ELSEVIER

Journal of Chromatography A, 728 (1996) 195–199

JOURNAL OF CHROMATOGRAPHY A

Flash chiral chromatography with cellulose tris(3,5-dimethylphenylcarbamate)-coated phases Improved resolution of basic analytes

S.J. Grieb[a], S.A. Matlin[a,*], A.M. Belenguer[b]

[a]Department of Chemistry, University of Warwick, Warwick, Coventry CV4 7AL, UK

[b]SB Pharmaceuticals, Old Powder Mills, Tonbridge, Kent TN11 9AN, UK

Abstract

The preparative resolution of some chiral basic analytes on a flash chiral chromatography column packed with a cellulose carbamate-coated phase is described. Compared with separations obtained, on a column packed with 20% (w/w) cellulose tris(3,5-dimethylphenylcarbamate) coated onto underivatised flash silica (40–63 μm Davisil, 150 Å), a substantial improvement was observed when the support medium was changed to octadecylated silica (37–55 μm Bondapak ODS, 300 Å). The ODS phase is extremely effective for the preparative separation of basic analytes such as β-blockers and the optimum conditions can be readily established by the use of a related high-pressure liquid chromatography column containing the chiral carbamate coated onto Nucleosil-ODS (5 μm, 300 Å).

Keywords: Flash chromatography; Enantiomer separation; Chiral stationary phases, LC; Preparative chromatography; β-Blockers; Orphenadrine; Halofantrine; Basic compounds

1. Introduction

Many chiral compounds display different biological activity with respect to their stereochemistry. This has intensified the demand for methods that cannot only monitor optical purity, but also provide small quantities of pure enantiomers for testing. High-pressure liquid chromatography (HPLC) using chiral stationary phases (CSPs) is a widely used technique for furnishing this need. However, whilst much work has been carried out on the development of efficient CSPs for analytical separations, relatively few of these phases are frequently used for chiral preparative separations [1] for several reasons including phase loadability and the high cost of the columns and the associated equipment.

Cellulose and amylose derivatives coated onto macroporous aminopropylated silica (APS) have proved to be some of the most effective and universally applicable phases available. Developed by Okamoto et al. [2–4], these columns are now widely available as Chiralcel and Chiralpak (Daicel) and Okamoto has reported [5] that about 80% of all racemates examined were resolved on such phases.

In view of their wide separation scope, we have extensively investigated these phases [6,7], and in particular have examined the influence of support structure and support surface chemistry on enantioselectivity. These results led us to investigate the use of inexpensive flash silica for preparative-scale appli-

* Corresponding author. Present address: Human Resource Development Division, Commonwealth Secretariat, Marlborough House, Pall Mall, London SW1Y 5HX, UK.

0021-9673/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved

SSDI 0021-9673(95)01027-0

cations in a flash chromatography mode. The method proved to be an extremely easy, rapid and inexpensive process for the resolution of tens to hundreds of milligrams of many chiral analytes [8]. In this paper, we demonstrate further improvements to the flash chiral chromatographic method which has allowed us to separate basic compounds much more efficiently.

2. Experimental

2.1. Chemicals and solvents

For the flash column, octadecylated (ODS) Bondapak irregular silica (Waters, USA) which had a particle size of 37–55 μm and a pore size of 300 Å was used. For the HPLC column, octadecylated Nucleosil spherical silica (Hichrom, UK) which had a particle size of 5 μm and a pore size of 300 Å was used. The cellulose (Avicel) was purchased from Merck (Darmstadt, Germany) and the 3,5-dimethylphenylisocyanate from Lancaster (Morecambe, UK). Racemic mixtures and enantiomers were purchased from either Sigma (Pool, UK) or Aldrich (Gillingham, UK), except halofantrine which was a gift from SmithKline Beecham Pharmaceuticals (Tonbridge, UK). Solvents (HPLC grade) were obtained from Rathburn (Walkerburn, UK).

2.2. Apparatus

For the flash separations, a glass flash chromatography column (50 cm×2.2 cm I.D.) which had been modified to allow on-line UV detection was used (see Fig. 1). The chiral separations were monitored using a Cecil CE-212 variable wavelength UV detector and a Kipp and Zonen (Delft, Netherlands) chart recorder.

