Mono S HR 10/10
Mono S HR 16/10

INSTRUCTIONS

Columns prepacked with Mono S™ are designed for fast, high resolution cation exchange separations of proteins, peptides, polynucleotides and other biomolecules.

Introduction

Mono S HR 10/10 (8 ml) and HR 16/10 (20 ml) cation exchange columns have been designed for operation with FPLC™ System. These instructions will help you obtain the best results from your column.

Other columns available in the series of MonoBeads™ are Mono Q™ (anion exchange) and Mono P™ (chromatofocusing).

Unpacking

Please check the delivery against the packing list.

Designation	Code No.	No. supplied
Mono S HR 10/10	17-0557-01	1
or		
Mono S HR 16/10	17-0507-01	1
Filter kit HR 10	18-3575-01	1 (10 filters)
or		
Filter kit HR 16	18-3585-01	1 (10 filters)
Filter tool	18-3590-01	1
Wrench	19-7481-01	1
Instructions		1

Quality control tests

To guarantee the quality of Mono S HR 10/10 and HR 16/10 each column is efficiency tested. Each media batch undergoes a function test to ensure reproducible results.

Connecting the column to FPLC System

1. The column is supplied with rubber tubing connecting the inlet to the outlet of the column. Remove this tubing and the connectors, but keep them for future storage of the column.
2. Connect the shorter preflanged tubing (the outlet) to the detector.
3. Connect the longer preflanged tubing (the inlet) to a valve which can be positioned for sample injection and elution e.g. GE Healthcare Valve V-7 or Motor Valve MV-7.
   When using the Valve PV-7 or the Motor Valve PMV-7, connect a Union, M6 female/1/16″ male (Code No. 18-3858-01) between the flanged tubing and the valve.
4. It is recommended to use a prefilter with the prepacked column (see “Spare parts and accessories”).

Connecting the column to HPLC systems

Columns prepacked with Mono S can be used with any HPLC system if the pump can provide precise and accurate flow at relatively low back-pressures. The column should be connected as described for an FPLC System via two unions which adapt the M6 connector to 1/16″ tubing (see “Spare parts and accessories”).

Important before use

The glass columns HR 10/10 and HR 16/10 are stable up to 5 MPa (50 bar, 750 psi) and 3 MPa (30 bar, 450 psi) respectively. Set the pressure limit control accordingly.

The gel is delivered in a 20% ethanol-water solution with sodium as the counter-ion, and should be equilibrated according to the following steps.

1. Wash away the packing solution with 40 ml (HR 10/10) or 100 ml (HR 16/10) of start buffer (low ionic strength).
2. Change to the desired counter-ion by washing with 80 ml (HR 10/10) or 200 ml (HR 16/10) of eluent B (high ionic strength).
3. Equilibrate with approx. 40 ml (HR 10/10 or approx. 100 ml /HR 16/10) of starting buffer. Before applying the sample, make sure that the ion exchange bed has reached equilibrium.

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This is done by pumping starting buffer through the column until the conductivity and/or the pH of the effluent is the same as for the ingoing buffer, the starting buffer.

To ensure long column life, always filter eluents and centrifuge or filter samples before applying them to the column.

Flow rates up to 6 ml/min (HR 10/10) and up to 10 ml/min (HR 16/10) are recommended depending on requirements and eluent viscosity.

Gel properties

Mono S is a strong cation exchanger based on a beaded hydrophilic resin with one of the narrowest particle size distributions available. The chemistry of the beads was developed at GE Healthcare and the monodispersity was accomplished through a unique process developed by Prof. John Ugelstad of SINTEF, Trondheim, Norway.

Mono S has a particle size of 10 µm. The absence of fines gives the packed columns large void volumes (40%) and therefore low back-pressures.

The charged group on the gel is $–CH_2–SO_3^-$. Ionic capacity of the gel is 0.14–0.18 mmol/ml. Separations of substances with molecular weights up to 10^7 have been carried out successfully. Protein capacity is normally in the range 20–50 mg/ml gel.

