Chromatography systems

ÄKTA™ avant

ÄKTA avant is a preparative chromatography system intended for method and process development (Fig 1). Available in two versions, ÄKTA avant systems have different flow rate/pressure specifications but share the same hardware setup. With flow rates up to 25 ml/min, ÄKTA avant 25 system is designed for chromatography media (resins) screening and method optimization. ÄKTA avant 150 system, with flow rates up to 150 ml/min, is designed for scaling up to larger columns.

UNICORN™ software is used to control automated processes on ÄKTA avant to increase productivity and efficiency. ÄKTA avant offers a complete solution for fast, high-quality protein separations while maintaining reliability and increasing process knowledge.

ÄKTA avant offers the following benefits:

- ÄKTA avant together with UNICORN software provides a platform for efficient process development
- ***Design of Experiments (DoE)***, an experimental design tool, provides time and cost savings by capturing more precise information in fewer experiments
- Integrated fraction collector with cooling functionality protects purified samples
- Automatic on-line buffer preparation using ***BufferPro*** reduces the time required for buffer blending and manual titration, increasing your productivity
- UNICORN software provides intuitive and flexible method creation, system control, and evaluation to simplify your purification task

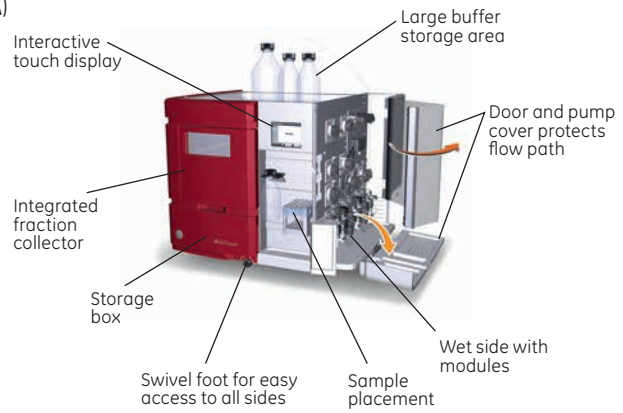
Fig 1. ÄKTA avant is a preparative chromatography system designed for process development, method optimization, and scale-up.

System components

The system consists of the ÄKTA avant instrument and UNICORN software. The instrument offers easy access to working areas using a swivel foot (Fig 2A). ÄKTA avant has a modular design, with all valves, monitors, and columns mounted on the wet side of the instrument. The wet side of the instrument allows easy interaction with the system, and has a door and pump cover for safer handling during runs (Fig 2A and 2B). A buffer tray on top of the instrument provides a large storage area for vessels and bottles. On the front side of the instrument, ÄKTA avant has a built-in, cooled fraction collector that provides secure sample storage.

An interactive display on the front panel informs you of the current instrument and method state, and the run can quickly be paused or continued from the display. To ensure reliability, the system performs self-diagnostic tests of appropriate settings at start-up.

(A)



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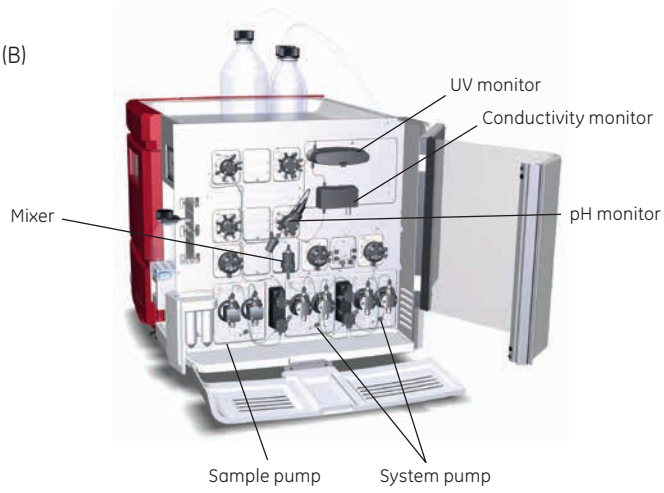

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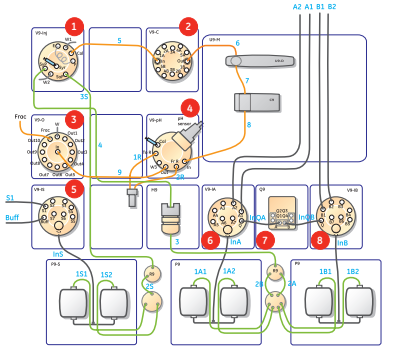


1 Injection valve
2 Column valve
3 Outlet valve
4 pH valve
5 Sample inlet valve
6 Inlet valve A
7 Quaternary valve
8 Inlet valve B

Fig 2. (A) Features of ÄKTA avant; (B) an illustration of the wet side; (C) a diagram showing the wet side.

The flow path is designed to minimize band-broadening effects, and wetted materials used in the flow path are biocompatible and resistant to commonly used solvents. ÄKTA avant system components are described in the following sections in more detail.

Pumps

ÄKTA avant system and sample pumps are based on well-known technology and robust construction. The pumps deliver reproducible flow rates at both low and high back pressures, allowing short separation times and the use of modern scale-up chromatography media. Figure 2B shows a view of the wet side of ÄKTA avant and the location of the pumps. There are two pressure sensors connected to the pumps for monitoring the pressure.

- **System pump:** Provides continuous and accurate flow rates, to give reproducible isocratic or gradient elutions. This pump consists of two pairs of pump heads, which deliver synchronized, low-pulsation flow to the mixer. For ÄKTA avant 25, the maximum operating pressure is 20 MPa (200 bar, 2900 psi) and the maximum working flow rate is 25 ml/min. For ÄKTA avant 150, the maximum operating pressure is 5 MPa (50 bar, 725 psi) and the maximum working flow rate is 150 ml/min. For column packing, ÄKTA avant 25 and 150 can be used at flow rates up to 50 ml/min and 300 ml/min, respectively.
- **Sample pump:** Dedicated pump for performing automatic sample application. The sample pump, consisting of two pump heads, is based on the same pump principle as the system pump. Due to its design, pump purging and air removal can easily be performed automatically. The air sensor protects columns by ensuring no air is introduced into the column. The pump can apply samples directly to a column or indirectly via a capillary loop or a Superloop™ injection device.

