

NLAB Saga®



NANOLOGICA

A New Generation  
Silica for Purification  
by Chromatography

# CONTENT

About Nanologica	3
About NLAB Saga®	4
Product information	6
Adsorptive properties	7
Mechanical stability	8
Loading capacity	9
Chemical stability	10
Example of insulin purification	13
Example of cost reduction	15
Order information	16
Contact	17

---

# ABOUT NANOLOGICA

Nanologica is a Swedish life science tools company that provides advanced consumables to pharmaceutical manufacturers.

The company was founded in 2004 and stems from research connected to Dr. Alfonso Garcia-Bennett's research on the synthesis, analysis, and characterization of new porous materials. With a foundation in materials science and nanotechnology, the company has developed an expertise in chromatography.

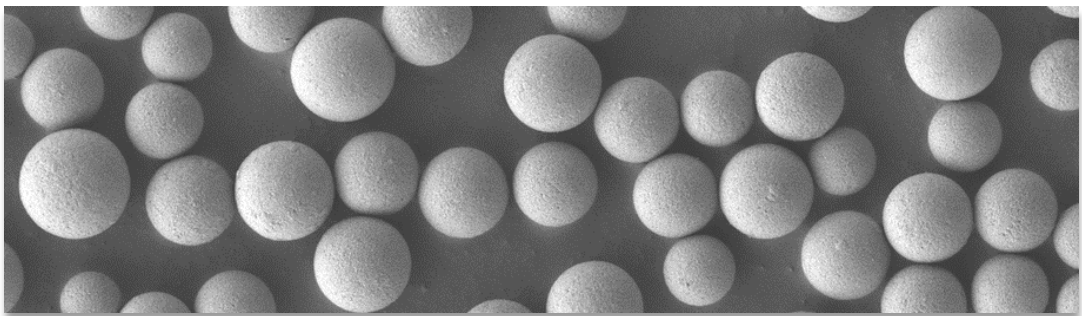
Today, Nanologica is one of the world-leaders in the development of nanoporous silica particles for purification by chromatography. Through a proprietary manufacturing method, we can precisely control the shape, size, porosity, and surface properties of silica particles. Full control of the silica manufacturing process also enables an exceptional batch-to-batch reproducibility with excellent properties for the purification of peptides.

Nanologica's main product, the NLAB Saga® silica media, has been especially developed for the purification of peptide drugs. Due to outstanding mechanical and chemical stability, NLAB Saga® is an excellent choice for the purification of insulin and GLP-1 analogues. By making the purification processes more efficient and having a long lifetime, NLAB Saga® can increase productivity and significantly reduce manufacturing costs for pharmaceutical manufacturers.

In addition to the silica media NLAB Saga®, Nanologica carries a range of other purification media, as well as analytical HPLC columns sold under the brand name SVEA®.

Nanologica's mission is to provide products that streamline workflows and reduce manufacturing costs for pharmaceutical manufacturers, with the goal of increasing the availability of cost-effective drugs to enable more patients around the world access to vital treatments for diabetes and obesity.

The company is headquartered in Södertälje, Sweden and Nanologica's share (NICA) is listed for trading on Nasdaq Stockholm Main Market.



