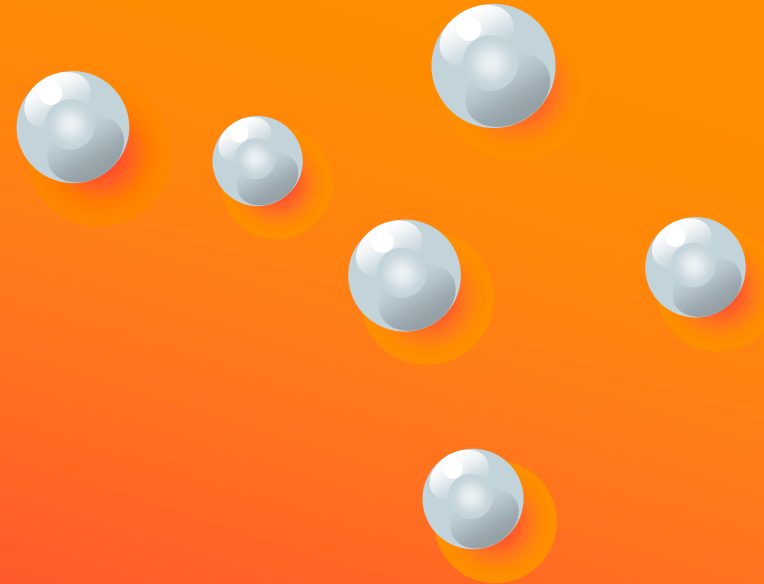


Kromasil Eternity™

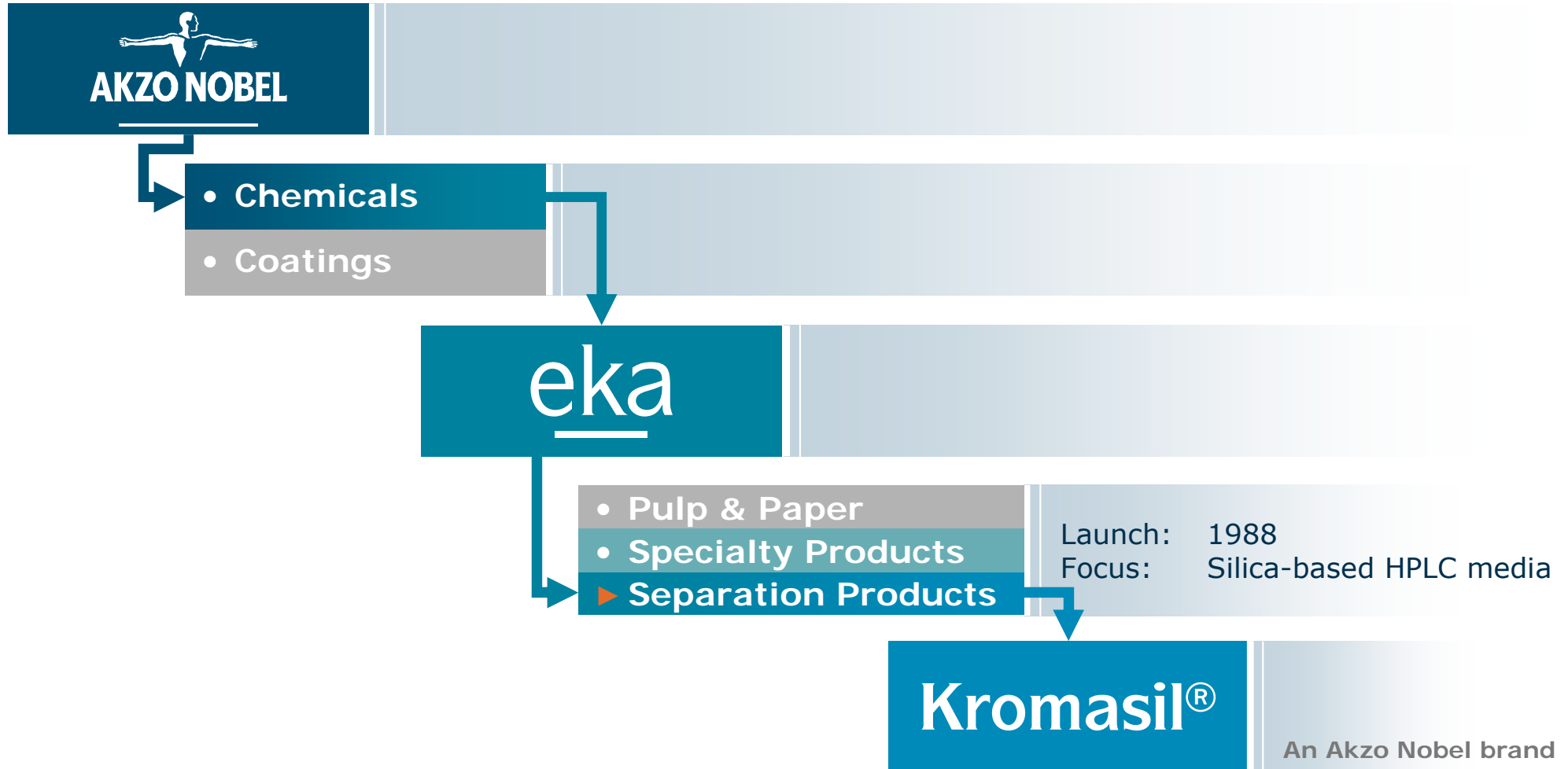
Designed for long life



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