For the HPLC analyses, separations were carried out at ambient temperature on a system comprising a Waters 510 pump, a Cecil CE-2012 UV detector, Rheodyne 7125 injector with a 10-μl loop and a J J Instruments CR650 chart recorder (Southampton, UK). A high-pressure slurry packer fitted with a Haskel 780-3 pump was used for column packing. Gel permeation chromatography (GPC) was carried out on a PL Modular System with Caliber software (Church Stretton, UK) using refractive index (R.I.) detection.

2.3. Chromatographic conditions

The flash and HPLC chromatography was performed at ambient temperature using the mobile-phase compositions listed at the foot of Table 1. For the flash column, a flow-rate of approximately 4 ml/min was used and samples (1 ml) of 20 mg/ml

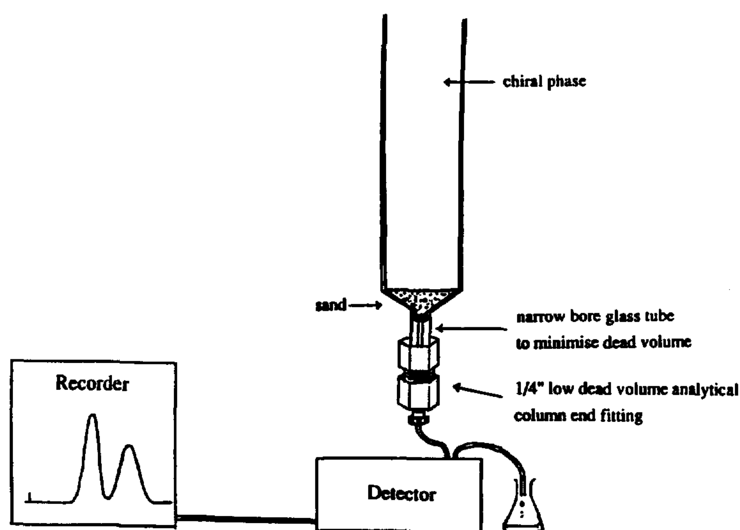

Fig. 1. Glass flash chromatography column which has been modified to allow on-line UV detection.

Table 1
Chromatographic parameters for analytical and preparative resolutions of some basic analytes

Analytes	HPLC column		Flash column	
	α	R_s	α	R_s
Alprenolol[a]	2.95	3.11	3.39	1.69
Propranolol[a]	1.98	3.10	2.36	1.71
Oxprenolol[a]	5.18	7.32	5.99	5.46
Orphenadrine[b]	2.01	4.18	2.14	2.63
Halofantrine[b]	1.77	2.46	2.28	1.20

[a] Mobile phase: hexane–2-propanol–diethylamine (80:20:0.1, v/v/v).
[b] Mobile phase: hexane–2-propanol–diethylamine (98:2:0.1, v/v/v).

dissolved in 2-propanol were injected. For the HPLC, a flow-rate of 0.5 ml/min was used and samples were injected as solutions (10 μg/ml) in the mobile phase. The racemates were detected at 273 nm and the dead time (t_0) of the columns was determined by an injection of 1,3,5-tri-*tert.*-butylbenzene.

2.4. Chromatographic calculations

The separation factor (α) was calculated as $\alpha = k'_2/k'_1$ and capacity factor (k') as $k'_1 = (t_1 - t_0)/t_0$ and $k'_2 = (t_2 - t_0)/t_0$, where t_1 and t_2 refer to the retention times for the first and second eluting enantiomers, respectively.

The resolution factor (R_s) was calculated by the formula: $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ where w_1 and w_2 are the peak widths for the first and second eluting enantiomer peak, respectively.

2.5. Preparation of the chiral columns

Cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) was prepared as previously reported [3]. The dried carbohydrate was refluxed for 24 h in dry pyridine. After cooling, 3.5 equivalents of 3,5-dimethylphenyl isocyanate was added and the mixture refluxed for a further 72 h. The cooled solution was poured into 1.5 l methanol and stirred for 1 h. The white solid was filtered, washed well with methanol and dried under vacuum at 50°C to constant weight. Elemental analysis $(C_{33}H_{37}N_3O_7)_n$, calculated: C 65.66, H 6.18, N 6.98; found: C 64.92, H 6.06, N 6.85. GPC analysis [two PL Mixed-C columns in series, elution with tetrahydrofuran (THF)] showed the product to have $M_n = 5.1 \cdot 10^4$ and $M_w/M_n = 4.18$, where M_n is number-average molecular mass and M_w is weight-average molecular mass.