---

# NLAB SAGA® - SILICA FOR PREPARATIVE CHROMATOGRAPHY

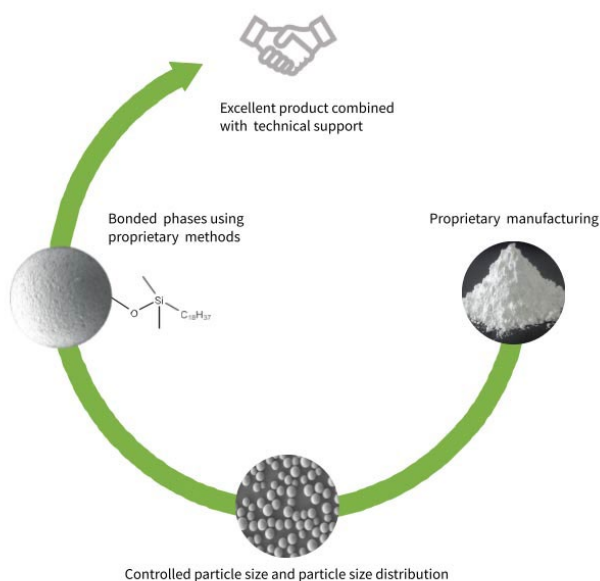
NLAB Saga® has been specifically developed to meet the strict requirements of industrial scale purification by chromatography. It has a superior mechanical and chemical stability together with a high available surface area and ligand density, in addition to narrow pore size distribution. This, combined with controlled particle size distribution, results in silica with a high loading capacity and low back pressure.

Due to its exceptional mechanical and chemical stability, NLAB Saga® is an excellent choice for the purification of peptides such as insulin, insulin analogues and GLP-1 analogues.

Nanologica's proprietary manufacturing process, combined with dedicated and experienced technical support, makes NLAB Saga® an excellent choice for your purification needs – it is a new generation of silica.

Nanologica has full control of the entire manufacturing process, from raw material to finished product. NLAB Saga® has been tested and used worldwide by pharmaceutical companies manufacturing APIs at industrial scale where quality, performance and durability are uncompromisable.

The high efficiency and long lifetime of the product enables lower production costs for an improved total economy for the manufacturer, making NLAB Saga® the most cost-effective alternative for large-scale purification of peptides.



---

# NLAB SAGA®

- Perfectly spherical, fully porous silica
- Exceptional chemical stability at high and low pH
- Superior mechanical stability
- Outstanding loading capacity
- Smooth surface with evenly distributed silanol groups
- Tightly controlled particle size
- High purity silica - very low metal content
- High carbon content
- Available in various particle sizes

ALL SUPPORTIVE  
DATA IS AVAILABLE  
IN THE NLAB SAGA®  
REGULATORY  
SUPPORT FILE



# PRODUCT INFORMATION

## NLAB Saga® 100-10-SIL

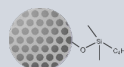
Pore size 100 Å  
Particle size 10 µm  
Phase Bare silica  
-  
USP L3



Article # S700S1

## NLAB Saga® 100-10-C4

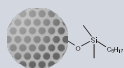
Pore size 100 Å  
Particle size 10 µm  
Phase C4  
Butylsilane  
USP L26



Article # C700S1

## NLAB Saga® 100-10-C8

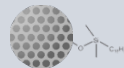
Pore size 100 Å  
Particle size 10 µm  
Phase C8  
Octylsilane  
USP L7



Article # B700S1

## NLAB Saga® 100-10-C18

Pore size 100 Å  
Particle size 10 µm  
Phase C18  
Octadecylsilane  
USP L1



Article # A700S1

## NLAB Saga® 100-13-SIL

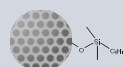
Pore size 100 Å  
Particle size 13 µm  
Phase Bare silica  
-  
USP L3



