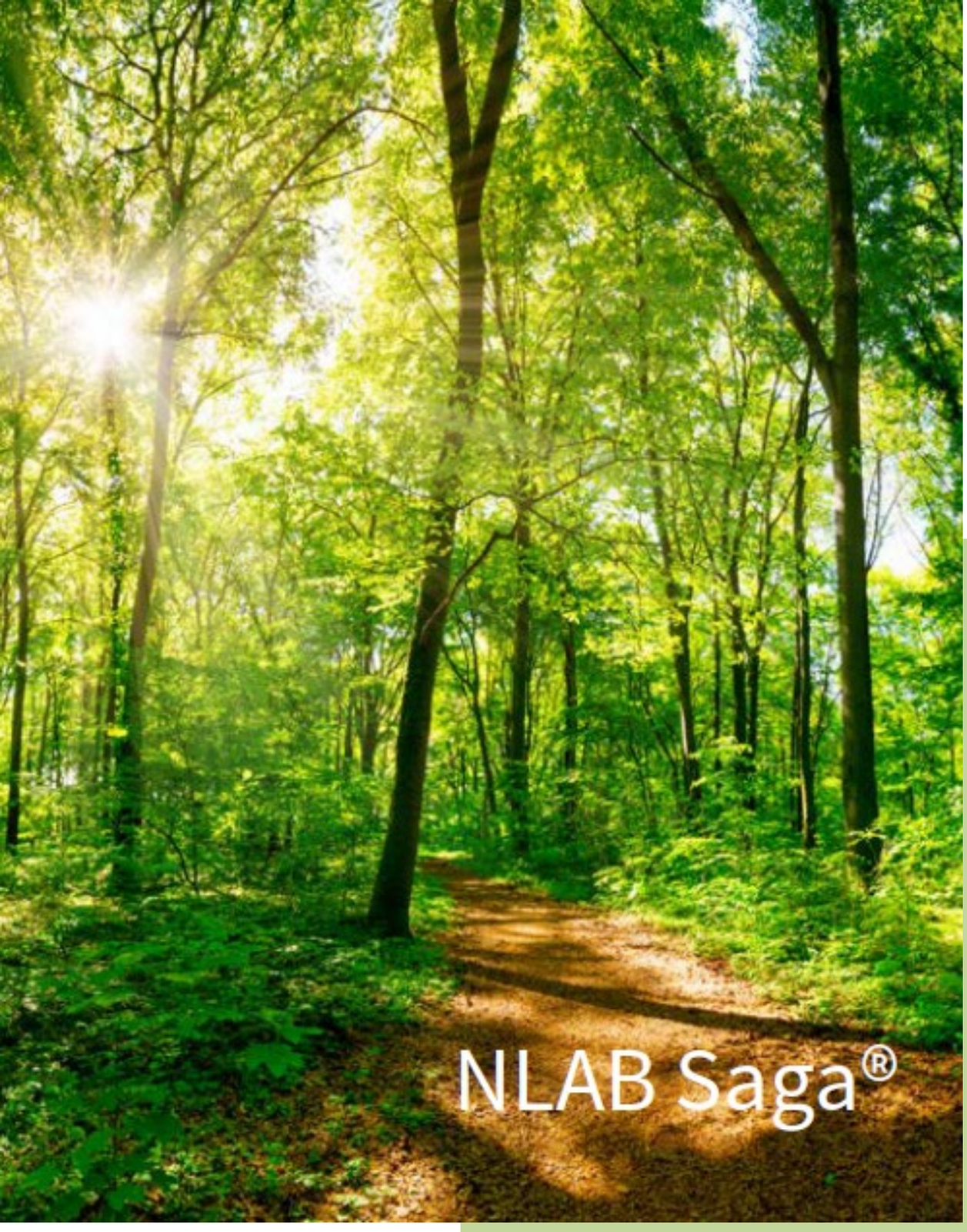


NLAB Saga®

A New Generation Silica for Purification by Chromatography

NANOLOGICA

CONTENT

ABOUT NANOLOGICA

Nanologica was founded in 2004 and is a Swedish nanotechnology company world-leading in the development of nanoporous silica particles for purification by chromatography.

Our mission is to increase the availability of cost-effective drugs, enabling more patients around the world access to vital treatments for diabetes and obesity.

