## **Installation and Operation Guide**









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Safety

Before installing, operating, or maintaining this equipment, it is imperative that all hazards and preventive measures are fully understood. While specific hazards may vary according to location and application, heed the following general warnings:

#### **⚠ WARNING**

Liquids associated with this instrument may be classified as carcinogenic, biohazard, flammable, or radioactive. Should these liquids be used, it is highly recommended that this application be accomplished in an isolated environment designed for these types of materials in accordance with federal, state, and local regulatory laws, and in compliance with your company's chemical/hygiene plan in the event of a spill.

#### **↑** WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired.

#### **⚠ WARNING**

If you are using flammable solvents or chemicals with this system, vapor concentration levels may exceed the maximum exposure levels as recommended by OSHA Guide 1910.1000. To reduce those levels to a safe exposure, Teledyne ISCO recommends that you place the system in a laboratory hood designed for the purpose of ventilation. This hood should be constructed and operated in accordance with federal state and local regulations. In the event of a solvent or chemical spill, your organization should have a plan to deal with these mishaps. In all cases, use good laboratory practices and standard safety procedures.

Hazard Severity Levels

This manual applies *Hazard Severity Levels* to the safety alerts. These three levels are described in the sample alerts below.

### **!** CAUTION

Cautions identify a potential hazard, which if not avoided, may result in minor or moderate injury. This category can also warn you of unsafe practices, or conditions that may cause property damage.

#### **⚠ WARNING**

Warnings identify a potentially hazardous condition, which if not avoided, could result in death or serious injury.

## **!** DANGER

DANGER – limited to the most extreme situations to identify an imminent hazard, which if not avoided, will result in death or serious injury.

Hazard Symbols

The equipment and this manual use symbols used to warn of hazards. The symbols are explained in the table below.

Hazard Symbols				
Warnings and Cautions				
$\triangle$	The exclamation point within the triangle is a warning sign alerting you of important instructions in the instrument's technical reference manual.			
4	The lightning flash and arrowhead within the triangle is a warning sign alerting you of "dangerous voltage" inside the product.			
Symboles de sécurité				
$\triangle$	Ce symbole signale l'existence d'instructions importantes relatives au produit dans ce manuel.			
4	Ce symbole signale la présence d'un danger d'électocution.			
Warnungen und Vorsichtshinweise				
À	Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören.			
4	Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sei vor "gefährlichen Spannungen" im Inneren des Produkts warnt.			
Advertencias y Precauciones				
$\triangle$	Esta señal le advierte sobre la importancia de las instrucciones del manual que acompañan a este producto.			
4	Esta señal alerta sobre la presencia de alto voltaje en el interior del producto.			

For Additional Information Technical assistance for the Teledyne ISCO Automation Modules can be obtained from:

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#### Section 1 Introduction

#### 1.1 Overview

The AutoInjector Module, AutoSampler Module, and Column Selector Module allow you to increase the productivity of the Combi*Flash*<sup>®</sup> EZ Prep by automating a number of tasks involved with high-performance liquid chromatography (HPLC). These modules are controlled by Teledyne ISCO's PeakTrak<sup>®</sup> software to maintain the ease-of-use you are familiar with and practically eliminate the need for training.

The three modules can be used separately or in conjunction with one another for even more automated operation.

 $AutoInjector\ Module$ 

The AutoInjector Module performs automated, repetitive compound injections completely unattended. This allows you to automatically purify larger amounts of compound than can be purified with a single separation run. Automating this tedious process increases productivity.

More information regarding the AutoInjector Module—including installation, setup, and operation—can be found in Section 4.

AutoSampler Module

The AutoSampler Module performs automated purifications on multiple samples. In addition, the module doubles the fraction collection capacity of the EZ Prep by adding two collection racks. For more flexibility, samples can consist of multiple injections. You can even choose between maximizing rack capacity by filling racks completely or by optimizing work flow on a shared system by having each sample sent to the next rack. The racks utilize the same RFID rack swapping technology as the EZ Prep, allowing you to remove completed racks and replace them with new racks for practically unlimited fraction collection capacity.

More information regarding the AutoSampler Module—including installation, setup, and operation—can be found in Section 3.

Column Selector Module

The Column Selector Module enables the EZ Prep to automatically access up to four prep columns. This allows you easy access to a variety of column sizes and chemistries. It also allows you to dedicate columns to both high and low pH separations. When combined with the AutoSampler Module, each sample can be separated on the column best suited for the job.

More information regarding the Column Selector Module—including installation, setup, and operation—can be found in Section 2.

### Section 2 The Column Selector Module

#### 2.1 Column Selector Module Installation

#### ✓ Note

Combi*Flash* EZ Prep software must be version 3.1.0 or higher to support the automation capabilities.

The Column Selector Module can be laid flat horizontally or stood on an edge vertically. If you are installing the Column Selector Module horizontally, it is recommended that you first install the columns before continuing with the Column Selector Module installation. If you are installing the module vertically, install the columns after installing the module.

## 2.1.1 Installing the Column Selector Module

Removing the housing from the EZ Prep

To install the Column Selector Module vertically, follow the steps below.

