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Solve your problems – Column Troubleshooting

KNAUER Webinar, 26.06.2012

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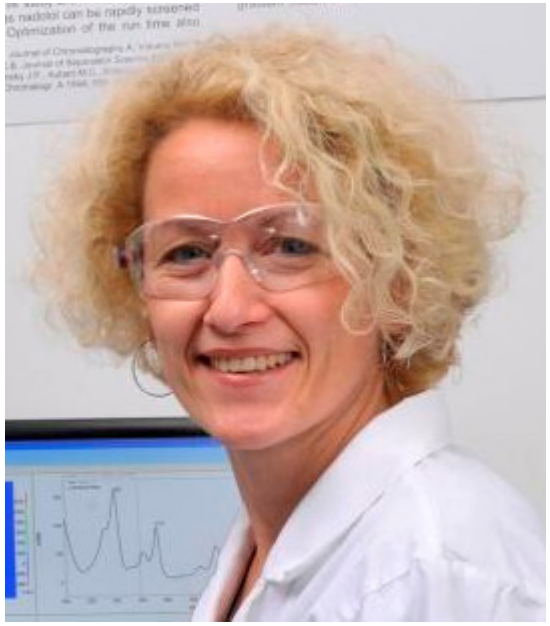
Solve your problems – Column Troubleshooting



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Applications and Columns Dep.

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Solve your problems…every time to do!

- What have I to do to follow up the changings of my column?

Test your column after installation!

Test your column periodically!

The two biggest pitfalls…

- What are the most common reasons for less column life time?

Inadequate cleaned samples

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The two biggest pitfalls…

- What are the most common reasons for less column life time?

Column:	Bluespher 100 – 2 Phenyl, 100 x 2 mm ID	
Conditions:	Eluent:	A: Methanol B: Water + 0.3% TFA
	Gradient:	0 – 5 min 62% A 5 – 9 min 62 – 80% A
	Flow:	0.5 ml / min
	Temp.:	50 °C
Detection:	UV at 210 nm	

Unsuitable method parameters

Inadequate cleaned samples…

- What can happen?

  Precipitation effects

  Deactivating silica gel and modification

  Chemical reactions

  Blockage's of the silica gel pores

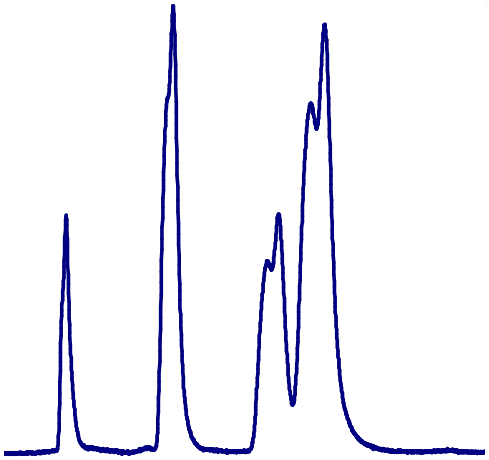
  Effects from ionic strength

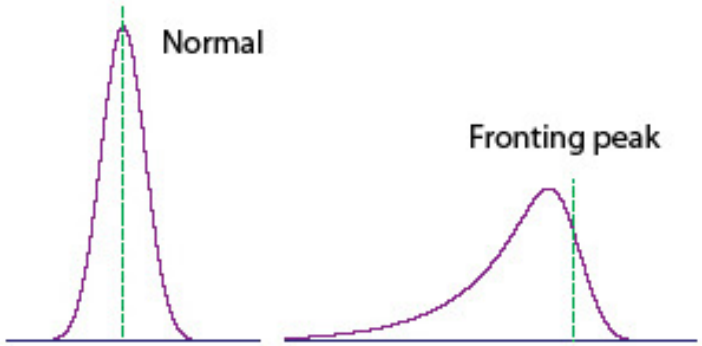
  Effects from solvent strength

Inadequate cleaned samples…

- How can I recognize that?