Valves

A series of motorized, multiport valves control the movement of liquid through the system (Fig 2C). The different types of valves are described below.

- **Injection valve:** Enables automatic sample application onto the column, without replumbing capillaries between different injection modes. It allows for a variety of sample application techniques including filling a capillary loop manually via a syringe or with the sample pump, filling a Superloop device manually with a syringe or sample pump, or using the sample pump for direct load onto a column.
- **Sample inlet valve:** Allows automatic change between different samples. This valve has an integrated air sensor, ensuring complete sample application. The valve has seven sample inlet positions plus a dedicated buffer inlet for filling the sample pump with solution before the sample is introduced, and for washing out the valve and pump between runs.

- **Column valve:** Connected to the injection valve and used to select the column to be used. Up to five columns can be connected to the column valve for automatic column scouting. The column valve has an integrated column bypass function, minimizing the risk of overpressure on the columns, for example, during system and pump wash procedures. To increase the yield and performance when using absorption techniques, the column valve also has a built-in reverse flow functionality. Reversed flow can be used during column cleaning or when eluting a sample to give sharper bands and a more concentrated target molecule eluent. The column valve also has two integrated pressure sensors that measure pre- and post-column pressure for the calculation of delta pressure (see Sensors and monitors).
- **Two inlet valves (A and B):** Enable automatic change between different buffers and wash solutions. The inlet valves have integrated air sensors (see Sensors and monitors).
- **Quaternary valve:** Used for ***BufferPro*** and for creating quaternary gradients. The valve has four buffer inlets that enable automatic buffer preparation with ***BufferPro***, using four stock solutions.
- **pH valve:** Includes an integrated pH electrode that enables in-line pH monitoring during the run. A flow restrictor is connected to the pH valve and can be automatically included in the flow path to generate a back pressure that prevents the formation of air bubbles in the UV flow cell. The pH valve is used to direct the flow to the pH electrode and flow restrictor, or alternatively, to bypass one or both. It is recommended to bypass the flow restrictor when using low pressure columns at high flow rates.
- **Outlet valve:** Mainly used to direct the flow to the fraction collector, waste, or other outlet ports. The valve has dedicated ports for the fraction collector and waste, as well as 10 other outlets for collecting large fractions.

Mixer

The mixer ensures homogeneous buffer composition during gradient runs (Fig 2B). The mixer chamber size depends on the flow rate and buffers used, with a larger mixer volume required for higher flow rates or difficult-to-mix buffers. Table 1 shows the mixer chamber sizes available for each instrument. The in-line filter on the mixer is easy to change, and has a pore size of 10 µm. The mixer is also easily changed by snapping it in or out of the mixer holder.

Table 1. Mixer chamber sizes available

System	Mixer chamber sizes
ÄKTA avant 25	0.6, 1.4, and 5 ml
ÄKTA avant 150	1.4, 5, and 15 ml

Sensors and monitors

Air and pressure sensors increase operational security and protect the system. The UV, conductivity, and pH monitors allow real-time data from the chromatographic run to be accurately measured.

- **Air sensors:** Enable exclusion of air from the system. Integrated air sensors are placed in the sample inlet valve and inlet valves A and B. When air is detected, the system is paused so that the air can be removed before further introduction into the flow path. During sample application, the air sensor detects when the sample has been completely injected so the method can continue to the next step without air being introduced into the flow path or column.
- **Pressure sensors:** Integrated into the column valve to protect the column and medium from overpressure. One pressure sensor measures the pressure before the column to protect the column hardware. Another sensor measures the pressure after the column and calculates the pressure difference (delta pressure [Δp]) over the packed medium bed (Fig 3). If one of the pressures exceeds the preset limit, the run is paused. Alternatively, the user can activate pressure flow regulated mode, which automatically decreases the flow rate when the pressure exceeds the preset limit. Pressure sensors are also connected to the system and sample pumps to protect the connected columns and instrument hardware.

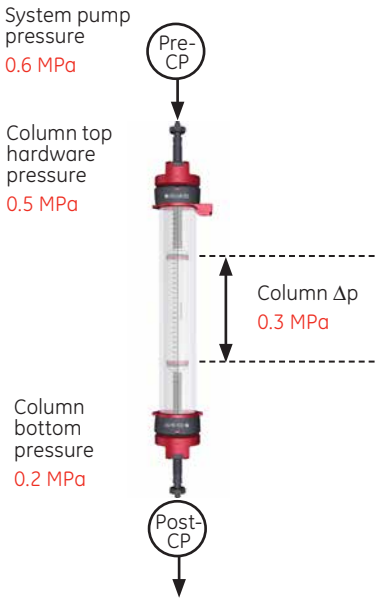


Fig 3. For increased operational safety, the column selection valve enables continuous measurement of precolumn (Pre-CP) and post-column pressure (Post-CP) during runs. The pressure difference over the packed medium bed (Δp) is calculated from the two signals.

- **UV monitor:** Provides real-time measurements of absorbance over the UV and visible range of 190 to 700 nm. To visualize protein separation at different wavelengths, the UV monitor has a flip-mode that allows monitoring of up to three wavelengths simultaneously. For optimum performance when purifying samples with different protein concentrations, there are three flow cell path lengths (0.5, 2 [default], and 10 mm). The optimal flow cell design, together with advanced fiber optics, provides a high signal-to-noise ratio without causing any local heating of the UV flow cell (especially critical when working with heat sensitive samples). The UV monitor contains a high intensity xenon lamp with a long lifetime that requires minimal start-up time. Every time the instrument is switched on, the UV monitor is automatically calibrated.
- **Conductivity monitor:** Measures conductivity of buffer and samples for on-line monitoring of the true gradient. The conductivity monitor also has an integrated temperature sensor for monitoring temperature. Temperature changes can lead to variation in conductivity, but the integrated temperature sensor corrects for and minimizes this type of variation.
- **pH monitor:** Continuously measures pH of buffer and samples. The pH electrode is integrated into the pH valve, and the built-in calibration port allows for convenient calibration-in-place without having to remove the pH electrode.

