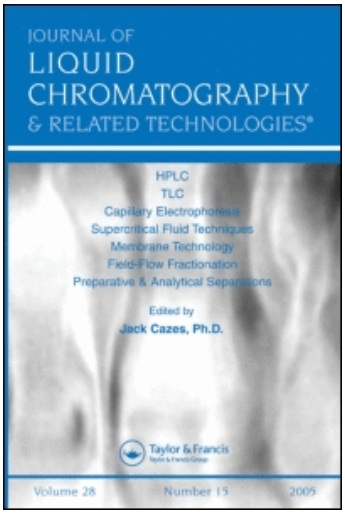

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DIRECT ENANTIOMERIC SEPARATION OF PHENGLUTARIMIDE BY CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY*

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ABSTRACT

Enantiomeric separation of racemic phenglutarimide (PG) by chiral high performance liquid chromatography is reported using cellulose tris (3,5-dimethylphenyl carbamate) known as Chiralcel OD chiral stationary phase. Maximum resolution (R_S) of 1.26 is obtained for the enantiomers of PG. The method could be applied for preparative scale chromatography and in the assay of PG in biological fluids.

I. INTRODUCTION

Phenglutarimide (PG), chemically known as (±)-3-(2-diethylaminoethyl)-3-phenyl-piperidine-2,6-dione, is an anticholinergic agent with actions and uses similar to those of

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benzhexol. It was formerly used also in the treatment of Parkinsonism [1]. Phenglutarimide possesses a chiral carbon at C_3 and is administered as a racemic mixture of (+)-S and (-)-R enantiomers as shown in Figure 1. Most of the pharmacological clinical efficacy and metabolism in rat and man has been studied on the racemic mixture and not on the individual enantiomers [2-10]. Recently, Lambrecht et al [11] reported the extremely high stereoselectivity and high affinities for the (+)-S-enantiomer PG as compared to the (-)-R-enantiomer to three muscarinic receptor subtypes namely, M_1, M_2 and M_3. It was found that the (+)-S-PG to be a potent M_1-selective antagonist as compared to (-)-R-PG.

Described here is a simple, isocratic method for the enantioseparation of the racemic mixture of PG. Effective resolution is achieved on cellulose tris (3,5-dimethylphenyl carbamate) chiral stationary phase (CSP), known as Chiralcel OD. Furthermore, preliminary evaluation of the chiral recognition-structure relationships, involved in the enantioseparation of structurally related drugs having piperidine-2,6-dione ring system on this cellulose CSP is discussed.

2. EXPERIMENTAL

2.1 Apparatus

The Waters Liquid Chromatography System (Waters Associates, Milford, MA, USA) consisted of a Model M-45 pump, a U6K injector, and a Lambda-Max Model 481 LC spectrophotometer UV detector operated at 257 nm. The stationary phase Chiralcel OD analytical column of cellulose tris-3,5-dimethylphenyl carbamate, (25cm x 0.46cm, i.d.; Daicel Chemical Industries, Tokyo, Japan) coated on silica gel with particle size 10 μm was used.

2.2 Chemicals

Racemic PG was obtained from Professor P. J. Nicholls, Welsh School of Pharmacy, Cardiff, U.K. and the corresponding (+)-S and (-)-R PG as hydrochloride salts were supplied by Professor Dr. J. Knabe, Institute of Pharmaceutical Chemistry, University of Saarland, Saarbrücken, Germany. HPLC grade hexane, 2-propanol were obtained from Fisher Scientific, Fairlawn, NJ, USA.

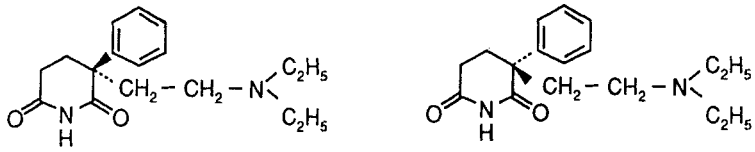

Fig. 1. The structures of the enantiomers of phenglutarimide.

2.3 Chromatographic Conditions

The maximum and symmetrical stereochemical resolution of PG was obtained using hexane and 2-propanol (70:30) on Chiralcel OD column. Flow rate was 1.0 ml/min. and chart speed was 0.5 cm/min. A temperature of 23°C was maintained throughout the experiment. Detection was obtained at UV 257 nm with a sensitivity range 0.01 aufs. Sample amount injected was 2.0 nmole for racemic PG and 1.0 nmol for its corresponding individual enantiomers.

2.4 Determination of Enantiomeric Elution Order

The enantiomeric elution was determined by chromatographing the individual enantiomers (-)-R-and (+)-S-PG separately under the same conditions. Thus, the peak that eluted with the lower capacity factor was identified as (+)-S-PG and the one that eluted with the higher capacity factor was identified as (-)-R-PG.

3. RESULTS AND DISCUSSION

The method described here is the first reported in literature where PG enantiomers can be directly resolved using cellulose (tris 3,5-dimethylphenyl carbamate) CSP. A typical chromatogram of the enantioseparation of racemic PG is shown in Fig. 2. By comparison the chromatogram and the capacity factor of individual enantiomers, the peak which eluted at a lower capacity factor ($k_1 = 1.11$) was identified as the (+)-S-enantiomer, (Fig. 3a) and the peak with the higher capacity factor ($k_2 = 1.66$) as the (-)-R-PG (Fig. 3b). The stereochemical separation factor (α) was 1.50. The maximum stereochemical resolution factor (R_S) was 1.26.