1. Remove the screw connected to the housing from inside of the EZ Prep using a #2 Phillips screwdriver (Figure 2-1).

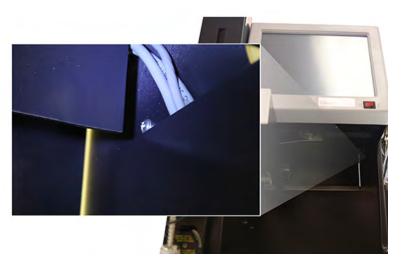


Figure 2-1 Location of the EZ Prep inner screw

2. Use a 1/4" nut driver to remove the nut from the bottom section of the housing (Figure 2-2).



Figure 2-2 Removing the bottom section housing nut

3. Use a 5/16" wrench to remove the nut located inside the EZ Prep column housing (Figure 2-3).



Figure 2-3 Removing the EZ Prep housing column inner nut

- 4. Use a screwdriver to remove the two screws from the back of the housing. At this point, the housing should come free from the EZ Prep with little effort (Figure 2-4).
- 5. Remove the mounting rail from the right side of the EZ Prep by removing the two nuts from the mounting rail using a 5/16" wrench (Figure 2-4).

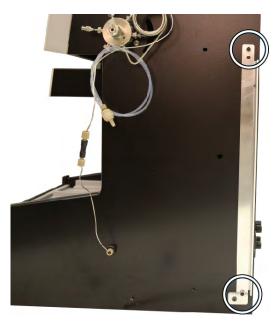


Figure 2-4 Location of the two EZ Prep mounting rail securing nuts

Mounting the Column Selector Module 1. Remove the three thumbscrews from the housing of the Column Selector Module by hand (Figure 2-5).



Figure 2-5 Location of the three Column Selector Module housing thumbscrews

2. Gently separate the housing of the Column Selector Module.

3. When the Column Selector Module is set vertically alongside the EZ Prep, two screws on the top of the module will be visible. Remove these two screws with a screwdriver (Figure 2-6).



Figure 2-6 Two screws on top of the Column Selector Modules

- 4. Remove the cover plate and set it aside.
- 5. Insert the mounting bracket into the designated slot located on top of the Column Selector Module, and secure it to the module with the attached screw (Figure 2-7).



Figure 2-7 Column Selector Module with mounting rail attachment configured

- 6. Replace the cover plate and screw it into place.
- 7. Connect the Column Selector Module mounting bracket to the EZ Prep using the provided thumbscrew (Figure 2-8).



Figure 2-8 Location of the Column Selector Module mounting rail attachment thumbscrew

8. Use the provided thumbscrew and washer to attach the Column Selector to the EZ Prep at the bottom of the module (Figure 2-9).

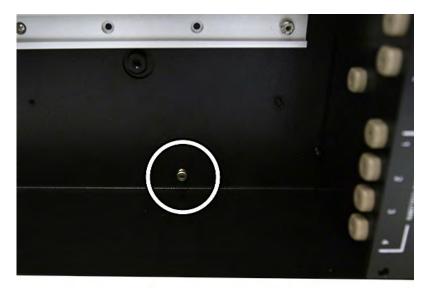


Figure 2-9 Location of the Column Selector Module lower attachment thumbscrew

#### 2.1.2 Establishing Column Selector Module Connections

1. Connect the Column Selector Module to the EZ Prep using the provided USB-to-RS232 cable (Figure 2-10).

#### ✓ Note

If you are also using an AutoSampler Module, first connect the Column Selector Module to the AutoSampler Module with the supplied USB cable. Then, connect the AutoSampler Module to the EZ Prep. The AutoSampler acts as a USB hub for multiple system modules.

If you are not using an AutoSampler but are using the Column Selector Module with an AutoInjector Module or CombiFlash Rf+ Purlon Mass Detector attached to the EZ Prep, you will need a USB hub (not provided).

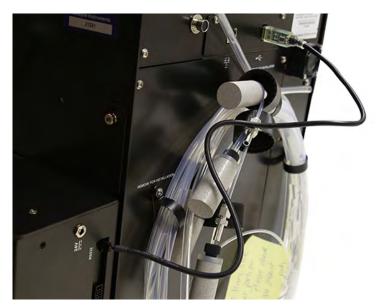


Figure 2-10 Connected USB-to-RS232 cable

- 2. Remove the stainless steel tubing which connects the EZ Prep inject valve to the column inlet.
- 3. Connect one end of the provided stainless steel tubing to the EZ Prep inject valve.
- 4. Connect the other end of the provided stainless steel tubing to the inlet port located on the back of the Column Selector Module (Figure 2-11).



Figure 2-11 Column Selector Module inlet port

- 5. Cut a piece of the provided green PEEK tubing long enough to reach from the outlet port of the Column Selector Module to the outlet port of the EZ Prep (Figure 2-12).
- 6. Connect the Column Selector Module outlet port to the inlet port on the EZ Prep using the provided tubing and hardware (Figure 2-13).

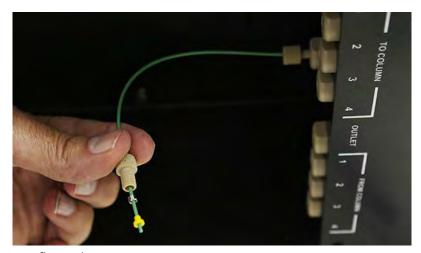


Figure 2-12 Proper outlet port tubing configuration



Figure 2-13 Connected Column Selector Module outlet port tubing

### ✓ Note

For instructions on assembling the tubing connections, please see the instruction sheet included with the hardware.

