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Short communication

Enantiomeric separation of chiral theophylline derivatives by liquid chromatography on cellulose-based sorbents

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Abstract

The enantiomeric resolution of seven closely related theophylline racemates by high-performance liquid chromatography on cellulose-based sorbents, in particular Chiralcel-OD, -OC and -OJ, is described. Although all chiral stationary phases (CSPs) are suitable for the enantioseparation of all racemates investigated, it is obvious that method development is different for each stationary phase. The screenings with the above-mentioned CSP included variation of mobile phase and temperature. It turned out that Chiralcel-OD should be used with 2-propanol in *n*-hexane as the mobile phase at higher temperatures, whereas Chiralcel-OC performed best with methanol at ambient temperature. Improved enantioseparations were observed for Chiralcel-OJ with increased modifier concentrations in *n*-hexane at increased temperature.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Theophylline derivatives

1. Introduction

Theophyllines belong to the pharmacological important class of selective phosphodiesterse (PDE) isoenzyme inhibitors. The in vivo effects of these compounds on the lung and on the heart are thought to be the consequence of the blockade of the different subclasses of this enzyme family in the body. MKS 492 [1], the enantiomer with the *R*-configuration of racemate 1 (the structure is given in Fig. 1), which was extensively investigated as an anti-asthma agent in preclinical [2,3] and clinical trials, belongs to the class of theophyllines that block very potently the subtype III of PDEs and that does not penetrate the blood–brain barrier.

Most of the C-8 derivatized theophyllines are chiral compounds. At the beginning of development, preparative chromatography on chiral stationary phases enabled us to obtain hundreds of milligrams of the pure enantiomers for characterization and first screening tests such as inhibition of different subclasses of PDE, adenosine binding, penetration of the blood–brain barrier, solubility, enantiomeric and chemical stability. The need for chromatographic tools for the enantioseparation on analytical and preparative scale is therefore obvious.

Although chiral theophyllines have been investigated for many years, only two papers deal with enantioseparation on a chiral stationary phase (CSP). Julien has reported on the baseline separation of tazifylline [4] enantiomers using an α-acid glycoprotein column, while Hermansson has demonstrated the successful application of silica-immobilized cellobiohydrolase 1 (CBH 1) for the enantiomeric resolution of proxyphylline [5]. Both racemates belong to the class of N-7-derivatized xanthines. To the best of our

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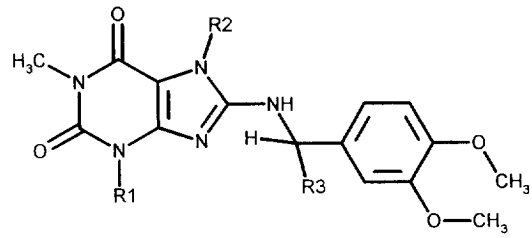

1,2,3,4,6,7

Rac.	R1	R2	R3
1	$-CH_3$	$-C_2H_4-O-CH_3$	$-CH_2-OH$
2	$-CH_3$	$-CH_3$	$-CH_2-OH$
3	$-CH_3$	$-C_2H_4-O-CH_3$	$-CH_2-OCON(CH_3)_2$
4	$-CH_2$-cyclopropyl	$-C_2H_4-O-CH_3$	$-COOC_2H_5$
6	$-CH_3$	$-C_2H_4-O-CH_3$	$-CH_2-O-CH_3$
7	$-CH_3$	$-C_2H_4-OH$	$-CH_2-O-CH_3$

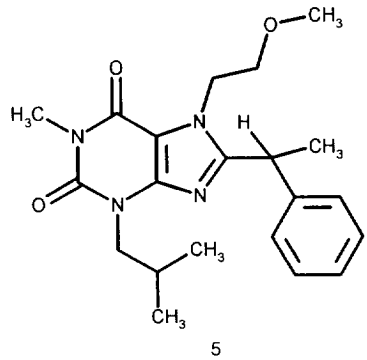

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Fig. 1. Structures of investigated theophyllines.

knowledge no publication deals with the enantiomeric separation of C-8-derivatized xanthines. This paper describes the enantioseparation of chiral theophyllines belonging to the class of C-8-derivatized xanthines on different cellulose-based sorbents. Since the alcohol component of the mobile phase is known to affect the enantiomeric separations obtained with cellulosic and amylosic CSPs [6–11] the influence of the mobile phase composition is investigated as well as the temperature dependence of the separations.

