Kromasil®
Eternity™

Kromasil EternityXT

Designed for extended lifetime

AkzoNobel

Easy handling of tough demands

Kromasil Eternity was introduced a few years ago, for analytical HPLC. Kromasil EternityXT is an extension of the Eternity portfolio, introducing a 10 µm C18 product aimed at preparative applications run under demanding conditions.

EternityXT is based on a patent-pending grafting technology (see description on the opposite page), where further development of the Eternity technology has produced a material with an extended lifetime even under extremely demanding conditions.

The pH window where EternityXT can be used is from 1 to 12, giving more flexibility than ever when optimizing for large-scale applications.

Chemical stability > **Longer lifetime** > ***Reduced costs***

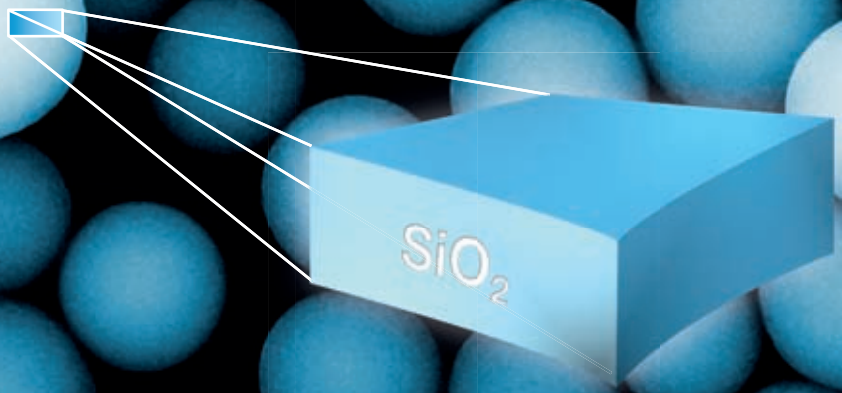
Chromatographic performance at high pH levels > **Better selectivity** > ***Loadability increase***

Loadability increase at high pH levels > **Improved productivity** > ***Reduced costs***

Mechanical stability > **Longer lifetime** > ***Reduced costs***

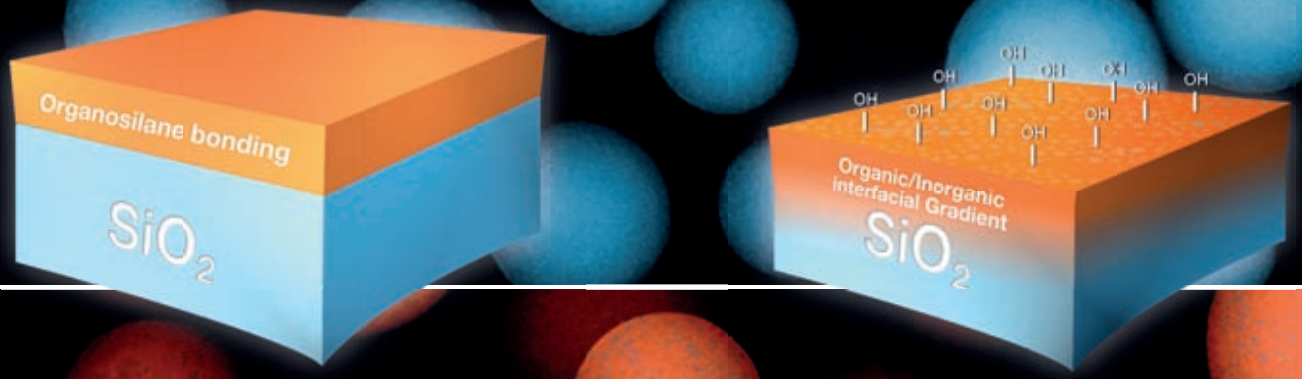
Makes your budget last longer

Kromasil EternityXT has an extremely long lifetime thanks to the combination of superior chemical and mechanical stability.



