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Short Communication

Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography*

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Introduction

Polysaccharide type chiral stationary phases (CSP) such as cellulose- and amylose-esters [1–3] and -phenylcarbamates [1] belong to the most commonly used packing materials for both, analytical- and preparative scale [2] LC enantioseparations. As recent studies show, there are some interesting new developments as in direction of elucidation of chiral recognition phenomena [4, 5], as well as in expanding area of application of these CSPs [1, 2, 4, 6–10]. On the other hand, miniaturization of the HPLC experiment is cost-effective [11] and, moreover, this technique offers some advantages that are especially important for pharmaceutical and clinical analysis.

As it can be seen from one of the basic equations for chromatographic peak resolution (R_s) one can reduce column length four times without any loss of the resolution, by only a 2-fold reduction of the column diameter. By this operation the column volume will be reduced by a factor of sixteen. This corresponds to a 16-fold reduction of the column packing materials and mobile phases needed.

A shortening of analysis time by shortening the column length is another obvious advantage of narrow-bore LC. The reduction of column size results in a lower dilution of the sample on the column and, thus, enhance the mass-sensitivity. This is very important for biopharmaceutical analysis where the mass of material is often very limited. Moreover, this technique is easy in handling and environmentally more safe. Due to lower flow rate the narrow-bore LC is more versatile in coupling to other analytical techniques such as mass-spectrometry, GC, capillary electrophoresis etc.

In spite of some abovementioned advantages applications of chiral narrow-bore and micro-columns are still scarcely published [3, 12, 13].

In the present study the use of commercially available polysaccharide phenylcarbamates, as well as some of recently described new derivatives [4, 9, 11] is demonstrated as CSPs for narrow-bore LC in enantioseparation of various chiral pharmaceuticals.

Experimental

Chemicals and drug substances

Microcrystalline cellulose (Avicel) was purchased from Merck (Darmstadt, Germany). Amylose B (MW = ca 16 000) was from Nakalai Tesque (Kyoto, Japan). 3,5-Dimethylphenyl isocyanate, 3-chloro-4-

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methylphenyl isocyanate and 4-chloro-3-methylaniline were reagent grade from Lancaster (Lancashire, UK). Triphosgene, [(±)-2,2,2-trifluoro-1-antrylethanol (**3**), (±)-benzoin (**4**), (±)-trans-stilbene oxide (**5**) and (±)-Tröger base (**6**) were purchased from Aldrich (Milwaukee, WI, USA). Racemic hexobarbital, methylphenobarbital and oxazepam were from the Sigma Chemical Company (St Louis, MI, USA). Racemic 1-N-benzoyl-5-ethyl-5-phenylbarbituric acid (Benzonal), 1-N-*o*-fluorbenzoyl-5-ethyl-5-phenylbarbituric acid (Halonal) and 1-N-benzoyl-5-ethyl-5-i-amyllbarbituric acid (Benzobamy) were gifts from Drug Design Laboratory in Tomsk Polytechnique (Tomsk, Russia). Some of other racemic drugs were gifts of manufacturers and some of them were extracted and purified by multiply recrystallization from the commercially available drug preparations. 4-Chloro-3-methylphenyl isocyanate was prepared from a corresponding amine by the conventional method using triphosgene. Wide pore silica gel (Nucleosil 1000-7 with pore diameter 1000 Å and particle size 7 μm) was obtained from Macherey-Nagel (Düren, Germany) and was silanized using 3-aminopropyl-triethoxysilane in benzene at 80°C before use. Hexane and 2-propanol as components of the mobile phase were of LC reagent grade from J.T. Baker (Deventer, the Netherlands).

Preparation of polysaccharide trisphenylcarbamate derivatives

The polysaccharide trisphenylcarbamate derivatives presented in Fig. 1 were prepared as described previously [4] by the reaction of cellulose (in case of **1a–c**) and amylose B (in case of **2a** and **b**) with an excess of the corresponding isocyanates in dry pyridine at ca 100°C and isolated as methanol unsoluble fractions. Almost complete substitution of the hydroxy groups with the carbamate moiety was confirmed by elemental analysis, IR and NMR spectra.

