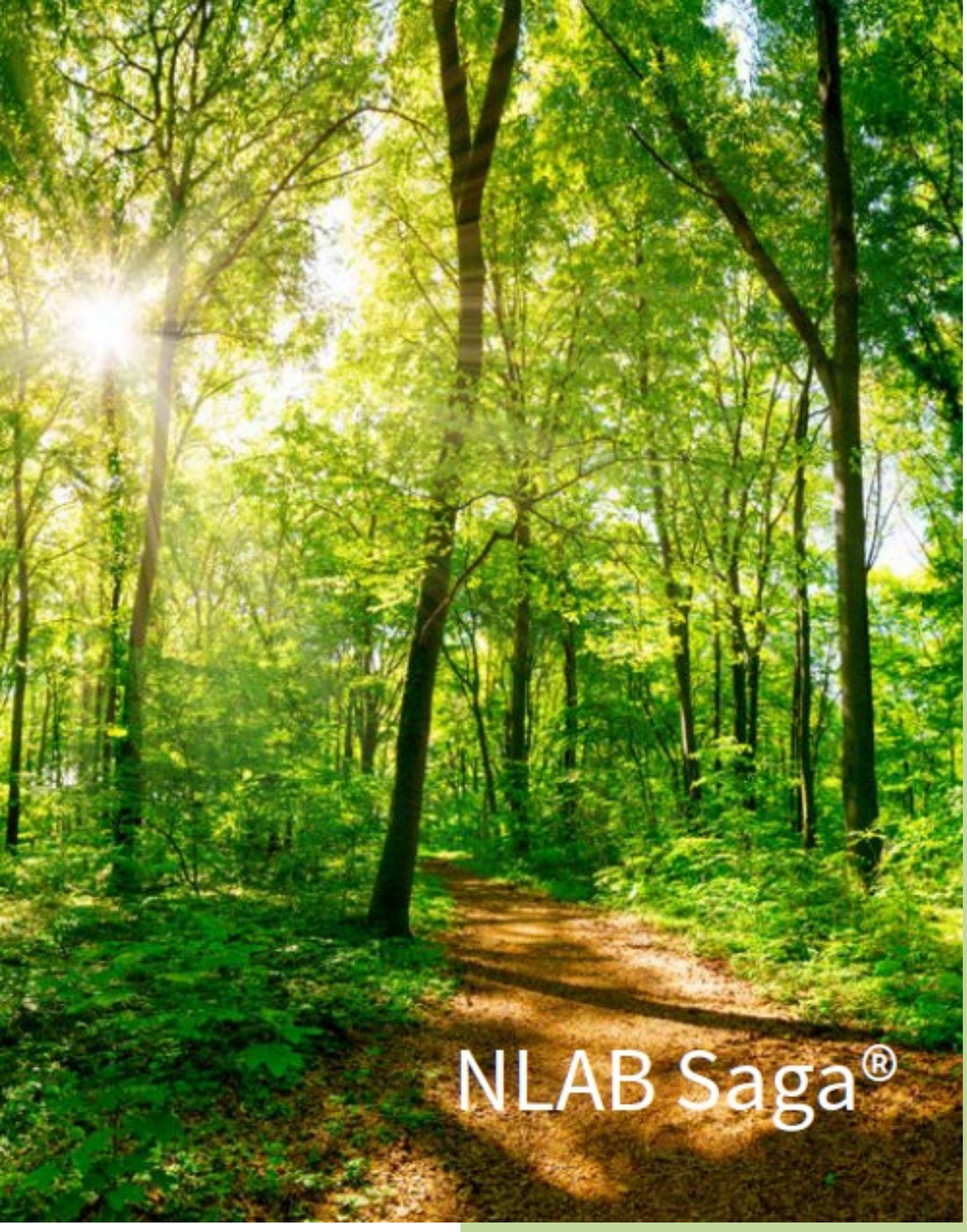


NANOLOGICA

Better and Cheaper Medicine Through Porous Silica

CONTENT

BETTER AND CHEAPER MEDICINE THROUGH POROUS SILICA

“We believe that all of us in the life science industry must strive towards either making medicines *better* – by significantly improving existing treatments or providing treatments where there are no available today – or *cheaper*, making them more widely available to patients in need. All with the overall purpose of providing treatment to more patients worldwide for the betterment of mankind.” – CEO Andreas Bhagwani

ABOUT NANOLOGICA

Nanologica was founded in 2004 and is a Swedish biotechnology company developing nanoporous silica particles for applications within life science. Nanologica is world-leading in controlling the shape, size, and type of porosity of silica particles. This knowledge is applied within two business areas; Drug Development and Chromatography. The company's mission is to contribute to better and cheaper treatments for patients worldwide.

