Kromasil Eternity™
Designed for long life
Outline of the presentation

► Objective for the “early project”

► Introduction of the new Kromasil Eternity
  - Modifying the silica
  - Functionalizing the silica

► Kromasil Eternity – The benefits

► Conclusions
Company orientation

AKZO NOBEL

- Chemicals
- Coatings

eka

- Pulp & Paper
- Specialty Products
  - Separation Products

Launch: 1988
Focus: Silica-based HPLC media

Kromasil®
An Akzo Nobel brand
Objective for the “early project”

- Develop an analytical stationary phase for extreme pH conditions.

- Optimize the surface chemical properties WHILE keeping key physical properties intact.
Let’s start from the beginning
The Kromasil Eternity platform – what is it?
Completely bonded interfacial gradient

Incomplete bonding
Kromasil Eternity C18
### Product characteristics:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligand:</strong></td>
<td>C18</td>
</tr>
<tr>
<td><strong>Particle sizes:</strong></td>
<td>2.5 µm &amp; 5 µm</td>
</tr>
<tr>
<td><strong>Pore Size:</strong></td>
<td>100 Å</td>
</tr>
<tr>
<td><strong>Surface Area:</strong></td>
<td>330 m²/g</td>
</tr>
<tr>
<td><strong>Carbon Load:</strong></td>
<td>14 %</td>
</tr>
<tr>
<td><strong>Endcapping:</strong></td>
<td>Proprietary</td>
</tr>
<tr>
<td><strong>pH Range:</strong></td>
<td>1 - 12</td>
</tr>
<tr>
<td><strong>USP:</strong></td>
<td>L1</td>
</tr>
</tbody>
</table>
Long term pH stability – a comparison

**Test conditions**
- **Columns:**
  - Kromasil Eternity-5-C18 4.6 x 250 mm
  - Waters XBridge, 5 μm, C18, 4.6 x 250 mm
- **Mobile phase A:** 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (90/10)
- **Mobile phase B:** 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (10/90)
- **Flow rate:** 1 ml/min
- **Temperature:** 45°C
- **Gradient:**
  - 0 min 100% A
  - 10 min 100% B
  - 15 min 100% B
  - 16 min 100% A
  - 20 min 100% A
- **Test amitriptyline:** 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (30/70)
- **Test prednisolone:** 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (70/30)
- **Test cycle:** 6 x gradient + tests = 172 min/cycle

**Amitriptyline**

- Percentage of initial efficiency
- Time range: 0 to 8000 min
- Comparison between Kromasil Eternity and Waters XBridge

**Prednisolone**

- Percentage of initial efficiency
- Time range: 0 to 8000 min
- Comparison between Kromasil Eternity and Waters XBridge
pH variation to control selectivity

Test conditions
- Column: Kromasil Eternity-2.5-C18 4.6 x 50 mm
- Mobile phase: acetonitrile/20 mM sodium phosphate pH 2.1, 7.2 and 11.0 respectively
- Gradient: 0-0.5 min: 10%, 5.5 min: 50% acetonitrile
- Flow rate: 1.5 ml/min
- Temperature: 25°C
- Detection: UV 254 nm
Scale-up or Scale-down

Kromasil Eternity, 5 µm, 21.2 x 50 mm
Kromasil Eternity, 5 µm, 4.6 x 50 mm
Kromasil Eternity, 2.5 µm, 4.6 x 50 mm

Test conditions
Sample: Mix of beta-blockers,
1 = atenolol
2 = pindolol
3 = metoprolol
4 = propranolol
5 = alprenolol
Mobile Phase: acetonitrile/50 mM triethylamine acetate, pH 11 (40/60)
Flow rate: 0.43 mL/min and 9.0 mL/min for 4.6 and 21.2 mm i.d. columns, respectively
Temperature: 20°C
Detection: UV 230 nm
Stationary Phase: Kromasil Eternity (2.5 and 5 µm)
Column length: 50 mm
Increase efficiency – Save time

