Superdex 30 Increase columns

SIZE EXCLUSION CHROMATOGRAPHY

Superdex™ 30 Increase prepacked columns (Fig 1) are designed for high-resolution, small-scale purification and analysis of peptides and other small biomolecules with molecular weights (M_r) from ~ 100 to 7000. These new generation size exclusion chromatography (SEC) columns replace their predecessors, Superdex Peptide columns, delivering improved performance in preparative and analytical purification.

Superdex 30 Increase columns provide:

- Small-scale preparative purification (sample volumes of 4 to 500 µL) and characterization of peptides and small biomolecules
- Higher resolution compared with Superdex Peptide for improved purity and analysis results
- Three times faster separations than Superdex Peptide with the same resolution
- Tolerance to repeated harsh cleaning procedures at high pH (1 M NaOH), giving long column life and minimal carry-over

Resin characteristics

Superdex 30 Increase resin is based on a high-flow agarose base matrix with good pressure/flow properties. The small bead size with narrow bead size distribution allows for high-resolution separations. In addition, low nonspecific interaction permits high recovery of biological materials. The characteristics of the Superdex 30 Increase resin are shown in Table 1.

Fig 1. Superdex 30 Increase 10/300 GL and Superdex 30 Increase 3.2/300 columns for high-resolution separation and analysis of peptides and small biomolecules.

Table 1. Characteristics of Superdex 30 Increase resin

Matrix	Composite of cross-linked agarose and dextran
Particle size, d_{50V} [1]	9 µm
Fractionation range	M_r ~100 to 7000
pH stability range	
operational[2]	3 to 12
cleaning-in-place (CIP)[3]	1 to 14
Temperature	
operational	4°C to 40°C
storage	4°C to 30°C

[1] Median particle size of the cumulative volume distribution
[2] pH range where resin can be operated without significant change in function
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

Separation of peptides and other small biomolecules

Superdex 30 Increase and the related SEC resins — Superdex 200 Increase, Superdex 75 Increase, and Superose™ 6 Increase — belong to the new generation of high-flow agarose matrices. These matrices utilize small beads for high-resolution applications. The resins have different selectivity to complement each other (Fig 2). Compared with Superdex 75 Increase (recommended fractionation range M_r 3000 to 70 000), Superdex 30 Increase gives improved separation in the lowest molecular weight range (Fig 3).

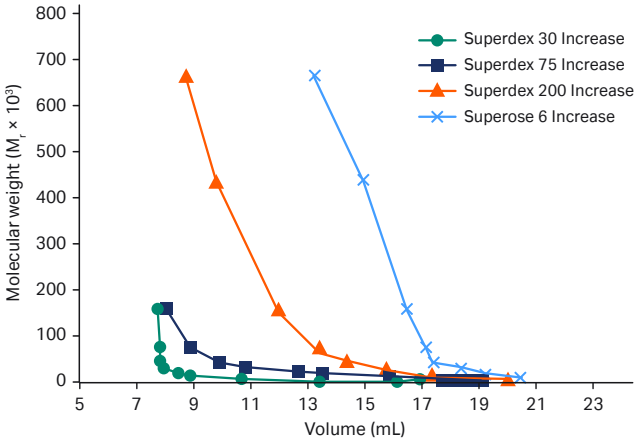


Fig 2. Selectivity of globular proteins and peptides of various molecular weights on Superdex 30 Increase 10/300 GL, Superdex 75 Increase 10/300 GL, Superdex 200 Increase 10/300 GL, and Superose 6 Increase 10/300 GL. Note that the whole fractionation range of Superose 6 Increase is not covered in this diagram.

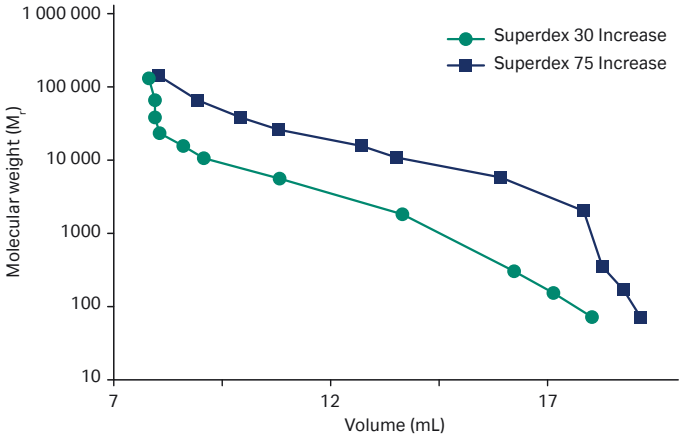


Fig 3. Selectivity of globular proteins and peptides on Superdex 30 Increase 10/300 GL compared with Superdex 75 Increase 10/300 GL column.

