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Chiral Separation of β-Blockers after Derivatization with a New Chiral Derivatization Agent, GATC

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A new chiral derivatization agent with sugar moiety, 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl isothiocyanate (GATC) was synthesized. Several β-blockers were investigated for the possible separation of the enantiomers by reversed-phase HPLC after derivatization with this new chiral derivatization agent (GATC). GATC was reacted readily with β-blockers at room temperature and the reaction mixture could directly be injected into the HPLC system. The corresponding diastereomers were well resolved on an ODS column with acetonitrile-ammonium acetate buffer as a mobile phase and monitored at UV 254 nm. The optimization of the derivatization procedure (concentration of GATC, reaction temperature and time) and HPLC conditions (pH and ionic strength of mobile phase) were investigated and compared with GITC.

Key words: Chiral separation, Chiral derivatization agent, β-blockers, HPLC

INTRODUCTION

Various approaches for the chromatographic separation of enantiomeric amine compounds have been studied. Two fundamentally different attempts have been established to resolve chiral amines by HPLC: the indirect method via covalent diastereomer formation using a chiral derivatizing agent (CDA) (Boppana *et al.*, 1992; Kim *et al.*, 1999; Peter *et al.*, 1999; Srinivas and Igwemeze, 1992) and the direct separation method (Baimer *et al.*, 1992; Lanchote *et al.*, 2000). The application of chiral derivatizing agent was the first widely-used method for the enantiomeric separation of optically active molecules in liquid chromatography. For amine compounds the chemically most selective CDAs are isothiocyanates such as 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), leading to the corresponding diastereomeric thiourea derivatives (Nishi *et al.*, 1900; Tian *et al.*, 1991), and the CDAs introduced for this reason include many isothiocyanate-based compounds.

In this paper we report the synthesis (Fig. 1) and application of a new sugar based isothiocyanate, 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl isothiocyanate (GATC). The reactivity of GATC towards chiral primary and secondary amines and amino alcohols of the β-blocker type was particulary evaluated. Chromatographic separations of the resulting diastereomeric thioureas were investigated by RP-HPLC and compared with those of some GITC derivatives of the same β-blockers.

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MATERIALS AND METHODS

Materials and equipment

Atenolol and pindolol hydrochloride were obtained from Il Dong Co. (Seoul, Korea), acebutolol hydrochloride from Rhone-Poluenc Rorer Korea Pharmaceuticals Ltd. (Seoul, Korea), betaxolol hydrochloride from Bukwang Pharm. Co. Ltd. (Seoul, Korea) and bisoprolol hemifumarate and metoprolol tartrate from Yuhan Co.(Seoul, Korea). 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate and triethylamine (TEA) were purchased from Sigma Chemical Co. (St. Louis, MO., U.S.A.). HPLC grade ethanol, isopropanol, n-hexane and acetonitrile were purchased from Duksan Pure Chemicals Co. (Ansan, Kyeonggi, Korea). Diethylamine as a analytical grade was obtained from Junsei Chemical Co. (Tokyo, Japan). Water was purified with a Milli-RO15 water purification system (Nihon milipore, Japan) and filtered through a 0.2 μm membrane filter. All other reagents were of analytical reagent grade. The mobile phase was degassed by sonication under vacuum before use it.

High performance liquid chromatograph was consisted of Shimadzu Model LC-9A pump, SPD-6AV spectrophotometric detector and C-R4AD chromatopac. The separations were carried out on a Mightysil RP-18 GP column (5 μm, 4.6 mm I.D. × 250 mm). Semi-preparative chiral chromatography was performed with Chiralcel OD column (10 μm, 10.0 mm I.D. × 250 mm) and Chiralcel OD column (10 μm, 4.6 mm I.D. × 250 mm) was used for optical purity test.

Synthesis of GATC

The reagent is readily synthesized after a straightforward three-step synthesis starting from the β-galactose (Fig. 1). The first reaction step was performed in pyridine with acetone as a acylating agent at room temperature for 6 days. The reacting product was treated with 38% hydrobromide in acetic acid at room temperature for 3 days. The resulting product was then treated with potassium thiocyanate in the presence of acetonitrile and heated at about 50°C.

