Kromasil®

Kromasil Phenyl

AKZO NOBEL

A new Kromasil product, Kromasil Phenyl, has been developed. The target has been high ligand coverage, therefore we have used a mono-functional silane, together with dense end-capping. Kromasil Phenyl gives unique selectivity for aromatic compounds and is in addition wettable, and therefore suitable in applications where loading or chromatography under 100% aqueous conditions is desired.

Silica

Kromasil Phenyl is based on Kromasil 100 Å silica which is known to have high chemical purity, and excellent mechanical stability.

Derivatization

In order to obtain a stationary phase with high chemical stability, high reproducibility and symmetrical peaks for basic compounds, a mono-functional silane was used in the derivatization followed by end-capping. A study of Kromasil Phenyl compared to "standard" Phenyl phases was performed using a mix of antidepressants which illustrates the low silanol activity of Kromasil Phenyl (Figure 1).

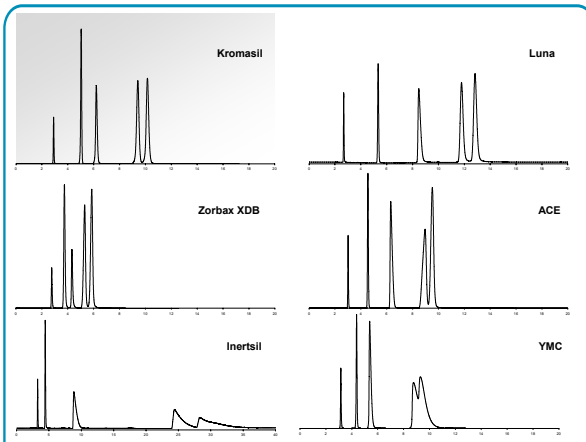

Figure 1. Comparison of Kromasil 100-5-Phenyl to competitor phenyl phases. Antidepressive substances: uracil, toluene (hydrophobic marker), nortryptiline, imipramine and amitriptyline. Mobile phase: MeOH/25 mM potassium phosphate pH 6.0, 80/20.

During the development of Kromasil Phenyl ^{29}Si CP/MAS NMR and elemental analysis, together with chromatographic evaluation, have been used in order to characterize the derivatization. The same derivatization procedure are used for all particle sizes, this is important in order to be able to easy scale-up from analytical to process scale. Figure 2 illustrates ^{29}Si NMR spectra for both 5 µm and 10 µm particles. The similarities are striking, showing high reproducibility. The NMR spectra corresponds with the chromatographic data; low silanol activity can be explained by the lack of the Q_2-signal and the low Q_3-signal. The surface coverage is 3.7 µmol/m^2.

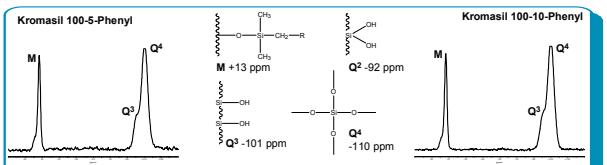

Figure 2. ^{29}Si CP/MAS NMR of Kromasil Phenyl.

Chemical stability

The chemical stability of the phase is determined by the quality of the silica, the surface coverage and the type of silane. Since Kromasil Phenyl is 100% wettable under aqueous conditions, the protection by the derivatization is even more important, and to produce a stable wettable phase is even more challenging than to produce a standard ODS phase. Several prototypes with varying carbon contents were manufactured during the development, and the stability were demonstrated to be very sensitive to this parameter. The optimal product, Kromasil Phenyl, was compared to Luna Phenylhexyl, since this product claims a very high chemical stability. The phases were tested for 6 days at pH 9.0, and the retention (rt) and the perfomance (N) of toluene were monitored (see Fig. 3). No large differences were observed. However after injecting Nortryptiline (pH 6.0) on both columns and compared to the asymmetry of Nortryptiline before they were exposed, the Kromasil column clearly shows a better performance.

The phases were tested for 6 days at pH 9.0, and the retention time (Rt) and the perfomance (N) of toluene were monitored (see Fig. 3). No large differences were observed. However, the Kromasil column clearly shows a better perfomance if the asymmetry of nortryptiline is compared before and after the stability study.

