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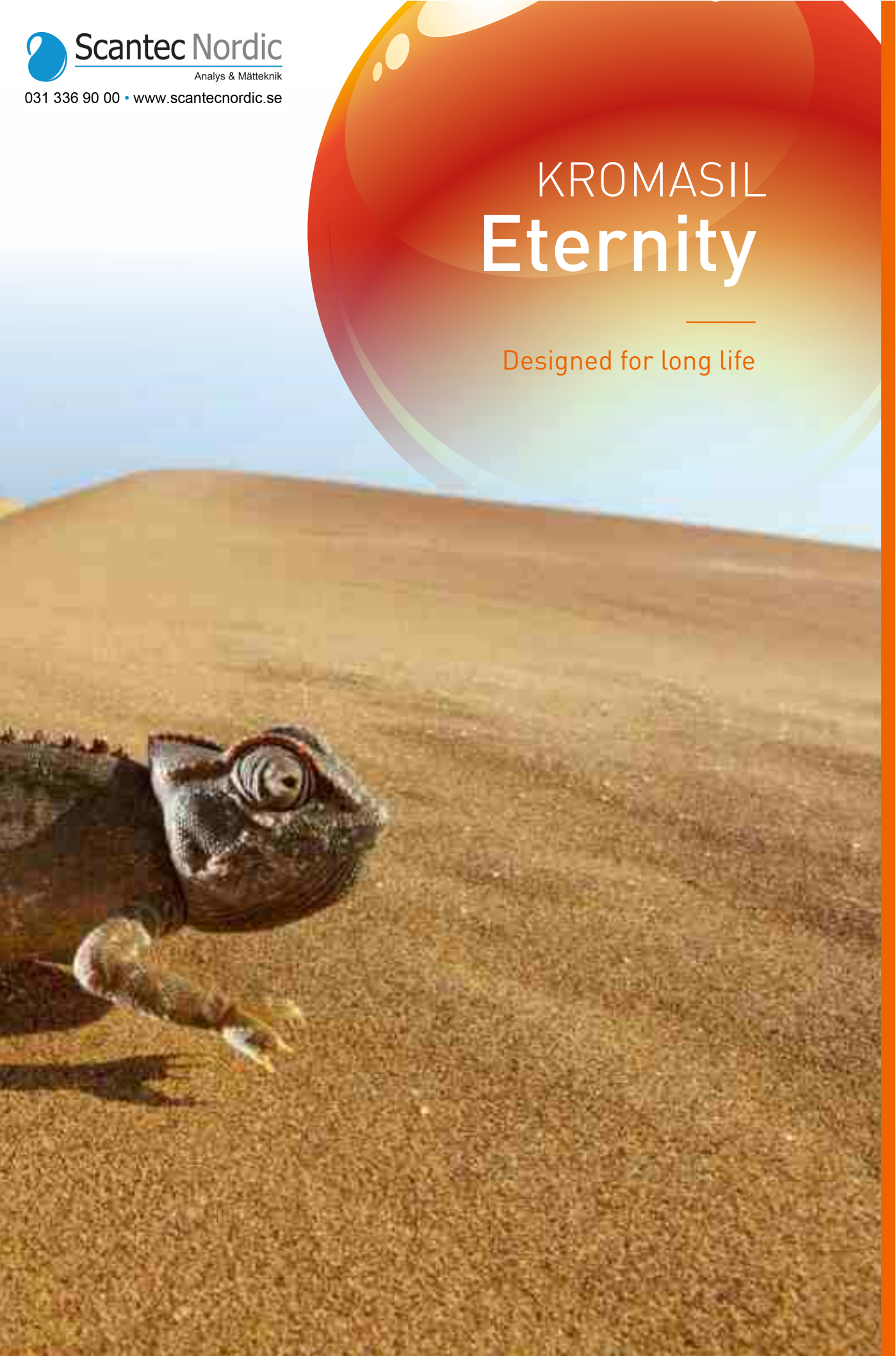
KROMASIL
Eternity