Article # S800S1

## NLAB Saga® 100-13-C8

Pore size 100 Å  
Particle size 13 µm  
Phase C8  
Octylsilane  
USP L7



Article # B800S1

## NLAB Saga® 100-5-SIL

Pore size 100 Å  
Particle size 5 µm  
Phase Bare silica  
-  
USP L3



Article # S500S1

## NLAB Saga® 100-3.5-SIL

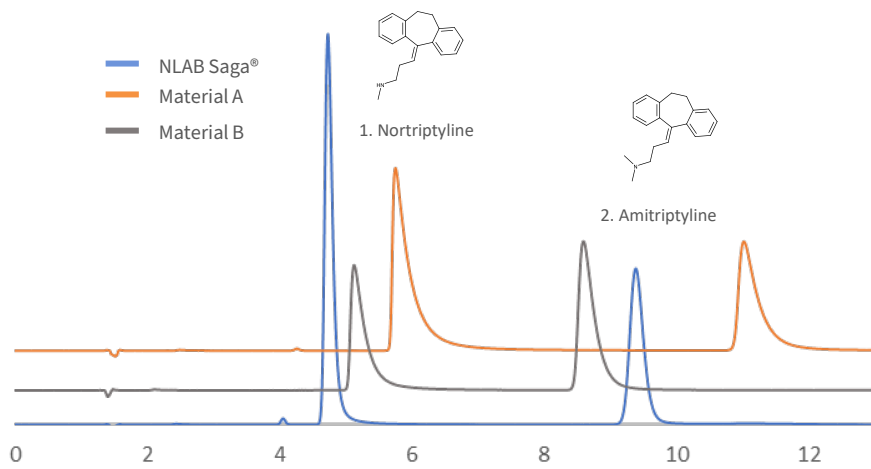
Pore size 100 Å  
Particle size 3.5 µm  
Phase Bare silica  
-  
USP L3



Article # S300S1

# ADSORPTIVE PROPERTIES

Chromatography reveals superior adsorptive properties for NLAB Saga® compared to other silica materials, due to NLAB Saga® having a fully homogenous and smooth surface, a high and evenly distributed ligand density as well as a low content of metals.



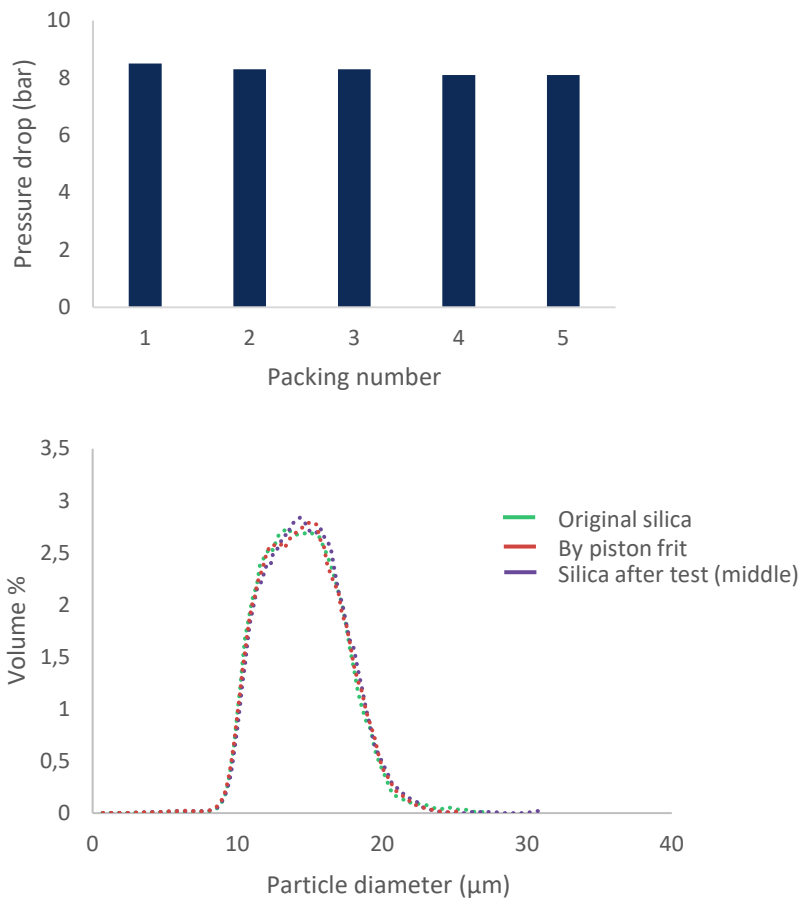
Mobile phase:	Methanol/25 mM K-phosphate pH 7.0 80/20	<b>Specifications</b>	<b><math>\alpha</math>(Nor/EB)</b>	<b>Tf(Nor)</b>	<b>Tf(Ami)</b>
Column:	150x4.6mm C18 5 $\mu$ m	NLAB Saga®	0.90	1.9	1.4
Flow rate:	1.0 ml/min	Material A	1.13	3.6	2.2
Temperature:	30 °C	Material B	1.33	3.4	2.2
Detection wavelength:	210 nm				

The primary cause of peak tailing (Tf) is the occurrence of more than one mechanism of analyte retention. Secondary analyte interactions, with ionized silanol groups on the silica surface, give rise to peak tailing. These interactions need to be minimized to achieve superior peak shapes and this study indicates that NLAB Saga® has minimal secondary interactions.