Fraction collector

The built-in fraction collector provides sample security, flexibility, and high throughput. The fraction collector has a cooling functionality to prevent sample overheating and protect purified samples. A variety of cassettes are available for tubes (3, 5, 8, 15, and 50 ml) as well as deep well plates (24-, 48-, and 96-well). Six cassettes can be loaded into the fraction collector in any combination that fits the user's needs (Fig 4). As an alternative to using six cassettes, loading capacity can be maximized by using a large tube rack for 50 ml tubes or a bottle rack for 250 ml bottles.

Upon loading, the type of cassette is automatically detected and the tube/bottle configuration is validated. Cassettes designed for tubes are equipped with a QuickRelease function that locks tubes into place when discarding liquid waste. Later, using QuickRelease, the tubes can be easily unlocked and discarded at the same time. The cassettes can also be used for convenient storage of fractions or holders for sample tubes.

ÄKTA avant has two beneficial features that minimize cross-contamination and spillage during fraction collection. DropSync can be used for flow rates up to 2 ml/min (ÄKTA avant 25 only) and minimizes spillage by timing fraction changes to occur between drops. At higher flow rates, the accumulator function temporarily holds the liquid flow during the time it takes to move to the next tube or well.

Fig 4. The built-in, cooled fraction collector holds up to six different cassettes. Cassettes are scanned, and the configuration is automatically validated.

Fraction collection can be based on time, volume, or automatic peak recognition. Automatic peak recognition minimizes cross-contamination and unwanted eluent can be diverted to the waste. Large fractions up to several liters can be collected using the outlet valve. By expanding the system with two extra outlet valves, 32 outlets can be used for fraction collection (see Optional components).

UNICORN software

UNICORN software gives you real-time control of your chromatography system. UNICORN consists of four modules: **Administration**, **Method Editor**, **System Control**, and **Evaluation**. This section describes some of the valuable tools included in UNICORN for increasing operational security, efficiency, and productivity.

Method Editor

The **Method Editor** module allows you to create or adjust methods to suit your application needs. It contains all the instructions used for controlling the run. The **Method Editor** includes built-in application support for chromatography runs. The interface provides easy viewing and editing of the run parameters. Figure 5 shows a screenshot of the **Method Editor** with customizable panes that provide a comprehensive overview of the run.

The **Method Editor** provides a choice of predefined methods for different chromatography techniques and maintenance procedures. Methods are built using phases. Each phase reflects a step in the run, such as sample application or wash. UNICORN includes a library of predefined phases for creating or editing your own methods. A method is created or edited by dragging-and-dropping phases from the ***Phase Library*** to the ***Method Outline***.

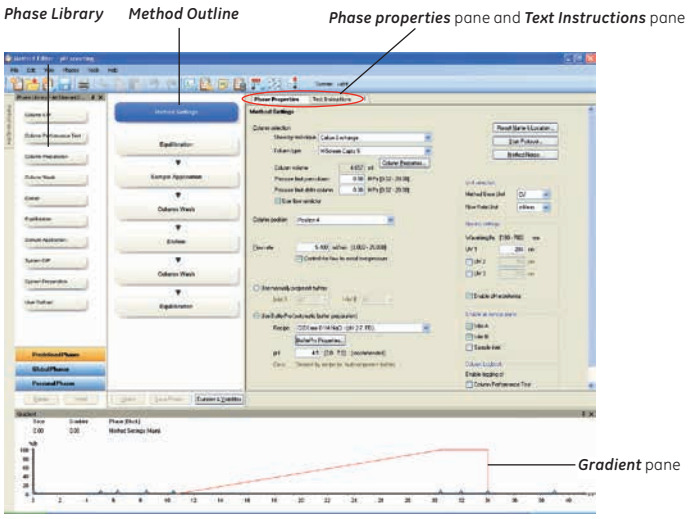


Fig 5. Method Editor has customizable panes that give a comprehensive overview of the method.

UNICORN includes a library of predefined GE Healthcare Life Sciences' columns. By selecting the column in the ***Phase Properties*** pane, column parameters (e.g., flow rate and pressure limits) are automatically programmed into the method. For added flexibility, advanced users can edit programming instructions directly in the ***Text Instructions*** pane.

System Control

The **System Control** module is used to start, view, and control a method run. The module consists of panes that provide an overview of the status of the run. The ***Run Data*** pane presents current data in numerical values, while the ***Chromatogram*** pane illustrates data as curves during the entire method run. The ***Process Picture*** pane displays the current flow path during the run and can be used to control the run (Fig 6). Color indication incorporated in the process picture shows the current open flow path with flow, or flow path with no flow. Real-time data from monitors are also displayed in the process picture pane.

Column Logbook

To increase operational safety, UNICORN software features the ***Column Logbook***. This practical tool keeps track of important column and run data to provide traceability and operational security. Most prepacked columns from GE Healthcare are barcode labeled, and individual columns are identified using a 2-D barcode scanner. The information can also be entered manually into UNICORN (Fig 7). UniTag sticker labels, with preprinted barcodes, are available for other columns (e.g., empty columns).

By tracking individual columns, information is recorded for each run regarding run data, such as total number of runs and maximum delta pressure. Notification limits can be set, for instance, to define the number of times the column may be run between cleanings, and the user is notified when it is time for column maintenance. The ***Column History*** function provides a list of all runs that have been performed with a particular column.

In addition to ***Column Logbook***, UNICORN offers security by utilizing electronic signatures, password protection, and audit trails. UNICORN is suitable for use in a regulated environment in a manner complying with FDA 21 CFR Part 11. For more detailed information, see UNICORN software data file (29135786).

Fig 7. Several prepacked columns are manufactured with labels containing barcodes, allowing individual columns to have unique IDs for traceability. For columns without barcode labels, UniTag labels are available.

