Spectrum HPCCC instrument

Operating Manual

For Technical Support Call:

Tel: + 44 (0) 1753 696979, Fax: +44 (0) 1753 696976

Contents

1.0 Delivery and First Checks

1.1 Unpacking the unit

When you have received the unit, before opening the delivery box please check for any signs of damage to the box or tampering with and if damage is observed make a photographic and written record of it. When trying to lift the unit out of the box, please bear in mind that the weight of the unit is in excess of 40Kg so we recommend you ideally use a mechanical lifting device or at least two people to lift.

1.2 Parts and accessories included in the delivery box

Spectrum HPCCC instrument

Power Cable

Interface Cable

Connectors and Jubilee Clips for attachment to Chilling unit

1.3 Safeguards

If the Spectrum HPCCC instrument is not operated in accordance with this document, the protection provided by the equipment may be impaired. The operator and those within the operator's organisation responsible for safety are responsible for operating the Spectrum HPCCC as specified in this document.

Key points to note:

1. *After opening the door, remove packing materials inside the chamber.*
2. *Before running the Rotor always check that the Flying Lead is installed correctly and its ends (which are outside the cabinet) are ALL CAPPED OR CONNECTED!*
3. *Check that the Rotor is free to rotate without any obstructions.*
4. *Only IEC approved equipment may be connected to the Signal Interface*
5. *It is important to always remember that this is a low-pressure device whose columns are designed with a pressure limitation of 200psi. Therefore, fine bore sections of tubing less than 0.8 mm internal diameter should not be used. The larger the bore of tubing used the lower the induced backpressure will be. The tubing used down stream of the Spectrum HPCCC instrument should also have the largest bore as is practical and be as short as is practical. This will reduce the backpressure through the columns in use and reduce the risk of bursting the tubing.*
6. *The Spectrum HPCCC instrument requires to be connected to a chilled water supply in order for the temperature control to operate. If the unit is not connected to a chilled water supply the over-temperature safety system will shut the units rotation down thus stopping the unit from operating.*
7. *The supply of chilled water should not be colder than 0℃. Temperatures below this will cause ice to form within the Spectrum's cooling system and stop it functioning correctly.*

2.0 Getting Started

2.1 Describing the Spectrum HPCCC Controls (Figure 1)

2.2 System Location, Start Up and Checks

The Spectrum HPCCC instrument should be ideally placed in a fumehood or well aerated location on a solid surface (not moving table or trolley). All power connections should be located well away from the unit to avoid risk of explosion.

When you have connected the power supply first switch on at the mains switch which will bring up the upper display (Rev counter) then turn the Temp. control On by turning clockwise which will bring up the temperature controller.

Then open the unit door by pressing the door interlock button and pushing the door slightly at the same time. Remove any packaging and padding from inside the unit and check that the column is moving freely without touching anything.

Next close the door and with rotational speed control turned completely anticlockwise to minimum speed, turn the Run Button clockwise. The rotor will start turning slowly with the speed displayed on the Rev. counter. Gradually turn up the speed to 1600 rpm listening for vibration.

Assuming everything is OK you can now switch off the unit and carry out the full installation.

2.3 Installation

The following instructions should be read and understood prior to full installation of the Spectrum HPCCC instrument:

2.3.1 General Installation Requirements

The Spectrum HPCCC is classed as a LABORATORY CENTRIFUGE and should be treated as such and operated in accordance with national safety requirements and any in-house safety requirements stipulated by your company.

The Spectrum HPCCC is only designed to operate safely under the following environmental conditions:

1. Indoor Use only;
2. Altitude up to 2000m;
3. Temperature 20^{o}C to 30^{o}C;
4. Maximum relative humidity 80% for temperatures up to 25^{o}C decreasing linearly to 50% relative humidity at 30^{o}C;
5. Mains supply voltage fluctuations not exceeding ±10% of the nominal voltage;
6. Installation Category (Over-Voltage Category) as specified in BS EN 61010-1:1993, Annex J. For mains supply the minimum and normal category is II.
7. Pollution Degree 2 in accordance with section 3.7.3 of BS EN 61010-1:1993.

Care should be taken when installing the Spectrum HPCCC to make allowances for its weight (40kg), both from the lifting/positioning and structural support point of view, and the instrument's cooling/ventilation requirements. The unit should be mounted on a substantial workbench, which is built to withstand the unit's heavy weight (40 kg) and any inadvertent out of balance when operating.

There should also be adequate space either side of the unit for auxiliary equipment such as pumps, detectors, fraction collectors and solvent reservoirs outside the Clearance Envelope defined below. The dimensions of the unit are: height 18.5 inches (470mm), depth 19 inches (485mm) and width 21 inches (535mm). Sub-clause 7.3.101 of BS EN 61010-2-020:1994 limits the permitted movement of a LABORATORY CENTRIFUGE to

300mm in any direction, in the event of a DISRUPTION. The laboratory management or those responsible for safety need to mark off an area around the Spectrum HPCCC to ensure that no person or any hazardous materials are within that boundary while the Spectrum HPCCC is operating. This marked off area is the clearance envelope.

2.3.2 Electrical Power Installation Requirements and Electrical Safety

The Spectrum HPCCC requires a single 240/110-volt 50/60-hertz alternating current (ac) supply capable of continuously supplying 8 amps. The electrical supply also needs a Protective Earth.

The maximum electrical power consumption of the Spectrum HPCCC is 0.75 kVA .

The only fuses that the operator may change are the 2 mains fuses (5mmx20mm, 8.0-amp, anti-surge (T), HBC, RS 390-5790). The operator may not change internal fuses. If after replacing external fuses, the Spectrum HPCCC fails to operate or continues to blow external fuses, please refer to the Spectrum HPCCC instrument Centrifuge Service Manual. **Before replacing any external fuses ensure that the Spectrum HPCCC is isolated from the mains supply.**

The Spectrum HPCCC is not fitted with an Emergency Stop. This is because the unit has safety features that will shut it down automatically if either an imbalance or over temperature situation occurs see section 2.3.4.

2.3.3 Noise Levels and Bearing Life of an Operating Spectrum HPCCC unit

The sound level that a Spectrum HPCCC emits is given in the table below:

Rotational Speed (rpm)	Sound Pressure Level at 0.1 metre (dBA)
1600	67
1200	61

This sound level is within the maximum requirement listed in BS EN 61010-1, which is 85 dBA at 1 metre or the operator’s normal position, the operator’s normal position is taken at 0.1 metres for the Spectrum HPCCC.

The service life of the bearings on a Spectrum HPCCC unit is 3 years given a usage of 40 hours per week for 3 years. It is recommended that the user maintains a log of the total number of hours that a Spectrum HPCCC instrument is operated.

2.3.4 Safety features

The Spectrum HPCCC has been fitted with a number of safety features:

- A door safety interlock, which disables the motor when the door is open and prevents the door being opened when the centrifuge is rotating. The door cannot be opened with the power off.
- A stop motion detector that prevents the door being opened until 10 seconds after rotation has ceased.
- Visual indications of rotational speed.
- An imbalance sensor that will shut down the Spectrum HPCCC if an imbalance occurs.
- Over-temperature protection that will shut down the Spectrum HPCCC if its temperature rises above 50°C.
- A Signal Interface, see Figure 1, which can be used to switch pumps and other equipment off if the Imbalance Sensor or Over-temperature protection shuts the Spectrum HPCCC down.

2.3.5 Chemical and Environmental Effects

The Spectrum HPCCC instrument has been designed to allow the pumping of solvents generally used in the chromatographic purification of products, through a continuous length of tubing. The tubing is divided into three lengths that are joined together by zero dead volume connectors. The middle length is the column that is wound on the Bobbin. The other two lengths are the flying leads. These lengths of PTFE tubing are very inert and will not be chemically attacked by the solvents commonly used. For a general list of the chemicals not to be used in a Spectrum HPCCC instrument see section 2.3.6.

2.3.6 Hazardous Substances

The following indicative list of materials may not be used in a Spectrum HPCCC:

1. Explosive materials.
2. Concentrated acids or alkalis.
3. Combinations of materials that chemically react vigorously.
4. Highly radioactive substances.
5. Substances containing live pathogenic micro-organisms.
6. Mercury.

2.3.7 Rotor and Bobbin Equipment Ratings and Solvent Density Limit

The maximum speed of the Spectrum HPCCC instrument is limited to 1600rpm. All of the components of the Spectrum HPCCC instrument Centrifuge have been designed to withstand this rotational speed. Any separate bobbins supplied by Dynamic Extractions will be designed on the same basis.

Bobbins supplied by other manufacturers will not and should not be fitted to the Spectrum HPCCC instrument rotor.

Solvent phases with a density greater than 5gram/ml (2000kg/m^3) should not be used in the Spectrum HPCCC.

2.3.8 Labelling of the Spectrum HPCCC instrument

The Spectrum HPCCC is labelled to provide information to the operator and to ensure safe operation. The mains fuses are labelled next to the mains input and forms part of the CE marking label, see section 2.3.2

Warning, consult accompanying documents symbol (Interface Connector) label:

The “Warning, consult accompanying documents” symbol above is used to indicate the Interface Connector. The Interface Connector is positioned directly below this symbol. For an explanation of the Interface Connector and its operation see section 4.4.

The Serial No. is unique to the Spectrum HPCCC to which the label is attached and should be quoted in any documentation related to the specific machine. For an explanation of the Electrical Supply, Max Power Consumption and Mains Fuse see section 2.3.2.

2.3.9 Full Installation (Figure 2)

This subject is covered in more detail in the DE quick start guide; **Developing and Optimising a Separation Method for HPCCC.**

In simple terms, the Spectrum HPCCC instrument is analogous to a preparative HPLC column and therefore should be treated in the same way. This means you need two isocratic HPLC pumps capable of at least 150 ml/min each, a 1/8 “ 6 way injection valve, a 6 way switching valve for reversing column flow, a UV/VIS detector (or DAD) with preparative flow cell and a fraction collector.

You will also need to connect the Spectrum HPCCC instrument to a recirculating chiller which is powerful enough to maintain the unit at a constant temperature of 30 – 32^{o}C during rotation. For this we suggest a unit capacity of 500W at 5ºC, containing a water/ethylene glycol mixture.

Figure 2 (overpage) describes how these components are connected together. This is an ideal fully automated system and several components such as the upstream and downstream switching valves and Nitrogen supply are useful but not essential. It is however, absolutely essential that all the tubing in the system is at least 0.8mm internal bore to avoid overpressure and column damage.

We recommend that the mode switching and injection valves are automatic so that they can be controlled by a HPLC computer program, however again this is not essential and manual ones can be used. These valves should ideally be 1/8” to avoid plugging from insoluble material. The water bath is a must if you are operating at room temperatures below 15^{o}C, otherwise

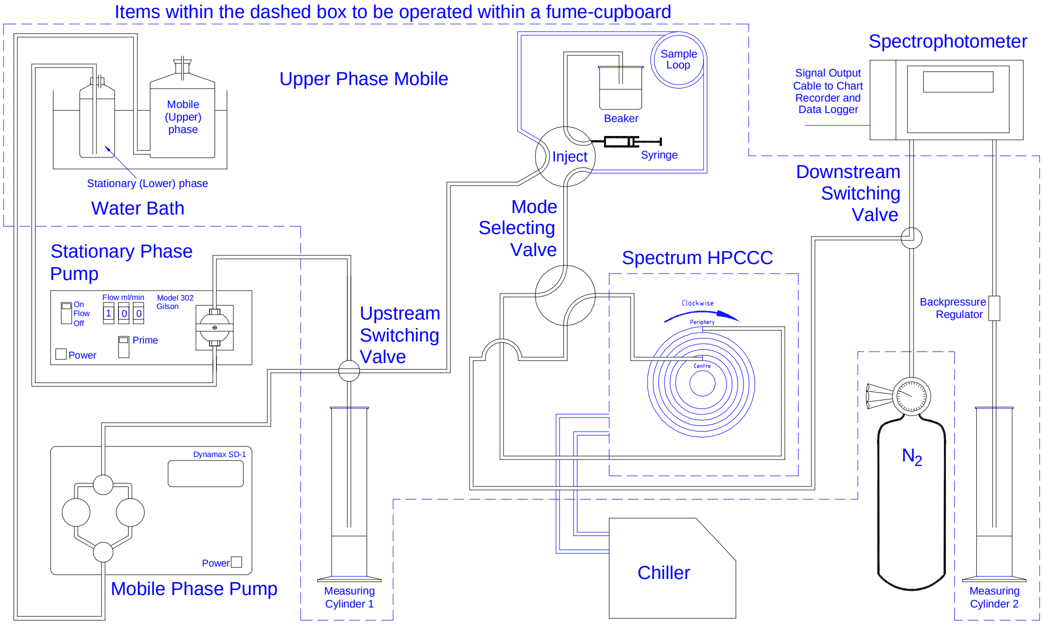

Figure 2: Full Installation Diagram for Spectrum HPCCC instrument

<u>3.0 Establishing an Equilibrium in the column and carrying out Separations</u>

3.1 Preparing the pumps

Once you have connected up the system as described in section 2.3.9 first determine which pump is going to be the organic only and which one is going to be aqueous only. Then with the solvent bottles full, prime the pumps until no air is being expelled and check that the flow rates are what you are expecting. Also make sure that you have set a back pressure limit on each pump (200 psi max.) to avoid any potential damage to the Spectrum HPCCC instrument columns.

3.2 Filling the column with Stationary Phase

First decide which solvent or phase you want to be the stationary phase (to do this refer to the DE publication, **Developing and Optimising a Separation Method for HPCCC**), for normal phase separations this will be the lower aqueous phase for reverse phase the upper organic is the stationary. Then decide which column or columns you want to use for your separation. You have a choice of the two analytical 11ml columns, 0.8mm ID or the two preparative 70ml, 1.6mm ID columns. Each column has two ends marked periphery and centre (marked AP and AC), for normal phase separations you need to connect the outlet from the injection valve to the periphery or for reverse phase connect to the centre.

Once connected, pump with the appropriate pump at a maximum of either 10 ml/min for the analytical columns or 50 ml/min for the preparative columns until the solvent appears in the outlet of the UV monitor. We recommend that the first time you use the unit you switch from upper to lower phase until you can see the two phases in your collection vessel. This will allow you to determine the total volume of the columns, volumes of the columns are given in an appendix, plus dead volume in your flow path. If you do switch remember to switch back to the stationary phase before starting to set up an equilibrium.

3.3 How to establish a Dynamic Equilibrium in your column

Once you have completely filled your chosen columns with stationary phase and all air has been expelled, start the rotation of the Spectrum HPCCC instrument at the minimum speed then gradually increase to 1600 rpm. Next start the recirculating chiller and adjust the temperature of the chiller until the temperature shown on the lower display of the Spectrum is stable at 30 – 32^{o}C. Once this is stable switch off the stationary phase pump and switch on the mobile phase pump at 0.5 – 2 ml/min for the analytical columns or 10 – 25 ml/min for the preparative columns. Again please refer to the DE publication, **Developing and Optimising a Separation Method for HPCCC**, on the choice of flow rates.

Collect the flow through from the column in a 50 ml measuring cylinder so that you can see the point at which a dynamic equilibrium is established (seen as the appearance of two layers in the cylinder). In the case of the analytical columns this should be after approximately 8ml of stationary phase has been extruded or 26 ml in the case of the preparative columns. If no equilibrium can be established you have either connected the columns the wrong way or you have a contaminant in the columns. To correct this stop the rotation and start the procedure again starting by refilling the columns with stationary phase. If this does not solve the problem check your connections to make sure you have attached the correct end of the column to the injector.

3.4 Carrying out a Separation

More details of this are written in the DE publication, **Developing and Optimising a Separation Method for HPCCC.** From an operational point of view the key points to look out for are the stability of your UV/VIS or DAD monitor baseline, bubbles appearing in the outlet, back pressure, temperature and any stationary phase loss. In terms of the proper functioning of your HPLC pumps you should have more than 40 psi back pressure upstream of the pumps and monitor. This back pressure allows the pumps to operate accurately at lower flow rates and stops degassing in the monitor. Air bubbles can normally be seen downstream of the monitor if there is a problem and will cause noise in the monitor baseline. If adding a 40 psi pressure regulator to the set up does not cure the noise then you have stationary phase stripping. Please refer to section 4.2.6 for the reasons and cures for this problem.

3.5 Emptying the column

To completely empty the columns all fluid, disconnect the pumps, set the rotation speed to 500 rpm and change the flow direction switch to empty. The contents of the columns will then come out immediately. If you want to analyse the stationary phase for retained compounds we suggest that you simply stop the column rotation while keeping on pumping the mobile phase. This will enable you to collect all the stationary phase and to determine the stationary phase retention. Once all the stationary phase has been collected you can then switch to the stationary phase pump to re-establish a dynamic equilibrium. This type of operation is called elution extrusion and is used to determine impurity retentates for mass balance or for purification in some cases.

3.6 Siphoning

It is important to be aware that the system can siphon unless a flow "on /off" tap is fitted to the outlet flying lead or a pressure regulator is used. Do not depend upon liquid flow stopping from outlet flying leads because the HPLC pump is switched off. If the outlet flying lead is below the inlet fluid reservoir level, liquids can siphon out of it, which can cause a spillage.

3.7 Connecting Columns in Series

Both the analytical and preparative columns can be connected in series to increase resolution. Connecting the columns in series will increase the resolution by a factor of 1.4 (the square root of two).

4.0 Safety Features

4.1 Imbalance Sensor, Over-Temperature Sensor and Signal Interface

The Signal Interface allows the Spectrum HPCCC to communicate with other equipment and power them down when there is an imbalance or an over-temperature condition. The imbalance sensor, over-temperature sensor and signal interface are safety devices that will isolate the power supply to the centrifuge drive and provide electrical signals to indicate that the power has been cut to the drive motor. **Only IEC approved equipment may be connected to the Signal Interface.**

Correct operation of the Imbalance Sensor, Over-Temperature and the Signal Interface should be checked once a year by a DE approved technician.

4.2 Imbalance Sensor Operation and Resetting

The imbalance sensor continuously measures the vibration generated by the Spectrum HPCCC and if the vibration level exceeds a set value the power to the drive motor is switched off and the rotation will slowly stop. A red LED flashes, see Figure 1, when the motor supply has been cut off. If the red LED is flashing ensure that the HPLC pumps connected to the Spectrum HPCCC are switched off and that all other equipment is switched off. The cause of the excessive vibration needs to be investigated and removed before normal operation can resume. Possible causes of excessive vibration are: the external end/s of the flying lead/s leaking due to removal of the end caps; a burst/broken flying lead inside the Cabinet; imbalance between Bobbins due to the operational set up, see section 3.2 and centrifuge rotational speed greater than 1600rpm. To detect the cause of the imbalance follow the steps below:

Check the external end of each flying lead to check that the end cap is still attached or is not leaking. If an end cap is missing or has leaked, the column attached to the leaking flying lead will need to be completely refilled to remove the imbalance. New end caps will also need to be fitted to both flying leads attached to the refilled column.

To detect a burst/broken flying lead first check to see if solvent is present underneath the Spectrum HPCCC (this will have drained from the Rotor Space through the drainage fitting located on the underside of the Spectrum HPCCC to the glass container). If solvent is present or is still draining from the Spectrum HPCCC collect the solvent in accordance with your company's safety procedures. The main door of the Cabinet can then be opened to allow access to the Rotor Space. The Rotor Space needs to be inspected with care as there may still be solvents present from a burst/broken flying lead. Inspect both flying leads for bursts or wear points. Any sign of leakage – the flying leads should be replaced immediately and the old leads inspected for damage and wear (see section 5.3.1 Care of flying leads).

Check the operational set up to ensure that a systematic error has not led to the imbalance.

Press the Imbalance Sensor reset button, the red LED will stop flashing, close the Cabinet door and switch the Rotor on again.

Monitor the speed of rotation to determine the steady state speed, if greater than 1600rpm reduce to 1600rpm.

If the Imbalance Sensor continues to trigger and shut the Spectrum HPCCC down check for other sources of vibration other than the Spectrum HPCCC, if no other sources can be found contact Dynamic Extractions Ltd.

Note: Each time the Imbalance Sensor cuts power to the motor the door interlock button, Figure 1, will need to be pressed and the door to the rotor space will need to be opened and closed before power can be re-supplied to the motor.

4.3 Over-Temperature Sensor Operation and Resetting

The over-temperature sensor will automatically reset once the unit has cooled down below 30°C. The door will need to be opened and closed to stop the red LED from flashing and allow the rotor to be spun.

4.4 Signal Interface

The interface connector has two connections. The first is a 5-volt dc signal output (that is not intended to supply power to other devices) used to switch other devices on or off. The second connection is a relay that is normally open and will close when the power to the motor is switched off (this relay is only intended to switch on control signals and should not be used to switch on power supplies). The 5-volt dc supply is connected between the black and red wires on the external alarm cable with the red wire at +5 volts when triggered and black wire 0 volts. The relay is connected across orange and brown (the maximum switch voltage is 60V dc and the contacts are rated at 500mA).

Note: The external lead supplied is less than 3 metres long for reasons of electrical safety and should not be extended beyond 3 metres. If the length is extended beyond 3 metres the operator does so at his/her own risk. Only IEC approved equipment may be connected to the Signal Interface.

5.0 Cleaning and Storing Spectrum columns

5.1 Instrument Cleaning

Cleaning of the Spectrum HPCCC should only take place once the electrical power is switched off and disconnected and the main door has been opened.

Regularly inspect the inside of the rotor space to ensure that the flying lead is not leaking or is broken. If there are small volumes of solvent or residue present, remove straight away.

The exterior of the equipment can be cleaned using an ordinary cloth and standard furniture polish.

5.2 Column Cleaning and Storage

It is important that the column should never be left without liquid.

At the end of use the columns should be emptied, cleaned with a mixture of water and methanol (1:1 v:v) by pumping several column volumes through the column, typically two, and then empty again and finally flushed with two column volumes of distilled water.

If stored, for a number of days, the columns should be filled with a 50:50 mixture of methanol and water.

5.3 Regular Maintenance

5.3.1 Care of the Flying Leads

It is important that no loss of fluid occurs from the tubing. All tubing connectors should be capped if not attached to external equipment. It is always prudent to run a fluid continuity check, where fluid pumped in, is balancing the quantity of fluid pumped out. This can be logged easily if using a fraction collector; otherwise the mobile phase needs to be collected in a graduated measuring cylinder. Fluid loss can lead to imbalance, vibration and loss of valuable samples.

Leaks can also be checked for by pressurising the flying lead and column to 200psi (13.5bar) using the nitrogen supply shown in figure 2 with the upstream switch valve closed so that no nitrogen can escape into measuring cylinder 1. Once the column and flying lead are pressurised closed the shut off valve on top of the nitrogen bottle. Monitor the pressure gages on the gas bottles regulator to see if the pressure drops. If the pressure remains constant over say half an hour there is not a leak. If the pressure drops check all joint fittings and valve for leaks using Snoop®. If a leaking fitting or valve is found tighten or replace as appropriate and repeat pressure test. If no leaks from valves or fittings are found replace the flying lead.

5.3.2 Procedure for Replacing Flying Leads

The flying leads for both the analytical and preparative columns use 1.6mm OD by 0.8 mm ID PTFE tubing.

Each bobbin has 1 analytical coil and 1 preparative coil. There are 2 bobbins giving a total of 4 coils, this means that there is a total of 8 flying leads. A flying lead is connected to a coil via a stainless steel connector block. These connector blocks are engraved to allow the operator to know how the coils are connected, see Appendix 1. The engraved labels for Bobbin 1 have a suffix “1” and for Bobbin 2 the suffix is “2”.

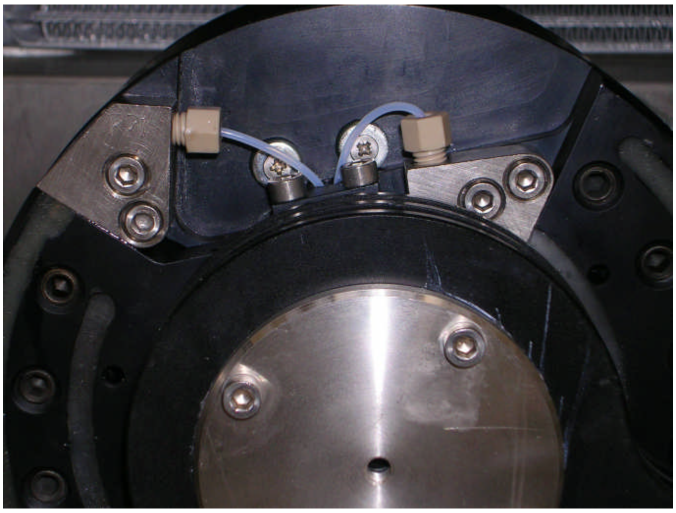

Figure 3 Front view of Spectrum Bobbin showing
a pair of stainless steel connector blocks

1) The flying lead connections are on the front of each bobbin. Rotate a bobbin so that the appropriate connections (analytical or preparative see Figure 3) are easily accessible.

2) A pair of flying leads consists of 2 leads each with a silver coloured gripper and a light brown hexagonal screw fitting. These parts are obtainable from Dynamic Extractions Ltd.

3) Ensure that the black circular flying lead clamps mounted on left-side of the centrifuge have been removed.

4) Remove any fittings and labels from the external ends of the appropriate flying leads.

5) Using an 8mm or 5/16" open ended spanner to unscrew the light brown screw fittings from the stainless steel connection blocks see Figure 3.

6) Remove all of the old flying leads from both bobbins, by gently pulling each one in turn from the appropriate bobbin. Remember to keep the light brown screw fittings as these will be used for the new flying leads.

7) To aid pushing the new leads through the guide tubes cut tapers on the opposite ends of the leads to the silver coloured gripper using a scalpel. Then slightly curl the tapered ends so that the cut surface is on the outside of the curve end.

8) **For Bobbin 1:** Remove the central flying lead guide attached to the front of the rotor by firstly unscrewing the 4 screws attaching it to the rotor and then detaching it from the liner, see Figure 4.

Figure 4 Detachment of Central Flying Lead Guide

9) Screw the light brown fitting of the 1st flying lead into the appropriate stainless steel connection block.

10) Using a syringe filled with silicone grease deposit approximately 1ml of grease into the entrance hole of the guide tube from which the old flying leads were removed, see step 6.

11) Originate the curved-tapered end of the lead so that it is pointing towards the **BACK** of the instrument and then push into the hole. Keep feeding the lead until it appears from the central hole in the rotor, see Figure 5. Pass the lead through the detached flying lead guide and then through the hole in the liner to the outside of the instrument, see Figure 6. Label this end of the lead with the same letters and numbers as those engraved on the stainless steel connection block to which it is connected.

12) Repeat from step 9 for the other 3 flying leads that are to be connected to Bobbin 1.

13) Reattach the central flying lead guide to the rotor taking care not to pinch the flying leads.

14) Gently pull the tapered ends of the leads to remove any slack between the connection blocks and the hole into which the leads have been pushed, see Figure 3. Fit the black circular external flying lead clamp.

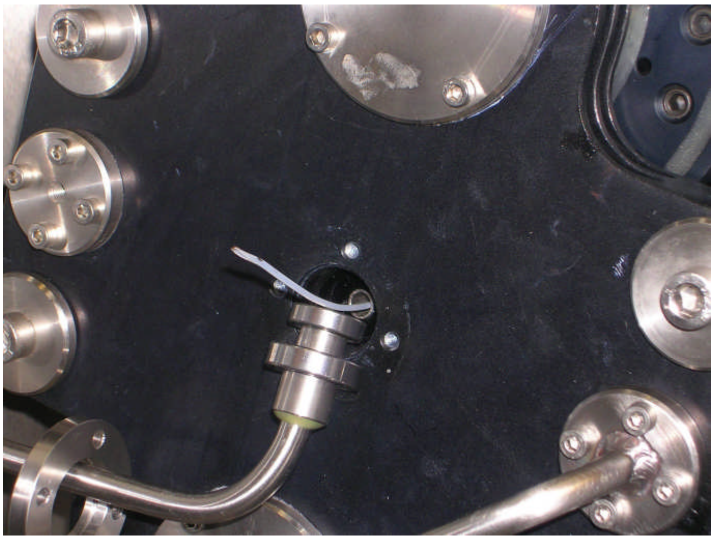

Figure 5 Shows a flying lead appearing from the central hole in the front of the rotor

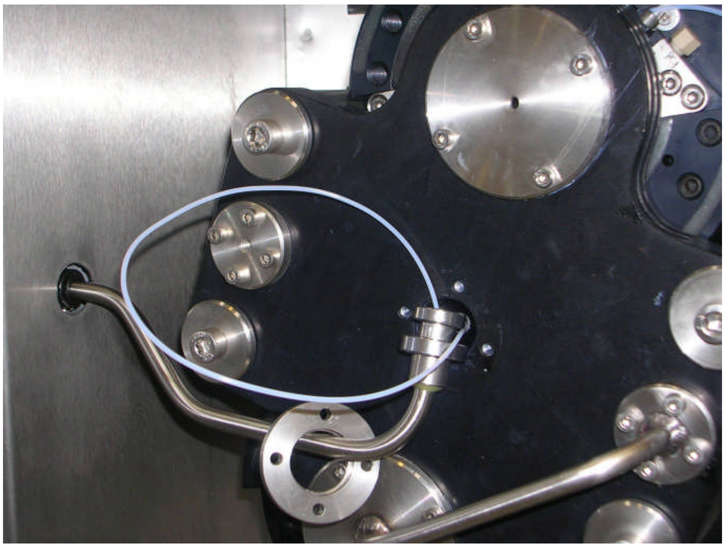

Figure 6 Shows the flying lead fed into the central flying lead guide

15) Cut-off the tapered ends of the leads beyond the point where silicone grease may have found its way along the inside of the tubing.

16) Using appropriate connectors seal each pair of flying leads together.

17) **For Bobbin 2:** Screw the light brown fitting of the 1st flying lead into the appropriate stainless steel connection block.

18) Using a syringe filled with silicone grease deposit approximately 1ml of grease into the entrance hole of the guide tube from which the old flying leads were removed, see step 7.

19) Originate the curved-tapered end of the lead so that it is pointing towards the **FRONT** of the instrument and then push into the hole. Keep feeding the lead until it appears from the rear hole on the left hand side of the instrument. Label this end of the lead with the same letters and numbers as those engraved on the stainless steel connection block to which it is connected.

20) Repeat from step 17 for the other 3 flying leads that are to be connected to Bobbin 2.

21) Gently pull the tapered ends of the leads to remove any slack between the connection blocks and the hole into which the leads have been pushed, see Figure 3. Fit the black circular external flying lead clamp.

22) Cut-off the tapered ends of the leads beyond the point where silicone grease may have found its way along the inside of the tubing.

23) Using appropriate connectors seal each pair of flying leads together.

The flying leads have now been replaced and the instrument is ready for use.

Note: It is important that no loss of fluid occurs from the tubing, so check that all tubing connectors are capped at all times if not attached to external equipment. It is always prudent to run a fluid continuity check, where fluid pumped in is balancing the quantity of fluid pumped out. This can be logged easily if using a fraction collector; otherwise the mobile phase needs to be collected in a graduated measuring cylinder. Fluid loss will lead to imbalance and loss of valuable samples.

5.3.3 Care of Columns (Columns).

Columns can be damaged by, rotation at speeds above 1600rpm and being over pressurised. The maximum speed of a Spectrum HPCCC is set at 1600rpm. Therefore the main cause of damage to columns will be over pressurisation. There are three main causes of over pressurisation.

1. Pumping mobile phase through a column at too high a flow rate (for analytical columns the maximum flow rate is 2ml/min and for the preparative columns the maximum flow rate is 10ml/min).
2. A blockage in the column caused by using unfiltered samples or precipitation of sample from solution.
3. Crystallisation of sample in a column or flying lead caused by not flushing with a minimum of three column volumes of distilled water after use (especially important if the column is not to be used for several days).

Note: If a column is burst or damaged it will need to be repaired or replaced to maintain the balance of the rotor assembly. The Spectrum HPCCC will need to be returned to the supplier so that the mass of old and new bobbins can be matched to maintain rotor balance. The centrifuge should only be returned after the solvents in the column have been replaced with distilled water see next paragraph.

5.3.4 Routine Maintenance and Servicing

The flying lead should be checked weekly for wear and leaks, see section 4.5. If worn, the leads should be replaced, see section 5.3.2. The position of the Spectrum HPCCC within the clearance envelope should be checked and the repositioned if necessary, see section 2.3.1.

Once a year the correct operation of the vibration sensor, over-temperature sensor and the signal interface should be checked by a trained DE representative. The condition of the Spectrum HPCCC's anti-vibration feet should be checked to ensure that the rubber has not been damaged.

On an annual basis the electrical systems should be inspected and tested to ensure correct operation and safe operation. The continuity of the protective earth should also be checked. The inspection and testing of the electrical systems should only be conducted by those qualified to do so. The flying lead should also be replaced even if no sign of wear can be detected.

Appendix 1: Column Volume and β-value range

Serial Number: Spectrum 70020101 (Spectrum HPCCC)

Column Volumes

Bobbin 1

Analytical column 1, connections AP1 and AC1, 13.0ml 0.8mmm bore with a β-value range from 0.64 to 0.81.

Preparative column 1, connections PP1 and PC1, 67.0ml 1.6mmm bore with a β-value range from 0.52 to 0.86.

Bobbin 2

Analytical column 2, connections AP2 and AC2, 12.5ml 0.8mmm bore with a β-value range from 0.64 to 0.81.

Preparative column 2, connections PP2 and PC2, 67.0ml 1.6mmm bore with a β-value range from 0.52 to 0.86.

Standard Flying Leads

Average volume per column = 1.1ml 0.8mm ID x 2.0 metres

Appendix 2: Nomenclature

Rotor Space, is the volume behind the main door in which the Rotor and Bobbin rotate.

Rotor is the structure upon which the Bobbin and counterweight are mounted.

Bobbin is a drum on which the columns are wound. There is one bobbin with one column wound on it.

β-value, is the ratio of the column radius to the rotor radius (r/R).

Column, are wound on the Bobbins in a clockwise direction from the centre to the periphery.

A Flying Lead is made from two lengths of PTFE tubing. One piece of tubing is connected to the centre of the column and the other to the outside of the column, the periphery. The flying lead attached to the Bobbin is routed through the Rotor and merges from the left hand side of the instrument. The Flying Lead carries the mobile and stationary phases to and from the column.

Direction of rotation, while performing a separation, regardless of normal phase or reverse phase, the centrifuge should be rotated in the clockwise direction and when emptying rotated in counter-clockwise direction. **Note:** Clockwise direction of rotation is defined when viewed through the door of the instrument.

Centre/Periphery, every column is wound from the centre to the periphery. The correct combination of the direction of rotation and the direction of pumping the mobile phase through the column will maximise the retention of stationary phase in the column. When using the upper phase as the mobile phase, pump from periphery to the centre and with lower phase mobile pump from centre to the periphery. The incorrect combination will reduce the retention of stationary phase and may also cause all of the stationary phase to be displaced from the column.