Through a proprietary production method, we create silica-based products that can purify more effectively and that last longer. This can reduce production costs and increase productivity for pharmaceutical manufacturers.

At Nanologica, we take great pride in the quality and performance of our products. They embody our core value – *Swedish Excellence in Nanoporous Silica*.

NLAB SAGA® -
SILICA FOR PREPARATIVE CHROMATOGRAPHY

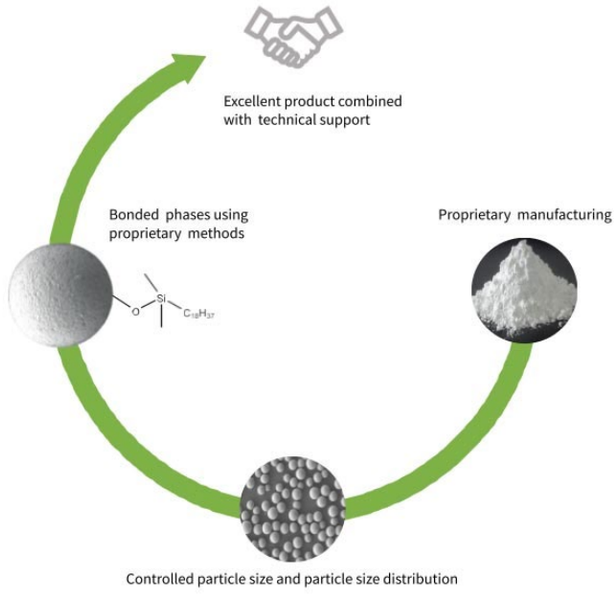
NLAB Saga® has been specifically developed to meet the strict requirements of industrial scale purification by chromatography. It has a superior mechanical and chemical stability together with a high available surface area and ligand density, in addition to narrow pore size distribution. This, combined 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 makes it possible to lower production costs for an improved total economy for the manufacturer, making NLAB Saga® the most cost-effective alternative to 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

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

NLAB SAGA® CHARACTERISTICS

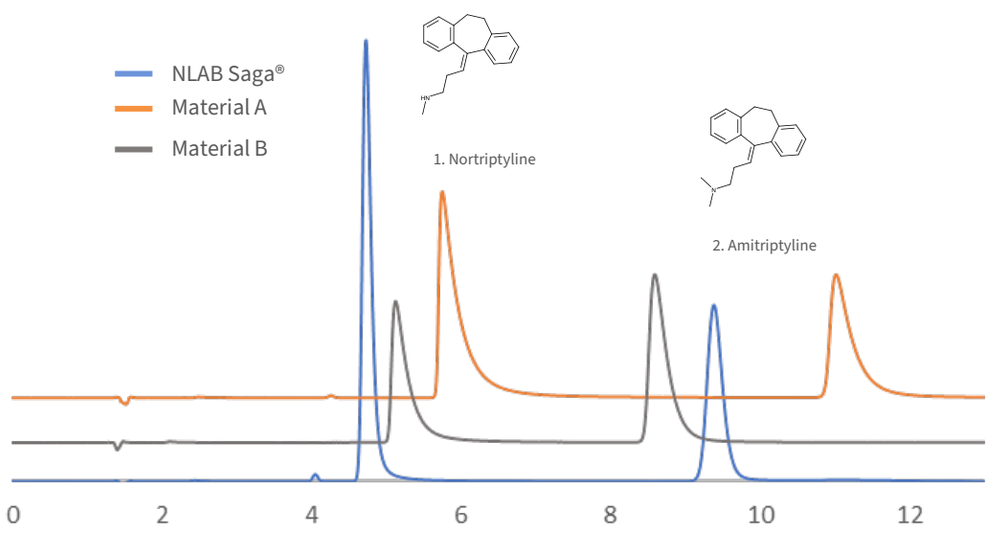
NLAB Saga® is produced at ton scale at Nanologica's CMO Sterling Pharma Solutions in the UK, using Nanologica's proprietary technology.

Current phases include SIL, C8 and C18. Other phases available upon request.