Derivatization procedures

Solutions of β-blockers (1 mM in acetonitrile) were prepared. To a 100 μL aliquot of this solution were added 100 μL of 20 mM GATC or GITC acetonitrile solution and 100 μL of 10 mM triethylamine acetonitrile solution. The resulting mixture was vigorously shaken and allowed to stand at room temperature for 30 min. After reaction, aliquots (5 μL) were injected into the HPLC using a 25 μL microsyringe (Fig. 2).

Chiral conversion test

Metoprolol racemate (10 mg) was dissolved in 1 mL of mobile phase. This solution was injected into the semi-preparative chiral HPLC system and resolved into each enantiomer on the Chiralcel OD column by n-hexane, ethanol and diethylamine (90/10/0.1, v/v/v) as a mobile phase at room temperature and flow rate of 3 mL/min monitoring at UV 276 nm. Fraction containg single enantiomer was collected and evaporated to dryness. Enantiomeric purity of single enantiomer was determined with chiral HPLC system using Chiralcel OD column(mobile phase; n-hexane/isopropanol/diethylamine (90/10/0.1, v/v/v), flow rate; 1.0 mL/min). (R)-(+)-metoprolol was derivatized with GATC (GITC) at room temperature for 30 min, followed by injected into the achiral HPLC system (column; Mightylsil RP-18 GP, mobile phase; 55% acetonitrile in 0.075M ammonium acetate buffer (pH=6.0), flow rate; 1.0 mL/min) and chiral conversion was examined.

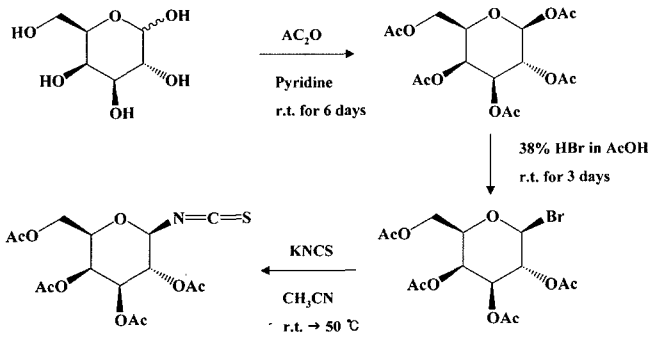

Fig. 1. Synthesis of GATC

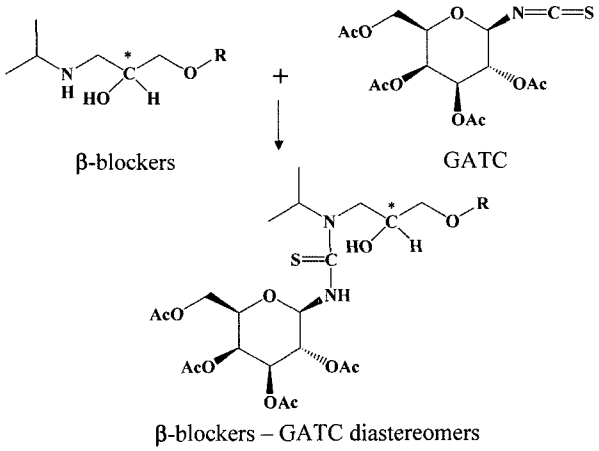

Fig. 2. Derivatization reaction of β-blockers using GITC and GATC

Optimization of the mobile phase

The effect of the changes in the mobile phase pH and ionic strength on the resolution of the diastereomers were investigated. pH of the mobile phase, 60% acetonitrile in 0.05M ammonium acetate buffer, was changed from pH 4.0 to 7.0. The concentration of the ammonium acetate in the mobile phase, 60% acetonitrile in ammonium acetate buffer (pH=6.0) was changed from 5 to 100 mM.

Optimization of derivatization

Metoprolol racemate was dissolved in acetonitrile. To 100 μL of this solution, 100 μL aliquots of various concentrations of GATC acetonitrile solution and 100 μL of 10 mM triethylamine acetonitrile solution were added and mixed. After standing for 30 min at room temperature, the resulting samples were injected into the achiral HPLC system and the peak areas were quantitated. The effects of time and temperature on the reaction were investigated. To 100 μL of 1 mM metoprolol racemate solution, 100 μL of 20 mM GATC acetonitrile solution and 100 μL of 10 mM triethylamine acetonitrile solution were added and reacted at room temperature, 45°C and 60°C for 0 min, 20 min, 40 min, 60 min, 90 min, 120 min, 180 min and 240 min. The resulting samples quantitated by achiral HPLC system.

