Kromasil AmyCoat, A New Polysaccharide-based Chiral Stationary Phase for Rapid and Efficient Chiral Resolution

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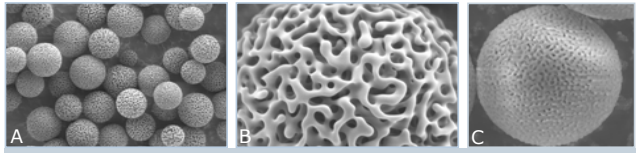
Background

Chromatographic analysis and purification of optically active compounds are still areas with large potential for improvement. In the analytical field, chiral phases with better performance giving enhanced resolution and shorter analysis time are desirable. Kromasil AmyCoat is a new fully back integrated chiral stationary phase from Kromasil, based on a tailor made silica coated with tris-(3,5-dimethylphenyl)carbamoyl amylose.

The Stationary Phase

The in-house developed wide pore silica is specially designed to minimize the amount of achiral interactions with the silica surface while maintaining the mechanical strength of Kromasil silica.
This mechanical strength allows for operating the columns without pressure restriction within HPLC range (≤400 bar).



FE-SEM pictures; **A** and **B:** pictures of uncoated Kromasil silica used for production of AmyCoat; **C:** Coated Kromasil silica

The selector used to coat the silica is tris-(3,5-dimethylphenyl)-carbamoyl amylose. This selector is well known for its ability to resolve a broad range of racemates. The unique coating technology ensures homogenous distribution of the selector and an optimal thickness which is important to generate a high-performing and stable product.

High efficient 3 µm particles

Kromasil AmyCoat is available in small particle sizes which gives high efficiency and consequently a high resolution. Table 1 illustrates the similarity in chiral recognition capability between Kromasil AmyCoat 3 µm and 5 µm. The higher resolution obtained using Kromasil AmyCoat 3 µm is a result of the higher plate count achieved with a smaller particle size. For difficult separations reducing the particle size could make the crucial difference between achieving baseline separation or not, as illustrated in figure 1.

Table1. Selectivity and resolution comparison of Kromasil AmyCoat 3 µm and 5 µm. Column size: 4.6 x 150 mm.

Racemate	AmyCoat 3 µm α	AmyCoat 3 µm R_s	AmyCoat 5 µm α	AmyCoat 5 µm R_s	Mobile Phase	Flow rate [mL/min]
Benzoin	1.3	6.5	1.3	4.4	heptane/2-propanol (90/10)	1
Bucetin	1.8	8.2	1.7	5.8	heptane/2-propanol (90/10)	2
Trifluoroanthrylethanol	1.4	6.4	1.4	4.2	heptane/2-propanol (90/10)	1
Hexobarbital	1.4	4.7	1.4	3.2	heptane/2- propanol (90/10)	1
Oxamniquine	1.2	3.2	1.2	2.3	heptane/2-propanol/DEA (90/10/0.1)	0.8
Alprenolol	1.6	5.3	1.7	4.4	heptane/2-propanol/DEA (90/10/0.1)	1
Metoprolol	1.5	3.2	1.4	2.0	methanol/DEA (100/0.1)	0.5

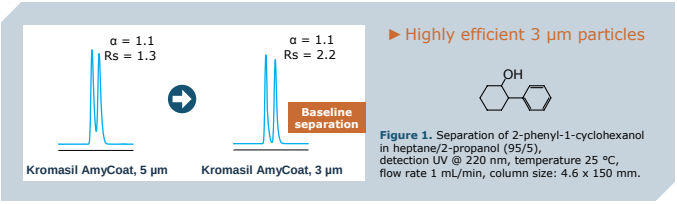


Figure 1. Separation of 2-phenyl-1-cyclohexanol in heptane/2-propanol (95/5), detection UV @ 220 nm, temperature 25 °C, flow rate 1 mL/min, column size: 4.6 x 150 mm.

High Speed Chromatography

The mechanical strength of Kromasil AmyCoat allows the columns to be operated at high flow rates. High flow rate combined with short column length provides very short analysis time. figure 2 shows high speed chromatography with baseline separation in less than 1 minute. Since the van Deemter plot is more flat for the smaller particle size, Kromasil AmyCoat 3 µm should be the first choice when running at elevated flow rates.