---

# MECHANICAL STABILITY

Mechanical strength tests were performed in a 50mm ID DAC column at 100 bar piston pressure. Analytical results show no mechanical degradation of the silica under high pressures. NLAB Saga® has an excellent mechanical stability due to our proprietary process and pore volume.

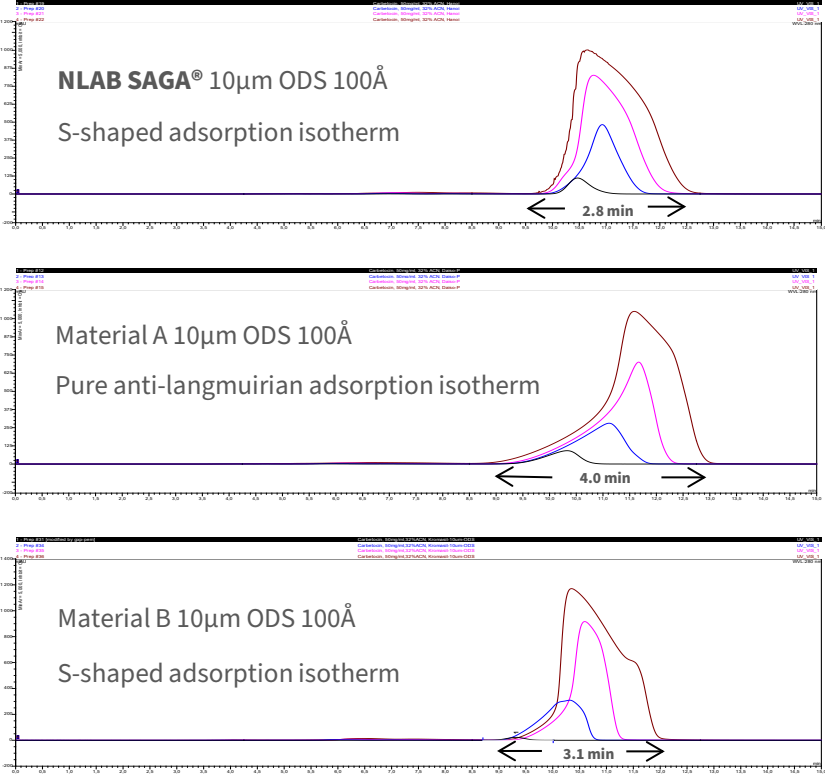


Packing and unpacking of silica in a DAC column at 100 bar is a generally accepted test of mechanical strength of silica. A DAC column with an internal diameter of 5 cm was used. 5 packings/unpackings to 100 bars were made with the same silica slurry. The mechanical stability test was performed on unbonded silica.

Minimum back pressure drop over packing cycles and unchanged particle size distribution indicates a high mechanical stability of NLAB Saga®. This is also shown by size distribution staying the same for native silica and used silica.

# LOADING CAPACITY

NLAB Saga® has an outstanding loading capacity due to a high available surface area and absence of micropores, as well as a homogenous surface with narrow pore volume distribution.



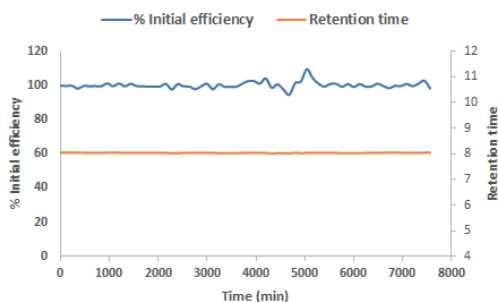
Loading comparisons from analytical up to preparative scale of an 8 A.A. cyclic peptide shows the narrowest band broadening for NLAB Saga®, indicating NLAB Saga® having the highest loading capacity.

