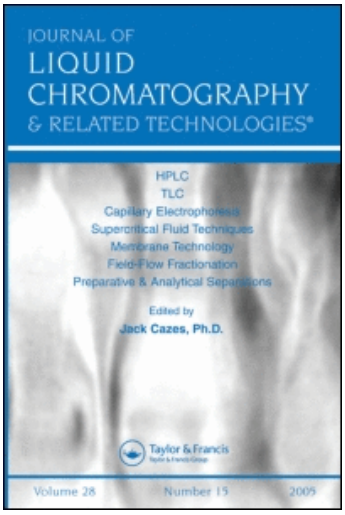

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DIRECT CHROMATOGRAPHIC RESOLUTION OF RACEMIC PYRIDOGLUTETHIMIDE USING TWO CELLULOSE-BASED CHIRAL STATIONARY PHASES

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ABSTRACT

Enantiomeric separation of racemic pyridoglutethimide by direct liquid chromatographic method was accomplished using Chiralcel OD and Chiralcel OJ columns without any derivatizations. Maximum resolution (R) of 0.96 was obtained for the enantiomers of pyridoglutethimide using Chiralcel OD column while maximum resolution (R) of 1.56 was obtained using Chiralcel OJ column.

INTRODUCTION

Aminogluthetimide [AG,(1)] is an agent which is clinically used for the treatment of hormone-dependent tumors acting through inhibition of the aromatase enzyme[1,2]. However, AG does produce several neurological side-effects e.g. sedation, ataxia among others, and also interferes with general steroid biosynthesis through inhibition of the desmolase enzyme system (inhibition of side-chain cleavage in cholesterol). Hence hydrocortisone must be administered as a replacement therapy[3].

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Pyridoglutethimide [PG,(2)] is a bioisoster of AG, chemically known as 3-ethyl-3(4-pyridyl) piperidine-2,6-dione, first synthesized by Foster et al[4]. Pyridoglutethimide exhibits a strong competitive inhibitory activity which is selective against the aromatase enzyme system while it does not inhibit the desmolase enzyme system, therefore there is no need for hydrocortisone administration. Furthermore PG produces little or no neurotoxic side effects[4,5].
Pyridoglutethimide is currently in clinical trials in post-menopausal women with hormone-dependent metastatic breast carcinoma and is considered a more effective and selective chemotherapeutic agent in this respect. Pyridoglutethimide is clinically administered as a racemic mixture, although it is reported that (+)-R enantiomer is most active than (-)-S enantiomer[6].

Chemical resolution of racemic PG was previously reported[6]. Recently, Boss et al[7] described a method for the direct resolution of racemic PG using a chiral stationary phase based on the (R,R)-tartaramide.

This paper describes a method for the direct resolution of racemic PG to their individual enantiomers using commercially available cellulose based chiral stationary phases namely Chiralcel OD (cellulose tris-3,5-dimethylphenyl carbamate, Scheme 1) and Chiralcel OJ (cellulose tris-4-methyl benzoate ester, scheme 2) columns. The optimum chromatographic conditions for this speration of racemic PG on these chiral stationary phases are also studied.

EXPERIMENTAL

Apparatus:

Waters LC system consisted of Model M-45 pump, a U6K injector, and a Lambda-Max Model 481 LC sepectrophotometer UV detector of 257 nm. The stationary phase of Chiralcel OD analytical column of cellulose tris-3, 5-dimethylphenyl carbamate and Chiralcel OJ analytical column of cellulose tris-(4-methylbenzoate) ester (25 cm x 4.6 mm, I.D., Daicel Chemical Industries, Tokyo, Japan) coated on silica gel with particle size 10 μm were used.

Chemicals:

Racemic pyridoglutethimide (±PG), (+)-R-pyridoglutethimide(+PG) and (-)-S-Pyridoglutethimide were kindly supplied by Dr. Michael Jarman of the

Institute of Cancer Research, Sutton, Surrey, U.K. HPLC grade 2-propanol and hexane were obtained from Fisher Scientific, Fairlawn, New Jersey, U.S.A.

Chromatographic Conditions:

The maximum and symmetrical stereochemical resolution of PG was obtained with hexane and 2-propanol (65:35) as eluent on chiral OD column. Flow rate was 0.7 ml/min and chart speed was 0.2cm/min. Temperature was maintained at 23°C throughout the experiment. Detection was obtained at UV 257nm with sensitivity range 0.01 AUFS. Sample amount injected was 2.8 nmole for racemic PG and 1.4 nmole for (+)-R-PG and (-)-S-PG enantiomers.

