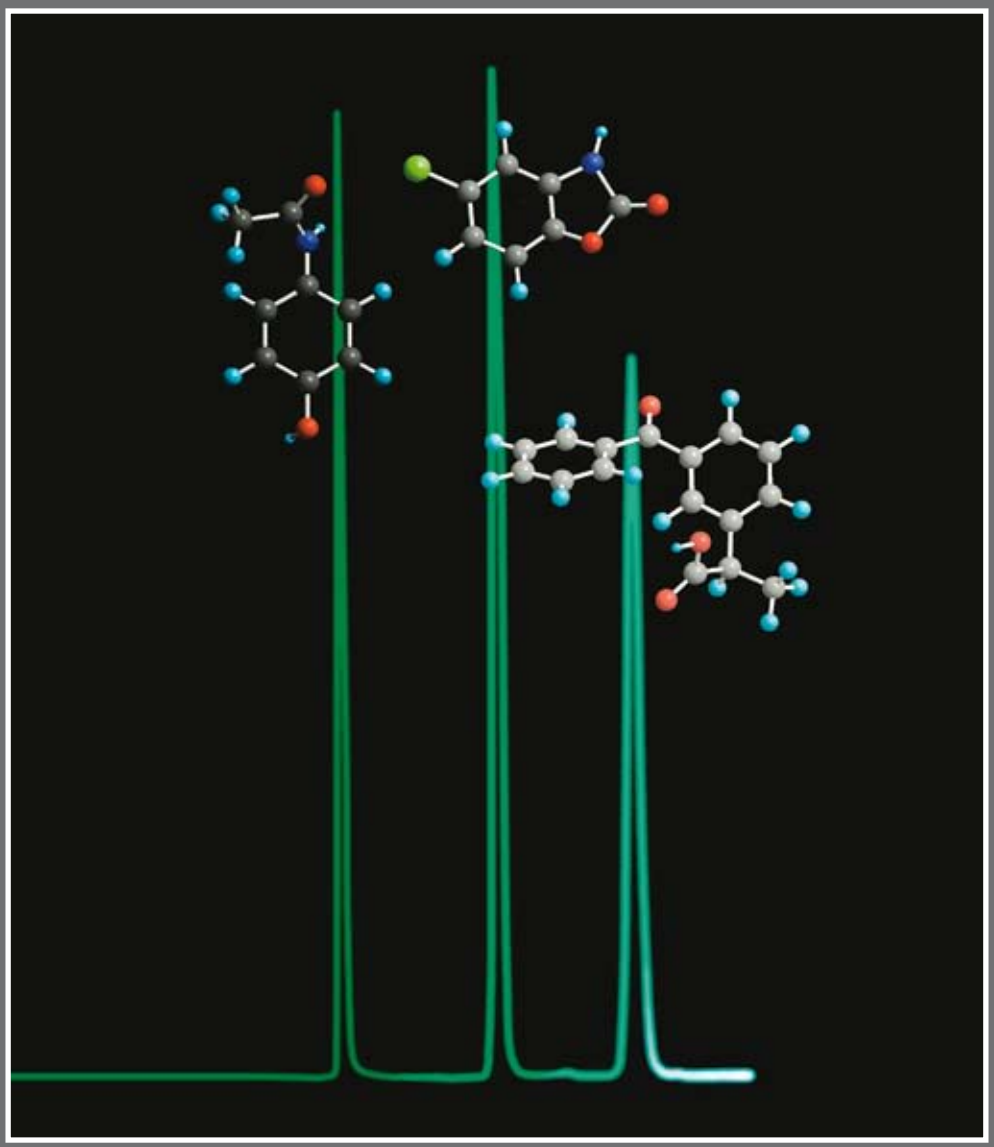
Kromasil®

The way to peak performance
in liquid chromatography



Kromasil –
for your analytical HPLC

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Kromasil is known, worldwide, for its high performance and excellent total economy in preparative and industrial scale HPLC

Let us show you, with facts and figures, how we have taken advantage of our High Performance Concept in large scale HPLC and made Kromasil the perfect choice also for your analytical HPLC separations.

Content

Kromasil silica matrix

There are several important properties to be taken into account in the development of a silica matrix for analytical HPLC. The following will have a fundamental impact on the overall performance in analytical HPLC:

- Particle shape
- Pore size and pore volume
- Particle size and particle size distribution
- Surface properties
- Metal impurities

Due to the number of properties, as well as superimposed effects of them, it is of utmost importance for us to be able to manufacture a silica with extremely high batch to batch reproducibility.

Kromasil silica is the perfect choice for your normal phase (NP) applications but also the ideal platform for derivatization of our new stationary phases.

Particle shape

Having perfectly spherical silica particles, like the Kromasil silica shown in figure 1, is not enough. At least not for us! Several silica's on the market are almost spherical but have cracks and rough surfaces. As you can see on the FE-SEM image the Kromasil silica has a smooth surface. Combining a perfectly spherical silica with a very smooth surface is a great combination when you are looking for a silica with a long lasting lifetime and ideal packing properties.