The amount of non-specific adsorption to MonoBeads is negligible. Recovery of enzyme activity is normally greater than 80% .

Chemical and physical stability

Mono S HR 10/10 and HR 16/10 columns can be used in aqueous media in the pH range 2–12. Aqueous solutions of urea, ethylene glycol and similar compounds may also be used.

Non-ionic or anionic detergents can be used but be sure to equilibrate the gel with the detergent solution beforehand. Cationic detergents should not be used with Mono S.

Mono S HR 10/10 and HR 16/10 columns are stable in alcohol/water solutions (C_1-C_4 alcohols). Dimethyl sulphoxide, dimethyl formamide, formic acid and similar solvents change the separation properties of the gel, so we do not recommend them. All oxidizing and other reactive substances should be avoided.

The glass columns HR 10/10 and HR 16/10 are stable up to 5 and 3 MPa respectively. Using an aqueous solution at a flow rate of 4.0 ml/min (HR 10/10) or 8.0 ml/min (HR 16/10), the operating pressure is generally less than 2 MPa.

Columns may be operated at temperatures between +4°C and +40°C.

Choice of elution conditions

Cation exchangers should be used with anionic or zwitterionic buffers, e.g. MES (2-[N-Morpholino] ethane sulphonic acid). Avoid cationic buffers since they bind to Mono S. Buffer concentrations should be at least 10 mM. Cationic detergents bind to Mono S and should not be used. Anionic (e.g. SDS) or non-ionic detergents (e.g. octylglucoside) may be used.

Table 1 gives recommended buffers for various pH intervals. This information has been determined with data from numerous runs peformed in our application laboratories. All values were determined at room temperature.

Sodium is the most common cationic counter-ion. A concentration of 1 M (e.g. NaCl) in buffer B is recommended to give a cleaning effect at 100%.

Twenty millitres/ml gel is a reasonable gradient volume. If the protein of interest is not eluted, then increase the gradient volume and the cation concentration at the end of the separation.

Table 1. Recommended buffers for various pH intervals.

pH interval	Buffer[3]	Concentration[1]	Anion	pKa (25°C)	$\frac{dpKa^2}{dT\ (°C)}$
3.8–4.3	formate	50 mM	Na^+, Li^+	3.75	+0.0002
4.3–4.8	succinate butanedioic acid	50 mM	Na^+	4.20	–0.0018
4.8–5.2	acetate	50 mM	Na^+, Li^+	4.76	+0.0002
5.0–6.0	malonate	50 mM	Na^+, Li^+	5.69	
5.5–6.7	MES	50 mM	Na^+, Li^+	6.15	–0.0110
6.7–7.6	phosphate	50 mM	Na^+	7.20	–0.0028
7.6–8.2	HEPES	50 mM	Na^+, Li^+	7.55	–0.0140
8.2–8.7	BICINE	50 mM	Na^+	8.35	–0.0180

1 Buffer concentration gradients may improve resolution.

2 When working at different temperatures, allow for changes in the pKa.

3 Buffers are made up with the acid form of the anions.

Eluent and sample preparation

Water should be of Milli-Q™ or corresponding quality. Use HPLC grade solvents, salts and buffers. Degas and filter all solutions through a 0.22 µm sterile filter. Either centrifuge (10 000 × *g* for 10 min) or filter samples through a 0.22 µm filter. Be sure to select a solvent resistant filter if samples are dissolved in organic solvents.

The samples should be fat-free. Turbid solutions can decrease the column lifetime.

When possible, dissolve the sample in start buffer. The buffer is easily exchanged by gel filtration with Sephadex™ G-25. For smaller volumes, Fast Desalting Column HR 10/10 (1 ml) or PD-10 columns (2.5 ml) may be used.

Note: Careful handling of solutions and samples increases the lifetime of the column considerably.

Column equilibration

To equilibrate the column for first-time use or for changing counter-ions, proceed according to steps 1–3 below.

