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Evaluation of the performance of commercially available polysaccharide-based chiral stationary phases after multicycle operation in multimodal elution mode

Gabriella Török *, Ludwig Goetelen, Raf Luyckx, Peter Van Broeck

Department of Chemical Process Control, Johnson & Johnson Pharmaceutical Research and Development, A Division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

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Abstract

The performance of four commercially available polysaccharide-based chiral stationary phases, Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS was evaluated after several cycles of extended multimodal operations. Acetonitrile, methanol, ethanol and their mixtures were chosen for polar-organic mobile phases, ethanol–*n*-heptane mixture and *n*-heptane were selected for normal-phase mode. Retention factor (k), selectivity (α), resolution (R_s) and theoretical plate count (N) were the chosen parameters to describe the column performance. One racemate for which all four columns have shown enantioselectivity was chosen as test compound. After 15 cycles of multimodal operations a slight decrease was seen in the retention factors (k) however, column efficiency, selectivity (α) and resolution (R_s) were maintained.
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Keywords: Chiral HPLC; Commercially available polysaccharide-based chiral stationary phases; Multimodal elution mode; Performance of the columns

1. Introduction

Enantiomers of chiral drugs may have differences in their biological- and pharmacological-effects and in their pharmacokinetic properties. Besides the effective isomer (eutomer), its counterpart (distomer) may be inactive or even toxic. Yet, before 1990 the great majority of the marketed synthetic drugs were racemic mixtures. Recently however, due to regulatory requirements [1,2] the great majority of innovative drug companies are focusing their activities on the development of single stereoisomeric drugs.

This led to an increased interest in suitable analytical methods allowing separation, determination, quantification and/or production of enantiomerically pure compounds both on analytical and preparative scale. Chromatographic techniques, particularly high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) are the most widely used analytical methods [3,4].

In the last decades tremendous number of new and improved CSPs were developed and commercialised [5]. Among the various kinds of commercially available CSPs polysaccharide-based phases, mainly benzoate or phenylcarbamate derivatives of cellulose or amylose, are the most extensively and successfully used CSPs. The range of enantiomers that can be separated by them is the broadest and most impressive of all the CSPs, cellulose derivatives being complementary to amylose derivatives.

The greatest limitation in the performance of the polysaccharide columns was, for a long time, that they could only be operated in one mode of elution, normal-phase mode [6] and the narrow range of solvents that could be used.

Previously several authors demonstrated that polysaccharide columns used in the normal-phase (NP) mode could also be switched to reversed-phase (RP) conditions [7–9]. Recently, studies on the applicability of these phases in multimodal elution mode, normal-phase, reversed-phase and

* Corresponding author. Tel.: +32 14 60 79 47; fax: +32 14 60 57 55.
E-mail address: gtorok@prdbe.jnj.com (G. Török).

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polar-organic (PO) modes, have been published [10–12]. Multimodality means that the same column can be used in different chromatographic modes.

In most of these investigations the columns were home-made and either separate columns were used for NP and RP applications or the columns were never switched back to NP conditions. Furthermore long (up to 6 h) and/or multiple equilibration steps, using a series of miscible solvents, of the columns were needed when changing elution mode. Andersson et al. [12] reported the only system that utilises commercially available polysaccharide based CSPs in multimodal elution with alkane/alcohol and methanol/ethanol mobile phases. Acetonitrile was left out from their mobile phases due to incompatibility with the alkane/alcohol mixtures and no data is available on column performance after several operating cycles.

Since chiral recognition and enantioselectivity of these CSPs strongly depend on the correct choice of mobile phase composition, extended multimodal operation in the PO and NP mode, covering the solvent polarity from acetonitrile to *n*-hexane or *n*-heptane would expand the applicability of these CSPs for routine chiral method development and analysis. Furthermore, it could lead to tremendous cost and time reduction.

To our knowledge no one has ever demonstrated that commercially available polysaccharide-based CSPs can be switched multiple times between different chromatographic modes with maintaining the performance of the columns.

The aim of this study was to evaluate the performance of four commercially available polysaccharide-based CSPs, Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS, after several cycles of extended multimodal operations. Acetonitrile, methanol, ethanol and their mixtures were chosen for polar-organic mobile phases, ethanol–*n*-heptane mixture and *n*-heptane were selected for normal-phase mode. Retention factor (k), selectivity (α), resolution (R_s) and theoretical plate count (N) were the chosen parameters to describe the column performance. One racemate for which all four columns have shown enantioselectivity was chosen as test compound.