Pore size and pore volume

What is the perfect pore size? It all depends on the size of the molecule you are trying to separate. Today, Kromasil offers the following pore sizes: 60 Å, 100 Å, 200 Å, and 300 Å.

The pore volume is optimized to give the highest available surface area without loosing mechanical strength. This combination is achieved by optimization of several different manufacturing steps.

Particle size and particle size distribution

A narrow and above all consistent particle size distribution leads to a column with maximum efficiency and bed stability. By having a narrow particle size distribution you automatically avoid high back pressure due to a low bed porosity.

Figure 1 | FE-SEM image of Kromasil 100 Å, 3.5 µm particles.

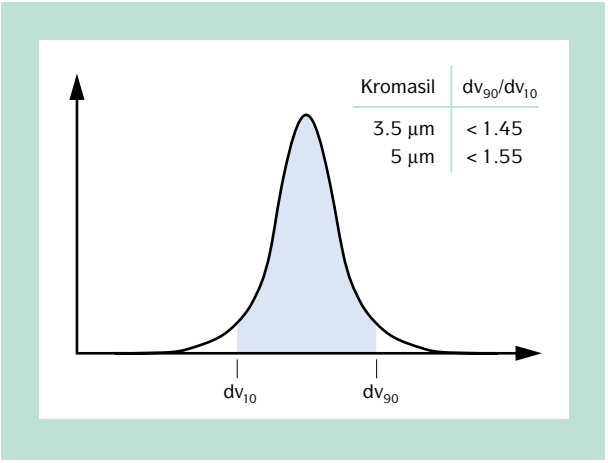

Figure 2 | Particle size distribution showing the dv_{90}/dv_{10} ratio.

In order to define and secure a narrow particle particle size distribution we use the dv_{90}/dv_{10} ratio in our quality control (QC) procedure, figure 2.

Particle size and particle size distribution are measured by the electrical sensing zone method. In order to obtain the true particle size, mass calibration is used to compensate for the porosity of the particles.

It is essential to understand this ratio and how it is calculated since it is an important factor for the overall HPLC performance. In the market place today several different definitions occur. In order to conceal a wide particle size distribution, quite often the dv_{90}/dv_{40} ratio is used. Always ask the manufacturer how the particle size distribution is measured and how they calculate it!

Surface properties

The Kromasil surface is topographically smooth and completely free from micro cavities. The surface silanol groups are evenly distributed and relatively neutral in their nature. These factors combined with the high reproducibility of the Kromasil silica surface is the foundation for a reproducible bonding process and derivatized product.

Metal impurities

Strongly bound metal ions present in the silica bulk and in the surface layers are in most cases the outcome from the silica manufacturing process. These metal ion species should be distinguished from adsorbed metal ion species, introduced in the final product due to use of metal ion containing solvents, chemicals etc. Adsorbed metal ion species are often possible to remove during a regeneration process, in contrast to the "built-in", strongly bound, metal ions, which are part of the final product.

It is well known that strongly electronegative metal ions (e.g. bivalent iron and trivalent aluminum), in the silica matrix, have the ability to enhance the acidity of silanols in their close proximity, figure 3. Increased acidity of silanols provides a higher possibility for ion-exchange interactions at any given pH. Moreover, metal ions present in the silica surface layer are able to interact directly with analytes having Lewis-base properties, figure 3. The direct metal-analyte interaction is most pronounced for chelating substances, but it also affects the chromatographic behavior of acids, alcohols, amines etc.

Three batches of Kromasil silica

Kromasil uses a proprietary manufacturing process. The metal content in all reagents and raw materials are minimized due to a rigorous QC procedure. In table 1 you will find information regarding the metal content in three typical batches.

Different levels of metal ions – a comparison study

In the case of a high purity and a high performing silica the accessibility of surface silanols as ion-exchange sites or the direct access to metal ions

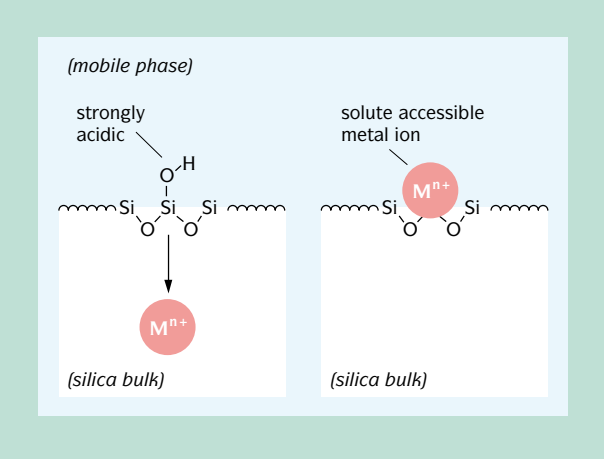

Figure 3 | The effect of metal ions in the silica matrix and in the silica surface layer.

Metal	Batch no. AT 9074 (ppm)	AT 9075 (ppm)	AT 9076 (ppm)
Na	6.0	5.5	5.1
Al	2.8	1.6	1.2
Fe	2.2	1.4	2.0

Table 1 | Metal content in three batches of Kromasil. The metal content is measured by ICP-SFMS.