1. Wash with 40 ml (HR 10/10) or 100 ml (HR 16/10) of start buffer (low ionic strength).
2. Change to the desired counter-ion by washing with 80 ml (HR 10/10) or 200 ml (HR 16/10) of eluent B (high ionic strength).
3. Equilibrate with 40 ml (HR 10/10) or 100 ml (HR 16/10) of the start buffer. Before applying a sample, equilibrate with start buffer until the base-line is stable.

Note: Be sure to equilibrate completely if using detergents.

Sample application

Make sure the sample is recently filtered or centrifuged before applying it to the column (see "Eluent and sample preparation"). The most convenient and reproducible sample injection is via the GE Healthcare valves V-7 , MV-7, PV-7 or PMV-7. Large volumes (up to 50 ml) can be applied from a Superloop (see "Spare parts and accessories"). Protein loading capacity is generally 20–50 mg/ml gel, or approx. 40 mg (HR 10/10) or 100 mg (HR 16/10) per single peak, depending on the sample. This loading usually gives good resolution but the capacity varies for different proteins.

Sample elution

Flow rates can be varied with little effect on resolution. A gradient volume of 20 ml/ml gel is generally sufficient. Larger gradient volumes generally improve resolution but also increase peak dilution.

Column re-equilibration

To re-equilibrate the column, inject 2 ml(HR 10/10) or 4 ml (HR 16/10) of a 1 M solution of the elution salt and equilibrate with the start buffer.
If changing counterions, follow the column equilibration procedure mentioned previously.

Method optimisation and scaling up

Sample elution is carried out by applying a concentration gradient to the column. Flow rates up to 6 ml/min (HR 10/10) or 10 ml/min (HR 16/10) are recommended. Optimal flow rate and gradient shape depend on the separation problem and the cation used. To save sample and buffer salts, it is recommended to optimise the separation on Mono S HR 5/5. The separation can then be directly scaled up to Mono S HR 10/10 or HR 16/10. Below are some general recommendations:

1. Best separating pH and buffer system for the sample can be determined by scouting on Mono S HR 5/5. High flow rates (up to 2 ml/min) and small gradient volumes (20 ml) may be used to save time. The elution ionic strength for each separated component is independent of the flow rate (Fig. 1).
2. Optimise the gradient volume to get the best resolution on Mono S HR 5/5. The resolution increases with increasing gradient volume (Fig. 2). At the same time, the elution ionic strength for the separated components is reduced (Fig. 3).
3. If necessary, resolution can be increased by reducing the flow rate (Fig. 4).
4. Determine the maximum loading that still provides acceptable resolution on Mono S HR 5/5 by increasing the sample amount.
5. Scale up the separation to Mono S HR 10/10 or HR 16/10 as below:

	HR 10/10	HR 16/10
Sample amount	× 8	× 20
Gradient volume	× 8	× 20
Flow rate	× 4 or up to 6 ml/min	× 8 or up to 10 ml/min

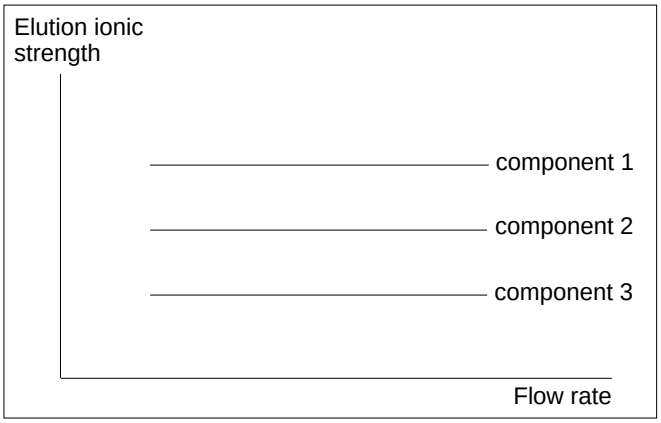


Fig. 1.