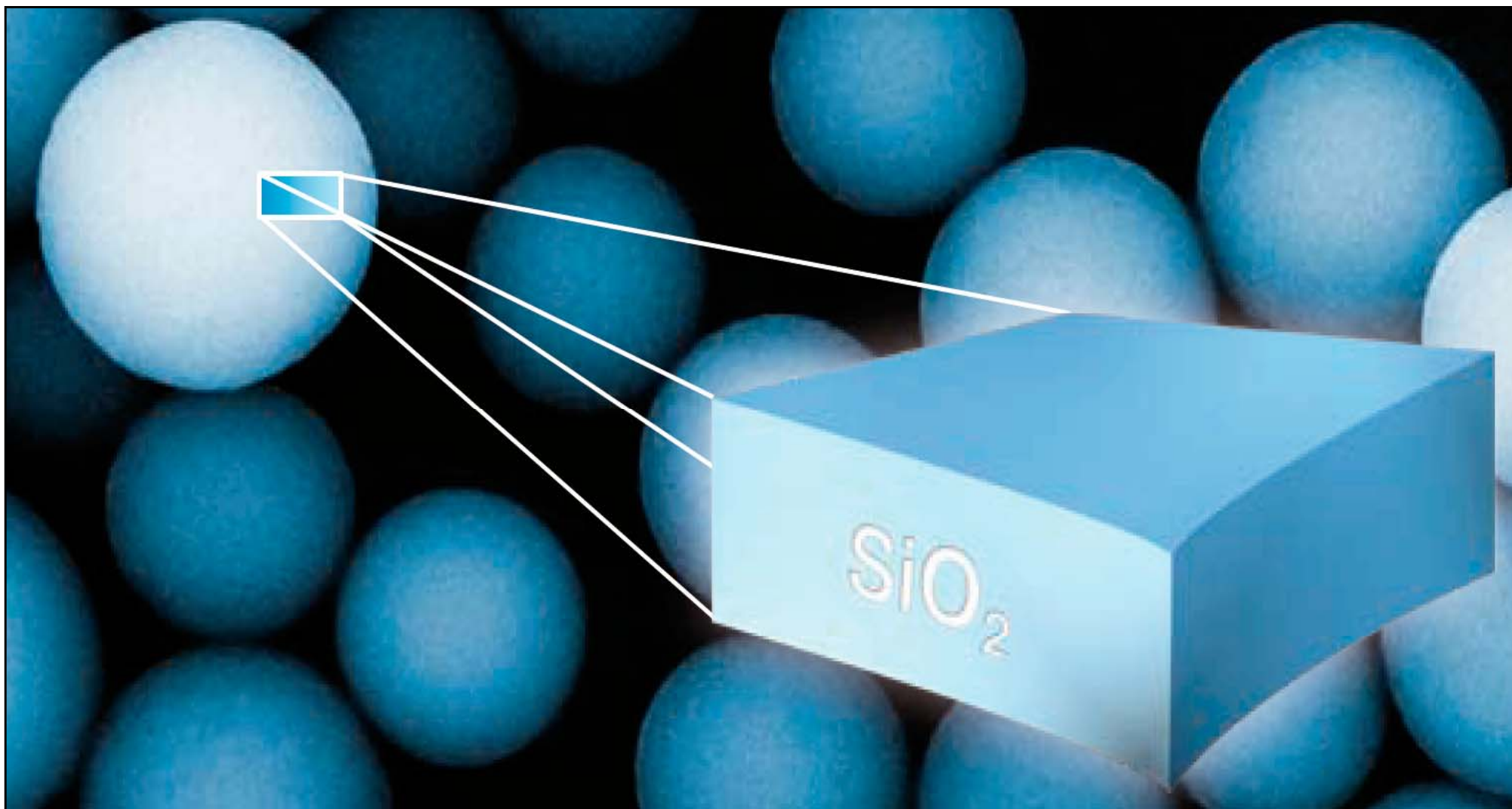
Company orientation



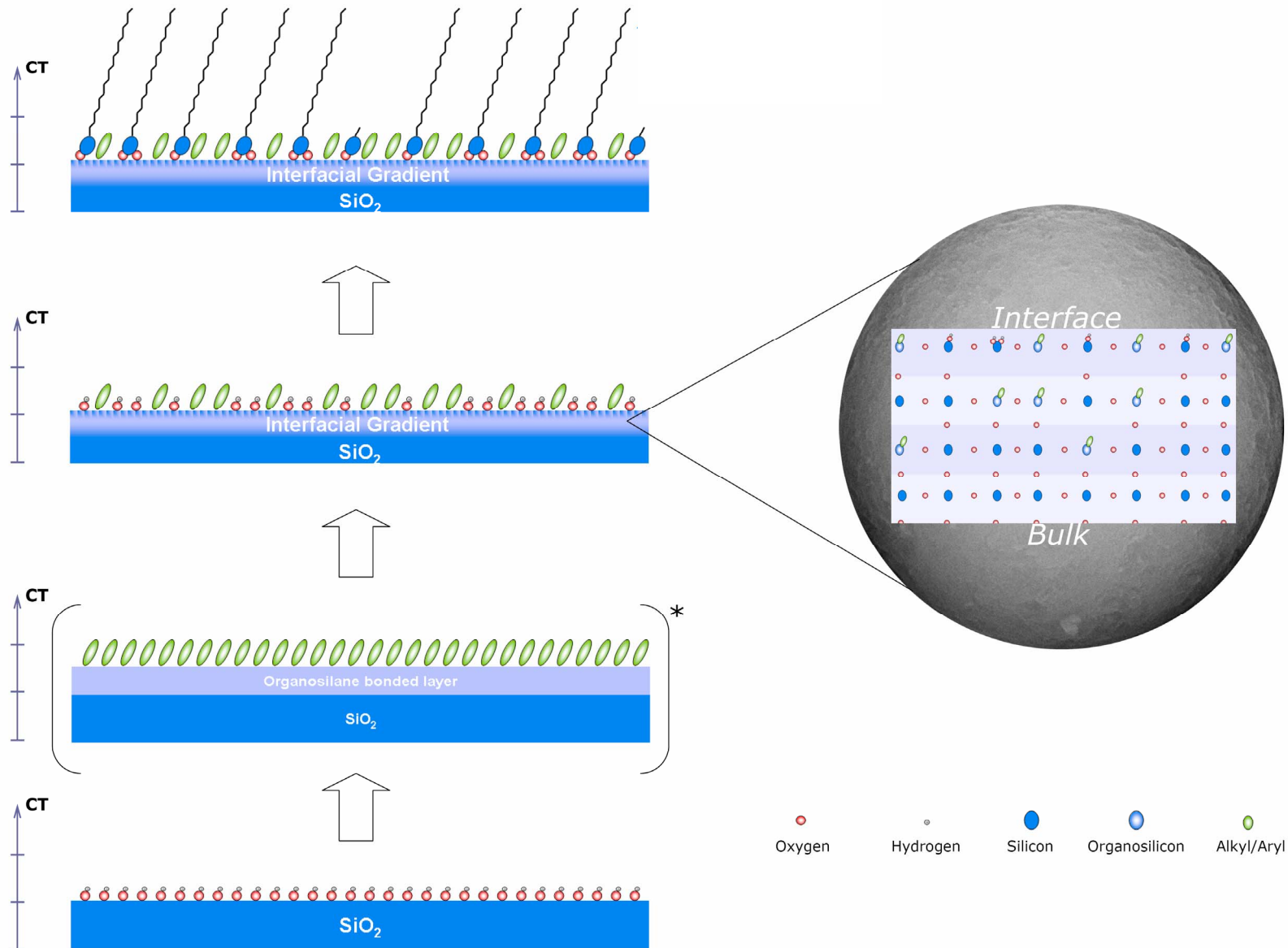
Objective for the “early project”

