BioProcess HPLC Columns

Maintenance Guide

um 56-3225-41 AA

Amersham Biosciences

Important user information

All users must read this entire manual to fully understand the safe use of the BioProcess HPLC columns.

CE Certification

This product meets all requirements of applicable CE directives. A copy of the corresponding declaration of conformity is available on request.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences, except for alterations described in this manual.

Terms and Conditions of Sale

Unless otherwise agreed in writing, all goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group that supplies them. A copy of these terms and conditions is available on request.

Should you have any comments on this product, we will be pleased to receive them at:

Amersham Biosciences AB
SE-751 84 Uppsala
Sweden

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Office Addresses

Amersham Biosciences AB

SE-751 84 Uppsala
Sweden

Amersham Biosciences UK Limited

Amersham Place
Little Chalfont
Buckinghamshire
England HP7 9NA

Amersham Biosciences Corp

800 Centennial Avenue
PO Box 1327
Piscataway NJ 08855
USA

Amersham Biosciences Europe GmbH

Munzinger Strasse 9
D-79111 Freiburg
Germany

Amersham Biosciences K.K.

Sanken Building
3-25-1 Hyakunincho, Shinjuku-ku
Tokyo 169-0073
Japan

Amersham Biosciences China Limited

13/F., Tower I
Ever Gain Plaza
88 Container Port Road
Kwai Chung, New Territories
Hong Kong

Safety Notices

This Maintenance guide contains safety notices prompting you on correct use of the column to avoid personal injury and/or damage of the equipment, or to present information that can optimize your use of the columns. The various types of safety notice include:

Warning notices

WARNING! The Warning notice highlights instructions that must be strictly followed in order to avoid personal injury. Do not proceed until the instructions are clearly understood and all stated conditions are met.

Caution notices

CAUTION! The Caution notice highlights instructions or conditions that must be followed to avoid damage to the product or to other equipment. Do not proceed until the instructions are clearly understood and all stated conditions are met.

Important notices

IMPORTANT! The Important notice highlights instructions or details relating to instructions that will help ensure optimal use of the column.

Notes

Note: Notes are used to indicate additional tips and information for trouble-free and optimal use of the product.

Contents

1 Safety

Please familiarize yourself with BioProcess™ HPLC Columns Instructions for Use before proceeding with any maintenance and service.

IMPORTANT! Whenever the column is used, always keep the Instructions for Use and Maintenance Guide for the column on hand.

IMPORTANT! The end user must ensure that all installation, maintenance, operation and inspection is carried out by qualified personnel who are adequately trained and understand the operating instructions.

IMPORTANT! A Health And Safety Declaration /Liability form must always be completed prior to maintenance/service by Amersham Biosciences personnel, or if the equipment is to be returned.

WARNING! The working pressure of the column should never exceed the design pressure, otherwise there is a risk of personal injury and damage to the column. A suitable safety device must be fitted.

WARNING! The working temperature range of the column should never fall outside the design temperature range.

WARNING! Do not use other chemicals than those proven not harmful to the wetted parts of the column.

WARNING! The wheel brakes must be activated at all times during operation or storage.

WARNING! Pay extra attention when moving the column to ensure that the column does not tip.

WARNING! Many of the procedures outlined in this chapter involving lifting heavy parts. It is therefore advisable to always have at least two people on hand, as well as a hoist, when performing column service and maintenance.

WARNING! Always use protective clothing appropriate with current application to ensure personal safety during operation.

Note: To retain sanitary surfaces, it is recommended that clean gloves be worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

2 Column disassembly and assembly

BioProcess HPLC columns are divided into three categories for the purposes of maintenance and service:

- Columns 50 mm diameter
- Columns 75 to 200 mm diameter
- Columns 250 mm diameter and over

This chapter describes how you can disassemble and reassemble the major components of the three column categories, for example to replace seals and O-rings, or for cleaning.

Note: The pictures shown in the steps below are representative images only. The characteristics of your column may vary from the images shown.

2.1 Extra equipment needed disassembly/assembly

- Crane or hoist
- Blocks for resting column components on
- Container of suitable size for column components, containing warm (50 °C) water
- Wrenches
- Allen keys
- 100% ethanol for cleaning and lubricating

2.2 Columns 50 mm diameter

2.2.1 Disassembling the column (50 mm)

WARNING! To avoid injury from explosion, ensure that there is no pressure in the column before dismantling.

Removing the base end plate and adapter (50 mm)

The base end plate and adapter are removed most easily as one unit. This step is sufficient for purposes of cleaning the column components

1. Rotate the column tube from its inverted upright position to its upright position, that is with the adapter rod at the top pushing down into the column tube.

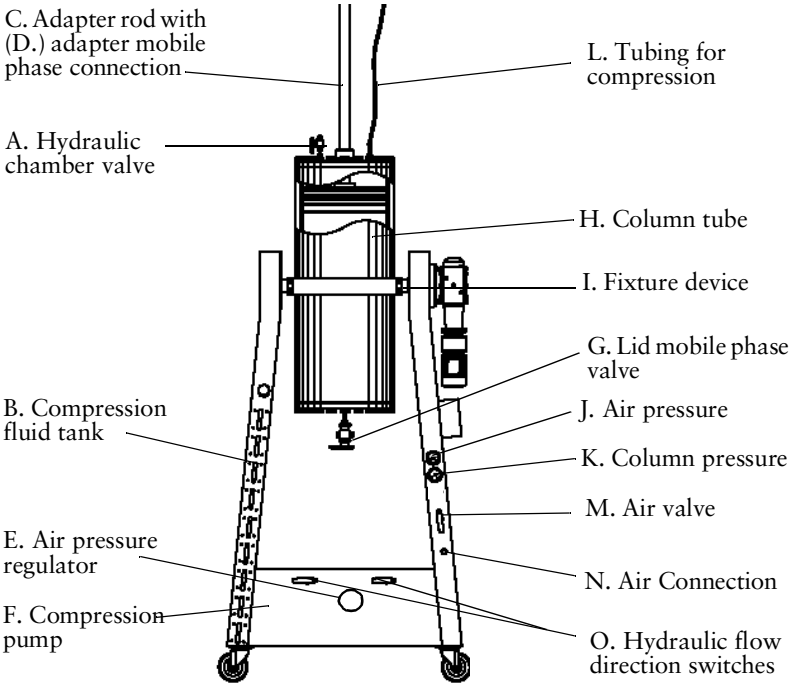

Fig 2-1. Schematic diagram of a BioProcess HPLC column in its upright position.

2. Withdraw the adapter to its most extreme position:

   Set both of the hydraulic flow direction switches (O) to the FROM COLUMN position.

CAUTION! Both hydraulic flow direction switches (O) must be moved to the same position before starting the pump. A high pressure can build up inside the tubing from the pump if the left switch is set to TO COLUMN and the right switch to FROM COLUMN.

   Set the AIR ON/OFF switch (M) to ON and use the air pressure regulator (E) to set the air pressure (J) to an appropriate pressure (e.g. 0.5 bar).

   The adapter will move out of the column by itself.

   When the adapter is fully extended turn off the air pump (M).

3. Use a wrench to remove the bolts that hold the base end plate in place on the column tube.

4. Pump fluid into the column via the lid mobile phase valve at a low flow rate. This will cause the adapter and base end plate to be pushed out of the column tube.

   Hold onto the adapter rod with both hands and lift free when the adapter and end plate are pushed out of the column tube. Carefully rest the adapter and base end plate on blocks.

5. Drain the fluid from inside the column via the lid mobile phase valve (G), or simply rotate the column tube and pour out the contents.

