ELSEVIER

Journal of Chromatography A, 697 (1995) 257–261

JOURNAL OF CHROMATOGRAPHY A

Rapid method development for the separation of enantiomers by means of chiral column switching

J.A. Whatley

Physical Methods Department, Roche Research Centre, Roche Products Limited, Welwyn Garden City, Herts. AL7 3AY, UK

Abstract

Some of the most extensive and versatile chiral stationary phases (CSPs) are those based on polysaccharide derivatives (cellulose and amylose) coated onto macroporous silica. Each derivative can exhibit subtly different enantioselectivity for a variety of chiral compounds.

Chiral column switching is a very useful analytical tool and ideally suited to these CSPs since they are all used with isopropanol–hexane or ethanol–hexane mixtures, with small additions of acid or base modifiers where appropriate. The technique lends itself to rapid method development where automated overnight runs can be set up to scout for a column and find separation conditions for racemic compounds.

The usefulness and potential of chiral column switching using twelve different CSPs arranged in two banks of six columns is illustrated with several chiral molecules. Examples where peak reversal has occurred are highlighted to show the advantages this presents the analyst in final quantitation and enantiomeric excess determination.

When scale-up is a consideration the main priority is to achieve high selectivity. Chiral column switching can be used to great effect to search for a column that will fulfil this need. This optimised separation can then be translated to a comparable preparative chiral column for isolation of milligram quantities of each enantiomer.

1. Introduction

During the last few years interest in the direct chromatographic resolution of enantiomers has increased dramatically, both analytically and preparatively. The efficiency, speed, wide applicability and reproducibility of HPLC has made it the instrument of choice for enantiomeric separations. Chiral column technology is now a rapidly expanding field in which well over fifty different phases are commercially available, with new ones appearing all the time [1]. This presents the chromatographer with a wide choice but also a difficult one in terms of which chiral stationary phase (CSP) to use for any given separation problem. Attempts to predict interaction mechanisms of chiral compounds with CSPs as a prelude to separation are often thwarted.

One of the most extensive and versatile CSPs are those based on polysaccharide derivatives (cellulose and amylose) coated onto macroporous silica [2]. The separations occur due to a multi-mode mechanism involving hydrogen bonding, $\pi-\pi$ interactions, dipole stacking and inclusion complexes. Each derivative shows subtly different characteristics and a definite enantioselectivity for a variety of compounds.

Chiral column switching is a very useful analytical tool and ideally suited to these CSPs since they are all used with isopropanol–hexane or ethanol–hexane mixtures, with small additions of

0021-9673/95/$09.50
SSDI 0021-9673(94)01030-7

acid or base modifiers where appropriate. The technique lends itself to rapid method development where automated overnight runs can be set up to scout for a column and find separation conditions for racemic compounds. Final optimisation of the best separation can then be undertaken if required.

More recently derivatised cyclodextrins have become available where derivatisation of the secondary hydroxyls has extended the enantioselective interactions not available on native cyclodextrins [3]. These columns can be used in normal-phase modes making them a useful addition to the chiral column switching range.

The Pirkle-type phases [4] and synthetic polymers such as OT(+) are examples of other chiral supports compatible with alcohol–hexane eluents which can be incorporated into column switching. Combination of all these CSPs gives the chromatographer a wide range of chiral columns to resolve racemic compounds.

2. Experimental

Chromatography was performed using a Kontron series 400 liquid chromatograph comprising an autosampler and dual-wavelength detector. Chiral columns were purchased from J.T Baker and Technicol. Hexane, isopropanol and ethanol (HPLC grade) were purchased from Rathburn. Polyether ether ketone (PEEK) tubing and Upchurch fittings were supplied by Anachem.

3. Results and discussion

This paper describes the usefulness and potential of chiral column switching using 12 different CSPs arranged in two banks of 6 columns. The column switching comprises two 6-way Valco valves, each housing 6 columns. The 8-way Valco valve directs the flow of solvent to each bank of 6 columns in turn and similarly directs the column eluent to the detector, via the 6-way zero-volume connectors. The column switching is integrated into the Kontron HPLC system where programming and commands for switching through the 12 columns is controlled via the data system incorporating an RS-232 multiport.

