

Kromasil Eternity™

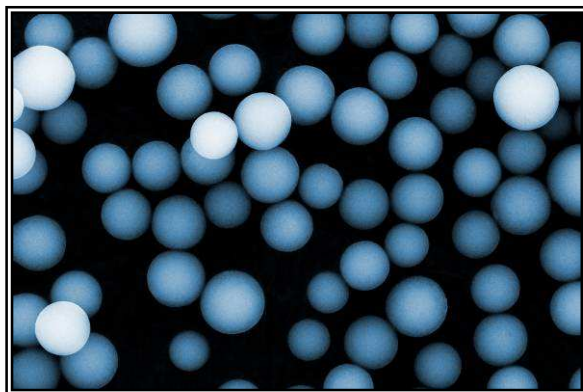
Designed for long life



Outline of the presentation

- ▶ Introduction of the Kromasil Eternity
 - /// Modifying the silica
 - /// Functionalizing the silica: C18 and PhenylHexyl
 - ▶ Product characteristics/assortment
 - ▶ Applications/Comparisons
 - /// Analytical HPLC
 - /// Preparative HPLC
 - ▶ Kromasil Eternity – The benefits
 - ▶ Conclusions
-
- ▶ Eternity Prep – Status information

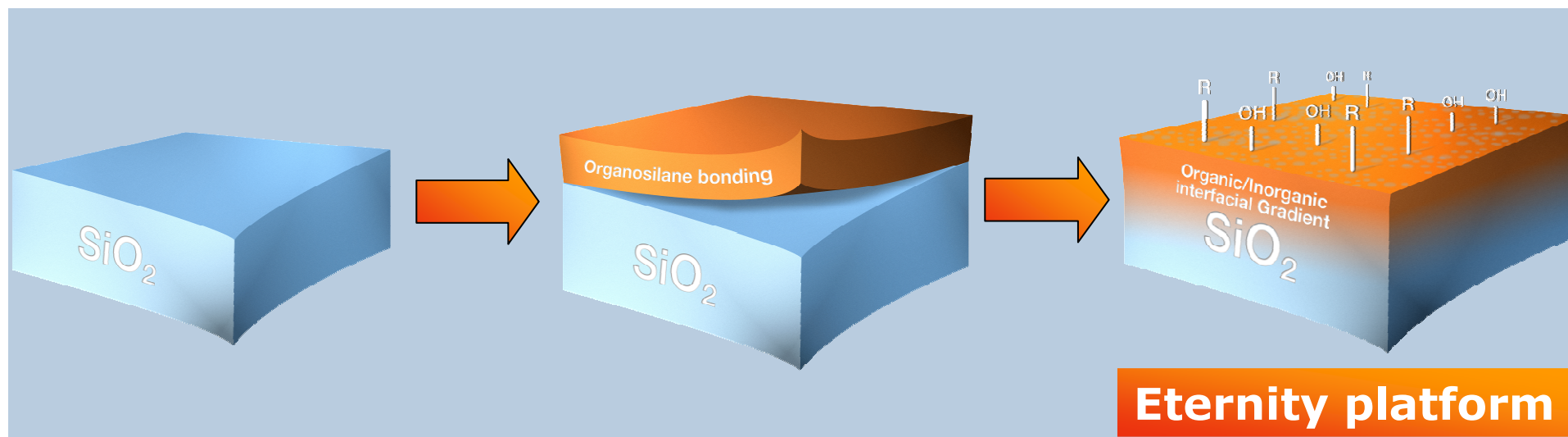
Kromasil Eternity platform



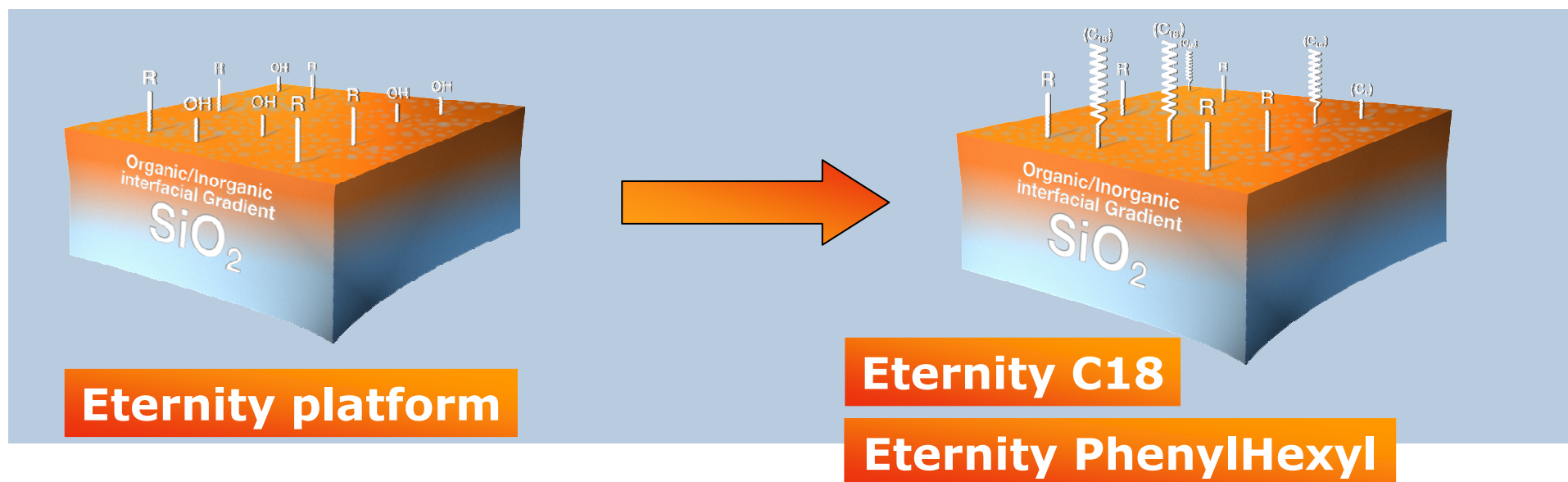
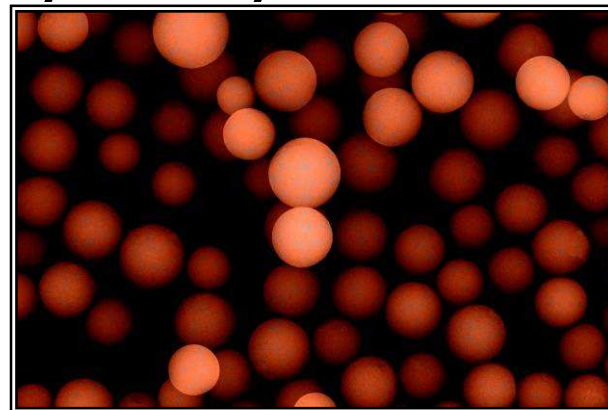
Particle sizes: 2.5 μm & 5 μm

Pore Size: 100 Å

Surface Area (porous): 330 m^2/g



Kromasil Eternity C18 & PhenylHexyl



Product characteristics – Eternity C18:

Ligand:	C18
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Particle sizes:	2.5 µm & 5 µm
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Pore Size:	100 Å
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Surface Area:	330 m²/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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Product characteristics – Eternity PhenylHexyl

Ligand:	PhenylHexyl
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Particle sizes:	2.5 µm & 5 µm
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Pore Size:	100 Å
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Surface Area:	330 m²/g
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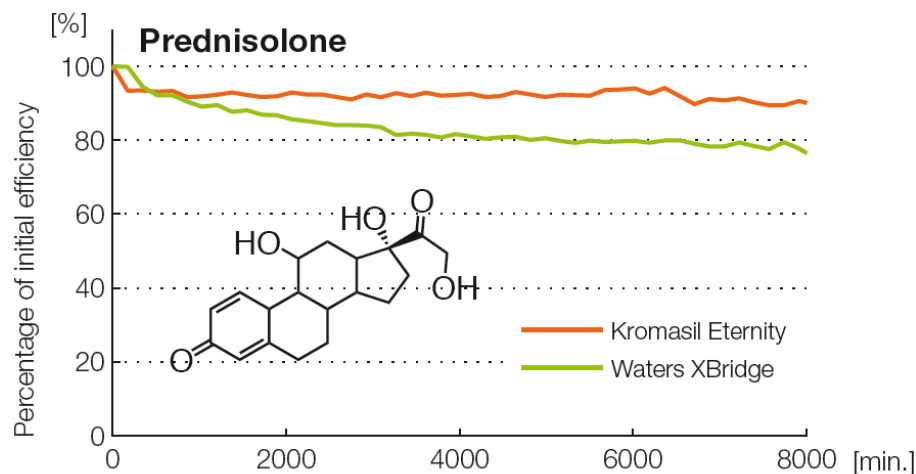
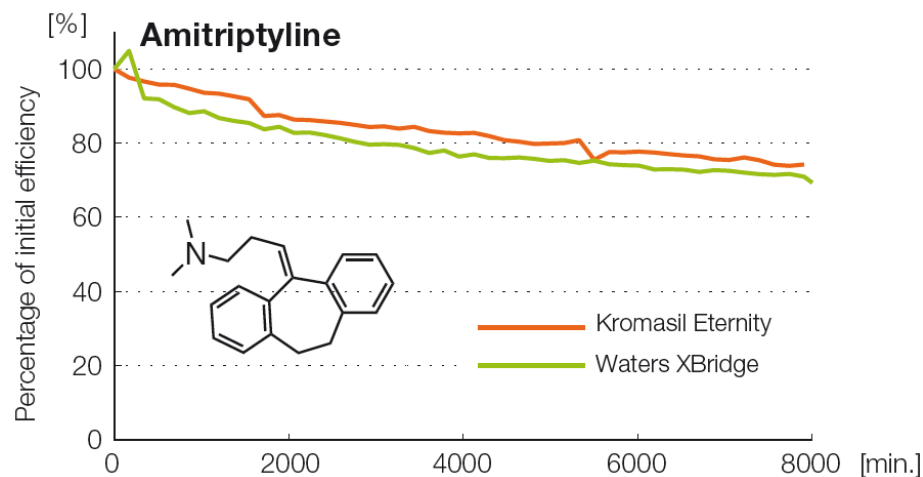
Carbon Load:	12 %
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Endcapping:	Proprietary
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pH Range:	2 - 12
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USP:	L11
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Long term high pH stability – a comparison



Test conditions

Columns: Kromasil Eternity-5-C18 4.6 × 250 mm
Waters X-Bridge, 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

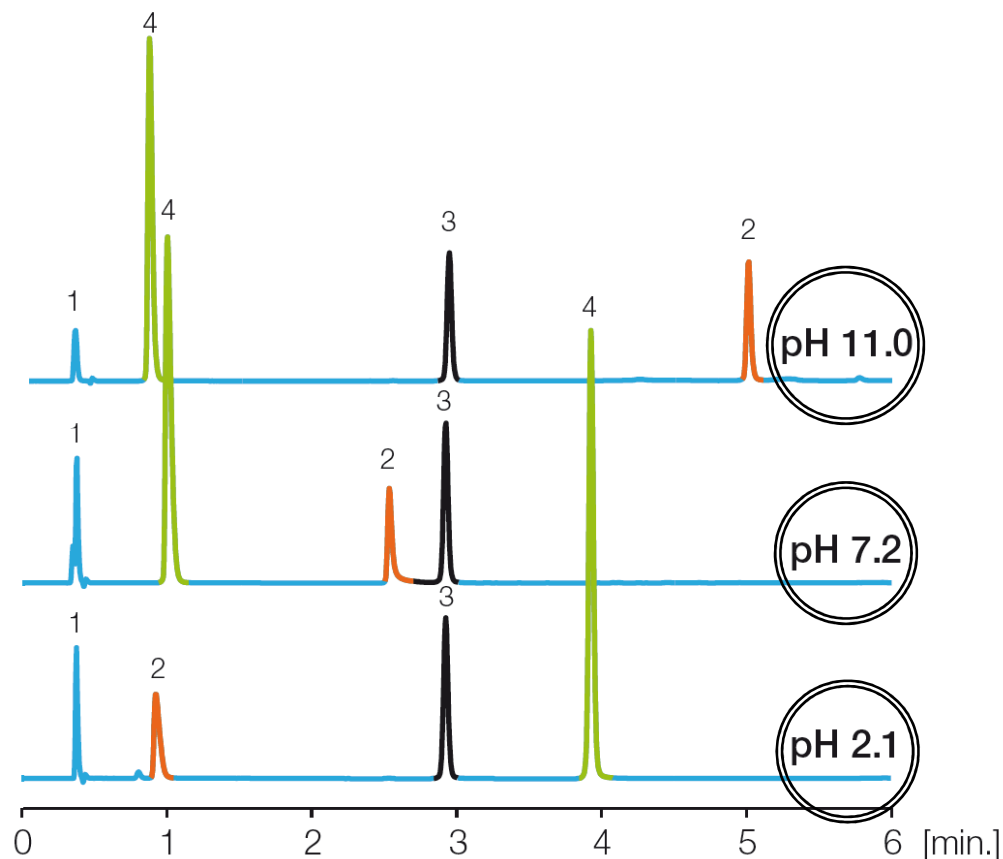
Gradient: 0 min 100% A
10 min 100% B
15 min 100% B
16 min 100% A
20 min 100% A