Removing the lid (50 mm)

To remove the lid:

1. Rotate the column in its inverted upright position, so that the lid is accessible from above.
2. Loosen and remove the bolts connecting the lid to the column tube.
3. Remove the lid.

Note: To help remove the lid you may need to insert jacking bolts into threaded holes on the lid. Screwing these in will help to release the lid.

2.2.2 Exchanging O-rings and seals (50 mm)

This section describes the exchange of O-rings and seals. You are recommended to change the O-rings and seals in your column once per year, particularly those that experience some form of movement in the column, for example the O-rings on the adapter.

Base end plate (50 mm)

The base end plate has a single O-ring round its outer circumference.

1. Use a sharp knife to cut the existing O-ring.

Fig 2-2. Base end plate with O-ring.

2. Set a new O-ring in its place. You may first need to warm the new O-ring in warm water (50 °C) and then place it on the base end plate.

Adapter (50 mm)

Changing the seals and O-rings on the adapter requires that adapter itself is disassembled. The adapter comprises an upper and a lower half. the upper half of the adapter is integral with the adapter rod, which is held in place by a screw-thread. Inside the adapter rod is an inner tube that is also connected to the lower half of the adapter by a screw.

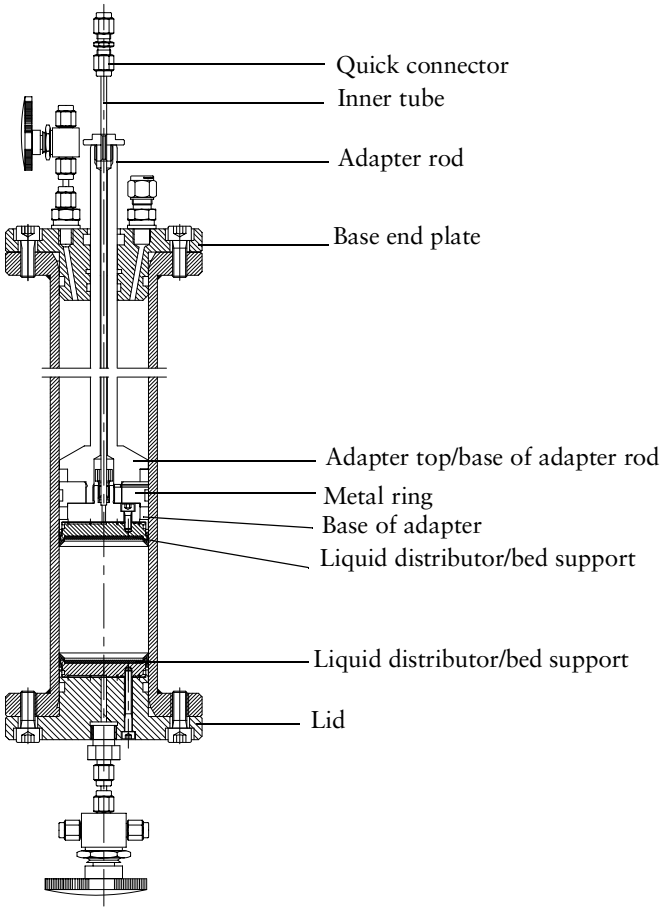

Fig 2-3. Longitudinal section of a 50 mm HPLC column.

1. Ensure that the adapter and adapter rod are resting on a stable surface.

2. Attach eye bolts to the base end plate and then connect a hoist/crane to these eye bolts. Gently lift the end plate so that it moves up the shaft of the adapter rod enough to be able to access the base of the adapter rod.
3. Hold the bottom of the adapter in place and unscrew the adapter rod.
4. Lift the unscrewed adapter rod and use a wrench to disconnect the inner tube from the top of the adapter surface.

Note: You cannot remove the adapter rod more than a few centimeters since it will not pass over the Quick Connector at the top of the inner tube.

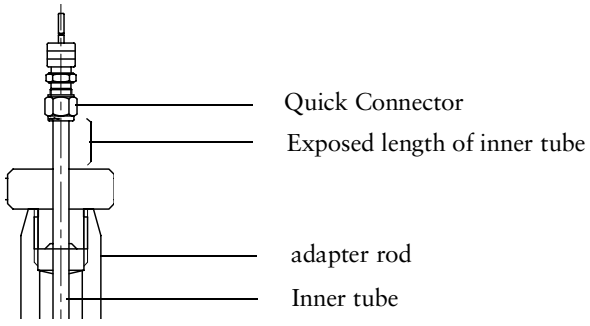

Fig 2-4. Longitudinal section through the tip of the adapter rod. Note that there is only a limited length of exposed inner tube along which to lift the adapter rod when it has been unbolted at its base.

5. Lift the base end plate and adapter rod away.
6. Replace the O-ring and seal on the upper section of the adapter (i.e. the base of the adapter rod).
7. Lift out the metal ring from the lower part of the adapter if this did not come out with the upper of the adapter.
8. Replace the O-ring and seal on the adapter bottom half of the adapter.
9. Reassemble the adapter and rod.

Base end plate center hole (50 mm)

There are only seals and excluders in the center hole of the base end plate through which the adapter rod passes. These should not normally need to be replaced on annual basis, and only when there is a leakage through this hole. To replace the seals and excluders the end plate must be separated from the adapter.

To separate the base end plate from the adapter:

1. Ensure that the adapter and adapter rod are resting on a stable surface.
2. Cut the inner tube at the top of the adapter rod as close to the Quick connection as possible.
3. Remove the locking nut.

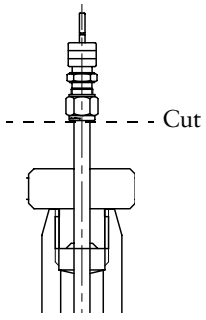

Fig 2-5. Longitudinal section through the tip of the adapter rod.

4. Carefully lift the base end plate up over the adapter rod.

To replace the seals and excluders in the center hole of the base end plate:

1. Remove the excluder. Use a sharp knife to cut the excluder if necessary.

CAUTION! Take care not to scratch any steel surfaces of the column when using a knife to remove the seals and O-rings.

2. Use a sharp knife to remove the seal.
3. Clean the inside of the center hole and lubricate the new seal and O-ring with ethanol.
4. Replace the seal.
5. Bend the O-ring/step seal as shown in the diagram. Avoid sharp bending.
6. Put on a new excluder.

Lid and liquid distributor (50 mm)

The lid and base end plate each have an O-ring that is in contact with the column tube. The lid also houses the liquid distributor and the bed support, which have O-rings that need replacing. The following description is given for the lid but can also be applied to the end plate.

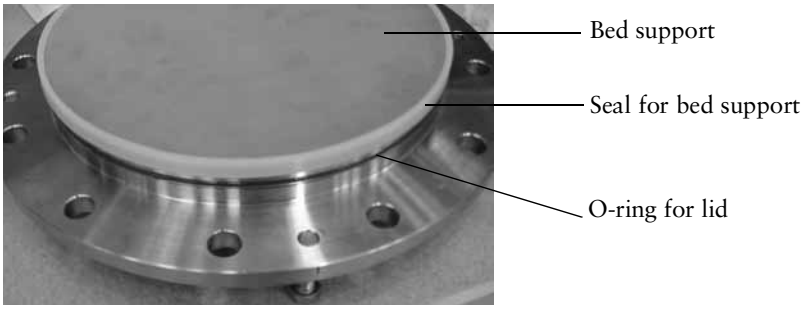

Fig 2-6. Lid with bed support fixed in place.