RESULTS AND DISCUSSION

Chiral semi-preparative HPLC of (R)-(+)- and (S)-(-)-metoprolol and chiral conversion test during the derivatization with GATC.

On the chiral semi-preparative HPLC system, (S)-(-)-metoprolol and (R)-(+)-metoprolol were fractionated (Fig. 3). When (R)-(+)-metoprolol was derivatized with GATC, no chiral conversion was found (Fig. 4).

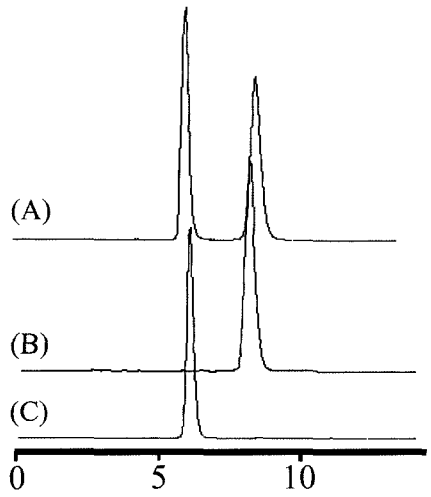

Fig. 3. Optical purity test of each enantiomers of metoprolol by chiral HPLC. (A) racemic metoprolol; (B) (S)-(-)-metoprolol; (C) (R)-(+)-metoprolol. Column, Chiralcel OD, 10 μm, 250 × 4.6 mm I.D.; mobile phase, n-hexane-ethanol-diethylamine (90:10:0.1, v/v/v); detector, UV 276 nm; flow rate, 1.0 mL/min.

Chromatographic behavior of the derivatives

GATC reacted selectively with β-blocker to form the corresponding diastereomeric thiourea. These diastereomers were well separable by RP-HPLC. Fig. 5 shows

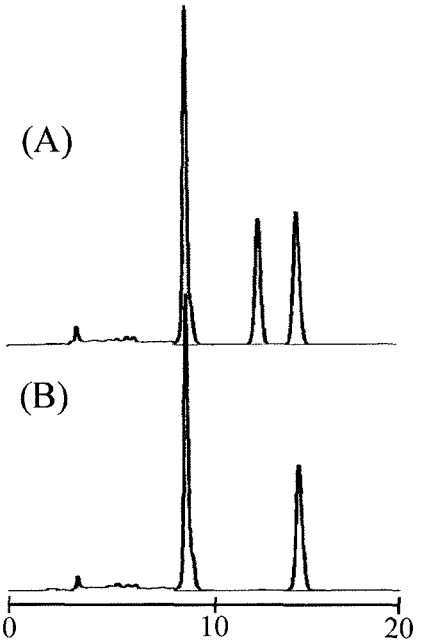

Fig. 4. Chiral conversion test of (R)-(+)-metoprolol after derivatization with GATC. (A) racemic metoprolol; (B) (R)-(+)-metoprolol. Column; Mightysil RP-18 GP, 5 μm, 250 × 4.6 mm I.D., mobile phase; 55% acetonitrile in 0.075M ammonium acetate buffer (pH=6.0), detector; UV 276 nm, flow rate, 1.0 mL/min.