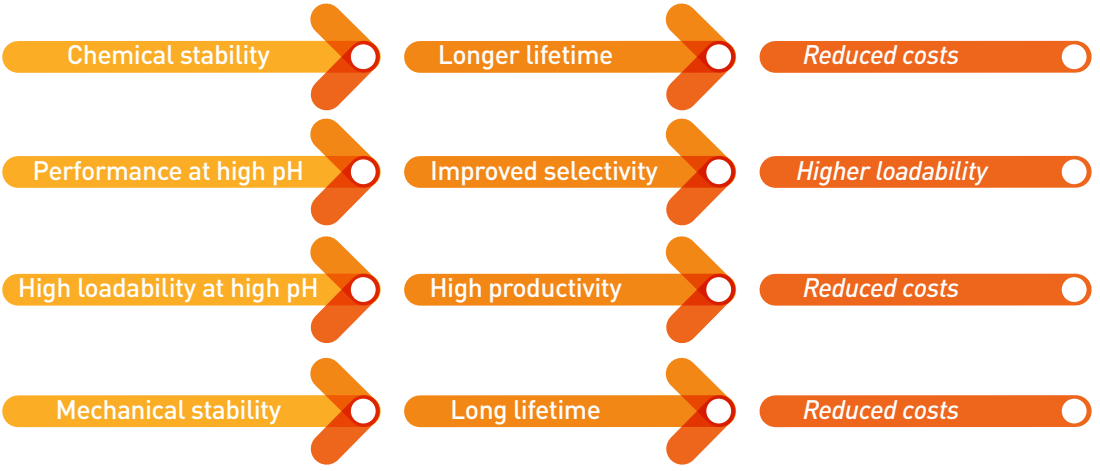
Designed for long life



Easy handling of tough demands

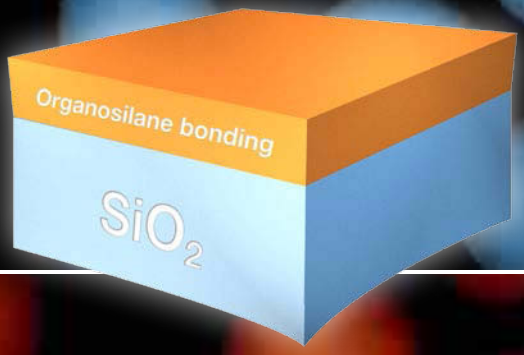
For regular silica-based stationary phases, exposure to extreme pH (especially basic) will have a negative impact on the chemical stability and therefore column lifetime. However, the silica/organosilane surface of the Kromasil Eternity platform offers a chemical stability that will secure a long-lasting stationary phase, even under tough pH conditions and higher temperatures.

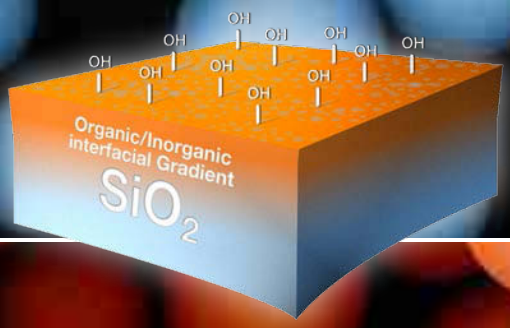
Summary of benefits for the Eternity platform



The silica matrix

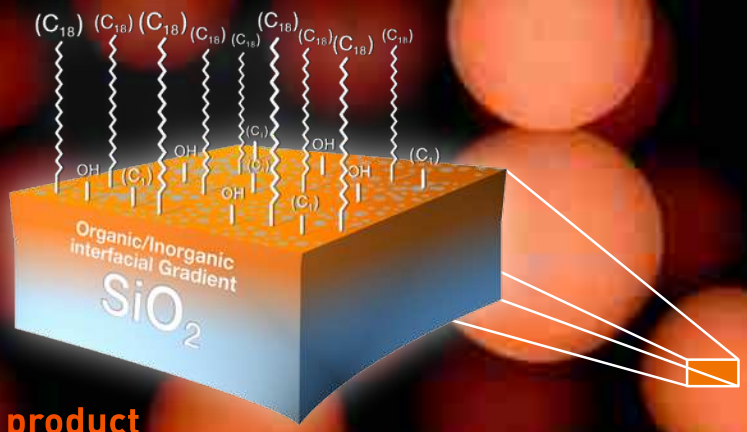
The Eternity platform is based on the Kromasil 100 Å silica matrix, well known for high mechanical stability, and a well-defined pore structure.





The organosilane interfacial gradient

The silica matrix is bonded using a patent-pending technology. An organosilane is immobilized on the silica, and, under certain proprietary conditions merged into an organic/inorganic interfacial gradient. The pores are virtually returned to their original size, resulting in a surface exhibiting both organic and inorganic moieties. This process step has been fine-tuned to give Kromasil EternityXT its extreme chemical stability, extending the pH range and packing lifetime.



The finished product

Finally the product is functionalized with various surface chemistries (C18 in illustration), followed by a proprietary endcapping process.

Excellent performance even at high pH values

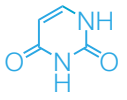
With the wide pH window, the Eternity platform gives users more flexibility to optimize selectivity and loading capacity compared to regular silica materials.

Optimizing resolution

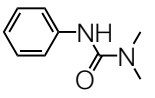
Substances with ionizable groups will exhibit significantly different retention times depending on their degree of ionization. Hence, by changing the pH, selectivity between substances can be altered so that resolution is optimized for a given separation.

In many cases, pharmaceuticals are basic. They are ionized at low or neutral pH, resulting in low retention, poor loadability and broad peaks. Being able to run at high pH means compounds become more retained with narrower peaks, revealing higher chances for better resolution and loadability.

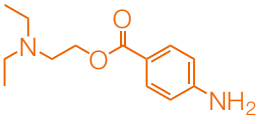
1 = uracil



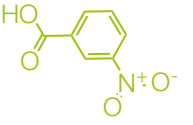
3 = fenuron



2 = procaine



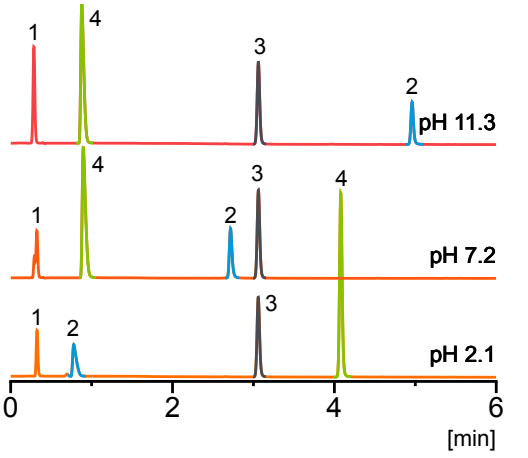
4 = 3-nitrobenzoic acid



Running at high pH

Basic pharmaceuticals become neutral at high pH and exhibit significantly sharper analytical peaks and higher loadability. Higher loadability means higher productivity, leading to a much more economical purification process. With EternityXT, large-scale separations can be run for an extended time, even at levels as high as pH 12.