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

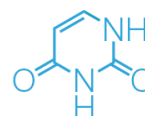
Test Prednisolone: 10 mM ammonium bicarbonate pH 10.5/acetonitrile (70/30)

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

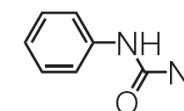
pH variation to control selectivity



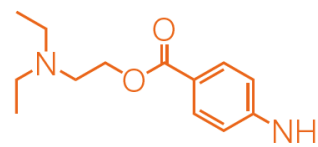
1 = uracil



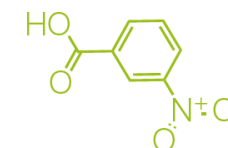
3 = fenuron



2 = procaine



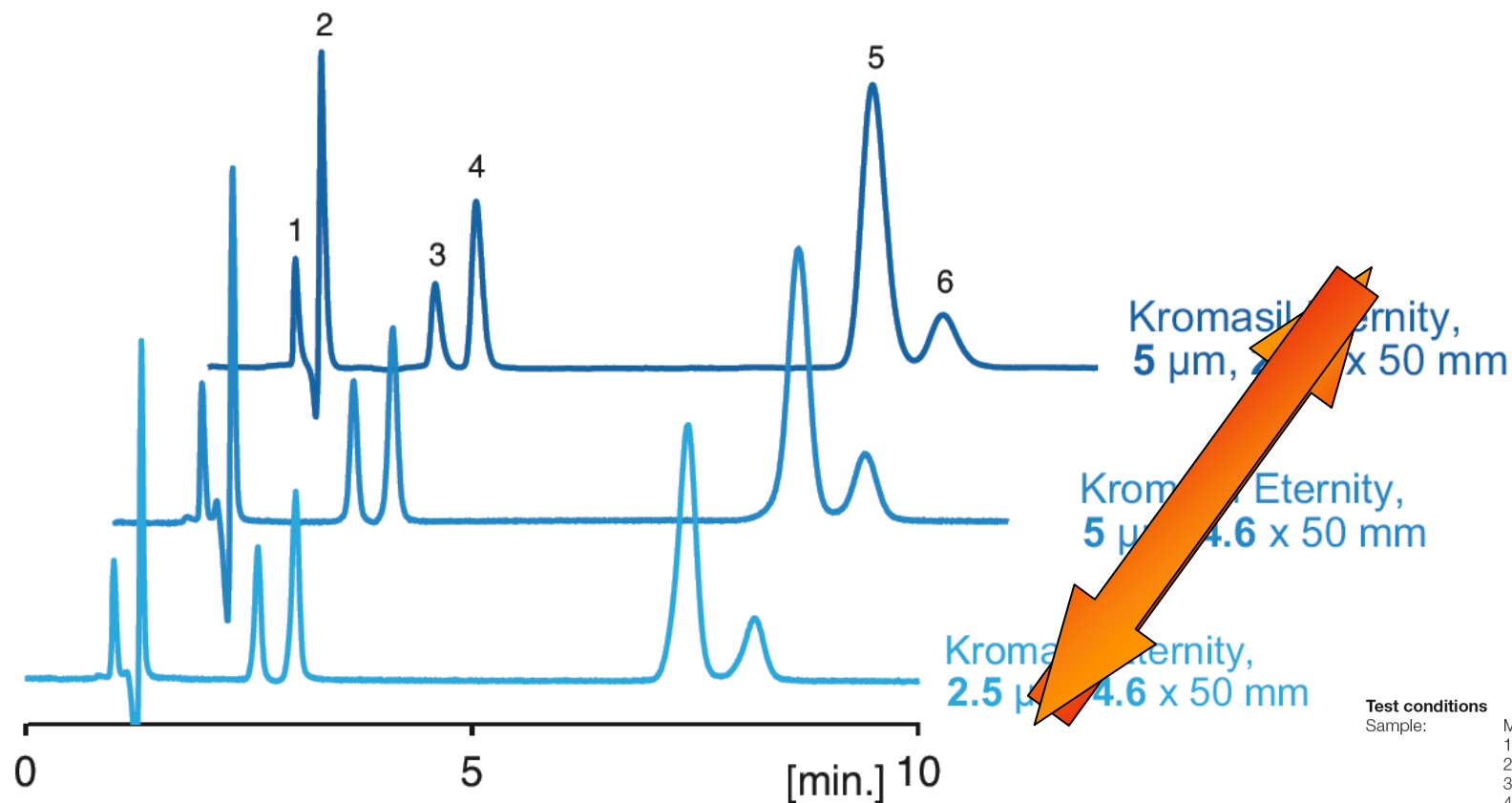
4 = 3-nitrobenzoic acid



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

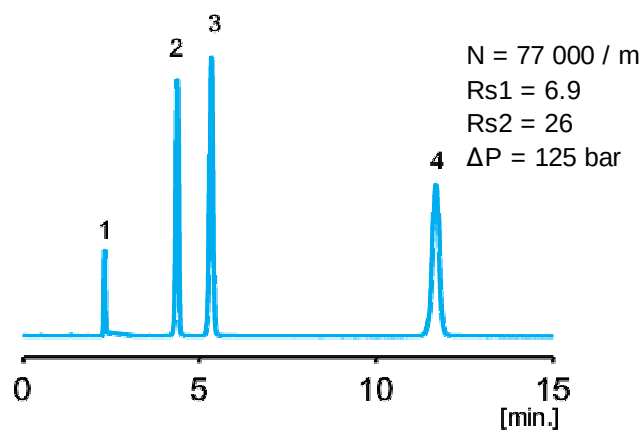
Detection: UV 230 nm

Stationary Phase: Kromasil Eternity (2.5 and 5 µm)

Column length: 50 mm

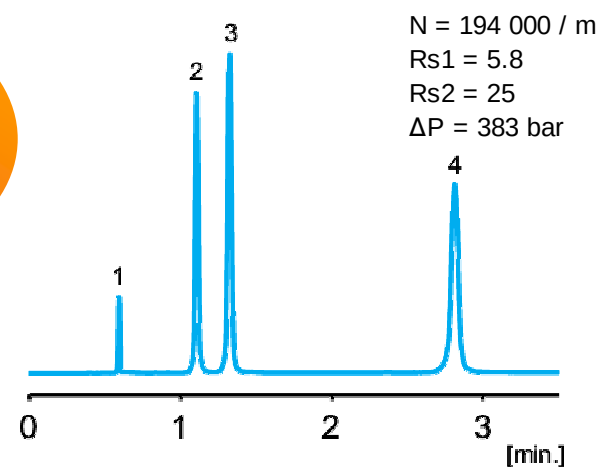
Increase efficiency – Save time (1/3)

START



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

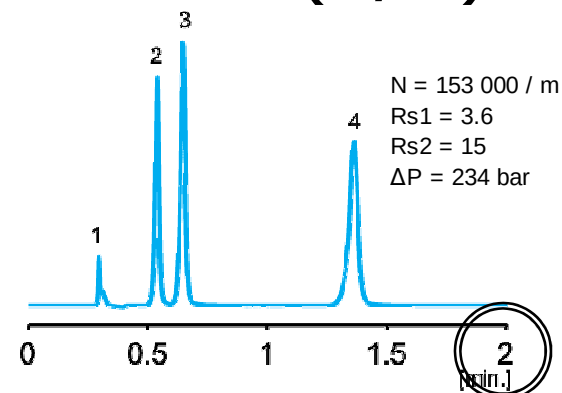


Stationary phase: Kromasil Eternity-2.5-C18
Column size: 4.6 x 100 mm
Flow rate: 1.6 ml/min

Increase efficiency – Save time (2/3)

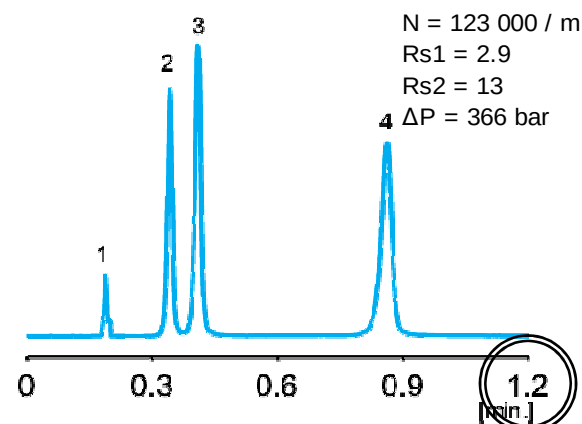
Save even more time
with shorter column
(1) and increased
flow rate (2)

1)



Stationary phase: Kromasil Eternity-2.5-C18
Column size: 4.6 x 50 mm
Flow rate: 1.7 ml/min

2)

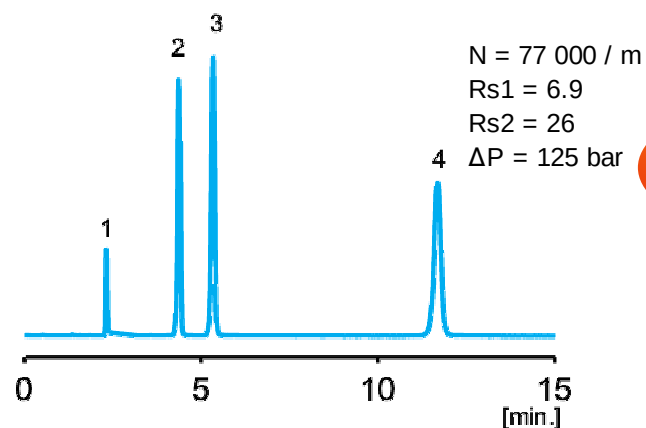


Stationary phase: Kromasil Eternity-2.5-C18
Column size: 4.6 x 50 mm
Flow rate: 2.7 ml/min

END

Increase efficiency – Save time (3/3)

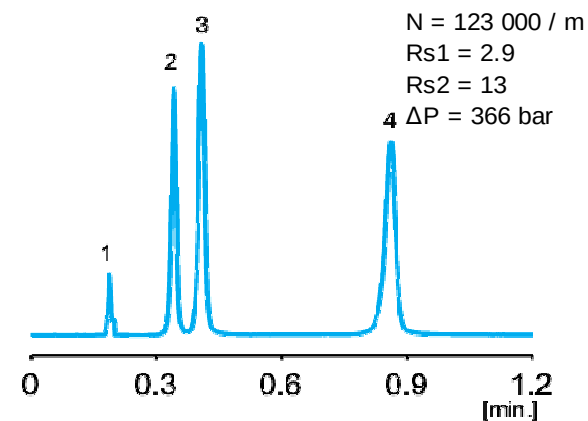
START



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

Run at 1/14 of
original analysis
time

END



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

Kromasil Eternity-C18 and Kromasil "classic"

▶ Benzoic acid derivatives

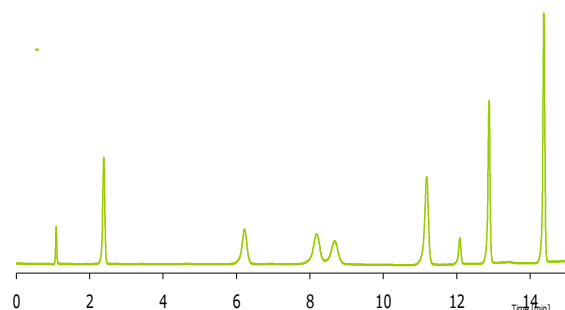
Conditions:

