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High-performance liquid chromatography of enantiomers of {2-[4-(3-ethoxy-2-hydroxypropoxy)phenylcarbamoyl]ethyl}-dimethylsulfonium *p*-toluenesulfonate (suplatast tosilate) on a cellulose tris-3,5-dimethylphenylcarbamate column

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Abstract

A high-performance liquid chromatographic method for the direct chiral resolution of suplatast tosilate (ST), {2-[4-(3-ethoxy-2-hydroxypropoxy)phenylcarbamoyl]ethyl}dimethylsulfonium *p*-toluenesulfonate and its decomposed products was developed using a cellulose tris-3,5-dimethylphenylcarbamate (Chiralcel OD-H) column eluted with a mixture of *n*-hexane, ethanol, trifluoroacetic acid and diethylamine. The method was applied to determination of the enantiomeric excess and chiral resolution of decomposed products of ST. The enantiomeric purity of the alternatively synthesized enantiomers was estimated to be 98.6% and 99.7% (enantiomeric excess) for (+)- and (−)-ST, respectively. No racemization of ST enantiomer was observed with decomposition at pH 6.8 and 37°C.

1. Introduction

Suplatast tosilate (ST), (±)-{2-[4-(3-ethoxy-2-hydroxypropoxy)phenylcarbamoyl]ethyl}dimethylsulfonium *p*-toluenesulfonate (under application for approval as a new anti-allergic drug by Taiho Pharmaceutical) shows effectiveness for the treatment of Type I allergy-related diseases such as bronchial asthma, allergic rhinitis, urticaria and similar maladies [1–6]. ST is a glycerol derivative containing a chiral centre and is available only as racemates in therapeutic formulations. In order to investigate the pharmacological, pharmacokinetic and toxicological properties of ST, enantiomers of ST were synthesized from enantiomers of 4-(3-ethoxy-2-hydroxypropoxy)aniline (D-2). Several physico-chemical properties of ST enantiomers were also examined. (+)-ST enantiomer showed a very small specific rotation, $[\alpha]_D^{25} = +1.7°$ (5% methanolic solution), which coincides well with the specific rotations of glycerol derivatives reported by Nelson et al. [7]. Although plasma levels of ST after oral administration in rats and dogs have been determined indirectly by measuring 4-(3-ethoxy-2-hydroxypropoxy)acrylanilide (D-1), a major metabolite which was produced quantita-

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tively by alkaline hydrolysis of ST, the analytical method was complicated [8].

Chiral resolutions of β-brocking drugs [9] and many other drugs [10–21] have been successfully accomplished by using a cellulose derivative column. This paper describes the direct separation of ST enantiomers using a cellulose tris-3,5-dimethylphenylcarbamate column. Applications of the method to the determination of the optical purity of the alternatively synthesized ST enantiomers and to stability studies of enantiomers and racemates in solution are described.

2. Experimental

2.1. Chemicals

ST was synthesized in Taiho Fine Chemical (Saitama, Japan). (±)-4-(3-Ethoxy-2-hydroxypropoxy)-3-(methylthio)propionanilide (D-3), enantiomers of ST and enantiomers of D-3 were synthesized from racemic or optically resolved D-2 at Taiho Pharmaceutical (Tokushima, Japan).

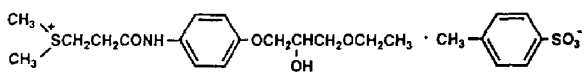
Compounds **1**–**6** and D-1 shown in Fig. 1 were synthesized at Taiho Pharmaceutical.

n-Hexane and ethanol of HPLC-grade and diethylamine (DEA) and trifluoroacetic acid (TFA) of analytical-reagent grade were obtained from Wako (Osaka, Japan) and used as received.

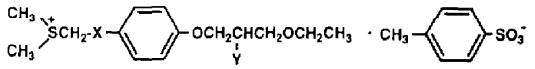
2.2. HPLC apparatus and conditions

The HPLC system used consisted of an LC-6A pump, an SPD-6A detector operating at 254 nm, a CR 4A integrator (Shimadzu, Kyoto, Japan), a Rheodyne Model 7125 valve injector with a 20-μl sample loop (purchased from GL Sciences, Tokyo, Japan) or a WISP Model 710 autosampler (Waters Chromatography Division, Millipore, Milford, MA, USA), and a CS-300C column cabinet (Chromato Science, Osaka, Japan).