- ▶ Develop an analytical stationary phase for extreme pH conditions.
- ▶ Optimize the surface chemical properties WHILE keeping key physical properties intact

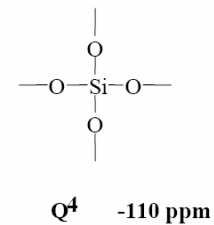
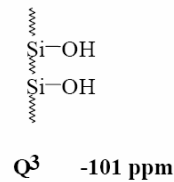
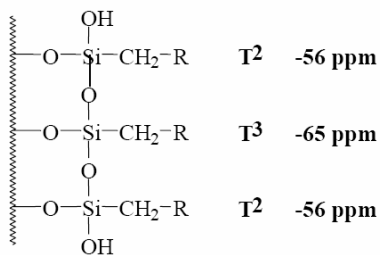
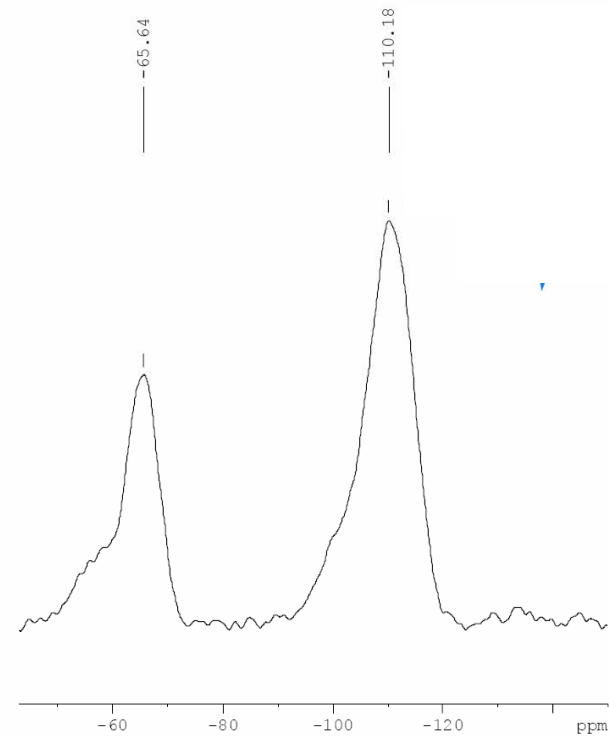
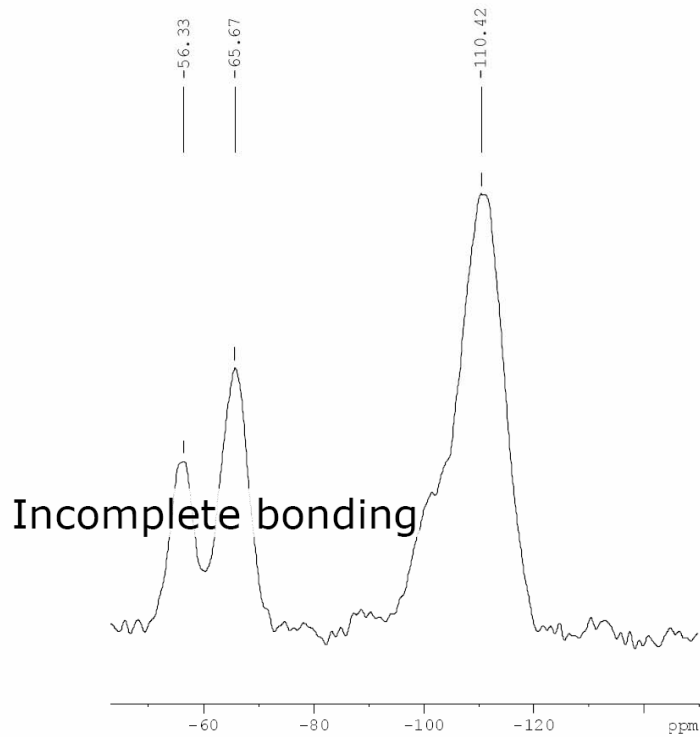
Let's start from the beginning