For the flash column, Bondapak ODS (40 g) was refluxed in THF (300 ml) for 30 min and allowed to cool. CDMPC (10 g, 20% w/w) was dissolved in 100 ml THF–N,N-dimethylacetamide (90:10, v/v) and added to the refluxed ODS. The solvent was removed under vacuum to dryness and the material was sieved (250 μm) to ensure a free-flowing powder suitable for packing. A portion of this material (40 g) was slurried in hexane–2-propanol (90:10, v/v) and packed into the glass chromatography column which had been adapted to allow on-line UV detection (Fig. 1). The packed column bed had a height of ca. 25 cm.

For the HPLC column, an identical method was used with scaled down quantities of reagents. The coated Nucleosil particles were sieved through a 38-μm mesh and the phase was high-pressure slurry packed (600 p.s.i.; 1 p.s.i.=6894.76 Pa) into a stainless-steel column (150×4.6 mm I.D.) in hexane–2-propanol (80:20, v/v).

3. Results and discussion

Recently, we have shown that efficient cellulose carbamate-coated phases can be prepared from widely available small pore (120 Å) supports, including underivatised silica [7]. For the majority of analytes, a 20% (w/w) CDMPC-coated underivatised silica (SI) phase gave better separation than CDMPC-coated aminopropylated or octadecylated silica supports. The only analytes which did not resolve as well on the CDMPC-coated SI phase were basic analytes containing non-sterically hindered basic groups. These analytes gave tailing peak shapes due to interactions with the silanol groups on the silica surface. However, a significant improvement in the resolution could be made by increasing the amount of diethylamine, a silanol suppressor, in the mobile phase from 0.1% to 1% (v/v).

The surprisingly efficient resolutions achieved when underivatised silica was used as the support medium led us to investigate the use of inexpensive

underivatised flash silica for preparative-scale applications in a flash chromatography mode [8]. The method worked extremely well and the resolution of many chiral analytes have been achieved. However, when some basic analytes containing non-sterically hindered basic groups were run on the CDMPC-coated underivatised flash silica column, the peak tailing was found to be worse than expected, even with 1% (v/v) diethylamine in the mobile phase. The separations of alprenolol and propranolol are shown in Fig. 2.

The poor resolution for these two analytes significantly limited the amount of pure enantiomer that could be collected. Therefore, since many chiral pharmaceuticals contain basic centres, a flash chiral column more suited to the separation of basic analytes was sought.

We re-examined the information obtained from the investigation into the effect of support surface chemistry on enantioselectivity [7]. Basic analytes were separated most efficiently on a 15% CDMPC-coated Hypersil ODS column, since the ODS support was able to shield the acidic silanol groups. However, it was also noted that the small-pored (120 Å) Hypersil ODS particle appeared to significantly exclude the CDMPC from the pore volume, causing peak broadening and reduced resolution for some analytes. Therefore, a Bondapak irregular ODS material (37–55 μm) with a pore size of 300 Å was chosen as a potentially suitable flash silica support. It was anticipated that the larger pore would more easily accommodate the CDMPC coating.

The Bondapak ODS readily accepted a 20% (w/w) CDMPC loading and the phase was packed into the flash chromatography column. This phase was found to be significantly better for the preparative resolution of alprenolol and propanolol (see Fig. 3).

An HPLC column packed with 20% (w/w) CDMPC coated on 5 μm Nucleosil ODS, 300 Å, was prepared in parallel with the flash column. The separation of three more basic analytes, oxprenolol, orphenadrine and halofantrine (20 mg), was examined on both the HPLC column and the flash column. The chromatographic results along with those for alprenolol and propranolol can be seen in Table 1.