Racemic pyridoglutethimide was also resolved using hexane and 2-propanol (50:50) as eluent on Chiralcel OJ column. Other chromatographic conditions were the same as those used for PG on OD column.

Determination of Enantiomeric Elution Order:

The enantiomeric elution order was determined by chromatographing the separate enetiomers under the same conditions. We have found in the case of resolution of PG enantiomers on Chiralcel OD column the peak that eluted with a lower capacity factor was identified as (-)-S-PG and the peak that eluted with a higher capacity factor was identified as (+)-R-PG. However, the elution order obtained for PG on OJ column was reversed. When both Chiralcel columns, OD-OJ or OJ-OD, were used in series, observed that the order of the columns did not change the elution order of the peaks, and it was similar to that of the OJ column, i.e. the peak with lower capacity factor was (+)-R-PG, and the peak with higher capacity factor was (-)-S-PG.

RESULTS AND DISCUSSION

The resolution of the enantiomers of pyridoglutethimide by chemical means has been reported[6]. Boss et al[7] recently, described a direct resolution of racemic pyridoglutethimide to determine the biological potency of the individual enantiomers. However, the authors prepared the chiral stationary phase derived from (R,R)-tartaramide according to Dobashi and Hara[8] for this purpose. The present work reports a method for the separation of pyridoglutethimide enantiomers using commercially available Chiralcel columns OD (cellulose tris-3,5-dimethylphenyl car-

bamate) column and Chiralcel OJ (cellulose tris-(4-methylbenzoate ester) column. The OD chiral phase column has been used successfully to directly separate several β-adrenergic blockers, e.g., timolol[9], penbutolol[10], celiprolol[11], carazolol[12], among others. The Chiralcel OJ column also has been used to separate different drugs such as muserine, methaqualone, mephobarbital and ethotoin[13]. Aboul-Enein and Islam[14] recently reported the separation of aminoglutethimide and glutethimide[15] enantiomers, which are structurally related drugs to PG, using OD and OJ columns.

These chiral stationary phases namely the Chiralcel OD and OJ were successfuly used to separate the racemic pyridoglutethimide into its corresponding enantiomers. Different concentrations of 2-propanol in hexane were used as mobile phase to optimize the separation. A chromatogram of enantiomer separation of pyridoglutethimide on Chiralcel OJ column is shown in Figure 1. Compared with the chromatograms and capacity factors of (+)-R-pyridoglutethimide (Figure 2), and (-)-S-pyridoglutethimide (Figure 3), the peak that eluted with lower capacity factor was identified as (+)-R-PG and the peak with a higher capacity factor was identified as (-)-S-PG. The maximum and symetrical stereochemical resolution (R) obtained was 1.56 using 50% 2-propanol in hexane (see Table 1). The separation of racemic pyridoglutethimide was also obtained successfully on Chiralcel OD column. Chromatogram of enantiomer separation on Chiralcel OD column is shown in Figure 4. Elution order was found to be the reverse of that obtained on Chiralcel OJ column as shown in Figures 5 and 6. However the stereochemical resolution (R) was 0.96 which was lower than the case of the Chiralcel OJ column. This indicates that Chiralcel OJ stationary phase is better in resolving racemic PG enantiomers. It is of interest to mention that the order of elution of the PG enantiomers did not change when these columns were used in series (either OD-OJ or OJ-OD). The mobile phase used consisted of hexane and 2-propanol (50:50) under the same chromatographic conditions as shown in Table 1.

CONCLUSION

Direct stereochemical separation of pyridoglutethimide was achieved using commercially available Chiralcel OD and Chiralcel OJ columns. This separation methods have advantages of being fast and need about 30 minutes to run, and direct as it required no derivatizations. Furthermore, these methods can be

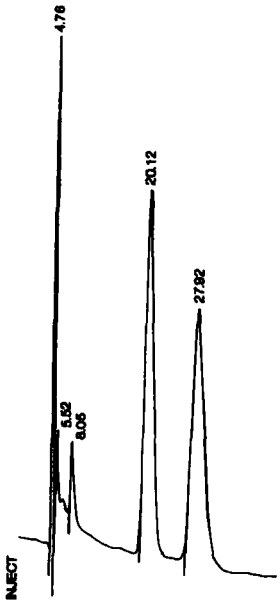

Fig. 1 LC separation of racemic Pyridoglutethimide. Column: Chiralcel OJ (250 x 4.6 mm I.D.); mobile phase: Hexane and 2-propanol (50:50; flow rate: 0.7 ml/min.; chart speed: 0.2 cm /min; temperature: 23°C; pressure: 250 psi; detector: UV 257 nm; 0.01 AUFS

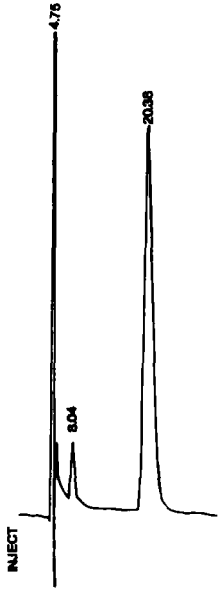

Fig. 2 Chromatogram of (+)-R-Pyridoglutethimide. Conditions were the same as Fig. 1, except sample amount was 1.4 nmole.