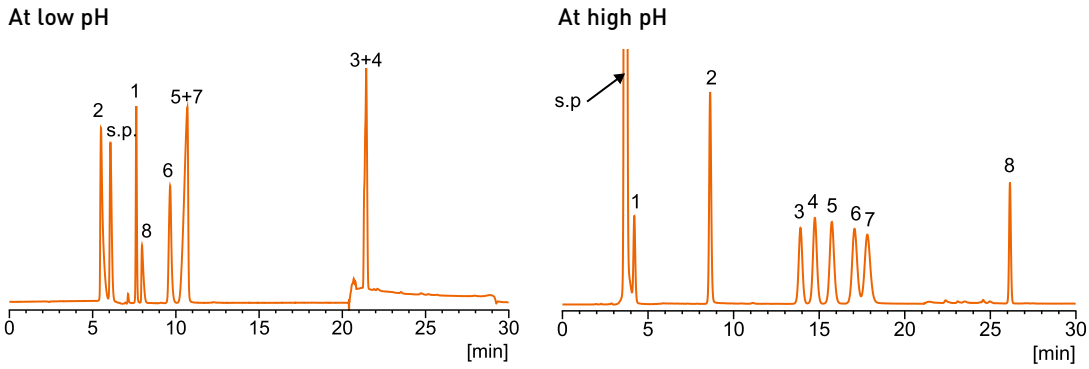
Choose selectivity by tuning pH



Conditions
Column: Kromasil EternityXT-2.5-C18 4.6 x 50 mm
Part number: XH2CLA05
Mobile phase: acetonitrile / 20 mM sodium phosphate pH 2.1, 7.2 and 11.3
Gradient 0-0.5 min: 10%, 5.5 min: 50% acetonitrile
Flow rate: 1.5 ml/min
Temperatur: 25°C
Detection: UV@254 nm

Substances: 1: uracil
2: procaine
3: fenuron
4: 3-nitrobenzoic acid

Improved resolution at high pH



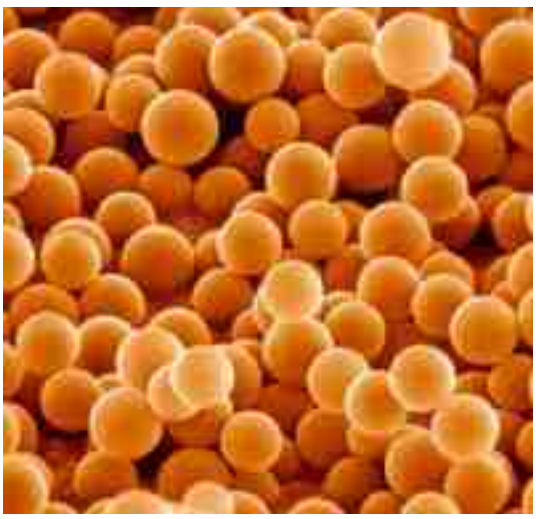
Conditions
Column: Kromasil EternityXT 10-C18, 4.6 x 250 mm
Part number: X10CLA25
Gradient: 0 min: 20%, 2 min: 29.5%, 16 min: 29.5%, 26 min: 90% acetonitrile
Flow rate: 1 ml/min
Temperature: ambient
Detection: UV @ 254 nm

Substances: 1: caffeine
2: aniline
3: 2-nitroaniline
4: 2.4-dinitroaniline
s.p.: solvent peak (acetone)
5: 2-etoxyaniline
6: 3.5-dimethylaniline
7: 3-ethylaniline
8: N,N-diethylaniline

At low pH
Mobile phase: acetonitrile / 10 mM potassium phosphate, pH 2.5

At high pH
Mobile phase: acetonitrile / 10 mM potassium phosphate, pH 10.5

The adjoining chromatograms showing separation of anilines illustrate the significant advantage of being able to use almost the entire pH range for developing a separation method. The low pH (pH = 2.5) chromatogram shows a non-favorable situation, with coelution of two pairs of peaks. However, at high pH (pH = 10.5), a chromatogram with well separated peaks can easily be obtained.



Stronger than ever

Kromasil EternityXT is based on the Kromasil 100 Å silica matrix, with exeptional mechanical stability as a result of the almost perfect spherical shape, combined with a proprietary process to further strengthen the matrix. In EternityXT, the new organic/inorganic platform reinforces the structure to an even higher level.

Columns for the lab

Kromasil Eternity HPLC columns come with particles down to 2.5 µm. EternityXT extends down to 1.8 µm to fit any UHPLC instrument for better efficiency and flexibility in the laboratory. Both can be used for reversed-phase separations and purifications that could demand harsh conditions, fast turnaround, easy method transfer and seamless scale-up from R&D to production.

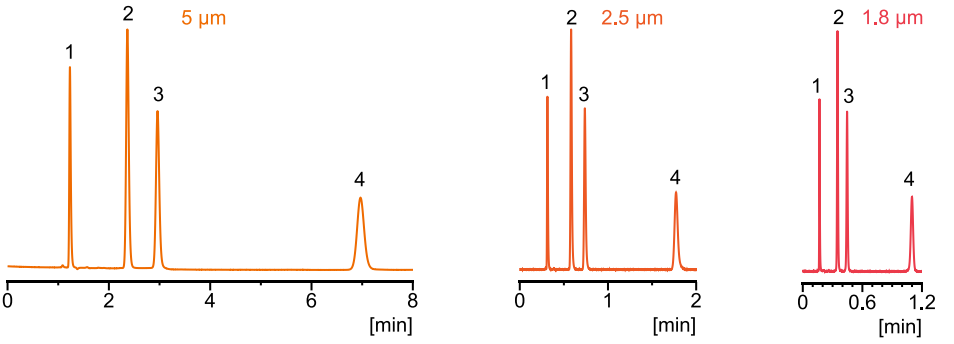
Work fast across the board

With columns built on the Eternity platform, users can now easily develop and validate UHPLC methods for synthetic and natural products, even under tough pH conditions. Method transfer to HPLC for characterization and quality control can be made seamlessly and, if required, scaled up directly for isolation and purification. Our extensive assortment of slurry-packed columns, combined with the wide range of particle sizes from 1.8 µm to 10 µm for the Eternity platform, help businesses improve productivity by using one stationary phase type across the entire company.