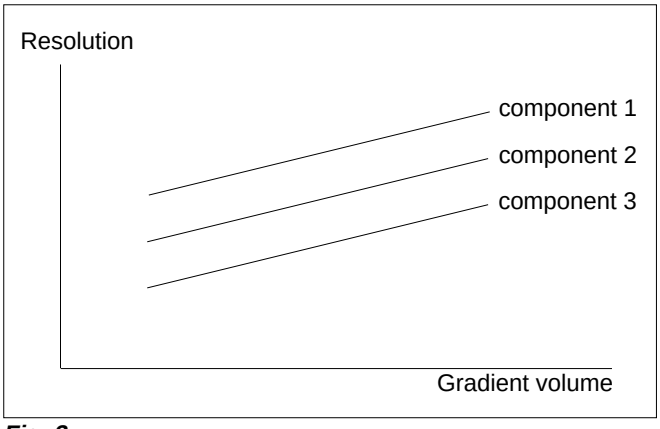


Fig. 2.

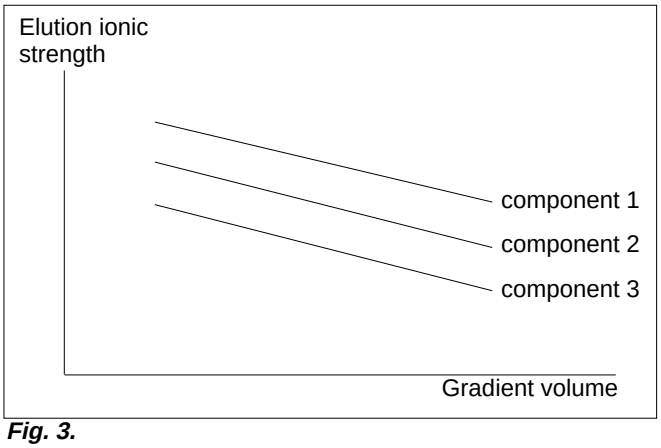


Fig. 3.

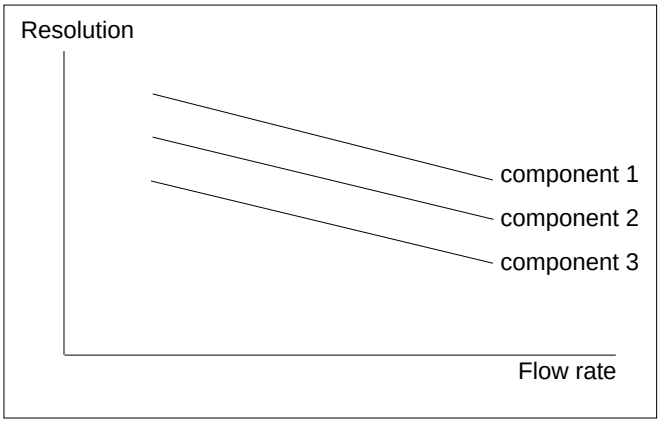


Fig. 4.

When using the GE Healthcare FPLC System, method development and scale up will be considerably simplified by using volume as the programming base in the controller.

Increased back-pressure

If increased back-pressure becomes a problem, perform the following steps in sequence until normal pressures are obtained (a good routine is always to record the back-pressure in your running protocols, e.g. just prior to sample injection).

1. Check the flanges of the tubings and reflange or exchange assemblies if damaged.
2. Turn the red adjusting ring on the top adaptor half a turn counter-clockwise. (The adaptor should still be close to the gel bed, without pressing against it, otherwise the back-pressure will be increased). Reverse the flow direction and pump 16 ml (HR 10/10) or 40 ml (HR 16/10) of buffer at 2 ml/min (HR 10/10) or 5 ml/min (HR 16/10). Return to normal flow direction and run for 4 min at 4 ml/min (HR 10/10) or 10 ml/min (HR 16/10). Readjust the top adaptor.
3. Check the top filter (Filter kit HR 10 or HR 16) and change if contaminated (see “Spare parts and accessories”).
4. If the problem persists, clean the column according to the procedure described under “Column cleaning” below.

Column cleaning

The following observations indicate that column washing be necessary.

