Important Aspects when Designing Chiral Preparative Separations using Coated Polysaccharide Stationary Phases

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Background

In preparative chromatography it is of immense importance to optimize the separation process in order to reduce the costs. Productivity is the most important parameter, often referred to as kg purified product per kg stationary phase per day. For large scale chiral separations, multi-column processes have proven to be very cost efficient.
Chiral stationary phases based on silica coated with derivatised amylose or cellulose are predominant on the market and in the literature, for enantiomeric separations. The presented study aimed at understanding these phases more thoroughly with the intention to improve and simplify the purification process using these CSP:s.

Importance of packing pressure

Kromasil CelluCoat and Kromasil AmyCoat are based on a wide pore silica in order to minimize the amount of achiral interactions with the silica surface while maintaining the mechanical strength. The importance of plate counts in preparative chromatography often diminishes with the scale. For batch chromatography using relatively small particles (i.e. 10 µm) a well packed column could be an important characteristic, especially for difficult separations. An analytical column packed with Kromasil CelluCoat/AmyCoat can be used up to the pressure limit of an ordinary HPLC (400 bar). In preparative chromatography DAC columns are used and this study showed that the achieved plate count is dependent on the packing pressure (see Figure 1). The maximum plate count is achieved around 70 bar. It should be noted that the packed density also increases with increased piston pressure and this causes higher back-pressures.

► Plate count and packed density vs. piston pressure

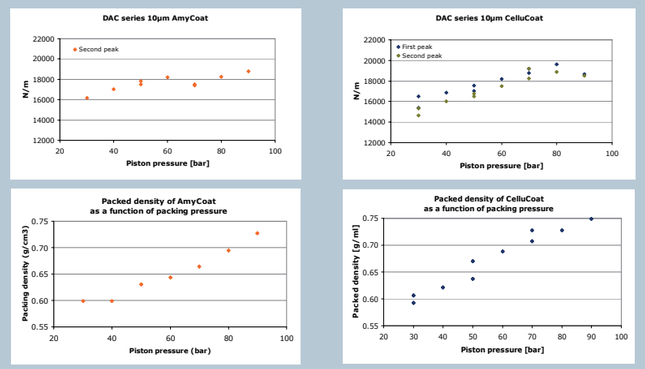


Figure 1. Plate counts and packed density as a function of piston pressure for DAC packing in a 5 cm column. Analyte: TFAE; Bed length (start) : ~50 mm; Mobile phase: heptane/2-propanol 90/10; Flow: 60 mL/min; UV @ 254 nm. Certain packing experiments are repeated in order to verify the results.

Mechanical strength is an important parameter in order to utilize the benefit of higher packing pressure. Another benefit is that a mechanically strong CSP survives repeated packing cycles in contrast to a mechanically weak support. Figure 2 illustrates the strength of Kromasil AmyCoat and CelluCoat. Also in screening using analytical columns mechanical stability is an important parameter enabling short run times due to high flow rates under high back-pressure

► Mechanical strength

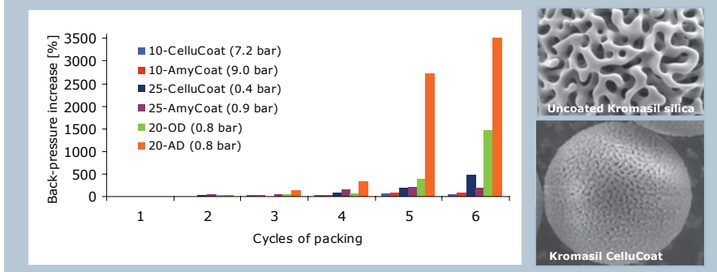


Figure 2. The test was designed to exert greater than normal mechanical stress on the chiral stationary phases, and is performed at a packing pressure above the one recommended by the manufacturer of Chiralcel OD and Chiralpak AD (50 bar).

The need for mobile phase additive

In preparative chromatography additives are undesirable since they complicate the solvent recovery process and they could also reduce the stability of the enantiomers in the mobile phase, particularly during evaporation. Analytical screening without additives with should however be made with careful considerations. Experiments using three substances clearly show that overloaded injections with and without additive are needed in order to decide if additive is needed or not in a preparative application (see figure 3 and 4). The results also clearly indicate that there is a large potential to exclude additives using Kromasil CelluCoat and to obtain improved results compared to a similar commercial CSP.