Property	**Method of Analysis**	**Value**	**Unit**
Available particle sizes	Coulter counter	10 13	µm
Particle size distribution d90/d10	Coulter counter	10µm ≤ 1.7 13µm ≤ 1.7	N/A
Pore volume	N_2 adsorption (BET)	0.90	ml/g
Surface area	N_2 adsorption (BET)	320	m^2/g
Pore size	N_2 adsorption (BET)	110	Å
Chemical purity	ICP	Al ≤ 10 Fe ≤ 10 Na ≤ 20	ppm
Carbon content	SS-EN 154707:2011	8 (C4) 12 (C8) 19 (C18)	% ds
Functional group density	Calculated	4.0 (C4) 3.9 (C8) 3.6 (C18)	$\mu mol/m^2$

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.



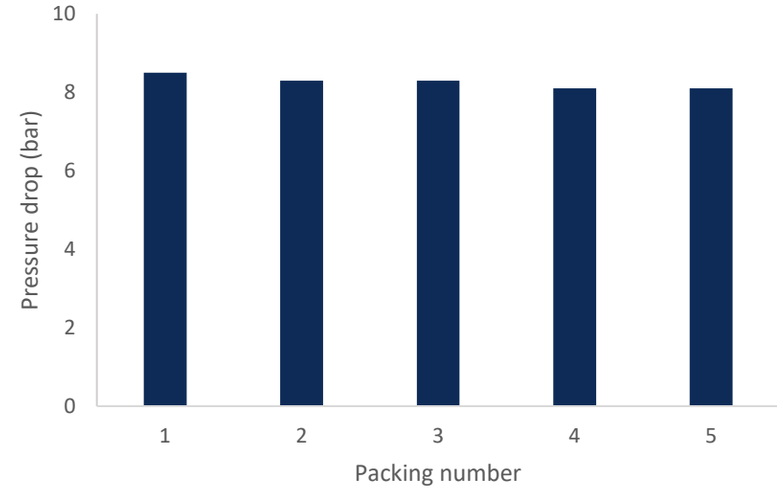
Mobile phase:	Methanol/25 mM K-phosphate pH 7.0 80/20
Column:	150x4.6mm C18 5µm
Flow rate:	1.0 ml/min
Temperature:	30 °C
Detection wavelength:	210 nm

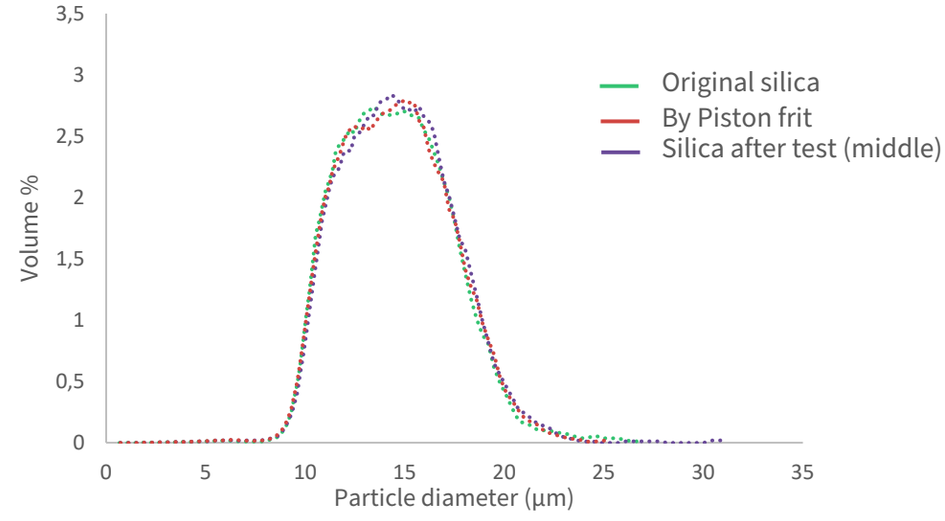
Specifications	α(Nor/EB)	Tf(Nor)	Tf(Ami)
NLAB Saga®	0.90	1.9	1.4
Material A	1.13	3.6	2.2
Material B	1.33	3.4	2.2

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 proprietary process and pore volume.