- increased back-pressure – please check the filters first
- colour change at the top of the column
- loss of resolution
- decreased sample recoveries

It is best to avoid these problems by washing the column routinely (e.g. every fifth or tenth run) following steps 1–9 below. More frequent washing may be necessary if complex samples are applied.

Steps 1–11 are guidelines. Use your knowledge about possible contamination to evaluate which cleaning method is suitable.

1. Connect the column inlet to the detector. Set the sensitivity to 0.2 AUFS.
2. Make sure there is no space between gel and adaptor. Start a reversed flow at a rate of 1–2 ml/min (HR 10/10) or 2–5 ml/min (HR 16/10). Carry out steps 3-9 in sequence,

ensuring each time that the monitored peaks are identical in size before proceeding to the next step.

3. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) 2 M NaCl solution. Rinse with water or buffer (A)
4. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) of 0.5% SDS in 0.5 M NaOH. Rinse with water or buffer (A). (To be performed at room temp. as SDS can crystallise at lower temperatures.)
5. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) 2 M NaCl solution. Rinse with water or buffer (A).
6. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) 2 M NaOH. Rinse with water or buffer (A).
7. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) 1 M HCl. Rinse with water or buffer (A).
8. Inject 2 ml (HR 10/10) or 5 ml (HR 16/10) 75% acetic acid or 1% TFA. Rinse with water or buffer (A).
9. Inject 4 ml (HR 10/10) or 10 ml (HR 16/10) 2 M NaCl solution or a 2 M solution which has the same counter-ion as the solution used for elution.
10. If the column performance is still not restored, try leaving the column overnight in a solution of 1 mg/ml pepsin, 0.1 M acetic acid and 0.5 M NaCl. (Instead of pepsin, other enzymes may be tried, e.g. DNase, depending on the contamination.) For enzymatic washing, the solution should be at a temperature of 37°C. After the enzymatic cleaning, perform the chemical scrubbing (steps 3–9) again.
11. As a last attempt to restore performance, suspend 2–3 mm of the gel top and remove it with a Pasteur pipette. Adjust the adaptor to eliminate the space above the gel bed.

Checking the column packing

A well packed column is essential for high performance chromatography even though high efficiency (that is, number of plates/column) is less critical in gradient techniques than isocratic techniques. For best performance, make sure there is no space between the top adaptor and the gel bed (adjust the adaptor by clockwise rotation of the red adjusting ring on the top of the column), and that the column is clean (see section "Column cleaning").

If you suspect column packing to the cause of reduced resolution, run a sample and note the shape of the sample zone. Cytochrome c (Sigma type 3) is a good test protein, since it is coloured and readily available.

1. Dissolve the cytochrome c, 1 mg/ml in 10 mM phosphate buffer, pH 7.0.
2. Apply approximately 500 µl (HR 10/10) or 1 ml (HR 16/10) of the cytochrome c solution to the column.
3. Elute with 10 mM phosphate buffer, containing 1 M NaCl.

Note the shape of the protein zone. When injected it should be a narrow, horizontal band at the top of the column. When eluted with 100% eluting buffer, the zone should move down the column as a band. If the band is wavy, diffuse or not horizontal during elution, adjust the top adaptor and, if not already done, clean the column.

Efficiency test

After column maintenance procedures, the efficiency of the column should be checked. Column efficiency, expressed as plates per metre (H^{-1}), is estimated using following equation:

$$H^{-1} = 5.54 \times (V_R/w_h)^2 \times (1\,000/L)$$

L = bed height (mm)
V_R = peak retention (elution) volume
w_h = peak width at half peak height
H^{-1} = number of theoretical plates/m

Sample:	Triglycine, 0.1 mg/ml
Sample volume:	200 µl (HR 10/10)
	500 µl (HR 16/10)
Eluent:	0.01 M H_2SO_4, pH 2.0 (KOH)
Flow rate:	2.0 ml/min (HR 10/10)
	5.0 ml/min (HR 16/10)
Detector:	214 nm, 0.12 AUFS
Chart speed:	2.5 cm/min

The number of theoretical plates per metre should be more than 125 000.

Function test

An alternative to the efficiency test is the function test described here.

