Data file 18-1130-00 AD

FineLINE 70/70L, FineLINE 100P/100LP, FineLINE 200P/200LP

The development of new generation chromatography media for high resolution, process scale separations has put increased demands on the engineering design of process scale columns. To meet these demands, GE Healthcare has developed the FineLINE™ column. FineLINE columns are specially designed for use with SOURCE™ 15 ion exchange, hydrophobic interaction (HIC) and reversed phase chromatography (RPC) media, as well as with SOURCE 30 ion exchangers. They incorporate a number of novel features, including a hydraulically adjusted adaptor and special nets in the end piece and adaptor. Materials of construction are electropolished stainless steel and EPDM. These columns are easy to operate to the high standards of hygiene applied in the production of biopharmaceuticals.

The key characteristics of FineLINE columns are:

- Easy to pack in about 10 minutes with axial compression
- Give stable and reproducible beds with more than 22 000 plates/m with SOURCE 15 media and more than 11 000 plates/m with SOURCE 30 media
- Can also be packed with MabSelect™ media (when using a 10 µm mesh)
- Easy to scale up
- Easy to adapt to eluents used with RPC media by changing O-rings (accessory kit available)
- All polymeric materials are approved according to USP class VI tests for toxicity

Fig 1. FineLINE columns are engineered to meet the special demands of modern, high resolution SOURCE separation media. The columns are primarily intended for scale-up, but are also well suited to small scale production. The FineLINE family also includes FineLINE Pilot 35, (Data File 18-1104-95) and the FineLINE 350 column.

General description

FineLINE columns (Fig 1) are developed for use with SOURCE 15 high resolution ion exchange, HIC and RPC media with 15 µm particle size diameters, and SOURCE 30 ion exchangers with 30 µm particle size diameters. The columns are primarily intended for scale up work, but are

also well suited to small scale production. The inner column diameters are 70 mm (FineLINE 70 and 70L) 100 mm (FineLINE 100P and 100LP) and 200 mm (FineLINE 200P and 200LP). The inner tube length is 350 mm for the standard versions and 700 mm for the extra long versions.

The columns are built according to the requirements of the European pressure equipment directive (PED) 97/23/EC or the directive for equipment in potentially explosive atmospheres (ATEX 100) 94/9/EC (for customized columns). They are individually pressure tested and inspected before delivery. A test certificate accompanies each column, and 100 and 200 size columns are also delivered with comprehensive documentation including material and calibration certificates, as well as welding and cleaning documentation.

The stainless steel components are electropolished. This technique removes a 10 to 15 µm layer of steel leaving a metallurgically clean surface with a very smooth microprofile. Electropolished stainless steel surfaces improve corrosion resistance and reduce friction and contamination. Studies have shown that electropolished surfaces reduce pyrogen contamination during the production of pharmaceuticals.

The design, materials and construction of FineLINE columns contribute to a high standard of hygienic operation. They are compatible with all liquids commonly used in process scale liquid chromatography. In routine use, FineLINE columns require little maintenance and are easy to keep clean and free from microbial contamination. Tubing connections can be made quickly and simply using hygienic sanitary clamp fittings. These minimize pockets and dead volumes where microbial attachment and growth could occur. See Sanitization study for further information.

Polymeric materials used in FineLINE columns have been tested for their biological reactivity according to USP class VI. The materials have been tested *in vitro* according to the "Elution Test" and *in vivo* according to the "Systemic Injection Test" and the "Intracutaneous Test". Sesame oil and 0.9% NaCl were used as samples in the *in vivo* tests.

Table 1 lists the main properties of FineLINE columns.

Table 1. Properties of FineLINE 100 and 200 column series

Column inner diameter	70 mm (FineLINE 70/70L) 100 mm (FineLINE 100P and 100LP) 200 mm (FineLINE 200P/200LP)
Max. column inner length	350 mm (FineLINE 70/100P/200P) 700 mm (FineLINE 70L/100LP/200LP)
Adaptor movement	0–350 mm, 50–700 mm
Stand foot print	48 × 48 (FineLINE 70/70L/100P/100LP) 59 × 59 (FineLINE 200P/200LP)
Inlet and outlet dimensions	4 mm (FineLINE 70/70L/100P/100LP) 6 mm (FineLINE 200P/200LP)
Recommended media	SOURCE 15 (ion exchange, HIC, RPC) SOURCE 30 (ion exchange)
Recommended bed heights for	
SOURCE 15	30–150 mm 50–300 mm (L versions)
SOURCE 30	50–150 mm 50–300 mm (L versions)
Flow rate range	50–1000 cm/h
Design pressure*	20 bar
Materials of construction**	
Column body	Electropolished stainless steel 316L
Gaskets	EPDM
Nets	Stainless steel 316L, 2 µm mesh
Autoclavable	121°C for 30 min

* Design pressure is the sum of the packing and operating pressures.

** All materials used conform to US Pharmacopoeia requirements.

The FineLine instruction manual contains full details of column components as well as descriptions of packing, testing, cleaning, and sanitizing procedures. A trouble-shooting guide and spare parts lists are also included.