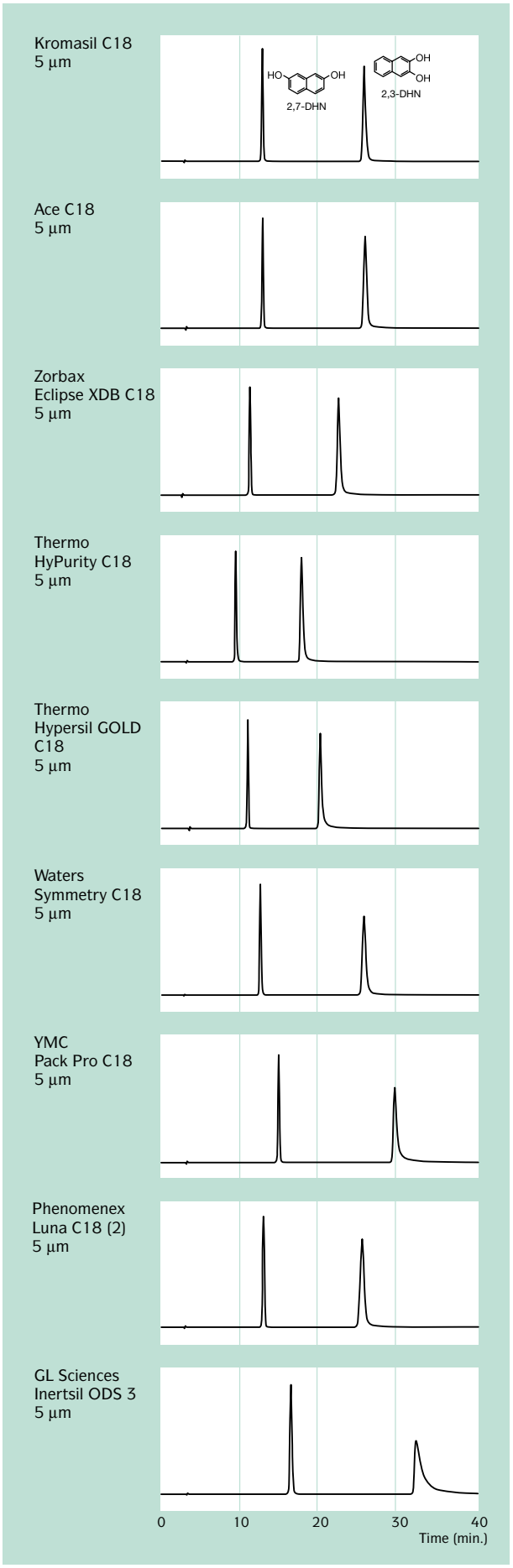

Figure 4 | Chromatograms from the DERT.

Figure 5 | Summary of the DERT-values.
DERT = $N_{2,7\text{-DHN}}/N_{2,3\text{-DHN}}$

Conditions: Column: 4.6 × 250 mm Silica: 100 Å, 5 µm, C18
Mobile phase: ACN/25 mM ammonium acetate pH 7.06 (20/80) w/w
Flow rate: 1.0 ml/min. Temperature: 20°C Detection: UV 254 nm
Injection sample: 2,7- and 2,3-dihydroxynaphthalene

in the silica surface layer are detrimental to the chromatographic performance.

There are several methods mentioned in the literature for determining the chromatographic effect of residual metal ions. Due to a displayed high sensitivity we have introduced the dihydroxy-naphtalene efficiency ratio test, abbreviated DERT in our QC procedure. This test is aimed at probing metal ions present for direct interaction. 2,3- and 2,7-dihydroxynapthalene (DHN) are injected. Of the two substances, 2,3-DHN has the ability to form chelating complexes with metal ions, while 2,7-DHN has not. The pronounced presence of metal ions for direct interaction with analytes is seen as a low performance and/or tailing of the 2,3-DHN peak, compared with the 2,7-DHN peak.

In order to visualize the difference in metal ion content and the impact thereof we investigated eight commercially available 5 µm-C18 phases. We performed the DERT and compared the outcome with a Kromasil 5 µm-C18 column. In figure 4 you will find the chromatograms and in figure 5 we have summarized the result by showing the DERT-value (= $N_{2,7\text{-DHN}}/N_{2,3\text{-DHN}}$) for the investigated phases. The plate count (N) between 2,3-DHN and 2,7-DHN should ideally be identical, i.e. the DERT-value should be close to 1!

Derivatization of the Kromasil silica

Even if many different new stationary phases are launched every year the ODS or C18 phase is still the most popular phase on the analytical market.

Extensive quality controls on every raw material together with several in process controls (IPC) throughout the manufacturing process ensure a reproducible final quality of our derivatized phases.

Surface coverage

In order to ensure a high chemical stability and an excellent chromatographic performance we have optimized the bonding step with respect to the surface coverage. Kromasil RP products are manufactured by using monofunctional silanes. This together with the Kromasil silica gives an outstanding batch to batch reproducibility and a high chemical stability. By using monofunctional silanes the reproducibility of the bonded phase is higher compared with bonded phases based on polyfunctional silanes.