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

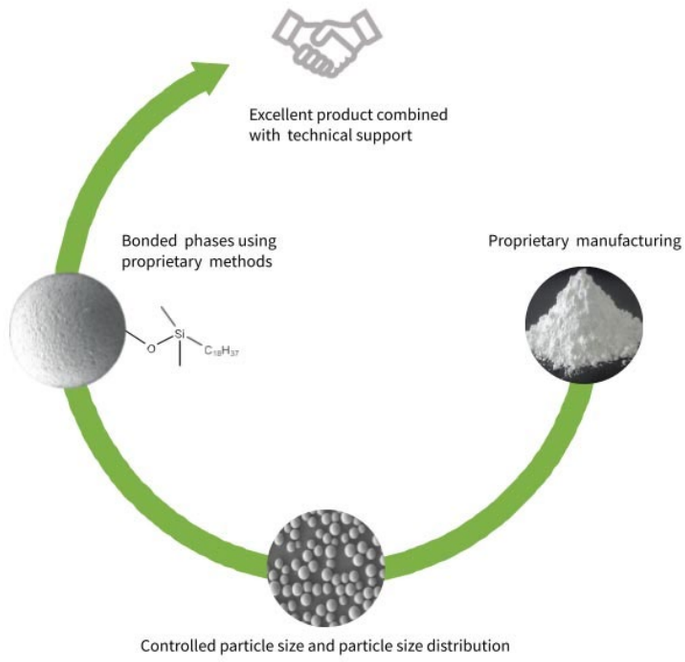
NLAB SAGA® -
SILICA FOR PREPARATIVE CHROMATOGRAPHY

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

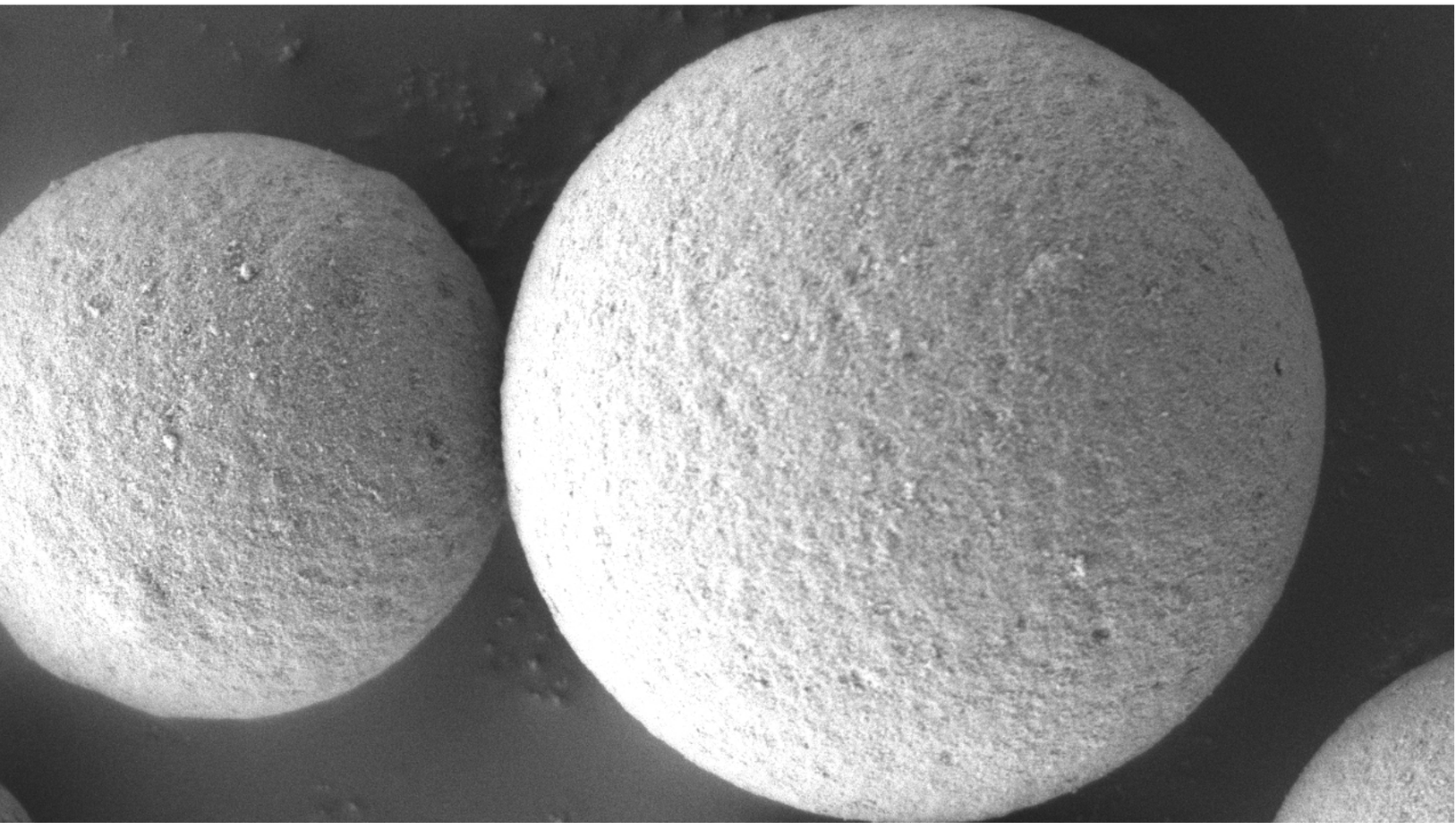
Nanologica has full control of the entire manufacturing process, from raw material to finished product. NLAB Saga has been tested and used by pharmaceutical companies worldwide manufacturing APIs at industrial scale where quality, performance and durability are uncompromisable. The high efficiency and long lifetime of the product makes it possible to lower production costs for an improved total economy for the manufacturer.