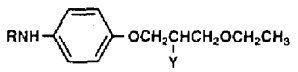
The following chiral HPLC columns were used: Chiralcel OD-H column, cellulose tris-3,5-dimethylphenylcarbamate coated on silica gel (25 cm × 0.46 cm I.D., particle size 5 μm); Chiralcel



Suplatast tosilate



	X	Y
Compound 1 : CH_2CONH		OCH_3
Compound 2 : CONH		OH
Compound 3 : CH_2CH_2CONH		OH
Compound 4 : CH_2O		OH



	R	Y
D-1	: CH_2=CHCO	OH
D-2	: H	OH
D-3	: $CH_3SCH_2CH_2CO$	OH
Compound 5 : CH_2=CHCO		OCH_3
Compound 6 : $CH_3SCH_2CH_2CO$		OCH_3

Fig. 1. Structures of suplatast tosilate and related compounds.

OG and Chiralcel OJ columns, cellulose tris-4-methylphenylcarbamate and cellulose tris-4-methylbenzoate, respectively, coated on silica gel (25 cm × 0.46 cm I.D., particle size 10 μm). All columns were obtained from Daicel Chemical Industries (Tokyo, Japan).

Racemic ST and related compounds were dissolved in methanol at concentrations of 1 mg/ml. ST enantiomers were dissolved in methanol at concentrations of 0.5 mg/ml. The volume injected was 10 μl and the flow-rate was 0.8 ml/min. The effects of mobile phase composition (ethanol, TFA and DEA contents), column temperature and sample size on the capacity factor (k'), enantioseparation factor (α), resolution (R_s) and tailing factor (T) were calculated from the recorded chromatograms as reported previously [22].

2.3. Decomposition of ST

About 0.20 g of ST or its enantiomers was weighed accurately, dissolved in 0.2 *M* phosphate buffer (pH 6.8) and kept at 37°C. A 3-ml volume of sample solution was withdrawn after 44 h and freeze-dried. The residue was dissolved in 3 ml of methanol and filtered. Each enantiomer of ST was determined by loading the filtrate under the optimized conditions.

3. Results and discussion

3.1. Optimization of chromatographic conditions

Preliminary studies showed that the presence of an amine in mobile phase was essential for the chiral separation of ST on a Chiralcel OD-H column. We found that the addition of an acid to the mobile phase was also necessary for the elution of ST from a silica gel column and that ST was extremely unstable in alkaline media. Thus, both TFA as an acid and DEA as an amine were added to mobile phase for the successful chiral separation of ST.

Effects of organic modifier

The influence of different types of alcohol in mobile phase on the enantioseparation factor, resolution, capacity factor and tailing factor of the second-eluted peak and peak-area ratios of enantiomers of ST is shown in Table 1 using a mixture of *n*-hexane and alcohol (75:25, v/v) containing 0.5% TFA and 0.1% DEA at 25°C as the eluent. With an increase in the alkyl chain length of the alcoholic modifier, longer retention times and greater peak broadening were observed. As the enantioseparation factor and resolution became smaller, the peak-area ratio of the enantiomers gradually deviated from 1.0, especially with *n*-butanol. In addition, the enantiomer peaks were not observed within 1 h when 2-propanol was used. When the concentration of 2-propanol was increased to 40%, however, the enantiomer peaks were observed, but all chromatographic parameters were unsatisfactory compared with the use of ethanol.

Effects of ethanol content in the mobile phase on the chromatographic parameters

The effects of ethanol content in the mobile phase were investigated at 25°C using a mixture of *n*-hexane and ethanol containing 0.5% TFA and 0.1% DEA. The results are summarized in Table 2. With an increase in ethanol content, the capacity factors of both isomers decreased. A favourable enantioseparation factor and resolution were obtained under all conditions, and (+)-ST was always eluted faster than (−)-ST. The optimum enantioseparation factor was determined at 25% or 30% ethanol content. The resolution became higher with a decrease in ethanol content. Because the second peak appeared 1 h after injection with a 15% of ethanol content, it was concluded that 25% ethanol was suitable.

