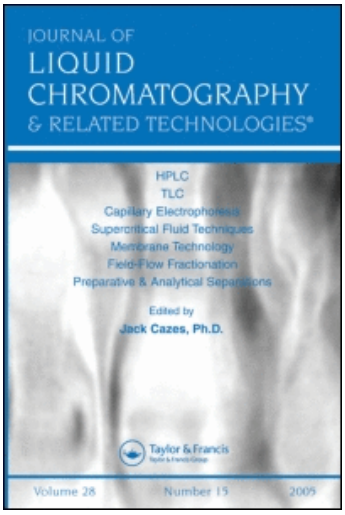

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SEPARATION OF THE DIASTEREOMERS OF BACLOFEN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING CYCLODEXTRIN AS A MOBILE PHASE ADDITIVE

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ABSTRACT

Baclofen (4-amino-3-p-chlorophenylbutyric acid), a skeletal muscle relaxant used in the treatment of spastic disorders, is administered clinically as a racemic mixture. This paper describes the separation of the diastereomers of baclofen, which was derivatized with (+)-1-(9-fluorenyl)ethyl chloroformate, (+)-1-(1-naphthyl)ethyl isocyanate, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide or 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate, by reversed-phase high-performance liquid chromatography using cyclodextrin as a mobile phase additive.

INTRODUCTION

Baclofen (4-amino-3-p-chlorophenylbutyric acid), a skeletal muscle relaxant used in the treatment of spastic disorders (Fig.

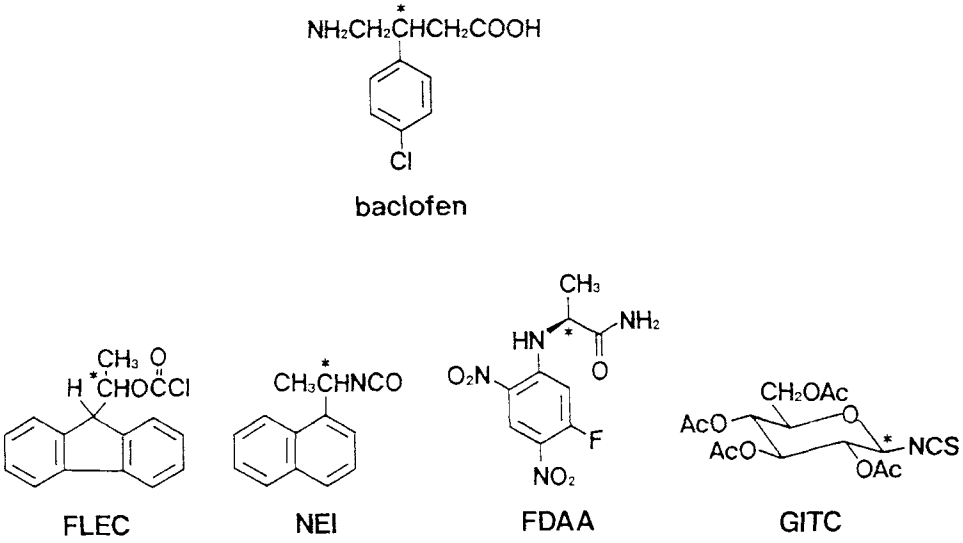

FIGURE 1. Structures of Baclofen and Derivatization Reagents

1), is administered clinically as a racemic mixture. The enantiomers of this compound differ in their pharmacodynamic and toxicological properties: the R-(-)-enantiomer is much more active but also more toxic than the S-(+)-enantiomer. The S-(+)-enantiomer interferes with the binding of the R-(-)-enantiomer, and has been proposed to act as an antagonist at the $GABA_B$-receptors. Because the kinetic disposition of the two enantiomers may also be different, the investigation of the pharmacokinetic behavior of both enantiomers is desirable. Several high-performance liquid chromatographic (HPLC)[1] and gas liquid chromatographic procedures for the determination of baclofen enantiomers have been described [2].

In previous papers, we reported that the much improved separation of steroids has been observed by the addition of a suitable cyclodextrin (CD) to the mobile phase in reversed-phase HPLC [3]. In addition, this inclusion chromatography has been used for the optical resolution of derivatized amino acids and dipeptides [4]. As a continuation of this work, the present paper deals with the separation of the diastereomers of baclofen which was derivatized using commercially available chiral reagents, (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC)[5], (+)-1-(1-naphthyl)ethyl isocyanate (NEI)[6], 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey's reagent, FDAA)[7] or 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)[8](Fig. 1), by reversed-phase HPLC using CD as a mobile phase additive.