1. Remove the seal holding the bed support in place and remove the bed support O-ring from underneath.

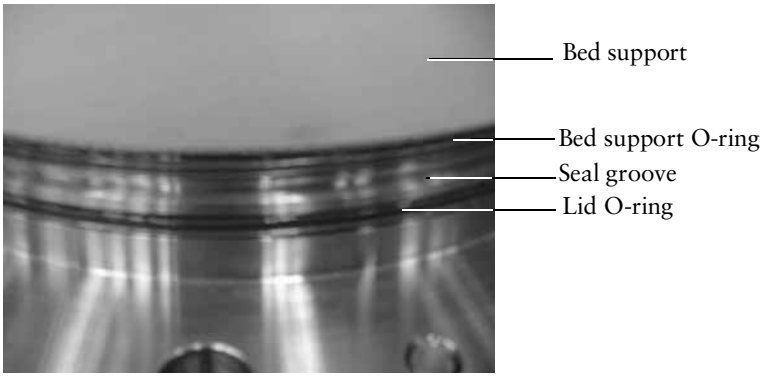

Fig 2-7. Lid with bed support seal removed to expose the bed support O-ring.

2. Remove the O-ring from the outer circumference of the lid.
3. Remove the liquid distributor by applying compressed air through the lid mobile phase inlet (H). The liquid distributor will move up.
4. Remove the O-ring from the liquid distributor and clean its surfaces with ethanol.
5. Place a new O-ring on the liquid distributor.
6. Place the liquid distributor back into the lid. The O-ring will keep it in place.
7. Place a new O-ring in its groove on the outer circumference of the lid.

8. Place a new bed support O-ring in the upper groove of the lid over which the seal for the bed support will sit.
9. Place the bed support on top of the liquid distributor and fix in place with the seal. You may first need to warm the seal in warm water (50 °C) before replacing.

IMPORTANT! The seal must be placed the correct way up. One edge of the seal is thicker than the other and this should be placed in face down to fit in the groove directly beneath where the bed support O-ring sits.

2.2.3 Assembling the column (50 mm)

Fitting the lid (50 mm)

1. Rotate the column in its inverted upright position, so that the lid can be fitted from above.
2. Gently lower the lid onto the column tube.
3. Use two or three jacking screws placed at opposite positions on the lid and screw these so that the lid fastens to the column tube evenly on all sides.
4. Replace the bolts connecting the lid to the column tube and tighten.

WARNING! There is a risk of injury to your fingers or other appendages should they become trapped between the lid and the column tube. Handle the lid with care.

Fitting the base end plate onto the adapter rod (50 mm)

1. Carefully lower the base end plate down along the shaft of the adapter rod.
2. Lower the base end plate until it rests on top of the adapter.
3. Screw the locking nut on to the tip of the adapter rod.
4. Attach a Quick Connector onto the exposed end of the inner tube.

Fitting the adapter and base end plate into the column (50 mm)

1. Rotate the column in its upright position, so that the base plate and adapter can be fitted from above.
2. Lift the base end plate and adapter above the column tube opening.
3. Carefully lower down into the column tube.

Note: You can lubricate the seals with ethanol.

4. Insert jacking bolts into the base end plate and into the column.

Successively screw the bolts by equal amounts to push the adapter and base end plate into the column.

5. When the adapter and base end plate are sufficiently inside the column, insert the bolts. Use a wrench to successively screw the bolts in by equal amounts.

2.3 Columns 75 to 200 mm diameter

2.3.1 Disassembling the column (75-200 mm)

WARNING! To avoid injury from explosion, ensure that there is no pressure in the column before dismantling.

Removing the base end plate and adapter (75-200 mm)

The base end plate and adapter are removed most easily as one unit. This step is sufficient for purposes of cleaning the column components

1. Rotate the column tube from its inverted upright position to its upright position, that is with the adapter rod at the top pushing down into the column tube.

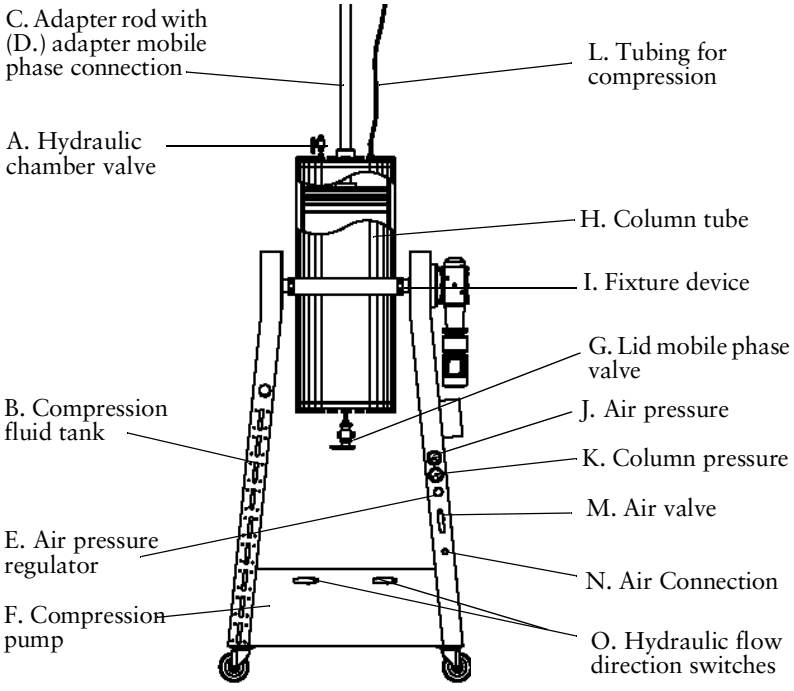

Fig 2-8. Schematic diagram of a BioProcess HPLC column in its upright position.

2. Withdraw the adapter to its most extreme position:

   Set both of the hydraulic flow direction switches (O) to the FROM COLUMN position.

CAUTION! Both hydraulic flow direction switches (O) must be moved to the same position before starting the pump. A high pressure can build up inside the tubing from the pump if the left switch is set to TO COLUMN and the right switch to FROM COLUMN.

   Set the AIR ON/OFF switch (M) to ON and use the air pressure regulator (E) to set the air pressure (J) to an appropriate pressure.

   The adapter will move out of the column by itself.

   When the adapter is fully extended turn off the air pump (M).

3. Use a wrench to remove the bolts that hold the base end plate in place on the column tube.
4. Attach the fittings from a crane or hoist to the eyes on the locking nut of the adapter rod.

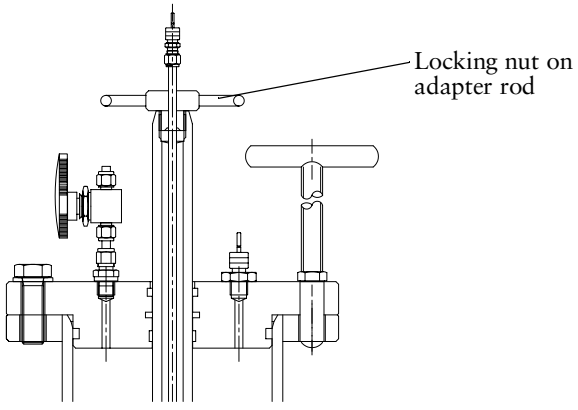

Fig 2-9. Longitudinal section through a column base end plate and adapter rod.

5. Pump fluid into the column via the lid mobile phase valve at a low flow rate. This will cause the adapter and base end plate to be pushed out of the column tube. Take up any slack in the hoist/ crane. Stop the pump when the adapter and base end plate are free. Immediately rest the adapter and base end plate on blocks.

6. Drain the fluid from inside the column via the lid mobile phase valve (G), or simply rotate the column tube and pour out the contents.

