Twelve years of silica-based HPLC purification with focus on peptides – an overview

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Kromasil®

The way to peak performance in liquid chromatography

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Abstract

The field of peptide purification in large scale is presented from an HPLC silica manufacturer's experience. The market development and the trend regarding equipment for HPLC is overviewed.

The importance of different cost factors in chromatographic processes is discussed as well as practical considerations in large scale HPLC.

Introduction

The business concept of Separation Products as shown in figure 1 is mainly focused on the preparative HPLC market. Our analytical grade silica is to a large extent sold through distributors. A large part is sold OEM often in other names.

Kromasil, our high performance HPLC silica, was introduced in 1988 just when the peptides market started to grow. Insulin was at that time purified by the use of smaller silica particles, say 10 up to 15 micron in size, in 30 cm diameter columns. This was the largest diameter of columns with so called dynamic axial compression for HPLC. World wide there were not many such columns the first being installed at Schering in Berlin.

In this very small niche of the silica based HPLC market we reached a world leading position a couple of years ago.The by far largest number of customers are in the peptides field and we now see a dramatic growth in number of substances and consumption of silica.

If I may extend the definition slightly to include insulin and growth factors in "peptides", more than half of the sales of high performance silica for industrial scale is in this market.

We sell Kromasil with a lot of technical support and have consequently learned much during the years. Part of what is not confidential I will share with you in this presentation.

High performing silica-based chromatography material in bulk and columns for best total economy in the purification of pharmaceuticals.

Figure 1 | Business concept, Separation Products.

Peptides purification

Examples of peptides including insulin that are purified by HPLC in large scale is shown in figure 2. Purification of insulin represents the by far largest use of industrial HPLC in the world. Now columns of sizes up to 60 cm in diameter are used and even larger are planned. The major players are Ely Lilly, Novo Nordisk, Aventis, Diosynth and Biobras.

For insulin 100 – 600 runs can be made on one packing of silica and the lifetime is extended by regeneration of the silica e. g. with 0.1 molar sodium hydroxide in alcohol or acetonitrile. For most peptides often tens of thousands of runs can be made without changing the silica.

In the next coming years the volume of produced insulin will increase dramatically. Activities are in late stages to introduce new products where insulin is administrated by spray inhalation by the patient. The dose then has to be 3–5 times larger depending on technique. Diabel (a joint venture between Aventis and Pfizer) is created to commercialize such a product.

Another example is growth factors like IGF and HGH. Not all commercial growth factors are purified by silica-based HPLC, but as demand of higher purity for new products is coming the use will increase.

There is a very large and increasing number of smaller peptide building blocks and final substances on the market and I can only show a few of them. On this list you can find both generics and new very interesting drug substances. Some newly developed drug substances are in stages where of course the information is confidential.

Insulin

- First large application: Lilly, Novo Nordisk, Aventis, Diosynth, Biobras
- 100 – 600 injections per batch of silica
- Regeneration to improve life time of the silica
- Strong growth

Growth factors

Examples IGFs and HGH.
Silica based HPLC not always used.

Other peptides

Desmopressin, Calcetonin and Somatostatin	UCB
Eptifibatide	COR Therapeutics
Abarelix API	Praecis
Decapeptyl, Lanreotide	Kinerton
Somatostatin, Oxytocin	PolyPeptide
Gosereline	AstraZeneca
Bivalirudin	The Medicine Company

Figure 2 | HPLC purification of peptides.

Peptide companies and capacities for custom purification

In figure 3 I have listed the most important companies involved in purification of peptides with capacity for high performance HPLC at a minimum scale of 20 cm diameter columns with dynamic axial compression. I present them in alphabetical order. Most of the companies listed have advanced peptide manufacturing technology. Very often an American company is behind a peptide drug and the substance is purified in Europe.

Columns diameter up to:

Avecia	20 cm
Diosynth	45 cm
Kinerton	30 cm
Mallinckrodt	30 cm
Peptisyntha	45 cm
PolyPeptide	45 cm
Schering AG	Can have spare purification capacity. Columns of diameter up to 45 cm
UCB	60 cm

Figure 3 | Companies specialized in peptides and/or custom purification (DAC columns).