High efficiency with small particles

When scientists need to get results fast and within an extended pH range, EternityXT columns can help achieve the desired laboratory efficiency.

With EternityXT columns you can maintain separation power across all dimensions and particle sizes. Here is an illustration of faster result turnaround with maintained resolution when using shorter columns with smaller particles.



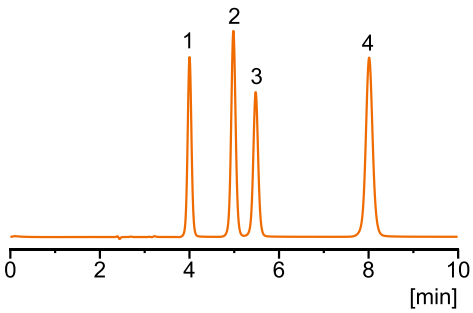
Conditions

Part numbers: X05CLA15, XH2CLAH7 and XF1CLA05, respectively
Stationary phase: Kromasil EternityXT, C18, particle sizes as in figures
Column size: 4.6 x 150 mm, 4.6 x 75 mm, 4.6 x 50 mm (respectively)
Mobile phase: acetonitrile / water/formic acid [25/75/0.1]
Substances: 1: uracil, 2: sulfathiazole, 3: sulfamerazin, 4: sulfamethoxazole
Flow rate: 1 ml/min, 2 ml/min, 2.8 ml/min (respectively)
Temperature: 25°C
Detection: UV @ 254 nm

Alternative separations

While C18 columns are the most commonly used for reversed-phase chromatography, PhenylHexyl is an alternative phase chemistry that provides additional interaction opportunities, especially when the analytes of interest contain an aromatic ring. Available for both Eternity and EternityXT.

Separation of xanthines on Kromasil EternityXT PhenylHexyl.



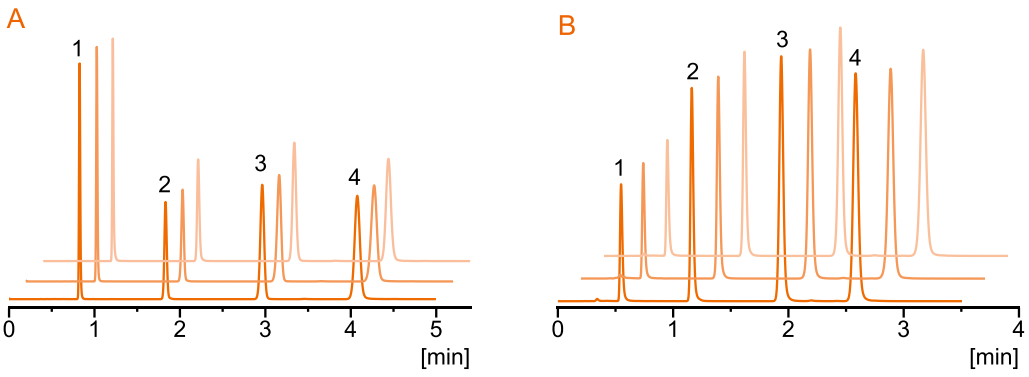
Conditions
Part number: X05PXA25
Column: Kromasil EternityXT, 5 µm, PhenylHexyl, 4.6 x 250 mm
Mobile phase: acetonitrile / water/formic acid [40/60/0.1]
Substances: 1: theobromine, 2: 1.7-dimethylxanthine, 3: theophylline, 4: caffeine

Flow rate: 1 ml/min
Temperature: 30°C
Detection: UV @ 254 nm

Consistent results between columns and batches

Since AkzoNobel controls the entire manufacturing process of the Eternity platform, from the initial production steps of the stationary phase to the finished packed columns, batch-to-batch as well as column-to-column reproducibility is assured.

Comparisions of three columns showing column-to-column (A) and batch-to-batch (B) reproducibility.



Conditions
Part numbers: XH2CLA10 and XH2CLD10
Column: Kromasil EternityXT, 2.5 µm, C18, A: 4.6 x 100 mm, B: 2.1 x 100 mm
Mobile phase: acetonitrile / water: A: [70/30], B: [65/35]
Substances: 1: dimethyl phthalate, 2: toluene, 3: biphenyl, 4: phenanthrene

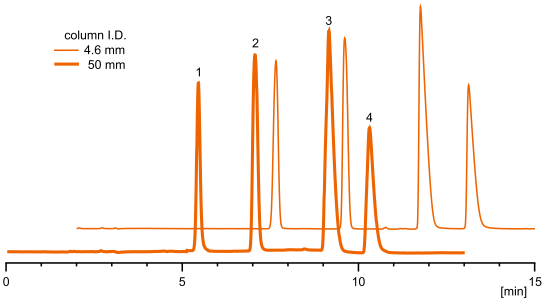
Flow rate: A: 1.7 ml/min, B: 0.65 ml/min
Temperature: A: 25°C, B: 35°C
Detection: UV @ 254 nm

Columns for the lab (cont.)

Scale-up with ease

As it is fairly straightforward to scale HPLC up or down, having the reproducible Eternity platform phases available on a broad range of particle and column sizes gives the user the key tools to carry out method scaling efficiently.