Mono S HR 10/10
Experimental:

Sample:	500 µl solution containing 1. Wheat germ lectin (GE Healthcare), 3 mg/ml 2. β-lactoglobulin, 1.5 mg/ml
Buffer A:	0.02 M Formic acid, pH 4.0 (NaOH)
Buffer B:	0.02 M Formic acid with 0.75 M LiCl, pH 4.0 (NaOH)
Flow rate:	4 ml/min
Gradient:	0.5–100% B in 20 min
Detector:	UV-M/UV-MII, 280 nm, 0.5 AUFS
Chart speed:	0.5 cm/min

Mono S HR 16/10
Experimental:

Sample:	500 µl solution containing 1. Wheat germ lectin (GE Healthcare), 3 mg/ml 2. β-lactoglobulin, 1.5 mg/ml
Buffer A:	0.02 M Formic acid, pH 4.0 (NaOH)
Buffer B:	0.02 M Formic acid with 0.75 M LiCl, pH 4.0 (NaOH)
Flow rate:	10 ml/min
Gradient:	0.5–100% B in 20 min
Detector:	UV-M/UV-MII, 280 nm, 0.2 AUFS
Chart speed:	0.5 cm/min

Storage and prevention of microbial growth

Before storing for long periods, wash the column sequentially with 100 ml (HR 10/10) or 200 ml (HR 16/10) of 0.5 M NaCl, 50 ml (HR 10/10) or 100 ml (HR 16/10) of water and 50 ml (HR 10/10) or 100 ml (HR 16/10) of 20% ethanol. The column should be stored between +4 and +30°C.

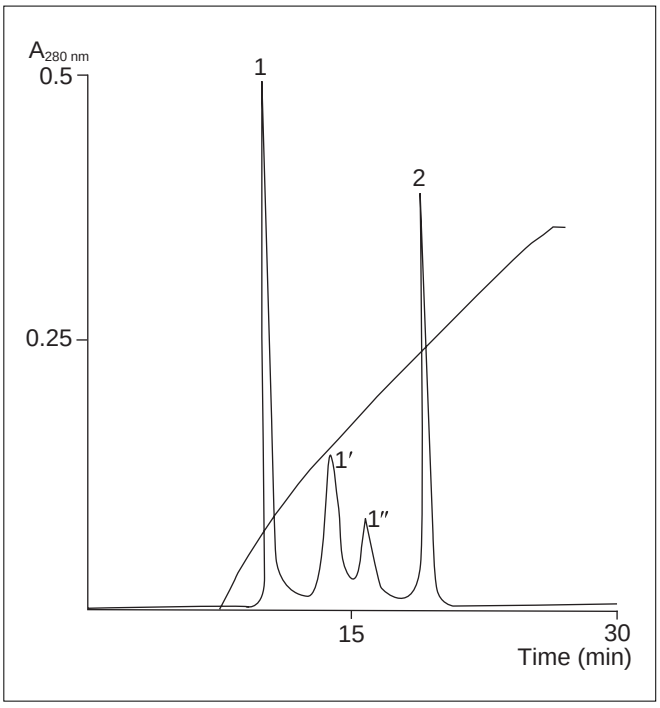


Fig. 5. Typical chromatogram from a function test of Mono S HR 10/10.