NLAB Saga was developed with a clear goal in mind – to increase the availability of better and cheaper peptide and oligonucleotide-based medicines to a larger number of patients across the world.



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



NLAB SAGA® CHARACTERISTICS

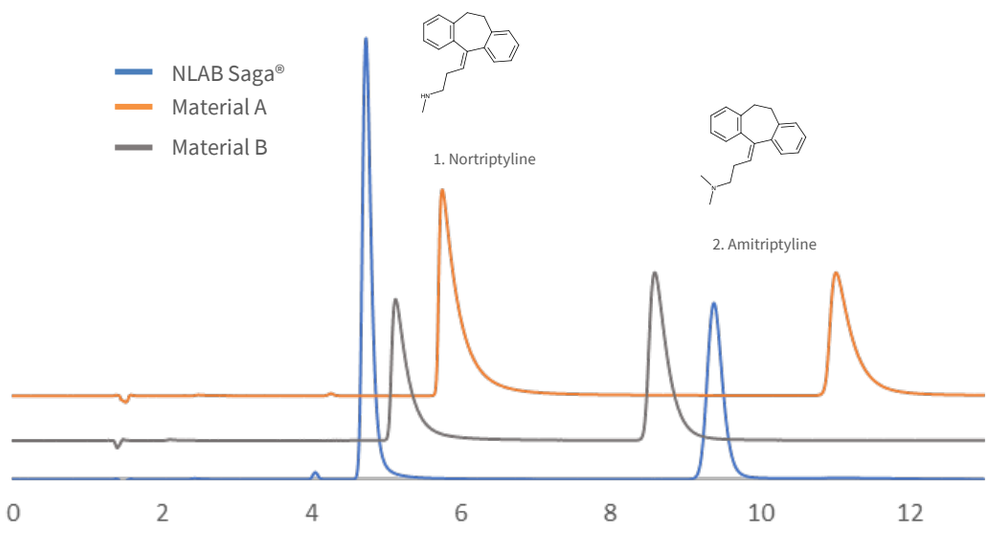
NLAB Saga is produced at ton scale at Nanologica's CMO Sterling Pharma Solutions in the UK, using Nanologica's proprietary technology.

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.