The Column Selector Module has multiple channels for retaining the column mounting clamps. To change the channels, unscrew the retainer at the end of the track, slide the column mounting clamp off the track, and slide it in place wherever required.

- 7. Place the columns into a clamp suitable for the column size.
- 8. Using the provided tubing and a tubing cutter, connect the column input port to the "TO COLUMN" port on the Column Selector Module. If a guard column is used, it can be plumbed directly before the individual column.
- 9. Connect the column output port to the "FROM COLUMN" port on the Column Selector Module.

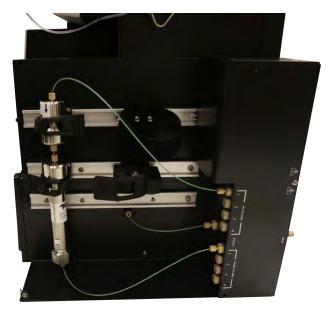


Figure 2-14 Proper column connections on the Column Selector Module

#### ✓ Note

Record the position of the columns, as you will need this information during your initial configuration. After the configuration is complete, the columns will be selected by name rather than by how they are connected.

- 10. If desired, secure the housing of the Column Selector Module back onto the module using the thumbscrews removed in Step 1 of *Removing the housing from the EZ Prep*.
- 11. If you are using an AutoSampler, a 24 V cable may provide power for the Column Selector module from the AutoSampler (See 3.1.3 *Making AutoSampler Electrical Connections*); otherwise, connect the Column Selector Module to the supplied power supply, and then connect the power supply to a power outlet.

### ✓ Note

If you are installing an AutoInjector Module, continue with the next Section 2.1.3; if installing an AutoSampler and AutoInjector continue to Section 3; otherwise, installation of the Column Selector Module is complete.

2.1.3 Mounting the
AutoInjector Module to
the Column Selector
Module
(If installing without
an AutoSampler)

The Column Selector Module can be used in conjunction with the AutoInjector Module. Begin by installing the Column Selector Module, and then install the AutoInjector Module using these steps:

- 1. Remove the cover plate from the top of the Column Selector Module by removing the two screws.
- 2. Attach the AutoInjector Module to the top of the Column Selector Module using the two screws supplied with the module (Figure 2-15).



Figure 2-15 Column Selector Module with attached AutoInjector Module

3. Remove the Luer fitting from the EZ Prep inject valve (Figure 2-16).

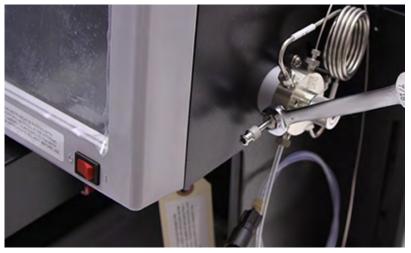


Figure 2-16 Removing the Luer fitting from the EZ Prep inject valve

4. Remove the waste line from the EZ Prep inject valve. Remove the check valve from this line and set it aside for later use (Figure 2-17). Discard the waste line.



Figure 2-17 Removing the waste line from the EZ Prep inject valve

### ✓ Note

If you have an AutoSampler Module or a CombiFlash Rf+ Purlon™ Mass Detector attached to the EZ Prep, you will need to obtain a USB hub to connect both modules.

- 5. Locate the supplied waste tubing and fitting package (PN 60-5234-653).
- 6. Unscrew the attached plastic fitting in this fitting package until the plastic fitting is almost completely detached from the steel fitting.
- 7. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 2-18 Waste tubing fitting attached to the EZ Prep inject valve

- 8. Turn the plastic fitting until it is attached hand tight to the valve.
- 9. Turn the steel fitting  $^1\!/_2$  turn tighter. If it is difficult to do so, loosen the plastic fitting slightly (Figure 2-18).
- 10. Remove the plastic fitting while leaving the steel fitting attached (Figure 2-19).



Figure 2-19 Waste fitting with the plastic fitting removed

11. Attach the supplied tubing from this package to the steel fitting by screwing the new plastic fitting into the steel fitting. Tighten the steel fitting finger tight (Figure 2-20).



Figure 2-20 Waste fitting with tubing attached

12. Connect the opposite end of this tubing to the left port of the AutoInjector Module (Figure 2-21).



Figure 2-21 Connection of the waste tubing from the EZ Prep inject valve to the left  $AutoInjector\ Module\ port$ 

13. Connect the supplied waste line (PN 60-5234-654) to the right port of the AutoInjector Module (Figure 2-22).



Figure 2-22 Waste line connected to the right AutoInjector Module port

14. Connect the check valve removed in Step 4 to the waste line from the AutoInjector Module. Make sure that the arrow on the check valve points away from the AutoInjector Module as shown in Figure 2-23.