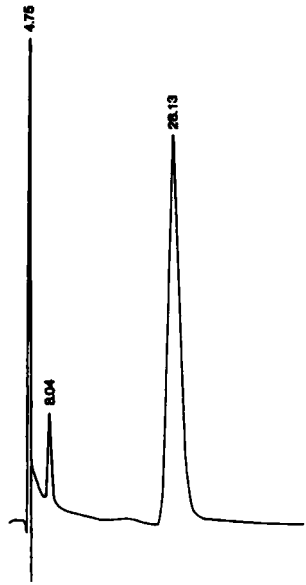

Fig. 3 Chromatogram of (-)-S-Pyridoglutethimide. Conditions were the same as in Fig. 1, except sample amount was 1.4 nmole.

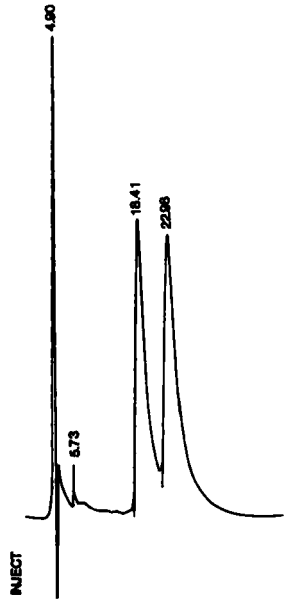

Fig. 4 LC Separations of racemic Pyridoglutethimide. Column: Chiralcel OD (250 x 4.6mm I.D.); mobile phase: Hexane and 2-propanol (65:35); sensitivity: 0.01 AUFS; sample amount: 2.8 nmole; other conditions were the same as Fig. 1.

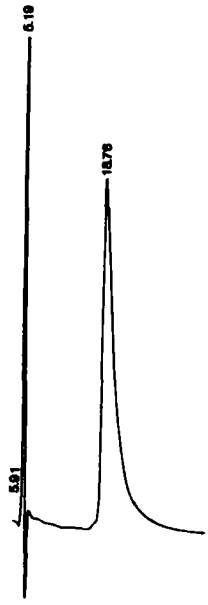

Fig. 5 Chromatogram of (-)-S-Pyridoglutethimide. Conditions were the same as in Fig. 4 except sample amount was 1.4 nmole.

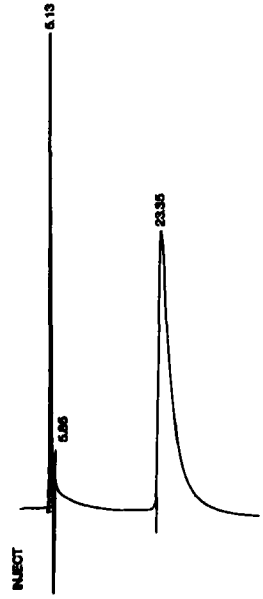

Fig. 6 Chromatogram of (+)-R-Pyridoglutethimide. Conditions were the same as in Fig. 4 except sample amount was 1.4 nmole.

Table I. Optimized parameters of capacity factor (k), stereochemical separation factor (α) and stereochemical resolution factor (R) of pyridoglutethimide on Chiralcel OD and Chiralcel OJ columns.

Compound	Solvent	Column	k'	k"	α	R
PG	A	OD	2.76	3.69	1.34	0.96
PG	B	OJ	3.15	4.74	1.50	1.56
PG	B	OD + OJ	2.70	3.30	1.20	0.96
PG	B	OJ + OD	2.93	3.46	1.18	1.0

Chromatographic Conditions:

Solvent System A = hexane: 2-propanol (65:35)

Solvent System B = hexane: 2-propanol (50:50)

Flow rate: 0.7ml/min.;temperature:23°C;detector:UV 257nm

k' = 1st eluted peak

k" = 2nd eluted peak

α = stereochemical separation

R = stereochemical resolution

applied for preparative chiral chromatography for the resolution of racemic pyridoglutethimide in large quantities. The method also can be used for optical purity determination of the individual enantiomers as well as analysis of the drug in biological fluids.

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