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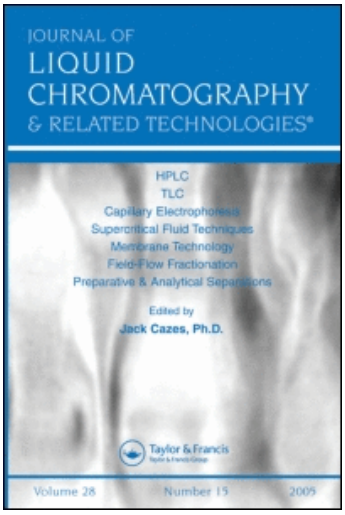

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DIRECT HPLC SEPARATION OF THALIDOMIDE ENANTIOMERS USING CELLULOSE TRIS-4-METHYLPHENYL BENZOATE CHIRAL STATIONARY PHASE

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ABSTRACT

A newly developed and commercially available cellulose tris-4-methylphenyl benzoate (Chiralcel OJ) chiral stationary phase was used for the enantiomeric resolution of thalidomide enantiomers. A simple isocratic and direct liquid chromatographic resolution of racemic thalidomide was accomplished without any derivatization. Solvent system was hexane and ethanol (50:50) with the flow rate 1 ml/min. at 23°C. The capacity factor (k') for the first eluted enantiomer R-(+)-thalidomide was 9.67 and separation factor (α) obtained was 1.54. The maximum stereochemical resolution factor (R) obtained was 15.05. Since a large stereochemical resolution with baseline separation was achieved, this method could be used for a large scale preparative separation and also for optical purity determination of the drug in bulk and formulation dosage forms.

INTRODUCTION

Thalidomide, chemically known as 2-(2,6-Dioxo-3-piperidinyl)-1H-isoindole-1,3,(2H)-dione (Scheme 1), a non-barbiturate hypnotic, was synthesised in Germany in 1953. It was reported that thalidomide had teratogenic effects

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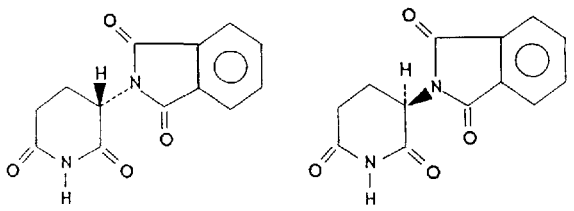

R-(+)-Thalidomide S-(-)-Thalidomide

Scheme 1. The absolute configuration of R-(+)-thalidomide and S-(-)-thalidomide.

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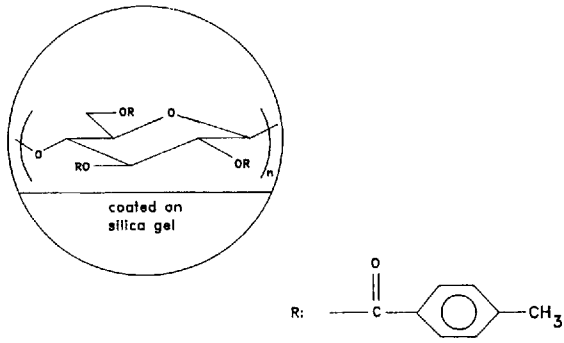
when administered to women early in pregnancy. Other toxic effects due to thalidomide also including paraesthesias and peripheral neuropathy. Thalidomide metabolites might act as folic acid antagonist and could be used as anti-neoplastics (1). Thalidomide has shown to have immunosuppressive activity. No evidence of neurotoxicity had been seen during the use of thalidomide to control erythema nodosum leprosum (ENL) and indeed thalidomide seemed particularly valuable in ENL neuritis (2). A patient with WeberChristian disease (relapsing non-suppurative panniculitis) not controlled by fluocortolone responded rapidly to thalidomide, without relapse (3). Though it was withdrawn from market because of association fetal abnormalities and teratogenic effect, thalidomide is currently used in treatment of certain and limited cases of leprosy and is administered as the racemic mixture. It is of interest to mention that teratogenic effect was due to the S-(-) enantiomer whereas the R-(+) form is responsible for the sleeping inducing effect. Blaschke et al (4) reported the resolution of thalidomide enantiomers using a chiral polymide column (80cm long) made of poly(S)-N-(1-cyclolhexylethyl) methacrylamide coupled with 1,2-ethandioldimethacrylate and a mobile phase consisted of benzene:dioxane (4:1). However, the column is not commercially available and elution was performed at 50°C.



Scheme 2. The structure of the chiral stationary phase (OJ-CSP) used in this study.

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The present communication describes the development of an isocratic and simple analytical method for the separation and identification of thalidomide enantiomers. This method utilizes the commercially available cellulose tris-(4-methylphenyl benzoate) ester (Chiralcel OJ) chiral stationary phase (Scheme 2).

EXPERIMENTAL

Apparatus

The water (Waters Associates, Millford, Massachusetts, 01757, U.S.A.) LC systems consisted of a Model 6000A pump, a U6K injector, and a Lamda-Max Model 481 LC spectrophotometer UV detector operated at 240 nm. A cellulose tris-(4-methylphenyl benzoate) ester known as Chiralcel OJ column (Daicel Chemical Industries, Ltd., Tokyo, Japan, 250mm x 4.6mm, I.D.) with particle size of 10 µm was used.

Chemicals

RS-Thalidomide (FDA-Lot A), R-(+)-Thalidomide (JCR-294401) and S-(-)-Thalidomide (JCR-294302) were supplied by Division of Drug Analy-

sis, Food and Drug Administration, St. Louis, MO, U.S.A. HPLC-grade hexane was obtained from Fisher Scientific, New Jersey, U.S.A. HPLC-grade ethanol was obtained from BDH Chemicals Ltd., Pool, England.