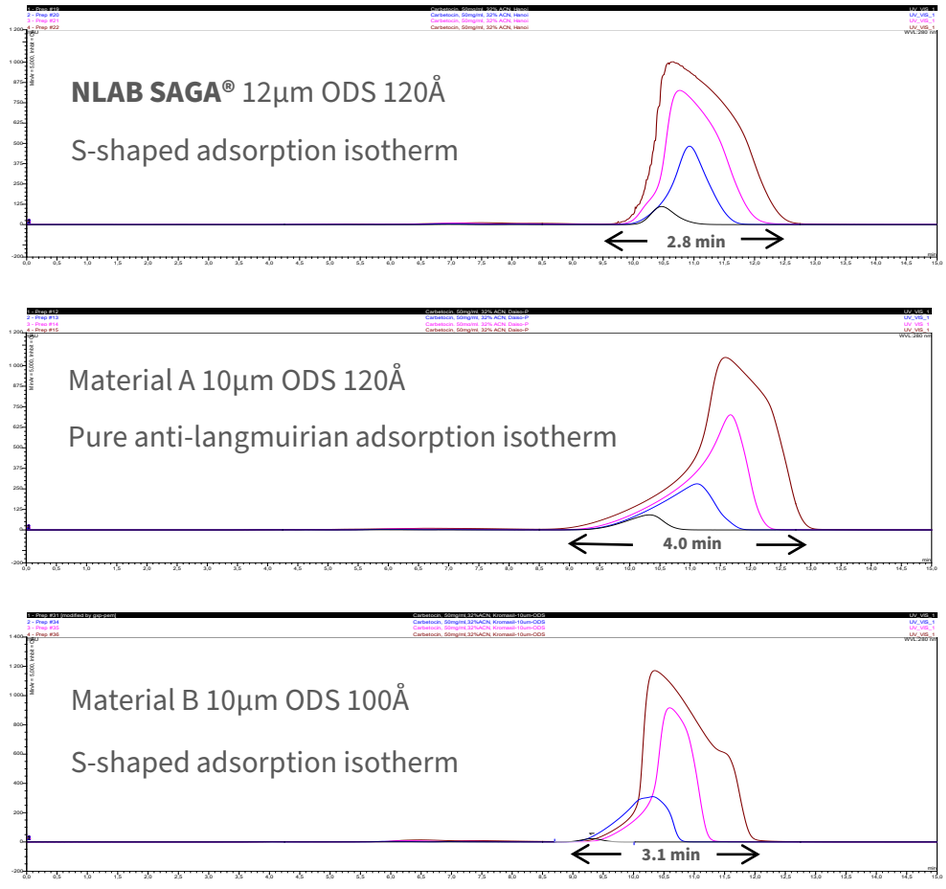




A DAC column with an internal diameter of 5 cm was used. 5 packings/unpackings to 100 bars were made with the same silica slurry. The mechanical stability test was performed on unbonded silica. Packing and unpacking of silica in a DAC column at 100 bar is a generally accepted test of mechanical strength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

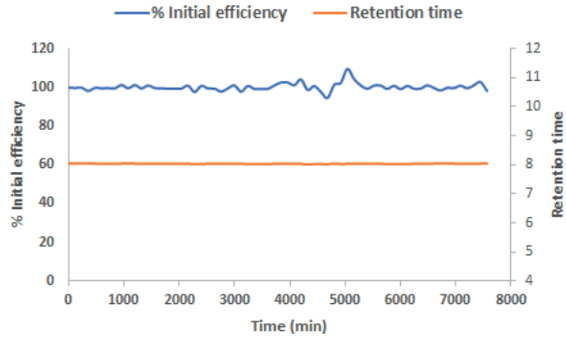
BASIC AND ACIDIC CHEMICAL STABILITY FOR C18

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

Column	SVEA® C18 Gold 100x4.6 mm 5 µm
Mobile Phase	A - 1% TFA in water, pH 0.9
	B - 1% TFA in acetonitrile
Flow Rate	1.0 ml/min
Temperature	60°C
Analyte	Ethylbenzene

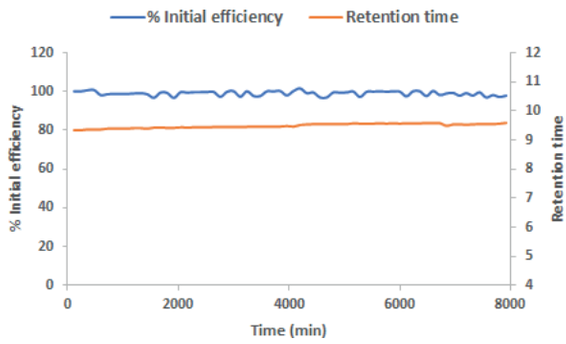
Gradient cycle	10-90% B in 5 min
	90% B for 2 min
	90-10% B in 1 min
	10% B for 2 min