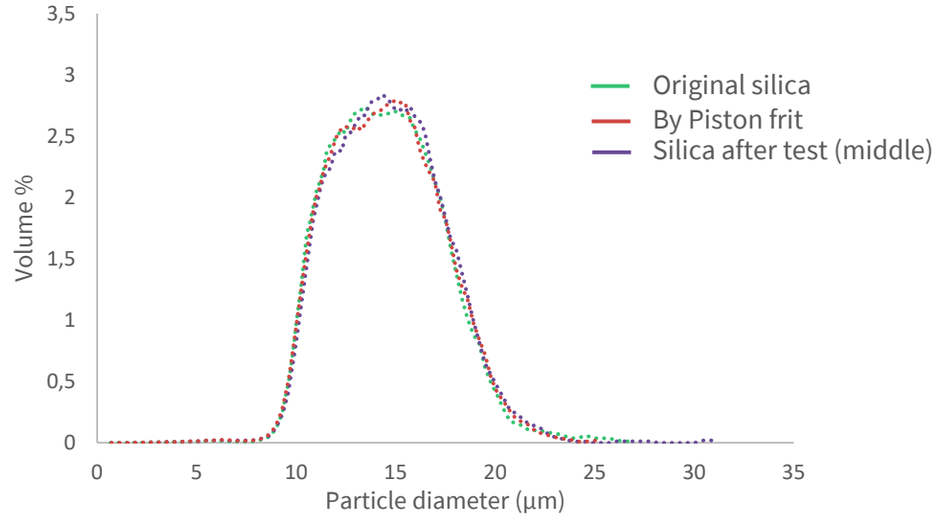
Mobile phase:	methanol/25 mM K-phosphate pH 7.0 80/20
Column:	150x4.6mm C18 5µm
Flow rate:	1.0 ml/min
Temperature:	30 °C
Detection wavelength:	210 nm

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

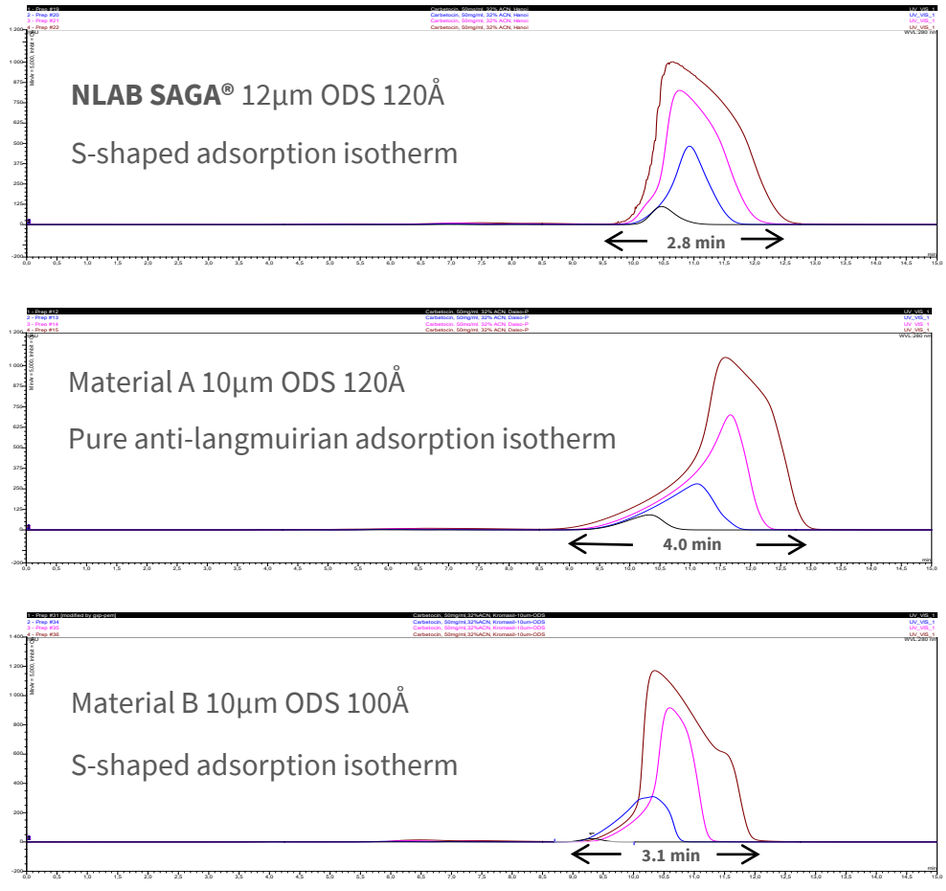
NLAB Saga has an excellent mechanical stability due to narrow pore size distribution and pore volume. Backpressure stays the same meaning there is no mechanical degradation of the silica. This is also shown by size distribution staying the same for native silica and used silica.