To minimise dead volume and band spreading the column switching was housed between the autosampler and the dual-wavelength detector. PEEK tubing, 1/16 in. × 0.010 in. I.D. (1 in. = 2.54 cm) and Upchurch fingertight fittings were used to make all connections between valves and columns. The flexibility of the PEEK tubing and ease of making connections rated to 4000 p.s.i. (1 p.s.i. = 6894.76 Pa) was seen as an advantage. Any column changes could be made quickly, without the need for spanners, and problems associated with cold welding of stainless-steel ferrules are avoided. All columns used in this configuration were 25 cm × 0.46 cm I.D. with inverted female end fittings.

A typical overnight program would inject one sample sequentially through 12 columns allowing 1 h analysis time per column, plus 20 min equilibration of mobile phase for each column prior to sample injection. This would give a total run time of 16 h, 20 min column conditioning and 60 min assay time. Alternatively, two compounds could be assayed through 6 columns over the same time span. The system is flexible enough to allow the analyst to select specific columns from the 12 to assay the racemic compound. This is made possible via the program file of the Kontron HPLC system whereby commands to initiate contact closures can be made to switch through to the chosen columns.

As an illustration of what can be achieved an intermediate in the potassium channel opener project, Ro 31-6905/000 (Fig. 1) was used as an example.

The sample used for the overnight run was

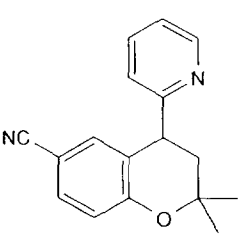

Fig. 1. Structure of potassium channel opener, Ro 31-6905/000.

made up to give an enantiomeric excess of isomers (approximately 2:1, *R*:*S*) to highlight peak reversal. The 12 chiral columns used in this switching program are as follows: Chiralcel OA, Chiralcel OB, Chiralcel OC, Chiralcel OD, Chiralcel OF, Chiralcel OG, Chiralcel OJ, Chiralcel OK, Chiralcel OT(+), Chiralpak AD, Whelk-01(R,R) and Pirkle DNBPG (covalent). Four of the 12 columns showed a separation, Chiralcel OG, Chiralcel OD, Chiralpak AD and Chiralcel OJ using a mobile phase of isopropanol–hexane (10:90) at a flow-rate of 0.7 ml min^{-1}. Fig. 2 compares the enantiomeric separation achieved on these four columns. When this program was repeated with the same racemic mixture using ethanol–hexane (8:92) at 0.7 ml min^{-1} only two columns separated the enantiomers, viz. Chiralcel OD and Chiralcel OJ. The separation factor α was lower using the ethanol–hexane solvent mixture.

Notable points of interest are the inversion of peak elution order of the enantiomers on amylose- and cellulose-derivatised supports and peak reversal on two cellulose-based columns, i.e. Chiralcel OG and Chiralcel OJ.

The significance of peak order reversal of enantiomers merits comment. The chromatographer is often faced with the situation where a trace of the unwanted enantiomer elutes on the tail of the main enantiomer, making accurate quantitation difficult. If a column can be found that will invert the elution order then a much more favourable method is available for quantitation (lower limit of detection) and assay validation. In addition, any preparative clean up is made much easier if the unwanted isomer elutes first. Thus on both these counts column switching can be advantageous in searching quickly for possible peak reversal.

The significance of peak reversal to the chromatographer is best illustrated by the compound Ro 31-8829/000 (Fig. 3), a key intermediate in the synthesis of protein kinase C inhibitors.

It was necessary to determine accurately the optical purity of this compound by chiral HPLC. A reasonable separation had been achieved on a Chiralcel OF column using isopropanol–hexane (1:99) (Fig. 4). However, the undesired enantiomer (*S* enantiomer) eluted on the tail of the required isomer and was not fully resolved, making quantitation difficult. When this compound was chromatographed through 12 chiral columns during an overnight run a better separation was achieved on the Chiralcel OD column, but more importantly the elution order of the enantiomers was reversed (Fig. 5). This enabled the analyst to quantify more accurately the percentage of unwanted isomer since good baseline resolution had been achieved.

When scale-up to preparative HPLC is required the main priority is to obtain high selectivity. Chiral column switching can be used to great effect to search for a column that will achieve this. The optimised separation can then be translated to a comparable preparative chiral column for isolation of milligram quantities of each enantiomer for biological testing. This can save the synthetic chemist many hours of work striving for a homochiral route.