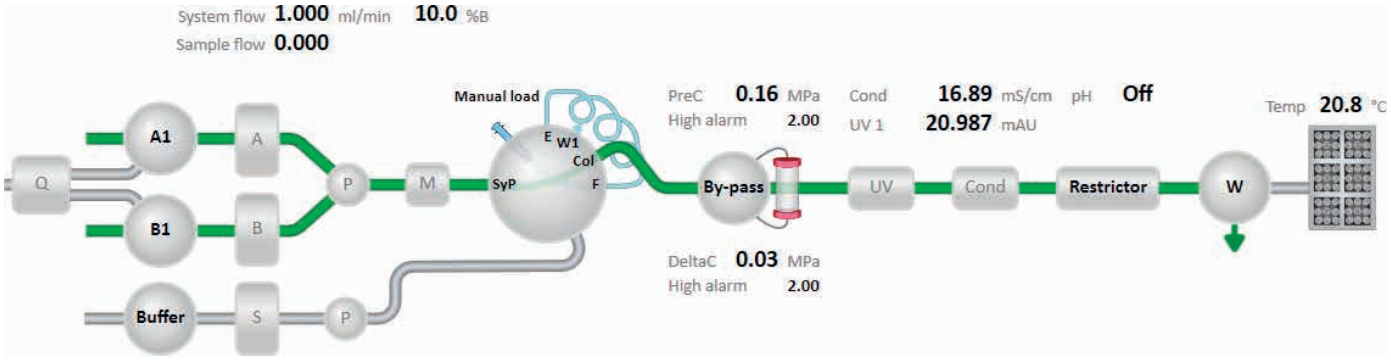


Fig 6. The UNICORN process picture shows currently active and inactive flow paths, and provides a fast and easy way to control the system.

BufferPro

Automatic buffer preparation with ***BufferPro*** increases productivity by minimizing the need for manual preparation of buffers. ***BufferPro*** can be used for automatic pH scouting to find optimal buffer compositions. For screening, a broad pH range is scanned by testing large pH steps. For further optimization and to test robustness, smaller pH steps are tested (Fig 8). ***BufferPro*** can be used at flow rates up to 25 ml/min on ÄKTA avant 25 and up to 40 ml/min on ÄKTA avant 150.

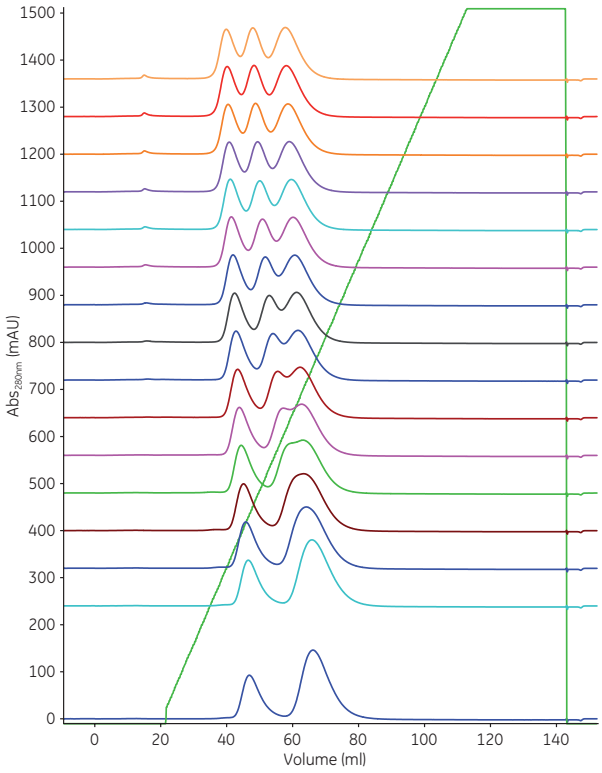


Fig 8. HiScreen™ Capto™ S column was run using the CIEX buffer from pH 4.0 to 5.5. The chromatogram shows sixteen runs using small (0.1) pH steps. The full pH range of the CIEX buffer is 2 to 7.

BufferPro eliminates time-consuming buffer preparation and manual titration for experiments requiring pH changes. Using ***BufferPro***, stable stock solutions can be prepared, stored, and used repeatedly. This saves time and reduces chemical waste compared to manual preparation. ***BufferPro*** delivers buffer compositions for large number of applications and features true buffer preparation using conjugated acid/base solutions for titration. This mimics the way manual buffer preparation is performed and increases buffering capacity and scalability.

Buffer is prepared in-line from four stock solutions (e.g., water, salt, buffer, and acid/base) and pH is monitored throughout the run, with changes in temperature compensated for automatically. ***BufferPro*** also compensates for the salt concentration in a gradient to achieve a more stable pH.

Buffer substances can be selected from the ***Buffer Library*** to change the pH range and create the required pH elution conditions. After mixing, ***BufferPro*** verifies the buffer properties by showing the actual mixing ratios. The accuracy of pH is crucial in many separations and BufferPro gives accurate and highly reproducible data.

Design of Experiments (DoE)

Included as standard for ÄKTA avant systems, UNICORN software has an integrated ***Design of Experiments (DoE)*** functionality. The ***DoE*** function is a powerful tool for an efficient approach to method optimization. In the traditional approach, optimal conditions can be determined by varying one parameter at a time while the rest of the parameters are kept fixed. Important information, such as interaction data between different parameters, might be missed. DoE is an organized, statistical approach that varies multiple factors simultaneously to significantly reduce the number of required experiments (Fig 9). The effect of all parameters and their interactions are detected and described in a validated statistical model. As the ***DoE*** tool is integrated seamlessly in UNICORN, scouting methods are automatically generated from DoE schemes, allowing fast and efficient method optimization. It also eliminates the need for data transfer between UNICORN and an external DoE software.

Experimental workflows in ***DoE*** include:

- Screening: to determine which factors are important in your process
- Optimization: to find the optimal factor settings for your process
- Robustness testing: adjusting different factors to investigate how robust your process is

By using the ***DoE*** tool, the entire experimental space can be explored efficiently by taking into account the important factors for your process, such as flow velocity and elution pH, and the appropriate range for each factor.