Mobile phase: ACN/H₂O, KPO₄ pH 2.5 (25/75)

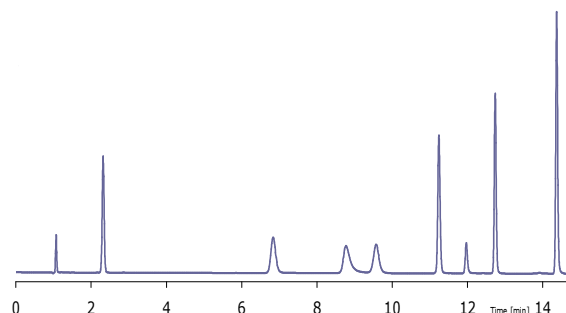
Gradient: 0 min (25/75), 7 min (25/75), 14 min (75/25)

Flow rate: 1.3 ml/min, Column size: 4.6 x 150 mm

Temperature: 20 °C, Detection: UV 254 nm



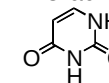
Kromasil Eternity-5-C18



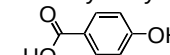
Kromasil100-5-C18 Classic

Substances

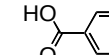
1-Uracil



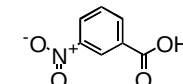
2- 4-hydroxybenzoic acid



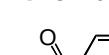
3- Benzoic acid



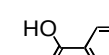
4- 3-nitrobenzoic acid



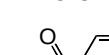
5- 3-Fluoro-benzoic acid



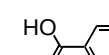
6- m-Toluic acid



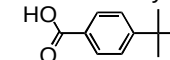
7- 3-Chloro-benzoic acid



8- 3,5-dimethylbenzoic acid

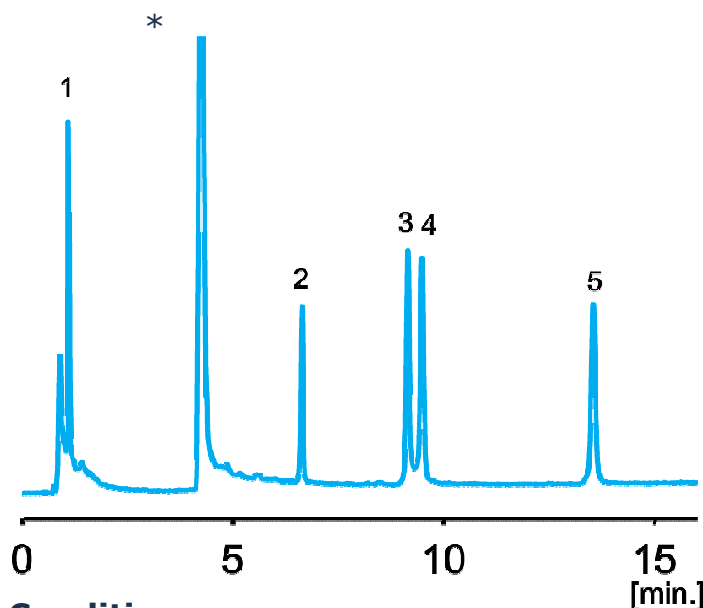


9- 4-tert-butylbenzoic acid



Applications at high pH

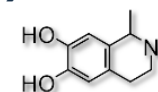
Basic substances at high pH



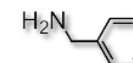
Conditions:

Stationary Phase: Kromasil Eternity-2.5-C18
 Column size: 4.6 x 100 mm
 Mobile Phase: methanol/water, 10 mM potassium phosphate buffer, pH 12.0
 Gradient (MeOH): 0 - 2 min 5 %; 2.1 min 60%; 15 min 80%
 Flow rate: 1.0 ml/min
 Temperature: 35 °C
 Detection: UV 254 nm

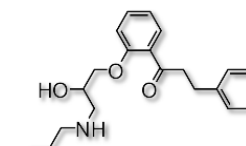
1) Salsolinol



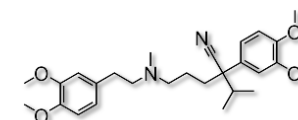
2) Benzylamine



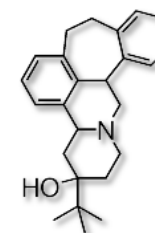
3) Propafenone



4) Verapamil



5) Butaclamol



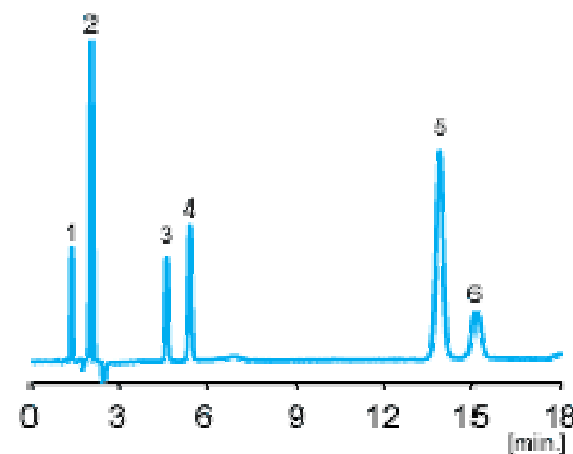
* Solvent front due to step gradient

Applications at high pH

Separation of some common β -adrenergic receptor antagonists

Test conditions:

Stationary Phase: Kromasil Eternity-5-C18
Column size: 4.6 x 250 mm
Mobile Phase: acetonitrile/water, 50 mM trimethyl-
amine acetate, pH 11 (40/60)
Flow rate: 1.3 ml/min
Temperature: 20°C
Detection: UV 230 nm



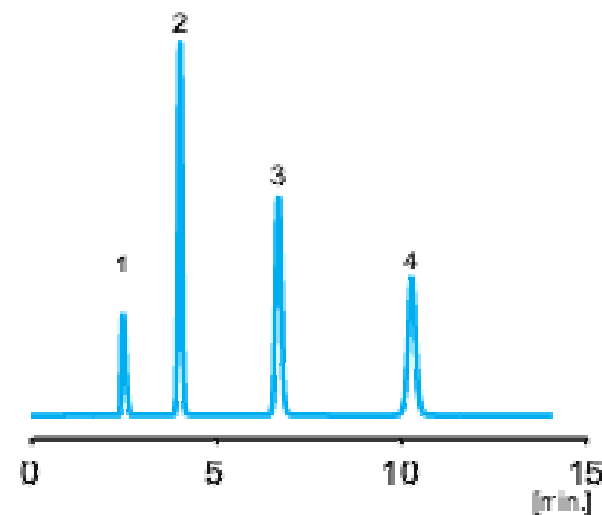
- 1 = Uracil
- 2 = Atenolol
- 3 = Pindolol
- 4 = Metoprolol
- 5 = Propanolol
- 6 = Alprenolol

Polar Application

Separation of caffeine and its metabolites

Test conditions:

Stationary Phase:	Kromasil Eternity-5-C18
Column size:	4.6 x 250 mm
Mobile Phase:	methanol/water (20/80)
Flow rate:	1.5 ml/min
Temperature:	30°C
Detection:	UV 273 nm



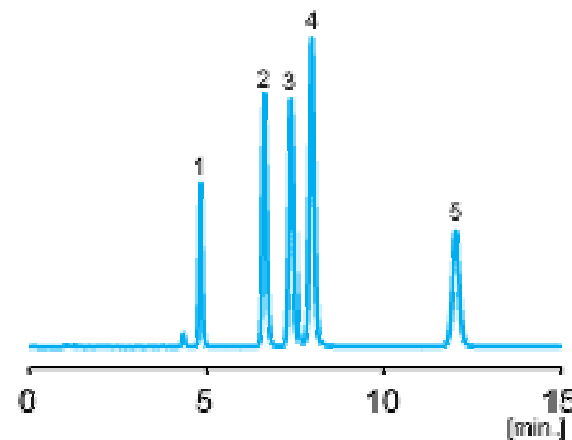
1 = Xanthine
2 = Theobromine
3 = Theophylline
4 = Caffeine

Hydrophobic Application

Separation of polycyclic aromatic hydrocarbons

Test conditions:

Stationary Phase: Kromasil Eternity-5-C18
Column size: 4.6 x 250 mm
Mobile Phase: acetonitrile/water (70/30)
Flow rate: 1.5 ml/min
Temperature: 25°C
Detection: UV 254 nm



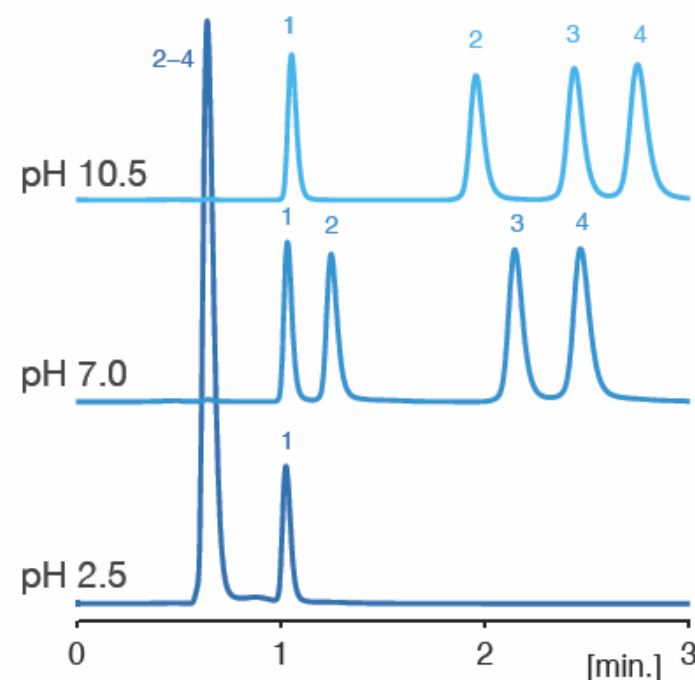
1 = naphthalene
2 = fluorene
3 = phenanthrene
4 = anthracene
5 = triphenylene



pH variation – the perfect tool for method development

Chromatographic conditions

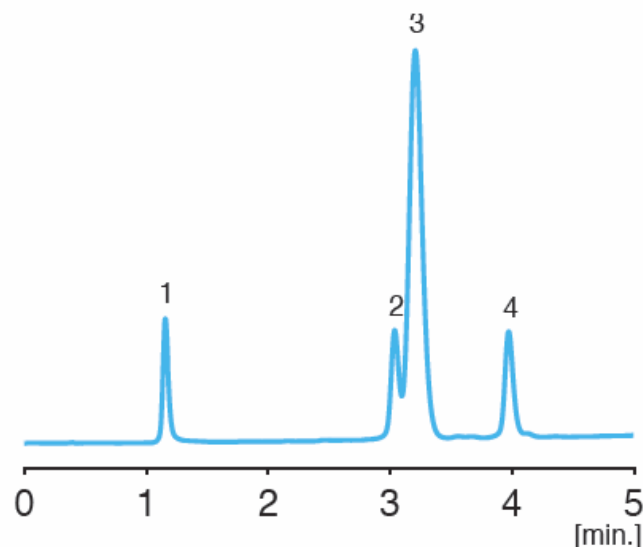
Column: Kromasil Eternity-2.5-Phenyl-Hexyl 2.1 x 50 mm
Sample: Antidep mix: 1 = toluene, 2 = nortriptyline, 3 = imipramine, 4 = amytryptiline
Mobile Phase: methanol/potassium phosphate buffer, 20 mM, pH 2.5, 7.0 and 10.5 respectively (80/20)
Flow rate: 0.35 ml/min
Temperature: 25°C
Detection: UV @ 215 nm



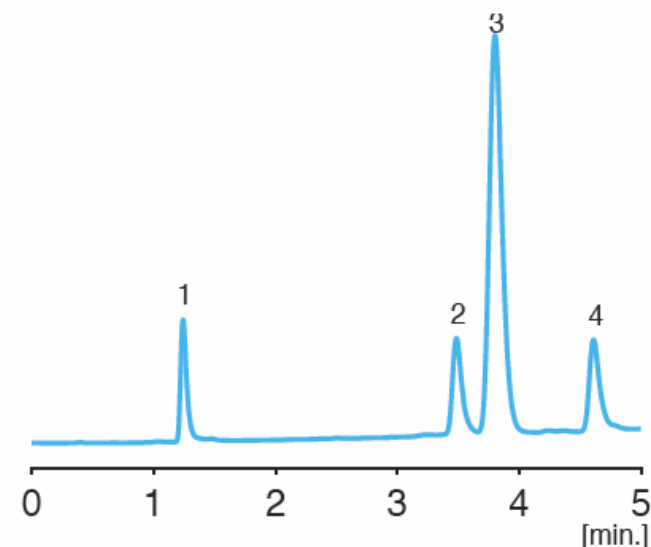


Alternate selectivity

Kromasil Eternity-2.5-C18



Kromasil Eternity-2.5-Phenyl-Hexyl



Common chromatographic conditions

Column size: 2.1 × 50 mm

Sample: Vitamines: 1 = p-aminobenzoic acid, 2 = biotin, 3 = vitamine B₁₂, 4 = vitamine B₁₂

Mobile Phase: methanol/potassium phosphate buffer, 20 mM, pH 2.5

gradient: methanol: 0 min 20%, 5 min 37%.