# BASIC AND ACIDIC CHEMICAL STABILITY FOR C18

NLAB Saga® shows excellent durability in harsh acidic as well as harsh basic conditions. Both efficiencies and retention times remain almost unaffected even after more than 7 000 column volumes, as shown in the stability tests below. Test were performed on SVEA® columns packed with NLAB Saga® C18.

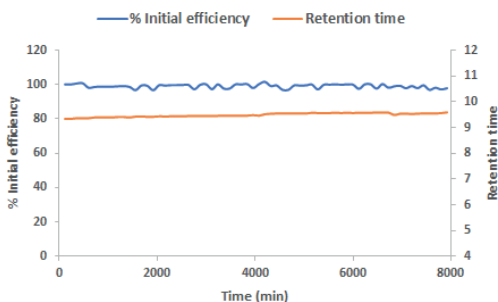
## ACIDIC CONDITIONS

<b>Column</b>	SVEA® C18 Gold 100x4.6 mm 5 µm	<b>Gradient cycle</b>	10-90% B in 5 min
<b>Mobile Phase</b>	A - 1% TFA in water, pH0.9 B - 1% TFA in acetonitrile		90% B for 2 min 90-10% B in 1 min 10% B for 2 min
<b>Flow Rate</b>	1.0 ml/min		
<b>Temperature</b>	60°C		
<b>Analyte</b>	Ethylbenzene		



## BASIC CONDITIONS

<b>Column</b>	SVEA® C18 Gold 100x4.6 mm 5 µm	<b>Gradient cycle</b>	10-90% B in 5 min
<b>Mobile Phase</b>	A - 10 mM ammonium bicarbonate, pH9.6 B - Acetonitrile		90% B for 2 min 90-10% B in 1 min 10% B for 2 min
<b>Flow Rate</b>	1.0 ml/min		
<b>Temperature</b>	45°C		
<b>Analyte</b>	Progesterone		



---

# BASIC CHEMICAL STABILITY FOR C8

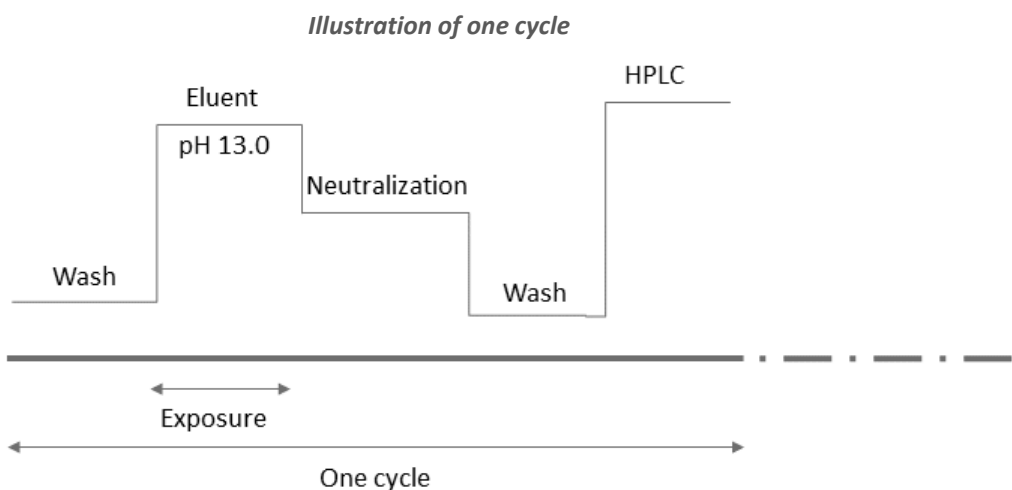
High chemical stability under basic pH is important in insulin manufacturing. During the purification cycles, silica becomes contaminated by fibrillated insulin which results in the increase of the backpressure and a decline in purity/yield.