Preparation of stationary phase

Column packing materials were prepared by dissolving the appropriate amount of the corresponding polysaccharide phenylcarbamate in tetrahydrofurane (**1a–c**) or in N,N′-dimethylformamide (**2a, b**) and coating on macroporous silica gel (Nucleosil 1000-7). The solvents were removed by drying of the sorbents at 60°C for 3 h under reduced pressure. The packing material was packed in 62 × 2 mm i.d. stainless-steel tubes by the conventional slurry packing technique using a Beckman 110 A HPLC pump (Beckman, Palo Alto, CA, USA) at 40 atm. The dead volumes of the columns were determined using 1,3,5-tri-tert.-butylbenzene as non-retained compound [14, 15] and the plate numbers of the columns for benzene were determined at 20°C using hexane–2-propanol (90:10, v/v) as the eluent with a flow-rate of 0.1 ml min^{-1}. A conventional size (250 × 4.6 mm i.d.) column was packed with packing material **2a** using high pressure slurry packing technique at 450–500 atm. The column dead volume and plate

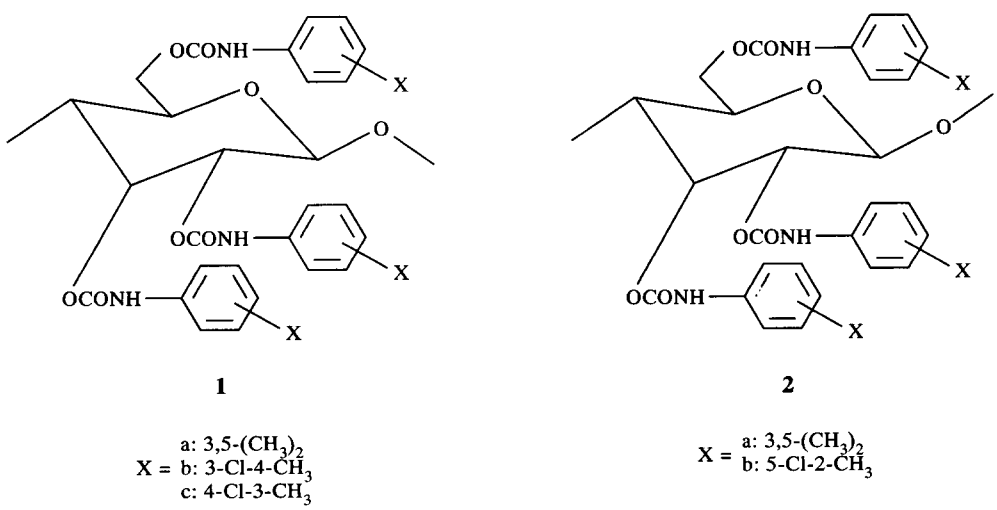
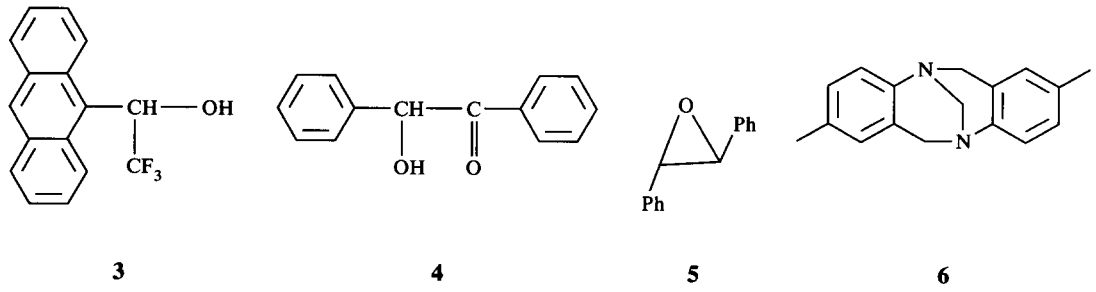


Figure 1
Structure of cellulose- (**1**) and amylose-(**2**) trisphenylcarbamates used as CSPs.



3 4 5 6

Figure 2
Structures of test racemic compounds.

number (ca 3500) were determined by the same way as for narrow-bore columns.

Apparatus

All narrow-bore chromatographic experiments were performed on a Milikhrom-1 microcolumn liquid chromatograph from Nauchpribor (Oryol, Russia), equipped with a syringe-type pump and variable wavelength UV detector unite. An injector was also syringe-type and operated at a stopped flow. A flow rate 0.1 ml min^{-1} and a detection wavelength of 254 nm were used in all narrow-bore experiments. LC separations with conventional-size column (250 × 4.6 mm i.d.) were carried out using a Beckman Model 110a isocratic pump, a Beckman Model 332 analytical optical unite (both from Beckman, Palo Alto, CA, USA) and a Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA).

All calculations (N, k_o, K', α, R_s) were carried out following equations commonly used in chromatography.

Results and Discussion

It is worth mentioning that some technical problems substantially restrict the more or less complete realization of abovementioned principal advantages of narrow- and microbore LC. Limitations, concerning the volume and mass of the sample to be injected, particle size of silica, void volume of a system used, and limited volume of detector cell (which means shorter optical pathway) should be emphasized first of all in this case.