As a starting point racemic compounds are put through the chiral column switching program using 10% isopropanol or ethanol in hexane, with modifiers where appropriate, at a flow-rate of between 0.5 to 0.7 ml min^{-1}. An analysis time of 60 min per column is considered adequate in most cases, combined with a 15-min equilibration period for each column prior to injection.

4. Conclusions

Chiral column switching offers the chromatographer a means of developing quickly separations of racemic compounds routinely and automatically during overnight runs. More often than not at least one column from the twelve will give a separation. Final optimisation of the separation can then be carried out if necessary.

Both isopropanol–hexane and ethanol–hexane mixtures can be used within the constraints of the maximum allowable alcohol percentages recommended by the manufacturers. Indeed when a separation is not obtained using isopropanol as the alcohol modifier, ethanol should also be tried. There are instances where a compound

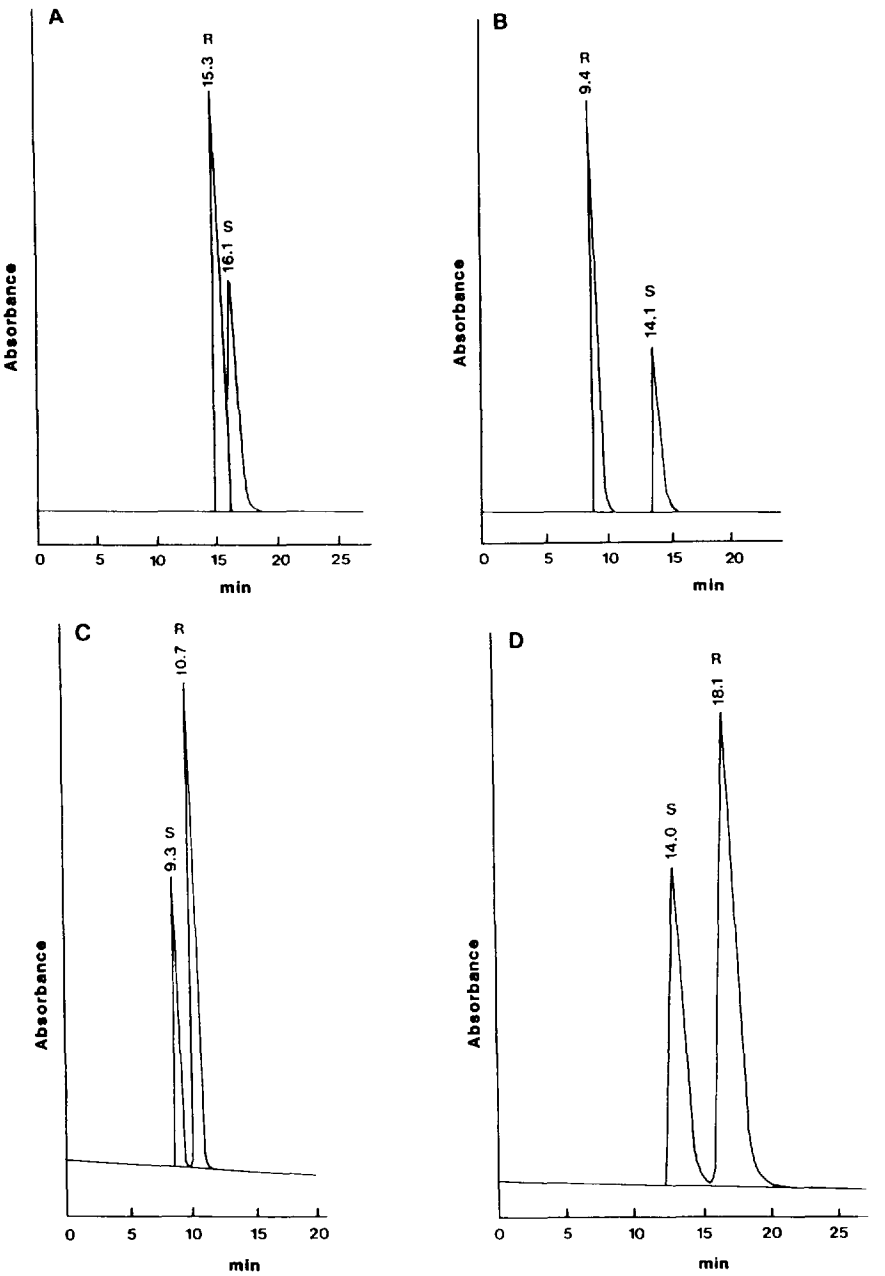

Fig. 2. Separation achieved on 4 out of 12 columns tried. (A) Chiralcel OG, (B) Chiralcel OD, (C) Chiralpak AD, (D) Chiralcel OJ. HPLC conditions: isopropanol–hexane (10:90), 0.7 ml min^{-1}; λ 215 nm, 2.0 AUFS; 1.0 mg ml^{-1}; 10 μl.

will not separate with isopropanol–hexane but will with ethanol–hexane and vice versa.