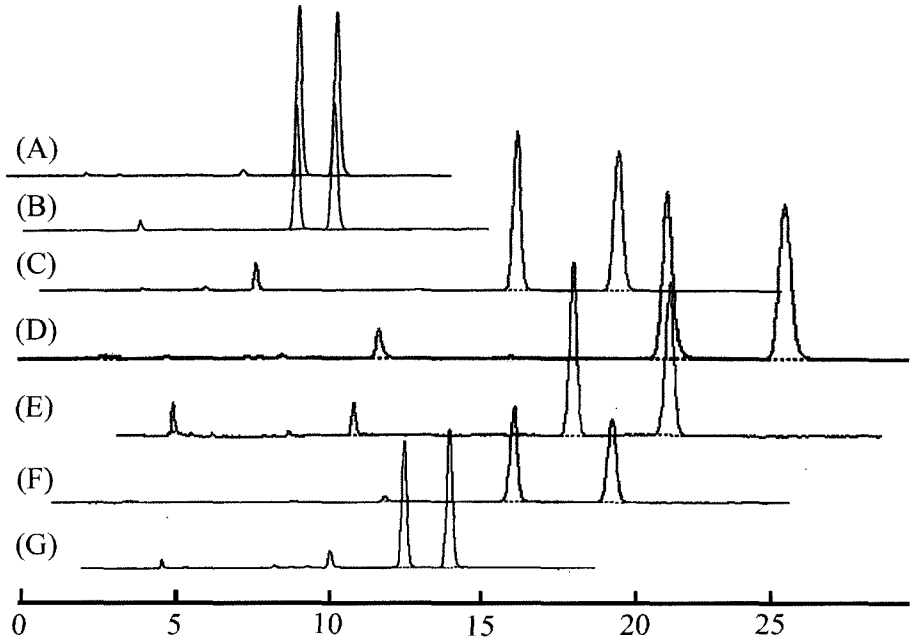

Fig. 5. Chromatograms of the diastereomers obtained from racemic β-blockers after derivatization with GATC; detailed chromatographic conditions see Table I. (A) Acebutolol, (B) Atenolol, (D) Betaxolol, (E) Bevantolol, (F) Bisoprolol, (G) Metoprolol, (H) Pindolol.

Table I. Chromatographic parameters of GATC- and GITC-derivatized β-blockers

β-blockers	Derivative	Mobile phase	Chromatographic parameters			
		50mM ammonium acetate buffer (pH=6)/acetonitrile	$k_1'^a$	$k_2'^a$	α^b	Rs^c
Acebutolol	GATC	50 / 50	3.58	4.16	1.16	3.37
	GITC		3.35	3.94	1.18	3.30
Atenolol	GATC	60 / 40	3.48	4.30	1.23	4.64
	GITC		3.39	3.98	1.18	3.45
Betaxolol	GATC	40 / 60	6.13	7.63	1.24	6.23
	GITC		6.11	7.19	1.18	4.70
Bevantolol	GATC	45 / 55	6.39	7.71	1.21	6.00
	GITC		5.68	7.58	1.15	3.99
Bisoprolol	GATC	45 / 55	7.03	8.72	1.24	6.00
	GITC		7.13	8.45	1.19	4.56
Metoprolol	GATC	45 / 55	4.97	6.23	1.25	5.94
	GITC		4.97	5.86	1.18	4.44
Pindolol	GATC	50 / 50	4.75	5.77	1.25	5.94
	GITC		4.71	5.52	1.17	4.14

a Capacity factor
b Selectivity
c Resolution

chromatograms of β-blockers derivatized with GATC. In Table I, chromatographic data for β-blockers derivatized with GATC and GITC are shown.

Optimization of the mobile phase

Changes in the capacity factors followed a similar pattern for the two derivatives metoprolol enantiomers. As the pH of mobile phase increased, the capacity factors and resolution factor were slightly increased (Fig. 6). At pH 6.0 best resolution and capacity factor were achieved. Fig. 7 shows that capacity factors almost were not changed as the ionic strength of mobile phase increased.

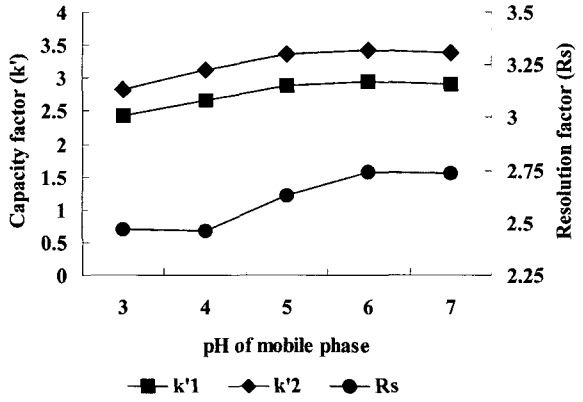

Fig. 6. The effects of changes in pH of mobile phase

In 75 mM of ammonium acetate the interference peak and the peak of the derivative of metoprolol were well resolved.