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. 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.

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.

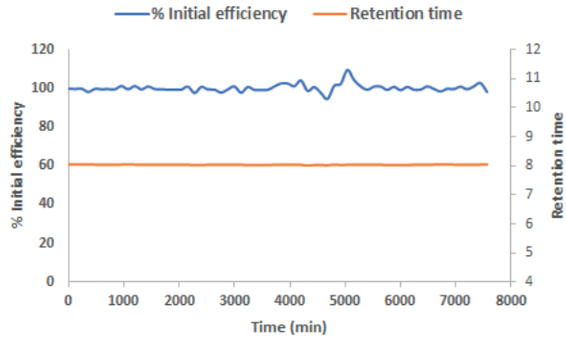
BASIC AND ACIDIC CHEMICAL STABILITY FOR C18

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, 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

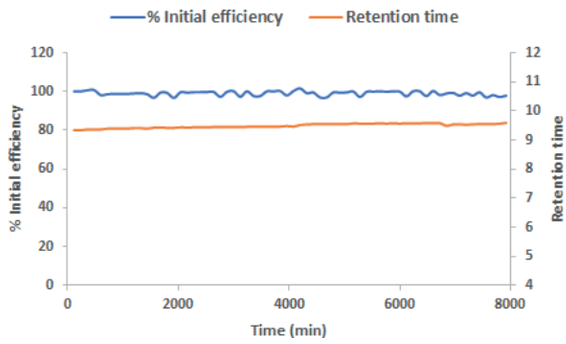
Gradient cycle	10-90% B in 5 min
	90% B for 2 min
	90-10% B in 1 min
	10% B for 2 min



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

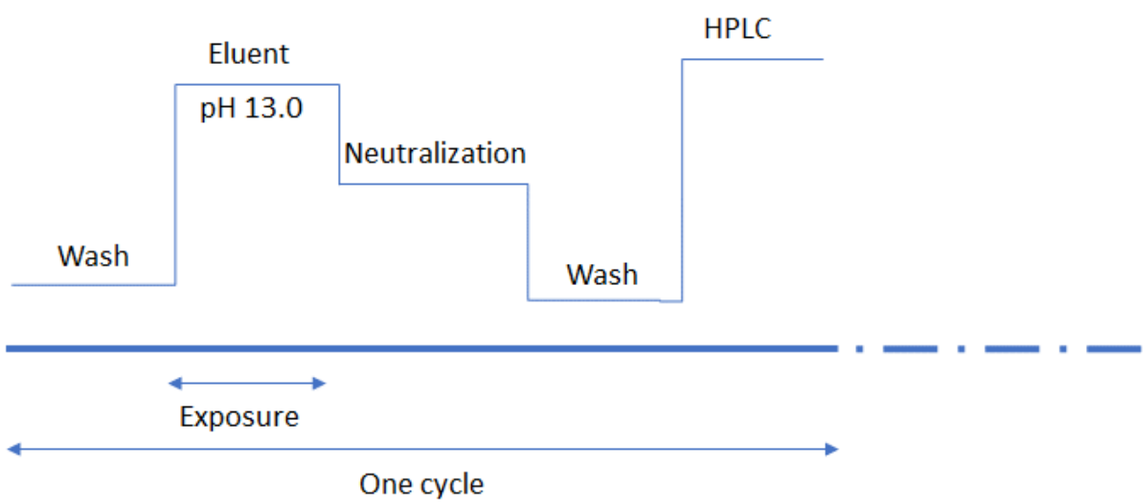
In large scale purification of proteins and peptides there will be depositions in the packing material. To avoid time-consuming packing and unpacking of the columns it is important to run cleaning in process. These cleaning steps are usually performed at a very high pH. Such conditions are harsh for 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