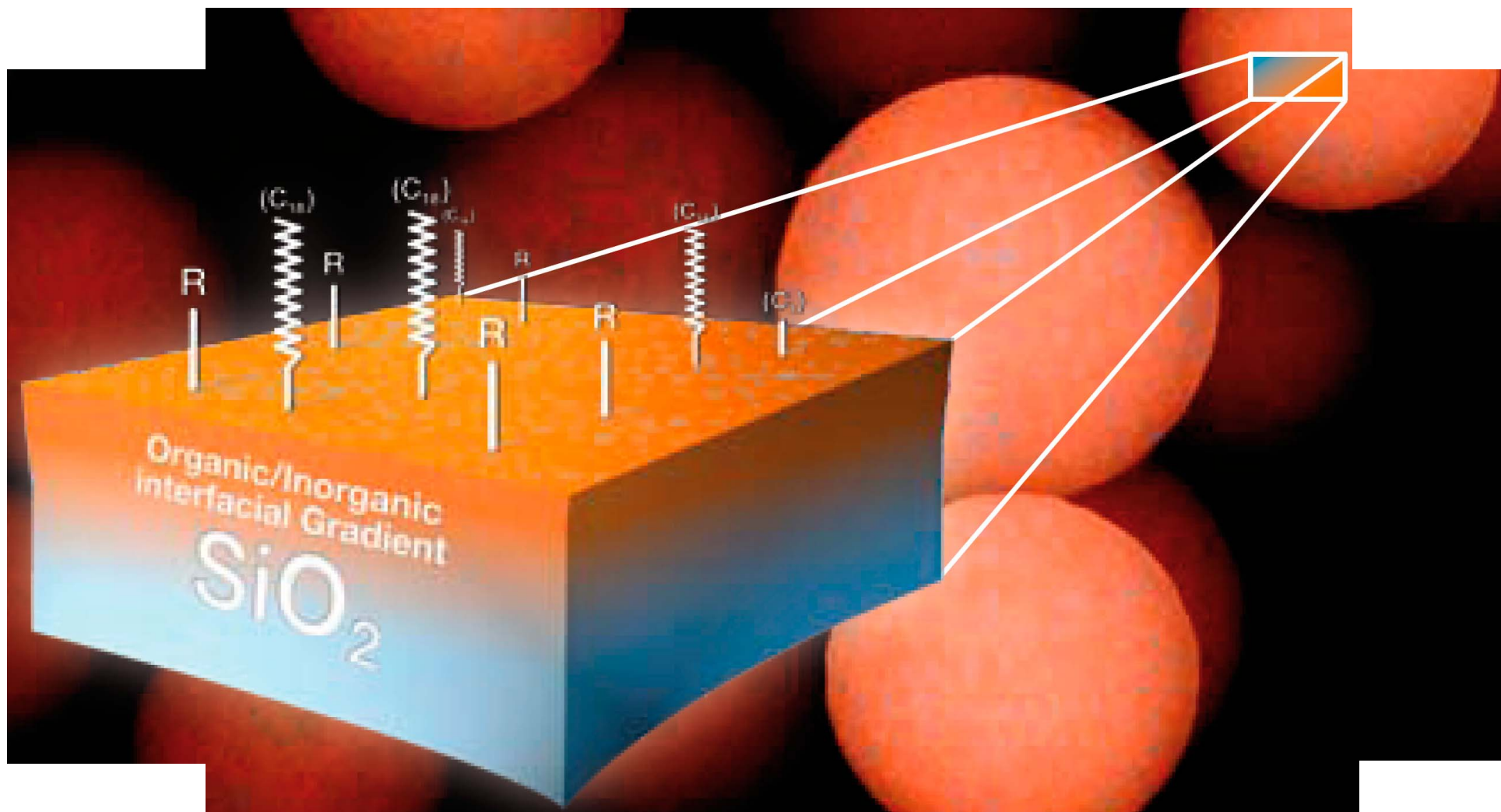
The Kromasil Eternity platform – what is it?



Completely bonded interfacial gradient



Kromasil Eternity C18



Product characteristics:

Ligand:	C18
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Particle sizes:	2.5 μm & 5 μm
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Pore Size:	100 \AA
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Surface Area:	330 m^2/g
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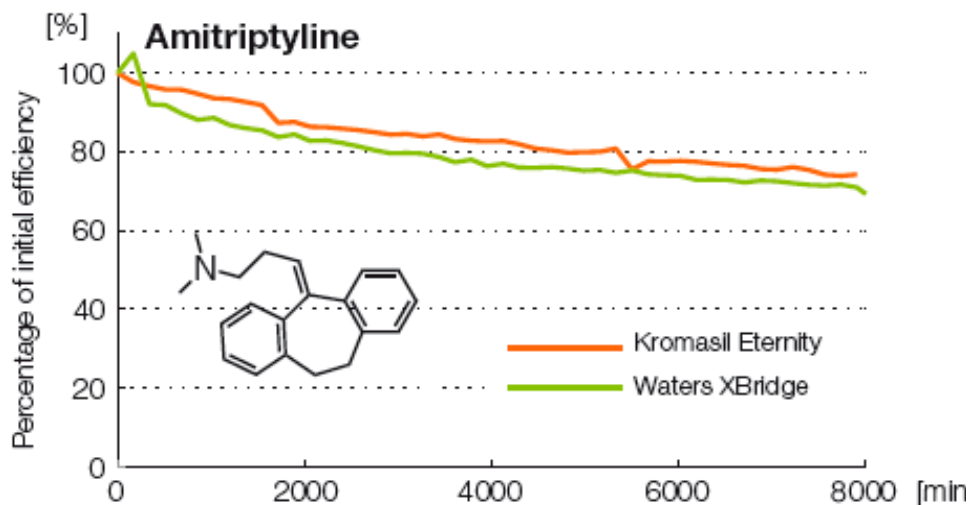
Carbon Load:	14 %
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Endcapping:	Proprietary
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pH Range:	1 - 12
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USP:	L1
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Long term pH stability – a comparison