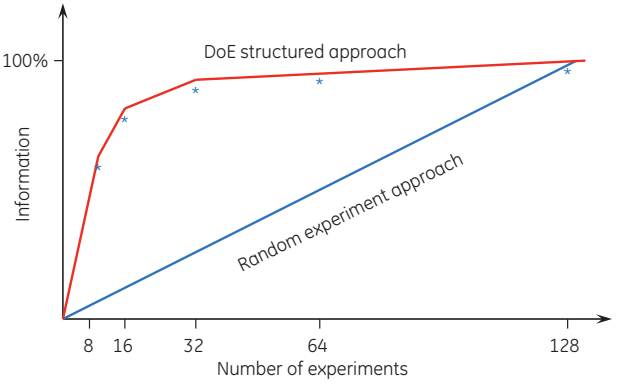


Fig 9. The UNICORN ***DoE*** tool is an efficient approach to optimization, capturing more information in fewer experiments.

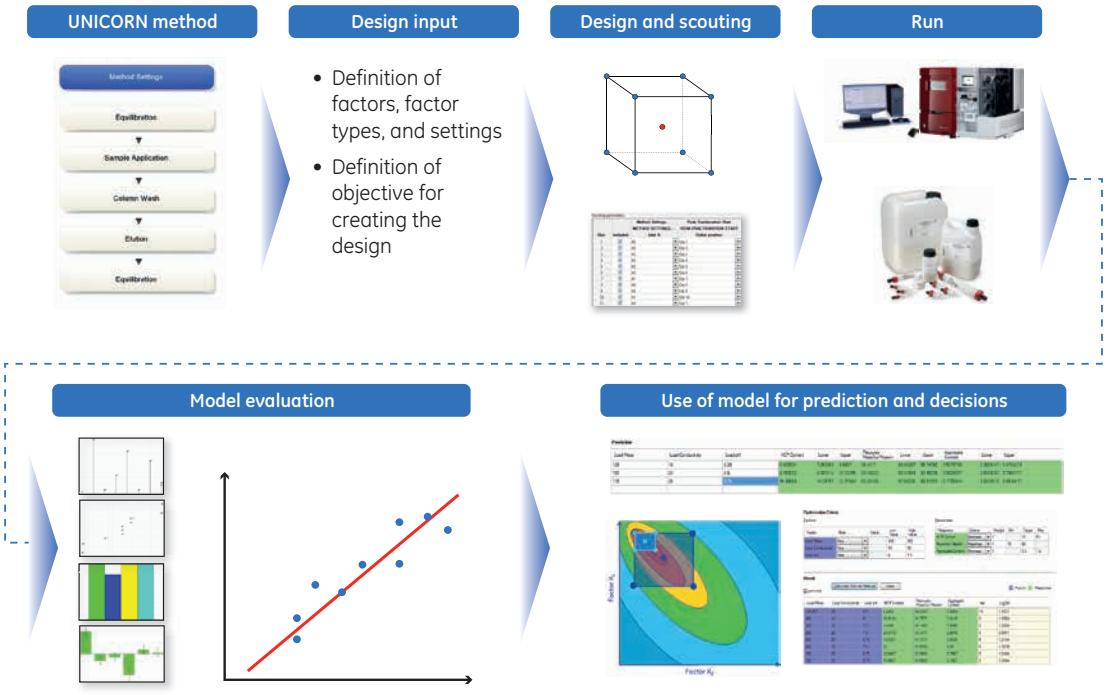


Fig 10. The basic ***DoE*** workflow is shown. In the ***DoE*** tool, multiple factors are varied simultaneously and the resulting data is used to generate a statistical model. The model is validated and used to produce maps that support decision making.

The resulting data or "responses" (e.g., yield and purity) are used to create a statistical model (Fig 10). This model is automatically generated in UNICORN and used to predict the response to changing a factor, and to produce maps (response contour plots) that support decision making. By using a DoE approach variability and noise, as well as interaction effects between different factors, can be estimated. This approach means that more precise information is obtained and better maps are generated, which can lead to better decision making.

Evaluation

With UNICORN 7, the **Evaluation** module provides a simplified user interface optimized for most commonly used workflows like quick evaluation, comparison of results, and work with peaks and fractions.

Applications

Rapid process development of a MAb purification

ÄKTA avant 25 system was used to develop a two-step chromatography process for purification of a monoclonal antibody (MAb). MabSelect SuRe™, a protein A-based medium was used for the initial capture step while a multimodal anion exchanger, Capto adhere medium, was used for reduction of impurities in a second polishing step. Table 2 summarizes the method development steps for the purification.

Table 2. Summary of the method development steps in a two-step MAb purification using ÄKTA avant 25 system

Step	Objective	Column
1	Determine elution pH on MabSelect SuRe	HiScreen MabSelect SuRe
2	Determine dynamic binding capacity with MabSelect SuRe	HiTrap™ MabSelect SuRe
3	MAb purification on MabSelect SuRe	HiScreen MabSelect SuRe
4	Prepare (scale-up) material for Capto adhere	XK 50/20 packed with MabSelect SuRe medium
5	Determine loading conditions on Capto adhere	HiScreen Capto adhere
6	Screen loading conditions on Capto adhere using DoE approach	HiTrap Capto adhere
7	Robustness study on Capto adhere using DoE approach	HiTrap Capto adhere

After determining the elution pH and dynamic capacity, MAb was purified on a HiScreen MabSelect SuRe column and resulted in 99% recovery (capture step, data not shown). For the polishing step, a DoE approach was used to optimize loading conditions by varying the following factors: sample pH, conductivity, and load. These factors were correlated to impurity levels in the MAb samples (i.e., host cell protein, antibody dimers, and aggregates) to determine the optimal loading conditions. After optimization, a robustness study, using a DoE approach, was performed to confirm that the process conditions were robust. For full experimental details, see application note "Rapid process development for purification of a MAb using ÄKTA avant 25" (28957347).

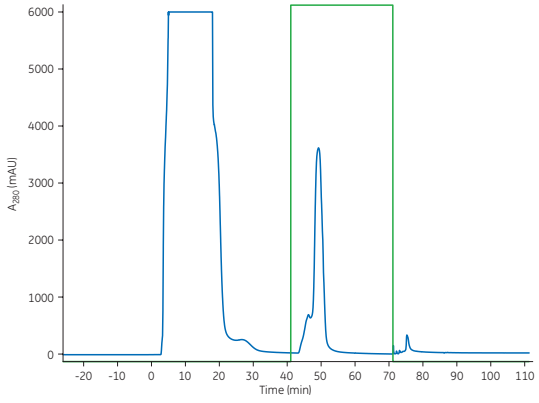
The feed material used for this study was challenging due to MAb aggregation and sample precipitation. Despite these challenges, high yield and purity of the target MAb was achieved. Using the integrated ***DoE*** functionality in UNICORN software together with prepacked HiScreen columns, optimization of the overall process was achieved in approximately one week.