Table 1
Effects of organic modifier in the mobile phase on the chromatographic parameters and peak-area ratio of (+)- and (−)-enantiomers of ST

Mobile phase	k'_2	α	R_S	T	r
n-Hexane–ethanol (75:25)	3.92	1.39	2.85	1.42	1.00
n-Hexane–*n*-propanol (75:25)	5.08	1.32	2.03	1.80	0.97
n-Hexane–*n*-butanol (75:25)	5.24	1.27	1.17	2.24	0.81
n-Hexane–2-propanol (60:40)	4.44	1.38	2.16	1.81	0.98

Chromatographic conditions: column, Chiralcel OD-H; mobile phase, as indicated, containing 0.5% TFA and 0.1% DEA; column temperature, 25°C; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_2 = Capacity factor of second-eluted peak; T = tailing factor of second-eluted peak; r = peak-area ratio of (+)- to (−)-enantiomer.

Table 2
Effects of ethanol on the retention and resolution of ST enantiomers

Ethanol (%)	k'_1	k'_2	α	R_S
15	8.42	11.39	1.35	3.71
20	4.70	6.37	1.36	3.06
25	2.89	3.95	1.37	2.63
30	1.96	2.70	1.37	2.39

Chromatographic conditions: column, Chiralcel OD-H; mobile phase, *n*-hexane–ethanol containing 0.5% TFA and 0.1% DEA; column temperature, 25°C; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second eluted peak.

Effects of TFA content in the mobile phase on the chromatographic parameters

The effects of TFA content in the mobile phase were investigated by adding 0.125–0.75% TFA to *n*-hexane–ethanol (75:25, v/v) containing 0.1% DEA at 25°C. The results are shown in Table 3. The mobile phase is basic with a 0.125% TFA content and acidic with 0.25% or more. With an increase in TFA content in acidic media, the capacity factors decreased. This was expected as ST is a tautomeric compound having an amide group. Therefore, the structural change of ST seems to be responsible for the observed variation of the capacity factor. Both the enantioseparation factor and resolution were not affected by TFA content, and 0.5% TFA content was chosen.

Table 3
Effects of TFA on the retention and resolution of ST enantiomers

TFA (%)	k'_1	k'_2	α	R_S
0.75	4.53	6.19	1.36	3.11
0.5	4.70	6.37	1.36	3.06
0.25	4.77	6.44	1.35	3.17
0.125	4.50	6.02	1.34	3.11

Chromatographic conditions: column, Chiralcel OD-H; mobile phase, *n*-hexane–ethanol (75:25) containing 0.1% DEA and TFA (variable); column temperature, 25°C; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second-eluted peak.

Effects of DEA content in the mobile phase on the chromatographic parameters

The effects of DEA content in the mobile phase were determined by adding 0–0.15% DEA to *n*-hexane–ethanol (75:25, v/v) containing 0.5% TFA at 25°C. The results are shown in Table 4. With an increase in DEA content, the capacity factors were decreased, and each enantiomer was incompletely resolved in the absence of DEA. The enantioseparation factor and resolution were not affected by the DEA content. Therefore, a 0.1% DEA content was selected.

Effects of column temperature

The effects of column temperature on the capacity factors, enantioseparation factor and resolution of ST were determined in the range 5–35°C using mobile phases A and B, which include 20 and 25% ethanol in *n*-hexane, respectively, and both containing 0.5% TFA and 0.1% DEA. The results are shown in Table 5. With an increase in the column temperature, the capacity factor, enantioseparation factor and resolution were decreased with both mobile phases, and a lower temperature resulted in good resolution. The resolution was almost the same when mobile phase A at 25°C (condition A) or mobile phase B at 15°C (condition B) were employed, but the enantioseparation factor was higher under the

Table 4
Effects of DEA on the retention and resolution of ST enantiomers

DEA (%)	k'_1	k'_2	α	R_S
0.15	4.39	5.97	1.36	2.98
0.1	4.70	6.37	1.36	3.06
0.075	4.89	6.66	1.36	3.07
0.05	4.98	6.78	1.36	3.14
0	6.07	7.95	1.31	1.18

Chromatographic conditions: column, Chiralcel OD-H; mobile phase, *n*-hexane–ethanol (75:25) containing 0.5% TFA and DEA (variable); column temperature, 25°C; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second-eluted peak.