High chemical stability

Superdex 30 Increase resin is stable in aqueous solutions over pH 3 to 12. The performance of the resin is not affected by exposure to solutions containing organic solvents (70% acetonitrile), chaotropic agents (6 M guanidine hydrochloride, 8 M urea), or detergents (1% SDS). Superdex 30 Increase also withstands the conditions used for CIP from pH 1 to 14. Short CIP cycles (approx. 3 h) with 0.1 M HCl, 1.0 M NaOH, or organic solvents have no significant influence on the chromatographic performance; however, the resin should not be stored in these solutions. See the column instructions for further details.

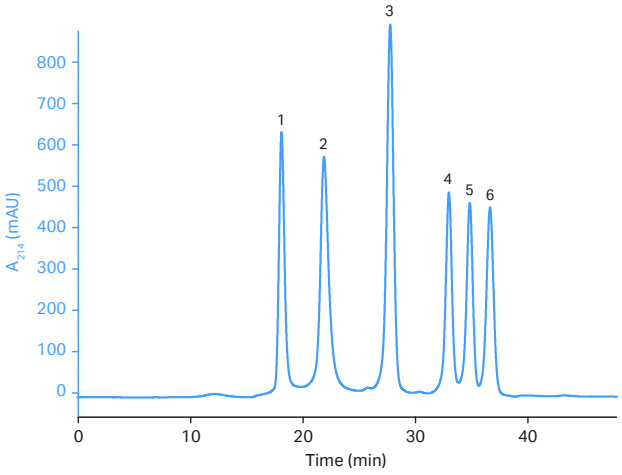
Improved resolution and reduced runtime compared with Superdex Peptide

Compared with its predecessor Superdex Peptide, Superdex 30 Increase has improved properties, both in terms of higher resolution and reduced runtime. Separation of a mix consisting of proteins and peptides showed that Superdex 30 Increase 10/300 GL had 25% to 55% higher resolution compared with Superdex Peptide 10/300 GL (Fig 4). Figure 5 shows that approximately the same resolution was achieved with three times faster separation using Superdex 30 Increase compared with Superdex Peptide.

For a series of oligomaltoses, resolution was improved on Superdex 30 Increase compared with Superdex Peptide (Fig 6).

Columns:	(A) Superdex 30 Increase 10/300 GL (B) Superdex Peptide 10/300 GL
Sample:	1. Cytochrome C (M_r 12 300), 0.2 mg/mL 2. Aprotinin (M_r 6500), 0.2 mg/mL 3. Gastrin (M_r 2126), 0.15 mg/mL 4. Hexaglycine (M_r 360), 0.2 mg/mL 5. Triglycine (M_r 189), 0.2 mg/mL 6. Glycine (M_r 75), 7 mg/mL
Sample volume:	100 µL
Buffer:	20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate:	0.5 mL/min
Detection:	214 nm
System:	High-performance liquid chromatography (HPLC)

(A) Superdex 30 Increase 10/300 GL, 0.5 mL/min



(B) Superdex Peptide 10/300 GL, 0.5 mL/min

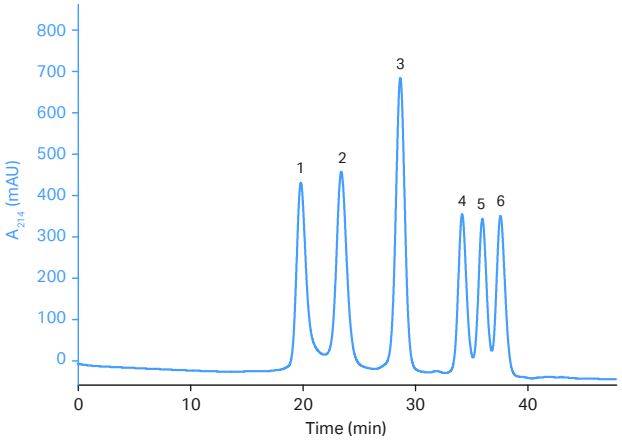
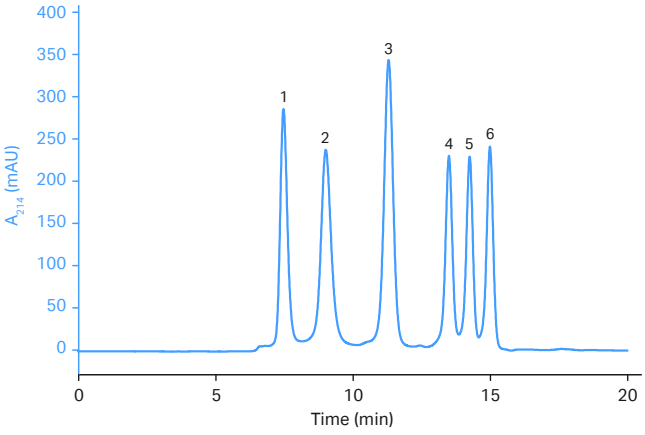


Fig 4. Chromatograms showing high-resolution SEC at 0.5 mL/min of a mix consisting of six proteins and peptides on (A) Superdex 30 Increase 10/300 GL and (B) Superdex Peptide 10/300 GL. In this comparison, the resolution improvement with the new generation column was 25% to 55%.