Seamless scalability

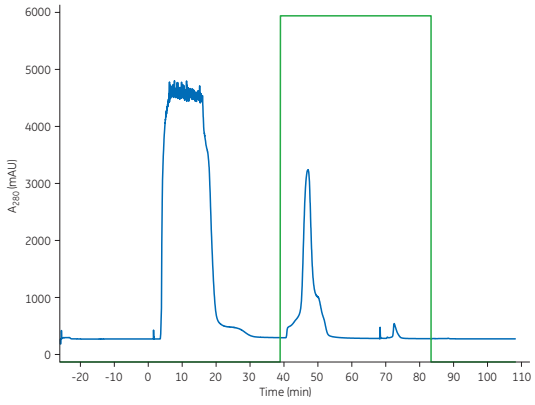
In UNICORN software, methods are quickly rescaled using a time-saving functionality that automatically converts methods between the two ÄKTA avant systems. Figure 11 shows a scale-up study that utilized this feature.

Insulin was first purified using ÄKTA avant 25 with prepacked HiScreen columns. The purification was conveniently scaled up to an AxiChrom™ 50 column using ÄKTA avant 150 system.

(A)
Column: HiScreen Capto MMC (two connected, 20 cm bed height)
Sample: Insulin*
Start buffer: 50 mM acetate, 8 M urea, 150 mM NaCl, pH 5.2
Elution buffer: 50 mM sodium phosphate, 8 M urea, 150 mM NaCl, pH 8
Flow rate: 1.86 ml/min
System: ÄKTA avant 25



(B)
Column: AxiChrom 50 packed with Capto MMC medium (20 cm bed height)
Sample: Insulin*
Start buffer: 50 mM acetate, 8 M urea, 150 mM NaCl, pH 5.2
Elution buffer: 50 mM sodium phosphate, 8 M urea, 150 mM NaCl, pH 8
Flow rate: 80 ml/min
System: ÄKTA avant 150



*Sample was kindly provided by Biomm SA (Brazil).

Fig 11. Predictable scale-up from a smaller column to a production-scale column using (A) ÄKTA avant 25; (B) ÄKTA avant 150.

Optional components

ÄKTA avant is a modular system that can be expanded to enhance the system capabilities and increase productivity. Due to the accessibility and design of the modules, they can be easily exchanged allowing quick and efficient servicing.

ÄKTA avant can be expanded with extra valves to obtain up to 28 inlets and 14 sample inlets to expand on buffer and sample inlet capacity, and up to 32 outlets for increasing number of fractions through the outlet valve. The Versatile valve is a general four-position valve that can be used, for example, for multistep purification schemes and incorporating external equipment into the flow path. Up to four versatile valves can be connected to the system. The Loop valve allows the use of up to five loops and can be used for collection of intermediate fractions when performing multistep purification or for automated purification of up to five different samples.

A second conductivity and UV monitor are also offered to increase detection points and to support multistep purification. The I/O-box provides a means of connecting external interfacing equipment such as detectors. The I/O-box receives analog or digital signals from, or transfers analog or digital signals to external equipment that needs to be incorporated in the system. For types of optional components available, see Ordering information.

BioProcess™ chromatography media

The BioProcess medium family comprises chromatography media widely used by biopharmaceutical manufacturers. Support for these products includes validated manufacturing methods, secure long-term medium supply, easy handling, and regulatory support files (RSF) to assist process validation and submissions to regulatory authorities.

In addition, the Fast Trak Training & Education team provides high-level hands-on training for all key aspects of bioprocess development and manufacturing.

ÄKTA avant has the flow rate and pressure specifications that support BioProcess media such as MabSelect™ and Capto. These high-flow media provide increased dynamic binding capacity at high flow rates. Using ÄKTA avant together with BioProcess media can reduce process time, increase productivity, and allow easy scale-up.

Columns

GE Healthcare offers an extensive range of prepacked columns that can be used with ÄKTA avant including HiTrap, HiPrep™, HiLoad™, and HiScreen columns. In addition to prepacked columns, empty HiScale™ columns are available for process development as well as AxiChrom columns for chromatography on a larger scale.

HiScreen prepacked columns

HiScreen columns are prepacked with a wide range of robust BioProcess media to allow repeated use with highly reproducible results. Designed for method optimization, HiScreen columns have a 10 cm bed height and can easily be connected in series to achieve a 20 cm bed height. To provide traceability, HiScreen columns have barcodes preprinted on their labels. For convenience, these columns can be connected to the ÄKTA avant system by easily snapping them into the Flexible column holder.

HiScale columns

HiScale is a family of pressure-stable columns designed for process development and preparative chromatography. The columns are optimized for BioProcess media and have several features that enable precise column packing, easy handling, and high reproducibility. HiScale columns are available in sizes of 16, 26, and 50 mm inner diameter (i.d.) and lengths of 20 and 40 cm.

AxiChrom columns

AxiChrom is a sanitizable column platform that simplifies column handling at all scales from process development to full-scale production. ÄKTA avant 150 supports Intelligent Packing of the smallest AxiChrom columns, 50 and 70 mm i.d. (Fig 12). Verified, preprogrammed packing methods are available to ensure optimally-packed beds and decrease operator dependence. AxiChrom columns have a purposeful design making them simple to operate. UNICORN software guides the user through method creation, setup, and maintenance. Scale-up using AxiChrom columns is straightforward and predictable.

Fig 12. AxiChrom columns provide intelligent packing, intuitive handling, and predictable scale-up.

Accessories

ÄKTA avant accessories include holders and clamps for attaching columns, flasks, and tubing to the system (Fig 13). For HiTrap columns, the Column holder rod is available for holding up to five columns simultaneously. Smaller columns such as HiScreen are easily snapped into the Flexible column holder. For a list of accessories, see Ordering information.