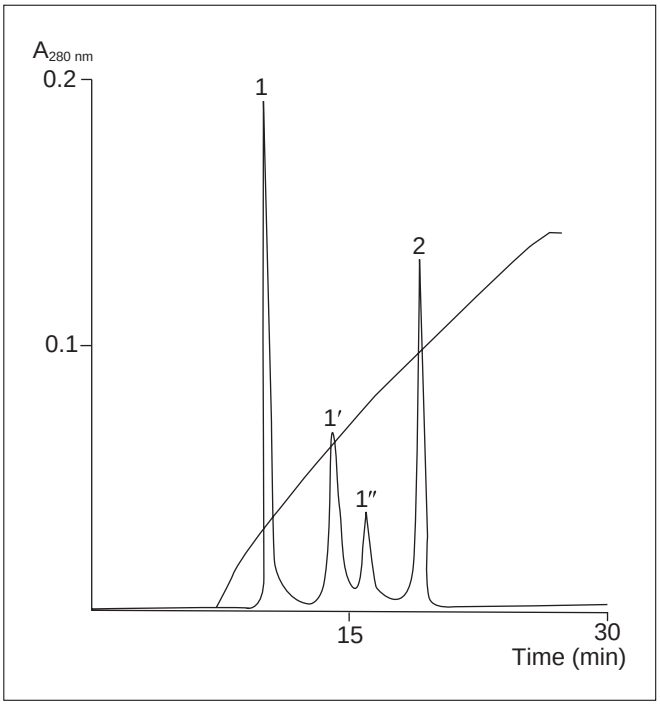
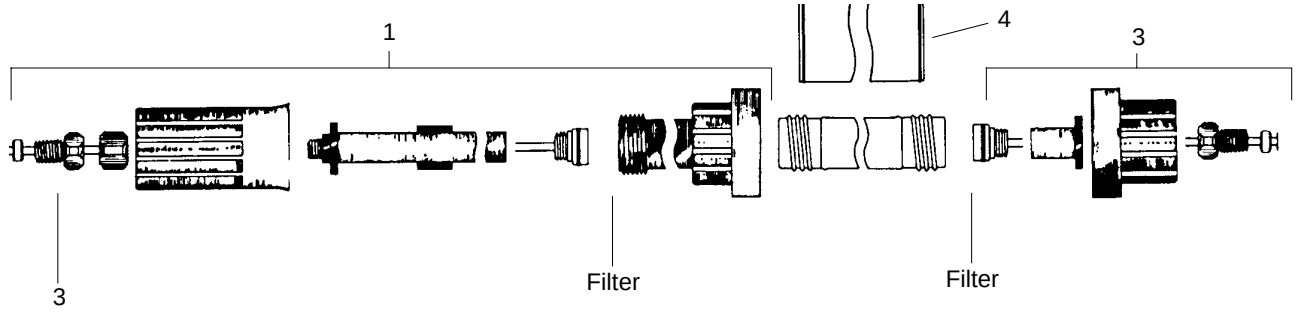


Fig. 6. Typical chromatogram from a function test of Mono S HR 16/10.

Spare parts and accessories

Pos.	Designation		Code No.	No. per pack	Pos.	Designation	Code No.	No. per pack
1	Top assembly	HR 10	18-1541-01	1		Sample loops 1 ml, 2 ml	18-5897-01	1 of each
		HR 16	18-1544-01	1		Superloop 10 ml	19-7585-01	1
2	Bottom assembly	HR 10	18-1542-01	1		Superloop 50 ml	19-7850-01	1
		HR 16	18-1545-01	1		Solvent resistant O-ring		
	Filter kit	HR 10	18-3575-01	1		(for the Superloop)	18-6300-01	1
		HR 16	18-3585-01	1		Union, M6 female/1/16″		
	Filter tool		18-3590-01	1		female, stainless steel		
3	Tubing connectors*		19-7476-01	5		(Waters compatible)	18-3405-01	1
4	Protective jacket					(Swagelok™ compatible)	18-3406-01	1
	HR 10/10		19-7449-01	1		Union, M6 female/1/16″		
	HR 16/10		19-7450-01	1		female, titanium		
	Capillary tubing					(Valco™ compatible)	18-3859-01	1
	(o.d. 1.8 mm, i.d. 0.5 mm)		19-7477-01	2 m		Union, M6 female/1/16″		
	Prefilter		19-5084-01	1		male, plastic		
	Filters + O-rings (prefilter)		19-5082-01	5+2		(Valco compatible)	18-3858-01	1
	Flanging/Start-Up kit					PD 10	17-0851-01	30
	120V		19-5079-01	1		Fast Desalting Column	17-0591-01	1
	220V		19-5090-01	1		HR 10/10		

* You need the Flanging/Start-Up kit to attach new tubing connectors.



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