Table 5
Effects of column temperature on the retention and resolution of ST enantiomers

Temperature (°C)	Mobile phase A[a]				Mobile phase B[b]			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
35	3.62	4.66	1.29	2.47	2.41	3.13	1.30	2.02
30	4.16	5.49	1.32	2.76	2.74	3.65	1.33	2.38
25	4.70	6.37	1.36	3.06	2.89	3.95	1.37	2.63
20	5.59	7.89	1.41	3.49	3.19	4.48	1.40	2.93
15	6.32	9.13	1.44	3.60	3.62	5.25	1.45	3.10
10	–	–	–	–	4.07	6.04	1.48	3.40
5	–	–	–	–	4.92	7.43	1.51	3.39

Chromatographic conditions: column, Chiralcel OD-H; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second eluted peak.
[a] *n*-Hexane–ethanol (80:20) containing 0.5% TFA and 0.1% DEA.
[b] *n*-Hexane–ethanol (75:25) containing 0.5% TFA and 0.1% DEA.

condition B than condition A. In addition, condition A needs a longer time for the analysis. Therefore, condition B is preferable for determination.

Effects of sample size

The effects of sample size on the capacity factors, enantioseparation factor, resolution, peak areas and peak-area ratios of the ST enantiomers were investigated using condition B. The results are shown in Table 6. With an increase in sample size, the capacity factors and resolution decreased, but the enantioseparation factor showed no dependence on the sample size.

Table 6
Effects of amount loaded on the retention and resolution of ST enantiomers

Amount loaded (μg)	k'_1	k'_2	α	R_s
10	3.92	5.67	1.45	3.11
7.5	3.93	5.69	1.45	3.29
5.0	3.97	5.75	1.45	3.55
2.5	4.01	5.77	1.44	3.88
1.25	4.13	5.92	1.43	3.85

Chromatographic conditions: column, Chiralcel OD-H; mobile phase, *n*-hexane–ethanol (75:25) containing 0.5% TFA and 0.1% DEA; column temperature, 15°C; flow-rate, 0.8 ml/min; detection wavelength, 254 nm. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second-eluted peak.

A chromatogram of ST under the optimized conditions is shown in Fig. 2. The plot of peak area against amount of ST loaded over the range 1.25–10 μg showed good linearity with correlation coefficients (r) of 0.9999 and 0.9998 for (+)- and (−)-ST, respectively. The peak-area ratios of two enantiomers were 1.00 for all sample sizes.

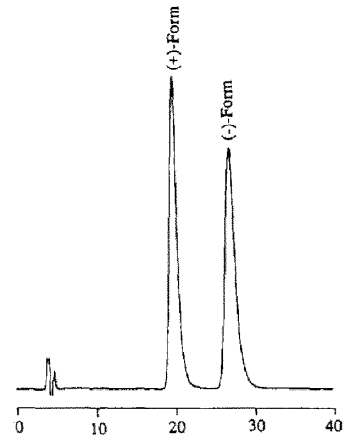


Fig. 2. Chromatogram of ST enantiomers on the Chiralcel OD-H column. Mobile phase, *n*-hexane–ethanol (75:25) containing 0.5% TFA and 0.1% DEA; flow-rate, 0.8 ml/min; detection wavelength, 254 nm; column temperature, 15°C; amount loaded, 5 μg.

This means that the optical purity of the enantiomer can be determined by the area percentage method.