► Separation of CBZ-Alanine, with/without TFA

► Separation of ibuprofen, with/without TFA

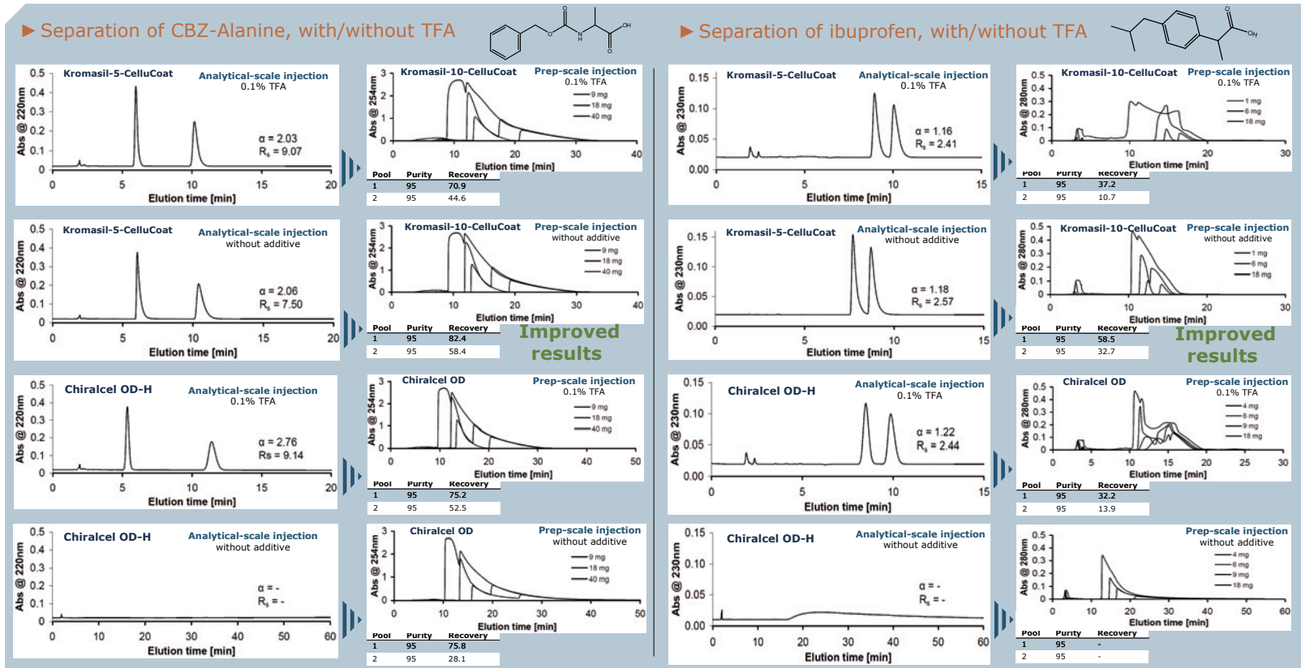
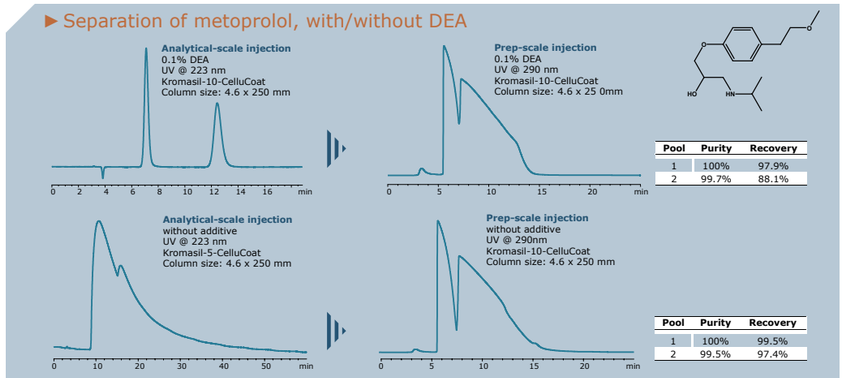


Figure 3. Analytical and preparative injections of ibuprofen and CBZ-Alanine with and without TFA as additive, heptane:2-propanol 90:10, 1 mL/min. Column size 4.6 x 150 mm for analytical injections and 4.6 x 250 mm for preparative injections.

► Separation of metoprolol, with/without DEA



► Stacked injections without additive – reproducible results

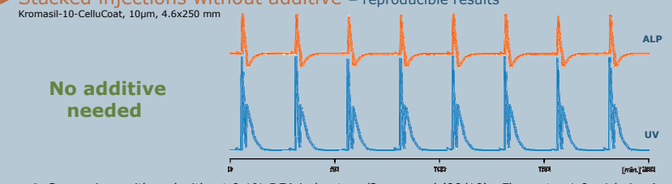


Figure 4. Comparison with and without 0.1% DEA in heptane/2-propanol (90/10). Flow rate: 1.0 mL/min; Analytical sample: Metoprolol 2 mg/mL in heptane/2-propanol (90/10); Analytical load: 5 µL, 10 µg; Preparative sample: Metoprolol 40 mg/mL in heptane/2-propanol (90/10); Preparative load: 500 µL, 20 mg

Conclusions

The study shows that there is a big potential to exclude additives in preparative purifications using Kromasil. The need for additive differs between manufacturers of CSP even though the phases are similar in theory (same selector supported on a silica matrix).

Overloaded injections are needed early in method development in order to decide if mobile phase additive is needed or not. A poor analytical separation without additive does not necessary mean a poor preparative separation if additive is excluded.

The high mechanical strength of Kromasil silica enables purifications with high plate counts in DAC columns.