Test conditions

Columns: Kromasil Eternity-5-C18 4.6 × 250 mm
Waters XBridge, 5 μm, C18, 4.6 × 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

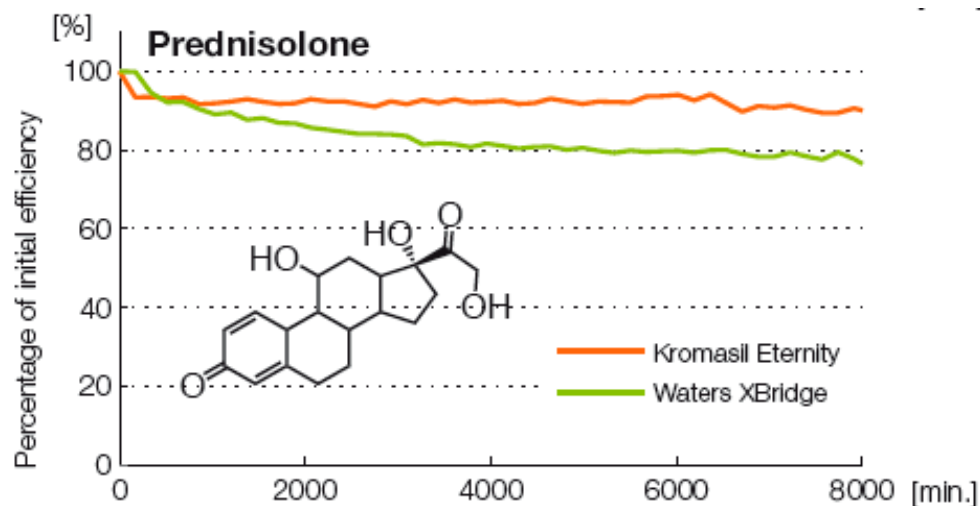
Temperature: 45°C

Gradient: 0 min 100% A
10 min 100% B
15 min 100% B
16 min 100% A
20 min 100% A

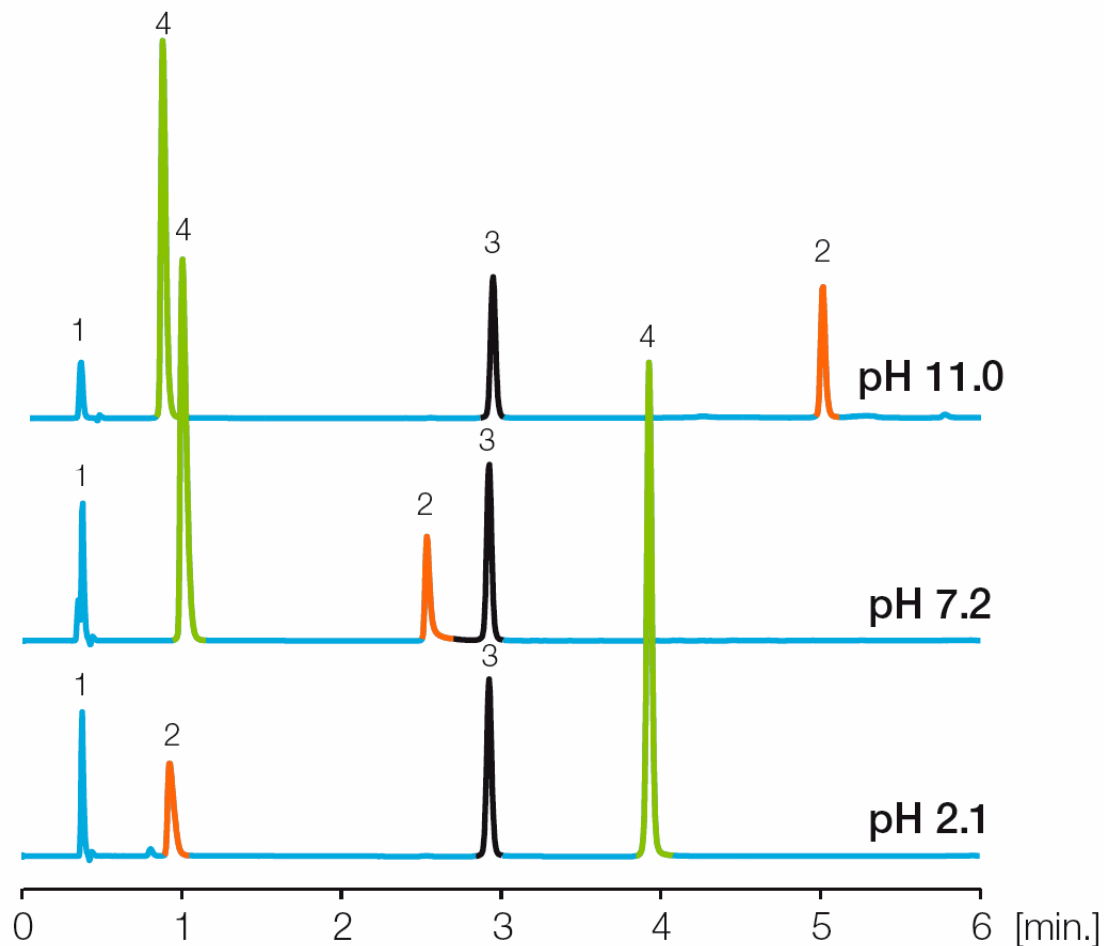
Test amitriptyline: 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (30/70)

Test prednisolone: 10 mM ammonium bicarbonate, pH 10.5/acetonitrile (70/30)

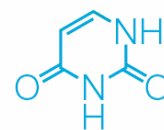
Test cycle: 6 × gradient + tests = 172 min/cycle