Removing the lid (75-200 mm)

To remove the lid:

1. Rotate the column in its inverted upright position, so that the lid is accessible from above.
2. Loosen and remove the bolts connecting the lid to the column tube.
3. Remove the lid using a suitable hoist or crane.

Note: To help remove the lid you may need to insert T-shaped jacking bolts into threaded holes on the lid. Screwing these in will help to release the lid.

2.3.2 Exchanging O-rings and seals (75-200 mm)

This section describes the exchange of O-rings and seals. You are recommended to change the O-rings and seals in your column once per year, particularly those that experience some form of movement in the column, for example the O-rings on the adapter.

Base end plate (75-200 mm)

The base end plate has a single O-ring round its outer circumference.

1. Use a sharp knife to cut the existing O-ring.

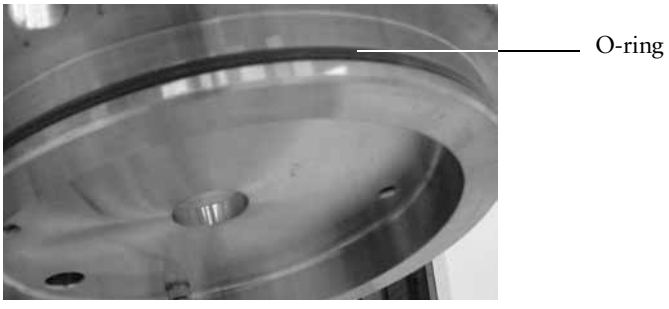

Fig 2-10. Base end plate with O-ring.

2. Set a new O-ring in its place. You may first need to warm the new O-ring in warm water (50 °C) and then place it on the base end plate.

Adapter (75-200 mm)

The adapter has two seals. Additionally, there is a seal holding the top bed support in place. The seals cannot be stretched over the adapter so this requires that the adapter is disassembled.

1. Ensure that the adapter and adapter rod are resting on a stable surface.
2. Attach a hoist/crane to the base end plate and gently lift so that it moves up the shaft of the adapter rod enough to be able to access the base of the adapter rod.
3. Use an Allen key to remove the bolts from the base of the adapter rod.
4. Lift the adapter rod and use a wrench to disconnect the inner tube from the top of the adapter surface. Lift the base end plate and adapter rod away leaving the adapter behind.

Note: You cannot remove the adapter rod more than a few centimeters since it will not pass over the Quick Connector at the top of the inner tube.

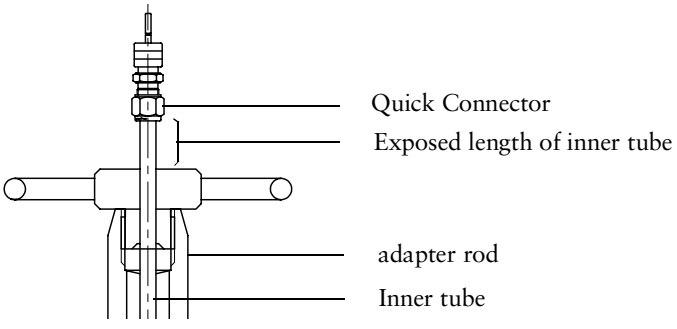

Fig 2-11. Longitudinal section through the tip of the adapter rod. Note that there is only a limited length of exposed inner tube along which to lift the adapter rod when it has been unbolted at its base.

5. The adapter is constructed from several parts. Unscrew the top of the adapter. There is a seal on the top of the adapter that you can replace.

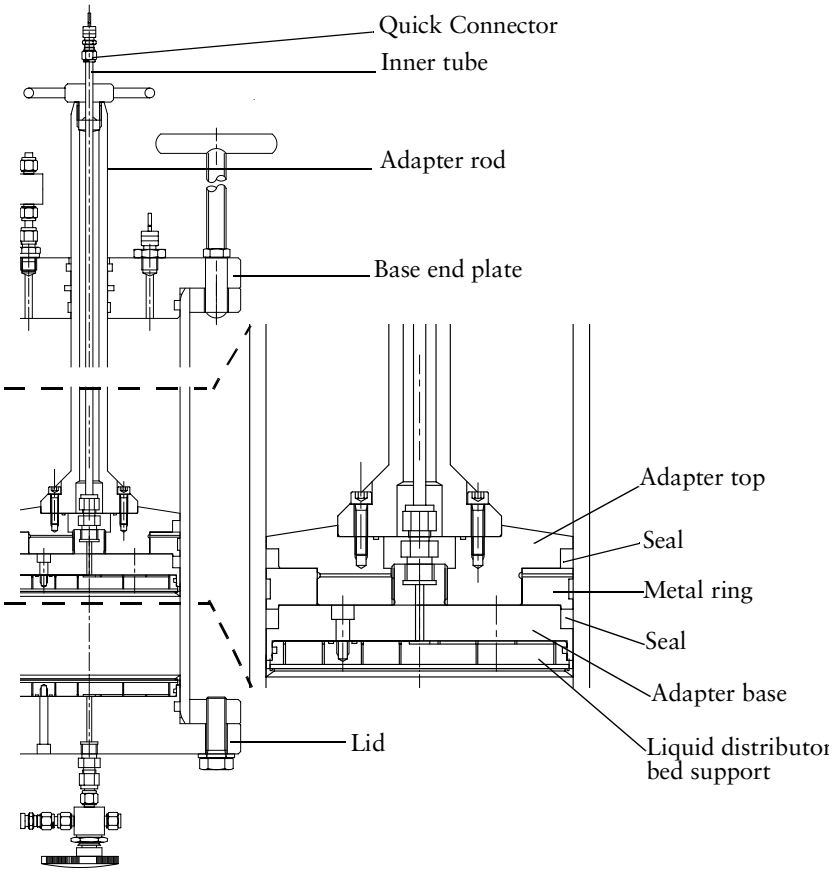

Fig 2-12. Longitudinal section of a 150 mm HPLC column.

6. Lift out the metal ring if this did not come out with the top of the adapter.
7. Replace the seal on the adapter base.
8. Reassemble the adapter.
9. Reconnect the adapter rod.

Base end plate center hole (75-200 mm)

There are O-rings and seals in a center hole of base end plate through which the adapter rod passes. In order to replace the O-rings and seals the end plate must be separated from the adapter.

To separate the base end plate from the adapter:

1. Ensure that the adapter and adapter rod are resting on a stable surface.

2. Cut the inner tube at the top of the adapter rod as close to the Quick connection as possible.
3. Remove the locking nut.

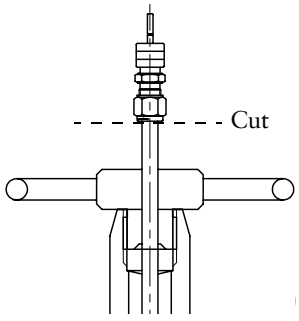

Fig 2-13. Longitudinal section through the tip of the adapter rod.

4. Carefully lift the base end plate up over the adapter rod.

To replace the O-rings and seals in the center hole of the base end plate:

1. Remove the excluder. Use a sharp knife to cut the excluder if necessary.

CAUTION! Take care not to scratch any steel surfaces of the column when using a knife to remove the seals and O-rings.

2. Use a sharp knife to remove the step seal protecting the O-ring.
3. Remove the O-ring by inserting some wire (20 mm length x 2 mm diameter) into the groove holding the O-ring and picking it out.
4. Clean the inside of the center hole and lubricate the new step seal and O-ring with ethanol.
5. Place the new O-ring into the groove in the center hole.
6. Place one edge of a new step seal onto the O-ring in the groove. Make sure that the step seal is the right way up according to the orientation in the cross section diagram below.