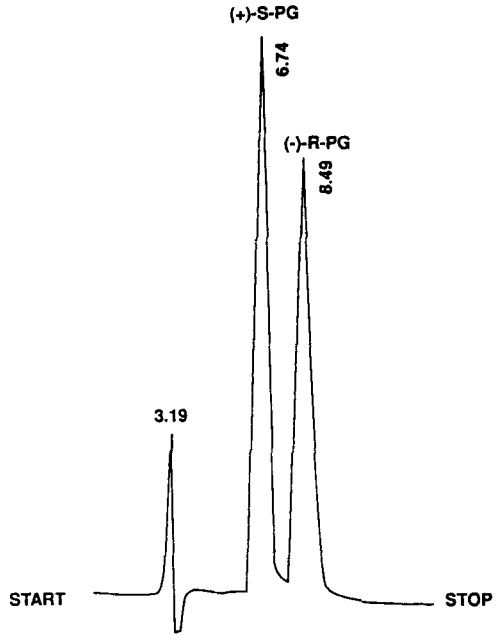

Fig. 2 Enantiomeric HPLC separation of racemic phenglutarimide. Column: Chiralcel OD (250 x4.6mm i.d.); mobile phase: hexane: 2-propanol (70:30); flow rate: 1 ml/min; chart speed: 0.5cm/min.; temperature: 23°C; detector: UV 257nm; sensitivity 0.01 AUFS; sample amount 2 nmol.

This cellulose-derived chiral stationary phase has been successfully used for direct separation of several piperidine-2,6-dione analogs namely aminoglutethimide [12], cyclohexylaminoglutethimide [13], 1991), pyridoglutethimide [14] and glutethimide [15]. It is of interest to mention that regardless of the absolute configuration of the substituents at C_3 of the piperidine 2,6-dione drug chromatographed the levorotatory enantiomers did elute first followed by the dextrorotatory enantiomer with the exception of PG where the (+)-S enantiomer did elute first followed by (-)-R enantiomer. This could be rationalized by the effect of non-aromatic basic substituent 2-diethylaminoethyl group at C_3 of the piperidine-2,6-dione ring system of PG which might have caused the reversal enantiomeric elution order of PG enantiomers contrary to the other analogs separated on the same CSP where the non-aromatic substituent at C_3 are more hydrophobic in nature being of hydrocarbon residue. It is obvious that the non-aromatic substituent and C_3 in this

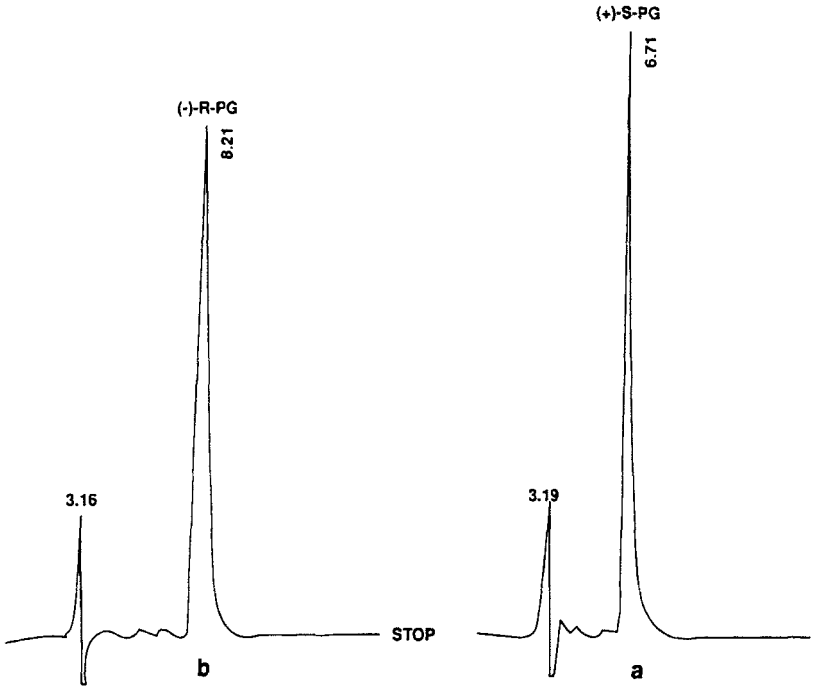

Fig. 3 (a) Chromatogram of (+)-(S)-phenglutarimide.

(b) Chromatogram of (-)-(R)-phenglutarimide Conditions were the same as in Fig. 1 except sample amount was1 nmol.

piperidine-2,6-dione series does play an important role as to the determination of the elution order of the individual enantiomers.

4. CONCLUSION

The direct stereochemical separation of racemic PG was achieved on commercially available cellulose 3,5-dimethylphenyl carbamate under isocratic conditions. This method could be applied on a preparative scale for the preparation of a large quantity of PG enantiomers by using the preparative Chiralcel OD column which is commercially available. Furthermore, since the method is simple and fast it can be adopted to quantitate the enantiomers of PG in biological fluids for further pharmacokinetic and pharmacodynamic studies which is in progress.

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