Hydrophobicity

The hydrophobicity of a RP-phase is related to the silica matrix, the silane used for modification, the surface coverage, and the surface distribution of functionalities. Generally, Kromasil RP-phases are considered having a high surface hydrophobicity. This high hydrophobicity has two major advantages:

- A high surface hydrophobicity provides a good separating power. The retention of analytes can be varied within greater range upon need.
- A high surface hydrophobicity provides a good long-lasting performance, i.e. high chemical stability.

Endcapping

Endcapping is used in order to minimize undesired interactions between residual silanols and the analytes. In the manufacturing process of Kromasil, we utilize our proprietary highly efficient technique to reduce these silanols.

Symmetrical peaks when using Kromasil

It is well known that residual silanol groups lead to severe peak tailing due to undesired interactions

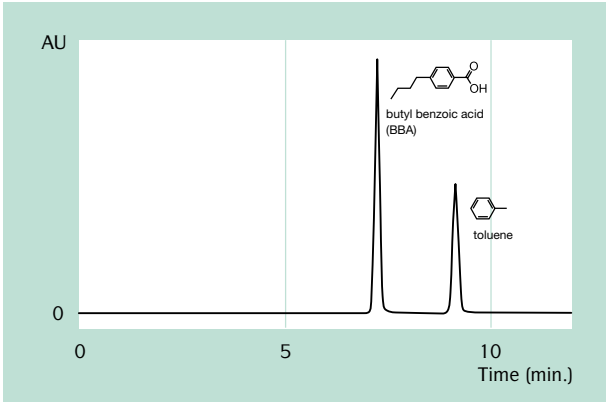

Figure 6 | Separation of butyl benzoic acid and toluene.

Conditions: Column: 4.6 × 250 mm Silica: KR100-5-C18
Mobile phase: ACN/25 mM potassium phosphate pH 3.2 (65/35) v/v.
Flow rate: 1.0 ml/min. Temperature: 20°C Detection: UV 254 nm
Injection sample: Butyl benzoic acid and toluene

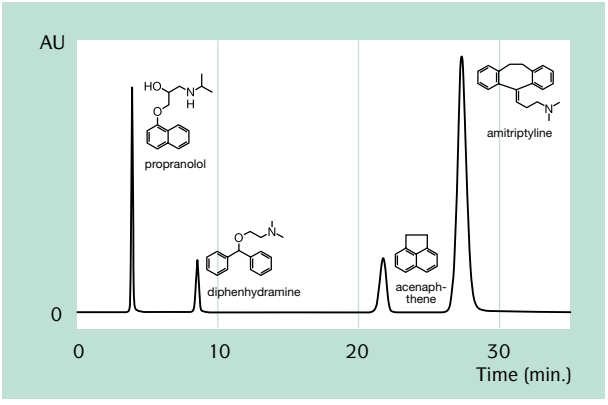

Figure 7 | Separation of propranolol, diphenhydramine, acenaphthene and amitriptyline.

Conditions: Column 4.6 × 150 mm Silica: KR100-5-C18
Mobile phase: MeOH/20 mM potassium phosphate pH 7.0 (65/35) v/v
Flow rate: 1.4 ml/min. Temperature: 20°C Detection: UV 240 nm
Injection sample: propranolol, diphenhydramine, acenaphthene, amitriptyline