Figure 2-23 Check valve connected to the waste line of the AutoInjector Module

- 15. Connect another length of tubing to the open end of the check valve and either run it to the waste container or tee into another line running to the waste container.
- 16. Locate the supplied sample probe tubing and fitting package (PN 60-5234-657).
- 17. Unscrew the attached plastic fitting in this fitting package until the plastic fitting is almost completely detached from the steel fitting.
- 18. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 2-24 Sample tube fitting attached to the EZ Prep inject valve

- 19. Turn the plastic fitting until it is attached hand tight to the valve.
- 20. Turn the steel fitting  $\frac{1}{2}$  turn tighter (Figure 2-24).
- 21. Remove the plastic fitting while leaving the steel fitting attached (Figure 2-25).



Figure 2-25 Sample tube fitting with the plastic fitting removed

22. Attach the supplied tubing from this package to the secured fitting by screwing the new plastic fitting into the secured fitting. Tighten the fitting finger tight with the wrench supplied in the package (Figure 2-26).



Figure 2-26 Sample tube fitting with sample tubing attached

2.1.4 Establishing
AutoInjector Module
Connections
(If installing without
an AutoSampler)

The AutoInjector Module comes with an attached USB-A cable. Connect this cable to an open USB hub that you previously connected the Column Selector Module to.

### ✓ Note

You will need a USB hub to connect the Column Selector Module and the AutoInjector Module.

Y-type Power Supply

A Y-type power supply (PN 69-5234-664) is supplied for using the Column Selector Module in conjunction with the AutoInjector Module. This power supply is used to power both of the modules from a single power source. Plug one end of the Y-type power cable into the Column Selector Module, and plug the other end into the AutoInjector Module. Plug the third end of the Y-type cable into the supplied power supply. Then connect the power supply to a wall outlet.



Figure 2-27 Y-Type power supply

2.2 Installing the Column Selector Module with the AutoSampler Module Using the Column Selector Module in conjunction with the AutoSampler Module does not affect the installation process for either module. Install each module in accordance with the instructions given in that module's section.

## ✓ Note

The USB cable for the Column Selector Module will be connected to the USB hub at the rear of the AutoSampler Module.

## ✓ Note

Power to the Column Selector Module will be provided by one of the 24 V power cables at the rear of the AutoSampler Module (See 3.1.3 *Making AutoSampler Electrical Connections*).

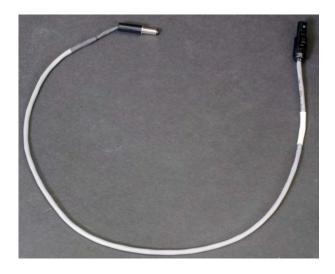




Figure 2-28 Power cable for the EZ Prep

#### 2.3 Column Selector Module Operation

#### 2.3.1 **Setup**

Before using the Column Selector Module for the first time, the CombiFlash EZ Prep must be configured to identify the column in each position:

- 1. Once the columns have been installed in the Column Selector Module, go to TOOLS > CONFIGURATION. The CONFIGURATION window opens.
- 2. There, select the PREP HPLC tab (Figure 2-29).
- 3. Then, define the parameters of each column so that a default method can be created for the column.

To create a new column definition, follow the steps below:

- 1. Select NEW and enter a COLUMN NAME.
- 2. Enter the Media Size, column Inside Diameter, and Length. If the default pressure limit of 3,500 is excessive, enter a new Pressure Limit as well.

These parameters are automatically entered into the RUN NOTE for each separation. The INSIDE DIAMETER is used to suggest a flow rate with a similar velocity to a 4.6 mm HPLC operating at 1 mL/min. This flow rate may be overridden at this time to decrease the time required for a separation. The column length along with the diameter is used to estimate a column's volume and to suggest a default gradient duration. This parameter can also be overridden to decrease run time.

3. The debubbler automatically operates if one of the names of the solvents contains the words "water", "H2O", "wasser", "eau", or "agua". The ENABLE DEBUBBLER field allows the user to enable the debubbler without having one of these words present.

- 4. To set other method parameters as default for the column being defined, or to create a new method with custom parameters, press Define Methods.
- 5. Once the column definition is set, press SAVE.
- 6. To identify the column in each position, select a previously defined column from each menu in the upper portion of the CONFIGURATION window. This allows you to select a column position by selecting the column name in the REDISEP menu or on the AutoSampler Module queue screen.



Figure 2-29 Configuring the Prep HPLC tab

## **Automation**

## Section 3 The AutoSampler Module

# 3.1 AutoSampler Module Installation

#### **✓** Note

CombiFlash EZ Prep software must be version 3.1.0 or higher to support the automation capabilities.

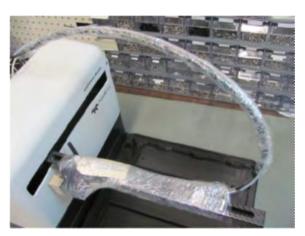
#### ✓ Note

Begin your installation on a clean workspace separate from the EZ Prep. You will be prompted to place the AutoSampler Module on the right side of the EZ Prep in 3.1.4 *Establishing Fluid Connections*.

# 3.1.1 Installing the AutoSampler Module

The AutoSampler Module includes an AutoInjector Module, unless you are installing to an existing unit already having an AutoInjector Module. To install the AutoSampler Module, follow the steps below:

1. Carefully unpack the Z-Drive and remove the packaging materials from the Y-axis arm (Figure 3-1).





**Z-Drive** 

Figure 3-1 Autosampler Module Y-axis arm and Z-Drive

2. Find the Y-axis carriage on the arm of the AutoSampler Module. The Z-Drive will be attached to this carriage (Figure 3-2).