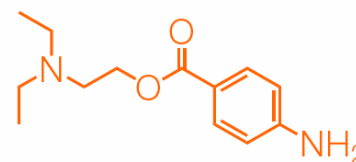
pH variation to control selectivity



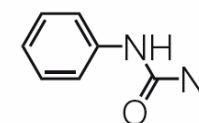
1 = uracil



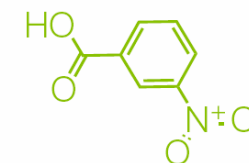
2 = procaine



3 = fenuron



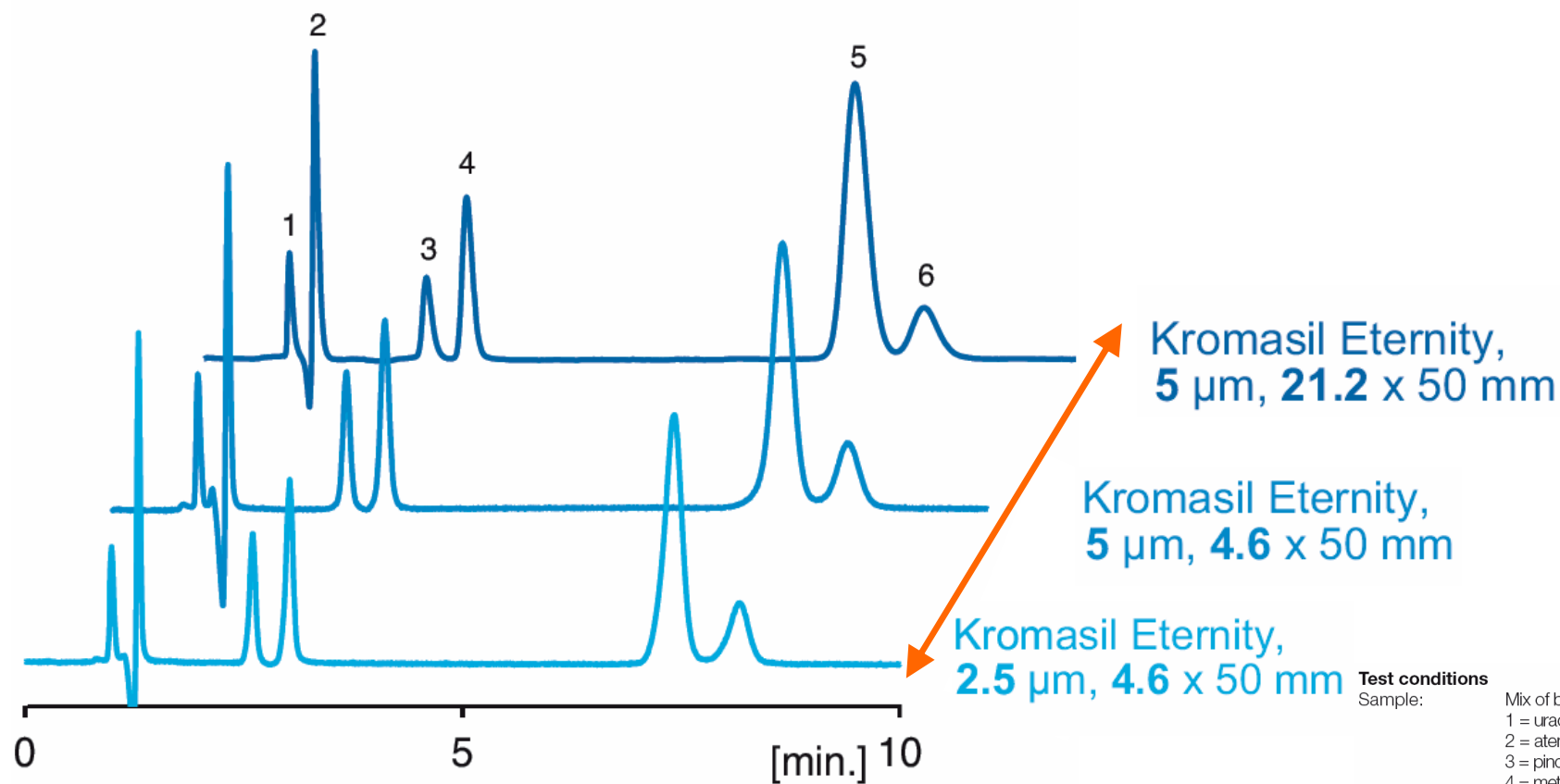
4 = 3-nitrobenzoic acid



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



Test conditions

Sample: Mix of beta-blockers,
1 = uracil
2 = atenolol
3 = pindolol
4 = metoprolol
5 = propranolol,
6 = 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

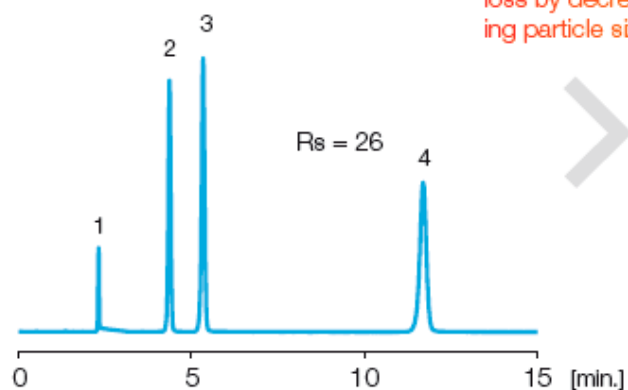
Mobile phase: acetonitrile/water/formic acid (25/75/0.1)