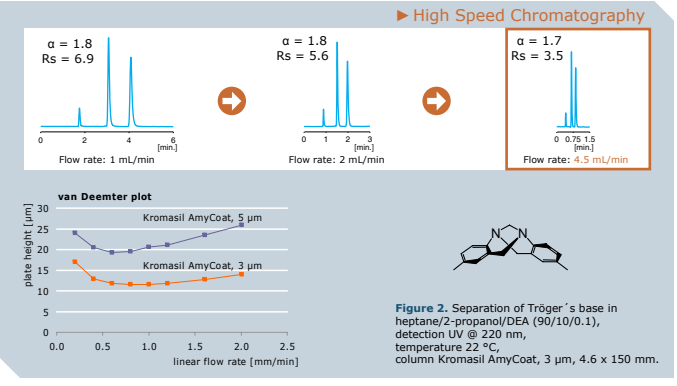


Figure 2. Separation of Tröger´s base in heptane/2-propanol/DEA (90/10/0.1), detection UV @ 220 nm, temperature 22 °C, column Kromasil AmyCoat, 3 µm, 4.6 x 150 mm.

In order to test the stability of the phase the chromatographic performance was evaluated before and after high flow rate conditions. As shown in figure 3, the column efficiency was maintained even after the harsh conditions of the sequence.

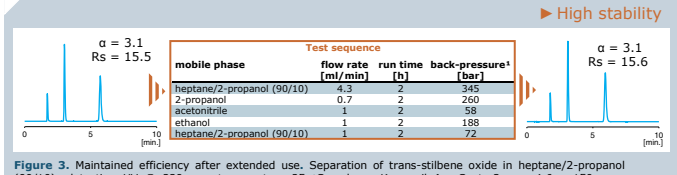


Figure 3. Maintained efficiency after extended use. Separation of trans-stilbene oxide in heptane/2-propanol (90/10), detection UV @ 229 nm, temperature 25 °C, column Kromasil AmyCoat, 3 µm, 4.6 x 150 mm.

1: total back-pressure over HPLC-system and column.

Additive Switching

In order to investigate memory effects after the use of additives in the mobile phase a basic substance was analyzed before and after the use of an acidic additive. As seen in figure 4, no memory effects were visible even after a short equilibration time.

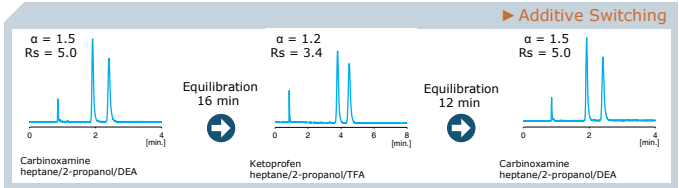


Figure 4. Separation of Carbinoxamine in heptane/2-propanol/DEA (90/10/0.1), detection UV @ 228 nm, Ketoprofen in heptane/2-propanol/TFA (90/10/0.1), detection UV @ 250 nm. For both substances: flow rate 2 mL/min, temperature 25 °C, column Kromasil AmyCoat, 3 µm, 4.6 x 150 mm.

Preparative Chiral Separations

Important aspects in preparative chiral separations are productivity, loadability, selectivity and solubility. Figure 5 illustrates one preparative application on Kromasil AmyCoat, 10 µm.

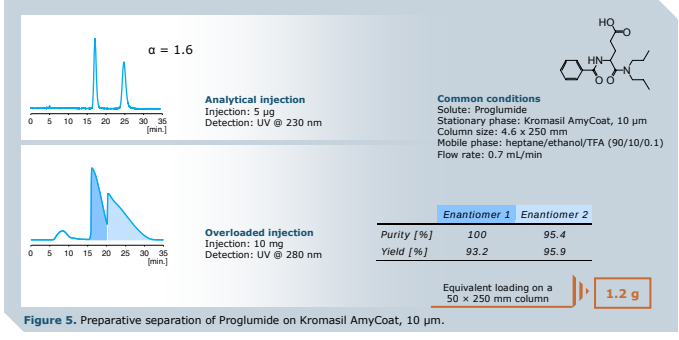


Figure 5. Preparative separation of Proglumide on Kromasil AmyCoat, 10 µm.

Summary

Kromasil AmyCoat is a new amylose based chiral stationary phase. The phase shows no degradation in performance when operated at high pressures and flow rates. There is also no loss in column performance when operated in different compatible mobile phases nor are any memory effects observed after the use of additives in the mobile phase. Furthermore, Kromasil AmyCoat displays a high capacity and is well suited for use in preparative applications.