2. Experimental

2.1. Chemicals

The racemate test compound (Fig. 1), *RS-N*-[4-[2-ethyl-1-(1H-1,2,4-triazol-1-yl)butyl]phenyl]-2-benzothiazolamine, used in this investigation was prepared in house [13]. Acetonitrile and methanol were of HPLC grade and purchased from Acros Organics (Belgium). Anhydrous ethanol (ACS grade) was obtained from J.T. Baker (Mallinckrodt Baker B.V., The Netherlands) and *n*-heptane (Spectranal) was a product of Riedel-de Haën (Sigma–Aldrich, Belgium).

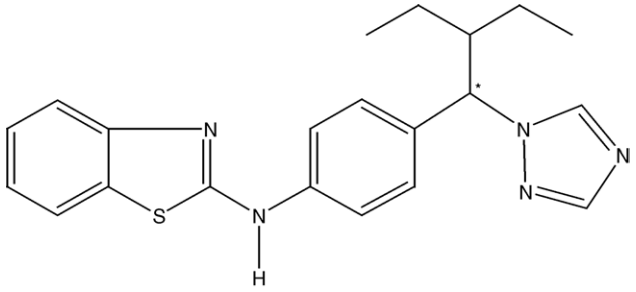

Fig. 1. Structure of *RS-N*-[4-[2-ethyl-1-(1H-1,2,4-triazol-1-yl)butyl]-phenyl]-2-benzothiazolamine.

2.2. Instrumentation and chromatographic conditions

The analyses were performed with a Waters-2695 Alliance HPLC separation module equipped with a photodiode-array detector (Waters 2996) (Waters Chromatography, Milford, MA, USA). A Mistral-Spark column oven with a built-in six-position valve was used to maintain the column temperature and for the automatic column selection.

Millennium^{32} Chromatography Manager version 4.00 data system (Waters Chromatography, Milford, MA, USA) was used for data collection and handling.

The columns, Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS, used in the study were 250 mm × 4.6 mm i.d. with 10 μm particle size and were purchased from (VWR International BVBA, Belgium).

The analyses were performed at 20 °C with a flow rate of 1 ml/min. The test compound was dissolved in ethanol to yield a concentration of 0.5 mg/ml and 6 μl of this solution was injected. Detection of the sample was done at 200 nm.

2.3. Calculations

Retention factor (k), selectivity (α), resolution (R_s) and theoretical plate count (N) were the chosen parameters to describe the column performance.

The acquired data was processed with Millennium^{32} Chromatography Manager data system version 4.00 and the parameters were calculated as follows:

- Retention factor (k)

$$k_i = \frac{t_{r_i} - t_0}{t_0}$$

where t_{r_i} is the retention time of the ith eluting enantiomer (first or second) and t_0 the retention time of an unretained compound.

- Selectivity (α)

$$\alpha = \frac{k_2}{k_1}$$

where k_2 and k_1 are the retention factors of the second and first eluting enantiomers, respectively.

- Resolution (R_s) according to US Pharmacopoeia (USP)

$$R_s = \frac{2(t_{r_2} - t_{r_1})}{w_2 + w_1}$$

where t_{r_2} and t_{r_1} are the retention times (in minutes) of the second and first eluting enantiomers, respectively, and w_2 and w_1 are the peak widths (in minutes) of the two enantiomer peaks on the chromatogram measured on the baseline.

- Theoretical plate count (N) according to US Pharmacopoeia (USP)

$$N = 16\left(\frac{t_r}{w}\right)^2$$

where t_r is the retention time (in minutes) of the enantiomers, and w the peak width (in minutes) measured on the baseline.

3. Results and discussion

In our department two automated chiral HPLC screening modules utilising commercially available polysaccharide based CSPs, Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS were routinely used. The first screening module was operated with polar-organic mobile phases, acetonitrile, methanol, ethanol and their mixtures, while the second was used under normal-phase conditions with *n*-heptane and its mixture with ethanol and 2-propanol. These systems were combined into one multimodal-screening module operating in the polar-organic and in the normal-phase mode while keeping a broad choice of solvents. Acetonitrile was one of the solvents chosen for the polar-organic mode due to its different properties (non-hydrogen bonding solvent) and polarity compared to alcohols. In the normal-phase mode often *n*-hexane is used. However, in this investigation *n*-heptane was used due to its less toxicity. Since the applied Waters Alliance HPLC separation module is only capable of handling four different solvents and acetonitrile and *n*-heptane were already selected, this leaves us two more possible solvents. Of the alcohols used in our separate screening modules methanol and ethanol were chosen. Both can be used in the polar-organic mode and ethanol can be used in the normal-phase mode in combination with *n*-heptane. Ethanol is also suitable for flushing the columns after normal-phase operations thus ensuring the return to polar-organic mode. The sequence of selected solvents and columns resulted in the new automated multimodal screening (Table 1). All four columns were conditioned at 1 ml/min flow rate for 15 min with one mobile phase composition and were subsequently used for analysis. In this study the same columns were used in the polar-organic and in the normal-phase modes.