Substances: Mix of sulfa-drugs

- 1 = uracil
- 2 = sulphathiazole
- 3 = sulphamerazin
- 4 = sulphamethoxazole

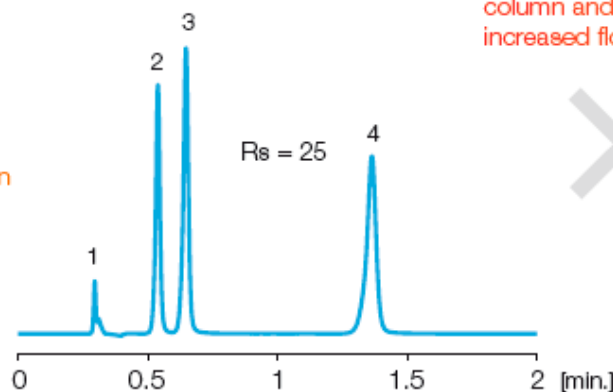
Temperature: 25°C

Detection: UV 254 nm



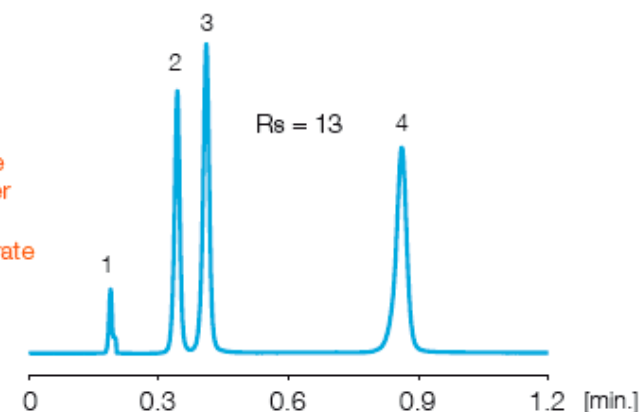
Stationary phase: Kromasil Eternity-5-C18
Column size: 4.6 x 250 mm
Flow rate: 1.0 ml/min

Save time
without resolution
loss by decreasing
particle size



Stationary phase: Kromasil Eternity-2.5-C18
Column size: 4.6 x 100 mm
Flow rate: 1.6 ml/min

Save even more
time with shorter
column and
increased flow rate



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

Run at 1/14 of original analysis time

Kromasil Eternity – Analytical scale

2.5 μm particle size

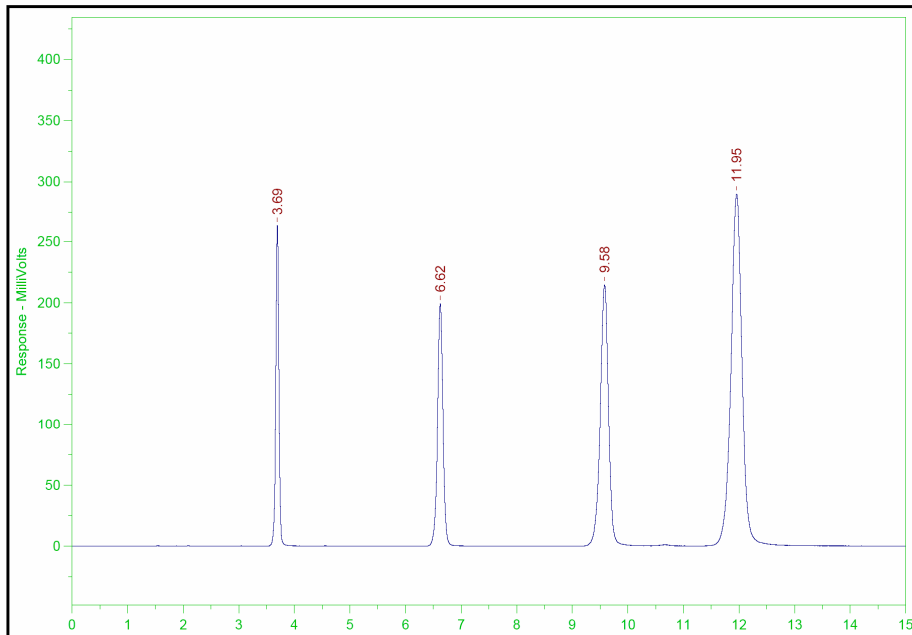
UHPLC and HPLC ($> 200\,000$ pl/m)

Easy to scale up to 5 μm

Test chromatogram

Eternity 5 μm 4.6 x 250 mm

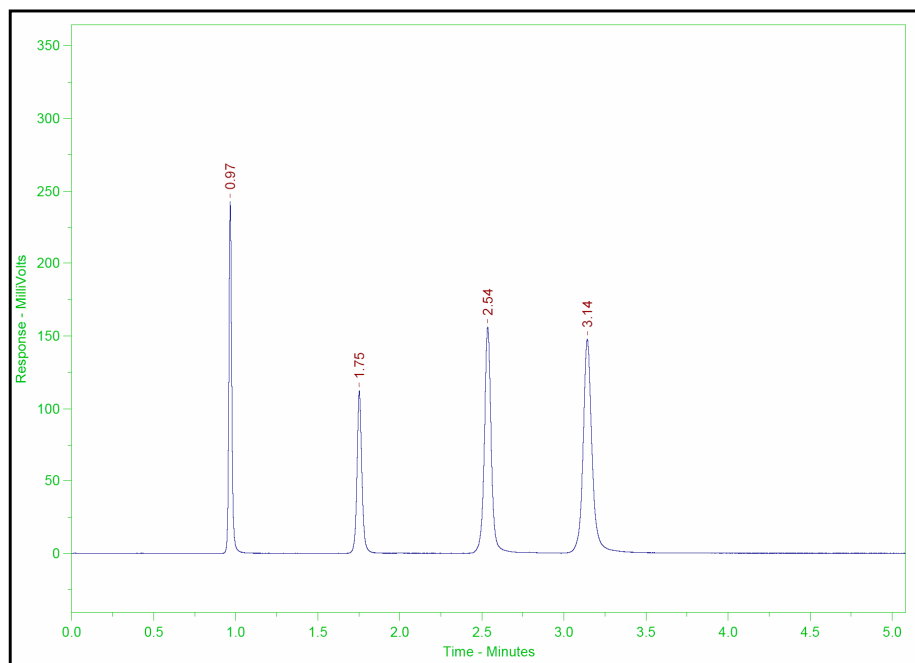
Packing:	Kromasil Eternity-5-C18	Serial Number:	82281
Length:	250 mm	Batch Number:	000007355
i.d.:	4.6 mm		
Mobile Phase:	Acetonitrile/Water (70/30)	Flow Rate:	1 ml/min
Storage Solvent:	Mobile Phase	Wavelength:	254 nm
Pressure:	74 bar		