Columns: (A) Superdex 30 Increase 10/300 GL
(B) Superdex Peptide 10/300 GL
Sample: 1. Cytochrome C (M_r 12 300), 0.2 mg/mL
2. Aprotinin (M_r 6500), 0.2 mg/mL
3. Gastrin (M_r 2126), 0.15 mg/mL
4. Hexaglycine (M_r 360), 0.2 mg/mL
5. Triglycine (M_r 189), 0.2 mg/mL
6. Glycine (M_r 75), 7 mg/mL
Sample volume: 100 µL
Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate: (A) 1.2 mL/min (B) 0.4 mL/min
Detection: 214 nm
System: HPLC

(A) Superdex 30 Increase 10/300 GL, 1.2 mL/min



(B) Superdex Peptide 10/300 GL, 0.4 mL/min

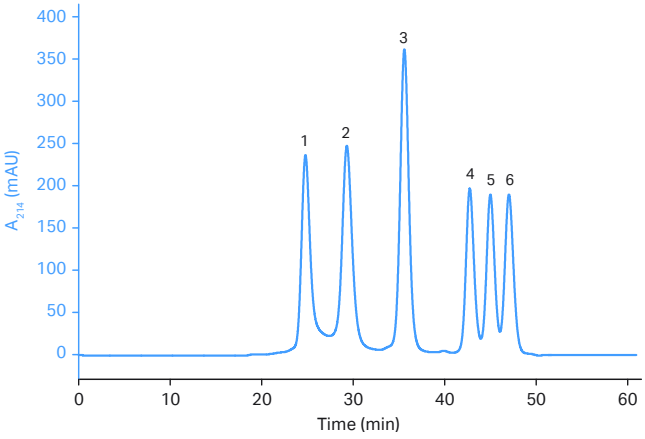
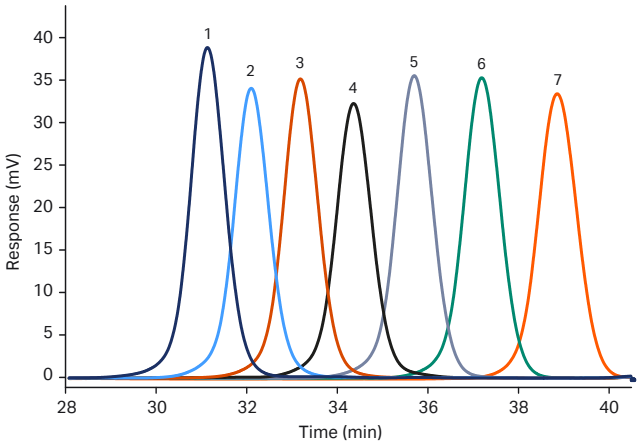


Fig 5. Chromatograms showing high-resolution SEC of a sample mix consisting of proteins and peptides on (A) Superdex 30 Increase 10/300 GL at 1.2 mL/min and (B) Superdex Peptide 10/300 GL at 0.4 mL/min. Note that approximately the same resolution was achieved with a three times faster separation using Superdex 30 Increase 10/300 GL.

Columns: (A) Superdex 30 Increase 10/300 GL
(B) Superdex Peptide 10/300 GL
Sample: 1. Maltoheptaose, M_r 1153
2. Maltohexaose, M_r 991
3. Maltopentaose, M_r 828
4. Maltotetraose, M_r 667
5. Maltotriose, M_r 504
6. Maltose, M_r 360
7. Glucose, M_r 180
Sample volume: 100 µL
Buffer: Milli-Q™ water
Flow rate: 0.5 mL/min
Detection: Refractive index detector (RID)
System: ÄKTA™ pure 25