Fig 13. ÄKTA avant accessories include holders and clamps for attaching columns, flasks, and tubing to the system. o.d. = outer diameter

System specifications

Control system	UNICORN software, version 6.3.2 or later
Dimensions (W × H × D)	860 × 660 × 710 mm
Weight (excluding computer)	116 kg
Power supply	100–240 V, ~50–60 Hz
Power consumption	800 VA
Enclosure protective class	IP 21, wet side IP 22

System pump

Pump type	Piston pump, metering type
Flow rate setting	ÄKTA avant 25: 0.001 to 25 ml/min (normal range) 0.001 to 50 ml/min (column packing flow) ÄKTA avant 150: 0.01 to 150 ml/min (normal range) 0.01 to 300 ml/min (column packing flow)
Pressure range	ÄKTA avant 25: 0 to 20 MPa (200 bar, 2900 psi) ÄKTA avant 150: 0 to 5 MPa (50 bar, 725 psi)
Viscosity range	ÄKTA avant 25: 0.35 to 10 cP ÄKTA avant 150: 0.35 to 5 cP

Sample pump

Pump type	Piston pump, metering type
Flow rate setting	ÄKTA avant 25: 0.01 to 50 ml/min ÄKTA avant 150: 0.01 to 150 ml/min
Pressure range	ÄKTA avant 25: 0 to 10 MPa (100 bar, 1450 psi) ÄKTA avant 150: 0 to 5 MPa (50 bar, 725 psi)
Viscosity range	0.7 to 10 cP

Mixer, valves, and fraction collector

Mixer and gradient formation

Mixing principle	Chamber with a magnetic stirrer
Mixer volume	ÄKTA avant 25: 0.6, 1.4, or 5 ml ÄKTA avant 150: 1.4, 5, or 15 ml
Gradient flow rate range	
Binary	ÄKTA avant 25: 0.25 to 25 ml/min ÄKTA avant 150: 1.0 to 150 ml/min
Quaternary	ÄKTA avant 25: 0.5 to 25 ml/min ÄKTA avant 150: 2 to 40 ml/min
Gradient composition accuracy	Binary: ± 0.6% Quaternary: ± 1%

Valves

Type	Rotary valves (except quaternary valve)
Quaternary valve[1] type	4-port solenoid actuated membrane valve
Optional valves[2]	Up to three optional valves can be installed in the system chassis (Second Inlet valve, Loop valve, Versatile valve, Second Column valve, Extra Outlet valve, Extra Inlet valve)

Number of inlets

Inlet A	7, expandable to 14
Inlet B	7, expandable to 14
Sample inlet	7, expandable to 14
Quaternary inlet	4

Outlet valve fractionation

Number of outlets	10, expandable to 32

Integrated fraction collector[3]

Number of fractions	up to 576 (6 × 96-well deep-well plates)
Vessel types	3, 5, 8, 15, or 50 ml tubes, 24-, 48-, or 96-well deep-well plates, 250 ml bottles
Fraction volumes	ÄKTA avant 25: 0.1 to 250 ml ÄKTA avant 150: 1 to 250 ml
Spillage-free modes	ÄKTA avant 25: DropSync or accumulator ÄKTA avant 150: accumulator
Cooling specification	6°C to 20°C, temperature control
Organic solvents	No
Delay volume (UV-dispenser head)	ÄKTA avant 25: 518 µl ÄKTA avant 150: 1807 µl

[1] The quaternary valve is not recommended for use with organic solvents.

[2] Using Extension boxes, 29110806, up to three additional modules can be installed outside the systems chassis enabling in total 6 optional modules.

[3] For safety reasons, organic solvents may not be used in the fraction collector during fractionation.

Sensors and monitors

Pressure and air sensors

Placement of pressure sensors	System pump, sample pump, pre-column, and post-column
Placement of air sensors	Inlet A, inlet B, and sample inlet
Optional placement	Before sample inlet valve, after injection valve
Sensing principle	Ultrasonic

UV monitor

Wavelength range	190 to 700 nm in steps of 1 nm, up to three wavelengths simultaneously
Absorbance range	-6 to 6 AU
Resolution	0.001 mAU
Linearity	within ± 2% at 0 to 2 AU
Drift	≤ 0.2 mAU; AU/h at 280 nm, 2 mm cell
Noise	< 0.08 mAU
Operating pressure	0 to 2 MPa (20 bar, 290 psi)
Flow cells	0.5 mm optical path length, 1 µl cell volume 2 mm optical path length, 2 µl cell volume 10 mm optical path length, 8 µl cell volume

Conductivity monitor

Conductivity reading range	0.01 to 999.99 mS/cm
Accuracy	± 0.01 mS/cm or ± 2%, whichever is greater, (within 0.3 and 300 mS/cm)
Operating pressure	0 to 5 MPa (50 bar, 725 psi)
Flow cell volume	22 µl
Temperature monitor range	0°C to 99°C
Temperature monitor accuracy	± 1.5°C within 4°C to 45°C

Temperature monitor

Reading range	0°C to 99°C
Accuracy	± 1.5°C within 4°C and 45°C

pH monitor

pH reading range	0 to 14
Accuracy	± 0.1 pH unit (within pH 2 and 12)
Operating pressure	0 to 0.5 MPa (5 bar, 72 psi)

Optional components

Second UV monitor U9-L

Wavelength range	280 nm
Flow cells	Optical path length 2 mm Cell volume 2 µl Optical path length 5 mm Cell volume 6 µl
Resolution	0.001 mAU
Linearity	± 5% within 0–2 AU
Drift	≤ 0.2 mAU; AU/h, 2 mm cell
Noise	< 0.1 mAU

Second Conductivity monitor

See specification for Conductivity monitor under System specification

Second Fraction collector F9-R

Number of fractions	Up to 175 per fraction collector
Vessels	175 (3 ml tubes) 85 (8 or 15 ml tubes) 40 (50 ml tubes)
Fraction volumes	0.1 to 50 ml
Spillage-free mode	DropSync
Flammable liquids	Yes
Dimensions (W × H × D)	320 × 250 × 400 mm
Weight	5 kg
Delay volume	Calculated based on tubing length and component volumes between the UV and fraction collector, see User Manual