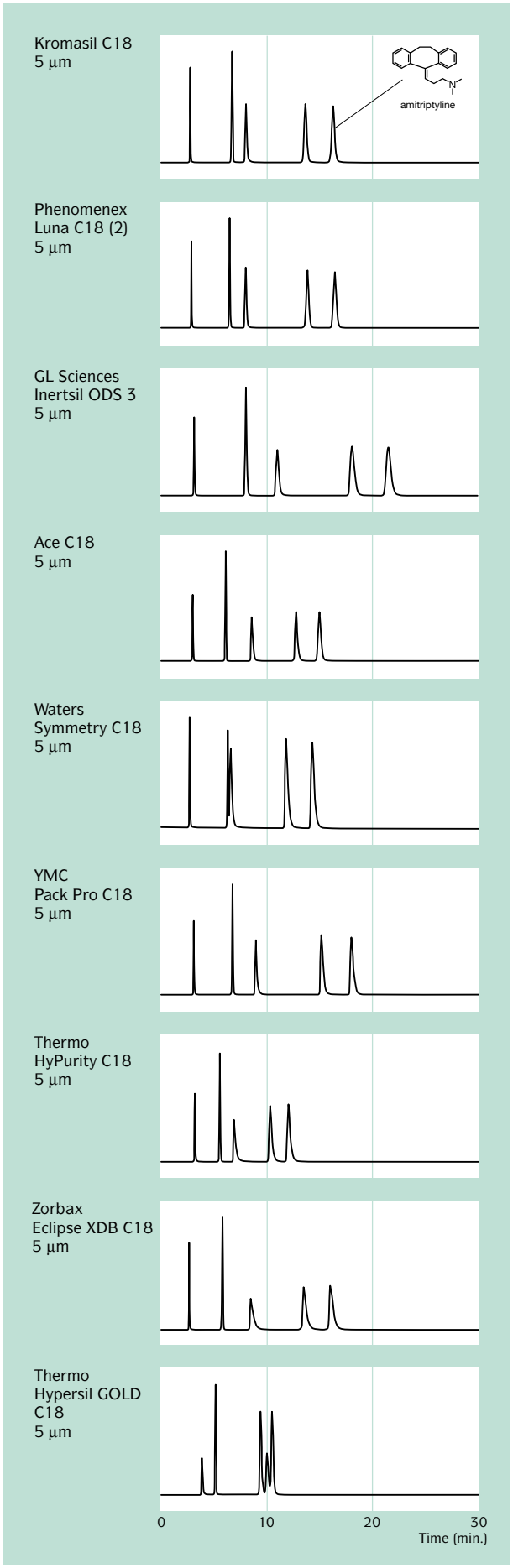

Figure 8 | Chromatograms from the test with anti-depressants (e.g. amitriptyline) at pH 7.

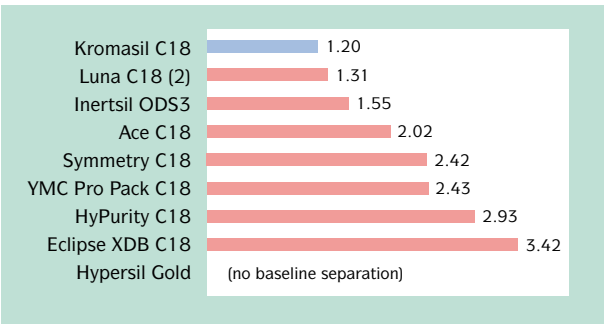

Figure 9 | Summary of the asymmetry factor ($As_{0,1}$) for amitriptyline at pH 7

Conditions: Column: 4.6 × 250 mm Silica: 100 Å, 5 µm, C18
Mobile phase: MeOH/25 mM potassium phosphate pH 7.0 (80/20) v/v
Flow rate: 1.0 ml/min. Temperature: 20°C Detection: UV 215 nm
Injection sample: phenylpropanolamine, toluene, nortriptyline, imipramine, amitriptyline

between the analyte and the stationary phase. Kromasil RP-phases show excellent peak shape for both acidic and basic compounds. Figure 6 shows the chromatogram of butyl benzoic acid and toluene. Figure 7 shows the chromatogram of propranolol, diphenhydramine, acenaphthene and amitriptyline.

Silanol activity – a comparison study

This test is part of our QC protocol, it is performed at pH 7 and the injected mix contains phenyl-propanolamine, toluene and three different tri-cyclic anti-depressants, nortriptyline, imipramine and amitriptyline. Under these conditions most of the acidic silanol groups are deprotonated. From a comparison perspective this is a perfect condition and a great indicator whether you have selected a high performing RP-phase or not.

Due to deprotonated silanol groups the main interaction causing peak tailing is ion exchange. Basic compounds, such as tricyclic anti-depress-ants, are extremely sensitive to such interactions.

The asymmetry factor for the amitriptyline peak is the perfect indicator and therefore we have used it in our comparison study. Figure 8 shows the chromatograms from the investigation and figure 9 shows a summary of the asymmetry factor ($As_{0,1}$) for amitriptyline at pH 7.

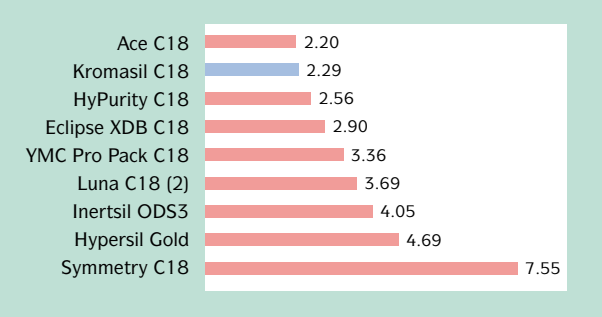

Figure 10 | Summary of the investigated ion exchange capacity

Conditions: Column: 4.6 × 250 mm Silica: 100Å, 5 µm, C18
Mobile phase: MeOH/H_2O/200 mM potassium phosphate pH 2.7 and pH 7.3 (34/90/10) w/w Flow rate: 1.0 ml/min. Temperature: 20°C
Detection: UV 254 nm Injection sample: uracil, benzylamine, phenol

Ion exchange interactions – a comparison study

This comparison study is an ion exchange capacity test performed at pH 2.7 and pH 7.3. In this test we have investigated the change in relative capacity factor ($r_{k'} = k'_{benzylamine}/k'_{phenol}$) between a base (benzylamine) and a slightly acidic compound (phenol), at two different pH.

At pH 7.3 most silanol groups are deprotonated, which results in ion exchange interactions with benzylamine (pKa = 9.3 i.e. positively charged below pH 9.3). The ion exchange interactions result in increased retention and tailing of the benzylamine peak. At pH 2.7 most silanol groups are protonated and the ion-exchange capacity therefore very low, leading to little retention of basic compounds like benzylamine.