MATERIALS AND METHODS

Materials

α-, β- and γ-CDs were kindly supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan). Heptakis-(2,6-di-O-methyl)-β-CD (Me-β-CD; 10.5 methyl residues/mol) was prepared and donated by Kao (Tokyo). Baclofen was kindly donated by Ciba-Geigy (Basel, Switzerland). FLEC and GITC were obtained from Wako (Osaka, Japan). The other derivatization reagents (NEI, FDAA) were purchased from Tokyo Kasei Kogyo (Tokyo).

Derivatization Procedure

The derivatization of R-(-)- or S-(+)-baclofen with FLEC, NEI, FDAA and GITC has been done using previously described procedures [5-8] to give the FLEC-, (+)-1-(1-naphthyl)ethyl carbamoyl (NEC)-, 2,4-dinitrophenyl-5-L-alanine amide (DNPA)- and GITC-baclofen diastereomers, respectively.

Apparatus

HPLC was carried out on a Shimadzu LC-6A chromatograph (Shimadzu, Kyoto, Japan) equipped with a Hitachi F-1050 fluorescence (FL)(Hitachi, Tokyo) or Shimadzu SPD-6AV UV (Shimadzu) detector. A YMC•GEL C_8 (5 μm) column (15 cm x 0.46 cm i.d.)(YMC, Kyoto) was used at ambient temperature at a flow rate of 1 ml/min, and the void volume was measured with $NaNO_3$ (UV 210 nm) or MeOH (λ_{ex} 280 nm, λ_{em} 320 nm). The pH of the mobile phase containing AcONa or KH_2PO_4 was adjusted with AcOH or H_3PO_4, respectively.

RESULTS AND DISCUSSION

Separation of the Diastereomers of Baclofen by the Conventional Reversed-phase HPLC

R-(-)- and S-(+)-baclofen were derivatized with FLEC, NEI, FDAA or GITC according to the reported procedure to give FLEC-, NEC-, DNPA- or GITC-baclofen, respectively. The obtained

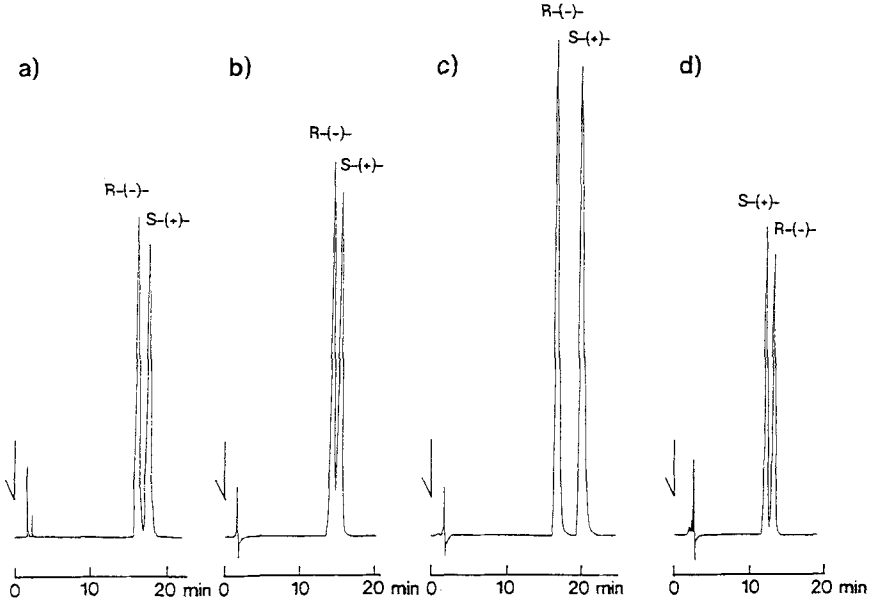

FIGURE 2. Separation of the Diastereomers of Baclofen

a) FLEC-derivatives b) NEC-derivatives

c) DNPA-derivatives d) GITC-derivatives.