(A) Superdex 30 Increase 10/300 GL



(B) Superdex Peptide 10/300 GL

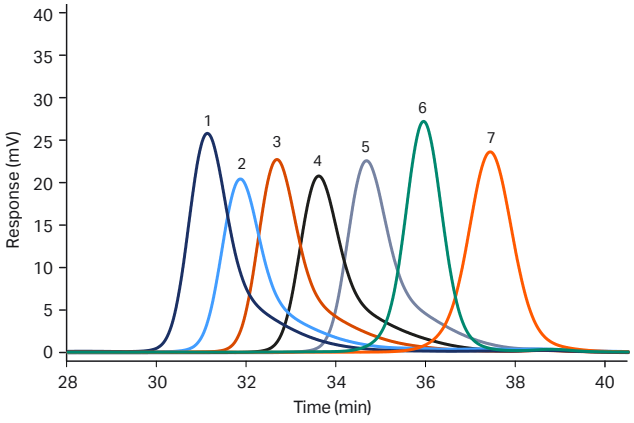


Fig 6. Chromatograms showing improved resolution of a series of oligomaltoses on (A) Superdex 30 Increase 10/300 GL and (B) Superdex Peptide 10/300 GL.

Column characteristics and selection

Two column dimensions to fit different needs

Superdex 30 Increase is available prepacked in high performance Tricorn™ 10/300 GL column and in 3.2/300 column format (Table 2).

Table 2. Characteristics of columns prepacked with Superdex 30 Increase

	Superdex 30 Increase 10/300 GL	Superdex 30 Increase 3.2/300
Bed dimensions diam. × height (mm)	10 × 300	3.2 × 300
Approximate bed volume (mL)	24	2.4
Column efficiency (theoretical plates m^{-1})	> 43 000	> 38 000
Recommended sample volume (µL)	25 to 500	4 to 50
Recommended operational flow rate (H_2O at 25°C, mL/min)[1]	0.80	0.075
Max. operational flow rate (H_2O at 25°C, mL/min)[1]	1.20	0.15
Typical pressure drop over column, (H_2O at 25°C, MPa)	3.0	2.0

[1] Flow rate needs to be decreased when working at a low temperature or with viscous solutions, see column instructions for more details

Both columns are made of glass to allow easier visual inspection of the packed bed. The glass tube is coated with a protecting plastic film or protected with a plastic sleeve. Each column has its own application purpose (Table 3). Superdex 30 Increase 10/300 GL is suitable for both small-scale preparative purification and for analytical applications. Superdex 30 Increase 3.2/300 is an excellent choice when working with very small sample volumes in high-resolution microscale separations.

Table 3. Column choice for different applications

Type of application/column	10/300 GL	3.2/300
Small-scale preparative runs (mg)	×	
Microscale preparative runs (µg) When sample amount is limited and low buffer consumption is important		×
High-resolution analysis (25 to 500 µL)	×	
High-resolution analysis (4 to 50 µL) When sample amount is limited and low buffer consumption is important		×

Excellent reproducibility and durability

Reproducible results are essential in all research. The long working life and high reproducibility of Superdex 30 Increase prepacked columns are the result of optimized design, stable properties of the resin, and controlled production procedures.

A total of 350 injections of a sample mix consisting of proteins and peptides with low concentration was performed on Superdex 30 Increase 10/300 GL column. Peak areas and resolution were essentially unchanged during the study (Fig 7A). The number of theoretical plates remained high over time (Fig 7B), confirming the repeatability of the column over multiple runs.

Column: Superdex 30 Increase 10/300 GL
Sample: 1. Cytochrome C (M_r 12 300), 0.16 mg/mL
2. Aprotinin (M_r 6500), 0.16 mg/mL
3. [Ile^7]-Angiotensin III (M_r 897), 0.08 mg/mL
4. Triglycine (M_r 189), 0.16 mg/mL
5. Glycine (M_r 75), 5.6 mg/mL
Sample volume: 50 µL
Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate: 0.8 mL/min
Detection: 214 nm
System: HPLC