Hardware for large scale HPLC

Figure 4 shows the largest and till date only 80 cm diameter high pressure column with dynamic axial compression. It was installed last October at BMS at Swords, Ireland and it gives 42,000 plates per meter with 10 micron silica. The symmetry is similar to what is achieved in analytical columns. Figure 5 summarizes the present situation on hardware.

Figure 4 | The BMS plant at Swords.

Dynamic axial compression always for diameters above 5 cm

Market share above 80%
Novasep/Prochrom
Own construction
Danprocess

High pressure slurry packed columns

5 cm diameter (and smaller)
Modcol

Figure 5 | Hardware for HPLC.

It is essential that for high efficiency silica a column with dynamic axial compression is used if the diameter is above 5 cm. The two market leaders, Prochrom and Novasep, merged recently and they have the by far largest share of the market. Another important supplier of systems with dynamic axial compression is Dan-Process. The key patents for dynamic axial compression have expired so we could expect more suppliers of this type of columns, one being ModCol.

Performance is often better in these columns compared to analytical ones.

It is very difficult to high pressure slurry pack 5 cm diameter columns with silica particles of about 10 micron size and it is not possible to pack wider columns. Till date ModCol is the only company with the ability to have nearly analytical performance in 5 cm diameter, but we hear about more companies to manage this.

High performance silica for large scale

Spherical silica with particle size depending on application of 10, 13 or 16 micron is dominating today. If the silica is destroyed by impurities in the feed or only to be used for, say 10 runs it is probably more economical to use a cheaper silica. We do not sell much 16 micron and most of our customers are using 10 micron.

Important features as shown in figure 6 are in priority order (and you need all of them):

Important features:

- Mechanical strength
- Chemical stability
- Surface properties
- Efficiency
- Accessible surface area
- Scalability
- Available in quantities

Figure 6 | High performance silica for industrial use.

- *Mechanical strength:* the wider the column the more important. The walls in analytical columns take up most of the pressure, but that is not the case above 5 cm in diameter. The larger the pores the more brittle is the silica and also the lower is the specific surface area. The key is to make a strong silica without sacrificing surface area. If the silica breaks down you get fines that clog the frit. This causes high back pressure, uneven flow profile and the silica fines can also contaminate the final product. With derivatized silica you can also get free silica surface causing tailing and leakage.

■ *Chemical stability:* Important for lifetime of the bed, but much more to avoid leakage that can contaminate the final product. We have a customer support file specifying very sensitive analytical methods and leakage data at different conditions. Specially regarding peptides you often like to start your gradient with pure water, which you cannot do with ordinary hydrophobic phases. You need about 5% of acetonitrile to wet the silica. There are now many "aqueous" or wettable phases on the market but as we found not yet anyone which is sufficiently stable. Chemical purity of the silica is very important for stability and also for surface properties.

■ *Surface properties:* you need of course selectivity and no tailing of the peaks.

■ *Efficiency:* of course you need plates also under heavy overload.

■ *Accessible surface:* if you do not have a narrow pore size distribution you will have micropores which are not accessible. There is always an optimal pore size for a certain molecular size. Use the smallest pores possible. Otherwise you loose surface area and get a too brittle silica. 100 Å is often the best choice for the purification of insulin.

■ *Scalability:* It is good if the silica is made in the same way in all particle sizes, the best if it is made in one batch and then fractionated. Many silica products are different for prep scale compared to analytical though they have the same brand name.

■ *Available in large quantities:* if you develop a method in smaller scale you have a lot of silica to compare. If it happens that the process is scaled up you need reliable supply of larger quantities. Otherwise you have to reevaluate the whole procedure. If it is very important you should visit the supplier also to make sure he is a manufacturer of the silica.

KromaGuide

When a chromatographic process is developed the most important part is the experimental work to find the best gradient/mobile phase and stationary phase. Alpha value and retention capacity has a very big impact on your costs.

Then it is to find the best running conditions and select size of column etc. To make this easier we have developed a software, KromaGuide where the evaluation is based on experiments in analytical scale, given restrictions for the HPLC system and the required purity and yield. KromaGuide can optimize economy or simulate importance of the different cost parameters.