3. Remove the two screws from the Y axis carriage. Slide the Z-Drive onto the arm until the two holes align with the matching holes in the Y-axis carriage.



Figure 3-2 AutoSampler Module Y-axis arm

4. Secure the Z-Drive to the carriage using the two plastic thumbscrews. Tighten the thumbscrews with your fingers (Figure 3-3).

Z-Drive



Figure 3-3 Z-Drive mounted on the Y-axis carriage

5. Rotate the Z-Drive rotor back and forth to ensure that the Z-Drive moves up and down freely (Figure 3-4).

## ✓ Note

If the rotor does not move freely, check that the other cables are not interfering with the movement of the green probe cable.



Figure 3-4 Z-Drive rotation

6. Mount the wash well by placing it into the receptacle located on the right-rear portion of the AutoSampler Module bed ( Figure 3-5).

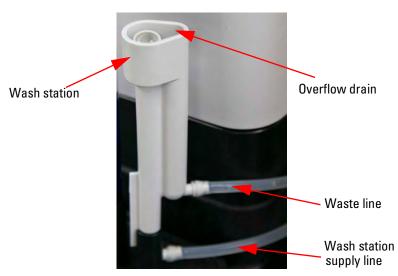


Figure 3-5 AutoSampler Module wash well with tubing attached (top tube is waste to port #2 while the bottom tube is supply port #3)

- 7. The wash well comes pre-plumbed to the peristaltic pump ports. If it is ever disconnected, it is important to connect the wash well waste tube (upper tube on wash well labeled 2) to port "2" of the peristaltic pump and connect the wash well supply tube (lower tube on wash well labeled 3) to port 3 of the peristaltic pump.
- 8. Connect the tube labeled "Wash Supply" to the tube labeled "1" connected to port "1" of the peristaltic pump on the back of the Autosampler Module. The free end of the

tubing can be placed in the wash solution container or tee'd into a solvent supply line (Figures 3-6 and 3-7).

### **✓** Note

The wash fluid peristaltic pump uses Pharm-A-Line™ tubing. Be sure to choose a wash fluid compatible with this tubing such as methanol.



Figure 3-6 AutoSampler Module peristaltic pump with tubing correctly attached





Figure 3-7 Wash and waste tubing connections and labels

## ✓ Note

The wash fluid peristaltic pump uses Pharm-A-Line tubing. Be sure to choose a wash fluid compatible with this tubing such as methanol.

# 3.1.2 Mounting the AutoInjector Module

The AutoInjector Module can either be installed onto the AutoSampler or onto the EZ Prep itself. When installing onto the EZ Prep

- if the unit has a Column Selector Module installed, follow mounting directions from Section 2.1.
- if it doesn't have a Column Selector module installed, follow mounting directions from Section 4.1.
- 1. Install the AutoInjector Module onto the AutoSampler Module by removing the right side mounting screws on the AutoSampler Module back panel (Figure 3-8).

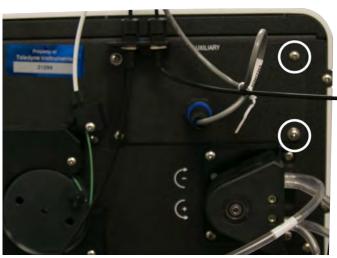


Figure 3-8 AutoSampler Module back panel with AutoInjector Module mounting screws removed

2. Place the AutoInjector Module on top of the AutoSampler Module, and fasten them together with the mounting screws removed in Step 7 (Figure 3-9).

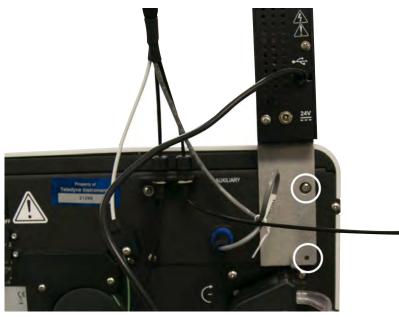


Figure 3-9 AutoInjector Module fastened to the AutoSampler Module

# 3.1.3 Making AutoSampler Electrical Connections

- 1. Connect the USB-A cable attached to the AutoInjector Module to one of the USB-A ports located on the back of the AutoSampler Module (Figure 3-10).
- 2. Connect a 24 V power cable from the rear panel of the AutoSampler Module to the AutoInjector Module.

## ✓ Note

The AutoSampler Module features two 24 V power ports. One is used to provide power to the AutoInjector Module. The other can be used to power the Column Selector Module (if applicable).

3. The short USB cable connecting the USB-B port on the top portion of the back panel of the AutoSampler Module to the USB-A port on the lower portion of the back panel of the AutoSampler Module should already be pre-installed. If not, make the connection (Figure 3-10, Figure 3-11).