I/O-box E9

Number of I/O boxes	2
Number of ports per box	2 analog in, 2 analog out 4 digital in, 4 digital out
Analog range	In ± 2 V Out ± 1 V

Ordering information

System and software[1,2]	Product code
ÄKTA avant 25	28930842
ÄKTA avant 150	28976337
ÄKTA avant 25/150 user manual[3]	29035184
UNICORN 7.0 workstation license for ÄKTA avant	29128120
UNICORN 7.0 remote license without DVD	29115426
UNICORN 7.0 dry license without DVD	29115427
UNICORN 7.0 DoE concurrent license[4]	29115440
UNICORN 7.0 Standalone Evaluation	29115454
UNICORN 7.0 Evaluation Classic[4]	29115456
UNICORN 7.0 Column Logbook lic[4]	29115441
UNICORN 7.0 manual package[5]	29127795

A range of computers, monitors, keyboards, printers, and cables are available, for details please contact your local GE Healthcare representative.

Optional components	Product code
Optional valves for ÄKTA avant 25	
Column valve V9-C2	28957236
Inlet valve V9-A2	28957221
Inlet valve V9-B2	28957223
Inlet valve V9-X1	28957227
Inlet valve V9-X2	28957234
Inlet valve V9-S2 (sample inlet)	28957225
Outlet valve V9-O2	28957238
Outlet valve V9-O3	28957240
Loop valve kit (V9-L)	29011358
Versatile valve (V9-V)	29011353
Optional valves for ÄKTA avant 150	
Column valve V9H-C2	28979330
Inlet valve V9H-A2	28979303
Inlet valve V9H-B2	28979315
Inlet valve V9H-X1	28979326
Inlet valve V9H-X2	28979328
Inlet valve V9H-S2 (sample inlet)	28979320
Outlet valve V9H-O2	28979332
Outlet valve V9H-O3	28979337
Loop valve kit V9H-L	29090689
Versatile valve V9H-V	29090691
Optional sensors	
Second UV monitor U9-L[6]	29011360
UV flow cell 2 mm for U9-L	29011325
UV flow cell 5 mm for U9-L	18112824
UV flow cell U9-0.5, 0.5 mm for U9-M	28979386
UV flow cell U9-2, 2 mm for U9-M	28979380
UV flow cell U9-10, 10 mm for U9-M	28956378
Second conductivity monitor C9n	29011363
Air sensor L9-1.2 mm[7]	28956502
Air sensor L9-1.5 mm[7]	28956500
Adapter for air sensor	28956342

Optional components	Product code
Fraction collector	
Fraction collector F9-R	29011362
I/O-box E9	
I/O-box E9	29011361

Accessories	Product code
Cassettes	
Cassette tray, holds up to six cassettes	28954209
Cassette, holds six 50 ml tubes (2-pack)	28956402
Cassette, holds fifteen 15 ml tubes (2-pack)	28956404
Cassette, holds twenty-four 8 ml tubes (2-pack)	28956425
Cassette, holds forty 3 ml tubes (2-pack)	28956427
Cassette, holds forty 5 ml tubes (2-pack)	29133422
Cassette, holds one 96-, 48-, or 24-well deep-well plate (2-pack)	28954212
Rack, holds fifty-five 50 ml tubes	28980319
Rack, holds eighteen 250 ml bottles	28981873
Tubing kits for ÄKTA avant 25	
Replacement tubing kit	28956606
Sample tubing kit for 7 inlets, i.d. 0.75 mm	28957217
Inlet tubing kit for 5 inlets	28957215
Outlet tubing kit for 10 outlets, i.d. 1.0 mm	28957219
Rinse system tubing (for ÄKTA avant 25 and 150)	28956504
Tubing kits for ÄKTA avant 150	
Replacement tubing kit	28979446
Tubing kit for 10 inlets (FEP i.d. 2.9 mm, o.d. 3/16")	28980987
Tubing kit for 10 outlets (FEP i.d. 1.6 mm, o.d. 1/8")	28980984
Barcode labels and scanner	
UniTag (1 sheet with 108 labels)	28956491
Barcode scanner 2-D with USB	28956452
Holders[8]	
Column holder	28956282
Column holder rod	28956270
Column clamp o.d. 10–21 mm	28956319
Tubing holder, spool, for small tubing (o.d. 1/8" and smaller)	28956274
Tubing holder, spool, for large inlet tubing (o.d. 3/16") for ÄKTA avant 150	29014283
Tubing holder, comb	28956286
Bottle holder	28956327
Flexible column holder	28956295
Multi-purpose holder	29011349
Rail extension	29011352
Loop Holder	29011350
Extension Box	29110806

[1] Computer ordered separately.
[2] For UNICORN upgrade packages please contact your local GE Healthcare representative.
[3] Electronic version of the ÄKTA avant user manual is supplied with the system on DVD.
[4] License is included in product code 29128120.
[5] Digital version of manuals are included on DVD with 29128120.
[6] Flow cells are ordered on separate code no.
[7] The 1.5 mm air sensor is placed before the inlet valves for the A, B, Q, and sample inlets; the position for the 1.2 mm air sensor is after the injection valve or before the sample inlet valve when 0.75 mm tubing is used.
[8] For more information about HiScreen, HiTrap, HiPrep, and HiLoad prepacked column formats, please visit www.gelifesciences.com/protein-purification.

Related literature	Product code
UNICORN 7.0 control software, Data file	29135786
Validation Support File UNICORN software, Data file	28962650
Good ÄKTA system practice, Cue card	29109616
Rapid process development for purification of a MAb using ÄKTA avant 25, Application note	28957347
Rapid method development for native protein purification using ÄKTA avant 25 chromatography system, Application note	28962337
Fast process development of a single-step purification using ÄKTA avant systems, Application note	28982780
Prepacked chromatography columns for ÄKTA design systems, Selection guide	28931778

A range of service agreements and validation support offerings are available. Please contact your GE Healthcare Sales or Service representative for details.

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