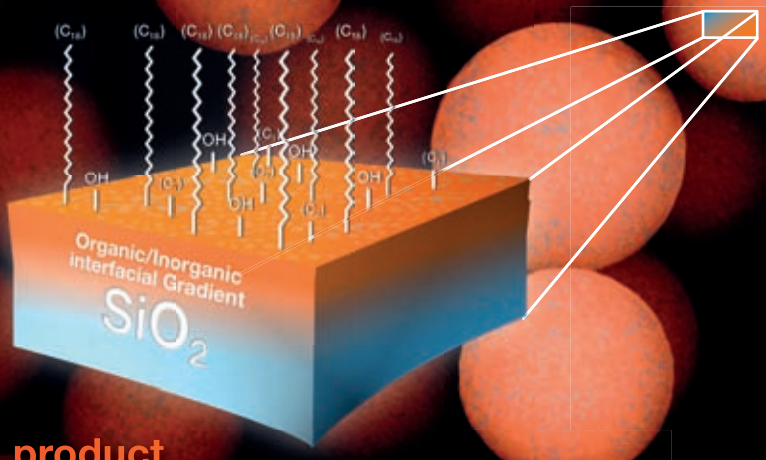
The silica matrix

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



The new EternityXT platform

The silica matrix is bonded using a new, 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 is what gives Kromasil EternityXT its extreme chemical stability, extending the pH range and column lifetime.



The finished product

Finally the product is functionalized with polymeric C18 followed by a proprietary endcapping process.

State-of-the-art stability

Traditional silica-based reversed phase materials very often have an upper limit for use at around pH 7.5-8.0. 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 conditions with even higher pH levels can be continuously used.

Up to pH 12

Introduction of Kromasil EternityXT, with the new EternityXT platform, and a merged organic/inorganic interfacial gradient combined with a proprietary C18 derivatization, has moved the pH level for continuous use up to pH 12. This gives you the flexibility to develop methods for isolation and large-scale purification between pH 1–12, for long-term use.

Flexibility at your fingertips

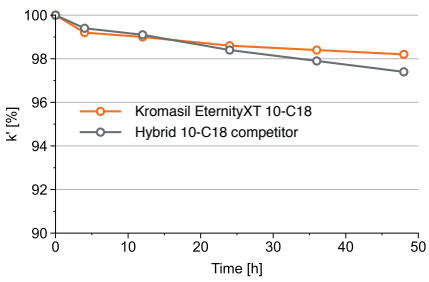
The main proportion of all synthetic pharmaceutical APIs are basic in nature, and will exhibit an increased loadability, and hence productivity, at a high pH. Basic peptides, oligos, PNAs, etc, will also benefit from high pH separation methods.

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.

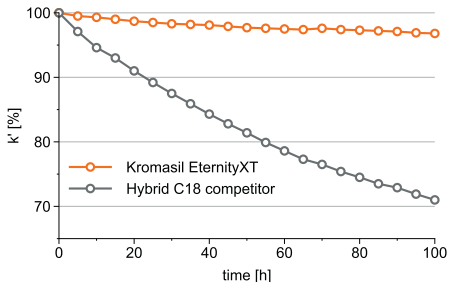
With Kromasil EternityXT you have the flexibility to develop separation methods for virtually the entire pH range, and to sanitize or regenerate using conditions previously reserved only for polymeric resins. This gives you the best of both worlds: highest performance and excellent stability at high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

Long-term chemical stability

Low pH



High pH



Conditions
Column size: 4.6 x 250 mm

Acidic hydrolysis
Mobile phase: methanol/water/TFA (5/95/0.1) **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

