

Analytical columns packed with both 3- and 5- μ m polysaccharide-based wide-pore chiral stationary phases (CSP) are shown to be stable to high operating pressures over a prolonged period.

Introduction

In the past, concerns have been expressed about the stability of columns packed with conventional wide-pore silica-based chiral phases to high operating pressures. There is a perception that wide-pore silicas are more fragile than their small-pore counterparts. Consequently, these columns are operated within moderate pressure limits. With the advent of high performance columns based on 3- μ m particles, such pressure limits can be restrictive on the development of rapid analytical methods and therefore a re-evaluation of the pressure stability of such phases has been undertaken.

Experimental

Agilent 1100 and 1200 HPLC systems, optimized for minimal extra-column volume, were used for the study. Columns packed with a variety of 3- μ m (150 x 4.6 mm) and 5- μ m (250 x 4.6 mm) Daicel polysaccharide-based chiral stationary phases (see Table 1) were employed. QC testing was carried out using *trans*-stilbene oxide as solute with 10% 2-propanol in hexane at a flow rate of 0.5 ml/min (5- μ m particles) or 1.0 ml/min (3- μ m particles). The 3- μ m columns were exposed to 200, 280, 360 and 430 bar for one week at each given pressure. Similar tests were carried out for 3- μ m reversed phase columns using aqueous acetonitrile as mobile phase. The 5- μ m columns were exposed to 360 bar for 10 days followed by 50 hours exposure to a cyclic pressure gradient from 90 to 435 bar with a 1 hour period.

Results

Table 1 shows the column efficiency measured before the tests together with the percent change in this parameter for the columns tested. Only very small changes in efficiency were seen, well within the limits of the experimental error over the testing period. No changes in retention or selectivity were seen and the pressure drop across the columns remained constant, indicating that the particles remained undamaged by the procedures.

Table 1

Column	Initial Efficiency		Change (%)	
	N ₁	N ₂	Δ N ₁	Δ N ₂
CHIRALPAK [®] AD-3	21230	15534	4.8	2.9
CHIRALCEL [®] OD-3	20570	16390	4.7	7.3
CHIRALPAK IA-3	25779	23293	-4.6	-1.5
CHIRALPAK IC-3	25175	22549	-0.8	-4.1
CHIRALPAK AD-3R	24190	24040	-0.4	1.5
CHIRALCEL OD-3R	22623	22436	5.7	3.0
CHIRALPAK AD-H	26340	24740	-2.1	0.0
CHIRALCEL OD-H	22880	18470	0.0	3.4
CHIRALCEL OJ-H	22470	21560	4.8	5.0
CHIRALPAK AS-H	26630	25120	1.0	2.6
CHIRALPAK AY-H	24480	19700	-0.7	3.7
CHIRALCEL OZ-H	21890	19340	1.0	2.3
CHIRALPAK IA	22660	17740	8.4	7.6
CHIRALPAK IB	28290	26900	4.9	5.6
CHIRALPAK IC	27520	26050	-3.6	-2.0

Figure 1 shows the chromatograms for CHIRALPAK AD-3R after the reversed phase pressure stability testing, while Figure 2 shows the chromatograms from the tests of CHIRALPAK IB.

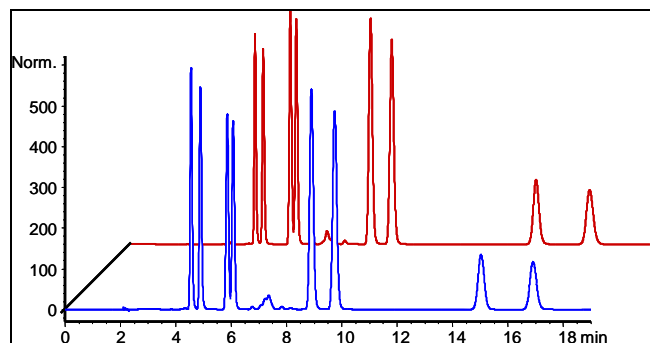


Figure 1. Chromatograms before (blue) and after (red) pressure testing of a CHIRALPAK AD-3R column (150 x 4.6 mm). Solutes: mephensin, 1-indanol, 1-(1-naphthyl)ethanol and 2-methyl-1-tetralone, 50% aqueous acetonitrile, 1 ml/min.

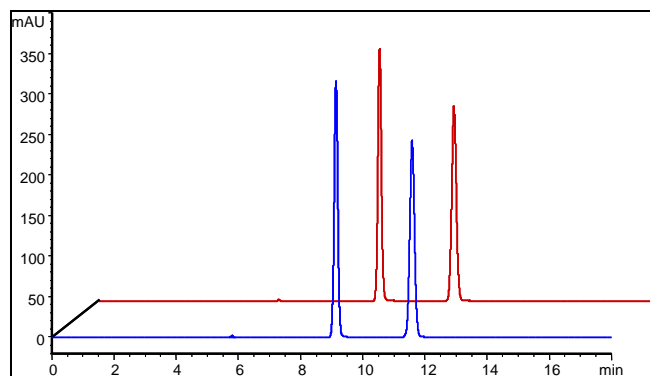


Figure 2. Chromatograms before (blue) and after (red) pressure testing of a CHIRALPAK IB column (250 x 4.6 mm). Solutes: *trans*-stilbene oxide, 10% 2-propanol in hexane, 0.5 ml/min.

Conclusions

Polysaccharide-based chiral HPLC and SFC columns using wide-pore silica have been shown to be stable to high pressures. These test results, attributable to improvements in silica and packing technologies, allow the operation of both 3- and 5- μ m analytical columns at all pressures attainable by conventional HPLC and SFC systems.