2. Experimental

2.1. Materials

The structures of all theophylline derivatives **1–7** investigated in this study are given in Fig. 1. All racemates are Sandoz active ingredients and their syntheses have been described elsewhere. The solvents used for the preparation of the mobile phases were of LiChrosolv grade from Merck (Darmstadt,

Germany). The mobile phase compositions and chromatographic parameters are given in Tables 1–3. Dead times (t_0) were estimated using 1,3,5-tri-*tert.*-butylbenzene. Capacity factors k' have been calculated according to the equation $k'=(t_r-t_0)/t_0$; enantioselectivities (α) are used according to $\alpha=k'_2/k'_1$; the resolution factors (R) are used according to $R=1.18(t_2-t_1)/(W_{1(0.5)}+W_{2(0.5)})$, where t_1 and t_2 refer to the retention time of the first and second eluted enantiomer and $W_{1(0.5)}$ and $W_{2(0.5)}$ represent the peak width of the corresponding peak at 50% of its height.

2.2. Liquid chromatography

A Kontron HPLC pump (Model 420) was used in conjunction with a Kontron variable-wavelength UV detector (Model 430). The column temperature was maintained with a Henggeler (Riehen, Switzerland) column thermostat. The following chiral stationary phases (column: 25 cm×0.46 cm I.D.) were purchased from Daicel Chemical Industries (Tokyo, Japan): Chiralcel-OD=cellulose tris(3,5-dimethylphenylcarbamate), Chiralcel-OJ=cellulose tris(4-methylbenzoate) and Chiralcel-OC=cellulose tris-

Table 1
Influence of modifier concentration and temperature on the enantioseparation of theophylline derivatized racemates on Chiralcel-OD (flow-rate, 0.5 ml/min; UV detection, 295 nm; modifier concentration and temperature as indicated in the table)

Racemate	2-Propanol (in hexane) (%)	Temperature (°C)	t_2 (min)	k'_2	α	R
1	10	20	-	-	-	-
	30	20	28.73	5.93	1.00	0.00
	10	40	-	-	-	-
	30	40	18.65	2.73	1.00	0.00
2	10	20	-	-	-	-
	30	20	43.95	6.54	1.19	0.63
	10	40	47.13	7.60	1.00	0.00
	30	40	28.75	4.75	1.15	0.85
3	10	20	-	-	-	-
	30	20	32.47	4.57	1.00	0.00
	10	40	-	-	-	-
	30	40	18.21 (*S*)	2.64	1.00	0.00
4	10	20	59.99	9.40	1.38	2.22
	30	20	17.64	2.03	1.29	1.55
	10	40	32.00	4.84	1.23	2.34
	30	40	11.52	1.30	1.14	0.82
5	10	20	25.39	3.40	2.96	4.70
	30	20	11.86	1.03	2.79	3.19
	10	40	17.60	2.21	2.43	4.72
	30	40	9.04	0.81	1.94	1.98
6	10	20	-	-	-	-
	30	20	23.18	2.98	1.09	0.56
	10	40	48.23	7.80	1.09	0.96
	30	40	14.48	1.90	1.08	0.65
7	10	20	-	-	-	-
	30	20	18.05	2.10	1.00	0.00
	10	40	68.15	11.44	1.09	0.67
	30	40	12.88	1.58	1.00	0.00

- no elution within 90 min.

Table 2
Influence of temperature on the enantioseparation of theophylline derivatized racemates on Chiralcel-OC (mobile phase: methanol with a flow-rate of 0.5 ml/min, UV detection: 210 nm)

Racemate	Temp. (°C)	t_2 (min)	k'_2	α	R
1	20	34.61	4.85	1.33	1.44
	40	20.43	2.82	1.14	0.43
2	20	41.88	6.07	1.54	2.13
	40	24.41	3.56	1.35	1.04
3	20	56.14	8.48	1.20	0.84
	40	30.34 (*R*)	4.67	1.11	0.44
4	20	28.11	3.75	1.22	0.81
	40	16.89	2.16	1.00	0.00
5	20	7.84	0.32	1.00	0.00
	40	7.42	0.39	1.00	0.00
*	20	13.32	1.68	1.44	1.36
6	20	34.25	4.79	1.09	0.41
	40	20.98	2.92	1.00	0.00
7	20	29.23	3.94	1.49	1.79
	40	16.88	2.16	1.14	0.23

* 30% 2-propanol in *n*-hexane was used as the mobile phase.

