Kromasil®

Kromasil Analytical Performance

AKZO NOBEL

Prerequisites

Besides the metal ion content, there are a number of important prerequisites of a good RP-HPLC stationary phase:

- Pore size and pore size distribution – narrow and adequate
- Pore volume – optimized for mechanical strength
- Particle size distribution – narrow for optimal performance
- Surface area and topography – for effective and functional modification
- Surface density and distribution of the modification – for effective chromatography

These factors all affect the final performance of an RP-HPLC column.

The chemical function of metal ions in silica

Strongly bound metal ions present in the silica bulk are in most cases introduced during the silica manufacturing process. Both the chromatographic performance and the long-term stability are affected by the presence of metal ions in the silica. The data presented here are focused on the chromatographic effect.

<u>The chromatographic effect</u>

The effect of metal ions is dependent on their acidity. Highly electronegative metal ion species (*e.g.* bi- and trivalent iron and trivalent aluminium) are detrimental to the chromatographic performance. This effect is displayed via two mechanisms as shown in figure 1.

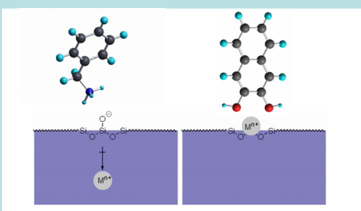

Figure 1. Interaction of analytes with metal ion effected interaction sites

Highly electronegative species inductively enhance the acidity of silanol groups in close proximity. The result is an increase in the amount of ion-exchange sites at lower pH. Moreover, if present in the surface layer of the silica, the metal ions have the ability to coordinate electron donating species (lewis bases), such as alcohols, ketones, acids and in some cases amines. Both effects can result in severe peak tailing and/or irreversible adsorption of certain analytes.

Tests to probe the effects of metal ions

To chromatographically investigate the presence of metal ions in the silica surface layer, and also strong ion-exchange sites/silanol activity, three different tests have been selected.

<u>Tests</u>

DERT - Metal ions directly available for interaction
Substances: 2,3- and 2,7-dihydroxynaphtalene
Mobile phase: ACN/0.025M NH_4Ac pH 7.05, 20:80 (w/w)

BAP - Ion-exchange capacity (employed at pH 2.7 and 7.3)
Substances: Benzylamine and Phenol (Uracil as t_0 marker)
Mobile phase: MeOH/H_2O/0.2M K-PO_4 at applied pH, 34:90:10 (w/w)

Anti-depressive substances - Silanol activity
Substances: Phenylpropanolamine, Toluene (hydrophobic marker), Nortriptyline, Imipramine, Amitriptyline
Mobile phase: MeOH/0.025M K-PO_4 pH 7, 80:20 (v/v)

Comparison of Kromasil to a metal-doped material

In figure 2, the effect of metal ions in the different tests can be seen. A Kromasil 100 Å, 5 μm, C_{18} phase is compared to a metal ion doped 100 Å, 5 μm, C_{18} material.

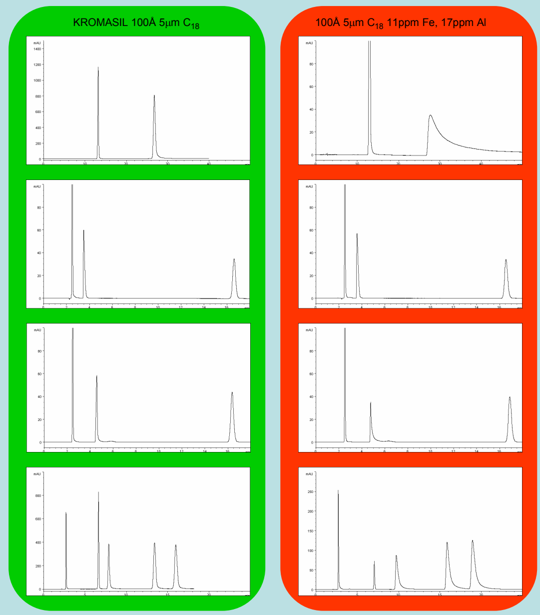

Figure 2. Comparison of Kromasil to a silica containing high levels of metal ions

First, from the DERT test, the direct interaction with metal ions is evident in the high-level metal ion material. The evaluated parameter $N_{2,7\text{-DHN}}/N_{2,3\text{-DHN}}$, also referred to as the DERT-value, should optimally be 1. The metal ions probed here are present in the silica surface layer. Second, with the use of benzylamine, the ion exchange capacity is investigated. The common parameter for comparing ion-exchange capacity in the benzylamine-phenol test is the ratio of $rk'_{\text{Benzylamine/Phenol}}$ at pH 7.3 and 2.7. Third, the use of a mixture of anti-depressants, reveals the performance under "real-life" conditions. In this test, the tailing of peaks ($As_{0.1}$ >1) is obvious in the high-level metal ion material. Regarding the DERT[1,2] and Benzylamine tests, these are often used in column characterizations.[3,4] Tests using anti-depressive substances are widely used under many different conditions. The asymmetry factors of anti-depressive substances are commonly used to quantify the silanol activity. Chromatographic data for especially Amitriptyline is frequently used[5,6]

In this study a wide range of tests have been evaluated and reduced to the above presented tests. These tests have been found to be the most sensitive, reproducible and reliable to investigate the effects of metal ions and silanol activity. In figure 3, a compilation of the output from the three tests is depicted. Nine commercially available materials are compared.

References

1. Cruz E. et al *J. Chrom. A* **1999**, *849*, 87-100
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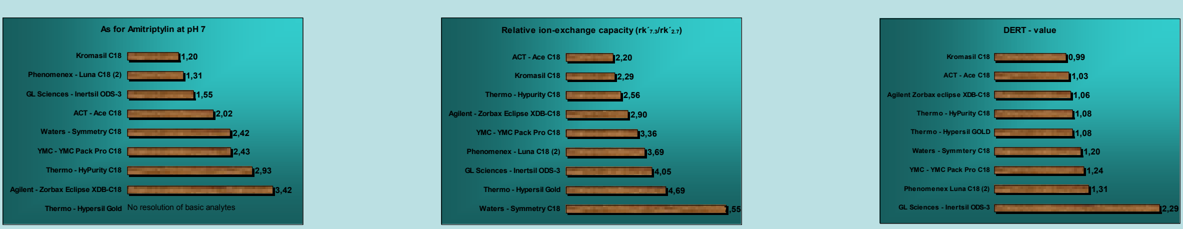
Commercially available materials – a comparison



The performance of Amitriptyline at high-silanol-activity conditions is a common comparison. This test is also ranked to be the most relevant for most chromatographic purposes.

The relative ion-exchange capacity at high and low pH reveals the presence of accessible silanols for ion-exchange interactions in the actual pH-interval. It should be added that all materials presented here would fall into the low ion-exchange capacity class.

The access to direct interaction with metal ions has the lowest importance. Most analytes do not have the ability of strong interaction (chelation) with metal ions.

Figure 3. A comparison of output from the tests performed on commercially available materials