Although the separation factors (α) observed on the flash column were slightly better than those obtained on the HPLC column, as expected the

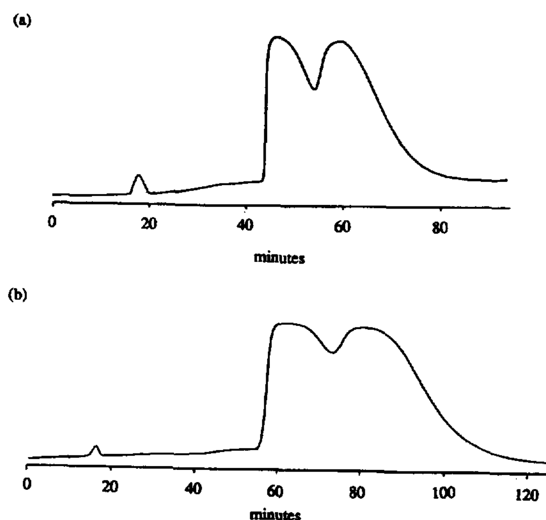

Fig. 2. Resolution of (a) alprenolol and (b) propranolol on flash chiral column packed with 20% w/w CDMPC on 40–63 μm Davisil (40 g). Mobile phase: hexane–2-propanol–diethylamine (80:20:0.1, v/v/v). Flow: 4 ml/min.

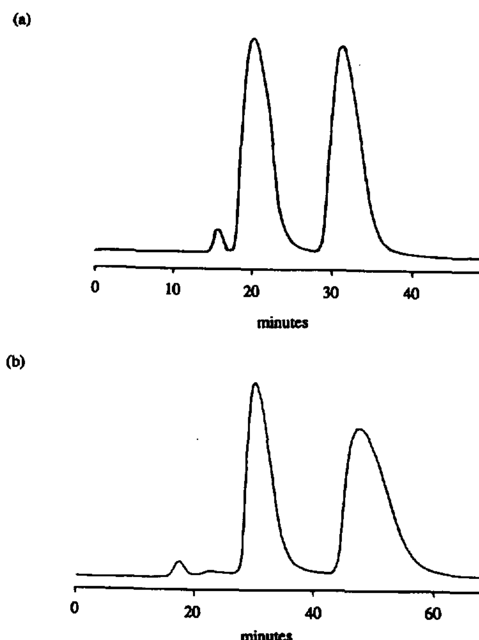

Fig. 3. Resolution of 20 mg of (a) alprenolol and (b) propanolol on flash chiral column packed with 20% w/v CDMPC on 37–55 μm Bondapak ODS (40 g). Mobile phase: hexane–2-propanol–diethylamine (80:20:0.1, v/v). Flow: 4 ml/min.

resolution on the coarser irregular flash silica was proportionally lower than on the HPLC column. The flash chiral method utilising the CDMPC-coated ODS gave excellent peak shapes for all the basic analytes examined and in most cases excellent baseline separations were obtained. The corresponding HPLC column can be used to identify quickly and efficiently the optimum mobile-phase composition required for a given application.

4. Conclusions

A CDMPC-coated ODS flash chiral column has been shown to give extremely efficient resolution of basic analytes with non-sterically hindered amine moieties. Using non-optimised mobile-phase conditions and a sample load of 20 mg (0.5 mg/g of chiral phase), baseline resolution was achieved for four of the five basic analytes. Therefore, although the sample-loading capacity will obviously depend on the extent of separation, this column has the potential to be able to resolve much larger quantities of sample. Mobile-phase conditions could be optimised using an HPLC column packed with a similarly coated analytical ODS phase. Further investigations of the scope and utility of flash chiral chromatography using carbohydrate carbamated phases are currently in progress.

Acknowledgement

This work was supported by the EPSRC, SmithKline Beecham Pharmaceuticals and Shandon HPLC.

References

[1] E. Francotte, J. Chromatogr. A, 666 (1994) 565.
[2] Y. Okamoto, M. Kawashima, K. Yamamoto and K. Hatada, Chem. Lett., (1984) 739.
[3] Y. Okamoto, M. Kawashima and K. Hatada, J. Am. Chem. Soc., 106 (1984) 5357.
[4] Y. Okamoto, M. Kawashima and K. Hatada, J. Chromatogr., 363 (1986) 173.
[5] Y. Okamoto and Y. Kaida, J. Chromatogr. A, 666 (1994) 403.
[6] S.J. Grieb, S.A. Matlin, J.G. Phillips, A.M. Belenguer and H.J. Ritchie, Chirality, 6 (1994) 129.
[7] S.J. Grieb, S.A. Matlin, A.M. Belenguer and H.J. Ritchie, J. Chromatogr. A, 697 (1995) 271.
[8] S.A. Matlin, S.J. Grieb and A.M. Belenguer, J. Chem. Soc. Chem. Commun., (1995) 301.