Easy to pack

FineLINE columns can be packed easily and reproducibly in as little as 10 minutes. Simple adjustments of a pressure relief valve connected to the column lid and a 4-port (2-way) valve mounted on the lower column outlet cause the hydraulic adaptor to automatically and smoothly move down the column at the correct pressure as the bed is packed (Fig 2). The adaptor is then mechanically locked in place when packing is complete.

Fig 2. The FineLINE column is easy to pack and operate to the high standards of hygiene applied in the production of biopharmaceuticals.

This procedure results in very stable and very uniform beds. Chromatograms of the same separation run at different scales are virtually identical (Fig 3).

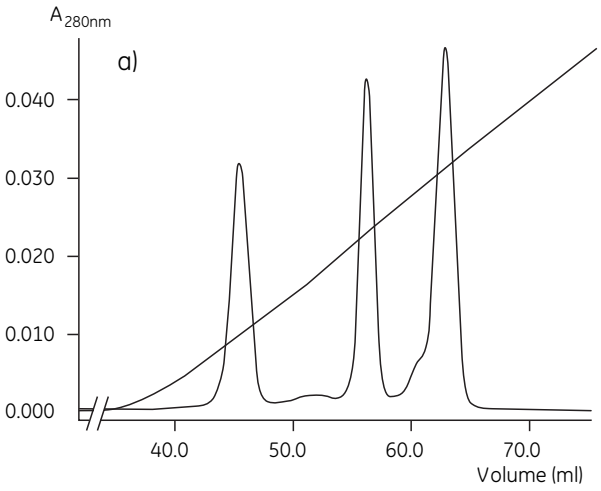
Detailed packing instructions are given in the instruction manual.

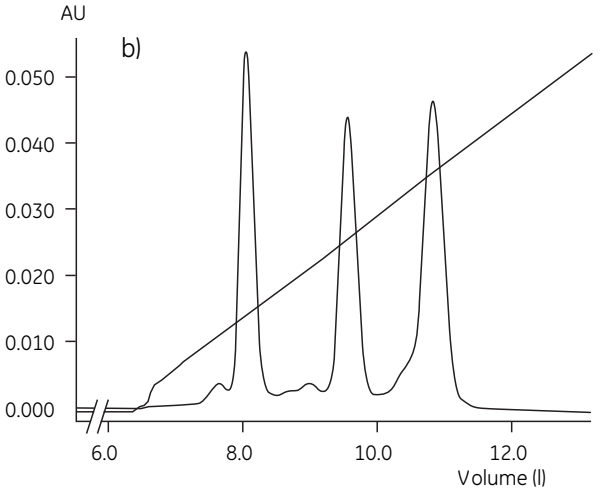
Reproducible scale-up

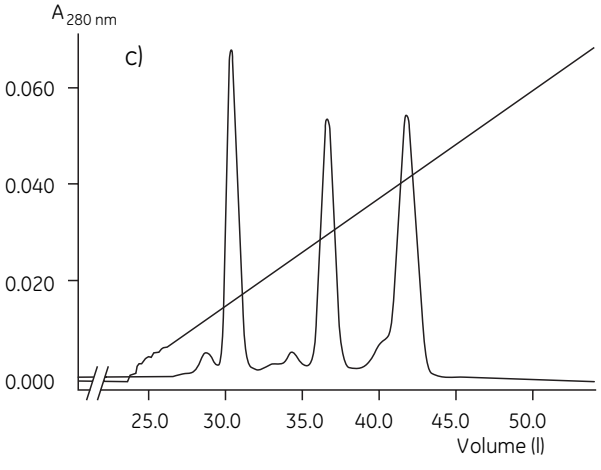
As noted above, the elution pattern obtained with a FineLINE column packed with SOURCE is essentially the same as that obtained when the same separation is run on a high resolution laboratory scale column. Figure 3 illustrates this characteristic.

Over 700-fold scale up with maintained performance

Medium:	SOURCE 30S
Column:	a) Laboratory column (7.5 mm i.d. × 50 mm, 2.2 ml) b) FineLINE 100 (100 mm i.d. × 50 mm, 393 ml) c) FineLINE 200 (200 mm i.d. × 50 mm, 1.57 l)
Sample:	Ribonuclease A, cytochrome C and lysozyme (3.75:1:1)
Sample load:	0.32 mg/ml gel
Buffer A:	20 mM sodium phosphate, pH 6.8
Buffer B:	20 mM sodium phosphate pH 6.8 + 0.4 M sodium chloride
Flow rate:	300 cm/h
Gradient:	0–100% B, 20 column volumes
System:	a) FPLC™ System, UNICORN™ control b) BioProcess™ System 8 mm, UNICORN control c) BioProcess System 8 mm, UNICORN control







Sanitization study

A sanitization study using microbial challenge testing was performed on a FineLINE 100 column. Sanitization efficiency was tested after the column. The column was packed with separation medium and infected with three strains of bacteria; *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Following infection, the column was sanitized with sodium hydroxide (NaOH) according to recommended sanitization methods. After the sanitization cycle had been completed, microbial samples were taken from the eluate at the column outlet, selected surfaces inside the column, as well as from the gel packed in the column. Table 2 shows the results of this sampling. No surviving organisms were found, confirming the hygienic design of the column and the effectiveness of the sanitization procedure.