The relative capacity factor is used to quantify the ion-exchange capacity at given conditions. In this comparison we study the increase in ion-exchange capacity when going from acidic to neutral pH. This provides a measure of the silanol groups available for ion-exchange interaction in the actual pH-range. The data presented is the ratio between the ion-exchange capacity at pH 7.3 and pH 2.7 ($rk'_{7.3}/rk'_{2.7}$), figure 10.

Chemical stability

Kromasil is well known for its high performance in large scale HPLC. Two critical factors in large scale HPLC are also important factors in analytical HPLC, chemical and mechanical stability. These two factors were fundamental cornerstones when Kromasil was developed. The chemical stability is together with mechanical stability the two most important factors for determining the lifetime of your column. Kromasil is the perfect choice for your difficult separations but at the same time stable enough to be your preferred column for your everyday routine analysis.

At low pH the bonded phase can be hydrolyzed, resulting in a less hydrophobic surface. At higher pH the silica matrix itself can be dissolved, which means that both silica and bonded phase are lost. These processes result in changed retention times and poor peak shape.

In order to show the chemical stability of Kromasil we have performed long term stability tests at pH 2 and pH 10. Both conditions were tested for a period of more than 22,000 column volumes, figure 11 and figure 12.

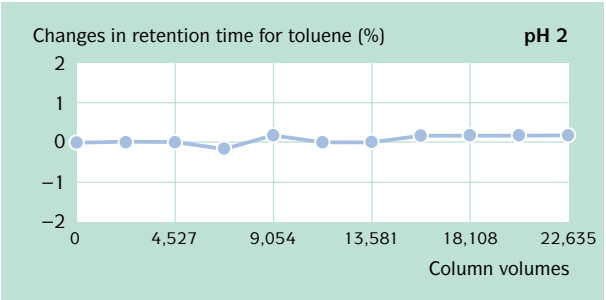

Figure 11 | Long term chemical stability at pH 2 – change in retention time for toluene.

Conditions: Column: 3 × 50 mm Silica: KR100-5-C18
Mobile phase: ACN/H_2O (50/50) v/v 0.1% triflouroacetic acid (TFA)
Flow rate: 1.0 ml/min. Temperature: 20°C Column volumes: 22,635

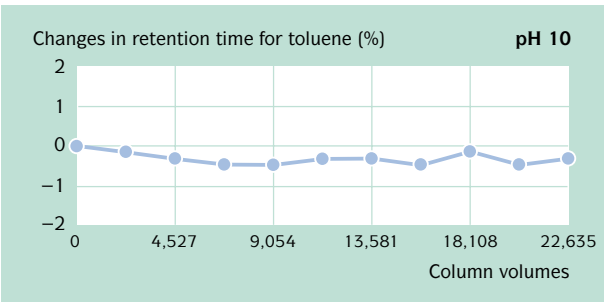

Figure 12 | Long term chemical stability test at pH 10 – change in retention time for toluene.

Conditions: Column: 3 × 50 mm Silica: KR100-5-C18
Mobile phase: ACN/H_2O (50/50) v/v 0,25% triethyl amine (TEA)
Flow rate: 1.0 ml/min. Temperature: 20°C Column volumes: 22,635

Column packing of Kromasil products

Each Kromasil column is separately packed under highest quality conditions. Every column is also individually inspected, tested and released according to our final column specification.

Since Kromasil has such a high mechanical stability, we are able to pack our columns with a very high packing pressure. This guarantees an extremely high column to column reproducibility but also a column with an excellent bed stability.

Kromasil column packing – reproducibility study

In order to visualize this we have packed 10 analytical columns with packing material from the same Kromasil 5 µm C18 batch, table 2.

Column	Retention time for toluene (min.)	$N_{0.5}$ for toluene (plates/m)	$As_{0.1}$ for phenanthrene
1	4.64	111,600	1.01
2	4.60	109,600	1.04
3	4.65	111,600	1.03
4	4.52	108,800	1.03
5	4.65	104,400	1.04
6	4.62	108,400	1.05
7	4.62	109,200	1.05
8	4.53	103,200	1.01
9	4.52	110,800	1.05
10	4.51	110,400	1.01

Table 2 | Packing result when packing ten 4.6 × 250 columns from the same Kromasil batch.

Conditions: Column 4.6 × 250 mm Silica: KR100-5-C18
Mobile phase: MeOH/H_2O (90/10) v/v
Flow rate: 1.0 ml/min. Temperature: 20°C Detection: UV 254 nm
Injection sample: dimethyl phthalate, toluene, biphenyl, phenanthrene

A long term study of the Kromasil bed stability

Chromatographers today run their methods with faster gradients and higher flow rates than ever before. The rapid change in viscosity due to a quick gradient requires a column with a stable bed.

In order to show the bed stability we have performed this test by repeating a short and steep gradient for a period of more than 22,000 column volumes. These conditions, 5 to 95% organic modifier over three minutes, are rather challenging. However, figure 13 and figure 14 show no evidence of change in retention time for toluene or asymmetry factor ($As_{0.1}$) for amitriptyline.