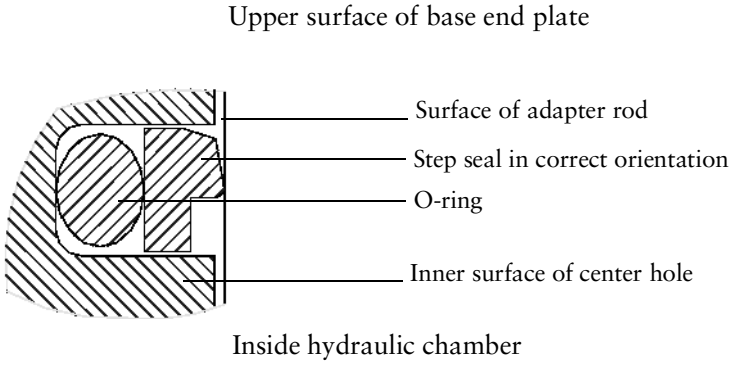

Fig 2-14. Cross section through the center hole showing the orientation of the step seal.

7. Bend the O-ring/step seal as shown in the diagram. Avoid sharp bending.

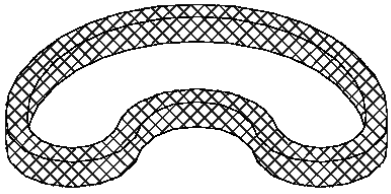

Fig 2-15. Bending of the step seal to facilitate fitting in the center hole.

8. Carefully press the folded step seal into place over the O-ring. A cross-section of the O-ring and packing seal should be as in the following diagram.
9. Put on a new excluder.

Lid and liquid distributor (75-200 mm)

The lid and base end plate each have an O-ring that is in contact with the column tube. The lid also houses the liquid distributor and the bed support, which have O-rings that need replacing. The following description is given for the lid but can also be applied to the end plate.

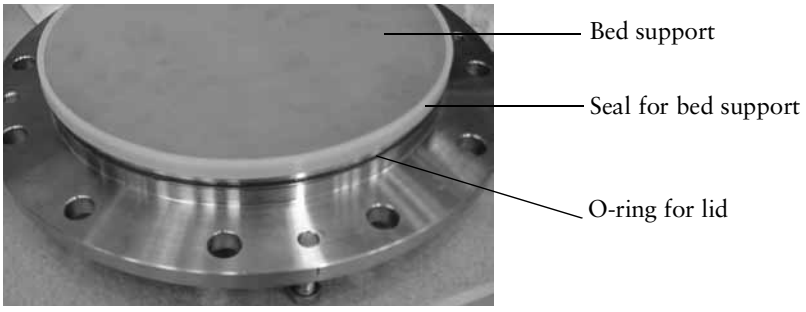

Fig 2-16. Lid with bed support fixed in place.

1. Remove the seal holding the bed support in place and remove the bed support O-ring from underneath.

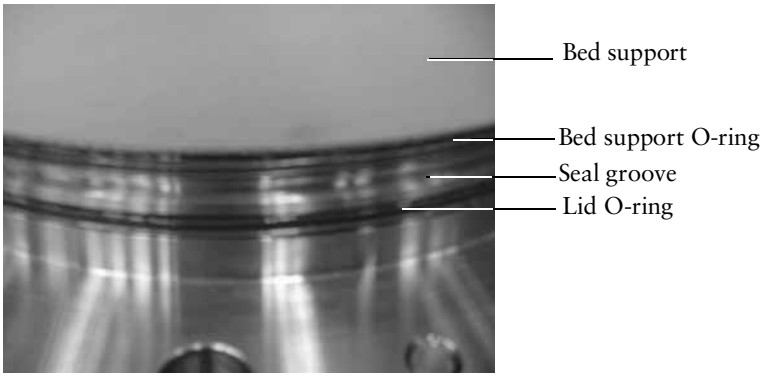

Fig 2-17. Lid with bed support seal removed to expose the bed support O-ring.

2. Remove the O-ring from the outer circumference of the lid.
3. Use an Allen key to remove the bolts on the outer surface of the lid that hold the liquid distributor in place.
4. Remove the liquid distributor by applying compressed air through the lid mobile phase inlet (H). The liquid distributor will move up.
5. Remove the O-ring from the liquid distributor and clean its surfaces with ethanol.

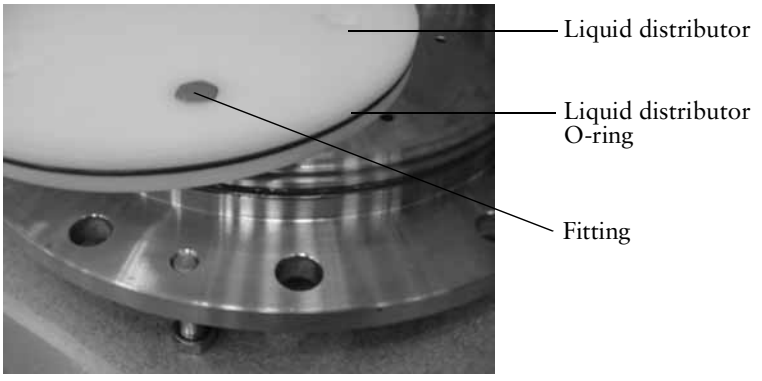

Fig 2-18. Liquid distributor.

Note: You can remove the fittings from the surface of the liquid distributor. These fittings hold the liquid distributor in place together with the bolts removed in step 3 above.

Fig 2-19. Bolts and fittings holding the liquid distributor in the lid.

6. Place a new O-ring on the liquid distributor and replace the fittings.
7. Place the liquid distributor back into the lid and bolt into place.
8. Place a new O-ring in its groove on the outer circumference of the lid.
9. Place a new bed support O-ring in the upper groove of the lid over which the seal for the bed support will sit.
10. Place the bed support on top of the liquid distributor and fix in place with the seal. You may first need to warm the seal in warm water (50 °C) before replacing.

IMPORTANT! The seal must be placed the correct way up. One edge of the seal is thicker than the other and this should be placed in face down to fit in the groove directly beneath where the bed support O-ring sits.

2.3.3 Assembling the column (75-200 mm)

Fitting the lid (75-200 mm)

1. Rotate the column in its inverted upright position, so that the lid can be fitted from above.
2. Use the hoist/crane to gently lower the lid onto the column tube.
3. Use two or three T-shaped jacking screws placed at opposite positions on the lid and screw these so that the lid fastens to the column tube evenly on all sides.
4. Replace the bolts connecting the lid to the column tube and tighten.

WARNING! There is a risk of injury to your fingers or other appendages should they become trapped between the lid and the column tube. Handle the lid with care.

Fitting the base end plate onto the adapter rod (75-200 mm)

1. Rotate the column in its upright position, so that the adapter and base end plate can be fitted from above.
2. Lift the base end plate using a crane/hoist and carefully lower onto the shaft of the adapter rod.
3. Lower the base end plate until it rests on top of the adapter.
4. Screw the locking nut on to the tip of the adapter rod.
5. Attach a Quick Connector onto the exposed end of the inner tube.

Fitting the adapter and base end plate into the column (75-200 mm)

1. Lift the base end plate and adapter above the column tube opening.
2. Carefully lower down into the column tube. Keep the fittings from the crane/hoist taught to prevent tipping.

Note: You can lubricate the seals with ethanol.

3. Insert T-shaped jacking bolts into the base end plate and into the column. Successively screw the bolts by equal amounts to push the adapter and base end plate into the column. You will need to periodically lower the crane/hoist.
4. When the adapter and base end plate are sufficiently inside the column, insert the bolts. Use a wrench to successively screw the bolts in by equal amounts.