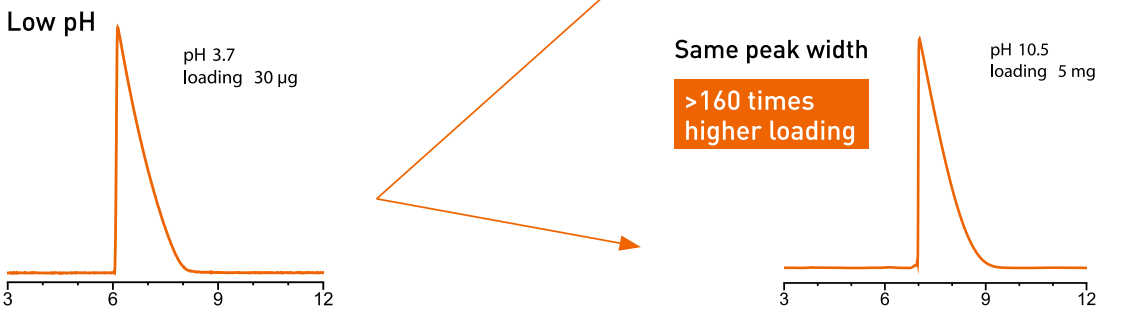
The separation of β-blockers illustrates the possibility to scale up your separation developed in analytical scale to larger scale chromatography, essentially without any loss of performance. Use 4.6 mm ID or 10 mm ID columns for the method development, and use the data obtained for predicting the performance in larger scale. With dynamic axial columns (DAC) it is possible to reproduce the performance obtained in analytical columns even in very large scale.



Conditions
Part number: X10CLA25
Column: Kromasil EternityXT 10-C18 4.6 x 250 mm
Mobile phase: acetonitrile / 10 mM ammonium hydrogen carbonate, pH 10.5
Substances: 1: sotalol, 2: nadolol, 3: pindolol, 4: metoprolol
Gradient: 0 min: 10%, 10 min: 90% acetonitrile
Flow rate: 1 ml/min
Temperature: ambient
Detection: UV @ 230 nm

Loadability increases at high pH

The loadability increase that can be obtained at high pH for basic compounds is illustrated in the adjoining chromatograms, where diphenhydramine is run at pH = 3.7 and 10.5, respectively. At low pH, the molecule is ionized, leading to a large band broadening even at very low loadings. The same loading at high pH (upper right chromatogram) produces a sharp peak without any tendency to broaden as a function of concentration overload. To obtain the same band broadening at high pH, the loading has to be increased more than 160 times. Hence, loading capacity is increased by a factor >160!



Conditions
Column: Kromasil EternityXT-10-C18, 4.6 x 250mm
Part number: X10CLA25
Flow rate: 1 ml/min
Detection: UV @ 254 nm

Low pH, low loading
Loading: 30 µg diphenhydramine
Mobile phase:
acetonitrile / 25 mM ammonium format, pH 3.7 (35/65)

High pH, low loading
Loading: 30 µg diphenhydramine
Mobile phase:
acetonitrile / 25 mM ammonium hydrogen carbonate, pH 10.5 (70/30)

High pH, high loading
Loading: 5 mg diphenhydramine
Mobile phase:
acetonitrile / 25 mM ammonium hydrogen carbonate, pH 10.5 (70/30)

State-of-the-art stability

Traditional silica-based reversed phase materials very often have an upper limit for use at neutral to slightly basic pH. At higher pH levels, the silica matrix starts to dissolve. With Kromasil Classic RP phases this limit has been moved up to pH 9.5, and in some cases, even higher. With the Eternity platform, the boundaries are moved beyond what could be expected from the strongest silica matrix.

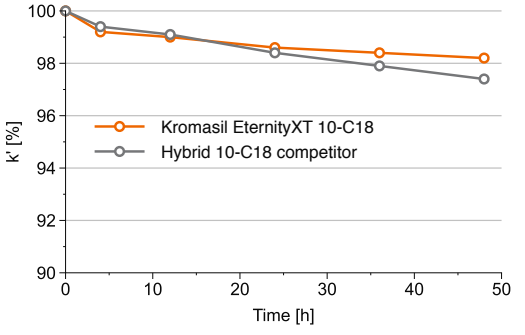
Up to pH 12

The first generation of Eternity C18 set a new standard for column lifetime expectations for hybrid materials. With EternityXT C18, users get the flexibility to develop methods for quick UHPLC analysis as well as isolation and large-scale purification between pH 1-12, for long-term use.

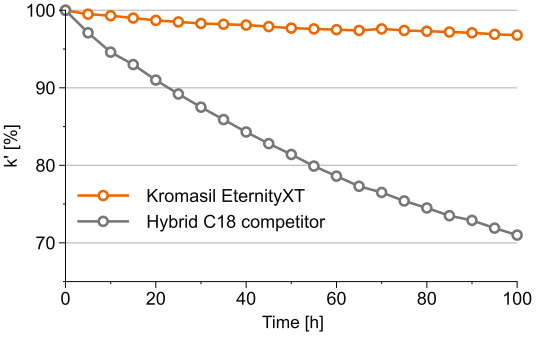
Long-term chemical stability

In the adjoining figures the long-term chemical stability at low and high pH is shown. Low pH conditions simulate a very long-term use by applying an elevated temperature and a highly aqueous mobile phase. The hybrid materials still show excellent stability, with very low shift in k' over time. High pH conditions also include highly aqueous buffer and elevated temperature. It has been shown that carbonate buffer is especially aggressive when used with silica-based packing materials, but it has little effect on the retention factor for EternityXT, due to the very dense C18 derivatization and the EternityXT gradient, protecting the silica matrix.