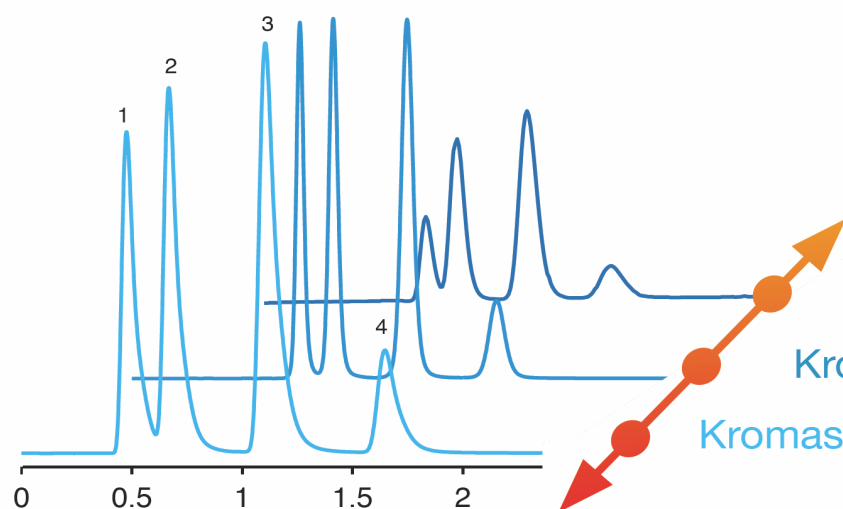
Flow rate: 0.35 ml/min

Temperature: 25°C

Detection: UV @ 210 nm



Scale-up or Scale-down



Kromasil Eternity Phenyl-Hexyl, **5 μ m**, **21.2 x 50 mm**

Kromasil Eternity Phenyl-Hexyl, **5 μ m**, **4.6 x 50 mm**

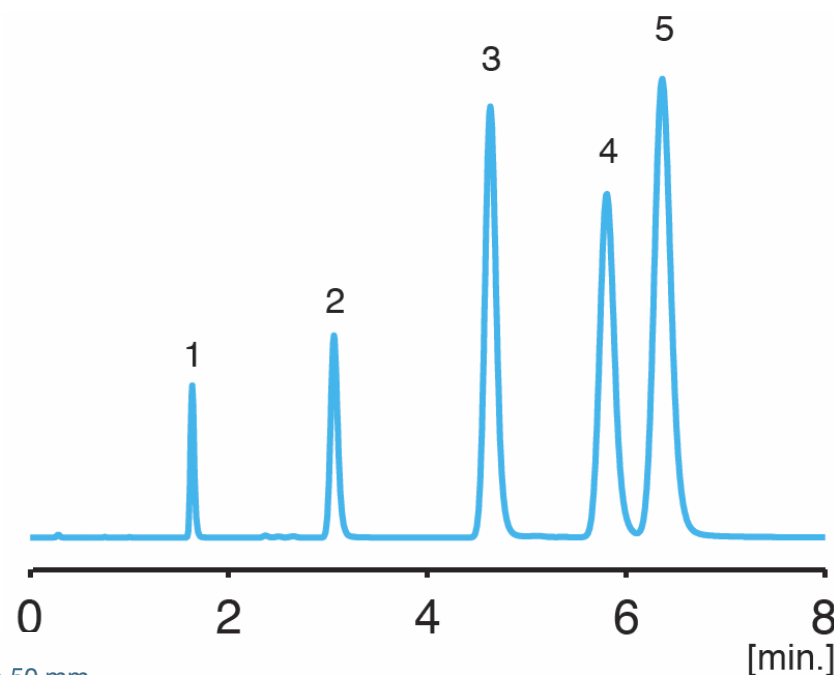
Kromasil Eternity Phenyl-Hexyl, **2.5 μ m**, **2.1 x 50 mm**

Common chromatographic conditions

Sample: 1 = 4-nitroaniline, 2 = 3-nitroaniline,
3 = 2-nitroaniline, 4 = 2,4-dinitroaniline
Mobile Phase: methanol/water (50/50)
Flow rate: **xx** ml/min, 1.7 ml/min and **xx** ml/min, respectively
Temperature: 20°C
Detection: UV @ 280 nm



Separations of aromatics

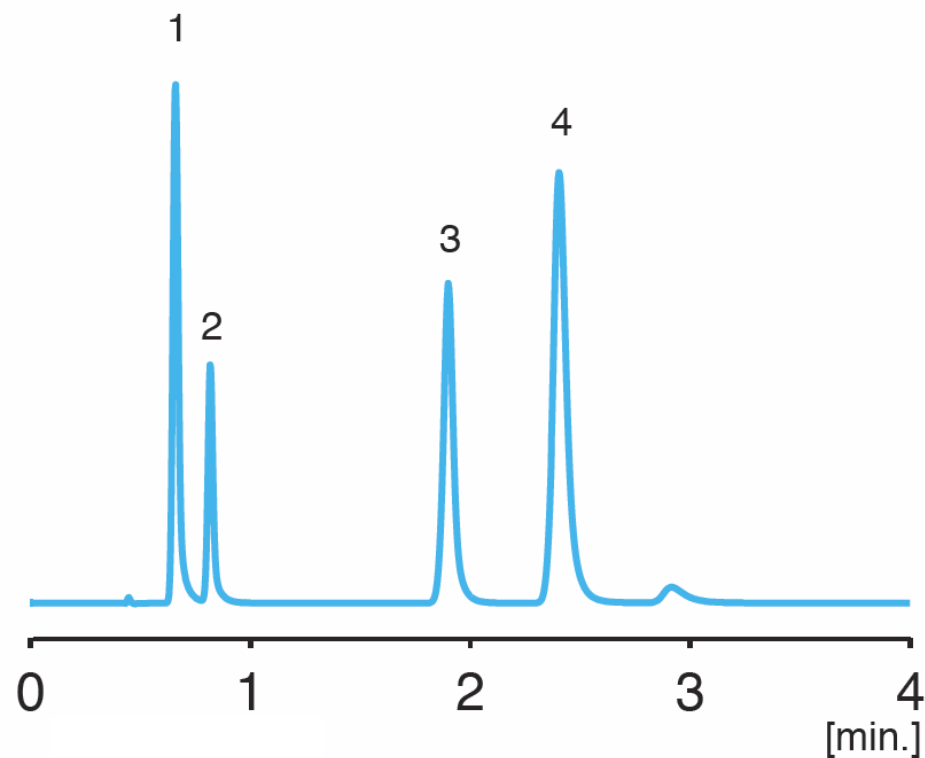


Chromatographic conditions

Column: Kromasil Eternity-2.5-Phenyl-Hexyl 4.6 × 50 mm
Sample: 1 = sodium nitrite, 2 = naphthalene, 3 = biphenyl,
4 = phenanthrene, 5 = anthracene
Mobile Phase: methanol/water (65/35)
Flow rate: 1.2 ml/min
Temperature: 30°C
Detection: UV @ 254 nm



Separation of nucleobases



Chromatographic conditions

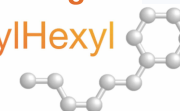
Column: Kromasil Eternity-2.5-Phenyl-Hexyl 4.6 × 50 mm
Sample: 1 = cytosine, 2 = uracil, 3 = thymine, 4 = adenine
Mobile Phase: potassium phosphate buffer, 25 mM, pH 7.85
Flow rate: 1.2 ml/min
Temperature: 30°C
Detection: UV @ 254 nm

Kromasil Eternity – Analytical scale

Kromasil® Eternity™ C18
Designed for long life

Eternity PhenylHexyl

For your HPLC and UHPLC



2.5 µm particle size

UHPLC and HPLC (> 200 000 pl/m)

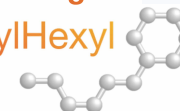
Easy to scale up to 5µm

Kromasil Eternity – Semi. prep. scale

Kromasil® Eternity™ C18
Designed for long life

Eternity PhenylHexyl

For your HPLC and UHPLC



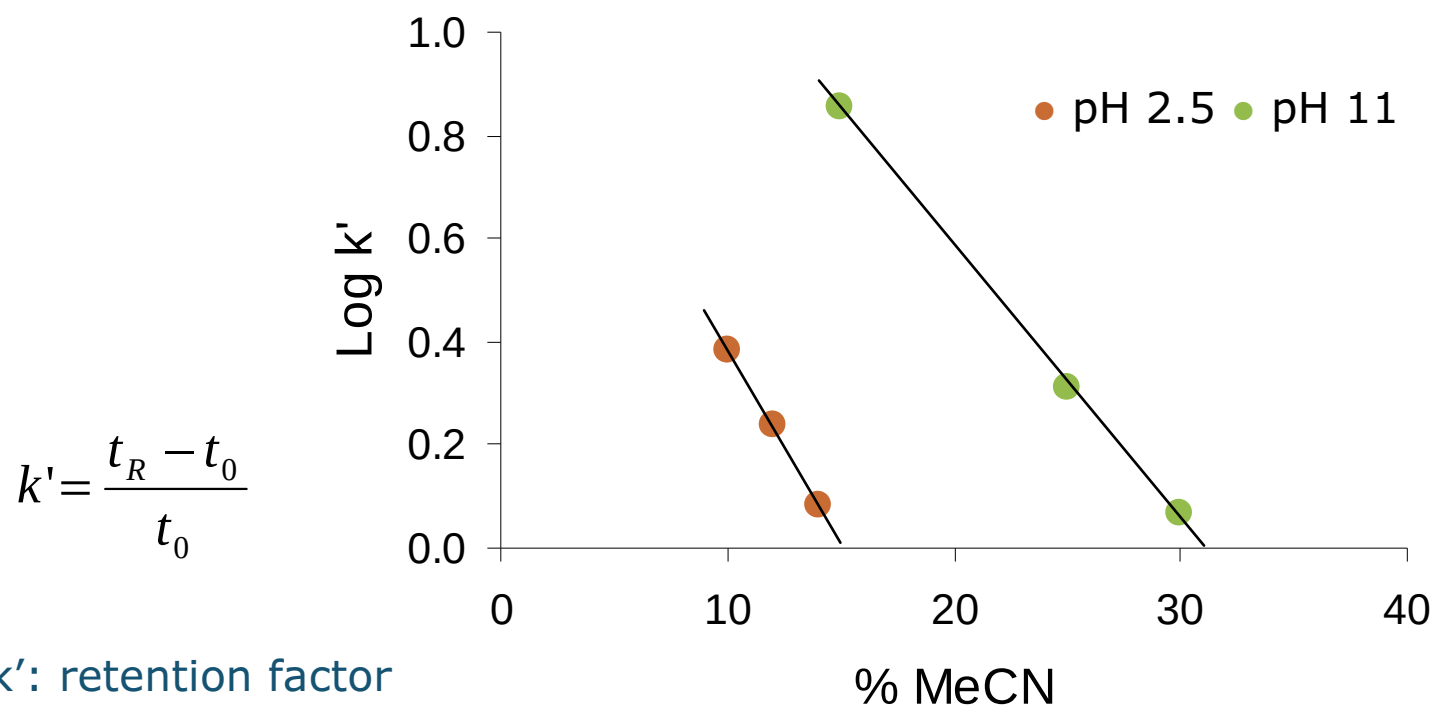
5 µm particle size

HPLC

Easy to scale down to 2.5µm

Prep application at low and high pH [1(3)]