(phenylcarbamate). The cellulose derivatives are coated on silica gel with a particle size of 10 μm.

3. Results and discussion

The ability of chemically modified cellulose to separate a variety of racemates has recently been reviewed by Okamoto [12]. Especially if modified as triesters or triscarbamates, the corresponding CSPs exhibit excellent resolution properties. With respect to their separation problems the authors claim a probability of 80% success if the racemate is screened in a first test with Chiralcel-OD, Chiralcel-OJ and Chiralpak-AD.

Table 3
Influence of temperature on the enantioseparation of theophylline derivatized racemates on Chiralcel-OJ (mobile phase: 30% 2-propanol in *n*-hexane with a flow-rate of 0.5 ml/min; UV detection: 295 nm)

Racemate	Temp. (°C)	t_2 (min)	k'_2	α	R
1	40	-	-	-	-
2	40	56.42	10.02	1.08	0.54
3	40	61.40 (*R*)	10.99	1.20	1.31
4	40	22.49	3.39	1.23	1.36
5	40	7.64	0.49	1.23	0.81
6	40	48.83	8.54	1.32	2.31
7	40	33.91	5.62	1.24	1.43

- no elution within 90 min.

In our first screening we have investigated Chiralcel-OD using 10% and 30% 2-propanol in hexane as the mobile phase at 20°C as well as at 40°C. The results of that screening are summarized in Table 1. As expected, decreasing retention times and separation factors are obtained with increasing amounts of 2-propanol and/or increasing temperature. Thus, all separations are enthalpy controlled. We have recently shown that increased separation factors at elevated temperatures are possible using cellulose-derivatized CSP [13,14]; nevertheless, it is accepted that the known examples are exceptions. Although for racemate **5** a separation factor of $\alpha=3$ has been observed, the general utility of Chiralcel-OD for the separation of theophillines is questionable. Most of the racemates are not baseline resolved and in the case of racemates **1** and **3** not even a partial separation has been obtained. The amount of 2-propanol seems not to influence the separation significantly, whereas there is a tendency to have better resolution at higher temperatures. Peak sharpening at 40°C overcompensates the slight decrease in enantioselectivity and yields higher resolution factors. In the case of racemate **4** the individual enantiomers were available, thus allowing the determination of the order of elution. The enantiomer with the *R*-configuration is eluted prior to its mirror image. Typical chromatograms are given in Fig. 2 Fig. 3. In Fig. 2 the influence of the modifier concentration is demonstrated for the separation of racemate **4**, whereas in Fig. 3 the influence of temperature is shown for the enantioseparation of racemate **5**. A recommendation for the separation of theophyllines on Chiralcel-OD would be as follows: adjust the amount of 2-propanol to obtain reasonable capacity factors and perform the separation at higher temperatures.

In our second screening all racemates have been chromatographed on Chiralcel-OC. Although a baseline separation is obtained for racemate **5** with 30% 2-propanol in hexane on Chiralcel-OC, the mobile phase was changed to methanol, since broad peaks in combination with long retention times and poor resolution were obtained for the remaining