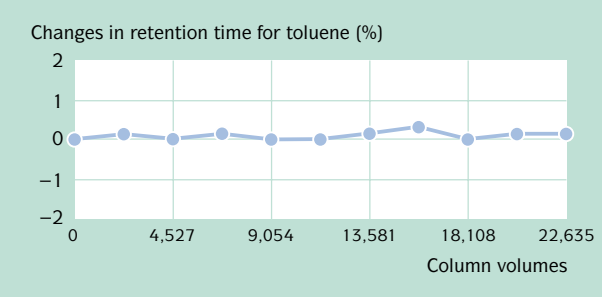

Figure 13 | Long term bed stability test at pH 6.8 – change in retention time for toluene.

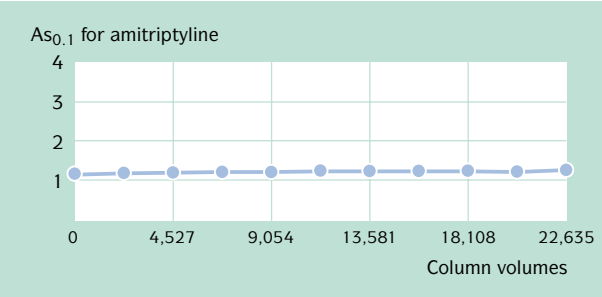

Figure 14 | Long term bed stability test at pH 6.8 – asymmetry factor ($As_{0.1}$) for amitriptyline.

Conditions, fig. 13 and 14: Column: 3 × 50 mm Silica: KR100-5-C18
Mobile phase, A: ACN/10 mM ammonium acetate pH 6.8 (5/95) v/v
Mobile phase, B: ACN/10 mM ammonium acetate pH 6.8 (95/5) v/v
Gradient: 5 – 95% organic modifier over 3 minutes repeated
Flow rate: 1.25 ml/min. Column volumes: 22,635

Column performance – information on each Kromasil column

Each Kromasil column is individually packed and tested according to our rigorous QC program. Every column is supplied with an individual test chromatogram. In figure 15 an example of such a test chromatogram is shown together with the certificate of analysis from that particular batch.

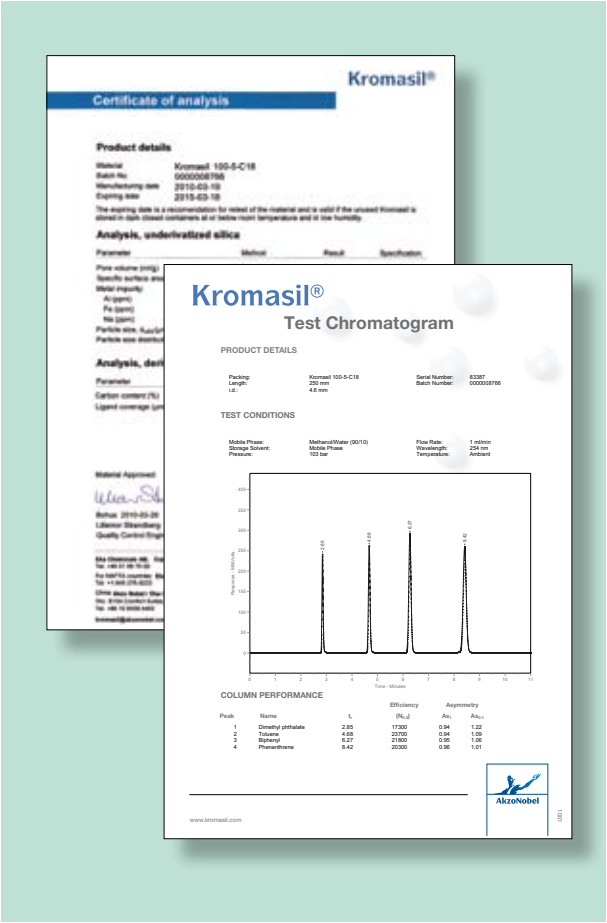

Figure 15 | Information regarding each column we pack, Certificate of Analysis (from the batch) and Test chromatogram.

Asymmetry and efficiency measurements

The test chromatogram shows the efficiency and asymmetry calculations for each compound in the test mix.

The efficiency for each compound is measured at 50% of peak height, figure 16.

$$N_{0.5} = 5.54\ (t_r/W_{0.5})^2$$

The asymmetry factor ($As_{0.1}$) is determined from the front (A) and the back (B) peak widths at 10% peak height, figure 16.

$$As_{0.1} = B/A$$

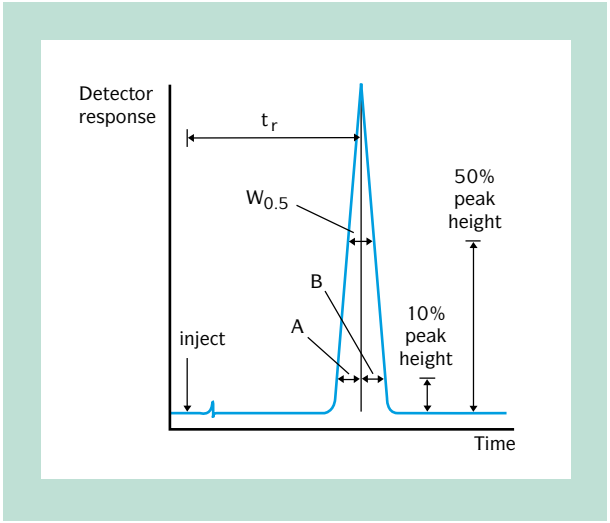

Figure 16 | Measurement of peak efficiency and peak asymmetry.