One hundred percentage (v/v) acetonitrile was the starting mobile phase composition followed by its mixture with methanol and ethanol (50/50%, v/v), respectively, then methanol–ethanol 50/50% (v/v) mixture and 100% (v/v) ethanol were used before applying the *n*-heptane containing mobile phases, *n*-heptane–ethanol 50/50% (v/v) and finally 100% (v/v) *n*-heptane.

Table 1
Sequence of mobile phases and columns in one multimodal operating cycle

Mobile phase (%, v/v)	Status	Time (min)	Column sequence
Acetonitrile (100)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
Acetonitrile/MeOH (50/50)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
Acetonitrile/EtOH (50/50)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
MeOH/EtOH (50/50)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
EtOH (100)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
EtOH/*n*-heptane (50/50)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
n-Heptane (100)	Conditioning	15	Chiralcel OD Chiralcel OJ
	Analysis	20	Chiralpak AD Chiralpak AS
EtOH (100)	Conditioning	30	Chiralcel OD Chiralcel OJ Chiralpak AD Chiralpak AS

Flow rate: 1 ml/min, temperature: 20 °C.

After operating the columns in 100% (v/v) *n*-heptane, but before starting a new cycle with 100% (v/v) acetonitrile all columns were conditioned with 100% (v/v) ethanol at 1 ml/min flow rate for 30 min. One complete cycle, screening of one analyte on all four columns with all seven mobile phase compositions including the 30 min conditioning takes 18.3 h.

All four columns were operated under the above-mentioned conditions during several cycles. To evaluate whether the performance of the columns are maintained when switched multiple times between the chromatographic modes we have chosen one racemate (Fig. 1) for which all four columns have shown enantioselectivity. Retention factor (k), selectivity (α), resolution (R_s) and theoretical plate count (N) were the selected parameters to describe the column performance. The results are summarised in Table 2. Representative chromatograms are shown in Fig. 2A–D.

Table 2
Performance of Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS columns in selected conditions after several operating cycles in multimodal elution modes, described by the retention factor (k), selectivity (α), resolution (R_s) and theoretical plate count (N)

Column	Mobile phase (%, v/v)	Cycle	k_1	k_2	α	R_s	N_1	N_2
Chiralcel OD	*n*-Heptane (100)	1st	4.09	4.75	1.16	1.10	1495	1305
		15th	3.90	4.51	1.16	1.10	1525	1321
Chiralcel OJ	MeOH/EtOH (50/50)	1st	1.02	1.48	1.46	2.29	2441	1766
		15th	1.01	1.47	1.46	2.26	2335	1715
Chiralpak AD	MeOH/EtOH (50/50)	1st	1.59	2.25	1.42	2.33	2416	1431
		15th	1.54	2.21	1.43	2.33	2330	1335
Chiralpak AS	MeOH/EtOH (50/50)	1st	0.69	0.81	1.17	0.75	2034	1383
		15th	0.67	0.80	1.19	0.75	2151	1587
	n-Heptane (100)	1st	3.69	4.82	1.31	1.34	817	579
		15th	3.54	4.64	1.31	1.32	807	522

Flow rate: 1 ml/min, temperature: 20 °C, k_1 and k_2 are the retention factors, N_1 and N_2 are the theoretical plate counts calculated for the first and second eluting enantiomer, respectively.