3.2. Chiral resolution of ST and related compounds

The capacity factors and enantioseparation factor of ST and related compounds were determined using mobile phase B and Chiralcel OD-H, Chiralcel OG and Chiralcel OJ columns at 15°C. The results are summarized in Table 7. On the Chiralcel OD-H column, ST enantiomers were resolved with an enantioseparation factor of 1.40, while the methylation of the hydroxyl group of ST (compound **1**) resulted in poor retentivity and enantioselectivity. The same was observed with D-1 and **5** and with D-3 and **6**. It is interesting that **2** and **3**, having shorter and longer alkyl chains, were not resolved under condition B. It was impossible to resolve **4**, which has no amide or amine group on the side-chain, on the Chiralcel OD-H column. However, on the Chiralcel OG column, the enantiomers of **4** were resolved with an enantioseparation factor of 1.19. The enantiomers of **2** and **3** were successfully resolved by using the Chiralcel OG column. On the Chiralcel OJ column, the compounds except **4** and **6** were not resolved at all; **4** and **6** were partly resolved with enantioseparation factors of 1.09 and 1.06, respectively. The chiral recognition is assumed to be due to the formation of inclusion complexes [23] and binding to the polar carbamate groups. The racemates interact with the carbamate group via hydrogen bonding with the NH and C=O groups and dipole–dipole interaction with C=O group [11,24,25]. Further, the hydroxyl group of the amino alcohol seems to be of importance for the chiral recognition process [17]. The results for the chiral resolution of ST and related compounds on the cellulose trisphenylcarbamate derivative column coincided well with these results. It was also found that the length of the carbamoyl side-chain was the important factor for chiral recognition of ST.

It is noteworthy that the elution times of (−)-D-1 and (−)-D-3 was shorter than those of (+)-D-1 and (+)-D-3 on the Chiralcel OD-H column, while (+)-ST was eluted rapidly under the same condition. The dimethylsulfonium group of ST might greatly affect the elution order. The

Table 7
Capacity factors and enantioseparation factor of ST and related compounds on Chiralcel OD-H, Chiralcel OG and Chiralcel OJ columns

Substance	Chiralcel OD-H			Chiralcel OG			Chiralcel OJ		
	k'_1	k'_2	α	k'_1	k'_2	α	k'_1	k'_2	α
ST	3.99 (+)	5.59 (−)	1.40	20.1 (+)	28.1 (−)	1.40	2.32	2.32	1.00
1	1.58	1.86	1.18	11.5	11.5	1.00	2.17	2.17	1.00
2	2.29	2.29	1.00	11.9	15.1	1.26	2.53	2.53	1.00
3	1.73	1.73	1.00	9.40	11.8	1.25	1.50	1.50	1.00
4	1.22	1.22	1.00	9.69	11.5	1.19	1.95	2.12	1.09
D-1	0.94 (−)	1.14 (+)	1.20	1.94 (+)	2.23 (−)	1.15	1.10	1.10	1.00
D-2	0.18 (−)	0.21 (+)	1.16	0.72	0.72	1.00	0.21	0.21	1.00
D-3	1.24 (−)	1.44 (+)	1.17	3.85 (+)	4.54 (−)	1.18	2.28	2.28	1.00
5	0.76	0.76	1.00	3.05	3.05	1.00	1.41	1.41	1.00
6	0.88	0.88	1.00	3.36	3.36	1.00	2.16	2.28	1.06

Mobile phase, *n*-hexane–ethanol (75:25) containing 0.5% TFA and 0.1% DEA; column temperature, 15°C. k'_1 = Capacity factor of first-eluted peak; k'_2 = capacity factor of second-eluted peak; (+) and (−) mean (+)-enantiomer and (−)-enantiomer for each substance.

elution order of enantiomers of D-1 and D-3 on the Chiralcel OD-H column was opposite to that on the Chiralcel OG column. These results suggest that the substituent on the aromatic ring of the chiral stationary phase greatly affects the order of elution of enantiomers.