**Test conditions**
- Mobile phase: acetonitrile/water/formic acid (25/75/0.1)
- Substances: Mix of sulfis drugs
  1 = uracil
  2 = sulphathiazole
  3 = sulphamerazin
  4 = sulphamethoxazole
- Temperature: 25°C
- Detection: UV 254 nm

Save time without resolution loss by decreasing particle size

Save even more time with shorter column and increased flow rate

**Run at 1/4 of original analysis time**

**Stationary phase:** Kromasil Eternity-5-C18
- Column size: 4.6 x 250 mm
- Flow rate: 1.0 ml/min

**Stationary phase:** Kromasil Eternity-2.5-C18
- Column size: 4.6 x 50 mm
- Flow rate: 2.7 ml/min

**Stationary phase:** Kromasil Eternity-2.5-C13
- Column size: 4.6 x 100 mm
- Flow rate: 1.6 ml/min
Kromasil Eternity – Analytical scale

- 2.5 µm particle size
- UHPLC and HPLC (> 200 000 pl/m)
- Easy to scale up to 5 µm
Test chromatogram
Eternity 5 µm 4.6 x 250 mm

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention Time</th>
<th>Concentration</th>
<th>Flow Rate</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phthalate</td>
<td>3.69</td>
<td>23200</td>
<td>0.83</td>
<td>0.95</td>
</tr>
<tr>
<td>Toluene</td>
<td>6.62</td>
<td>23900</td>
<td>0.83</td>
<td>1.00</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>9.58</td>
<td>21600</td>
<td>0.83</td>
<td>0.89</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>11.95</td>
<td>19200</td>
<td>0.82</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Packing: Kromasil Eternity-5-C18
Serial Number: 82281
Batch Number: 000007355

Length: 250 mm
I.d.: 4.6 mm

Mobile Phase: Acetonitrile/Water (70/30)
Storage Solvent: Mobile Phase
Pressure: 74 bar
Flow Rate: 1 ml/min
Wavelength: 254 nm
Test Chromatogram
Eternity 2.5 µm 4.6 x 100 mm

Packing: Kromasil Eternity-2.5-C18
Length: 100 mm
ID: 4.6 mm

Serial Number: K0160
Batch Number: PDG435

Mobile Phase: Acetonitrile/Water (70/30)
Storage Solvent: Mobile Phase
Pressure: 243 bar

Flow Rate: 1.5 ml/min
Wavelength: 254 nm

<table>
<thead>
<tr>
<th>Compound</th>
<th>tR</th>
<th>RI</th>
<th>ε</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phthalate</td>
<td>0.97</td>
<td>17100</td>
<td>0.82</td>
<td>1.35</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.75</td>
<td>19100</td>
<td>0.79</td>
<td>1.24</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>2.54</td>
<td>20700</td>
<td>0.79</td>
<td>1.04</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>3.14</td>
<td>19200</td>
<td>0.73</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Kromasil®
AkzoNobel
Tomorrow’s Answers Today
Kromasil Eternity – Semi. prep. scale

- 5 µm particle size
- HPLC
- Easy to scale down to 2.5 µm
**Purification of crude amitrityline**

Initial Purity: 62%
Load: 420 mg (36 mg/g)

**Final Purity: 100%**
Yield: 96%

- **Column:** Kromasil Eternity 5 μm C18 (21.2 x 50 mm),
  Mobile phase: 25 mM NH4HCO3 pH 10.5/ACN,
  Gradient: 45-80% ACN/10 min.
  Flow: 21 mL/min, 25°C, 254 nm

- **Purity assessment of fraction pool:**
  Column: Kromasil Eternity 2.5 μm C18 (4.6 x 50 mm),
  Mobile phase: 10 mM NH4HCO3 pH 10.5/MeCN (45/55)
  Flow rate: 1.7 mL/min, 25°C, 254 nm

N_{amitr.}: 179’000 pl/m
Kromasil Eternity – The Benefits

Modified silica ➔ Long-lasting columns ➔ Better economy

Small particles (2.5 μm) ➔ High efficiency ➔ Faster analyses

Modified silica ➔ pH 1 to 12 ➔ Easier method development
Thank you for your attention!