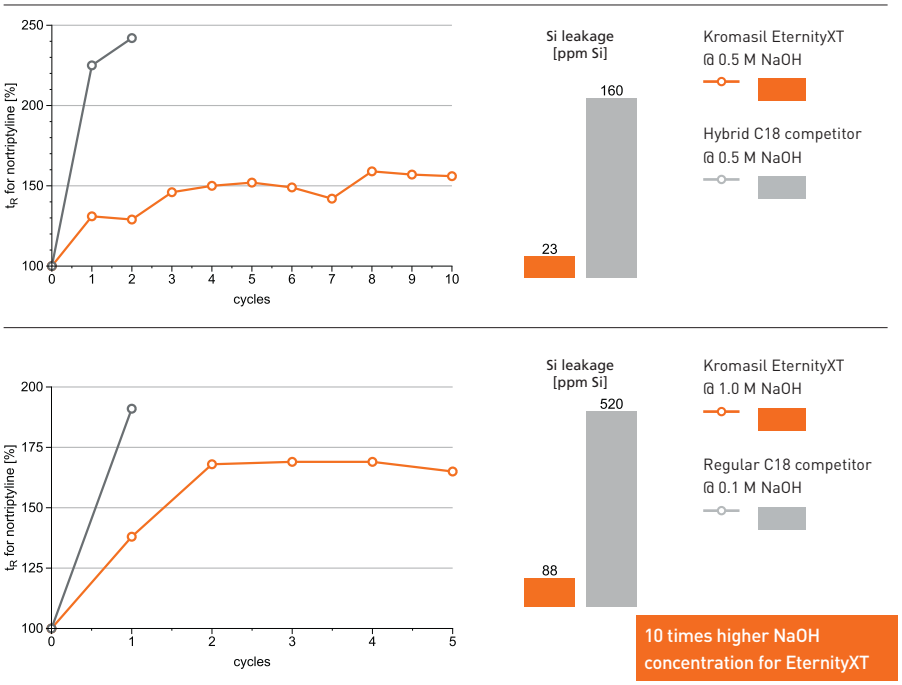
Chromatographic test conditions
Test compound: phenanthrene
Mobile phase: acetonitrile/water (70/30) **Flow rate:** 1 ml/min **Detection:** UV @ 254 nm

In the adjoining figures the long-term chemical stability at low and high pH is shown.

Low pH conditions simulate very long-term use by applying an elevated temperature of 80°C, and a highly aqueous mobile phase, 95% water, with 0.1% TFA (Trifluoroacetic acid), pH ≈ 1.9. The hybrid materials still show excellent stability, with very low shift in k' over time.

High pH conditions include highly aqueous carbonate buffer at pH 10.5, at an elevated temperature of 60°C. 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 Eternity XT, due to the very dense C18 derivatization and EternityXT platform, protecting the silica matrix.

Chemical stability – CIP conditions



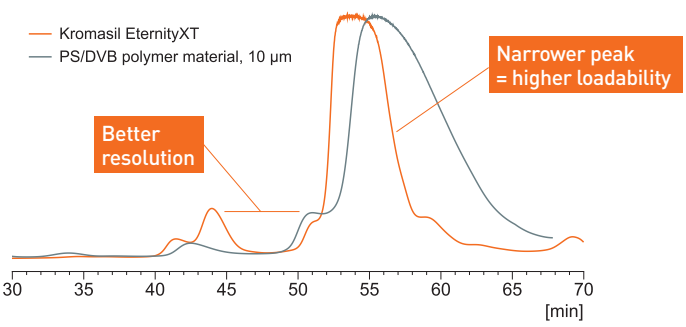
Conditions
Column size: 4.6 x 250 mm
Mobile phase: 10 column volumes of NaOH solution/ethanol (50/50)
Flow rate: 1 ml/min, for 10 column volumes (contact time 41.5 min)
Temperature: ambient
Test compound: nortriptyline at pH 7.0

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 C18, a regular competitor, is quickly impaired already at ten times lower hydroxide concentration, i.e. 0.1 M. NaOH.

Chromatographic performance – EternityXT vs polymeric packing



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

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.

Excellent performance even at high pH values

Kromasil EternityXT, with a pH user window from 1 to 12, gives you more flexibility to optimize selectivity and loading capacity than ever before.

Performance bandits

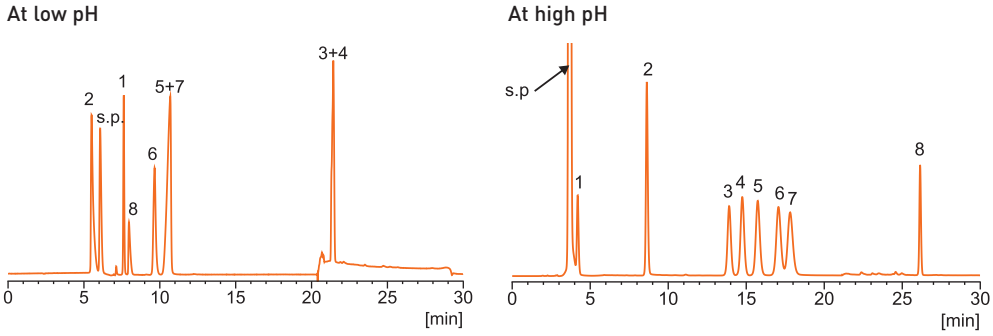
Pharmaceuticals with ionizable groups will exhibit significantly different retention times depending on the degree of ionization. Hence, by changing pH the selectivity between different pharmaceuticals can be altered so that selectivity is optimized for the separation.