  - Appreciable higher back pressure
  - Less separation performance
  - Matrix effects / Coelution
  - Split peaks
  - Fronting / Tailing / Broad peaks





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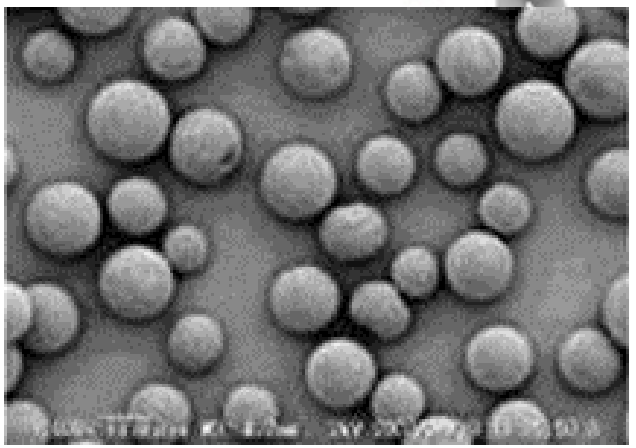
A detailed view…

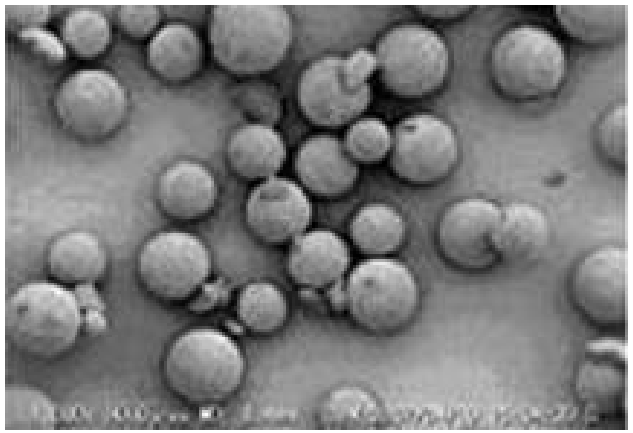
- To high back pressure…

  Damages in wide bore silica material

  Pressing polymeric material

  Column dead volume





Reverse column flow direction

READ COLUMNS USE INSTRUCTIONS

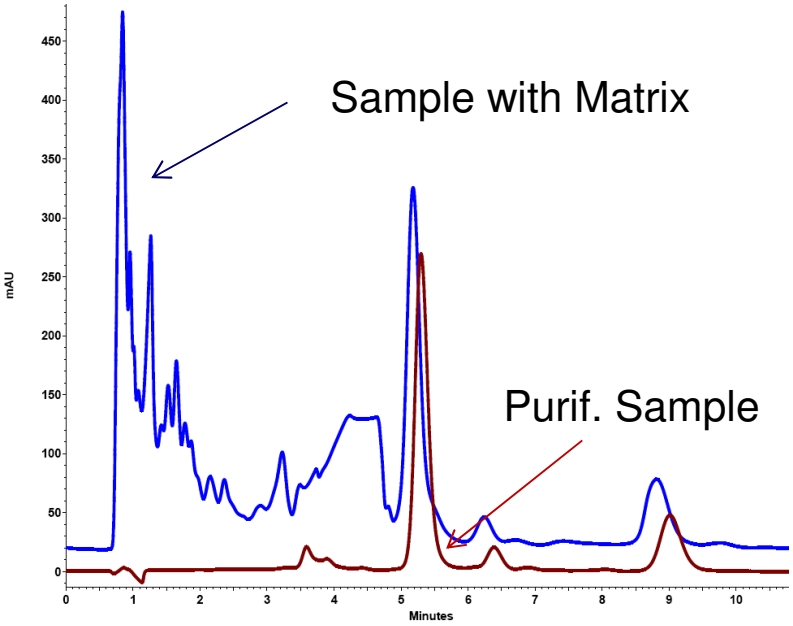
Inadequate cleaned samples…

- How can I avoid that?

Sample filtration / Inline filter

Falling out of proteins (Carrez etc..)

Online / Offline SPE



Using guard columns

Think about column with packing material with higher particle and pore size

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Inadequate cleaned samples…

- How can I rescue a damaged column?