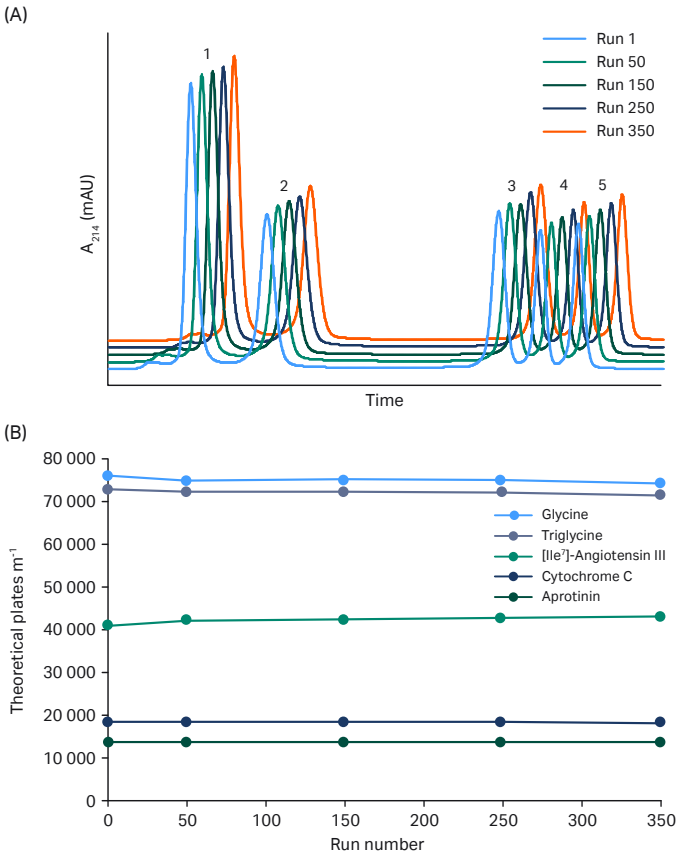


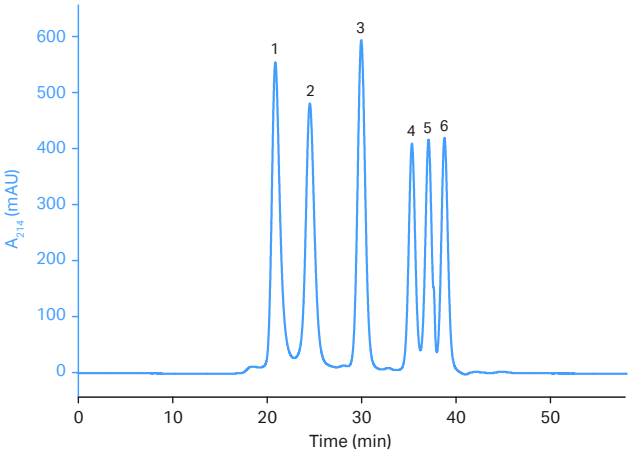
Fig 7. (A) Repeated injections of a sample mix consisting of proteins and peptides on Superdex 30 Increase. Results from run 1, 50, 150, 250, and 350 are shown. Peaks of the proteins and peptides are labeled 1 to 5; (B) The plate number per meter (N/m) of five proteins and peptides plotted against the injection number.

High sensitivity with small sample volumes

Superdex 30 Increase 3.2/300 gives high resolution and good sensitivity for small sample volumes. To compare column sizes, a 10 µL sample mixture of proteins and peptides was applied to both Superdex 30 Increase 3.2/300 and 10/300 GL columns. As shown in Figure 8, the detection signal from the run with Superdex 30 Increase 3.2/300 (Fig 8A) was considerably higher compared with the more diluted peaks from Superdex 30 Increase 10/300 GL (Fig 8B).

Columns:	(A) Superdex 30 Increase 3.2/300 (B) Superdex 30 Increase 10/300 GL
Sample:	1. Cytochrome C (M_r 12 300), 0.2 mg/mL 2. Aprotinin (M_r 6500), 0.2 mg/mL 3. Gastrin (M_r 2126), 0.15 mg/mL 4. Hexaglycine (M_r 360), 0.2 mg/mL 5. Triglycine (M_r 189), 0.2 mg/mL 6. Glycine (M_r 75), 7 mg/mL
Sample volume:	10 µL
Buffer:	20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate:	(A) 0.05 mL/min; (B) 0.5 mL/min
Detection:	214 nm
System:	HPLC

(A) Superdex 30 Increase 3.2/300, 0.05 mL/min



(B) Superdex 30 Increase 10/300 GL, 0.5 mL/min

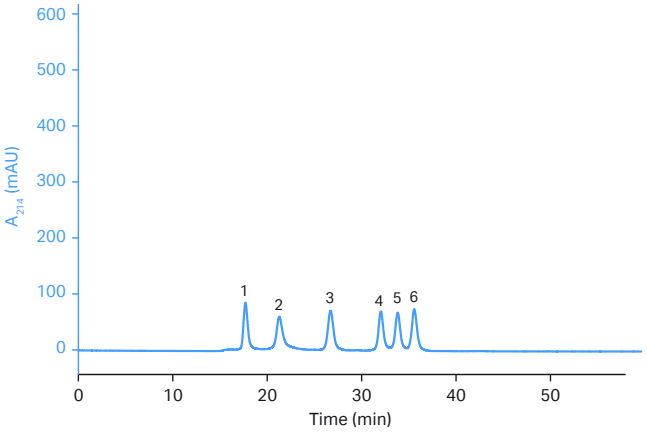


Fig 8. A small volume (10 µL) of a sample mixture of proteins and peptides was separated on (A) Superdex 30 Increase 3.2/300 column and (B) Superdex 30 Increase 10/300 GL column. Both graphs show the same scale for UV detection.