Dimethyl phthalate	3.69	23200	0.83	0.95
Toluene	6.62	23900	0.83	1.00
Biphenyl	9.58	21600	0.83	0.89
Phenanthrene	11.95	19200	0.82	0.96

Test Chromatogram

Eternity 2.5 μm 4.6 x 100 mm



Packing:	Kromasil Eternity-2.5-C18	Serial Number:	K0160
Length:	100 mm	Batch Number:	PDG435
i.d.:	4.6 mm		
Mobile Phase:	Acetonitrile/Water (70/30)	Flow Rate:	1.5 ml/min
Storage Solvent:	Mobile Phase	Wavelength:	254 nm
Pressure:	243 bar		

Dimethyl phthalate	0.97	17100	0.82	1.35
Toluene	1.75	19100	0.79	1.24
Biphenyl	2.54	20700	0.79	1.04
Phenanthrene	3.14	19200	0.73	1.19

Kromasil Eternity – Semi. prep. scale

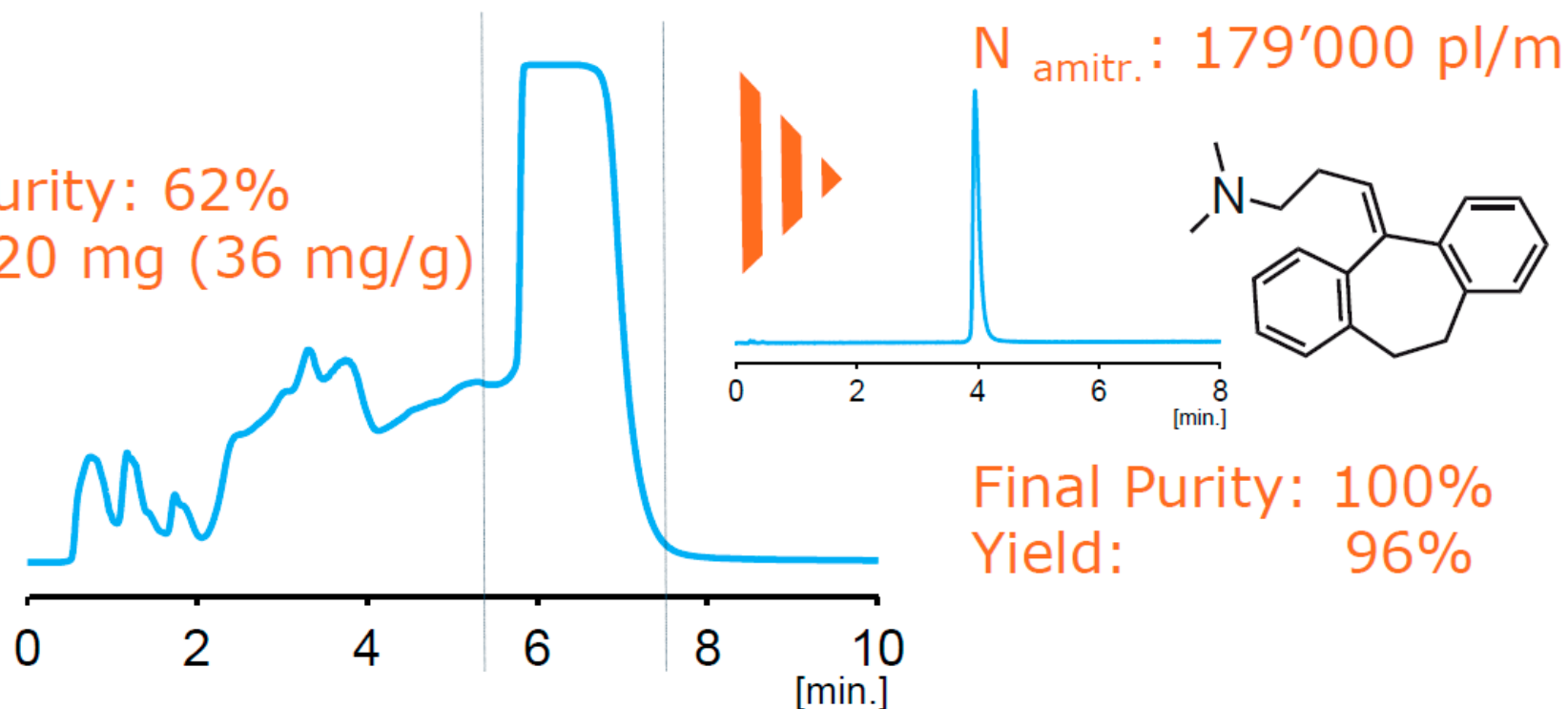
5 μm particle size

HPLC

Easy to scale down to 2.5 μm

Purification of crude amitriptyline

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



- ▶ Column: Kromasil Eternity 5 μm C18 (21.2 x 50 mm),
Mobile phase: 25 mM NH_4HCO_3 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 NH_4HCO_3 pH 10.5/MeCN (45/55)
Flow rate: 1.7 mL/min, 25°C, 254 nm

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!