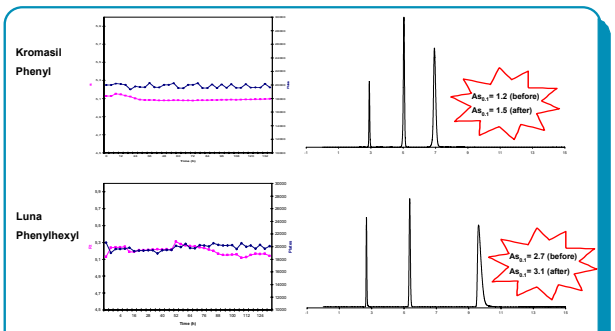

Figure 3. Stability study at pH 9.0 (MeOH/10 mM NH_4Ac, 80/20) for 6 days. Retention time (Rt) and Plates (N) were monitored for toluene, and the chromatograms shows uracil, toluene and nortryptiline at pH 6.0 after the stability study.

Applications

Phenyl phases are known to have enhanced selectivity compared to standard alkyl phases (C4-C18) for aromatics, due to π-π interactions resulting from a simple overlap of the π-orbitals in the phenyl stationary phase and in the analyte.[1,2] π-π Interactions can also involve a charge transfer of electrons from electron-rich to electron poor aromatic rings.[3] The retention is especially strong for strong π-acids, and the retention increase in the following order: aliphatics (least) < benzene derivatives < polycyclic aromatic hydrocarbons ≈ nitro-substituted benzene derivatives (most).[1] The wettability of Kromasil Phenyl also makes it suitable for purification and analysis of small aromatic-containing peptides. For application examples see figures 4-6.

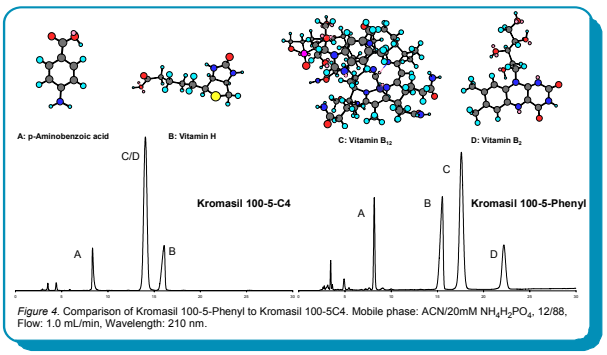

Figure 4. Comparison of Kromasil 100-5-Phenyl to Kromasil 100-5C4. Mobile phase: ACN/20mM $NH_4H_2PO_4$, 12/88, Flow: 1.0 mL/min, Wavelength: 210 nm.

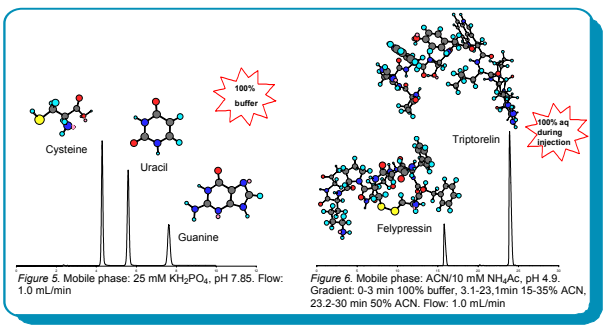

Figure 5. Mobile phase: 25 mM KH_2PO_4, pH 7.85. Flow: 1.0 mL/min

Figure 6. Mobile phase: ACN/10 mM NH_4Ac, pH 4.9. Gradient: 0-3 min 100% buffer, 3.1-23,1min 15-35% ACN, 23.2-30 min 50% ACN. Flow: 1.0 mL/min

References

[1] Snyder, L. R., et. al., *J. Chromatogr. A*, 1062 (2005) 65.
[2] Tanaka, N., et. al., *J. Chromatogr.*, 239 (1982) 761.
[3] Reubsaet, E.; et al., *J. Chromatogr. A*, 841 (1999) 147.