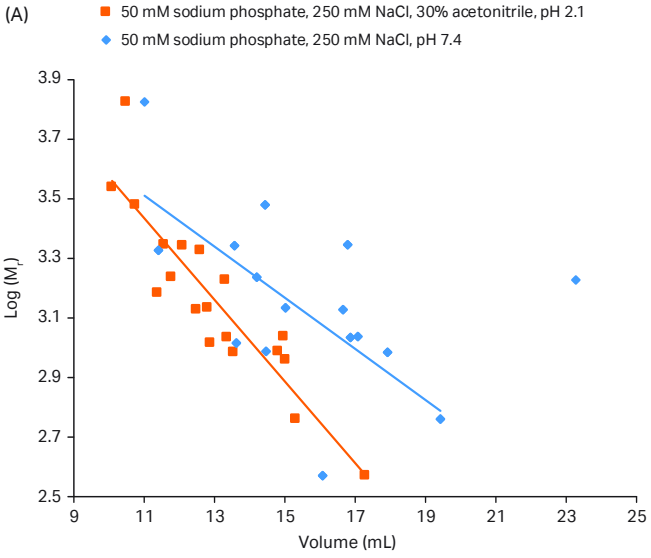
Operation

Choice of eluents

An eluent that ensures the sample is fully soluble and that will simplify downstream applications should be selected.

As with all SEC resins, weak nonspecific interaction can occur between certain molecules and the resin. Peptides and small biomolecules are more susceptible than larger proteins to interactions with SEC resins, as peptides display very little or no tertiary structure leaving the amino acid side chains more exposed. Minor differences in shape also affect the retention. To minimize undesirable interactions and obtain a size-based separation for peptides, a mobile phase with low pH and 150 to 300 mM NaCl often works well. For hydrophobic peptides, addition of organic solvent (e.g., 30% acetonitrile) is beneficial. The effect of mobile phase on elution volume in Superdex 30 Increase 10/300 GL is exemplified in Figure 9.

(A)



(B)

Peptide	M_r
Aprotinin	6511
Growth hormone releasing factor 1-29 amide	3359
Adrenocorticotropic hormone fragment 1-24	2933
Dynorphin A	2147
Gastrin	2127
Bovine β-casein, monophosphopeptide	2062
Neurotensin	1673
Somatostatin	1638
Mastoparan	1480
Met-Lys-Bradykinin	1320
Angiotensin I	1296
[Lys^8]-Vasopressin	1057
Angiotensin II	1046
[Ala^{28}]-Amyloid β 25-35	1003
Arg-Lys-Arg-Ala-Arg-Lys-Glu	943
Angiotensin III	931
Trp-His-Trp-Leu-Gln-Leu	882
Leucine enkephalin	556
Hexaglycine	360

Fig 9. (A) Effect of mobile phase on elution volume on Superdex 30 Increase 10/300 GL; (B) Table showing the peptides used in the experiments.

Sample volumes, flow rates, and system dead volumes

When working with protein concentrations below 10 mg/mL, the SEC separation becomes less dependent on sample concentration. To achieve high resolution, the sample volume should be less than 2% of the column volume (CV). Sample volumes between 0.1% and 1.0% of the column volume give the highest resolution. By decreasing the flow rate, an increasing resolution can be achieved from a given column. For maximal resolution in SEC, extra-column volumes should be minimized. This applies to volumes both before the column (sample injector -> column inlet) and after the column (column outlet -> UV cell or fraction collector). Decrease the volumes by using a suitable small-volume column valve (or no valve) and use short, narrow-diameter capillaries. For further information, see cue card 29181181 and other literature sources under "Ordering information". See also the column instructions for optimization examples.

Applications

Separation of enzymatically degraded hyaluronan

Hyaluronan is a natural linear polysaccharide that can retain large amounts of water and has become important in a number of medical and esthetic applications such as eye surgery and dermal filling. To improve mechanical properties and slow down degradation *in vivo*, hyaluronan is cross-linked to form hydrogels.

SEC is a useful tool for characterization of hyaluronan. When differently cross-linked hyaluronans are subjected to enzymatic degradation, oligosaccharides with different sizes are obtained. These oligosaccharides need to be separated by SEC before analysis by nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS), which give information about cross-linking efficiency and the position of the substitutions. In such analyses, well-characterized oligosaccharide standards are used.

Figure 10 illustrates the separation of such standards on Superdex 30 Increase 10/300 GL.