Chromatographic Conditions

The maximum and symmetrical stereochemical resolution of thalidomide was obtained using hexane and ethanol (50:50) on chiralcel OJ (Cellulose Tris-4-methylphenyl benzoate chiral stationary phase column, 250mm x 4.6mm, I.D.) column. Flow rate was 1 ml/min., and chart speed was 0.25 cm/min. Temperature was maintained at 23°C. Detection was obtained at UV 240 nm with sensitivity range 0.01 AUFS. Sample amount injected was 2 nmole for racemate thalidomide, 1 nmole for R-(+)-thalidomide and S-(-)-thalidomide separately.

Determination of Enantiomeric Elution Order

The enantiomeric elution order was determined by chromatographing the separate enantiomers under the similar conditions. Thus the peak that eluted with a lower capacity factor was identified as R-(+)-thalidomide and the peak that eluted with a higher capacity factor was identified as S-(-)-thalidomide.

RESULTS AND DISCUSSION

The method described here is the separation of thalidomide enantiomers using isocratic conditions and a newly developed cellulose tris-4-methylphenyl ben-zoate chiral stationary phase column (Chiralcel OJ). This cellulose derived chiral phase has been successfully used to directly separate several drugs (5,6). Chromatogram of enantiomeric separation of thalidomide is shown in Figure 1. The comparison of the chromatograms and

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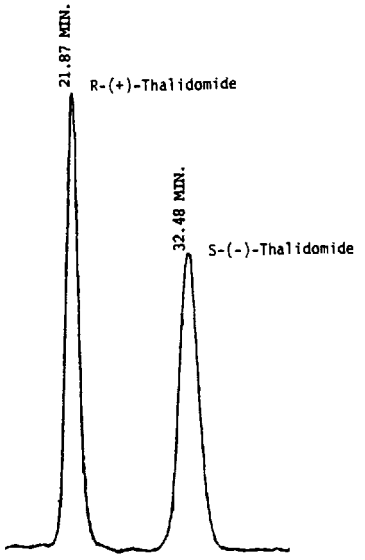

Fig. 1. Enantiomeric HPLC separation of racemic thalidomide. Chromatographic conditions:- Column: Chiralcel OJ (250 X 4.6 mm, I.D.); mobile phase: hexane & ethanol (50:50); flow rate: 1 ml/min.; chart speed: 0.25 cm/min.; temperature: 23 °C; detector: UV 240 nm; sensitivity: 0.01 AUFS; sample amount: 2 nmole.

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capacity factors of R-(+)-thalidomide (Fig. 2a) and S-(-)-thalidomide (Fig. 2b) with Figure 1, indicated that the peak eluted with a lower capacity factor was that of R-(+)-thalidomide while the peak that eluted with a higher capacity factor was that of S-(-)-thalidomide. The capacity factor (k) for the first eluted peak was 9.67, the stereochemical separation factor (α) obtained was 1.54, and stereochemical resolution factor (R) obtained was 15.05. The method described here has advantages over the Blaschke et al (4) method since it can be performed on a commercially available column, less time consuming (required less than 35 minutes to perform), and was done at room temperature, thus avoiding the possible

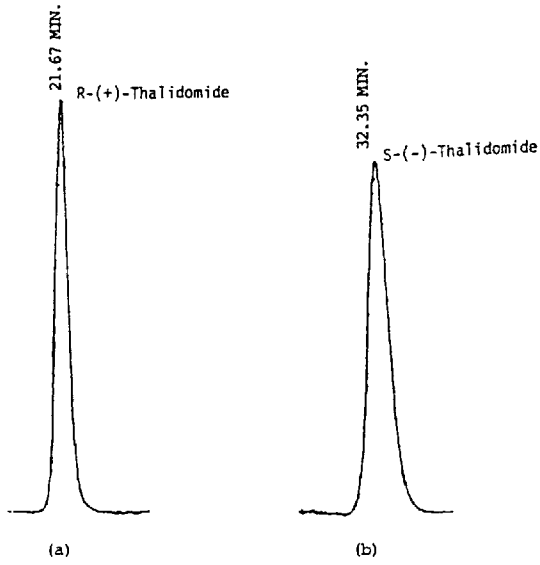

Fig. 2. (a) Chromatogram of R-(+)-Thalidomide.
(b) Chromatogram of S-(-)-Thalidomide.
Conditions were same as in Fig. 1, except the sample amount was 1 nmole.

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occurrence of racemization that might be accelerated at a higher temperature.

CONCLUSION

This simple and direct stereochemical separation of thalidomide was achieved on a commercially available Chiralcel OJ analytical column using hexane and ethanol (50:50) as a mobile phase in an isocratic condition at room temperature. The maximum stereochemical resolution (R) obtained in this study could be due to the chiral recognition mechanism explained by Wainer and Stiffin (7) which involves a combination of attractive interactions and inclusion of the analyte in a chiral chiral cavity. Since a large stereochemical resolution (R) 15.05 was achieved with a baseline

separation and preparative column (500mm x 22.2mm, I.D.) in this chiral stationary phase is also commercially available, so this method could be directly applied for preparative separation of thalidomide enantiomers. It could also be used for the determination of R-(+)-thalidomide and S-(-)-thalidomide in biological fluids which is presently in progress in this laboratory. This method has the advantage being fast as it requires about 35 minutes to perform.

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