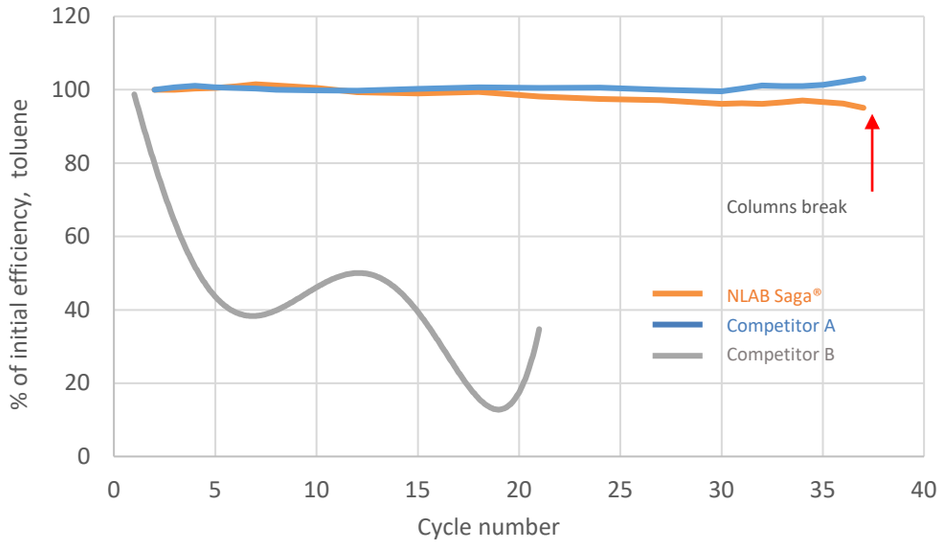
Test conditions:

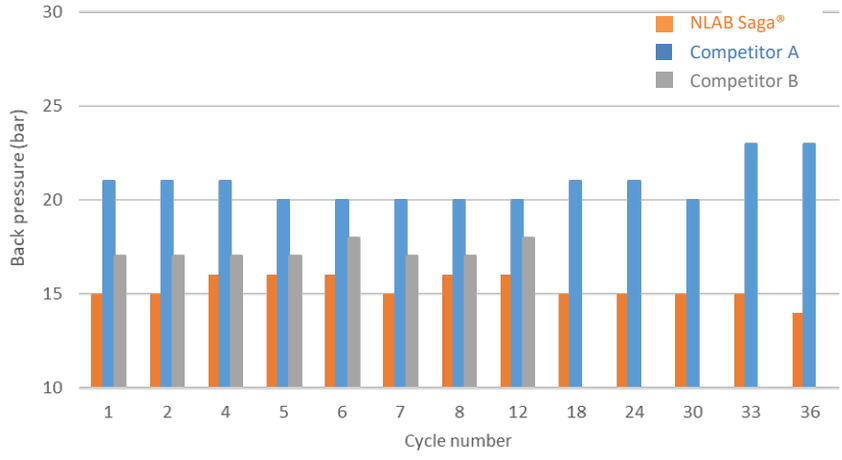
- Wash: 95/5 MeOH/H_2O, 10 CV
- Eluent: 60/40 MeOH/100mM NaOH, pH 13.0, 12 CV
- Temperature: Ambient cycle 1-30. **From cycle 30 and onwards 50ºC**
- Neutralization: 90/10 MeOH/1%AcOH, 10CV
- Flow rate: 1ml/min
- Chromatographic evaluation

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>

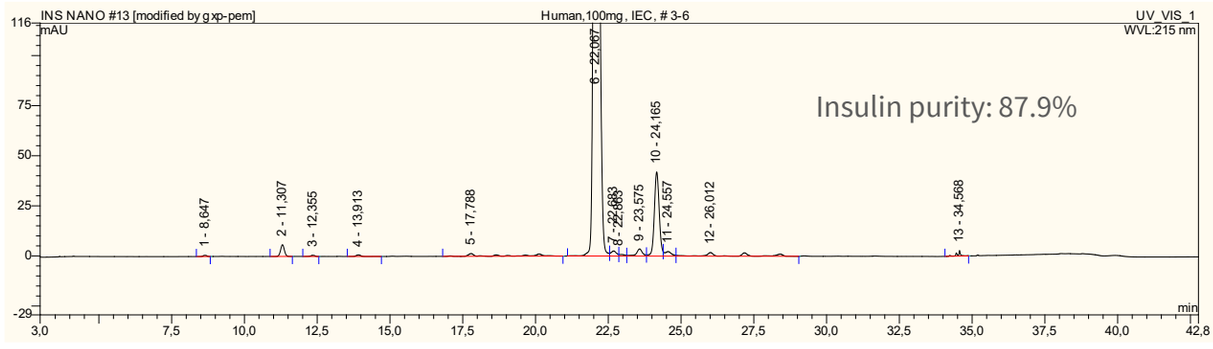
Mobile phase:	80/20 MeOH/25 mM K-phosphate, pH 7.0
Flow rate:	1 ml/min
UV:	210 nm
Temperature:	30ºC, after 30 cycles the temperature was raised to **<u>50ºC</u>** to speed up the experiment
Analytes:	Uracil, Toluene

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

The target for the experiment was to reach the purity threshold of 99.2% as set per the USP. Insulin purity was measured before and after one step of reversed-phase purification.