To regenerate the column, CIPs (Cleaning In Place) procedures are used. These cleaning steps are usually performed at a very high pH. Such conditions are harsh for the silica. Therefore, it is of great importance that the silica survive under high pH conditions in order to have a long-lasting product.

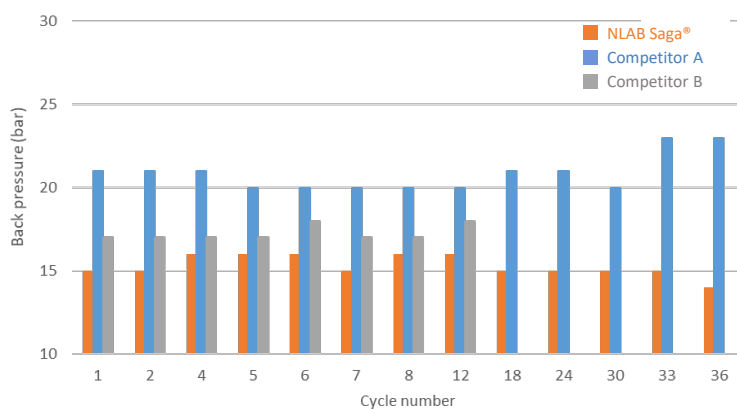
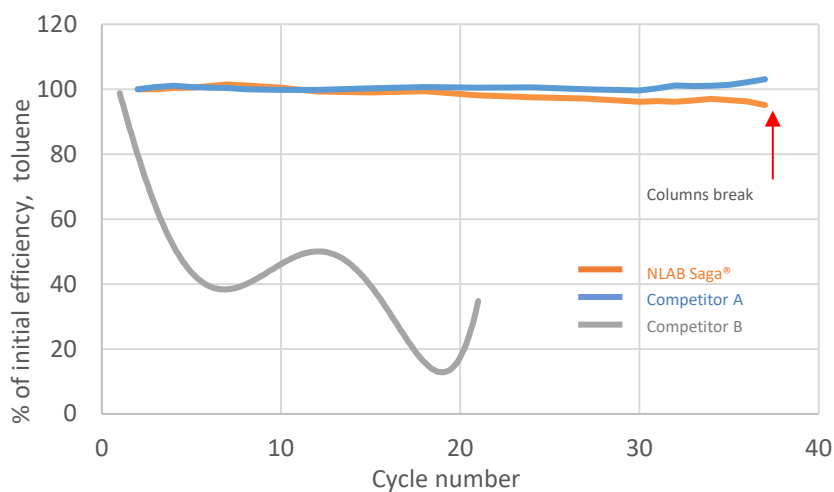
## Alkaline regeneration test designed to mimic wash cycles

### Test conditions:

- Wash: 95/5 MeOH/H<sub>2</sub>O, 10 CV
- Eluent: 60/40 MeOH/100mM NaOH, pH 13.0, 12 CV
- Temperature: Ambient cycle 1-30. **From cycle 30 and onwards 50°C**
- Neutralization: 90/10 MeOH/1%AcOH, 10CV
- Flow rate: 1ml/min
- Chromatographic evaluation



Comparison of NLAB Saga® 13 µm C8, Competitor A 13 µm C8 and Competitor B 10 µm C8 in terms of alkaline stability shows that NLAB Saga® and Competitor A performs consistently well and withstand the harsh experimental conditions, while Competitor B is falling behind.