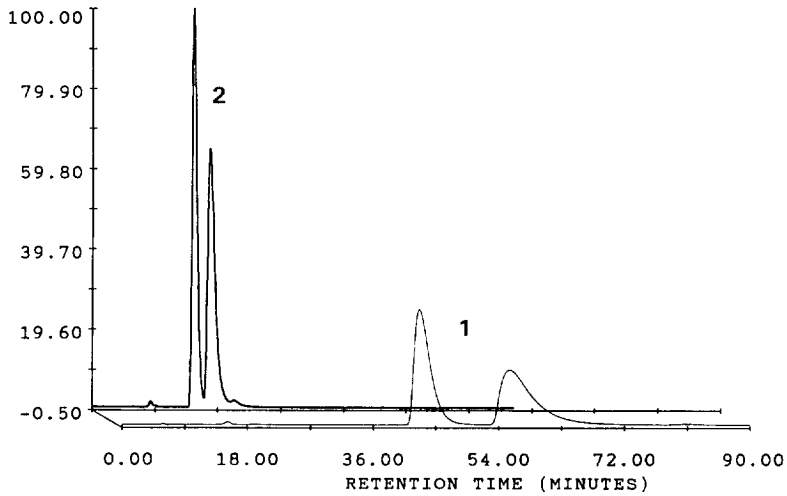

Fig. 2. Effect of 2-propanol concentration on the enantiomeric separation of racemate **4** on Chiralcel-OD at 20°C (curve 1: 10% 2-propanol; curve 2: 30% 2-propanol); chromatographic parameters are given in Table 1.

racemates. The loss of enantioselectivity for this racemate when chromatographed with methanol as mobile phase seems, therefore, to be the result of the small capacity factors instead of a poorer chiral discrimination property of the CSP. The results of that screening are summarized in Table 2. Again, decreasing retention times and enantioselectivities are obtained with increasing temperature. In comparison with the separations on Chiralcel-OD, it turned out that Chiralcel-OC seems to be more applicable for the separation of chiral theophillines. The separation factors are all in the same order of magnitude ($\alpha = 1.2$) and are somewhat lower in comparison with the best separation on Chiralcel-OD; nevertheless, all racemates could be resolved on Chiralcel-OC. Interestingly, in the case of racemate **4** the order of elution has changed in comparison with Chiralcel-OD (see Table 1). Now the enantiomer

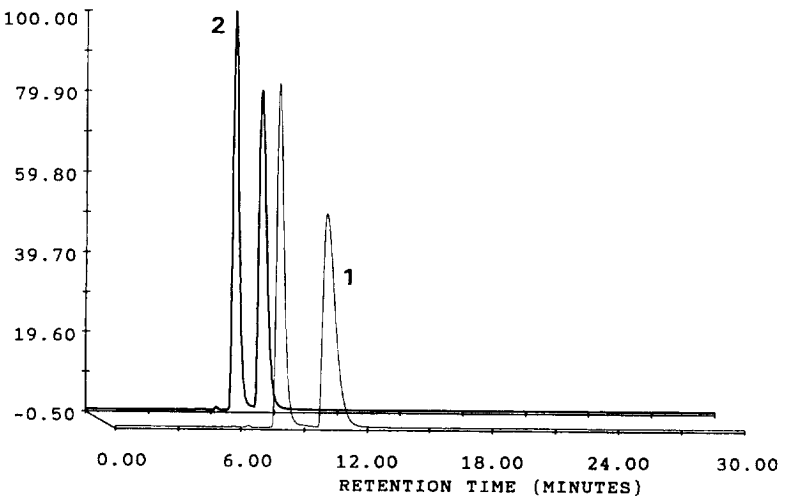

Fig. 3. Effect of temperature on the enantiomeric separation of racemate **5** on Chiralcel-OD with 30% 2-propanol in *n*-hexane (curve 1: 20°C; curve 2: 40°C); chromatographic parameters are given in Table 1.