BASIC CONDITIONS

Column	SVEA® C18 Gold 100x4.6 mm 5 µm
Mobile Phase	A - 10 mM ammonium bicarbonate, pH 9.6
	B - Acetonitrile
Flow Rate	1.0 ml/min
Temperature	45°C
Analyte	Progesterone

Gradient cycle	10-90% B in 5 min
	90% B for 2 min
	90-10% B in 1 min
	10% B for 2 min



BASIC CHEMICAL STABILITY FOR C8

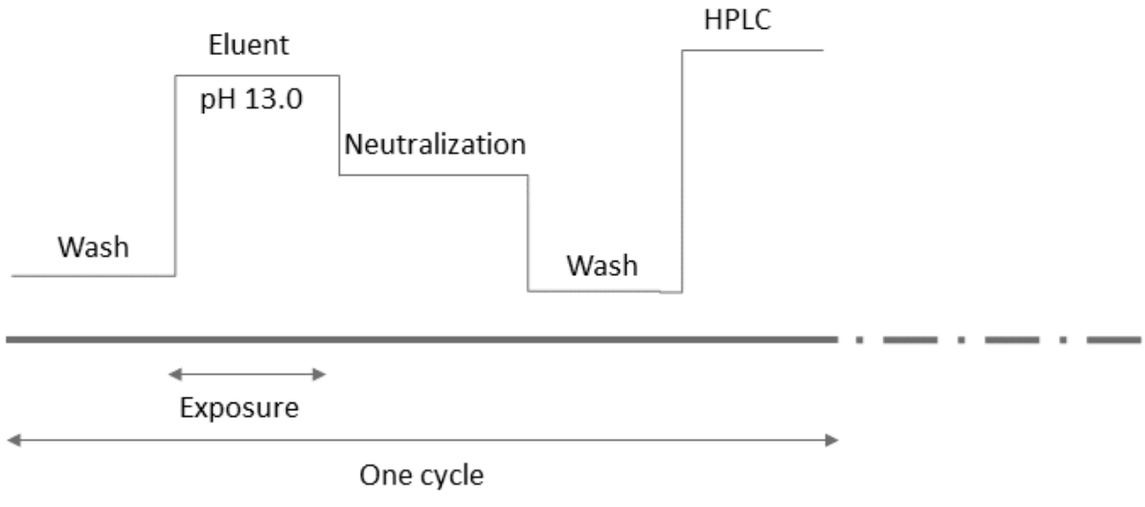
High chemical stability under basic pH is important in insulin manufacturing. During the purification cycles, silica gets contaminated by aggregated insulin which results in the increase of the backpressure and a decline in purity/yield. To regenerate the column, CIPs (Cleaning In Place) procedures are 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 these conditions in order to have a long-lasting product.

Alkaline regeneration test designed to mimic wash cycles

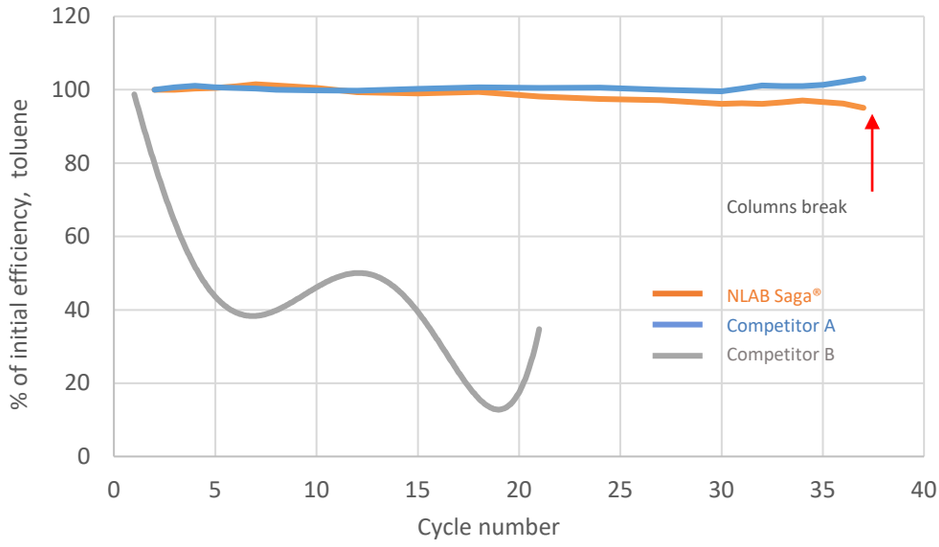
Test conditions:

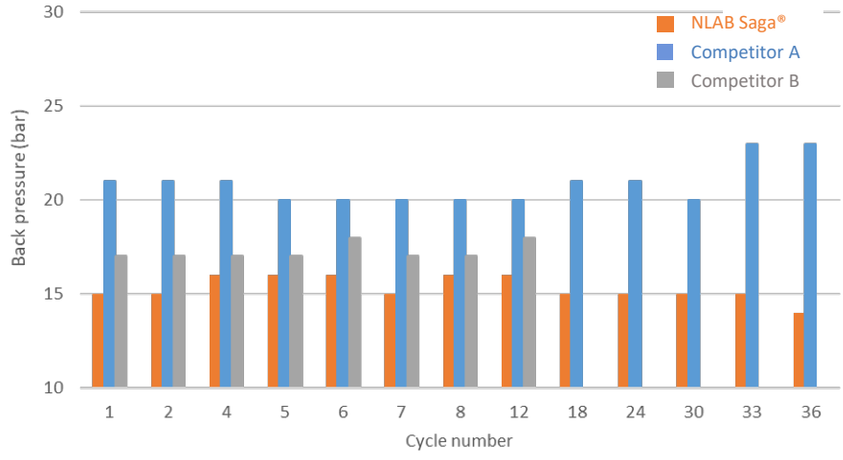
- Wash: 95/5 MeOH/H_2O, 10 CV
- Eluent: 60/40 MeOH/100mM NaOH, pH 13.0, 12 CV
- Temperature: Ambient cycle 1-30. **From cycle 30 and onwards 50°C**
- Neutralization: 90/10 MeOH/1%AcOH, 10CV
- Flow rate: 1ml/min
- Chromatographic evaluation

Illustration of one cycle



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.





<u>Chromatographic test conditions:</u>

Mobile phase:	80/20 MeOH/25 mM K-phosphate, pH 7.0
Flow rate:	1 ml/min
UV:	210 nm
Temperature:	30ºC, after 30 cycles the temperature was raised to **<u>50ºC</u>** to speed up the experiment
Analytes:	Uracil, Toluene

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

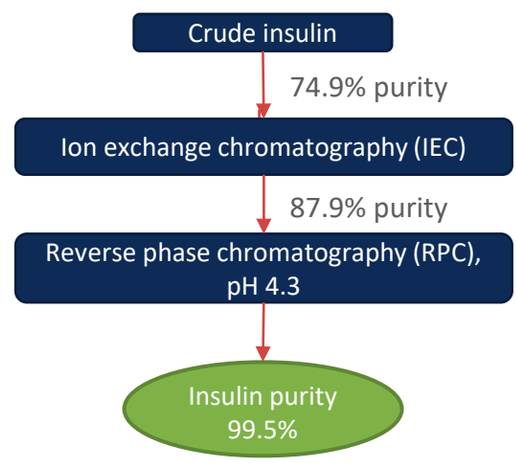
EXAMPLE OF INSULIN PURIFICATION

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.

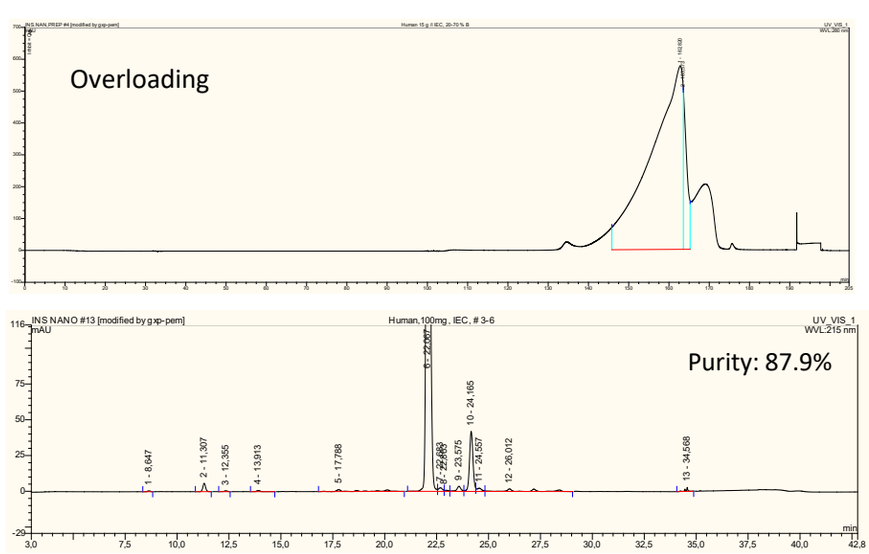
As a first step, ion exchange purification was performed. Ion exchange has an orthogonal selectivity to reversed phase chromatography purification and removes impurities, which are difficult or cannot be removed by the latter step.