Figure~3--10 Auto Sampler~Module~USB-B~connection



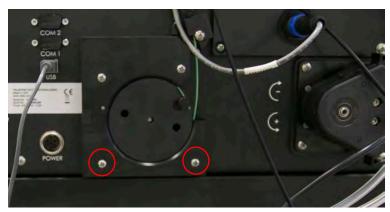
Figure~3-11 Auto Sampler~Module~USB-A~connection~(center)

4. Locate the AutoSampler Module's power supply bracket as show in Figure 3-12.

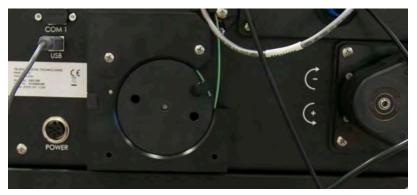


Figure 3-12 AutoSampler Module power supply bracket shown removed from the AutoSampler Module

5. Remove the two bottom screws from the AutoSampler Module's rotor (Figure 3-13, Figure 3-14).



Figure~3-13 Auto Sampler~Module~rotor~before~removing~the~bottom~screws



Figure~3-14 Auto Sampler~Module~rotor~after~removing~the~bottom~screws

6. Place the power supply bracket so the two holes in the bracket align with the two lower holes on the AutoSampler

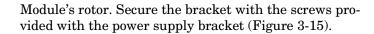




Figure 3-15 Power supply bracket secured to the AutoSampler Module

7. Slide the power supply into the bracket (Figure 3-16).



Figure 3-16 Inserting the power supply into the power supply bracket

8. Plug the power supply into the power port immediately above the power supply, and then connect the power supply to a suitable power source (Figure 3-17).



Figure 3-17 AutoSampler Module with power connection made

9. Connect the 24 V power supply cable (Figure 3-18a) to the 24 V port on the back of the AutoSampler (Figure 3-18b) and connect the other end to the Injection Valve. If a column select valve is installed, repeat the connection for the column select valve.





Figure 3-1824 V Power supply cane and the port on the back of the EZ Prep

10. Connect the long provided USB cable from the USB-A port on the back of the EZ Prep to the USB-B port on the lower portion of the back panel on the AutoSampler Module (Figure 3-19, Figure 3-20).



Figure 3-19 EZ Prep USB-A connection



Figure 3-20 AutoSampler Module USB-B connection

## 3.1.4 Establishing Fluid Connections

To establish the necessary connections, follow the steps below:

- 1. Place the AutoSampler Module on the right side of the EZ Prep.
- 2. Disconnect the tubing at the back of the EZ Prep from the port labeled DIVERTER VALVE WASTE (Figure 3-21).



Figure 3-21 Diverter Valve Waste port with no connection

3. Connect the shorter length of tubing from the AutoSampler Module to the port labeled DIVERTER VALVE WASTE at the back of the EZ Prep (Figure 3-22).



Figure 3-22 Diverter Valve Waste port with AutoSampler Module tubing connection

- 4. Route the longer length of tubing from the AutoSampler to waste. This can be done by connecting the tube to an existing waste line with the supplied tee or by placing it into a waste container using a weight (PN 209-016-351).
- 5. Remove the Luer fitting from the EZ Prep inject valve (Figure 3-23).

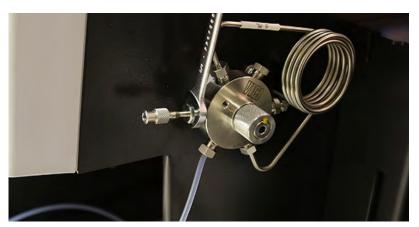


Figure 3-23 Removing the default EZ Prep injector port

- 6. Locate the supplied sample probe tubing and fitting adapter package.
- 7. Unscrew the attached plastic fitting in this fitting package until the plastic fitting is almost completely detached from the steel fitting.
- 8. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 3-24 Sample probe tube fitting connected to the EZ Prep inject valve

- 9. Turn the plastic fitting until it is attached hand tight to the valve.
- 10. Turn the steel fitting  $^1\!/_2$  turn tighter. If it is difficult to do so, loosen the plastic fitting slightly (Figure 3-24).
- 11. Remove the plastic fitting while leaving the steel fitting attached (Figure 3-25).



Figure 3-25 Sample probe tube fitting with the plastic fitting removed

12. Attach the supplied sample probe tubing from this package to the steel fitting by screwing the new plastic fitting into the steel fitting. Tighten the steel fitting finger tight with the wrench supplied in the package (Figure 3-26).



Figure 3-26 Sample probe tube fitting with sample probe tubing attached

13. Secure the probe tubing to the side of the EZ Prep by removing the housing screw, fitting the tubing bracket to the screw, and replacing the screw (Figure 3-27).

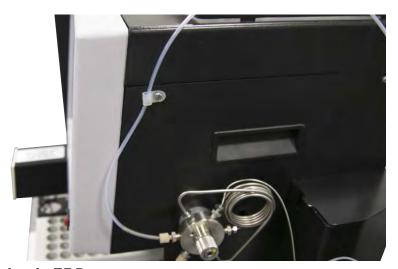


Figure 3-27 Sample probe tubing secured to the EZ Prep

### ✓ Note

The tubing bracket should be affixed to the EZ Prep so that the probe tubing threads vertically through the bracket. This ensures that the probe tubing will not be obstructed by other end of the probe attachment.

- 14. Locate the supplied waste tubing and fitting package (PN 60-5234-653).
- 15. Unscrew the attached plastic fitting in this fitting package until the plastic fitting is almost completely detached from the steel fitting.

16. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 3-28 Waste tubing fitting attached to the EZ Prep inject valve

- 17. Turn the plastic fitting until it is attached hand tight to the valve.
- 18. Turn the steel fitting  $\frac{1}{2}$  turn tighter. If it is difficult to do so, loosen the plastic fitting slightly (Figure 3-28).
- 19. Remove the plastic fitting while leaving the steel fitting attached (Figure 3-29).



Figure 3-29 Waste fitting with the plastic fitting removed

20. Attach the supplied tubing from this package to the fitting just installed. Tighten the fitting finger tight (Figure 3-30).



Figure~3--30~Waste~fitting~with~tubing~attached

21. Connect the opposite end of this tubing to the left port of the AutoInjector Module (Figure 3-31).



Figure 3-31 Connection of the waste tubing from the EZ
Prep inject valve to the left AutoInjector
Module port

22. Connect the supplied waste line (PN 60-5234-654) to the right port of the AutoInjector Module (Figure 3-32).

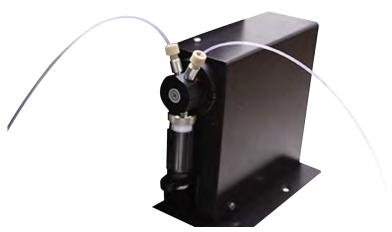


Figure 3-32 Waste line connected to the right AutoInjector Module port

23. Connect the check valve removed previously to the waste line from the AutoInjector Module. Be sure that the arrow on the check valve points away from the AutoInjector Module as shown in Figure 3-33.



Figure 3-33 Check valve connected to the waste line of the AutoInjector Module

- 24. Connect another length of tubing to the open end of the check valve and either run it to the waste container or tee into another line running to the waste container.
- 25. Install the included 18 mm test tube in the receptacle at the right rear corner of the AutoSampler platform just to the left of the wash well (Figure 3-34). Fluid in this tube will be used for bracketed solvent injections. In this technique, the injected sample is separated from the chromatographic solvents by a small amount of air and a "bracket" solvent during the sample loading and injection process.

Refer to Figure 3-35, which shows a completed setup of an EZ Prep with an AutoInjector mounted on the AutoSampler.

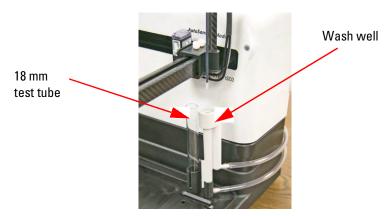


Figure 3-34 Location of the test tube and wash well on the AutoSampler



Figure 3-35 EZ Prep system with the AutoSampler

## 3.2 AutoSampler Module Operation

#### 3.2.1 Power Cycling

Before the AutoSampler Module can be used with the CombiFlash EZ Prep, the EZ Prep must auto-sense the AutoSampler Module. This process takes place during the power-up sequence of the EZ Prep. Be sure to power up the AutoSampler Module before powering up the EZ Prep. If you have not done this, cycle the power on the EZ Prep by turning the EZ Prep off, waiting ten seconds, and turning it on again.

To Power on the System

The AutoSampler gets its power from the EZ Prep.

1. Turn ON the power switch on the AutoSampler (Figure 3-36).



Figure 3-36 On switch for the AutoSampler

2. Turn ON the power to the EZ Prep. A red light on the AutoSampler light ups, the arm moves to the HOME position, and then the light turns blue (Figure 3-37).



Figure 3-37 Power switch for the EZPrep

## 3.2.2 Fraction Collection Capacity

The AutoSampler Module doubles the fraction collection capacity of the Combi*Flash* EZ Prep. They are treated as an extension of the racks present in the EZ Prep. In general, if racks are placed in both the EZ Prep and AutoSampler, available racks will used in this order: EZ Prep left rack (A), EZ Prep right rack (B), AutoSampler left rack (C), AutoSampler right rack (D).

The RFID capabilities of these additional racks are supported in the same way as the racks for the EZ Prep. If any racks are missing or identified as containing fractions from a previous separation, the system moves to the next available rack in the order mentioned above. When a rack is full, the system will automatically advance to the next available rack. If a rack is replaced with a fresh rack, this will be sensed by the system software, and the new rack will be made available for use. The sample racks also have RFID tags, which allow for the recognition of vial locations.

When the system moves from the EZPrep fraction racks to the AutoSampler fraction racks, the fluid uses an alternative path. This leaves a portion of tubing with fluid remaining for the fraction collector previously used. To prevent the loss of any compound, the software monitors for a portion of the separation with no peaks while using the last available row of the fraction collection rack on that module. If a portion without a peak to collect is identified, the system moves to the next rack on the next module (the AutoSampler or EZ Prep). This may leave a few tubes unused in the previous rack. If the system is mid peak or separation peak collection is set to ALL, the fluid remaining in

the tubing is placed into one of the tubes in the last row of the rack before the systems moves to collect any undetected compound and then advances to the next rack.

### **✓** Note

The sample rack cannot be swapped during a separation sequence.

#### 3.2.3 Configuration

Before using the AutoSampler Module for the first time, the EZ Prep should be configured for the proper injection loop volume.

- 1. Go to TOOLS > CONFIGURATION. The CONFIGURATION window opens. Select the PREP HPLC tab.
- 2. Enter a LOOP VOLUME (Figure 3-38).