Another common problem in RP chromatography of pharmaceuticals is that the loading capacity, and hence the productivity, is too low to create an economic production process. The explanation is that in many cases the pharmaceuticals are basic, and at low or neutral pH these pharmaceuticals will be protonized. This means that the substances are present in a positively charged form, resulting in very low loadability. Even low loadings will generate extremely broad peaks.

Reaching a higher level

The solution would be to run at a high pH level, where the basic pharmaceuticals become neutral, and hence exhibit significantly 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 pH levels as high as pH 12.

Improved resolution at high pH



Conditions
Column: Kromasil EternityXT 10-C18, 4.6 x 250 mm
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

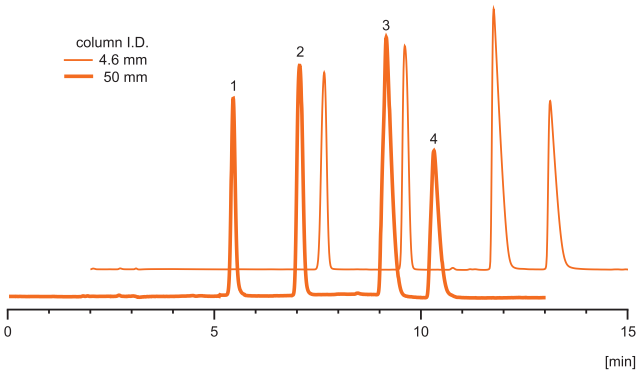
Substances: 1: caffeine
2: aniline
3: 2-nitroaniline
4: 2,4-dinitroaniline
s.p.: solvent peak (acetone)
5: 2-etoxyaniline
6: 3,5-dimethylaniline
7: 3-ethylaniline
8: N,N-diethylaniline

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 big 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.

Scale-up of separation of ß-blockers

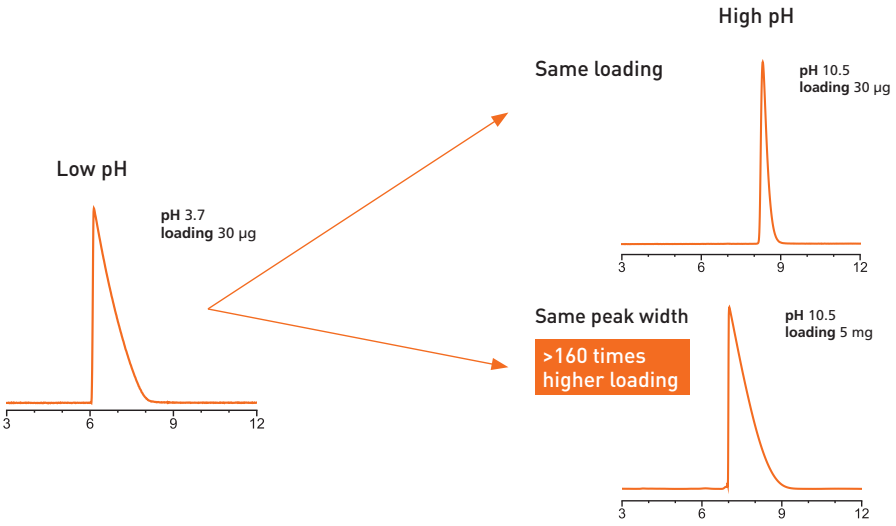


Conditions
Column: Kromasil EternityXT 10-C18 4.6 x 250 mm
Temperature: ambient
Mobile phase: acetonitrile/10 mM ammonium hydrogen carbonate, pH 10.5
Gradient: 0 min: 10%, 10 min: 90% acetonitrile

Flow rate: 1 ml/min
Detection: UV @ 230 nm
Substances: 1: sotalol
2: nadolol
3: pindolol
4: metoprolol

The separation of ß-blockers illustrates the possibility to scale up your separation developed in analytical scale to larger scale chromatography, essentially without any loss of performance. We recommend using 4.6 mm ID or 10 mm ID columns for the method development, and using 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.