**Chromatographic test conditions:**

**Mobile phase:** 80/20 MeOH/25 mM K-phosphate, pH 7.0

**Flow rate:** 1 ml/min

**UV:** 210 nm

**Temperature:** 30°C, after 30 cycles the temperature was raised to **50°C** to speed up the experiment

**Analytes:** Uracil, Toluene

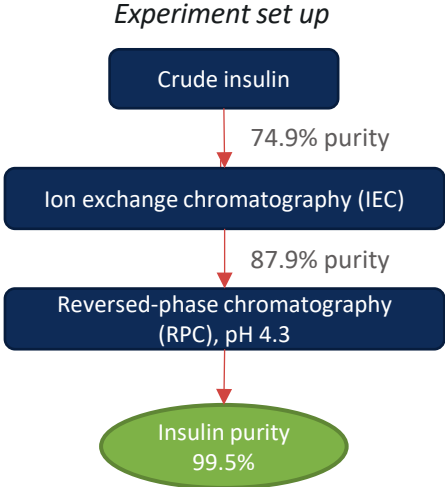
*Agilent 1100 system used for chromatographic evaluations and Shimadzu LC-20AD stand alone pump were used for regeneration simulation. The tests were performed at Nanologica's lab in Södertälje, Sweden.*

# EXAMPLE OF INSULIN PURIFICATION

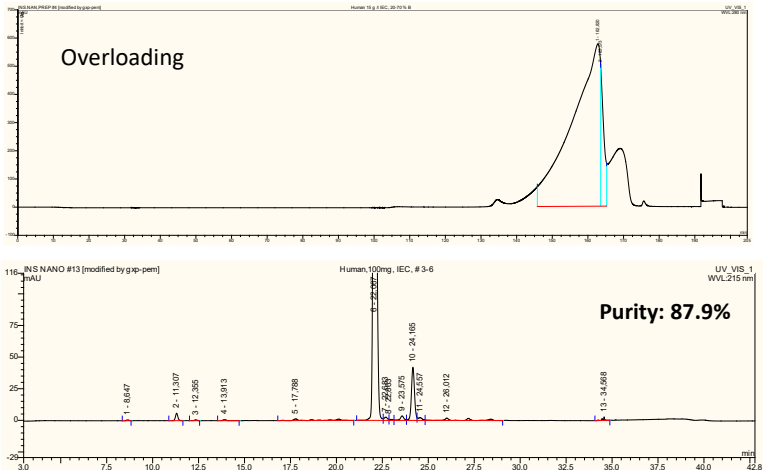
A comparison experiment between Nanologica’s NLAB Saga® C8 13µm and three competitors (A, B, and C) was run. The target for the experiment was to reach the required purity threshold of 99.2% as set per the USP. The same quality of crude insulin was used in all trials. Overloading conditions as well as loading amounts, particle size and functionality were kept the same for all silica materials.

As a first step, ion exchange purification was performed. Ion exchange has an orthogonal selectivity to reversed-phase chromatography purification and removes impurities that are difficult or cannot be removed by reversed-phase chromatography.

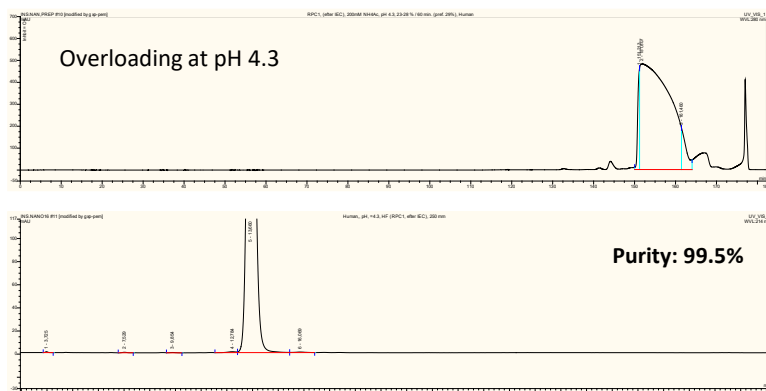
Insulin purity was measured before and after one step of reversed-phase purification.