Quality assurance and quality control of Kromasil products

The QA department takes an active roll in the manufacturing of the Kromasil products. The overall ambition is to continuously develop the process, in order to make better products.

The Kromasil organisation, located in Bohus Sweden, is operating in accordance to ISO 9001:2000 and ISO 14001.

Quality control of raw materials

Each raw material is checked and released according to their specification. The specifications for the critical raw materials are more detailed and the level of impurities much more in focus. A close dialogue is always kept with the supplier of each critical raw material.

IPC – In Process Controls

In order to ensure and maintain the high quality, several in process controls (IPC) have been introduced.

Releasing the final product

Prior to releasing the final product the QA department checks the batch protocol, the deviation report and every specification point on the final specification. The QA department always actively releases the final product with a signature

Batch to batch reproducibility – the evidence

In figure 17 – 20, we have summarized a few critical quality parameters from 50 different batches of Kromasil 5 µm C18.

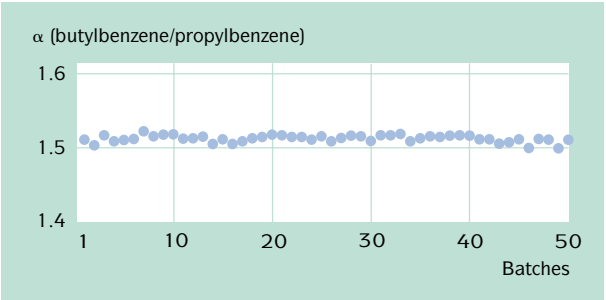

Figure 17 | Batch to batch reproducibility of 50 batches of Kromasil 5 µm C18 with respect to α.

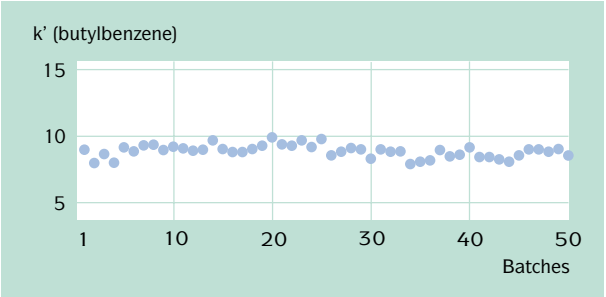

Figure 18 | Batch to batch reproducibility of 50 batches of Kromasil 5 µm C18 with respect to k'.

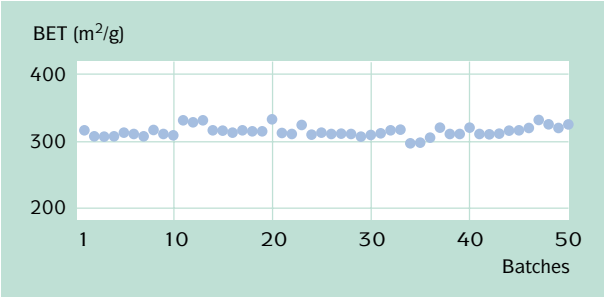

Figure 19 | Batch to batch reproducibility of 50 batches of Kromasil 5 µm C18 with respect to surface area, (BET).

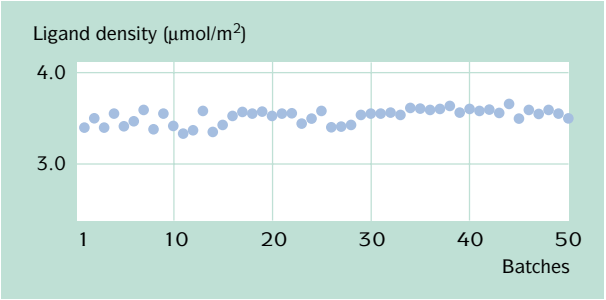

Figure 20 | Batch to batch reproducibility of 50 batches of Kromasil 5 µm C18 with respect to ligand density.

Conditions, figure 17 and 18: Column: 4.6 × 250 mm Silica: 100 Å, 5 µm, C18 Mobile phase: ACN / H_2O (70/30) v/v Flow rate: 2.0 ml/min. Temperature: 20°C Detection: 254 nm

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 60 years, Eka Chemicals has 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, or in high-pressure slurry-packed columns. The development, production and marketing of Kromasil are ISO 9001 certified.

Eka Chemicals is a global company with 2,900 people and production in 18 countries. It is a business unit within AkzoNobel, one of the world's largest chemical groups, with more than 60,000 employees in 80 countries.

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