3.3. Determination of enantiomeric excess of ST

The enantiomeric excess (e.e.) of alternatively synthesized (+)- or (−)-ST was determined by the proposed method using *n*-hexane–ethanol (75:25, v/v) containing 0.5% TFA and 0.1% DEA as the eluent and a Chiralcel OD-H column at 15°C. The values obtained for (+)- and (−)-ST were 98.6 and 99.7% e.e., respectively.

3.4. Chiral resolution of decomposition products of ST

It has been reported that the decomposition of ST in acidic media results in the formation of D-2 together with a small amount of D-1 and D-3, and D-1 in alkaline media. The chromatogram of ST and its decomposed products is shown in Fig. 3. ST was separated completely from three decomposition products, and it was found that this HPLC condition could be used for the chiral resolution of the decomposition products of ST. For the complete separation of enantiomers of D-1, D-2 and D-3, we used a mobile phase with a low ethanol content (8.5%) and the chromatogram of the three decomposition products of ST is shown in Fig. 4. Although the resolution of D-2 enantiomers was incomplete under the conditions in Fig. 4, D-1 and D-3 enantiomers were completely resolved. For D-2, complete resolution was achieved when the mobile phase was changed to *n*-hexane–2-propanol (60:40, v/v) using the same column (Fig. 5). No racemization of ST enantiomer was observed with decomposition at pH 6.8 and at 37°C, and (+)- and (−)-STs yielded (+)- and (−)-decomposition products, respectively.

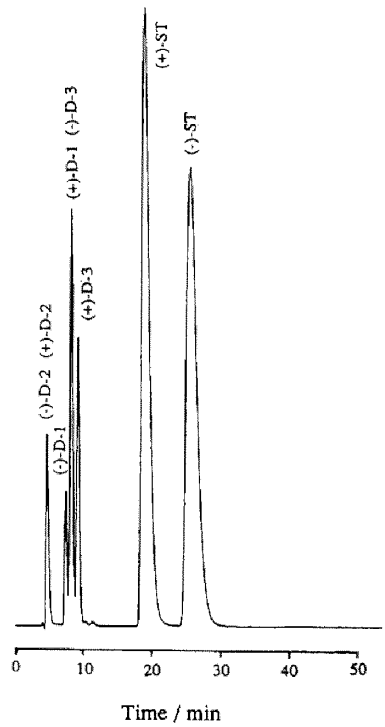


Fig. 3. Chiral resolution of ST and related compounds on the Chiralcel OD-H column. Mobile phase, *n*-hexane–ethanol (75:25) containing 0.5% TFA and 0.1% DEA; flow-rate, 0.8 ml/min; detection wavelength, 254 nm; column temperature, 15°C; amount loaded, 25 μg for ST and D-2 and 2.5 μg for D-1 and D-3.

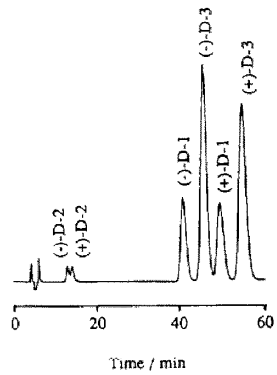


Fig. 4. Chromatogram of racemic D-1, D-2 and D-3 on the Chiralcel OD-H column. Mobile phase, *n*-hexane–ethanol (915:85) containing 0.5% TFA and 0.1% DEA; flow-rate, 0.8 ml/min; detection wavelength, 254 nm; column temperature, 15°C; amount loaded, 5 μg each.

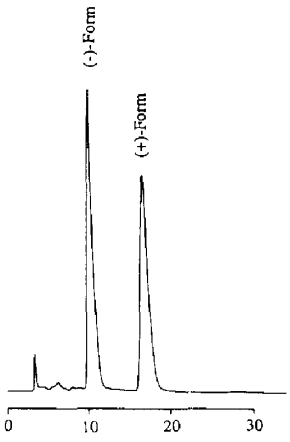


Fig. 5. Chromatogram of racemic D-2 on the Chiralcel OD-H column. Mobile phase, *n*-hexane–2-propanol (60:40); flow-rate, 0.8 ml/min; detection wavelength, 254 nm; column temperature, 15°C; amount loaded, 5 μg.

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