Low pH



High pH



Conditions
Column size: 4.6 x 250 mm

Acidic hydrolysis
Mobile phase: methanol / water/trifluoroacetic acid (5/95/0.1), pH ≈ 1.9
Flow rate: 0.2 ml/min
Temperature: 80°C

Basic hydrolysis
Mobile phase: acetonitrile / 10 mM ammonium carbonate, pH 10.5 (10/90)
Flow rate: 0.2 ml/min
Temperature: 60°C

Chromatographic test conditions
Test compound: phenanthrene
Mobile phase: acetonitrile / water (70/30)
Flow rate: 1 ml/min
Detection: UV @ 254 nm

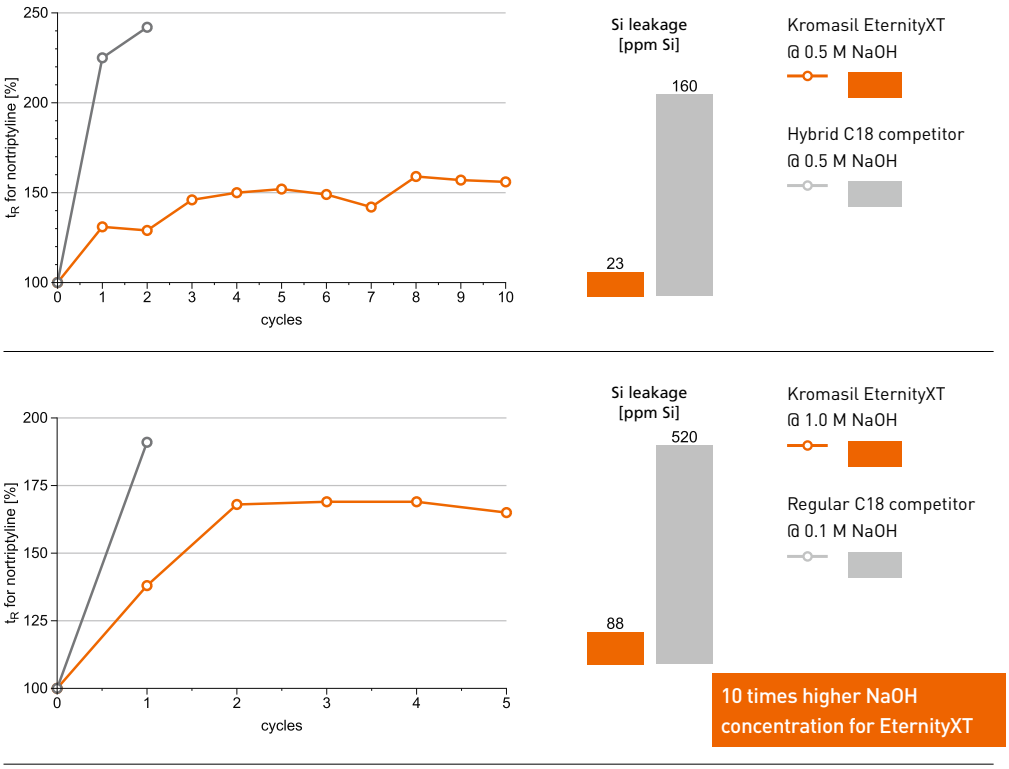
Flexibility at your fingertips

The main proportion of all synthetic pharmaceutical APIs are basic in nature, and will exhibit an increased loadability, and hence productivity, at a high pH. Basic peptides, oligos and PNAs will also benefit from high pH separation methods. In addition, it is possible to sanitize or regenerate Kromasil EternityXT in-column (cleaning in place, or CIP) even using 1 M NaOH when necessary. 1 M NaOH is a standard in biochromatography for polymeric resins.

With Kromasil EternityXT, users have the flexibility to develop analytical and separation methods for virtually the entire pH range, and to sanitize or regenerate the column using conditions previously reserved only for polymeric resins. This gives scientists the best of both worlds: highest performance and excellent stability at high pH.

Chemical stability – CIP conditions

In purification of polypeptides and proteins it is common to use high pH CIP processes (cleaning-in-place) to remove irreversibly adsorbed depositions on the packing material. The figures show retention time change after a number of CIP cycles, and the leakage of silicon during the process. For 0.5 M NaOH it can be seen that the leading hybrid C18 competitor exhibits a much lower stability compared to EternityXT, both in terms of retention time change and leakage of silicon. At 1.0 M NaOH, i.e. standard cleaning conditions for polymeric materials, EternityXT still shows very high chemical stability, while a regular C18 competitor is quickly impaired already at ten times lower hydroxide concentration, i.e. 0.1 M NaOH.

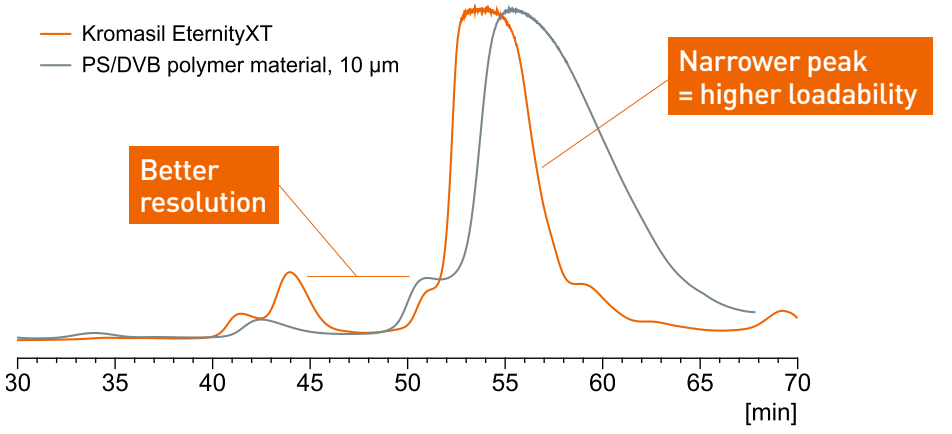


Conditions
Column size: 4.6 x 250 mm
Mobile phase: 10 column volumes of NaOH solution / ethanol (50/50)
Flow rate: 1 ml/min, for 10 column volumes (contact time 41.5 min)
Temperature: ambient
Test compound: nortriptyline at pH 7.0

State-of-the-art stability (cont.)