Relationship of 1-phenylpiperazine retention with mobile phase strength and pH

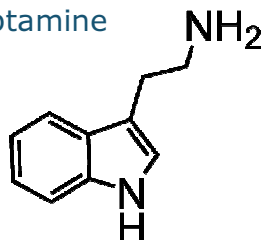


k' : retention factor
 t_R : retention time
 t_0 : retention time of non-retained compound

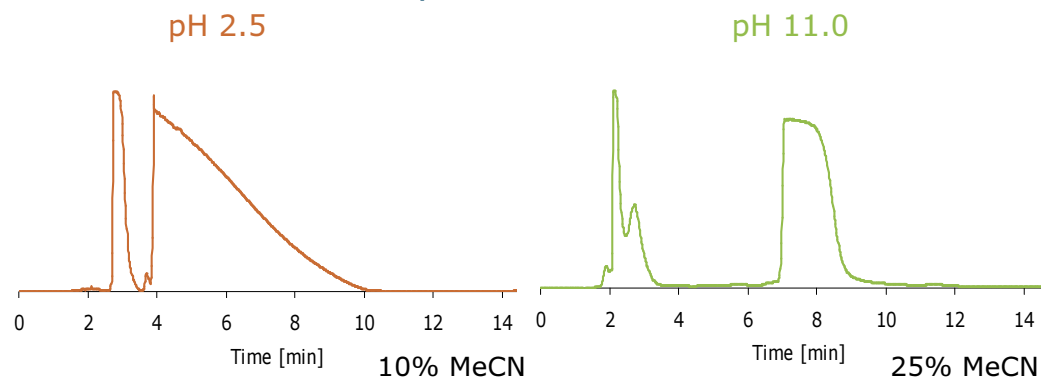
Prep application at low and high pH [2(3)]

Development of preparative separation methods for two basic crude compounds at acidic and basic pH

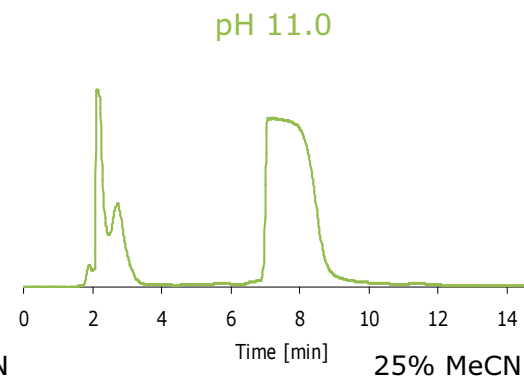
tryptamine



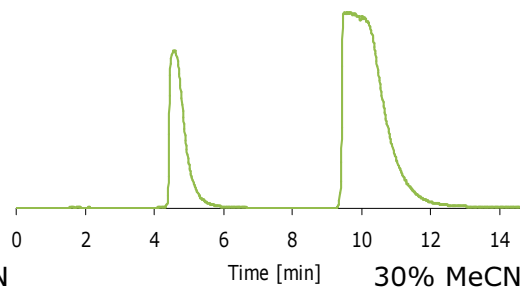
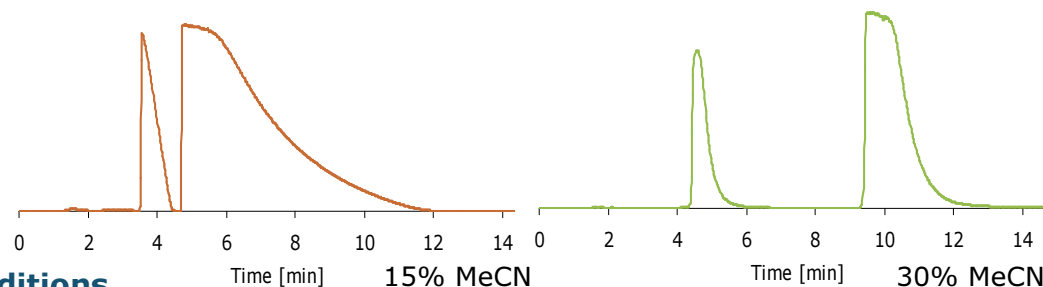
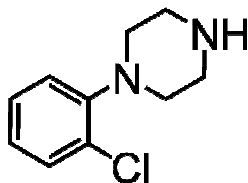
pH 2.5



pH 11.0



1-(2-chlorophenyl)
piperazine



Chromatographic conditions

Sample: crude tryptamine and 1-(2-chlorophenyl)piperazine

Injection: 2.5 mg (0.5 ml of 5 mg/ml sample solution)

Column: Kromasil Eternity 5-C18 4.6 x 150 mm

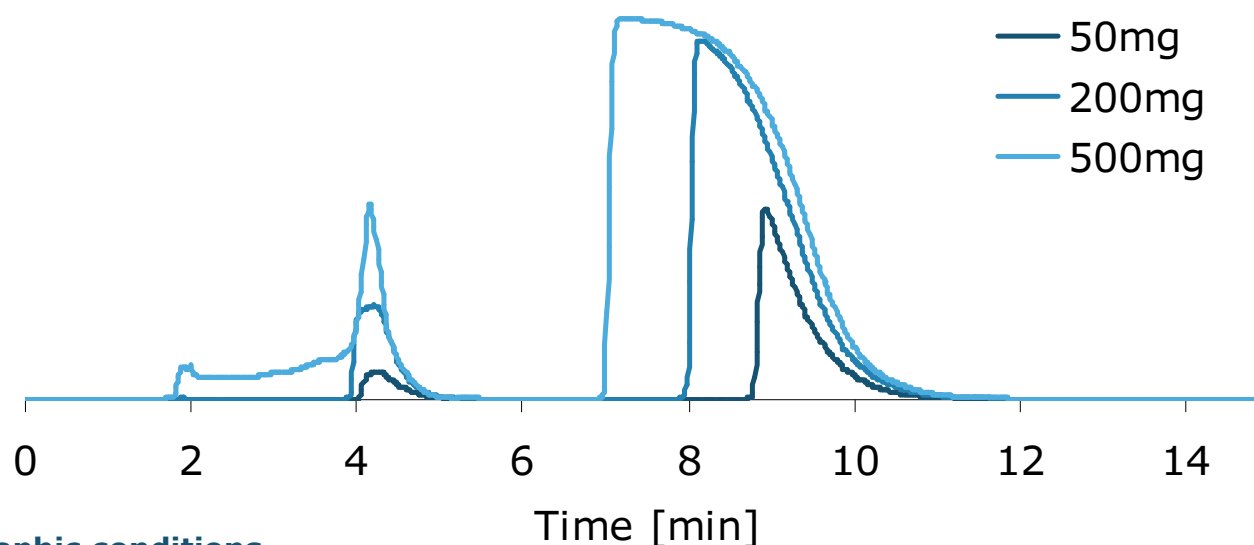
Mobile phase: Acetonitrile/0.1 M potassium phosphate pH 2.5 or pH 11.0

Flow rate: 1 ml/min

Detection: UV @ 300 nm and 280 nm

Prep application at low and high pH [3(3)]

Scale up of column dimension followed by 10 times
load increase without reaching touching bands



Chromatographic conditions

Sample: crude 1-(2-chlorophenyl)piperazine

Injection: 10 ml of 5, 20, and 50 mg/ml sample,
sample dissolved in acetonitrile/0.1 M potassium phosphate pH 13 30/70

Column: Kromasil Eternity 5-C18 21.2 x 150 mm

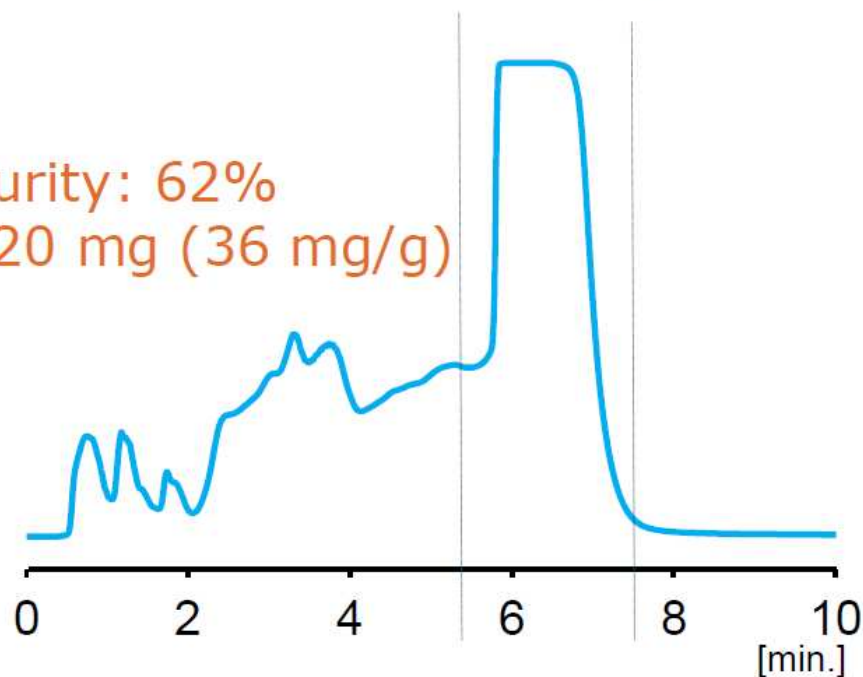
Mobile phase: Acetonitrile/0.1 M potassium phosphate pH 11 30/70 v/v

Flow rate: 21 ml/min

Detection: UV @ 280 nm

Purification of crude amitriptyline

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



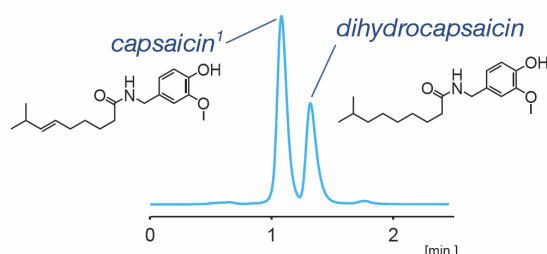
- ▶ Column: Kromasil Eternity 5 μ m C18 (21.2 x 50 mm),
Mobile phase: 25 mM NH_4HCO_3 pH 10.5/ACN,
Gradient: 45-80% ACN/10 min.
Flow: 21 mL/min, 25°C, 254 nm
- ▶ Purity assessment of fraction pool:
Column: Kromasil Eternity 2.5 μ m C18 (4.6 x 50 mm),
Mobile phase: 10 mM NH_4HCO_3 pH 10.5/MeCN (45/55)
Flow rate: 1.7 mL/min, 25°C, 254 nm



Preparative application

Separation of capsaicin from dihydrocapsaicin

Analytical injection



Common chromatographic conditions

Columns: *Analytical and fraction analysis:*
Kromasil Eternity-2.5-PhenylHexyl 2.1 × 50 mm

Prep injections:

Kromasil Eternity-5-PhenylHexyl 4.6 × 250 mm

Kromasil Eternity-5-PhenylHexyl 21.2 × 250 mm

Mobile Phase: methanol/water (70/30)

Flow rates: 0.4 ml/min, 1 ml/min and 21.2 ml/min, respectively

Temperature: 20°C

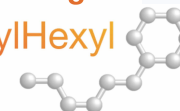
1: Capsaicin is the active component in chili peppers and has analgesic properties.

Kromasil Eternity – The Benefits

Kromasil® Eternity™ C18
Designed for long life

Eternity PhenylHexyl

For your HPLC and UHPLC



Modified silica > Long-lasting columns > Better economy

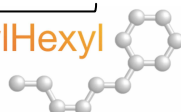
Small particles (2.5 µm) > High efficiency > Faster analyses

Modified silica > **pH 1 to 12**
pH 2 to 12 > Easier method development

Kromasil® Eternity™ C18
Designed for long life

Eternity PhenylHexyl

For your HPLC and UHPLC



ETERNITY IN PREP - STATUS

Present Development Work

- ▶ Kromasil Eternity Prep, 10 µm, C18
 - /// First Eternity Prep, more phases will follow
- ▶ Different possibilities for the final chemistry are tested:
 - /// Objectives are different in analytical vs. prep
- ▶ Optimization of properties important in prep HPLC:
 - /// Chemical stability
 - /// Mechanical stability
 - /// Loadability
- ▶ Current status shown in the following slides ...