In order to evaluate the suitability of the microcolumn chromatographic system used in this study for enantioseparation, the attempt was made to resolve chiral compounds (Fig. 2), successful resolution of that was reported recently using conventional-size columns [8]. Particularly, (±)-2,2,2-trifluoroantrylethanol (**3**) and (±)-benzoin (**4**) were resolved on the narrow-bore column, packed with chiral sorbent on the basis of **1a**, and (±)-trans-stilbene oxide (**5**) and (±)-Tröger base (**6**) on the same dimension column, packed with chiral sorbent on the basis of **2a**. Baseline resolutions of the enantiomers were achieved in all instances (Fig. 3). Fluctuations in enantioselectivity (α) observed between conventional size and narrow-bore columns are reasonable in all cases taking into account that linear flow rates of mobile phase, charges of polysaccharide phenylcarbamates used and sources of silica were different.

For more reliable comparison between a narrow-bore and conventional size columns,

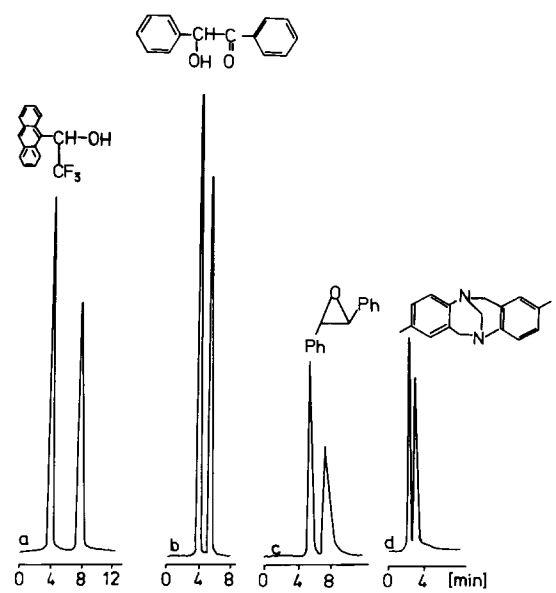


Figure 3
Chiral separation of test racemic compounds using narrow-bore (62 × 2 mm i.d.) columns packed with cellulose-(a,b) and amylose-(c,d) tris(3,5-dimethylphenyl carbamate)s **1a** and **2a**, respectively, on silica gel. Mobile phase: hexane–2-propanol 90:10 (v/v); flow rate 0.1 ml min^{-1}.

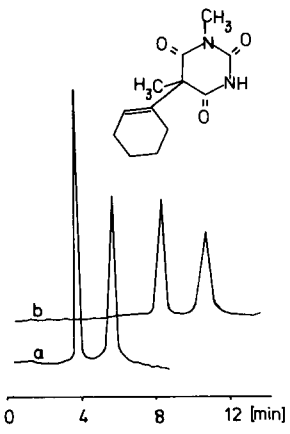


Figure 4
Chiral separation of racemic hexobarbital using narrow-bore (62 × 2 mm i.d.) (a) and conventional size (250 × 4.6 mm i.d.) (b) columns packed with amylose tris(3,5-dimethylphenylcarbamate) (**2a**) on silica gel. Mobile phase: hexane–2-propanol 90:10 (v/v); flow rate: (a) 0.1 ml min^{-1} and (b) 1.0 ml min^{-1}. Detection at 254 n. Injected volumes 2 μl (a) and 20 μl (b) of 1 ml ml^{-1} solution of hexobarbital in mobile phase.

completely identical packing material on the basis of the chiral selector **2a**, coated on Nucleosil 100-7, was packed in both conventional size (250 × 4.6 mm i.d.), and narrow-bore (62 × 2 mm i.d.) columns. The enantio-separation of (±)-hexobarbital was carried out on these columns using a same mobile phase (Fig. 4). Linear velocity of the mobile phase is ca. twice higher in case of narrow-bore column, which results in shorter retention times in this case. Selectivity of enantio-separation also decreases slightly for narrow-bore column, most probably due to the same reason, but peak resolution (R_s) in the later case is higher, than in the former one. Higher mass-sensitivity is also apparent in case of narrow-bore column. Thus, advantages of a narrow-bore LC, such as shorter analysis time, higher peak resolution and higher mass-sensitivity, in chiral analysis is obvious from Fig. 4.