Chromatographic performance – EternityXT vs polymeric packing

It is well known that polystyrene/divinylbenzene (PS/DVB)-based packing materials exhibit very high chemical stability at high pH, allowing cleaning steps involving for example 1 M NaOH. However, the material can unfortunately not compete with silica-based packing materials in terms of chromatographic performance. The graph shows a typical comparison between a silica- and a polymer-based packing material: EternityXT and the market leader for PS/DVB-based packings, where identical conditions have been used. The chromatogram shows a preparative separation of insulin, where it can be seen that the silica-based material, EternityXT, has markedly sharper peaks, with roughly only 50% of the band broadening seen on the PS/DVB-based material. Both analytical efficiency and loading capacity is significantly better for EternityXT. With Kromasil EternityXT it is possible to obtain the high separation power associated with silica-based materials, and at the same time experience very high chemical stability at high pH, as can be seen in the figures.



Conditions

Column size: 4.6 x 250 mm
Temperature: 25°C
Mobile phase: ethanol / ammonium acetate 0.2 M

Flow rate: 0.7 ml/min
Detection: UV @ 280 nm
Gradient: for EternityXT, 0 min: 30%, 60 min: 38% ethanol
for PS/DVB, 0 min: 34%, 60 min: 42% ethanol

Withstands pressure time and time again

Kromasil Classic changed the world of large-scale and industrial-scale chromatography by combining a high available surface area with great mechanical stability. Kromasil EternityXT builds upon this legacy and further enhances the performance of preparative chromatography.

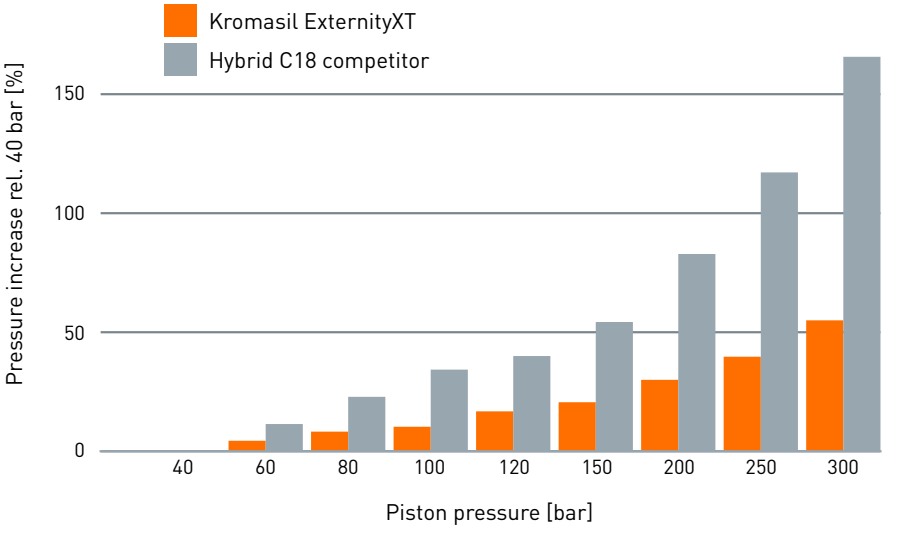
High loading capacity

Kromasil Classic is a packing material with very high loading capacity, and hence high productivity, as it can withstand the high mechanical stress the packing is exposed to in a dynamic axial compression (DAC) column. Kromasil EternityXT is a preparative packing material with exceptional physical and chemical properties. It takes mechanical stability to the next level by exhibiting even higher mechanical stability, with the same high available surface area, and hence loading capacity.

Based on the Kromasil 100 Å silica matrix, Kromasil EternityXT has exceptional mechanical stability as a result of the spherical shape and a proprietary process that further strengthens the matrix. In EternityXT, the new organic/inorganic platform reinforces the structure to an even higher level.

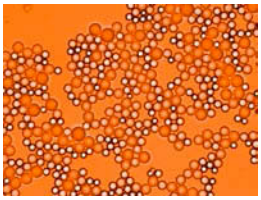
Pressure over packed bed during mechanical stability test

To simulate a repeated packing procedure without emptying the column, a test method with a successive increase of piston pressure was applied. The back pressure increase is a measure of the degree of densification and degradation of the material after repeated packings.

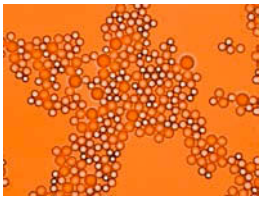


Test conditions
The test material is packed in a 50 mm ID DAC column, and the pressure is increased stepwise, from 40 bar up to 300 bar. The backpressure is monitored during the process using ethanol as the mobile phase. The backpressure monitored during the pressure increase cycle is shown in the diagram.