Chemical Stability at High pH

Column: 4.6 x 50 mm
Mobile Phase A: 10 mM NH_4HCO_3 pH 10.5 / MeCN 95/5
Mobile Phase B: 10 mM NH_4HCO_3 pH 10.5 / MeCN 5/95
Flow: 1.7 mL/min
Temp: 60°C
Gradient: 0 min 100%A
10 min 100% B
10.2 min 100% A
15 min 100% A

Equilibration for test:

5 min at 1.7 mL/min with 10 mM NH_4HCO_3 pH 10.5 / MeCN 70/30

Test :

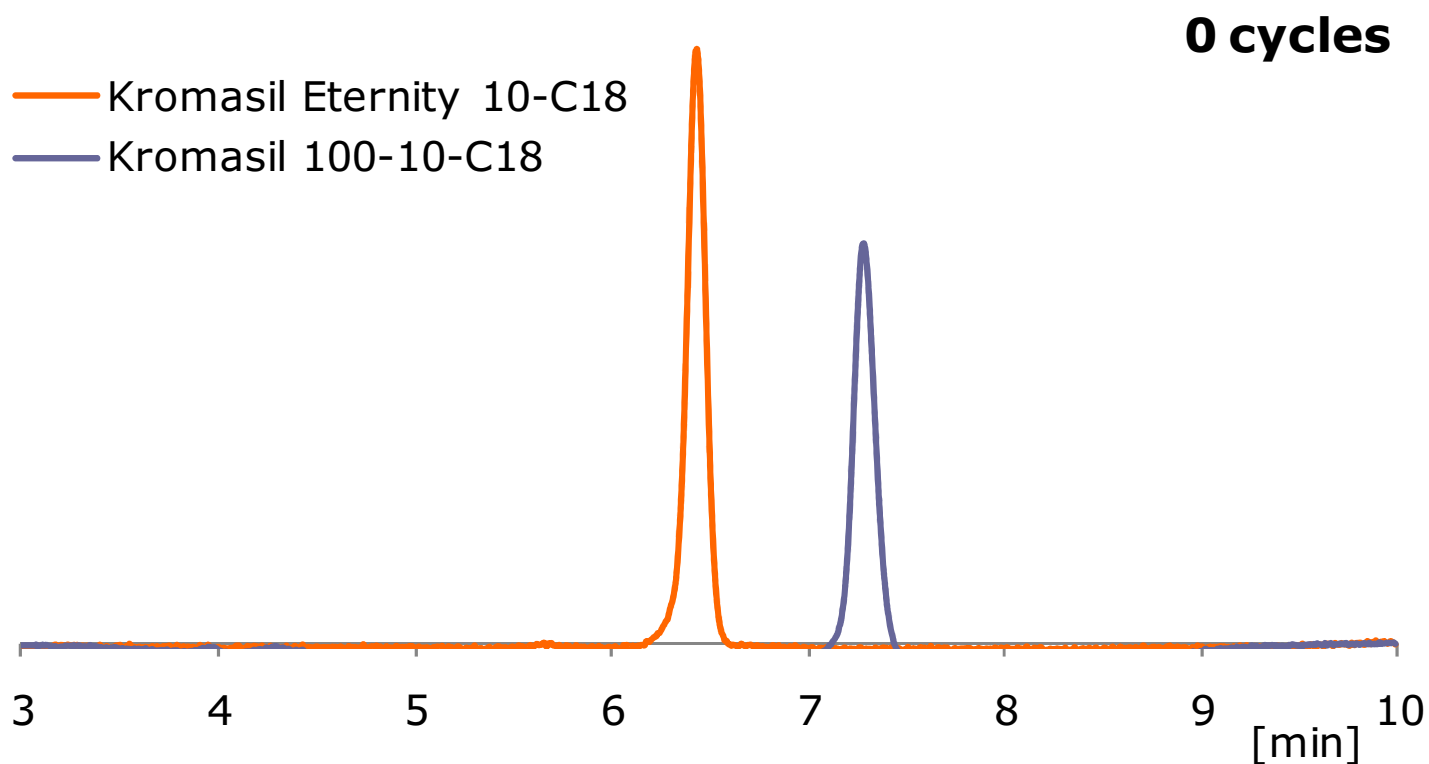
Amitriptyline at 60°C, 1.7 mL/min with 10 mM NH_4HCO_3 pH 10.5 / MeCN Gradient: 30-80% MeCN/10 min

Test Cycles:

20 x gradient + equilibration for test (5 min) + test (15 min)

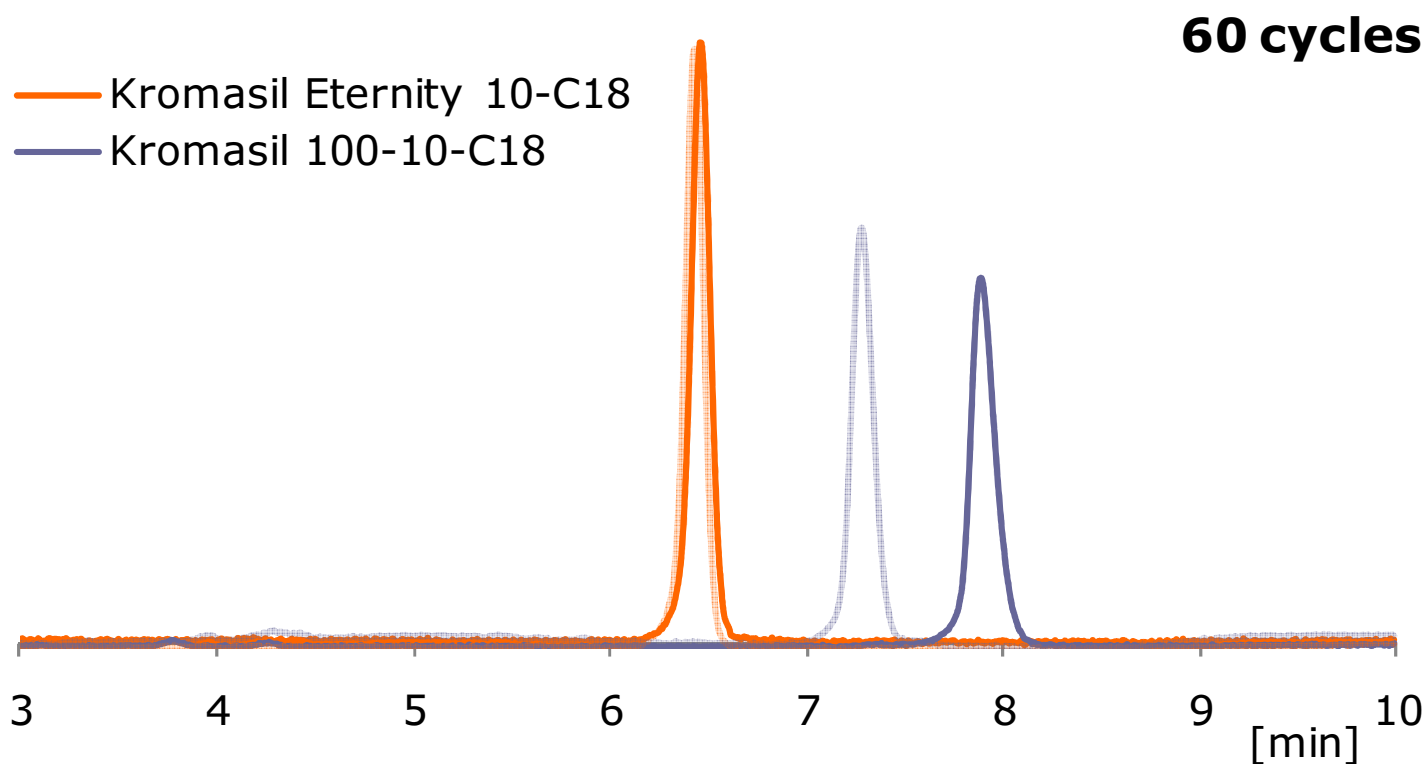
Chemical Stability at High pH

- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline



Chemical Stability at High pH

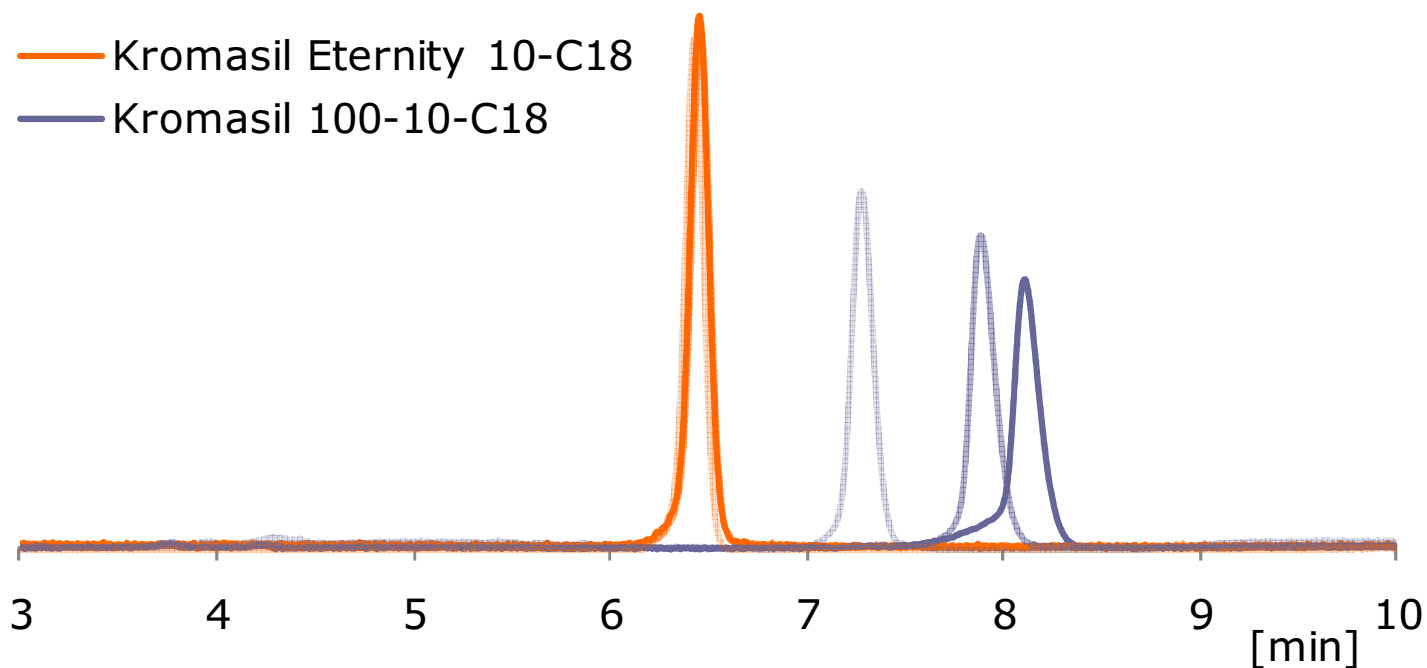
- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline



Chemical Stability at High pH

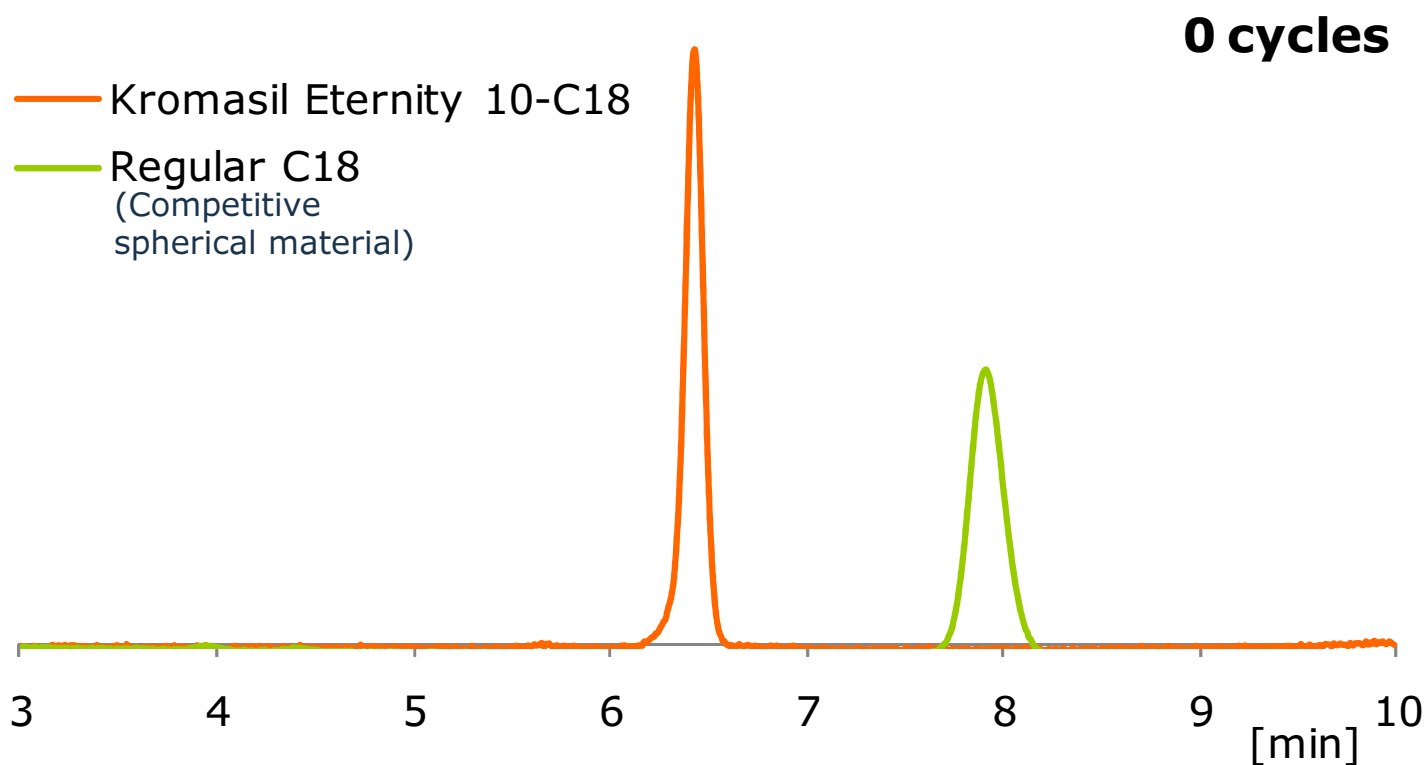
- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline

120 cycles



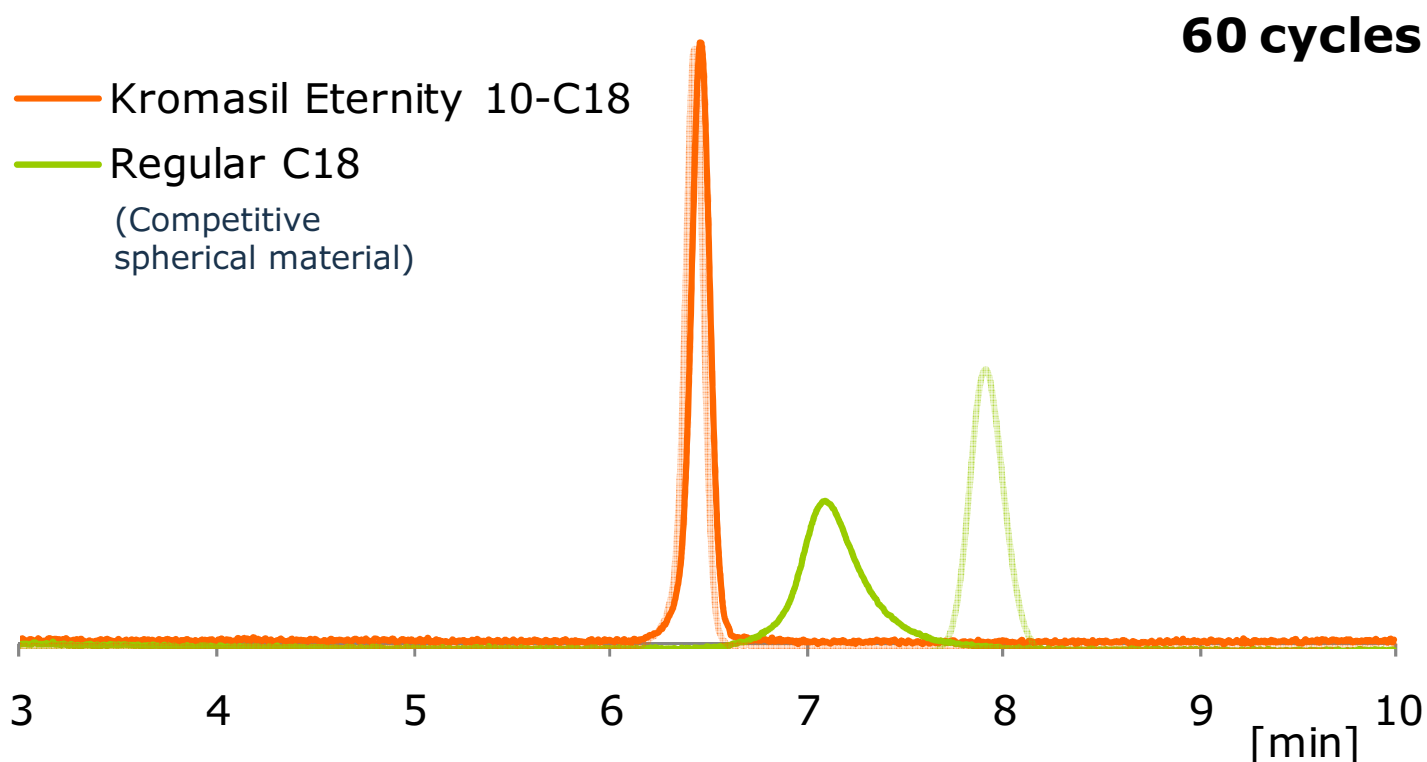
Chemical Stability at High pH - Comparison

- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline



Chemical Stability at High pH - Comparison

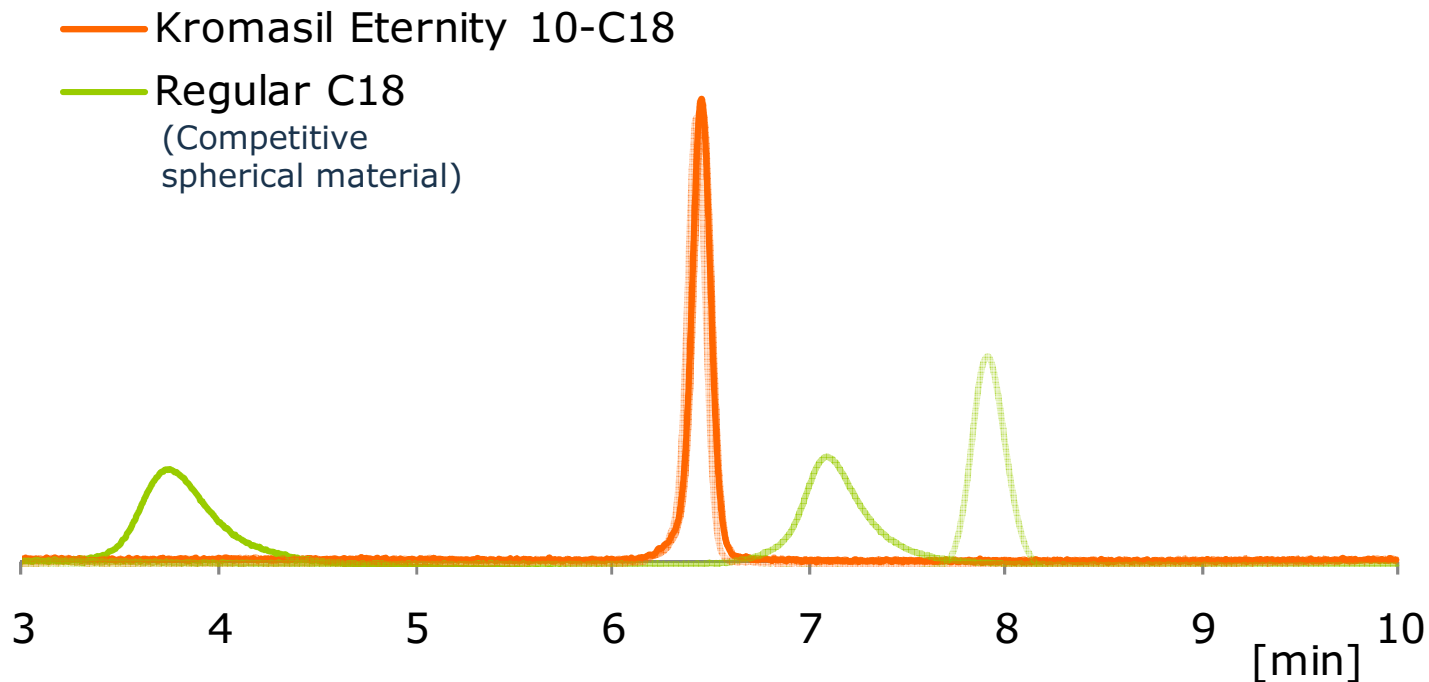
- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline



Chemical Stability at High pH - Comparison

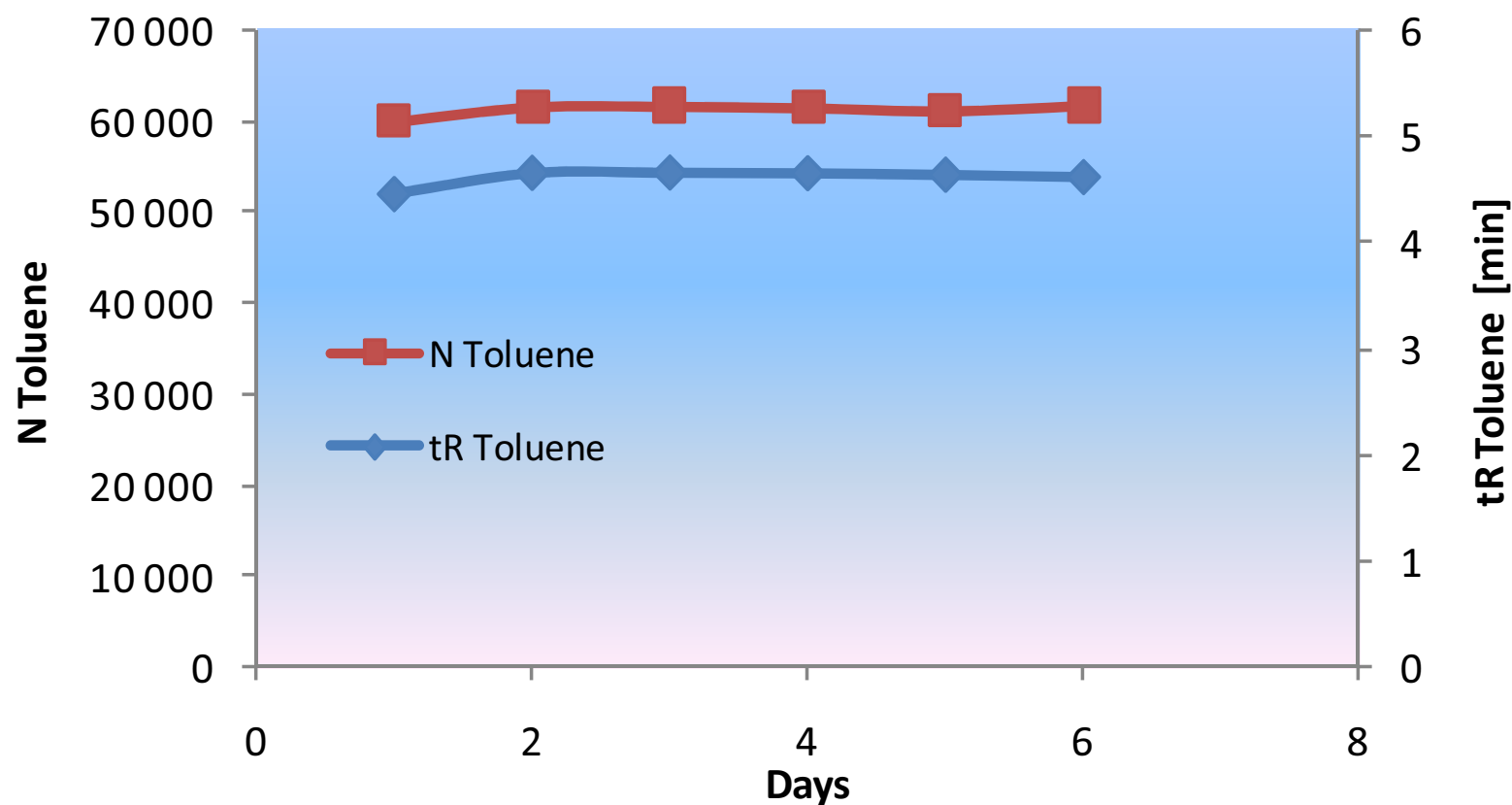
- ▶ NH_4HCO_3 / MeCN gradient at pH 10.5, 60°C
- ▶ Test substance: Amitriptyline