Columns:	Superdex 30 Increase 10/300 GL
Samples:	Hyaluronan oligosaccharide standards, HA-4 (M_r 821), HA-6 (M_r 1222), and HA-10 (M_r 2025) from hyaluronidase-degraded hyaluronan purified on Q Sepharose™ Fast Flow
Sample volume:	50 µL
Buffer:	10 mM sodium phosphate, 80 mM sodium sulfate, pH 7.0
Flow rate:	0.5 mL/min
Detection:	205 nm
System:	HPLC

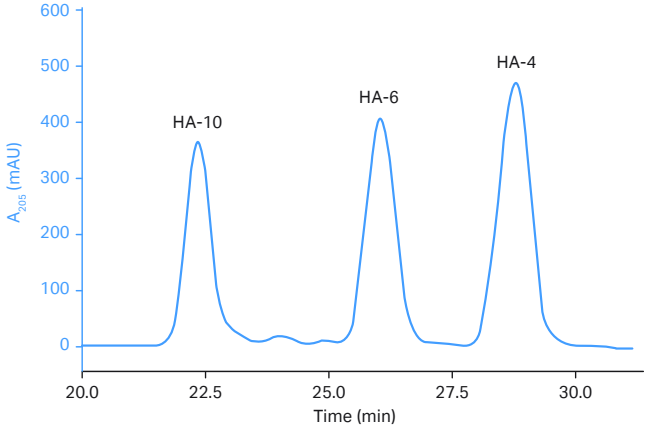


Fig 10. Separation of hyaluronan standards on Superdex 30 Increase 10/300 GL.

Analysis of size distribution of a protein hydrolysate

There is a growing interest in protein hydrolysates. It has been shown that short bioactive peptides can have higher nutritive value and act as antioxidants, for example.

In the characterization of protein hydrolysates, it is important to analyze the size distribution. This is challenging with techniques such as reversed phase chromatography (RPC) or SDS-PAGE, as small peptides are not separated efficiently according to size. Superdex 30 Increase, on the other hand, offers size separation of small proteins and peptides under native conditions.

Figure 11A shows SDS-PAGE analysis of proteins extracted from lentils (lane 2) and a tryptic hydrolysate obtained from the extract (lane 3). The protein extract has been hydrolyzed into small peptides, but these could not be separated efficiently.

The hydrolysate sample was separated on Superdex 30 Increase, Superdex 75 Increase, and Superdex 200 Increase columns. Figure 11B shows that the hydrolysate was most efficiently separated on Superdex 30 Increase with its smaller fraction range (M_r 100 and 7000). On the other hand, Superdex 75 Increase and Superdex 200 Increase are more suitable for separation of larger molecules such as the proteins extracted from the lentils.

Columns: Superdex 30 Increase 10/300 GL
Superdex 75 Increase 10/300 GL
Superdex 200 Increase 10/300 GL
Sample: Tryptic hydrolysate from lentils
Sample volume: 50 µL
Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate: 0.75 mL/min
Detection: 214 nm
System: HPLC

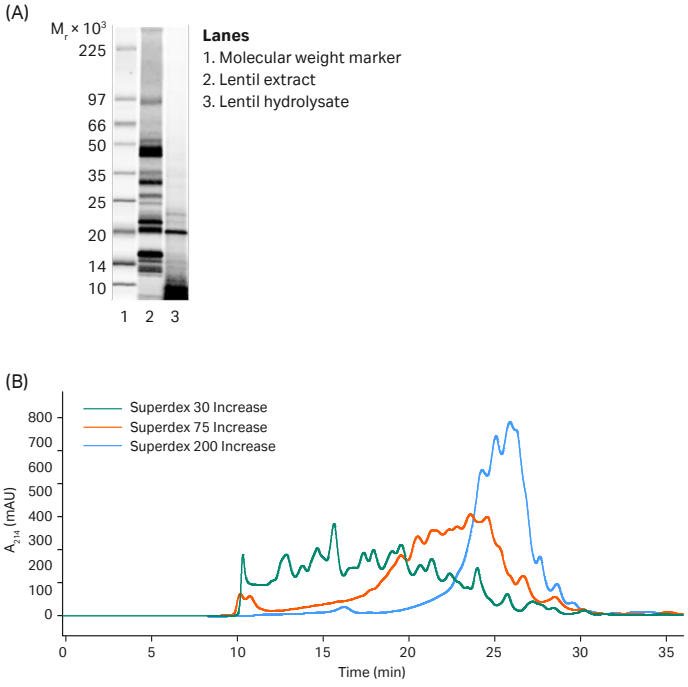


Fig 11. (A) SDS-PAGE analysis of lentil extract, lane 2, and lentil hydrolysate, lane 3; (B) Separation of a lentil hydrolysate sample on Superdex 30 Increase, Superdex 75 Increase, and Superdex 200 Increase.