Loadability increases at high pH



Conditions
Column: Kromasil EternityXT-10-C18, 4.6 x 250mm
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)

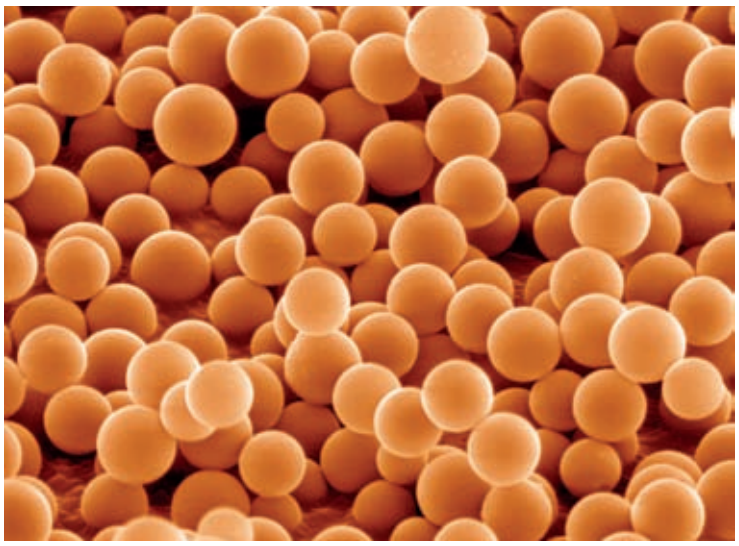
The loadability increase that can be obtained at high pH for basic compounds is illustrated in the adjoining chromatograms, where diphenhydramine is run at pH = 3.7 and 10.5, respectively. At low pH, the molecule is ionized, leading to a large band broadening even at very low loadings. The same loading 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!

Withstands pressure time and time again

In the late 1980s, Kromasil Classic revolutionized large-scale and industrial-scale chromatography by combining a high available surface area with great mechanical stability. Kromasil EternityXT builds upon this legacy and takes performance even further.

Athletic power

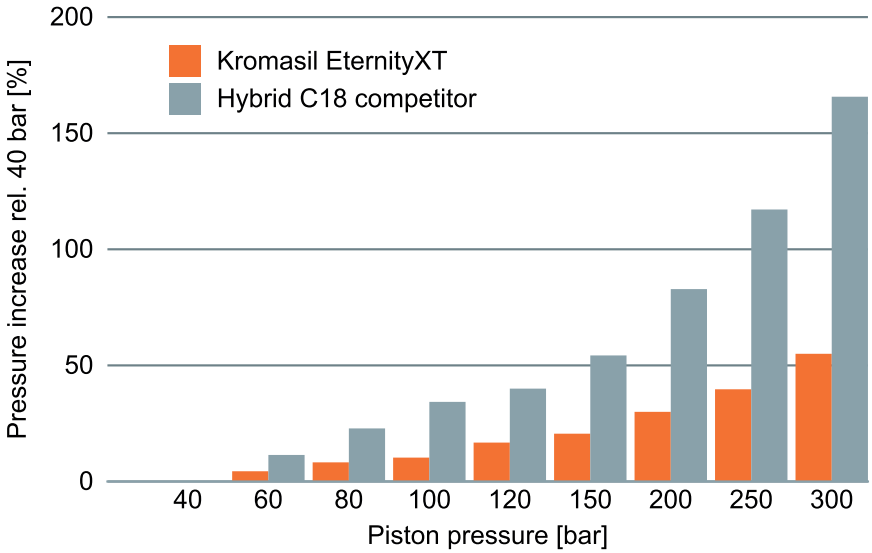
Kromasil Classic is a packing material giving very high loading capacities, and hence productivity, at the same time as it can withstand the high mechanical stress the packing is exposed to in a DAC (Dynamic Axial Compression) column. Kromasil EternityXT takes mechanical stability to the next level by exhibiting even higher mechanical stability, with the same high available surface area, and hence loading capacity. What you get is a preparative packing material with exceptional physical and chemical properties.