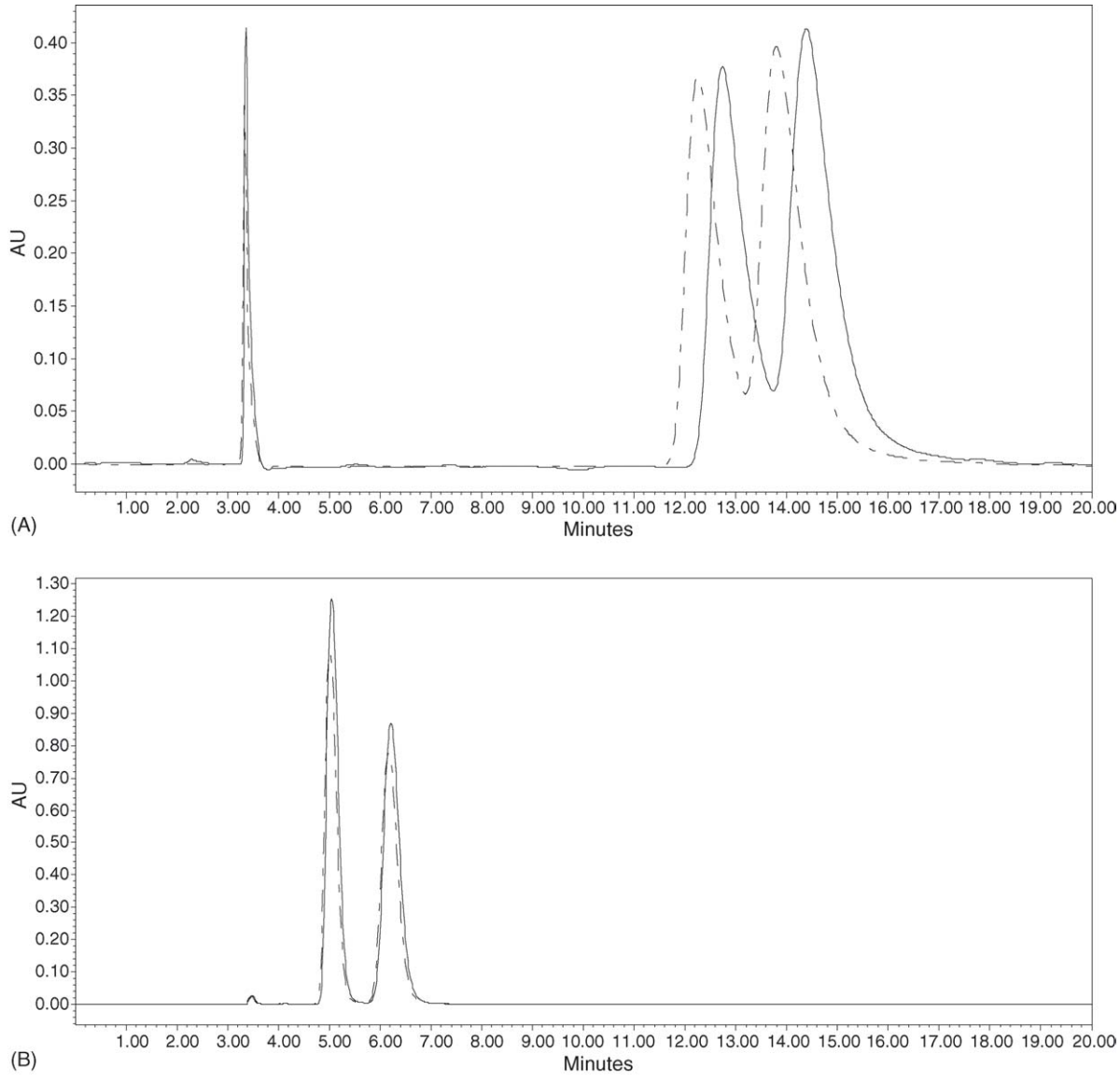

Fig. 2. Representative chromatograms on Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS columns in selected conditions after several operating cycles in multimodal elution modes. Column: (A) Chiralcel OD, (B) Chiralcel OJ, (C) Chiralpak AD, (D) Chiralpak AS; mobile phase: (A, D) *n*-heptane 100% (v/v), (B, C) MeOH/EtOH 50/50% (v/v); flow rate: 1 ml/min; temperature: 20 °C; solid line: 1st cycle; dashed line: 15th cycle.