Conditions: mobile phase, a) MeCN-0.5% AcONa(pH 6.5)(7:12), b) MeOH-0.5% AcONa(pH 6.7)(11:8), c) MeOH-0.5% KH_2PO_4(pH 4.0)(11:8), d) MeOH-0.5% phosphate buffer(pH 6.5; KH_2PO_4/K_2HPO_4)(1:1); detection, a) FL (λ_{ex} 260 nm, λ_{em} 315 nm), b) FL (λ_{ex} 235 nm, λ_{em} 333 nm), c) UV 340 nm, d) UV 250 nm.

diastereomers of baclofen were subjected to the conventional reversed-phase HPLC using the YMC•GEL C_8 column. The selection of the best organic modifier (MeOH or MeCN) and buffer (AcONa, KH_2PO_4 or KH_2PO_4/K_2HPO_4; including pH adjustment) allowed the separation of each pair of diastereomeric FLEC-, NEC-, DNPA- and GITC-baclofens as shown in Fig. 2 and the resolutions (Rs) of these pairs were 1.75, 1.30, 3.44 and 2.07, respectively. The DNPA-derivatives showed the most satisfactory Rs value of all these diastereomeric pairs. The GITC-S-(+)-baclofen was eluted earlier than the corresponding R-(-)-baclofen derivative, but all the other derivatized R-(-)-baclofens were eluted earlier than the corresponding diastereomers.

Effect of CD on the Separation of the Diastereomers of Baclofen

The addition of α-, β-, γ-CD or Me-β-CD (each 5 mM), which was used instead of β-CD due to its solubility, to the mobile phase did not have much effect on the separation of the NEC- or DNPA-baclofen diastereomers. On the contrary, the addition of γ-CD improved the separation of the FLEC-baclofen diastereomers as shown in Fig. 3. The Rs value was much improved from 1.75 to 3.47 by the addition of γ-CD (10 mM). This phenomenon was also observed during the separation of the diastereomers of GITC-baclofen by the addition of α-CD as shown in Table 1. The other CDs were ineffective for the separation of the diastereomers of FLEC- or GITC-baclofen. The addition of glucose (5 mM) to the

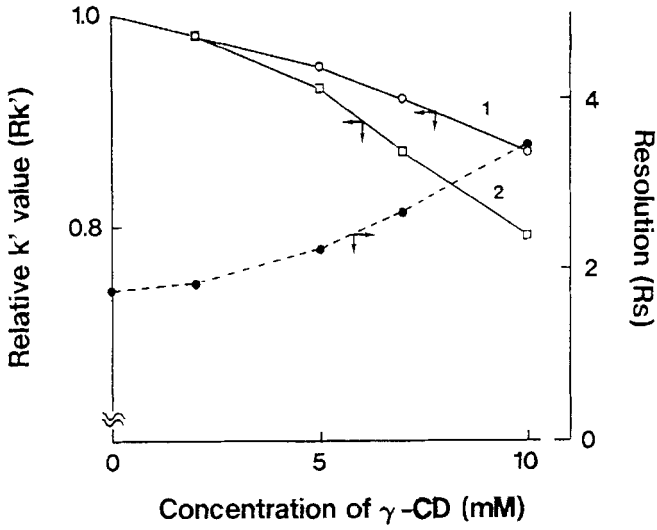

FIGURE 3. Effect of γ-CD on the Resolution of the Diastereomers of FLEC-baclofen

1: FLEC-S-(+)-baclofen 2: FLEC-R-(-)-baclofen.

Rk': The k' value obtained without CD was taken as 1.0.

Conditions: mobile phase, MeCN-0.5% AcONa(pH 6.5) (7:12) containing γ-CD as indicated. Other conditions are the same as in Fig. 2a.

mobile phase was also ineffective for the separation of the diastereomers of FLEC-baclofen. These evidences showed that the inclusion phenomenon played an important role during the separation of the diastereomers using this type of chromatography.

TABLE 1. Effect of α-CD on the Separation of the Diastereomers of GITC-Baclofen[a)]

Baclofen	0 mM		5 mM		7 mM	
	k'	Rs	Rk'[b)]	Rs	Rk'	Rs
R-(-)-	7.96		0.94		0.93	
S-(+)-	7.14	2.07	0.92	2.41	0.91	2.58

a) Conditions: mobile phase, MeOH-0.5% phosphate buffer (pH 6.5; KH_2PO_4/K_2HPO_4)(1:1) containing α-CD as indicated, t_0 1.86 min; detection, UV 250 nm.

b) The k' value obtained without CD was taken as 1.0.

CONCLUSION

R-(-)- and S-(+)-baclofen were derivatized using commercially available chiral reagents and the obtained pairs of diastereomers were separated by reversed-phase HPLC. The diastereomers obtained using Marfey's reagent (FDAA) gave the most satisfactory results in the conventional HPLC. The inclusion chromatography using CD as a mobile phase additive was effective for the separation of the diastereomers of FLEC- or GITC-derivatives.

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