  Reverse flow direction

  Flushing column with high elution eluent

    salt accumulations? inject (3 x) 0,01 M HNO_3 or diluted NH_3

    organic accumulations? inject THF, DMSO or DMF

  Replace contaminated fittings

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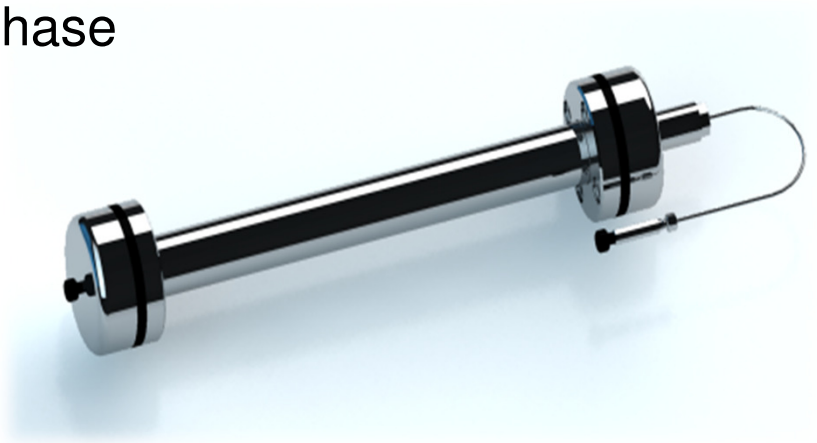
Unsuitable method parameters…

- What are the most important parameters?
  - Combination column – mobile phase
    - Polarity
    - pH
    - Stability of modification
  - Temperature
  - Flow



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Unsuitable method parameters…

What can happen?

Deactivating of modification

Polarity and Ionic strength of mobile phase

Reactions with or destroying of silica gel modification

Kind and pH of mobile phase

Dissolving or destroying of the stationary phase carrier

Kind and pH of mobile phase

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Unsuitable method parameters…