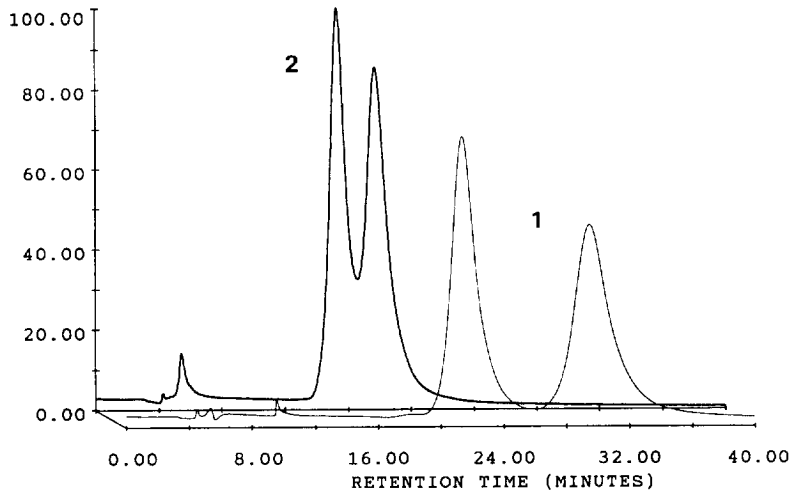

Fig. 4. Effect of temperature on the enantiomeric separation of racemate **7** on Chiralcel-OC (curve 1: 20°C; curve 2: 40°C); chromatographic parameters are given in Table 2.

with the *S*-configuration is eluted first. Typical chromatograms are shown in Fig. 4 for the separation of racemate **7** indicating the sensitivity of temperature on the separation. Even though a near baseline resolution is achieved at 20°C a significant loss on enantioselectivity is observed at 40°C. A recommendation for the separation of theophyllines on Chiralcel-OC would be as follows: use methanol as the mobile phase at low temperatures.

In the last screening the separation capability of Chiralcel-OJ was investigated. It was necessary to run the screening with 30% 2-propanol in hexane at 40°C, since no elution within 90 min occurred with lower 2-propanol concentrations (at 40°C). Even under these conditions racemate **1** was not eluted. As in the case of Chiralcel-OD and Chiralcel-OC, decreasing retention times and separation factors are obtained with increasing temperature. In the case of racemate **4** the order of elution is the same as with Chiralcel-OC (see Table 2) and, thus, is opposite in

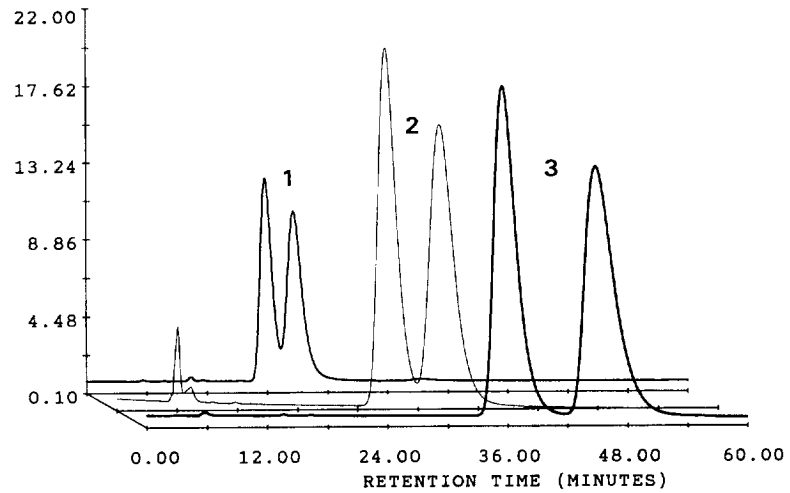

Fig. 5. Enantiomeric separations of racemates **4** (curve 1), **7** (curve 2) and **6** (curve 3) on Chiralcel-OJ; chromatographic parameters are given in Table 3.

comparison with Chiralcel-OD (see Table 1). The enantiomer with *S*-configuration is eluted first. Typical chromatograms are shown in the overlay plot in Fig. 5 for the separations of racemates **4**, **7** and **6**, providing baseline separations for all mentioned racemates. A recommendation for the separation of theophyllines on Chiralcel-OJ would, therefore, be as follows: increase the amount of 2-propanol until reasonable capacity factors are obtained at higher temperatures.

In summary, we have demonstrated for the first time that CSPs based on chemically modified cellulose are suitable for the enantiomeric separation of C-8 derivatized xanthines. It seems likely that some separations can be further improved by changing the organic modifier (e.g. replacement of 2-propanol by ethanol etc.) or by careful optimization of the solvent strength. Nevertheless, in routine analysis, it is time and capacity saving, if all samples can be treated automatically under the same conditions. From our results it was not possible to identify one column with a corresponding set of chromatographic parameters; nevertheless, the number of possibilities has been significantly reduced while considering the above-mentioned strategies.

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