A FineLINE 200 column has also been tested using this method and the same result obtained – no surviving organisms were found.

Table 2. Results of the sanitization tests performed on a FineLINE 100 column

	Concentration (m.o/ml) of infecting organism		
Number of organisms found in:	***Escherichia coli* 3.2 × 105 m.o/ml**	***Pseudomonas aeruginosa* 5.8 × 105 m.o/ml**	***Staphylococcus aureus* 5.3 × 105 m.o/ml**
Eluate before infection	0	0	0
Eluate after sanitization	0	0	0
Gel	0	0	0l
Gasket under bottom plate	0	0	0
Welded area, bottom plate (swab)	0	0	0
Welded area, adaptor plate (swab)	0	0	0
Adaptor plate (swab)	0	0	0
Rinse through the bottom plate	0	0	0

Ordering information

FineLINE columns are delivered with one fixed end-piece, one hydraulically adjustable adaptor, and valves for the hydraulic system. The pressure relief valve and column stand are not included and must be ordered separately. As the pressure relief valve is only required when packing the column, only one valve will generally be needed, irrespective of the number of columns in use.

Column	Code No.
FineLINE 70	18-1152-98
FineLINE 70L	18-1152-99
FineLINE 100P	11-0027-98
FineLINE 100LP	11-0027-99
Stand 70/100	18-1031-10
FineLINE 200P	11-0031-14
FineLINE 200LP	11-0031-15
Stand 200	18-1031-20
Pressure relief valve	18-1105-36

Common spare parts and accessories

Description	Quantity per pack	Material	10/70L	Code No. FineLINE 100LP	Code No. FineLINE 200LP
Bed support Adaptor Complete 2 µM	1	316 L	18-1153-61	11-0034-04	11-0034-06
Bed support End piece Complete 2 µM	1	316 L	18-1153-62	11-0034-05	11-0034-07
Bed support Adaptor complete 10 µM	1		18-1153-67	11-0034-72	11-0034-74
Bed support End piece complete 10 µM	1		18-1153-68	11-0034-73	11-0034-75
O-ring 104.37 × 3.53	3	EPDM		18-1103-89	
O-ring 104.37 × 3.53	1	PTFE		18-1105-50	
O-ring 202.79 × 3.53	2	EPDM			18-8489-01
O-ring 202.79 × 3.53	1	PFR			18-1106-30
O-ring 64.5 × 3	1	EPDM		18-1105-48	18-1105-48
O-ring 91.67 × 3.53	2	EPDM		18-1103-91	
O-ring 91.67 × 3.53	1	PFR		18-1105-49	
O-ring 187.3 × 6.99	1	EPDM			18-1106-26
O-ring 187.3 × 6.99	1	PFR			18-1106-29
Piston seal	1	EPDM	18-1139-56	18-1139-56	18-1106-28
O-ring Kit RPC*			18-1155-43	18-1105-45	18-1106-23
Air trap complete	1			18-1102-96	18-1102-97
Manometer kit 10 bar	1			18-1031-07	18-1031-07
Valve 4-port, 2-way	1		18-5757-01	18-5757-01	18-5757-01
Valve 4-port, 4-way	1		18-5758-01	18-5758-01	18-5758-01

* 4 different O-rings to replace EPDM O-rings when working with RPC. EPDM = Ethylenepropylene diene, PTFE = polytetrafluoroethene, Simriz = perfluorcarbon rubber.

Chemical resistance of materials in FineLINE columns

Substance	Concentration by volume	60–90 days
Acetic acid	10%	OK
Acetic acid	25%	see note[3]
Acetonitrile	5%	OK
Acetonitrile	50%	see note[1]
Acetone	10%	OK
Ethanol	100%	see note[1]
Ethylene glycol	50%	OK
Formaldehyde	1.5 M	OK
Formic acid	10%	OK
Glycerol	100%	OK
Hydrochloric acid	0.1 M	see note[2]

Substance	Concentration by volume	60–90 days
Isopropyl alcohol	100%	see note[1]
Methanol	100%	OK
Nitric acid	0.1 M	OK
n-Propanol	100%	OK
Phosphoric acid	25%	OK
Sodium chloride	0.5M	see note[4]
Sodium hydroxide	2 M	see note[3]
Trifluroacetic acid	0.1%	OK
Triton™ X-100	100%	OK
UREA	8M	OK

1 Change to Simritz O-rings.

2 Not recommended. The stainless steel will be affected.

3 Not longer than 4 hours.

4 Can be used under normal running conditions. Do not use NaCl in storage solutions. Please note that NaCl can cause corrision on stainless steel in acid storage (pH below 4.0).

Note: Do not use the following chemicals in FineLINE columns:

- Extreme oxidizers (such as peroxides)
- Fluorine and halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters (such as acetates)
- Aromatic hydrocarbons (such as toluene)
- High concentrations of strong acids