Over the past few years we have chromatographed a large number of racemic compounds using 12-column chiral switching methodology with a success rate of approximately 90%. A successful separation is one where at least one column has produced a baseline or near-baseline

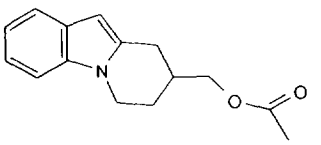

Fig. 3. Structure of protein kinase C inhibitor, Ro 31-8829/000.

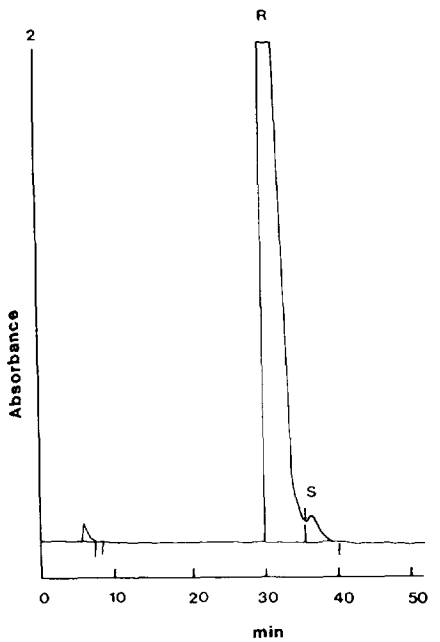

Fig. 4. Enantiomer separation of Ro 31-8829/000. HPLC conditions: Chiralcel OF; isopropanol–hexane (1:99), flow-rate 0.5 ml min^{-1}; λ 220 nm, 1.0 AUFS; 2.0 mg ml^{-1}; 10 μl.

separation. Quite often several columns demonstrate enantioselectivity.

Our experience has shown that in terms of successful applications using polysaccharide columns the Chiralcel OD and Chiralpak AD are by far the most versatile; approximately 75% of racemates could be separated on one or both of these columns. The Chiralcel OJ and Chiralcel OG follow on as the next most useful columns for our work.

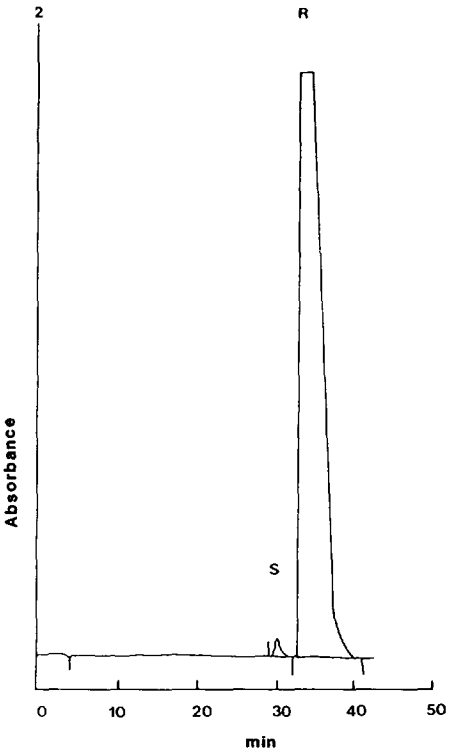

Fig. 5. Enantiomer separation of Ro 31-8829/000, peak reversal. HPLC conditions: Chiralcel OD; isopropanol–hexane (1:99), flow-rate 0.5 ml min^{-1}; λ 220 nm, 1.0 AUFS; 2.0 mg ml^{-1}; 10 μl.

References

[1] I. Wainer, *Trends Anal. Chem.*, 6 (1987) 125.
[2] T. Shibata, I. Okamoto and K. Ishii, *J. Liq. Chromatogr.*, 9 (1986) 313.
[3] D. Armstrong and W. Demand, *J. Chromatogr. Sci.*, 22 (1984) 411.
[4] V.R. Meyer, *Chromatographia*, 24 (1987) 639.