- How can I recognize tha

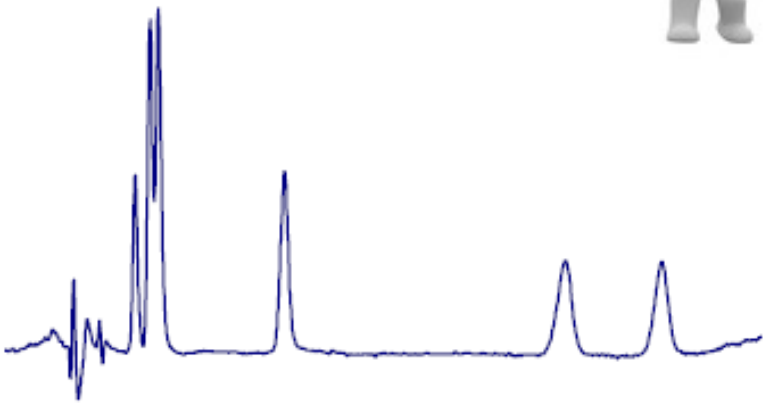
No or less separation performance

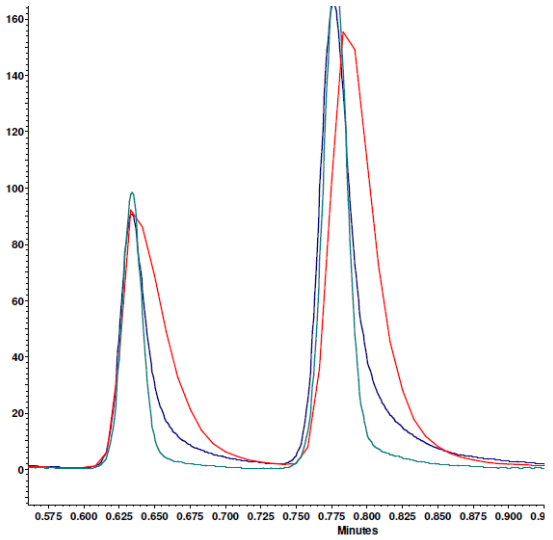
Less reproducibility

Split peaks

Fronting / Tailing / Broad peaks

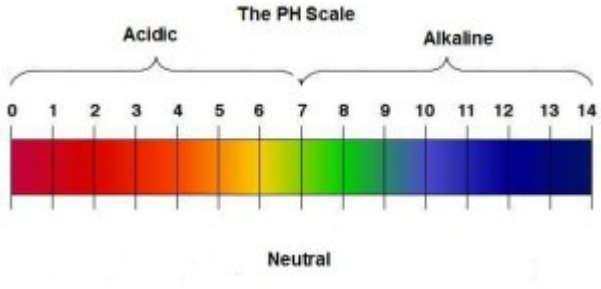
High column bleeding / Noisy baseline





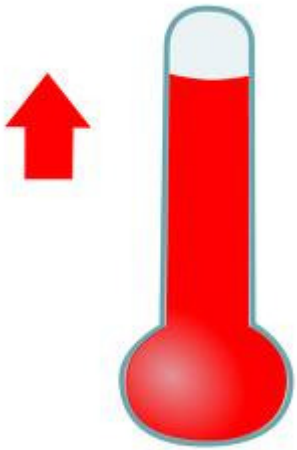
A detailed view…

- pH and temperature…



To high pH → dissolving silica gel material

To low pH → dissolving modification



High temperature?

→ 4 – 40 °C o.k.

→ 45-50 °C short term

→ 60 – 80 °C avoid / short term !!!

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Unsuitable method parameters…

- How can I avoid that?

  Read columns use guidelines

  Use HPLC grade solvents

  Try to develop non critical methods

  Using guard and victim columns

  Using of special columns (high endcapping / high temperature)

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Unsuitable method parameters…

- How can I rescue a damaged column?

Reverse flow direction

Flushing column with intermediate elution media (isopropanol)

Follow regeneration guidelines

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Help is coming…

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Application Note

► HPLC Troubleshooting Guide

Category	Troubleshooting
Matrix	-
Method	HPLC, UHPLC
Keywords	Troubleshooting, HPLC and UHPLC problems, column care and use
Analytes	-
ID	V000003N, 09/11

Summary

In HPLC or UHPLC numerous problem can arise. In comparison to former days, technology and instrumentation have been improved but typical problems still occur. Especially for inexperienced HPLC users but also for advanced learners, help in isolating, identifying and correcting typical problems is needed.

available as .pdf
www.knauer.net

Help is coming…

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KNAUER HPLC Column Selection Guide

KNAUER HPLC and UHPLC Columns Selection Flow Chart for Small and Large Molecules

Reversed-phase HPLC and UHPLC are still some of the most often used key analysis techniques that can be applied for the determination of ionic and non-ionic analytes. Therefore, this KNAUER Columns Selection Guide will focus on reversed-phase columns. Just follow the outline below for the easy selection of a reversed-phase column for method development.

This flow chart provides information on choosing an initial column for method development of small molecule and protein or peptide samples including decisions on the stationary phase and column configuration.

Small Molecules MW < 2000		Large Molecules MW > 2000
Packing Pore Size 100 Å/120 Å/180 Å	The pore size of the packing material is firstly chosen based on the size of the analytes. Small molecules can diffuse easily in and out of standard packings with 100 Å pore size. Larger molecules like proteins and peptides may not diffuse in and out of these small pores and therefore, 300 Å packings are recommended.	Packing Pore Size 300 Å
Starting Column Bonded Phase Eurospher/Eurospher II C18	C 18 is the recommended starting material for a wide range of reversed phase applications. It maximizes retention for moderately polar to non polar compounds. Other phases with shorter chain modifications should be used if resolution can not be	Starting Column Bonded Phase Eurosil Bioselect C18/C18A

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Euroline

▶ Column Care and Use

1. Silica based phases

Column Usage and Column Care

The proper care of an HPLC column is extremely important for the lifetime of the column and, consequently, for the quality of your HPLC analysis. The following pages will give you some guidelines for the use, cleaning and storage of HPLC columns. These guidelines will depend on the nature of the chromatographic support (silica, polymers or others) and on the surface chemistry of the corresponding stationary phase.