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.

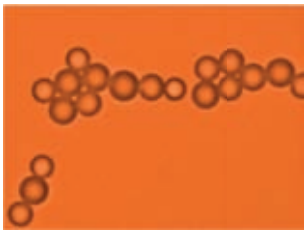
Pressure over packed bed during mechanical stability test



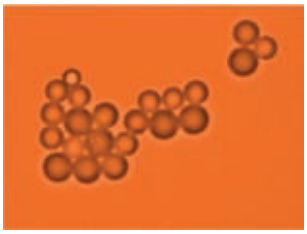
Test conditions
The test material is packed in a 50 mm ID DAC column, and the pressure is increased step-wise, from 40 bar up to 300 bar. The back-pressure is monitored during the process using ethanol as the mobile phase. The back-pressure monitored during the pressure increase cycle is shown in the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

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.

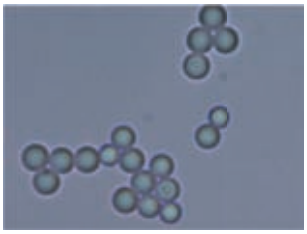
EternityXT before



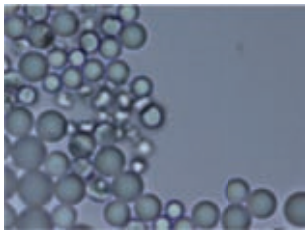
EternityXT after



Competitor before



Competitor after



Get to know more

In addition to the physical and chemical properties of Kromasil EternityXT it is important to know some other facts. Our manufacturing starts with the silica raw material, and runs all the way through to the finished packing material. Since we control the total manufacturing process we can guarantee the highest quality of the final product. Kromasil has been ISO 9001 certified since the early nineties.

The market leader

The Kromasil group not only supplies packing material, but is also a leader in the field of developing and optimizing methods for the separation process. Our team can advise on how to pack columns, select optimal running conditions, or optimize large-scale separations.

With broad experience of separating complex mixtures around the world, our experts can perform a method development for your application, and optimize the running parameters to give you the most economic separation process possible.

Available anytime, anywhere

Kromasil is manufactured in a state-of-the-art, multi-ton plant in very large batches, assuring high reproducibility. The manufacturing of a modern hybrid material such as Kromasil EternityXT is a complicated process, demanding many steps in the production process. For Kromasil, all intermediate products are kept in stock in large quantities, ensuring that production and delivery times even for large volumes can be kept at a minimum.

Registering the final separation step in the production of a pharmaceutical is a way of securing problem-free production.

Availability of Kromasil EternityXT

Bulk

Particle size: 10 µm,

Phase: C18,

Part number: X10CLblk

Columns

Part numbers for selected columns prepacked with Kromasil EternityXT 10-C18

Diameter [mm]	Column length [mm]	
	150	250
4.6	X10CLA15	X10CLA25
10	X10CLP15	X10CLP25
21.2	X10CLQ15	X10CLQ25
30	X10CLR15	X10CLR25
50	X10CLT15	X10CLT25


Kromasil
SPHERICAL SILICA
FOR HPLC
Kromasil EternityXT
Particle size: 10 µm
Phase: C18
Batch: 000001697
Quantity: 8 kg
Part number: X10CL80

The moment you adopt our Kromasil High Performance Concept, you join thousands of chromatographers who share a common goal: to achieve better separations when analyzing or isolating pharmaceuticals or other substances.

Not only will you benefit from our patented silica technology, but you gain a strong partner with a reliable track record in the field of silica products. For the past 70 years, we have pioneered new types of silica. Our long experience in the field of silica chemistry is the secret behind the development of Kromasil, and the success of our Separation Products group. Kromasil is available in bulk and in high-pressure slurry-packed columns. The development, production and marketing of Kromasil are ISO 9001 certified.

Kromasil is a brand of AkzoNobel, the largest global paint and coatings company and a major producer of specialty chemicals with headquarters in Amsterdam, the Netherlands. With 50 000 people in more than 80 countries around the world, we are committed to sustainability, excellence and delivering Tomorrow's Answers Today™.

PUBBetxt_1301

www.kromasil.com

Kromasil®