Optimization of derivatization

The effect of the concentration of GATC added was shown in Fig. 8. An increase of the concentration of GATC led to a general increase in formation of the diastereomers. Derivatization of metoprolol were increased upto 20 times molar excess of GATC and reached a plateau. In the final analytical conditions, the GATC concentration was chosen 20 times molar excess in order to provide an adequate excess of reagent. As the reaction temperature was increased from room temperature to 45°C or 60°C, the peak areas of diastereomers were increased but the range of change was narrow. The formation of the derivatives of metoprolol increased with the reaction time up to 20 min at room temperature and reached a plateau (Fig. 9).

The described three-step synthesis protocol to obtain GATC is simple and straightforward, leading to a stable CDA. This CDA is suitable for the indirect resolution of chiral primary and secondary amines and amino alcohols using RP-HPLC. During reaction process, no racemization was observed.

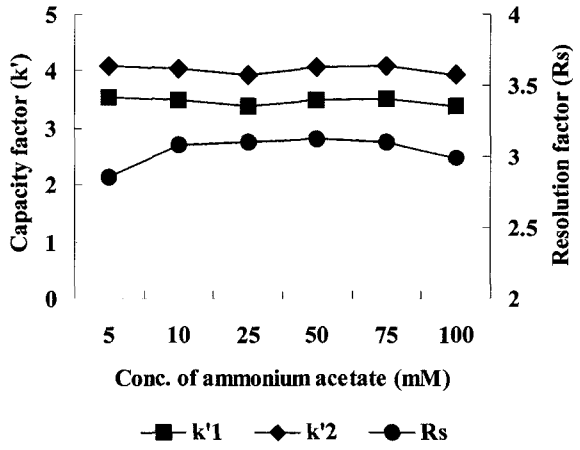

Fig. 7. The effects of changes in ionic strength of mobile phase

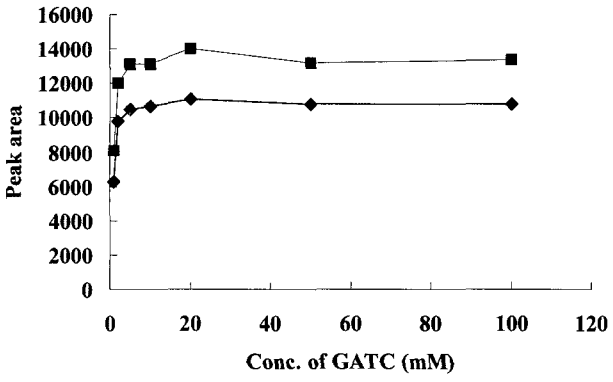

Fig. 8. The effect of concentration of GATC on derivatization reaction

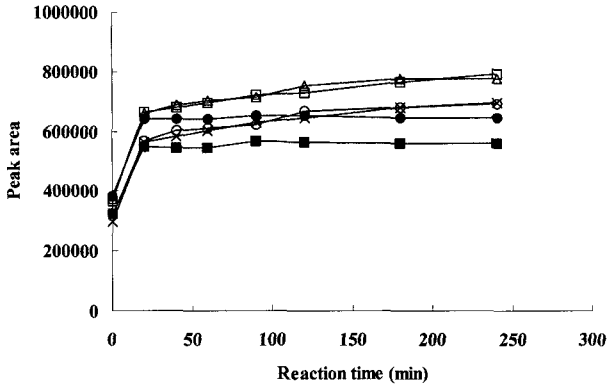

Fig. 9. The effect of reaction time and temperature on derivatization reaction. ■; GATC-(S)-(-)-metoprolol derivative at room temperature, ●; GATC-(R)-(+)-metoprolol derivative at room temperature, ○; GATC-(S)-(-)-metoprolol derivative at 45°C, △; GATC-(R)-(+)-metoprolol derivative at 45°C, x; GATC-(S)-(-)-metoprolol derivative at 60°C, □; GATC-(R)-(+)-metoprolol derivative at 60°C.

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