A number of other racemic pharmaceuticals were analysed using the same dimension

Table 1
Resolution of chiral pharmaceuticals using narrow-bore columns packed with polysaccharide CSPs

Pharmaceutical	Column	Eluant	k_1'	α	R_s
Hexobarbital	1b	a	0.30	2.30	1.2
	2a	a	2.10	1.63	1.5
	1a	a	3.90	1.34	1.0
Mephobarbital	1a	a	2.40	1.82	3.7
	2a	a	3.80	1.80	3.0
Benzonal	1a	a	3.00	1.51	2.0
Halonal	2a	a	2.80	1.23	0.8
	1c	a	0.40	2.52	1.5
	1a	a	3.00	1.22	1.2
Benzobamyl	1b	a	3.00	1.22	0.9
1-N-Methyl-5-ethyl-5-propylbarbital	1b	a	1.40	1.21	0.6
Dithiazem hydrochloride	1c	c	3.0	1.81	4.0
Pindolol	1c	c	2.10	1.37	4.0
	1b	a	9.50	2.14	0.8
Acebutolol	1c	d	25.7	1.12	0.8
Propranolol	1c	d	3.30	1.21	1.2
Aminogluthetimide	1c	c	25.00	1.20	1.3
Mesuximide	1c	a	4.10	1.12	1.4
Bifonazole	1c	b	3.6	1.56	2.0
	1b	a	4.6	1.80	2.0
Nisoldipine	1c	a	4.3	1.18	2.0
	1b	a	3.4	1.13	2.0
Oxazepam	1c	a	18.30	1.25	0.7
	1b	a	9.40	1.25	1.8
Prilocain	2b	f	2.50	1.38	1.5
Carticain	2b	f	2.90	1.30	1.6
	1c	f	5.00	1.48	4.1
Doxapram	1c	a	9.10	1.24	1.8
	2b	f	9.10	1.67	1.6
Ambucetamid	2b	f	3.80	1.26	0.9
Chlorphenoxamin	1c	e	2.30	1.19	1.5

Eluant (a) hexane-2-propanol 90:10; (b) hexane-2-propanol 85:15; (c) hexane-2-propanol-diethylamin 80:20:0.1; (d) hexane-2-propanol-diethylamin 90:10:0.1; (e) hexane-2-propanol-diethylamin 98:2:0.1 and (f) hexane-2-propanol-diethylamin 95:5:0.1.

narrow-bore columns packed with various polysaccharide phenylcarboamate CSPs (Table 1). Enantioseparation of some pharmaceuticals (for example, halonal, benzobamyl, 1-N-methyl-5-ethyl-5-propylbarbital, bifonazol, carticain, doxapram and ambucetamid) given in Table 1 are described first time, at least best for our knowledge, using polysaccharide CSPs. New type of recently described [4, 9, 10] mixed chloro- and methylphenylcarbamates of polysaccharides (columns **1b**, **c** and **2b**) seems to be promisable in scope of broadening the number of chiral substances which can be resolved using polysaccharide phenylcarbamates as CSPs.

References

[1] Y. Okamoto and Y. Kaida, *J. Chromatogr. A* **666**, 403–419 (1994).
[2] E. Francotte, *J. Chromatogr. A* **666**, 565–601 (1994).
[3] J. Chen, D. Steenackers and P. Sandra, in *Proceedings of the 15th International Symposium on Capillary Chromatography* (P. Sandra, Ed.), pp. 1163–1169. Hütig (1993).
[4] B. Chankvetadze, E. Yashima and Y. Okamoto, *J. Chromatogr. A* **670**, 39–49 (1994).
[5] E. Yashima, M. Yamada and Y. Okamoto, *Chem. Lett.* 579–582 (1994).
[6] K. Ikeda, T. Hamasaki, H. Kohno, T. Ogawa, T. Matsumoto and J. Sakai, *Chem. Lett.* 1089–1093 (1989).
[7] A. Ishikawa and T. Shibata, *J. Liq. Chromatogr.* **16**, 859–878 (1993).
[8] Y. Okamoto, Y. Kaida, R. Aburatani and K. Hatada, in *Chiral Separations by Liquid Chromatography* (S. Ahuja, Ed.), pp. 101–113. American Chemical Society, Washington, DC (1991).
[9] B. Chankvetadze, E. Yashima and Y. Okamoto, *Chem. Lett.* 745–748 (1993).
[10] B. Chankvetadze, E. Yashima and Y. Okamoto, *Polymer Preprints, Japan* (English Edition), **42**, E228 (1993).
[11] R.P.W. Scott, in *Techniques in Liquid Chromatography* (C.E. Simpson, Ed.), pp. 79–96. Wiley, Chichester (1982).
[12] A. Marsch, J. Maier-Rosenkranz and E. Grom, *Labor Praxis* **16**, 120–123 (1992).
[13] W. Hu and H. Haraguchi, *Bull. Chem. Soc. Jpn* **66**, 1967–1970 (1993).
[14] H. Koller, K.H. Rimböck and A. Manschreck, *J. Chromatogr.* **282**, 89–92 (1993).
[15] W.H. Pirkle and C.C.J. Welsh, *J. Liq. Chromatogr.* **14**, 1–8 (1991).

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