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

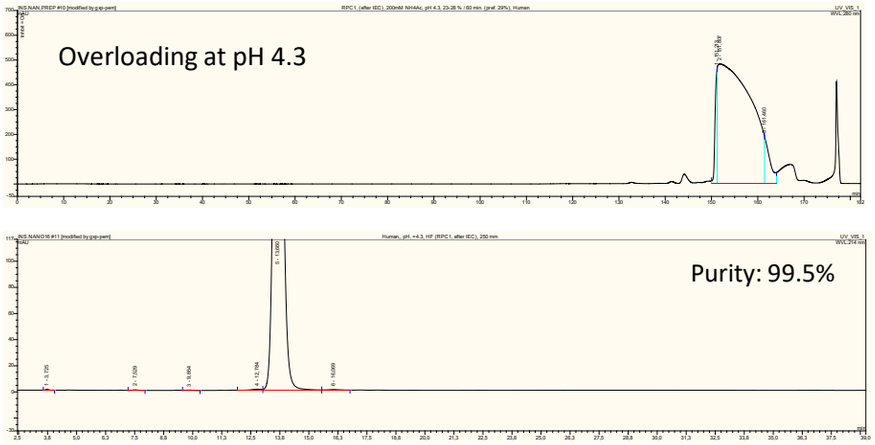
Experiment set up



Ion exchange purification



Reversed phase chromatography pH 4.3



Chromatographic test conditions:

- Loading: 0.37 g/cm^2 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 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.

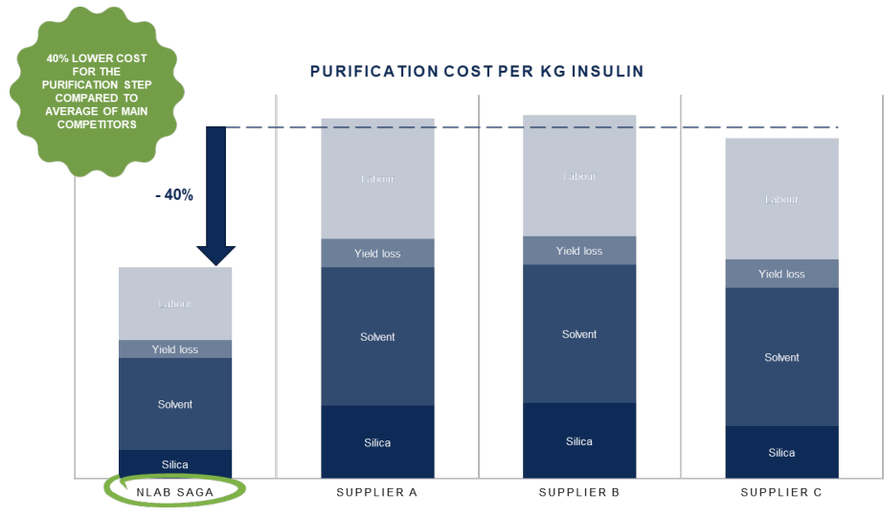
Experimental results were used in the cost calculation model. The cost model has been developed describing the flow in the manufacturing process for the purification of insulin, including use of material (e.g., solvent) and labour cost**. The cost saving in the purification step has then been applied 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 faster than competitors
- ✓ Higher lifetime and lower manufacturing cost

** NLAB Saga® saves: 41% vs. Supplier A, 42% vs. Supplier B, 38% vs. Supplier C*
*** Two FTEs*
**** BMJ Glob Health 2018;3:e000850. doi:10.1136/ bmjgh-2018-000850*

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