120 cycles

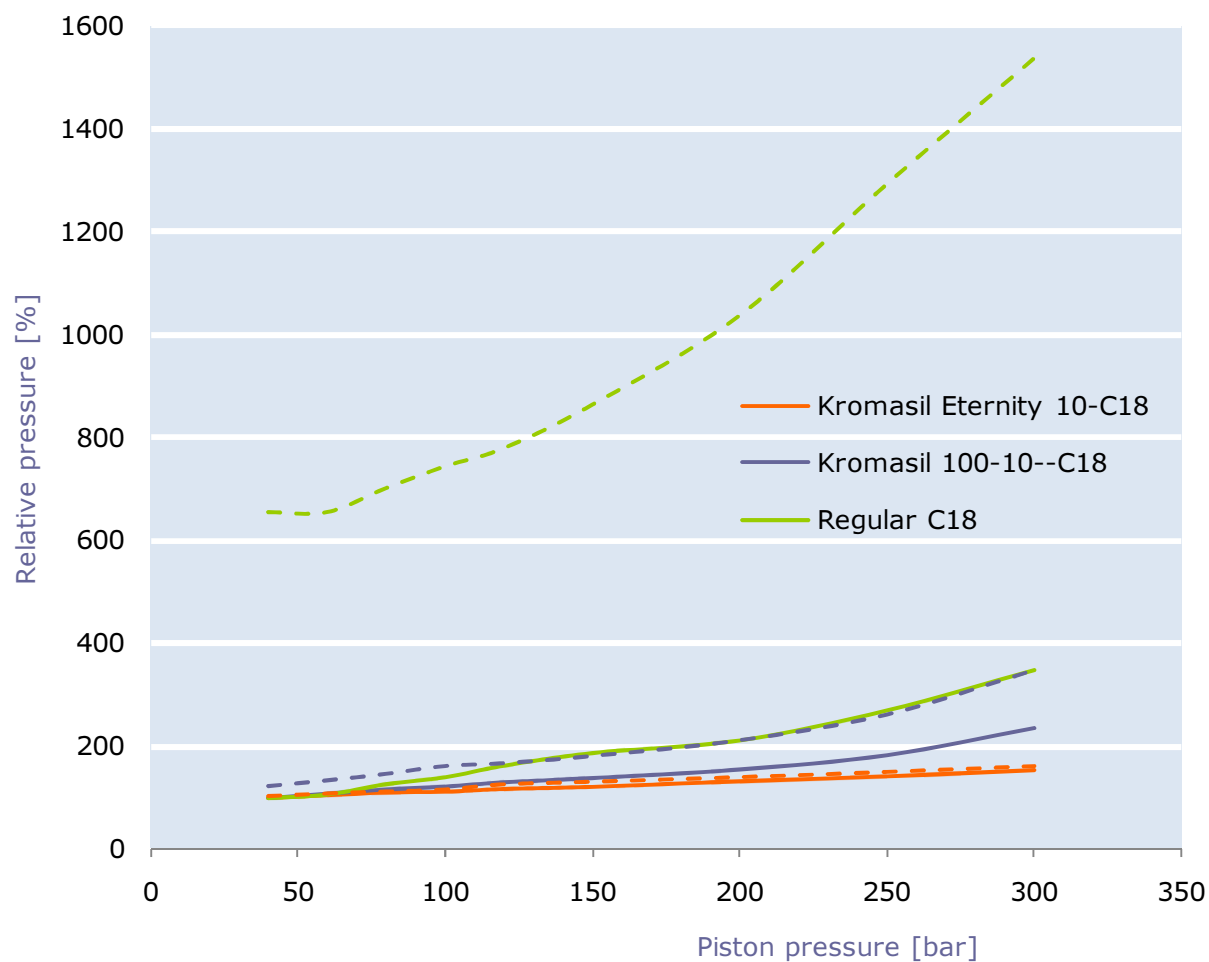


Results: Bicarbonate at pH 10.5 / 25°C

Long Tern Stability at 10 mM NH_4HCO_3 pH 10.5 / 25°C



Mechanical Stability in DAC column



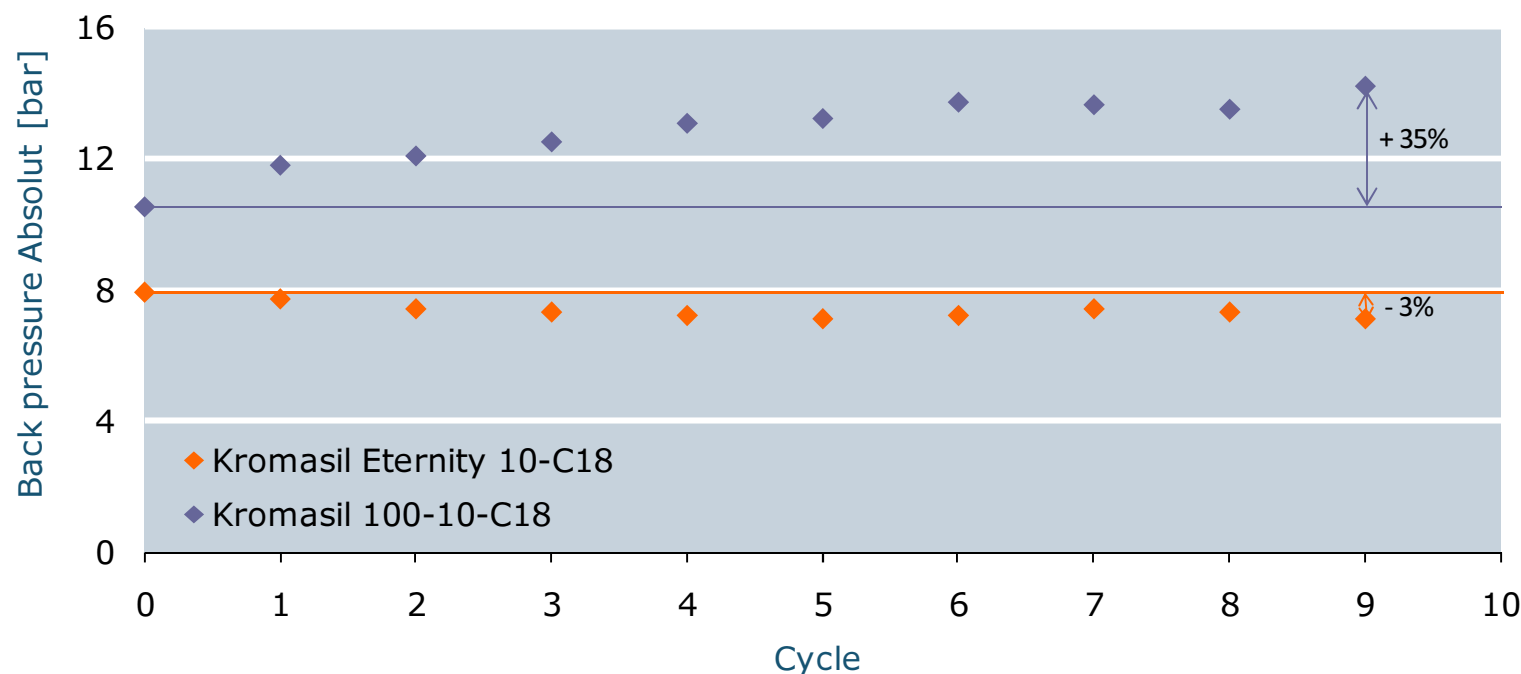
Test Procedure

1. Measure backpressure at increasing piston pressure (solid lines)
2. Unpack the column, re-slurry, and re-pack the column
3. Measure backpressure again at increasing piston pressure (dashed lines)

Mechanical stress at high pH

NaOH regeneration conditions

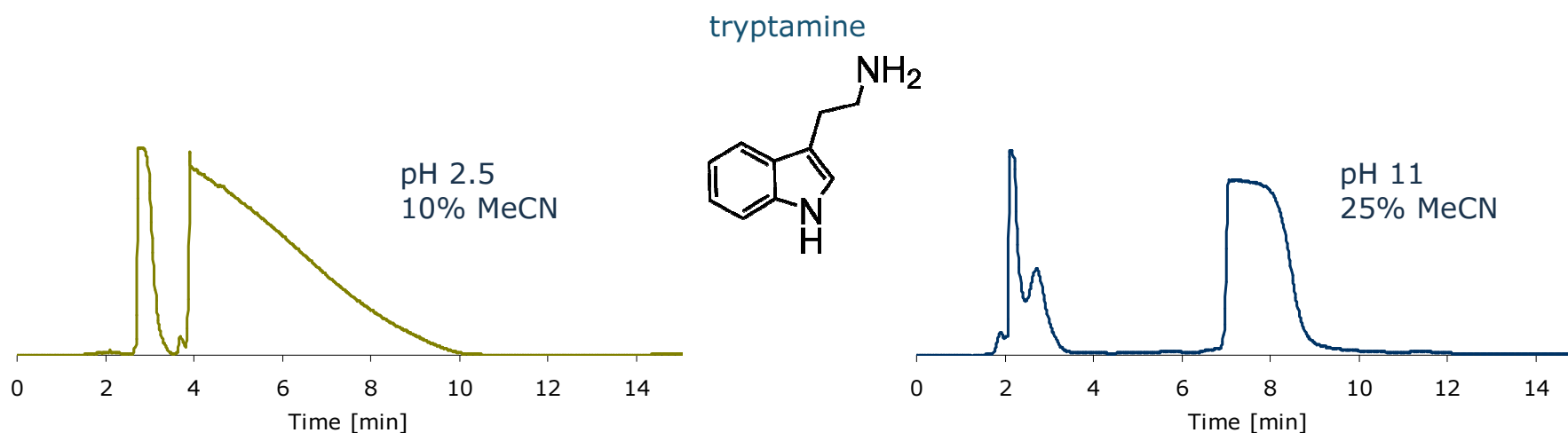
NaOH regeneration cycles



1. 50 g of packing material are packed into a 50 mm ID DAC column, using 100% n-propanol
 2. The back pressure is measured at 100 mL/min with n-propanol
 3. The bed is purged with 0.1 M NaOH/n-propanol 50/50 for 40 min at 40 mL/min
 4. Equilibration with 100% n-propanol for 5 min at 100 mL/min
- Steps 2-4 are repeated 9 times

Loadability

- ▶ Loadability was tested with vitamine B12, tryptamine, and 1-(2-chlorophenyl)piperazine
- ▶ Similar loadability compared to KR100-C18 at neutral pH
- ▶ Higher loadability at high pH for basic solutes (Kromasil Classic should not be used above pK_a of bases, $\approx$ pH 10)



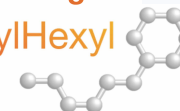
Eternity Prep – summary status

Kromasil Eternity Prep 10µm-C18

- ▶ Material shown in the presentation available as "Special Grade"
- ▶ Presently tested by key customers; feedback used in R&D work

Kromasil® Eternity™ C18
Designed for long life

Eternity PhenylHexyl
For your HPLC and UHPLC



Thank you for your attention!



Kromasil®

AkzoNobel
Tomorrow's Answers Today