Insulin purity measured to 87.9% <u>BEFORE</u> reversed-phase purification



USP analytical method conditions

Flow rate:	1.2 ml/min
UV:	214 nm
Temperature:	40°C
Eluent:	A: 200mM Na_2SO_4/H_3PO_4 (pH=2.3)
	B: Acetonitrile/ 200mM Na_2SO_4/H_3PO_4 (pH=2.3) 45/55
Gradient:	0-30 min / 57%B; 30-44 min /57-89%B; 44-50 min / 89%B

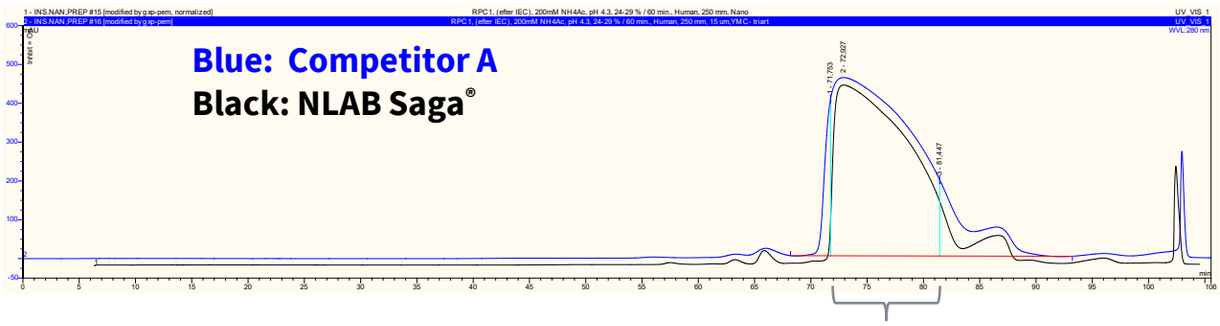
Experimental set up

The mass overload experiments were performed under the same conditions for NLAB Saga and the competitor.

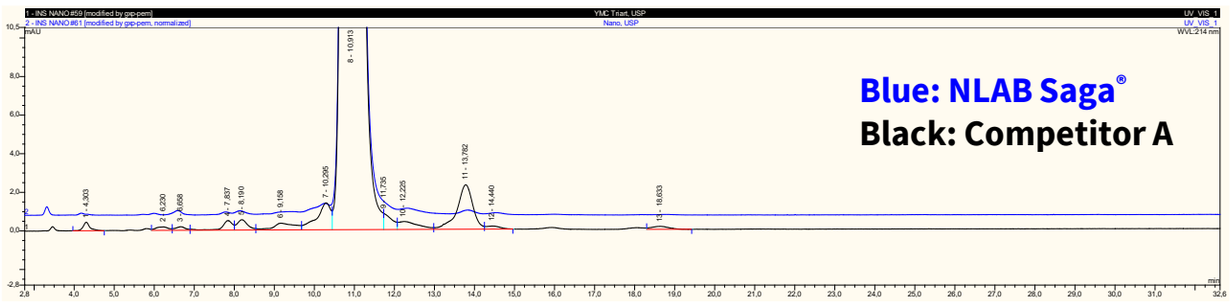
Process parameters which were kept constant:

- **Loading**: 0.37 g/cm^2 column cross sectional area (15 g/litre)
- **Yield**: 90 % ± 1 % in all steps
- **Slope of the gradient:** (0.083 % / minute, pH= 4.3)
- **Linear flow rate**: 180 cm/h
- **Organic solvent**: Acetonitrile
- **Feed**: Insulin with 87.9 % purity

Mass overload - the exact same fraction from the insulin purification for both Competitor A and NLAB Saga



Insulin purity measured to 99.5% <u>AFTER</u> two step of reversed-phase purification



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 two steps of reversed-phase purification. The Competitors A, B and C do not reach the USP target, meaning they would 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.

NANOLOGICA

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