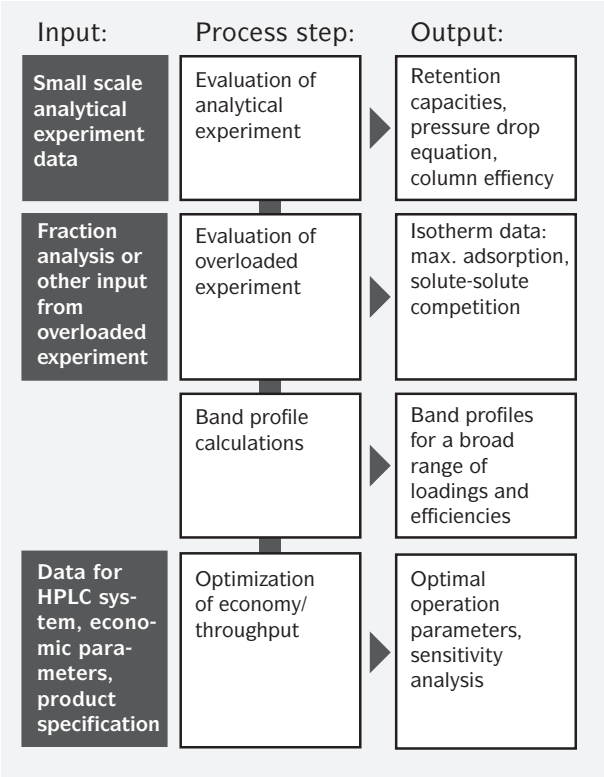

Figure 7 | KromaGuide HPLC optimization process.

KromaGuide is mainly applied to isocratic HPLC but gives good indications also for gradient runs. You need three analytical injections with different flow rates. This gives you pressure/flow rate relations, retention capacities and efficiency.

The software is demonstrated in figure 7. Two or three runs around the expected optimal loading give you information about self-displacement (where different components in the feed compete at the same sites on the silica) and the isotherms.

If you then put in restrictions of your hardware KromaGuide can calculate how much to load, when to cut fractions, flow rate, bed length and give you estimated costs divided on the different cost factors.

KromaGuide is not for sale but used in our customer support for Kromasil.

Sensitivity analysis

Figure 8 demonstrates a typical sensitivity analysis. It is a 90:10 feed with k'(1) 1.5 , first compound purified to 99.5% with 13 micron silica in a 30 cm diameter column. It is 100% self-displacement. You can see that solvent as nearly always represents more than half of the costs.

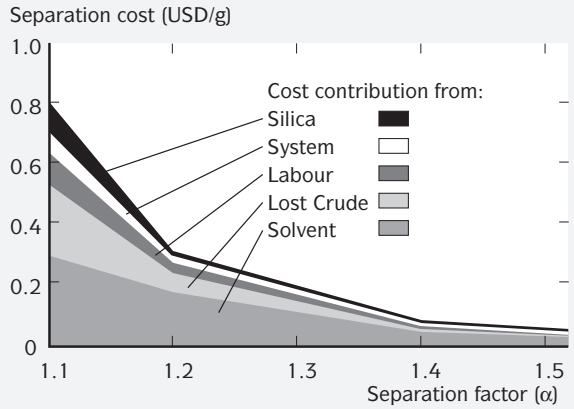

Figure 8 | Separation factors influence on cost.

The silica, also as nearly always, represents a very small fraction of the costs. This means of course that it is worth selecting the best silica because it will strongly influence all other costs. The alpha value is very important and you can see from figure 8 that if you increase alpha from 1.3 to 1.4 you reduce your costs to less than half in this example.

Practical aspects

The performance of the column is very important. Make a test with toluene for RP to be sure that you have the plates and symmetry. Figure 9 shows the test we performed after having packed the 80 cm diameter Prochrom column at BMS, at Swords.

The most common mistake when purifying peptides is to condition the column with water or to start the gradient with 100% water. Always have at least 5% organic modifiers when you start the gradient in RP mode (C4, C8 or C18). Otherwise you will have wetting problems and can even have break-through (no retention) of the compounds. The reason for this breakthrough is that the mobile phase does not enter into the pores.