## *Ion exchange purification*



## Reversed-phase chromatography pH 4.3



### Chromatographic test conditions:

- Loading: 0.37 g/cm<sup>2</sup> column cross-sectional area (15 g/liter)
- Yield: 90% ± 1% in all steps
- Slope of the gradient: (0.083% /minute)
- Linear flow rate: 180 cm/h
- Organic solvent: Acetonitrile

### Summary of results

Company	Phase	USP method Purity (%)	Backpressure* (Bar)
Nanologica	NLAB Saga® C8	99.50	15.5
Competitor A	C8	97.08	10.2
Competitor B	C8	98.56	19.8
Competitor C	C8	98.82	21.5

NLAB Saga® surpasses the set USP target by reaching a purity of 99.5% after one step of reversed-phase purification. The competitors A, B and C do not reach the USP target, meaning they need one more step of purification to pass the USP threshold.

Thus, NLAB Saga® needs fewer steps to reach the USP purity target, leading to a lower manufacturing cost for the insulin manufacturer.

# EXAMPLE OF COST REDUCTION

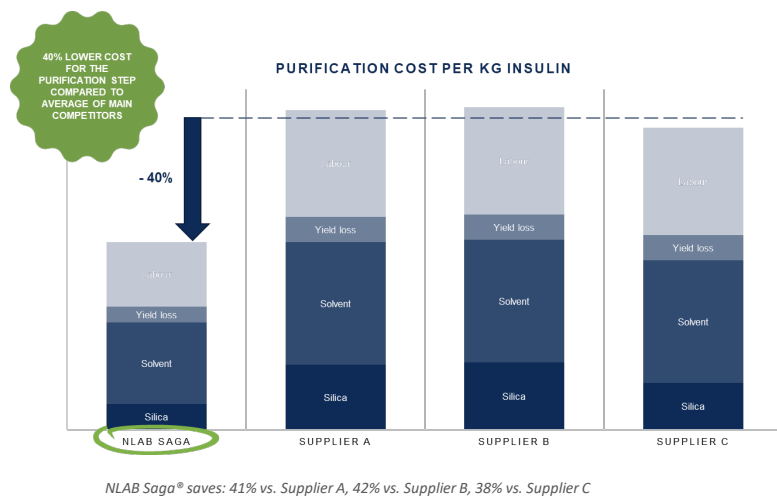
Results of reversed-phase chromatography (RPC) insulin purification experiments showed that by using NLAB Saga®, insulin targeted purity was achieved after one RPC step, whereas for competitors A, B, and C one more RPC step was required.

Experimental results were used in the cost calculation model. The cost model has been developed describing the flow in the manufacturing process for the purification of insulin, including use of material (e.g., solvent) and labour cost\*. The cost saving in the purification step has then been applied to the total manufacturing cost for insulin to show the overall cost saving potential. Assumptions are based on published\*\* and internal data.

## Results of RPC insulin purification experiments

	NANOLOGICA	COMPETITOR A	COMPETITOR B	COMPETITOR C
Phase	NLAB® C8	C8	C8	C8
Insulin purity (%), according to USP method	99.50%	97.08%	98.56%	98.82%
		Do not reach purity threshold of 99.2%		

## Cost comparison using a cost calculation model



- ✓ Excellent performance compared to competitors
- ✓ Target purity of final product was reached with fewer purification cycles than competitors
- ✓ Higher lifetime and lower manufacturing cost

# NLAB SAGA® ORDER INFORMATION

<i>Product</i>	<i>Pore Size</i>	<i>Particle Size</i>	<i>Phase</i>	<i>Article Number</i>
NLAB Saga 100-10-SIL	100 Å	10 µm	SIL	S700S1
NLAB Saga 100-10-C4			C4	C700S1
NLAB Saga 100-10-C8			C8	B700S1
NLAB Saga 100-10-C18			C18	A700S1
NLAB Saga 100-13-SIL		13 µm	SIL	S800S1
NLAB Saga 100-13-C8			C8	B800S1
NLAB Saga 100-5-SIL		5 µm	SIL	S500S1
NLAB Saga 100-3.5-SIL			3.5 µm	SIL



NANOLOGICA



Website

Nanologica AB  
Forskargatan 20 G  
SE-151 36 Södertälje  
Sweden  
+46 8 410 749 49



Main contact