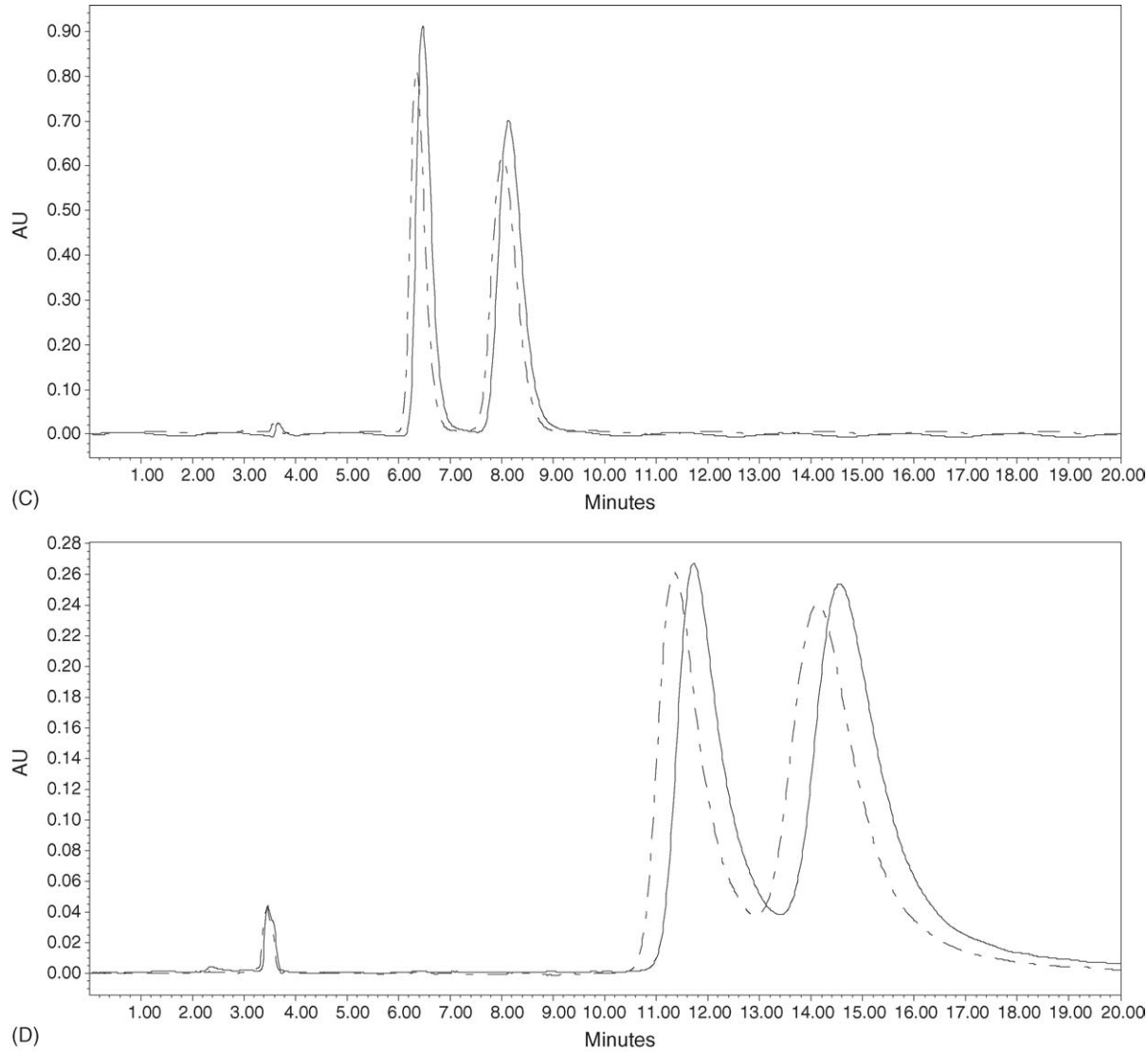

Fig. 2. (*Continued*).

After 15 cycles of multimodal operations the efficiency of the columns has not changed significantly, the performance of the columns was still maintained. A slight decrease was seen in the retention factors (k) however, selectivity (α) and resolution (R_s) were not affected.

4. Conclusions

In this investigation it was demonstrated that in multimodal, polar-organic and normal-phase elution cycles acetonitrile could be included in the mobile phases and combined with alkane/alcohol mixtures with choosing the appropriate sequence of mobile phases and a short conditioning of the columns with ethanol at the end of the operating cycle.

The study proved also that the performance of four commercially available polysaccharide-based CSPs, Chiralcel OD, Chiralcel OJ, Chiralpak AD and Chiralpak AS, was maintained after 15 cycles of multimodal operation, when switching from polar-organic solvents (acetonitrile, methanol, ethanol and their mixtures) to normal-phase mode (ethanol–*n*-heptane mixture and *n*-heptane) than back to polar-organic mode (acetonitrile) after short conditioning with ethanol. Selectivity and resolution were not affected.

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