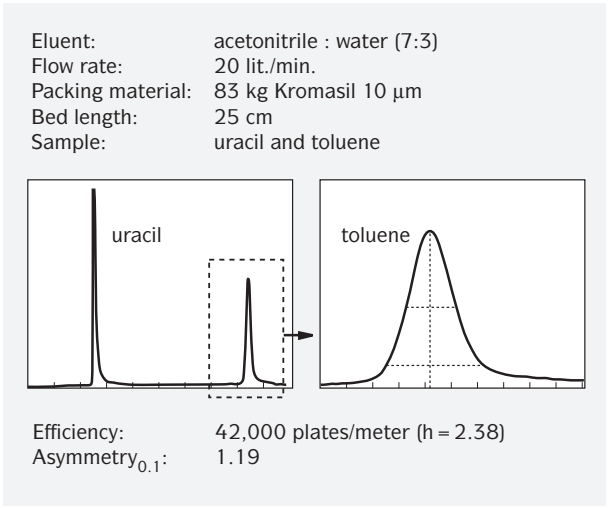

Figure 9 | Test of the column at BMS, Swords.

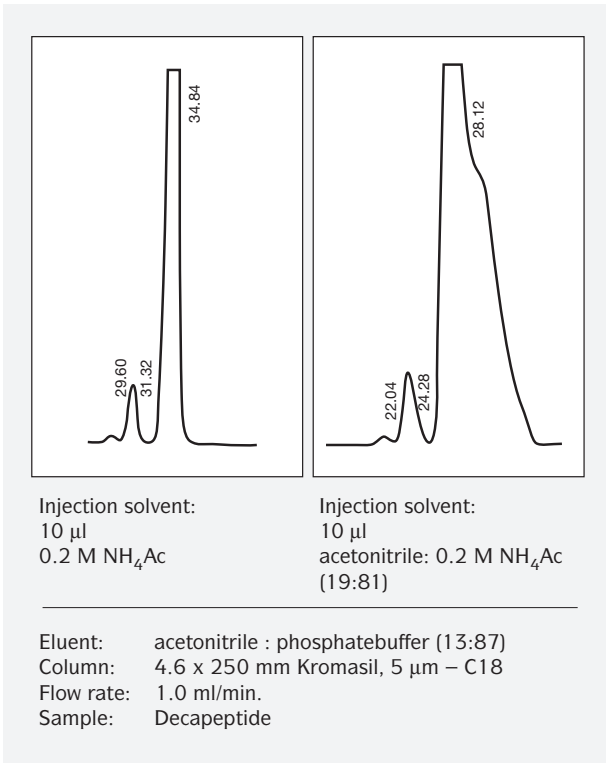

Figure 10 | Solvent injection effect.

We have also seen clogging of the outlet frit due to precipitation of a substance. In the column the compound is in equilibrium between the stationary and mobile phase. When it leaves the column the concentration goes up and a precipitation can occur.

Another very common cause of bad performance is solvent injection effects. If you use a solvent for your feed which is too strongly eluting the peaks can easily be destroyed as is shown in figure 10. Here the retention is shorter and you

also have a double peak when using too much acetonitrile as injection solvent.

Thermal effects on peak shape are also common but more pronounced at diameters around 5 cm. A temperature gradient radially can cause double peaks due to uneven flow distribution.

Not so common is volume overload. It should of course not happen since it is so easy to calculate the maximum volume of solvent to load with the sample.

Recommendations

Finally figure 11 summarizes our recommendations for problem free large scale HPLC:

Always DAC columns – and pack them with instruction from the silica manufacturer, for example at the highest possible pressure and in a stable slurry.

Check the mechanical strength of the silica, you do not see anything in narrow columns but could experience pressure problems after having scaled up.

Pancake chromatography, short beds and frequent loadings.

Remember for example the impact on cost of the alpha value. Experimental work in small scale pays off.

Avoid solvents for your feed with too high elution strength.

A small impurity in the front is often pushed out by self-displacement from the main component and therefore it pays off to increase loading in this case. A small component in the tail is dragged in under the main peak (so called tag-along) and you have to avoid too heavy overload.

Recommendations

1. DAC for columns of diameter 5 cm and larger
2. Mechanically very rigid silica particles, spherical with narrow particle size distribution
3. Silica particle size 10 µm, 13 µm or 16 µm, depending on separation factor
4. Bed lengths 20 to 50 cm
5. A carefully performed experimental work. α, k', c_s^{max}
6. Caution when injecting the sample in other solvents than the mobile phase
7. Utilizing positive displacement effects and avoiding negative ones

Figure 11 | Recommendations for problem free large scale HPLC.

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 3,000 people in 30 countries. It is a business unit within Akzo Nobel, one of the world's largest chemical groups, with more than 67,000 employees in 80 countries.

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