2.4 Columns 250 mm diameter and over

2.4.1 Disassembling the column (250+ mm)

WARNING! To avoid injury from explosion, ensure that there is no pressure in the column before dismantling.

Removing the base end plate and adapter (250+ mm)

The base end plate and adapter are removed most easily as one unit. This step is sufficient for purposes of cleaning the column components

1. Rotate the column tube from its inverted upright position to its upright position, that is with the adapter rod at the top pushing down into the column tube.

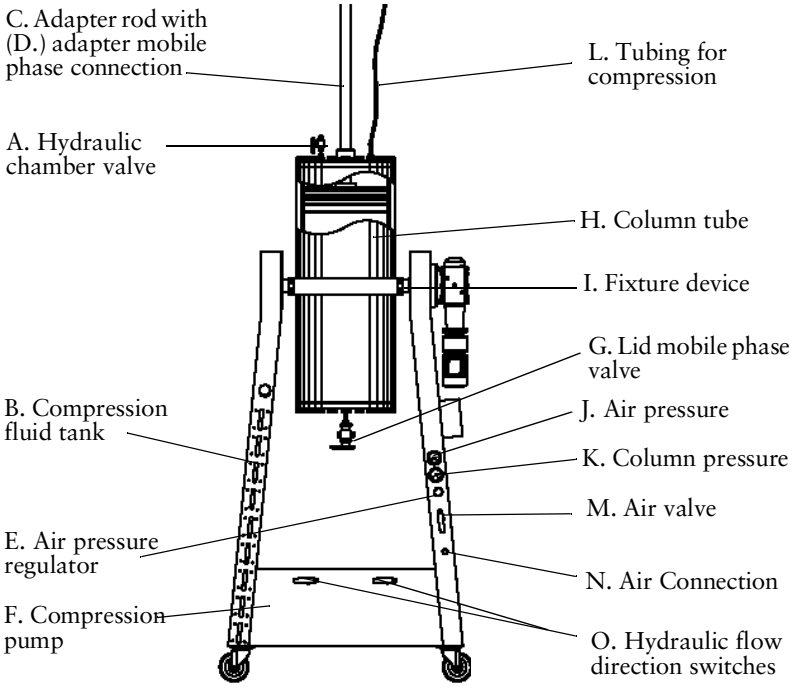

Fig 2-20. Schematic diagram of a BioProcess HPLC column in its upright position.

2. Withdraw the adapter to its most extreme position:

   Set both of the hydraulic flow direction switches (O) to the FROM COLUMN position.

CAUTION! Both hydraulic flow direction switches (O) must be moved to the same position before starting the pump. A high pressure can build up inside the tubing from the pump if the left switch is set to TO COLUMN and the right switch to FROM COLUMN.

   Set the AIR ON/OFF switch (M) to ON and use the air pressure regulator (E) to set the air pressure (J) to an appropriate pressure.

   The adapter will move out of the column by itself.

   When the adapter is fully extended turn off the air pump (M).

3. Use a wrench to remove the bolts that hold the base end plate in place on the column tube.
4. Attach the fittings from a crane or hoist to the eyes on the locking nut of the adapter rod.

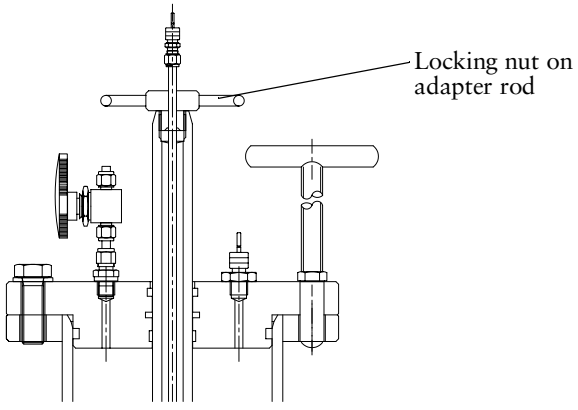

Fig 2-21. Longitudinal section through a column base end plate and adapter rod.

5. Pump fluid into the column via the lid mobile phase valve at a low flow rate. This will cause the adapter and base end plate to be pushed out of the column tube. Take up any slack in the hoist/ crane. Stop the pump when the adapter and base end plate are free. Immediately rest the adapter and base end plate on blocks.

6. Drain the fluid from inside the column via the lid mobile phase valve (G), or simply rotate the column tube and pour out the contents.

Removing the lid (250+ mm)

To remove the lid:

1. Rotate the column in its inverted upright position, so that the lid is accessible from above.
2. Loosen and remove the bolts connecting the lid to the column tube.
3. Remove the lid using a suitable hoist or crane.

Note: To help remove the lid you may need to insert T-shaped jacking bolts into threaded holes on the lid. Screwing these in will help to release the lid.

2.4.2 Exchanging O-rings and seals (250+ mm)

This section describes the exchange of O-rings and seals. You are recommended to change the O-rings and seals in your column once per year, particularly those that experience some form of movement in the column, for example the O-rings on the adapter.

Changing the O-rings on the base end plate and on the adapter do not require that you separate the these two components. However, changing the O-ring in the packing house, if for example there is a leak at the center of the end plate, requires that you separate the base end plate from the adapter.

Base end plate (250+ mm)

The base end plate has a single O-ring round its outer circumference.

1. Use a sharp knife to cut the existing O-ring.

Fig 2-22. Base end plate with O-ring.

2. Set a new O-ring in its place. You may first need to warm the new O-ring in warm water (50 °C) and then place it on the base end plate.

Adapter (250+ mm)

The adapter has two O-rings protected by seals, and a seal protecting the leakage detection system hole. Additionally, there is a seal holding the top bed support in place.

1. Remove the seal protecting the leakage detection system hole. This is easy to remove since it has a split end. Save this to one side.

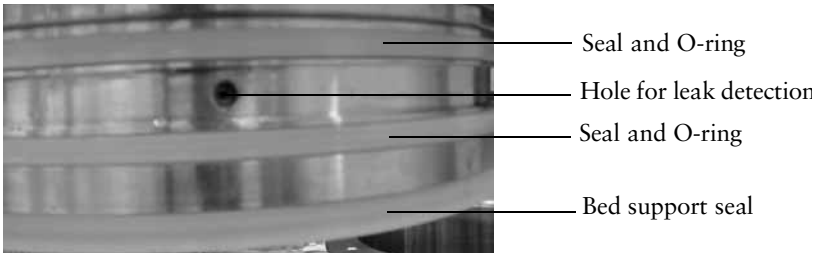

Fig 2-23. Seals on the adapter with the protective seal for the hole to the leak detection system.

2. Carefully remove the top bed support by pulling off the seal holding it in place.
3. Remove the liquid distributor by unscrewing the attachments and sliding the distributor out. Replace the O-ring on the distributor.
4. Use a sharp knife to cut through the seals for the two O-rings.
5. Use a knife to cut through the O-rings.
6. Replace the O-rings and the seals. You may first need to warm the new O-rings and seals in warm water (50 °C) before placing them on the adapter.
7. Replace the seal protecting the leakage detection system hole.
8. Replace the upper bed support. You may first need to warm the seal in warm water (50 °C) before replacing.

Adapter rod (250+ mm)

There is an O-ring to be replaced under the adapter rod where it connects with the adapter.

1. Ensure that the adapter and adapter rod are resting on a stable surface.
2. Attach a hoist/crane to the base end plate and gently lift so that it moves up the shaft of the adapter rod enough to be able to access the base of the adapter rod.
3. Use an Allen key to remove the bolts from the base of the adapter rod.

