: 1 ml/min.

The enantiomeric purities of the pooled fractions were calculated from the reconstructed displacement chromatogram and are shown in Fig. 4 as a function of the recovered amount (bottom axis) and the production rate (top axis). Forward pooling was used for the less retained *R*-enantiomer, backward pooling for the more retained *S*-enantiomer. It can be seen that about 11 mg of the *R*-enantiomer and 7 mg of the *S*-enantiomer can be collected at an enantiomeric purity of better than 99.9%. When the purity requirement is relaxed to 95%, almost identical amounts (12.5 mg vs. 12.6 mg) can be collected for both enantiomers.

Production rates (shown on the top axis) were calculated from the actual separation times, with no allowance for column regeneration. When 100% enantiomeric purity is required, about 820 μg/min can be produced of the *R*-enantiomer. For the *S*-enantiomer, the production rate at the

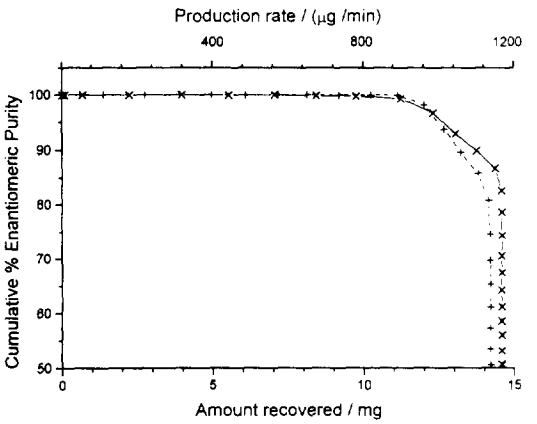

Fig. 4. Plot of cumulative % enantiomeric purity vs. amount of enantiomer recovered (bottom axis) and production rate (top axis) plots for the displacement chromatographic separation shown in Fig. 3. Symbols × = *R*-VP; + = *S*-VP.

100% purity level is lower, about 520 μg/min. Lowering the target purity to 95% boosts the production rates for both enantiomers to the same, higher level: 920 and 930 μg/min, respectively.

The recovery values for both enantiomers are very encouraging: about 78% of the *R*- and 48% of the *S*-enantiomer loaded onto the column can be recovered at 100% enantiomeric purity. For a lower, 95% target enantiomeric purity, 88% of the *R* and 87% of the *S*-enantiomer can be recovered.

4. Conclusions

It has been found that the enantiomers of VP, a pharmaceutical intermediate, could be separated on a cellulose-based Chiralcel OD stationary phase with a modest selectivity coefficient of 1.34, and moderate retention (capacity factor of the more retained enantiomer is 1.85). PD, the starting material used in the synthesis of VP, is more retained than VP itself, has a competitive, non-linear adsorption behavior, and can be readily used as a displacer for the separation. Under the selected conditions, up to 28 mg of racemic VP could be loaded onto the two 4.6 mm I.D. analytical columns connected in series. The good enantiomeric purity and high yield of the separated fractions demonstrates that though the Chiralcel OD column contains a physically coated chiral stationary phase, it can be used in the normal-phase mode to achieve preparative displacement chromatographic separations of pharmaceutically interesting enantiomers.

Acknowledgements

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