Purity of a peptide: different lots of [Ile^7]-Angiotensin III

Peptides are attracting increasing interest as therapeutics since they are considered potent and specific, but also relatively safe and easy to manufacture. Characterization of different types of impurities is important not only for release of a drug, but also for initial functionality studies in early drug discovery phases. Analytical SEC offers quantitative assessment of aggregates, multimers, and other size homogeneities of the active peptide in native conditions.

Figure 12 illustrates the separation of a peptide from two different lots using Superdex 30 Increase 10/300 GL. The amount of main impurity (peak 1) was 1.2% of the total peak area for lot A and 0.1% for lot B. Total impurity was 3.2% and 0.7% for lots A and B, respectively. Additional mass spectrometry analysis showed that the main impurity (peak 1) contained peptide with a mass suggesting acetylation of an amino acid and that an impurity in peak 2 contained peptide with a mass suggesting a missing amino acid in the sequence.

Column: Superdex 30 Increase 10/300 GL
Sample: Different lots of [Ile^7]-Angiotensin III (M_r 897)
Sample volume: 100 µL
Buffer: 20 mM phosphate buffer, 280 mM NaCl, pH 7.4
Flow rate: 0.5 mL/min
Detection: 214 nm
System: HPLC

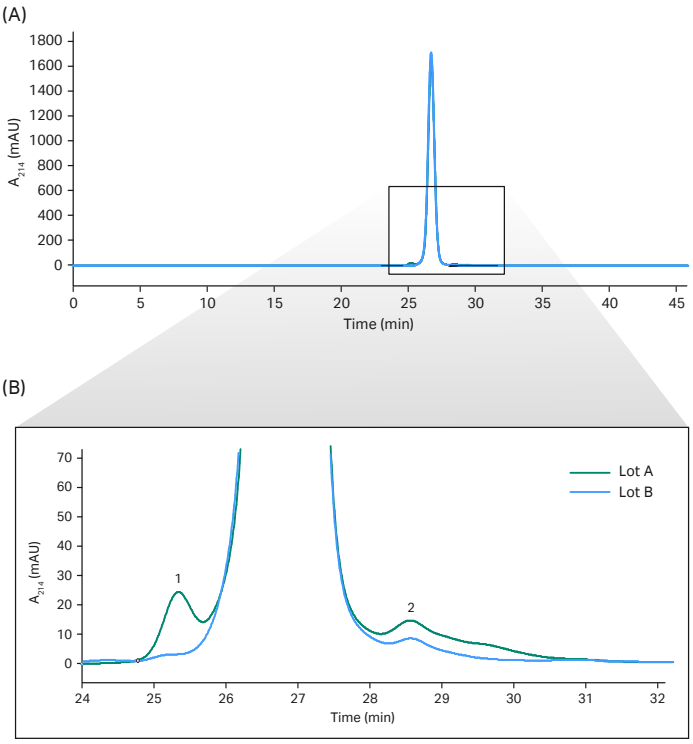


Fig 12. (A) Separation of two different lots of [Ile^7]-Angiotensin III on Superdex 30 Increase; (B) Magnified chromatogram showing impurity profile.

Ordering information

Product	Quantity	Product code
Superdex 30 Increase 10/300 GL	1	29219757
Superdex 30 Increase 3.2/300	1	29219758
Related products		
Gel Filtration LMW Calibration Kit	1	28403841
Gel Filtration HMW Calibration Kit	1	28403842
Superdex 75 Increase 10/300 GL	1	29148721
Superdex 75 Increase 5/150 GL	1	29148722
Superdex 75 Increase 3.2/300	1	29148723
Superdex 200 Increase 10/300 GL	1	28990944
Superdex 200 Increase 5/150 GL	1	28990945
Superdex 200 Increase 3.2/300	1	28990946
Superose 6 Increase 10/300 GL	1	29091596
Superose 6 Increase 5/150 GL	1	29091597
Superose 6 Increase 3.2/300	1	29091598
Accessories		
Tricorn 10 Filter Kit	1	29053612
Filter Tool	1	18115320
Fingertight connector, 1/16" male	10	18111255
Tricorn storage/shipping device	1	18117643
Related literature		
Handbook: Size exclusion chromatography, principles and methods	1	18102218
Selection guide: Prepacked chromatography columns for ÄKTA systems	1	28931778
Instrument management handbook: ÄKTA laboratory-scale chromatography systems	1	29010831
Procedure: Maintenance and cleaning of size exclusion chromatography columns	1	29140760
Cue card: Optimal configuration of ÄKTA pure 25 for small-scale SEC	1	29181181

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