Fig 2-24. Base of the adapter rod.

4. Carefully lift the adapter rod upwards a few centimeters to expose the inner tube.

Note: You cannot remove the adapter rod more than a few centimeters since it will not pass over the Quick Connector at the top of the inner tube.

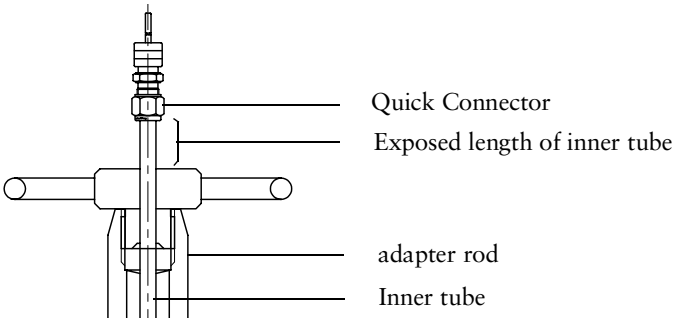

Fig 2-25. Longitudinal section through the tip of the adapter rod. Note that there is only a limited length of exposed inner tube along which to lift the adapter rod when it has been unbolted at its base.

5. Use a wrench to unscrew the inner tube at its base so that it is totally detached from the adapter.

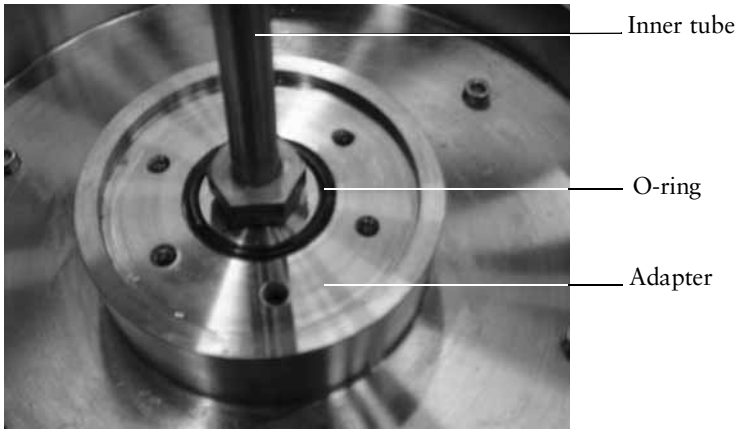

Fig 2-26. Adapter rod connection with the adapter.

6. Exchange the O-ring.
7. Reconnect the inner tube, lower the adapter rod and replace the bolts using an Allen key.

Packing house (250+ mm)

The packing house is located on the upper surface of the base end plate and creates the opening through which the adapter rod passes. In order to access the packing house the adapter must first be separated from the base end plate.

To separate the adapter from the base end plate:

1. Ensure that the adapter and adapter rod are resting on a stable surface.
2. Cut the inner tube at the top of the adapter rod as close to the Quick connection as possible.
3. Remove the locking nut.

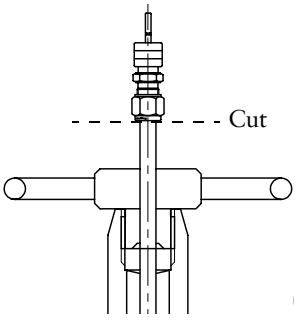

Fig 2-27. Longitudinal section through the tip of the adapter rod.

4. Carefully lift the base end plate up over the adapter rod.

Note: If you wish to totally separate the lid from the adapter you must first disconnect the leak detection tubing between the base and plate and the adapter.

To replace the O-rings and seals in the packing house of the base end plate:

1. Remove the excluder. Use a sharp knife to cut the excluder if necessary

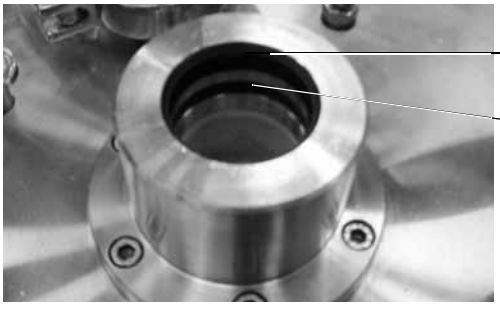

Fig 2-28. Packing house on the base end plate.

CAUTION! Take care not to scratch any steel surfaces of the column when using a knife to remove the seals and O-rings.

2. Use a sharp knife to remove the step seal protecting the O-ring.
3. Remove the O-ring by inserting some wire (20 mm length x 2 mm diameter) into the groove holding the O-ring and picking it out.
4. Clean the inside of the packing house and lubricate the new step seal and O-ring with ethanol.
5. Place the new O-ring into the groove in the packing house.
6. Place one edge of a new step seal onto the O-ring in the groove. Make sure that the step seal is the right way up according to the orientation in the cross section diagram below.

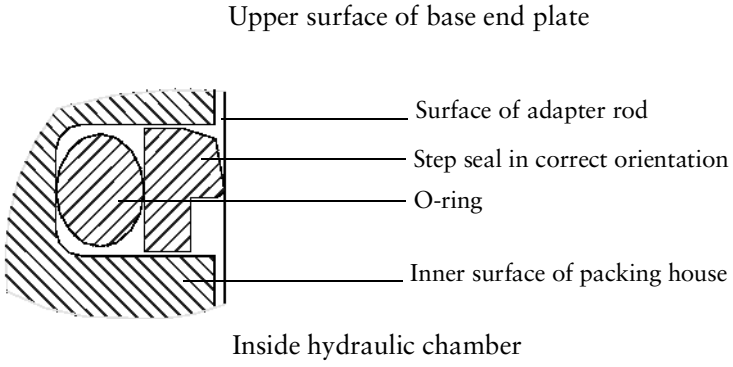

Fig 2-29. Cross section through the packing house showing the orientation of the step seal.

7. Bend the O-ring/step seal as shown in the diagram. Avoid sharp bending.

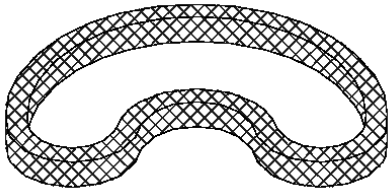

Fig 2-30. Bending of the step seal to facilitate fitting in the packing house.

8. Carefully press the folded step seal into place over the O-ring. A cross-section of the O-ring and packing seal should be as in the following diagram.
9. Put on a new excluder.

Lid and liquid distributor (250+ mm)

The lid and base end plate each have an O-ring that is in contact with the column tube. The lid also houses the liquid distributor and the bed support, which have O-rings that need replacing. The following description is given for the lid but can also be applied to the end plate.

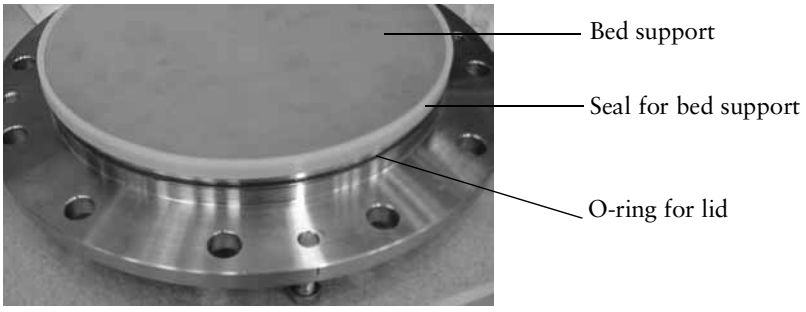

Fig 2-31. Lid with bed support fixed in place.

1. Remove the seal holding the bed support in place and remove the bed support O-ring from underneath.

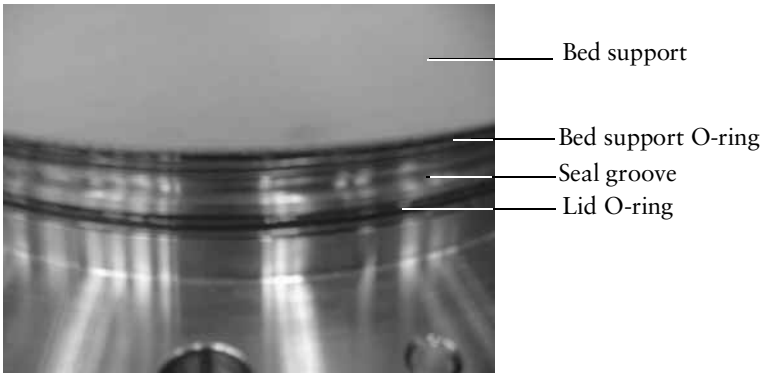

Fig 2-32. Lid with bed support seal removed to expose the bed support O-ring.

2. Remove the O-ring from the outer circumference of the lid.
3. Use an Allen key to remove the bolts on the outer surface of the lid that hold the liquid distributor in place.
4. Remove the liquid distributor by applying compressed air through the lid mobile phase inlet (H). The liquid distributor will move up.
5. Remove the O-ring from the liquid distributor and clean its surfaces with ethanol.

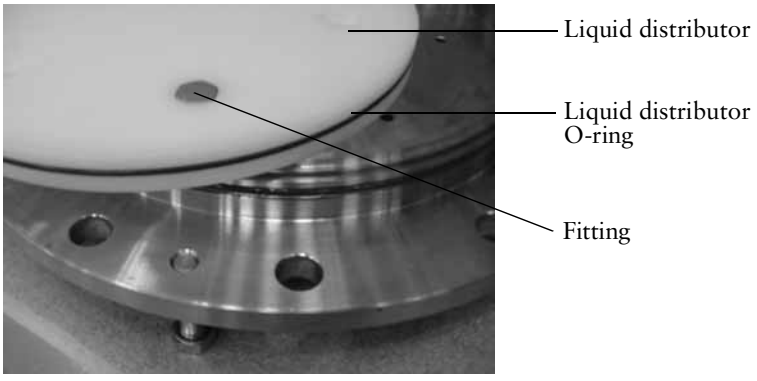

Fig 2-33. Liquid distributor.

Note: You can remove the fittings from the surface of the liquid distributor. These fittings hold the liquid distributor in place together with the bolts removed in step 3 above.

Fig 2-34. Bolts and fittings holding the liquid distributor in the lid.

6. Place a new O-ring on the liquid distributor and replace the fittings.
7. Place the liquid distributor back into the lid and bolt into place.
8. Place a new O-ring in its groove on the outer circumference of the lid.
9. Place a new bed support O-ring in the upper groove of the lid over which the seal for the bed support will sit.
10. Place the bed support on top of the liquid distributor and fix in place with the seal. You may first need to warm the seal in warm water (50 °C) before replacing.

IMPORTANT! The seal must be placed the correct way up. One edge of the seal is thicker than the other and this should be placed in face down to fit in the groove directly beneath where the bed support O-ring sits.

2.4.3 Assembling the column (250+ mm)

Fitting the lid (250+ mm)

1. Rotate the column in its inverted upright position, so that the lid can be fitted from above.
2. Use the hoist/crane to gently lower the lid onto the column tube.
3. Use two or three T-shaped jacking screws placed at opposite positions on the lid and screw these so that the lid fastens to the column tube evenly on all sides.
4. Replace the bolts connecting the lid to the column tube and tighten.

WARNING! There is a risk of injury to your fingers or other appendages should they become trapped between the lid and the column tube. Handle the lid with care.

Fitting the base end plate onto the adapter and adapter rod (250+ mm)

1. Lift the base end plate using a crane/hoist and carefully lower onto the shaft of the adapter rod.
2. If relevant reattach the leak detection tubing between the base end plate and the adapter.
3. Lower the base end plate until it rests on top of the adapter.
4. Screw the locking nut on to the tip of the adapter rod.
5. Attach a Quick Connector onto the exposed end of the inner tube.

Fitting the adapter and base end plate into the column (250+ mm)

1. Lift the base end plate and adapter above the column tube opening.
2. Carefully lower down into the column tube. Keep the fittings from the crane/hoist taught to prevent tipping.

Note: You can lubricate the seals with ethanol.

3. Insert T-shaped jacking bolts into the base end plate and into the column. Successively screw the bolts by equal amounts to push the adapter and base end plate into the column. You will need to periodically lower the crane/hoist.
4. When the adapter and base end plate are sufficiently inside the column, insert the bolts. Use a wrench to successively screw the bolts in by equal amounts.

3 Troubleshooting

This troubleshooting guide has been organised as a series of questions to help direct the operator to the cause of the problem. If after having consulted this guide, the problem cannot be solved, contact your local Amersham Biosciences representative for advice.

3.1 High back-pressure

1. Are all the valves between the pump and the collection vessel open?
2. Are all the valves clean and free from internal blockage?
3. Has the media been packed too hard?
4. Are the column bed supports blocked? Air may be trapped in the bed support. Always wet before use.
5. Could there be tightly bound sample material on the chromatography medium?
6. Has the correct valve for the flow rate been selected?
7. Is there any equipment in use either before or after the column, which could generate back pressure? For example, is an adequate size of flow cell being used? Are the sample and collection vessels at approximately the same level as the pump or are they higher?
8. Are there differences in internal diameter between the tubing on the column, the tubing from the pump, or anywhere else in the system?
9. Is the pressure gauge showing correct values?
10. Do the gaskets and the tubing have the same inner diameter?

3.2 Leakage

1. Is the adaptor properly positioned with respect to the column tube?
2. Are the connections to and from the pump correct and tightened?
3. Is there medium in the buffer return line? Check that the bed support is correctly fastened to the adaptor/bottom unit. Are the O-rings and seals intact?

3.3 Chromatograms not as expected

1. Are the recorder speed and/or signal correctly set, or has the sensitivity been changed?

2. Is the linear flow rate as intended?

3. Has back-mixing occurred anywhere in the column set-up? For example, could the sample have passed through an air trap?

4. Is the adaptor correctly positioned in close contact with the bed?

5. Has the column been correctly equilibrated?

6. Has there been a change in how the sample is handled prior to its application to the column? For example, are there sample batch variations? In the process, have there been any changes to the following: nets, separation media, time intervals between different operations, buffer constituents, precipitation procedures, chromatographic system hardware? Any such changes should be analysed for their effect on the chromatogram.

7. Consider the dilution, filtration, age, temperature and preparation of the sample and buffers.

8. Are all the buffers properly prepared according to the pre-defined specifications?

9. Were all dead volumes correctly filled with appropriate buffers and sample before running.

10. Is there a gap between the packed bed and the bed support? This may be due to a running flow rate that is higher than the packing flow rate, or to a running pressure that is too high.

3.4 Air trapped in the column

1. Do the buffers have the same temperature as the column?

2. Are all connections correctly fitted together?

3. Are all closed valves tightly shut?

4. If the air trap is used, is the volume of the air trap adequate?

5. In general, if air has entered the column, the column must be re-packed. However, if only a small amount of air has been trapped in the column, the air can be removed: Pump a solution with a temperature a few degrees Celsius above the media upwards through the column. The column should be tested again prior to use.

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