General guidelines

Silica is the ideal support for HPLC columns. It offers good mechanical stability, excellent physicochemical surface properties, a wide range of bonding chemistry and is compatible with a broad range of organic solvents. However, the following points are extremely important when working with silica based HPLC columns.

pH stability

In general silica based HPLC columns are stable within a pH range of 2 to 8. When measuring pH, the measurement should be done in the aqueous media before mixing the eluent with organic solvents. This will give a more accurate and consistent measurement of pH than taking a measurement in a mixed aqueous/organic media. Some modern HPLC columns can be used outside that pH range. New bonding chemistry allows for operating as low as pH 1 with some stationary phases. However, you should check vendors product information first before using a silica based column outside the pH range of 2 to 8. Stationary phases

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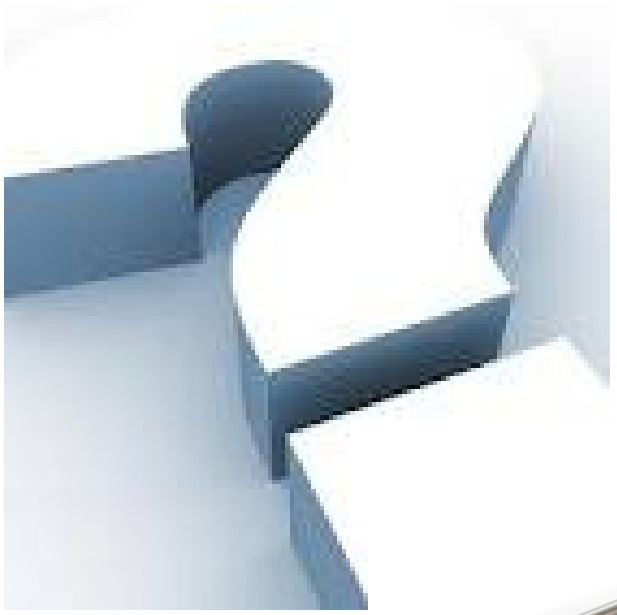
Troubleshooting…

- Time for Questions



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Troubleshooting…

- Contact:

Further questions and answers via mail

applications@knauer.net

columns@knauer.net

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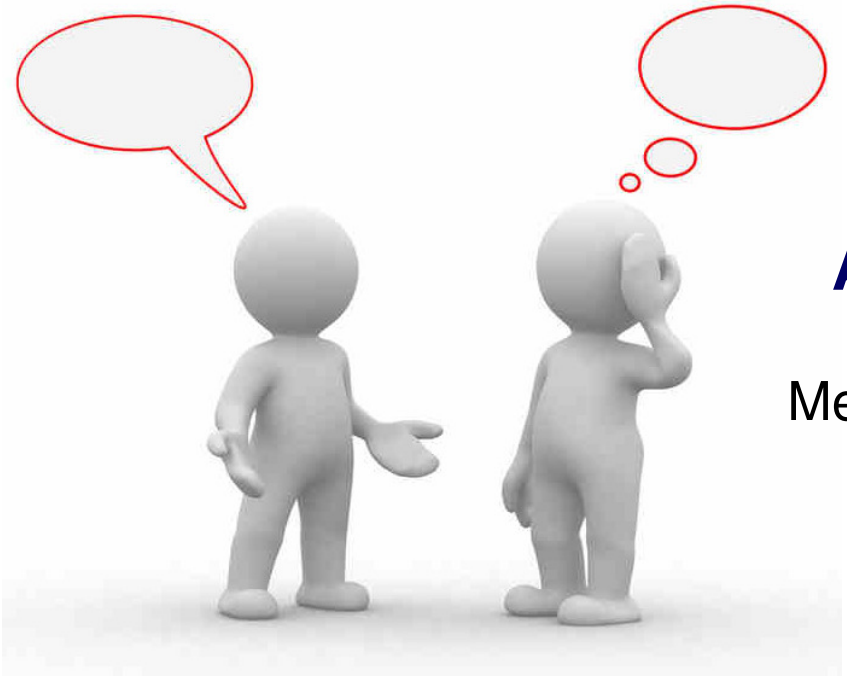
Next Webinar???



Applications and Columns

From small to large –
Method development from the column point of view!

27.11.2012