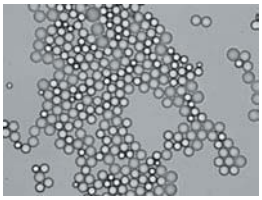
EternityXT before



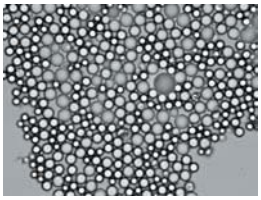
EternityXT after



Competitor before



Competitor after



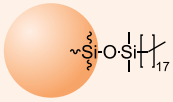
Key characteristics

In addition to the physical and chemical properties of Eternity and EternityXT, it is important to know some other facts. Manufacturing starts with the silica raw material and runs all the way through to the finished packing material. Controlling the total manufacturing process means the highest quality of the final product is guaranteed. All Kromasil products are manufactured in an ISO 9001 certified facility.

Product characteristics

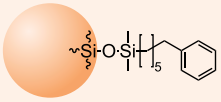
C18

	Eternity	EternityXT
ligand	octadecyl silane	
USP	L1	
pore size	100 Å	
particle size	2.5 and 5 µm	1.8, 2.5, 5 and 10 µm
surface area	330 m^2/g	
carbon load	14%	17%
endcapping	proprietary	
pH range	2-12	1-12



PhenylHexyl

	Eternity	EternityXT
ligand	6-phenylhexyl	
USP	L11	
pore size	100 Å	
particle size	2.5 and 5 µm	1.8, 2.5 and 5 µm
surface area	330 m^2/g	
carbon load	12%	15%
endcapping	proprietary	
pH range	2-12	



Availability

Please check the tables with part numbers in the availability part of this guide.

Kromasil Eternity columns

Kromasil Eternity, 2.1 mm i.d. columns

			column size, i.d. × length [mm]		
Family	**Phase**	**particle size [µm]**	**2.1 × 50**	**2.1 × 100**	**2.1 × 150**
Eternity	**C18**	**2.5**	EH2CLD05	EH2CLD10	
Eternity	**C18**	**5**	E05CLD05		E05CLD15
Eternity	**PhenylHexyl**	**2.5**	EH2PXD05	EH2PXD10	
Eternity	**PhenylHexyl**	**5**	E05PXD05		E05PXD15
EternityXT	**C18**	**1.8**	XF1CLD05	XF1CLD10	
EternityXT	**C18**	**2.5**	XH2CLD05	XH2CLD10	
EternityXT	**C18**	**5**	X05CLD05		X05CLD15
EternityXT	**PhenylHexyl**	**1.8**	XF1PXD05	XF1PXD10	
EternityXT	**PhenylHexyl**	**2.5**	XH2PXD05	XH2PXD10	
EternityXT	**PhenylHexyl**	**5**	X05PXD05		X05PXD15

Kromasil Eternity, 4.6 mm i.d. columns

			column size, i.d. × length [mm]			
Family	**Phase**	**particle size [µm]**	**4.6 × 50**	**4.6 × 100**	**4.6 × 150**	**4.6 × 250**
Eternity	**C18**	**2.5**	EH2CLA05	EH2CLA10		
Eternity	**C18**	**5**	E05CLA05	E05CLA10	E05CLA15	E05CLA25
Eternity	**PhenylHexyl**	**2.5**	EH2PXA05	EH2PXA10		
Eternity	**PhenylHexyl**	**5**	E05PXA05	E05PXA10	E05PXA15	E05PXA25
EternityXT	**C18**	**2.5**	XH2CLA05	XH2CLA10		
EternityXT	**C18**	**5**	X05CLA05	X05CLA10	X05CLA15	X05CLA25
EternityXT	**C18**	**10**				X10CLA25
EternityXT	**PhenylHexyl**	**2.5**	XH2PXA05	XH2PXA10		
EternityXT	**PhenylHexyl**	**5**	X05PXA05	X05PXA10	X05PXA15	X05PXA25

Kromasil Eternity, 10 mm i.d. columns

Family	Phase	particle size [µm]	column size, i.d. × length [mm]		
			10 × 100	10 × 150	10 × 250
Eternity	**C18**	**5**	E05CLP10	E05CLP15	E05CLP25
Eternity	**PhenylHexyl**	**5**	E05PXP10	E05PXP15	E05PXP25
EternityXT	**C18**	**5**	X05CLP10	X05CLP15	X05CLP25
EternityXT	**C18**	**10**	X10CLP10	X10CLP15	X10CLP25
EternityXT	**PhenylHexyl**	**5**	X05PXP10	X05PXP15	X05PXP25

Kromasil Eternity, 21.2 mm i.d. columns

Family	Phase	particle size [µm]	column size, i.d. × length [mm]		
			21.2 × 100	21.2 × 150	21.2 × 250
Eternity	**C18**	**5**	E05CLQ10	E05CLQ15	E05CLQ25
Eternity	**PhenylHexyl**	**5**	E05PXQ10	E05PXQ15	E05PXQ25
EternityXT	**C18**	**5**	X05CLQ10	X05CLQ15	X05CLQ25
EternityXT	**C18**	**10**	X10CLQ10	X10CLQ15	X10CLQ25
EternityXT	**PhenylHexyl**	**5**	X05PXQ10	X05PXQ15	X05PXQ25

Kromasil Eternity, 30 mm i.d. columns

Family	Phase	particle size [µm]	column size, i.d. × length [mm]		
			30 × 100	30 × 150	30 × 250
Eternity	**C18**	**5**	E05CLR10	E05CLR15	E05CLR25
Eternity	**PhenylHexyl**	**5**	E05PXR10	E05PXR15	E05PXR25
EternityXT	**C18**	**10**	X10CLR10	X10CLR15	X10CLR25