Care and Use of Kromasil® Coated Chiral HPLC Columns

Please read this information carefully before using the column. All Kromasil columns are individually manufactured and tested to meet strict specification criteria. The following measures will maintain their performance and lifetime.

PRODUCTS INTENDED
Those instructions are applicable for the following Kromasil coated chiral phases:
- for normal phase chromatography (NP):
  Kromasil AmyCoat™
  Kromasil CelluCoat™
- for reversed phase chromatography (RP):
  Kromasil AmyCoat RP
  Kromasil CelluCoat RP

IMPORTANT
The entire HPLC system, including solvent lines, injector, loops or autosampler has to be purged with a solvent compatible with the Kromasil coated chiral column. A thorough instrument purge with 2-propanol is recommended. Solvents commonly used for other normal phase separations, such as e.g. THF, dichloromethane, DMSO, DMF, ethyl acetate, acetone or chloroform could severely damage the Kromasil coated chiral column even at residual quantities. Changing mode (from RP to NP or vice-versa) on a coated chiral phase column may permanently impair its performance.

COLUMNS INSTALLATION
Shipping solvent: The normal and reversed phase columns are shipped in heptane/2-propanol (90/10) and acetonitrile/water (40/60), respectively

System dead volume: Reduce dead volume in the system to a minimum by using small internal diameter connection tubing, for analytical columns 0.010". Keep the tubing length between injector, column and detector as short as possible.

Column connection: The column should be mounted according to the flow direction indicated on the column. For optimum performance, it is important that the tubing used to connect the column to the injector or detector is swaged into position such that it abuts the internal shoulder of the fitting. Excessive tightening of the column end and fittings will result in damage to the column tubing and/or fitting.

Performance Testing: It is recommended that the performance of columns is tested upon arrival and at periodic intervals during use. The test conditions are described on the test chromatogram, the calculations of plates and symmetry as described below.

Plates: \( N = \frac{5.54 \cdot (t/W_{0.5})^2}{2} \)
Peak asymmetry factor: \( A_{5/2} = B/A \)

Mechanical damage: Protect the column from mechanical chock. Dropping or banging a column can impair its performance.

Storage: Wash out all additives from normal phase columns with a neutral mobile phase such as heptane/2-propanol (90/10) and buffer from reversed phase columns with a salt-free mobile phase such as acetonitrile/water (40/60). Close the column openings with the end-caps in order to prevent the packing from drying out and keep the column at ambient temperature (15-25°C).

OPERATIONAL GUIDELINES
Flow rate and pressure limitation: There are no specific pressure drop limits for the Kromasil coated chiral columns. They can be operated up to the common pressure drop limits of most HPLC instruments, 400 bar.

Temperature limits: Generally, Kromasil coated chiral columns can be operated between 0 and 40°C, except for buffered basic mobile phases. See compatibility table on next page for actual limits.
COMPATIBLE MOBILE PHASES

If you wish to use solvents, buffers or additives others than mentioned on this page, please consult the Kromasil technical support team first (kromasil@eka.com).

Changing mode (from RP to NP or vice-versa) on a coated chiral phase column may permanently impair its performance.

Switching between acetonitrile and methanol in the mobile phase on coated chiral phases may irreversibly damage the coating. When switching between mobile phases containing these solvents, run an intermediate wash with 100% ethanol for Kromasil AmyCoat / AmyCoat RP or 2-propanol for Kromasil CelluCoat / CelluCoat RP.

Compatible normal phase mobile phases:
Possible combinations and compositions for Kromasil AmyCoat and Kromasil CelluCoat are described in the tables below:

| Kromasil AmyCoat and Kromasil CelluCoat ¹ | alkane/2-propanol 100/0 to 0/100 | alkane/ethanol 100/0 to 0/100 | alkane/methanol ² 100/0 to 0/100 | alkane/MTBE 100/0 to 50/50 | ethanol/methanol 100/0 to 0/100 | (SFC) CO₂/alcohol 100/0 to 50/50 |

| Kromasil AmyCoat only ¹ | acetonitrile/methanol 0/100 to 15/85 85/15 to 100/0 | acetonitrile/2-propanol 100/0 to 0/100 | ethanol/MTBE 100/0 to 70/50 |

| Kromasil CelluCoat only ¹ | acetonitrile/methanol 85/15 to 100/0 | ethanol/MTBE 100/0 to 50/50 |

Mobile phase additives for normal phase: For basic samples we recommend the addition of 0.1% (< 0.5%) DEA and for acidic samples the addition of 0.1% (< 0.5%) TFA.

Switching between mobile phases on normal phase columns. When switching between non-miscible solvents, use 100% 2-propanol as a transition mobile phase. Kromasil AmyCoat will also need a subsequent wash with 100% ethanol.

When switching from 100% polar mode to alkane/ethanol run a transition wash with 100% ethanol for Kromasil AmyCoat or 2-propanol for Kromasil CelluCoat.

Otherwise, no intermediate column wash is necessary. An adequate equilibration time is depending on the column dimension. By increasing the flow rate the equilibration time can be reduced. However, stable base line should always be reached before a separation is started.

Compatible reversed phase mobile phases:
Possible combinations and compositions for Kromasil AmyCoat RP and Kromasil CelluCoat RP are described in the bottom table.

To avoid salt precipitation, a wash with acetonitrile/water (40/60)³ is recommended when switching from buffer to 100% organic modifier.

<table>
<thead>
<tr>
<th>Aqueous solution</th>
<th>Organic modifiers</th>
<th>Organic part ¹</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>acetonitrile ³</td>
<td>10-100 %</td>
<td>5-60°C</td>
</tr>
<tr>
<td>potassium phosphate buffer 0-0.5 M, pH 2.0-8.0 Suggestions: 50 mM at pH 2.0 20 mM at pH 8.0</td>
<td>methanol ³</td>
<td>pH &lt; 7: 5-40°C</td>
<td></td>
</tr>
<tr>
<td>phosphoric acid, aqueous solution at pH 2.0 Suggestions: 100 mM at pH 2.0 50 mM at pH 5.0</td>
<td>ethanol, 2-propanol</td>
<td>pH &gt; 7: 5-25°C</td>
<td></td>
</tr>
<tr>
<td>sodium hexafluorophosphate aqueous solution</td>
<td></td>
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<tr>
<td>sodium borate buffer 0-0.2 M, pH 7.5-9.0 Suggestions: 20 mM at pH 9.0</td>
<td>acetate acid, 0.1% ³</td>
<td>5-25°C</td>
<td></td>
</tr>
</tbody>
</table>

¹: volume by volume.
²: due to limited miscibility of methanol in alkanes, ethanol should be added as a mediator when exceeding 5% methanol.
³: Read the red-boxed note about these solvents.

Chemical acronyms: