

GlycoChrom™ HPLC Analyzer

Hardware Installation and System Test

P/N 410-5004

BIO-RAD

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Section 1

Introduction

The GlycoChrom automated HPLC analyzer is operated from expert software built around Bio-Rad's HPLC column technologies. It analyzes sugars, organic acids, organic bases, preservatives, metabolites, water soluble vitamins, proteins, polysaccharides, and other associated compounds on Aminex® HPLC columns and Bio-Silect® SEC columns. Manual versions of the system are available to users who want only a manual injector (Basic GlycoChrom analyzer) and users who need automation but want to collect data via a dedicated integrator or strip chart recorder (GlycoChrom automated analyzers). GlycoChrom systems are available in six basic configurations. Installation and operating instructions for each configuration are given in this manual.

- Basic GlycoChrom analyzer, for use with Aminex columns
- Basic SEC analyzer, for use with Bio-Silect SEC columns
- GlycoChrom automated analyzer, for use with Aminex columns
- GlycoChrom automated analyzer, for use with SEC columns
- GlycoChrom automated analyzer, with expert software
- GlycoChrom automated analyzer, with Expert software and 486 computer

Operating instructions for software and individual components are enclosed with each module. Refer to these instructions for specific operation of each unit.

- Model 1350 (1350T) Pump Instructions (Basic or Automated system)
- Model 1755 RI Monitor Instructions (Basic or Automated system)
- Model 1800 UV Monitor Instructions (Basic or Automated system)
- Column Heater Instructions (Basic System)
- Manual Injector HPLC Valve Assembly (Basic System)
- AS-96C Automatic Sampler Instructions (Automated System)
- Expert Software GlycoChrom PC Software Package (Automated)

Section 2

Basic GlycoChrom Analyzer

catalog number 125-0590

2.1 Analyzer Installation

The basic analyzer (stainless steel injector & solvent path), catalog number 125-0590, is for used with Aminex HPLC columns. The analyzer includes components for solvent delivery, sample injection, and refractive index detection in separate modules. The components of the analyzer are:

- Accessories—Column Kit and Instructions, catalog number 125-1536
- Model 1350 Soft Start® pump, catalog number 125-1350
- Model 1755 RI Monitor, catalog number 167-0458
- Valve System Organizer Assembly (manual injector), catalog number 125-0324
- Column Heater, catalog number 125-0425
- Guard Holder, catalog number 125-0131
- HPLC Fittings Kit, catalog number 125-0010

Carefully remove the units from their shipping containers. Inspect the serial number on the back of each unit to insure that it corresponds to the number on the inspection certificate. A relatively level space 72 inches (1.8 m) wide and 30 inches (0.76 m) deep is recommended for

set-up, although smaller spaces can be used. Three outlets are required (or one power strip) supplying 120 volts of AC power. For the best performance, select a space free of drafts (such as drafts due to an air conditioning or heater vent) and out of direct sunlight. Allow at least 1 inch on each side for proper ventilation. If any components are missing, contact Bio-Rad Laboratories Customer Service Department immediately.

Caution: The analyzers are shipped from the factory in the 120 VAC setting. For installation with 100 VAC power, the pump input voltage must be changed. For installation with 220 or 240 VAC power, the voltage and fuses must be changed on each component.

Installing the Injector and Placing The Modules

1. Bolt the Valve System Organizer to the right or left side of the HPLC pump. Use the side that is most convenient for the operator. Refer to Valve System Organizer manual for specific instructions.
2. Set the RI monitor next to the pump and set the column heater next to the pump. See pump and detector manuals for specific instructions.

Preparing Modules for Power-Up

Attach the main power cords to the backs of the pump, column heater, and detector, and plug them into a suitable power source. Each unit may be powered up by switching on its power switch. However, while making solvent tubing connections for the system, leave the power switches in the off position. After the stainless steel tubing connections have been swaged together, follow the procedures for priming and purging the solvent system.

Connecting the Solvent Path Tubing

1. Place small diameter plastic waste tubing on the outlet side of sample and reference cells on the RI detector (only one tubing from UV monitor) to a waste receptacle. Stainless steel tubing or plastic tubing ID 0.010" or larger may be used.

Where solvent outgassing (bubble spikes on the detector) is a problem with the solvent system, 12" long ID 0.010" tubing should be used with the waste receptacle for the tubing set above the pump heads to reduce the outgassing of small air bubbles from pure water solvents.

2. Place the pump solvent inlet line, with inlet filter attached, into a reservoir containing filtered and degassed HPLC water.
3. Use the draw-off valve on the front of the pump to slowly fill the inlet line and prime the pump (see pump manual for specifics).
4. When the solvent reservoir is placed above the pump heads, the eluant should flow from the connections, removing air bubbles that may be trapped in the pump. If air has become trapped in the system, it may be necessary to loosen each outlet check valve and the connection from the outlet T on the pump to prime the pump and clear the air. If no liquid appears, loosen the inlet tubing connections and the inlet check valves. Re-tighten all check valves and connections after air has been removed and the pump is properly primed with solvent.
5. Connect the stainless steel tubing shipped with and already attached to the pump transducer to number 2 port on the injector
6. Using the 0.010" tubing, plumb the number 3 port of the injector to the HPLC column. For shorter HPLC columns use tubing lengths that provide the shortest route for the column

flow path to the detector. Disconnect the fitting on the inlet side of the HPLC column so that air may be purged from system tubing before establishing solvent flow to the column.

7. At this point, power up the pump. The pump's Stop Flow LEDs should now be lit, and the flow rate should be displayed at a value of 0 ml/min.
8. On the pump, push Δ and RAPID together until the display reads 5.0 ml/min. Press RUN. The Stop Flow LEDs should turn off, and after a few seconds, the Pumping LEDs should light continuously. Let the pump run for 2 to 3 minutes. A steady stream of liquid should be flowing from the stainless steel tubing. Direct the stream into a waste container. If there is not a steady stream, try re-priming the pumps, or loosening the outlet check valves until no air bubbles appear. Dirty inlet filters will restrict fluid flow, and cause cavitation (air bubbles forming in the pump head).
9. Reduce the flow to 0.1 ml/min and reconnect tubing to the HPLC guard holder with the cartridge in place. Plumb the guard holder outside the column compartment. Using 3" PEEK tubing swage a connector piece from the guard cartridge to the Aminex HPLC column.
10. Before connecting the tubing to the guard cartridges and HPLC column, purge the system with water, then purge the system with the correct solvent for the column.
11. Connect the guard cartridge and column and start solvent flow at 0.1 or 0.2 ml/min (for analytical columns) before adjusting column heater temperature above 50 °C. After temperature reaches the adjusted temperature (allow 30 minutes or longer for higher temperatures), the flow rate may be adjusted to operating levels.

Connecting the Tubing to the Detector

1. Before operating the column, connect outlet side of column to detector. Using the plastic nut-ferrule combinations packed with the detector, insert solvent tubing into inlet side of sample port. Run sample-out tubing to waste receptacle.
2. On reference inlet, connect syringe (or purge line from injector) to reference inlet and connect plastic tubing to the outlet side of the reference cell. Fill the reference cell with operating buffer according to directions for the detector. When the prime-purge outflow from the manual injector is plumbed to the inlet reference cell, the RI detector reference cell can be purged automatically. Open the prime-purge valve, slowly, one full turn counterclockwise and increase the flow rate from the pump to 1 ml/min for 2 to 3 minutes. Reduce the flow rate to 0.1 ml/min and close the prime-purge valve (clockwise). The reference cell is now filled with fresh mobile phase.

Preparing Analyzer for Samples

1. Clear and prime injector by drawing off 1 ml of column eluant from the pump priming assembly with a syringe and inject the eluant slowly into the injector port (in the load position) through the 22 gauge needle with luer hub (DDI water may be used for this step). Follow this with 1 ml of air.
2. Switch the injector to the inject position and repeat rinsing with 1 ml eluant or DDI water and 1 ml air. When finished rinsing the injector, switch injector back to the load position.

Connecting Integrator or Strip Chart Recorder

Connect recording device to integrator or recorder connections on detector according to type of recorder used. See individual manuals for each of these devices. Generally a strip chart recorder uses the recorder output on the detector and integrators use the 1 volt Integrator output connections.

Connecting Automatic Start Signal Cable

Set up the HP 3395A integrator or chart recorder to start or plot results. Use detector cable connection recommended by recording device. The start cable (HP 3395A General Purpose Cable) can be connected to start input on the integrator from injector contact leads on Valve System Organizer so that the integrator does not have to be started manually.

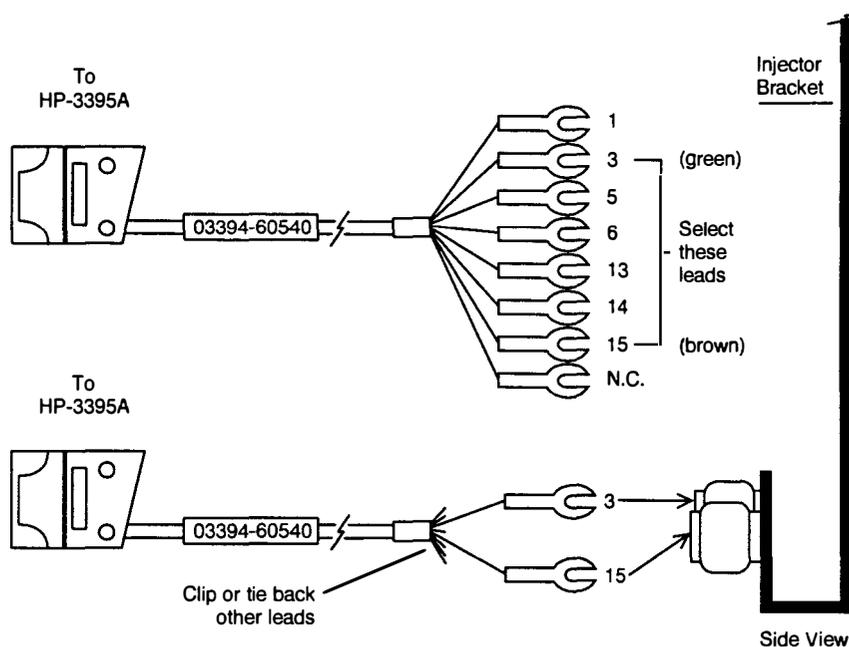


Fig. 2.1. Cable Connections.

Operating the HPLC Column

See Bio-Rad's applications manuals for operation of Aminex columns (bulletins 1312, 1157, 1147, or 1833) or Bio-Sil SEC columns (Bulletins 1833 or 1737).

2.2 Test Procedure For Basic GlycoChrom Analyzer

This section describes the test procedure for the Basic GlycoChrom Analyzer using an RI monitor. The test procedure checks that the system modules are operating correctly as a unit.

Eluant Preparation For Cation H⁺ Cartridges, HPX-87H Columns, And Fermentation Monitoring Columns

1. Prepare a 1 M H₂SO₄ stock solution by carefully diluting 56 ml of concentrated reagent grade sulfuric acid, 18 M, with distilled, deionized water (use HPLC grade water) to make a final volume of 1 liter in a volumetric flask (use recommended safety procedures for diluting concentrated acids). Filter the stock solution through a 0.45 μm membrane filter.
2. Prepare a 0.004 M H₂SO₄ eluant by diluting 4 ml of the 1 M H₂SO₄ stock solution to a 1 liter final volume with distilled, deionized water. Filter the eluant through a 0.45 μm membrane filter and degas prior to use.
3. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. The vacuum flask can also be used as the eluant reservoir. Use of a stir bar and a magnetic stir plate will improve degassing.

Eluant Preparation for Carbo C Cartridges and HPX-87C Columns

1. Solvent for HPX-87C and HPX-87P column is filtered and degassed DDI water. Prepare DDI (HPLC grade) water by filtering water through 0.45 μm membrane filter prior to use.
2. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. The vacuum flask can also be used as the eluant reservoir. Use of a stir bar and a magnetic stir plate will improve degassing.

Sample Preparation

Weigh out approximately 100 mg of reagent grade glucose. Make up a 5 ± 1 mg/ml solution of sugar by dissolving the sugar into 20 ml DDI water.

Column Preparation

Place a guard cartridge into the guard holder. (See Micro-Guard[®] cartridge installation instructions.) Connect the outlet side of the holder to the detector, but as yet **do not connect the inlet tubing** from the injector to the guard holder.

Equilibration

The analyzer flow path must be primed with properly prepared eluant and equilibrated before the analyzer can be tested for performance.

1. Place the inlet filter and tubing into the eluant reservoir. Pull 5 ml of eluant through the inlet tubing draw-off valve with a syringe to remove any air present in the tubing.
2. Increase the flow to 1 ml/min and allow the sulfuric acid eluant (or water eluant in the case of Carbo C cartridge) to travel through the system for several minutes (the tubing is not yet connected to the guard holder).
3. Reduce the pump flow to 0.1 ml/min and drip the solvent into the inlet side of the guard holder. After solvent fills the inlet, connect the nut/ferrule to the holder and tighten (needs two 1/4" wrenches for stainless steel nuts but only one 1/4" wrench for plastic finger-tight nut/ferrule combinations). Place cartridge holder in the column heater, set the temperature to 50 °C for Cation H+ cartridge or 60 °C for Carbo C cartridge, and turn on the column heater. (During normal operations with analytical column in place, the holder is placed outside the column heater.)

Note: Do not operate the guard holder above 60 °C. During normal carbohydrate column operations, the column is heated to 85 °C, but the holder is left outside the column heater.

4. When the prime-purge outflow from the manual injector is plumbed to the inlet reference cell, the RI detector reference cell can be purged automatically. Open the prime-purge valve, slowly, one full turn counterclockwise, and increase the flow rate to 1 ml/min for several minutes. Reduce the flow rate to 0.1 ml/min and close the prime-purge valve (clockwise). The reference cell is now filled with fresh mobile phase. Where a syringe is used to purge the reference cell, swage a nut-ferrule onto the needle or place a finger-tight nut ferrule onto the 1/16" OD purge needle (see RI detector accessory kit). Connect the purge syringe to the inlet side of the reference cell. Push 2 or 3 ml of mobile phase solvent through the needle. The detector is now ready to have solvent flowing through the sample cell.
5. Slowly increase the flow rate to 0.6 ml/min. Allow the test cartridge to equilibrate to temperature and flow rate for 15–20 minutes.

6. Zero the detector balance (using the zeroing knob and then the auto-zero button. See detector instructions for details).
7. After equilibration to the proper temperature and flow rate, load the 20 μ l loop of the injector with the sugar standard using a 1 ml sample syringe. Smoothly and rapidly switch to the inject position. The integrator will automatically begin if the microswitch is connected. If the microswitch is not connected, simultaneously push start button on integrator .
8. The chromatogram will start to show a peak after approximately 2 minutes. The apex of the peak will elute at approximately 3 minutes. After completion of the chromatogram, check for smoothness of baseline and symmetry of peak. See Figure 2.2.

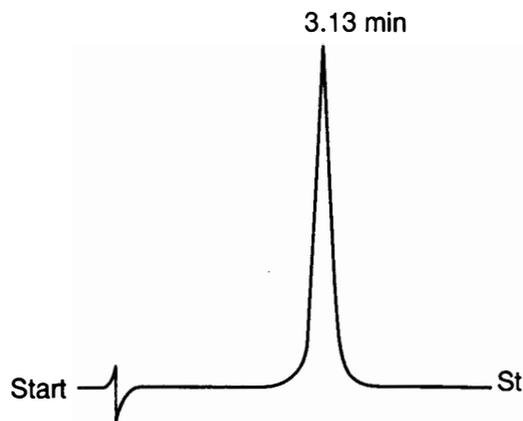


Fig. 2.2. Representative chromatogram.

9. Before injecting another sample and with the valve in the inject position, slowly flush the injector port with 1 ml of eluant, followed by 1 ml of air. Turn the injector back to the load position and repeat the solvent purging of the load port.
10. If the peak has a decidedly square top, increase the attenuation number by one or two factors and inject sample again. (See Figure 2.3.) The squared peak indicates sample overload or too sensitive attenuation of the recorder or integrator. Diluting the sample 1:1 will have the same effect and increasing attenuation settings. Repeat adjustments until peak is approximately 2/3 height of the recorder paper.

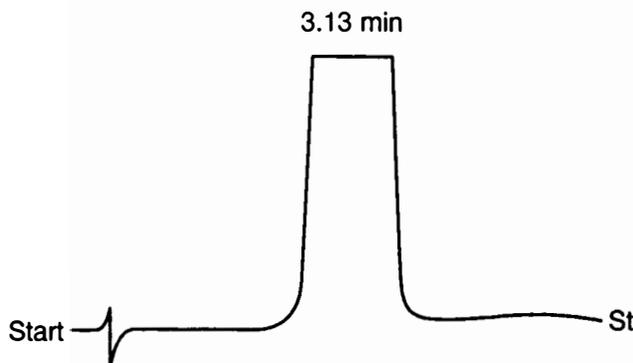


Fig. 2.3. Chromatogram indicating sample overload or too sensitive attenuation.

Section 3

Basic SEC Analyzer, catalog number 125-0591

3.1. Analyzer Installation

The Basic SEC Analyzer consists of a titanium injector with PTFE and titanium tubing. It is intended to be used with Bio-Sil® or Bio-Silect® SEC columns. The analyzer includes components for solvent delivery, sample injection, and UV detection in separate modules. The components of the basic SEC analyzer are:

Accessories—SEC Column, and Instructions, catalog number 125-1538
Model 1350 Soft Start pump, catalog number 125-1350 or 125-1352
Model 1800 UV Monitor, catalog number 167-0960
Valve System Organizer Assembly, (manual titanium injector),
catalog number 125-0325
Protein System Fittings Kit, catalog number 125-0018

Carefully remove the units from their shipping crate. Inspect the serial number on the back of each unit to insure that it corresponds to the number on the inspection certificate. A relatively level space 72 inches (1.8 m) wide and 30 inches (0.76 m) deep is recommended for set-up, although smaller spaces can be used. Three outlets are required (or one power strip) supplying AC power. For the best performance, select a space relatively free of drafts (such as drafts due to an air conditioning or heater vent) and out of direct sunlight. Allow at least 1" on each side for proper ventilation. If any components are missing, contact Bio-Rad Laboratories Customer Service Department immediately.

Caution: The analyzers are shipped from the factory in the 120 VAC setting. For installation with 100 VAC power, the pump input voltage must be changed. For installation with 220 or 240 VAC power, the voltage and the fuses must be changed on each component.

Installing the Injector and Placing the Modules

1. Bolt the Valve System Organizer to the right or left side of the HPLC pump. Use the side that is most convenient for the operator. Refer to Valve System Organizer instruction manual for specific instructions.
2. Set the UV monitor next to the pump. See pump and detector manuals for specific instructions.

Preparing Modules For Power-Up

1. Attach the individual main power cords to the backs of the pump and detector and plug them into a suitable power source. Each unit may be powered up by switching on its power switch. However, while making solvent tubing connections for the system, leave the power switches in the off position. After the titanium tubing connections have been swaged together, follow the procedures for priming and purging the solvent system.

Connecting the Solvent Path Tubing

1. Place small diameter plastic waste tubing on the outlet side of sample cell to a waste receptacle. Plastic tubing ID 0.010" or larger may be used.

Where solvent outgassing (bubble spikes) is a problem with the solvent buffer system, a 12" long ID 0.010" tubing may be used with the waste receptacle for the tubing set above the pump heads to reduce the outgassing of small air bubbles into detector.

2. Place the pump solvent inlet line, with inlet filter attached, into a reservoir containing filtered and degassed buffer.
3. Use the draw-off valve on the front of the pump to slowly fill the inlet line and prime the pump (see pump manual for specifics).
4. When the solvent reservoir is placed above the pump heads, the eluant should flow from the connections, removing air bubbles that may be trapped in the pump. If air has become trapped in the system, it may be necessary to loosen each outlet check valve and the connection from the outlet T on the pump to prime the pump and clear the air. If no liquid appears, loosen the inlet tubing connections and the inlet check valves. Re-tighten all check valves and connections after air has been removed and the pump is properly primed with solvent.
5. Connect the existing titanium tubing from the pump transducer to number 2 port on the injector.
6. Using the 0.010" tubing, plumb the number 3 port of the injector to the HPLC column. Disconnect the fitting on the inlet side of the HPLC column so that air may be purged from system tubing.
7. At this point, the pump should be on. The pump's Stop Flow LEDs should now be lit, and the flow rate should be displayed at a value of 0 ml/min.
8. On the pump, push Δ and RAPID together until the display reads 5.0 ml/min. Press RUN. The Stop Flow LEDs should turn off, and after a few seconds, the Pumping LEDs should light continuously. Let the pump run for 2 to 3 minutes. A steady stream of liquid should be flowing from the titanium tubing. Direct the stream into a waste container. If there is not a steady stream, try re-priming the pumps, or loosening the outlet check valves until no air bubbles appear. Dirty inlet filters will restrict fluid flow, and cause cavitation (air bubbles forming in the pump head).
9. Reduce the flow to 0.1 ml/min and connect tubing to SEC guard column. Using 3" tubing swage a connector piece to be used to connect the guard column with the SEC analytical column.
10. Before connecting the tubing to the guard and HPLC column, purge the system with the correct buffer for the SEC column.
11. Connect the guard column and analytical column and start solvent flow at 0.1 or 0.2 ml/min. Then the flow rate may be adjusted to operating levels.

Connecting Tubing to Detector

Before operating the column, connect outlet side of column to detector sample inlet port using 0.010" ID tubing. Run sample-out tubing to waste receptacle.

Set Up the Integrator or Chart Recorder

Set up integrator or chart recorder to start or plot results. Use detector cable connection recommended by recording device. The start cable (HP 3395A General Purpose Cable) can be connected to start input on the integrator from injector contact leads on Valve System Organizer so that the integrator does not have to be started manually. (See Step 7 of previous section for details and drawings.)

Preparing Injector for Samples

1. Clear and prime injector by drawing off 1 ml of column eluant from the pump priming assembly with a syringe and inject slowly into the injector port (in the load position) through the 22 gauge needle with luer hub. Follow this with 1 ml of air.
2. Switch the injector to the inject position and repeat rinsing with 1 ml of eluant and 1 ml air. When finished rinsing the injector, turn injector handle back to the load position.

Operating the HPLC Column

See Bio-Rad's applications manuals for operation of Bio-Sil and Bio-Silect SEC columns.

3.2 Test Procedure for Basic SEC Analyzer

This section describes the test procedure for the Basic GlycoChrom SEC analyzer with titanium and PTFE solvent tubing using a UV monitor. The test procedure checks that the system modules are operating correctly as a unit.

Eluant Preparation for Bio-Sil and Bio-Silect SEC Columns

1. Prepare a sodium phosphate buffer solution with distilled, deionized water (use HPLC grade water) to make a final volume of 1 liter. See Bio-Sil or Bio-Silect SEC column instruction manual, packed with the SEC column. Filter solution through a 0.45 μm membrane filter.
2. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. The vacuum flask can also be used as the eluant reservoir. Use of a stir bar and a magnetic stir plate will improve degassing.

Test Sample Preparation

Make up protein standard according to instruction with SEC column. Dissolve completely and set aside.

Column Equilibration

The analyzer flow path must be primed with properly prepared eluant and equilibrated before the analyzer can be tested for performance.

1. Place the inlet filter and tubing into the eluant reservoir. Pull 5 ml of eluant through the inlet tubing draw-off valve with a syringe to remove any air present in the tubing.
2. Increase the flow to 1 ml/min and allow the buffer to travel through the system for several minutes (the tubing is not yet connected to the guard column).
3. Reduce the pump flow to 0.1 ml/min and drip the solvent into the inlet side of the guard inlet (or column inlet side, if guard is not used). After solvent fills the inlet, connect the nut/ferrule to the holder and tighten (needs 1/4" wrenches for stainless steel nuts but plastic finger-tight nut/ferrule combinations can be used).
4. **Note: the UV monitor has electronic referencing and does not use a solvent reference system.** Open the prime-purge valve on the injector one full turn counterclockwise and increase the flow rate to 1 ml/min for several minutes. This will help purge the solvent flow path of any residual air bubbles. Reduce the flow rate to 0.1 ml/min and close the prime-purge valve (clockwise). The detector is now ready to have solvent flowing through the sample cell.
5. Slowly increase the flow rate to operating flow rate for the column. (For standard analytical size SEC columns, 1 ml/min is the recommended flow rate.) Allow the guard col-

umn (if plumbed into system) and analytical column to equilibrate for 15 to 20 minutes. Refer to instructions for the column.

6. Zero the detector balance using the zeroing knob.

Standard Injection

1. After equilibration to the proper flow rate, load 20 μ l of protein standard into the injector with a 1 ml sample syringe. Smoothly and rapidly, switch to the inject position. Integrator will automatically begin if micro switch is connected to integrator start signal, or simultaneously push start button on integrator if micro switch is not connected.
2. Chromatogram will start to show a peak after approximately 5 minutes. The apex of the first peak will arrive at approximately 6 minutes. After completing the chromatogram, check for smoothness of baseline and symmetry of peaks. If the buffer recommended in column instruction manual is used, the chromatogram should approximate the test chromatogram for the SEC column.

Section 4 GlycoChrom Automated Analyzer, Catalog Number 125-0592, And GlycoChrom Automated SEC Analyzer, Catalog Number 125-0593

4.1 Analyzer Installation

The analyzers include components for solvent delivery, sample injection, and refractive index detection (or UV detection in the case of SEC system) in separate modules.

The components of the automated analyzer, to be used with Aminex HPLC columns, are:

Accessories—Column Kit, and Instructions, catalog number 125-1536
Model 1350 Soft Start Pump, catalog number 125-1350
Model 1755 RI Monitor, catalog number 167-0458
AS-96C Autosampler, catalog number 167-1165
Stacking Partition with Bottles, catalog number 125-0599
Guard Holder, catalog number 125-0131
HPLC Fittings Kit, catalog number 125-0010

The components of the automated SEC analyzer, to be used with Bio-Sil SEC and Bio-Silect SEC columns are:

Accessories—SEC Column, and Instructions, catalog number 125-1538
Model 1350 Soft Start Pump, catalog number 125-1352
Model 1800 UV Monitor, catalog number 167-0960
AS-96CP Biocompatible Automatic Sampler
Stacking Partition with Bottles, catalog number 125-0599
Protein System Fittings Kit, catalog number 125-0018

Carefully remove the units from their shipping containers. Inspect the serial number found on the back of each unit to insure that it corresponds to the number found on the inspection certificate. A relatively level space 72 inches (1.8 m) wide and 30 inches (0.76 m) deep is recommended for installation, although smaller spaces can be used. Three outlets are required (or one power strip) supplying AC power. For the best performance, select a space free of drafts (such as drafts due to an air conditioning or heater vent) and out of direct sunlight. Allow at least 1" on each side for proper ventilation. If any components are missing, contact Bio-Rad Laboratories Customer Service Department immediately.

Caution: The analyzer modules are shipped from the factory in the 120 VAC setting. For installation with 100 VAC power, the pump input voltage must be changed. For installation with 220 or 240 VAC power, the voltage and fuses must be changed on each component.

Installing the AS-96C Automatic Sampler and placing the modules

1. Place the pump and detector side by side facing the front edge of the bench.
2. Place the stacking partition (thin beige metal organizer with solvent bottle holders) on the pump and detector so that the solvent bottle holders are on the right side. The area behind the detector and next to the pump will not have support but the partition will remain level under these conditions. The partition will fit over the top of the pump and RI monitor but the UV monitor will protrude beyond the partition.
3. Set the automatic sampler on top of and even with the front of the pump and detector. Route the solvent tubing from the pump's transducer underneath the automatic sampler, above the partition, and up the back of the automatic sampler so that it may be plumbed into the injector. (See automatic sampler instructions for connections.)

Preparing Modules For Power-Up

Attach the power cords to the backs of the pump, automatic sampler, and detector, and plug them into a suitable power source. Each unit may be powered up by switching on its power switch. However, while making solvent tubing connections for the system, leave the power switches in the off position. After the stainless steel tubing connections have been swaged together, follow the procedures for priming and purging the solvent system.

Connecting The Solvent Path Tubing

1. Place small diameter plastic waste tubing on the outlet side of sample and reference cells on the RI detector (only one tubing from UV monitor) to a waste receptacle. Yellow coded stainless steel tubing or plastic tubing ID 0.010" or larger may be used.

Where solvent outgassing (bubble spikes) is a problem with the solvent system, a 12" long ID 0.010" tubing may be used with the waste receptacle for the tubing set above the pump heads to reduce the outgassing of small air bubbles from pure water solvents.

2. Place the plastic pump solvent inlet line, with inlet filter attached, into plastic reservoir containing filtered and degassed HPLC water. Place the solvent bottle in its holder on the stacking partition (organizer divider).
3. Use the draw-off valve on the front of the pump to slowly fill the inlet line and prime the pump (see pump manual for specifics).
4. When the solvent reservoir is placed above the pump heads (*i.e.*, in its holder), the eluant should flow from the loosened connections, removing air bubbles that may be trapped in the pump. If air has become trapped in the system, it may be necessary to loosen each outlet check valve and open the connection from the outlet transducer on the pump to prime the pump and clear the air bubbles from the system. If no liquid appears, loosen the inlet tubing connections and the inlet check valves. The tubing should fill with solvent from the eluant reservoir. Re-tighten all check valves and connections after air has been removed and the pump is properly primed with solvent.
5. Connect the existing stainless steel tubing (titanium tubing on SEC system) from pump transducer to number 6 port on the injector (see section number 4.2. of AS-96C Automatic Sampler instructions).

6. Using the 0.010" PEEK tubing, plumb the number 5 port of the injector to the HPLC column using stainless steel or titanium fittings for 30 cm long columns (both Aminex and SEC columns). Use shortest tubing lengths that fit the size of the column in the column heater. If shorter columns are to be used, any suitable combination of 0.010" tubing and connectors is acceptable. **Disconnect the fitting on the inlet side of the HPLC column so that air may be purged from system tubing.**
7. At this point, power up the pump. The pump's Stop Flow LEDs should now be lit, and the flow rate should be displayed at a value of 0 ml/min.
8. On the pump, push Δ and RAPID together until the display reads 5.0 ml/min. Press RUN. The Stop Flow LEDs should turn off, and after a few seconds, the Pumping LEDs should light continuously. Let the pump run for 2 to 3 minutes. A steady stream of liquid should be flowing from the tubing. Direct the stream into a waste container. If there is not a steady stream, try re-priming the pumps, or loosening the outlet check valves until no air bubbles appear. Dirty inlet filters will restrict fluid flow, and cause cavitation (air bubbles forming in the pump head).
9. Reduce the flow to 0.1 ml/min and connect tubing to HPLC guard holder with cartridge in place. (In the SEC system, either an SEC guard column or SEC analytical column will be used.) Plumb the guard outside the column compartment. Using 2 to 3 inches of PEEK tubing swage a connector piece to be used to connect guard column with the SEC analytical column.
10. Before connecting the tubing to the guards and HPLC column, purge the system with water to clear out packing solvent. Then purge the system with correct solvent for the column.
11. Connect the guard holder and column and start solvent flow at 0.1 or 0.2 ml/min before adjusting column heater temperature above 50 °C. (For SEC system, use SEC guard column or SEC analytical column). After the temperature arrives at adjusted temperature (allow 30 minutes or longer for higher temperatures), the flow rate may be adjusted to operating levels. (In the case of SEC columns, the temperature can be set between 25 and 30 °C if the column is installed in the heater compartment.)

Connecting Tubing to Detector

1. Before operating the analytical column, connect outlet side of column to detector using PEEK tubing with metal nut ferrule combination on the outlet side of the column. Using the finger-tight nut-ferrule combinations packed with the detector, insert tubing into inlet side of sample port. Run sample-out tubing to waste receptacle.
2. For RI monitor reference inlet (RI monitor uses reference cell), connect syringe to reference inlet using needle and syringe packed with the RI monitor. Using the syringe needle and nut/ferrule combination, install the needle in the inlet reference port. Connect a drain line to the outlet side of reference cell using hubless needle, plastic nut/ferrules and plastic tubing packed with RI monitor. Using the syringe without the needle in place, draw off several ml of solvent from the solvent draw-off valve on the pump (see pump instructions if necessary). Install the nut/ferrule combination onto the syringe and fill the reference cell with operating solvent. Operating solvent must remain in contact with reference cell for detector to operate correctly.
3. The UV monitor references the sample electronically, so no reference cell is used. In the case of the UV monitor plumb sample in and sample out ports with either finger-tight or metal nut/ferrule combinations.

Preparing Automatic Sampler for Samples

See section 4.3 in AS-96C Automatic Sampler manual for injector pre-flush and sample loading procedures.

Connecting Recording Device

Connect recording device to detector's integrator or recorder leads according to type of recorder used. See individual manuals for each of these connections. Generally a strip chart recorder is connected to the recorder output on a detector and an integrator is connected to the integrator output on that same detector.

Installing HP-3395A Integrator as Data System for Glycochrom Automated Analyzer

The solvent delivery system, detector, and AS-96C Automatic Sampler can be individually programmed for automated operation. If the data collection system for the analyzer is an HP 3395A integrator purchased from Bio-Rad or a third-party integrator, the integrator can be connected to the automatic sampler for unattended operation through the P1 connector strip on the back of the automatic sampler to allow inject signal input via TTL connector. The HP integrator has a general purpose cable equipped with spade lugs to make the necessary connection with the AS-96C.

1. Using cable number 03394-60540, the general purpose cable packed with the HP 3395A integrator, select spade lugs number 3 and number 15 to connect to P1 connector strip on AS-96C at number 8 and number 15 connections by modifying the spade lugs on the integrator cable.
2. Using a pair of wire cutters, clip the spade lugs for wire number 3 and wire number 15 at the shank of the spade lugs. The resultant cylindrical connector will then fit into the P1 connector for the autosampler.

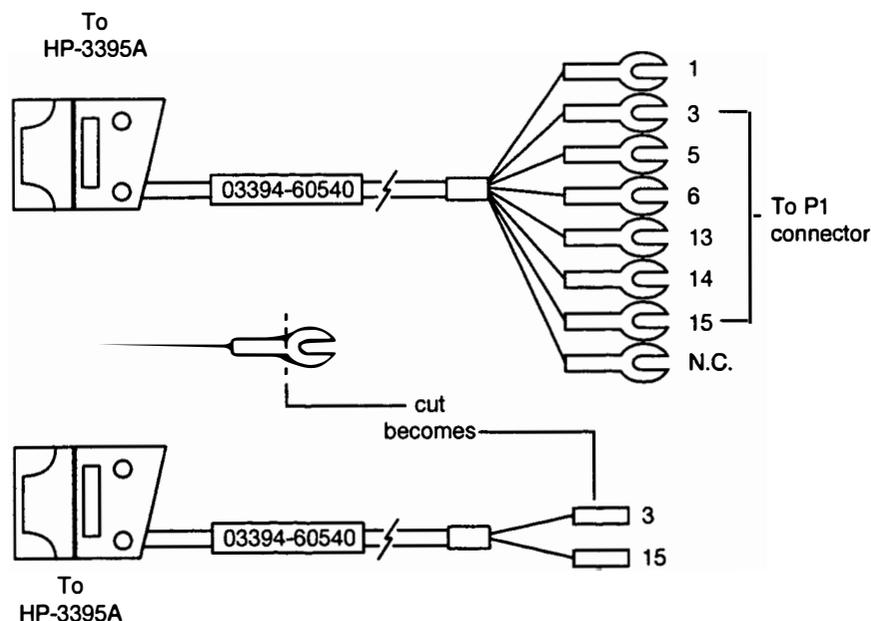


Fig. 4.1. Integrator Installation.

3. Plug integrator cable wire number 3 (green coded) into number 8 port of connector and plug cable wire number 15 (brown coded) into port number 15 of connector. Plug connector into autosampler as described by AS-96C manual. See section 4.2 for control I/O in AS-96C manual for details.

Programming AS-96C Automatic Sampler

The AS-96C Automatic Sampler may be programmed from its front panel according to instructions found in Chapter 5 for AS-96C Automatic Sampler. With integrator in ready mode, the automatic sampler will give the integrator the inject signal to start the analysis.

Operating the HPLC Column

1. See Bio-Rad's applications manuals for operation of Aminex columns or Bio-Sil SEC columns.
2. See Bio-Rad's SEC instruction manual and Bulletin 1737 for SEC column operation.

4.2 Test Procedure for Glycochrom Automated Analyzer or Automated SEC Analyzer

This section describes the test procedure for the Automated GlycoChrom Analyzer using the RI monitor or UV monitor. The test procedure checks that the system modules are operating correctly as a unit.

Eluant Preparation for Cation H⁺ Cartridges and HPX-87H Columns and Fermentation Monitoring Columns

1. Prepare a 1 M H₂SO₄ stock solution by diluting 56 ml of concentrated reagent grade sulfuric acid, 18 M, with distilled, deionized water (use HPLC grade water) to make a final volume of 1 liter in a volumetric flask. Filter the stock solution through a 0.45 µm membrane filter.
2. Prepare a 0.004 M H₂SO₄ eluant by diluting 4 ml of the 1 M H₂SO₄ stock solution to a 1 liter final volume with distilled, deionized water. Filter the eluant through a 0.45 µm membrane filter and degas prior to use.
3. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. Use of a stir bar and a magnetic stir plate will improve degassing.

Eluant Preparation for Carbo C Cartridges and HPX-87C Columns

1. Solvent for HPX-87C and HPX-87P columns is DDI water. Prepare DDI (HPLC grade) water by filtering water through 0.45 µm membrane filter prior to use.
2. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. Use of a stir bar and a magnetic stir plate will improve degassing.

Eluant Preparation for Bio-Sil and Bio-Silect SEC Columns

See Section 2.1 and 2.2 of Bio-Sil and Bio-Silect SEC instructions to set up and operate SEC columns from Bio-Rad. The SEC column should be connected according to SEC instructions and in the case of the AS-96C and AS-96CP instructions, use port number 6 on the automatic sampler to connect to the inlet side of the column using PEEK 0.010" ID tubing. The column may be plumbed in external to the column oven or using stainless steel or titanium nut/ferrules to swage onto PEEK tubing, the column can be installed in the heating compart-

ment of the automatic sampler. When salt buffers are used with the automatic sampler, be sure to wash the system free of buffers by flushing with DDI water after runs are completed.

Sample Preparation for Aminex Columns, Cartridges, and SEC Columns

If the analytical column is to be tested, make up a glucose solution according to Basic GlycoChrom system instructions. In the case of the Automated SEC system, make up the protein standard packed with the SEC column. Follow the test procedure by making up the buffer solution suggested in the instructions for the respective analytical column.

Column Preparation

Place Aminex or SEC column in-line between the injector and detector. (See Micro-Guard cartridge instructions for cartridge installation.) If a guard system is to be plumbed into the system, connect the outlet side of the holder or guard column to the analytical column, but as yet do not connect the inlet tubing from the injector to the column(s).

Equilibration

The analyzer flow path must be primed with properly prepared eluant and equilibrated before the analyzer can be tested for performance.

1. Place the inlet filter and tubing into the eluant reservoir. Pull 5 ml of eluant through the inlet tubing draw-off valve with a syringe to remove any air present in the tubing.
2. Increase the flow to 1 ml/min and allow the eluant to travel through the system for several minutes. (The tubing is not yet connected to the columns.)
3. Reduce the pump flow to 0.1 ml/min and drip the solvent into the inlet side of the guard holder or column if guard is not plumbed into the system. After solvent fills the inlet, connect the nut/ferrule to the holder and tighten. Place column in the column heater, and set the temperature to appropriate temperature for the column. (During normal operations the guard holder is plumbed outside the column heater. SEC columns can be plumbed outside the heater compartment.)

Note: Do not operate the guard holder above 60 °C. During normal carbohydrate column operations, the column is heated to 85 °C, but the holder is left outside the column heater.

4. Following instructions in AS-96C Automatic Sampler instruction manual found in Section 4.3, set-up and test the system.
5. Slowly increase the flow rate to 0.6 ml/min (1 ml/min for SEC columns). Allow the test column or column/cartridge combination to equilibrate to temperature and flow rate for 15-20 minutes.
6. Zero the detector balance (using the zeroing knob and/or auto-zero button).

Set Up the Integrator or Chart Recorder

Set up your integrator or chart recorder to start or plot results. Use detector cable connection recommended by recording device. The signal/start cable can be connected to start input on integrator from automatic sampler so that the integrator does not have to be started manually. (See automatic sampler manual for specific instructions.)

Standard Injection

1. After equilibration to the proper temperature and flow rate, operate the automatic sampler according to instructions in sections 5.4, 5.5, and 5.6 of AS-96C manual.

2. Chromatogram will start to show a peak after time shown on test chromatogram packed with column. After completion of the chromatogram, check for smoothness of baseline and symmetry of peak. See Figure 4.2 for Cation H⁺ cartridges using glucose as test sample.

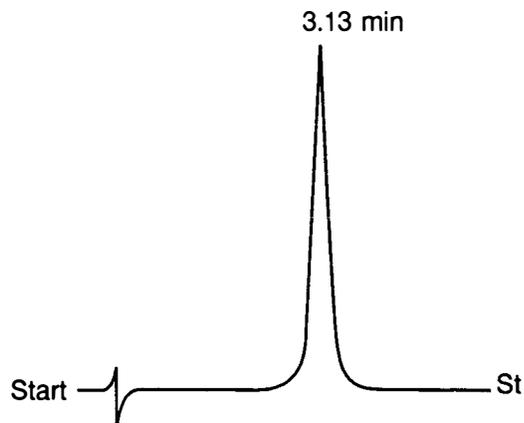


Fig. 4.2. Glucose test sample analysis on Cation H⁺ cartridge.

3. If the peak has a decidedly square top, increase the attenuation number on the integrator by one or two factors and inject sample again. (See Figure 4.3.) Repeat adjustments until peak is approximately 2/3 height of the recorder paper.

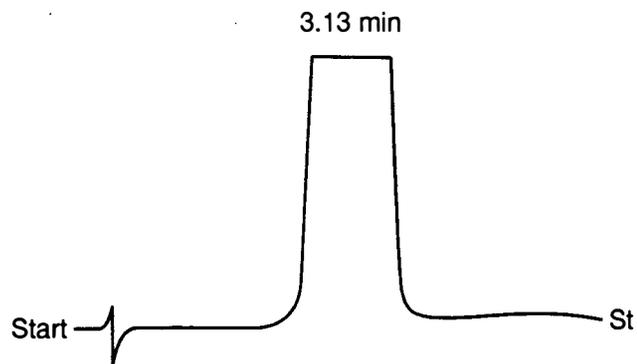


Fig. 4.3. Glucose test sample at wrong attenuation setting.

Section 5

GlycoChrom Automated Analyzer With Expert Software, Catalog Number 125-0594 and GlycoChrom Automated Analyzer With Expert Software and 486 Computer, Catalog Number 125-0595

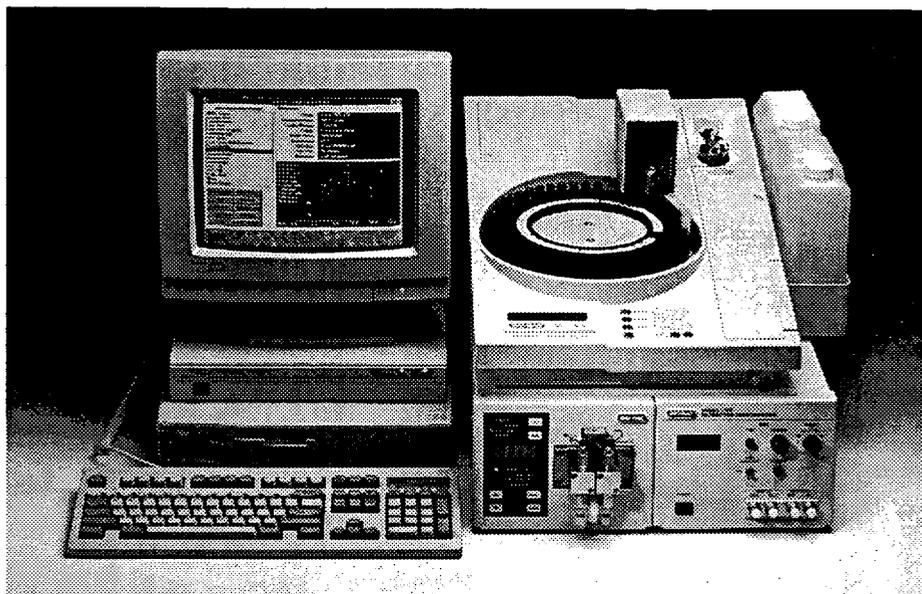


Fig. 5.1. Automated analyzer.

5.1 Analyzer Installation

The automated analyzers with Expert Software include components for solvent delivery, sample injection, and refractive index detection in separate modules. The components of the analyzers are:

Accessories- Software, Column Kit, Instructions, and SW Manual,
catalog number 125-1537

Model 1350 Soft Start Pump, catalog number 125-1350

Model 1755 RI Monitor, catalog number 167-0458

AS-96C Automatic Sampler, catalog number 167-1165

Stacking Partition with Bottles, catalog number 125-0599

Guard Holder, catalog number 125-0131

HPLC Fittings Kit, catalog number 125-0010

(486 Computer with monitor, keyboard, and mouse, included with system,
catalog number 125-0595)

Carefully remove the units from their shipping cartons. Inspect the serial number on the back of each unit to insure that it corresponds to the number on the inspection certificate. A relatively level space 72 inches (1.8 m) wide and 30 inches (0.76 m) deep is recommended for installation, although smaller spaces can be used. Six outlets are required (or one power strip) supplying AC power. For the best performance, select a space free of drafts (such as drafts due to an air conditioning or heater vent) and out of direct sunlight. Allow at least 1" on each side

for proper ventilation. If any components are missing, contact Bio-Rad Laboratories Customer Service Department immediately.

Caution: The analyzer modules are shipped from the factory in the 120 VAC setting. For installation with 100 VAC power, the pump input voltage must be changed. For installation with 220 or 240 VAC power, the voltage and fuses must be changed on each component.

Installing the AS-96C Automatic Sampler and Placing the Modules

1. Place the pump and detector side by side and facing the front edge of the bench.
2. Place the stacking partition (thin beige metal organizer with solvent bottle holders) on the pump and detector so that the solvent bottle holders are on the right side facing the front of the bench. The area behind the detector and next to the pump will not have support but the partition will remain level under these conditions. The partition will fit over the top of the pump and RI monitor.
3. Set the automatic sampler on top of and even with the front of the pump and detector. Route the solvent tubing from the pump's transducer underneath the autosampler, above the partition, and up the back of the automatic sampler so that it may be plumbed to the injector. See automatic sampler instructions for connections.

Installing the Software

See GlycoChrom software manual, catalog number 400-5002.

Preparing Modules for Power-Up

1. Attach the power cords to the backs of the pump, automatic sampler, and detector, instrument interface, and computer, and plug them into a suitable power source (a power strip is suggested). Each unit may be powered up by switching on its power switch. However, while making solvent tubing connections for the system, leave the power switches in the off position. After the stainless steel tubing connections have been swaged together, then follow the procedures for priming and purging the solvent system. Attach the communication cables for each of the modules according to the diagram below:

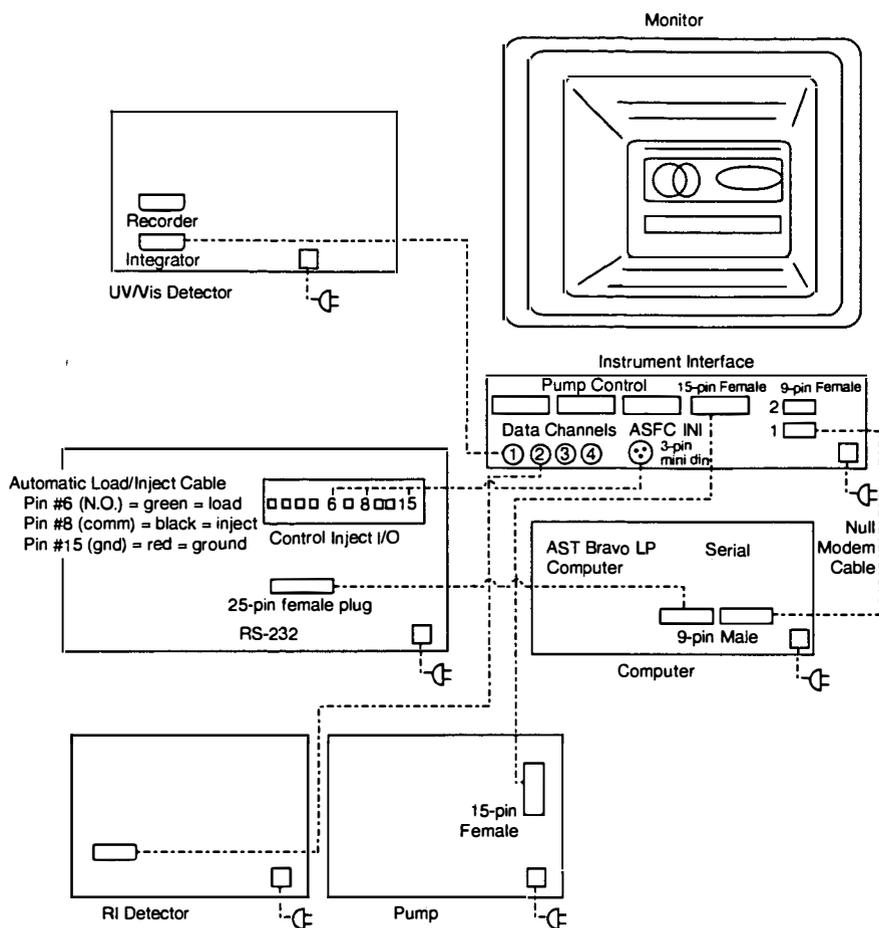


Fig. 5.2. Cable connections diagram (view from rear of system) components. Not drawn to scale.

Connecting the Solvent Path Tubing

1. Place small diameter plastic waste tubing on the outlet side of sample and reference cells on the RI detector to a waste receptacle. Yellow coded stainless steel tubing or plastic tubing ID 0.010" or larger may be used.

Where solvent outgassing (bubble spikes) is a problem with the solvent system, a 12" long ID 0.010" tubing may be used with the waste receptacle for the tubing set above the pump heads to reduce the outgassing of small air bubbles from pure water solvents.

2. Place the plastic pump solvent inlet line, with inlet filter attached, into plastic reservoir containing filtered and degassed HPLC water. Place the solvent bottle in its holder on the stacking partition (organizer divider).
3. Use the draw-off valve on the front of the pump to slowly fill the inlet line and prime the pump (see pump manual for specifics).
4. When the solvent reservoir is placed above the pump heads (*i.e.*, in its holder), the eluant should flow from the loosened connections, removing air bubbles that may be trapped in the pump. If air has become trapped in the system, it may be necessary to loosen each outlet check valve and open the connection from the outlet transducer on the pump to

prime the pump and clear the air bubbles from the system. If no liquid appears, loosen the inlet tubing connections and the inlet check valves. The tubing should fill with solvent from the eluant reservoir. Re-tighten all check valves and connections after air has been removed and the pump is properly primed with solvent.

5. Connect the existing stainless steel tubing from pump transducer to number 6 port on the injector (see section number 4.2 in AS-96C Automatic Sampler instructions).
6. Using the 0.010" PEEK tubing, plumb the number 5 port of the injector to the HPLC column using stainless steel fittings for 30 cm long columns. Use shortest tubing lengths that fit the size of the column in the column heater. If shorter columns are to be used, any suitable combination of 0.010" tubing and appropriate connectors is acceptable. Disconnect the fitting on the inlet side of the HPLC column so that air may be purged from system tubing.
7. At this point, power up the pump. The pump's Stop Flow LEDs should now be lit, and the flow rate should be displayed at a value of 0 ml/min. With all cable connections in place, including the pump cable from serial cable A on Instrument Interface to 15-pin connection on the connector above the line cord on the back of the pump, the pump control can be accessed from pump control button in the operating software. (With the with integrator pin connection on the pump disconnected, the pump may be operated from the pump's own face plate.)
8. From pump control panel set flow rate to 5.0 ml/min. Press RUN. The Stop Flow LEDs should turn off, and after a few seconds, the Pumping LEDs should light continuously. Let the pump run for 2 to 3 minutes. A steady stream of liquid should be flowing from the tubing. Direct the stream into a waste container. If there is not a steady stream, try re-priming the pumps, or loosening the outlet check valves until no air bubbles appear. Dirty inlet filters will restrict fluid flow, and cause cavitation (air bubbles forming in the pump head).
9. Reduce the flow to 0.1 ml/min and reconnect tubing to HPLC guard holder with cartridge in place.
10. Before connecting the tubing to the guard and HPLC column, purge the system with water to clear out packing solvent then purge the system with correct solvent for the column.
11. Connect the guard holder and column and start solvent flow at 0.1 or 0.2 ml/min before adjusting column heater temperature above 50 °C. Column heater controls may be accessed from AS-96C software controller under the Program pop-up menu. After temperature arrives at adjusted temperature (allow 30 minutes or longer for higher temperatures) flow rate may be adjusted to operating levels.

Connecting Tubing to Detector

1. Before operating the analytical column, connect outlet side of column to detector using PEEK tubing with metal nut ferrule combination on the outlet side of the column. Using the finger-tight nut-ferrule combinations packed with the detector, insert tubing into inlet side of sample port. Run sample-out tubing to waste receptacle.
2. For RI monitor reference inlet (RI monitor uses reference cell), connect syringe to reference inlet using the needle and syringe packed with the RI monitor. Using the syringe needle and nut/ferrule combination, install the needle in the inlet reference port. Connect a drain line to the outlet side of reference cell using hubless needle, plastic nut/ferrules and plastic tubing packed with RI monitor. Using the syringe without the needle in place, draw off several ml of solvent from the solvent draw-off valve on the pump (see pump instructions). Install the nut/ferrule combination onto the syringe and fill the reference

cell with operating solvent. Operating solvent must remain in contact with reference cell for detector to operate correctly.

Preparing Automatic Sampler for Samples

See Section 4.3 in AS-96C Automatic Sampler manual for injector pre-flush and sample loading procedures.

5.2 Test Procedure for Automated Analyzer With Software or Automated Analyzer With Software And Computer

This section describes the test procedure for the Automated GlycoChrom analyzer using the RI monitor. The test procedure checks that the system modules are operating correctly as a unit.

Eluant Preparation for Cation H⁺ Cartridges and HPX-87H Columns and Fermentation Monitoring Columns

1. Prepare a 1 M H₂SO₄ stock solution by diluting 56 ml of concentrated reagent grade sulfuric acid, 18 M, with distilled, deionized water (use HPLC grade water) to make a final volume of 1 liter in a volumetric flask. Filter the stock solution through a 0.45 µm membrane filter.
2. Prepare a 0.004 M H₂SO₄ eluant by diluting 4 ml of the 1 M H₂SO₄ stock solution to a 1 liter final volume with distilled, deionized water. Filter the eluant through a 0.45 µm membrane filter and degas prior to use.
3. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. Use of a stir bar and a magnetic stir plate will improve degassing.

Eluant Preparation for Carbo C Cartridges and HPX-87C Columns

1. Solvent for HPX-87C and HPX-87P columns is DDI water. Prepare DDI (HPLC grade) water by filtering water through 0.45 µm membrane filter prior to use.
2. To degas the eluant, use a vacuum flask and a vacuum apparatus that will pull a vacuum of at least 28" of mercury. Use of a stir bar and a magnetic stir plate will improve degassing.

Sample Preparation for Aminex Columns and Cartridges

If the analytical column is to be tested, make up a glucose solution according to Basic GlycoChrom system instructions. Follow the test procedure by making up the solvent solution suggested in the instructions for the respective analytical column.

Column Preparation

Place Aminex column in-line between the injector and detector. (See Micro-Guard Cartridge installation Instructions for cartridge installation.) If a guard system is to be plumbed into the system, connect the outlet side of the holder or guard column to the analytical column, but as yet do not connect the inlet tubing from the injector to the column(s).

Equilibration

The analyzer flow path must be primed with properly prepared eluant and equilibrated before the analyzer can be tested for performance.

1. Place the inlet filter and tubing into the eluant reservoir. Pull 5 ml of eluant through the inlet tubing draw-off valve with a syringe to remove any air present in the tubing.
2. With pump control cable disconnected from the pump, the pump flow may be controlled from the pump face. With the pump control cable installed and all other cable connections in place, the stop/start and pump flow rates are controlled from the pump control button in the software program. Increase the flow to 1 ml/min and allow the eluant to travel through the system for several minutes. (The tubing is not yet connected to the columns in-line.)
3. Reduce the pump flow to 0.1 ml/min and drip the solvent into the inlet side of the guard holder or column if guard is not plumbed into the system. After solvent fills the inlet, connect the nut/ferrule to the holder and tighten. Place column in the column heater, set the temperature to appropriate temperature for the column and turn on the column heater from the computer's AS-96C control panel. Open AS-96C "Program" and set temperature in pop-up menu. (During normal operations guard holder is plumbed outside the column heater.)

Note: Do not operate the guard holder above 60 °C. During normal carbohydrate column operations, the column is heated to 85 °C, but the holder is left outside the column heater.

4. Select the desired application from the face plate (CarboC or CationH application) and follow the directions on the face plate as to column, solvent, flow rate, temperature, etc. Place a 1 ml solution of 3% glucose in an autosampler vial and place capped vial in well number 1.
5. Set up and load autosampler vials in autosampler. On AS-96C operator plate, select new from file menu. (If a file has already been set up, then select open to view and select a previous file)
 - a. Pull down Program and select Run Parameters. Number the first vial 1, the last vial 1 and the number of injections (1–9), and set analysis time to 6 minutes, 1 minute longer than the method being employed in the application.
 - b. Pull down Program panel and select temperature control. Turn on column oven and type in correct temperature for the method. (Do not set temperature for the second oven temperature unless second oven temperature is needed.)
 - c. Pull down Control menu and send parameters (send param).
 - d. From the pump control panel, slowly increase the flow rate to 0.6 ml/min and touch the set button. Close the pump control plate. Allow the test cartridge to equilibrate to the correct temperature. (Time to equilibrate will be 15 to 60 minutes depending on set temperature of the oven. The higher the temperature, the longer to equilibrate. Zero the detector after column equilibration.
 - e. Push the Run button on the main screen (above the Pump control button) to start the method.
 - f. When Status Panel I appears and waiting for Injection shows in statement box, pull up Control menu on AS-96C panel and press Start. This will then start the method and will also start the sequence table to inject the entire series of runs specified in the sequence table. (The start button only has to be touched at the start of the run series.)
6. If necessary, zero the detector balance (using the zeroing knob and/or auto-zero button).

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