### ✓ Note

Figure 3-38 depicts what is shown when a Column Selector Module is installed. If it is not installed, the CONFIGURATION window may appear differently.

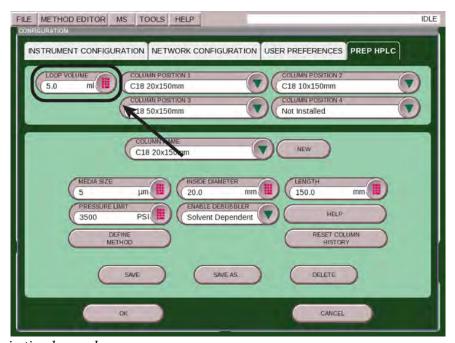


Figure 3-38 Configuring the injection loop volume

The system software assumes that the loop should only be 50% filled for maximum compound recovery. If the parameters entered for the individual injection exceed a 50% loop fill, a warning message recommending a change in the parameters appears. This advice can be ignored at your discretion.

#### 3.2.4 Setup for Operation

Prime AutoSampler Wash Station If you have installed the system to recirculate wash fluid, check that the container holds sufficient clean wash fluid for the planned separations.

- Be sure that the wash station supply line is placed into a suitable solvent (typically the strong chromatographic solvent) and that the waste line is routed to a suitable container.
- 2. Go to TOOLS > AUTOMATION MANUAL CONTROL, and select START WASH (Figure 3-39).

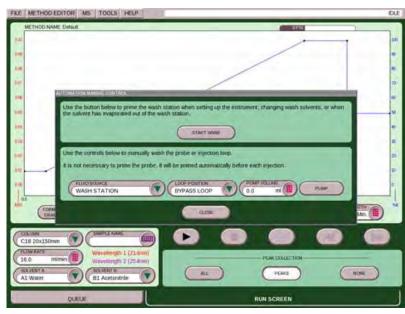


Figure 3-39 The Automation Manual Control window: Start Wash

3. Be sure that the wash solvent is pumped into the center portion of the wash station and, when full, the fluid drains into the overflow drain (Figure 3-38).

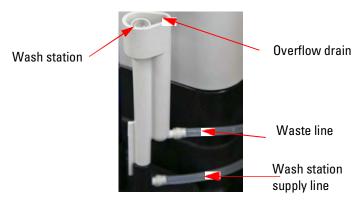


Figure 3-40 Wash station and overflow drain

Additionally, if the separations will use a solvent bracketed injection, be sure that there is sufficient bracket solvent for the planned separations. This solvent is placed into an 18 mm test

tube located to the left of the wash station (Figure 3-33). The system uses ~100  $\mu L$  per injection. The probe can only aspirate fluid down to the level of the top of the tube holder, so the tube must be filled to a level above that point.

## 3.2.5 Separation Using an AutoSampler Module

To begin a separation sequence with the AutoSampler Module, create a sample queue. After the AutoSampler Module has been installed and the EZ Prep is in the PREP HPLC mode, the software displays two tabs at the bottom of the screen. Accessing the Run tab results in a screen and operation much like the standard EZ Prep (without an installed AutoSampler Module). Pressing PLAY displays the Run Requirements window. There, you can perform a manual injection by removing the Luer fitting from the inject loop, or you can perform multiple injections on a single sample from the AutoSampler Module without creating a queue. Selecting the QUEUE screen allows you to set up a separation sequence.

### ✓ Note

If you are performing a manual injection, the Luer fitting or a needle port must be reinstalled on the injection valve.

#### 3.2.6 Using the Queue Tab

Follow the instructions below for further information on operating the AutoSampler Module:

- 1. Install a sample vial rack before opening the QUEUE tab. This allows the software to limit sample programming to the positions available for sample vials.
- 2. Open the QUEUE tab.

Follow the instructions below for further information on operating the AutoSampler Module:

≡ - Allows you to start the separation from the Queue tab, to remove a single row of an existing queue, clear all rows of the queue, or enable or disable a scouting pause. The scouting pause halts the system after the first separation is completed for the sample. This allows you to adjust the separation parameters based on the results of the initial separation. This is especially useful to allow adjustment of the quantity of sample injected for each separation to optimize the loading on the column. After the initial pause, you can modify the method as needed and disable the pause for the remaining injections, or leave it enabled to allow a second condition scouting separation.

**Sample Name –** Allows you to name each sample. Naming is optional. If left blank, the sample is named based on the date and time the separation was started.

Column (drop down menu) – If a Column Selector Valve Module (CSV-4) is installed, this menu allows you to select a separation column and associated method for use. If a CSV-4 is not installed, this menu allows you to select different methods that may be associated with the configured and installed separation column.

**Method** – Once a column has been selected, or if there is only one column defined on the system, the default method created for that column is automatically loaded. To change this method:

- Touch the method name. A list of methods associated with the column appears. These methods are listed in the order they were created.
- Alternatively, select CUSTOMIZE CURRENT METHOD or BROWSE to populate this field.

Select Customize Current Method to create a modified version of the method for use in the current queue. To use this modified method:

- Exit the Method Editor and select SAVE when prompted. A new method named "Temporary 1" will be created.
  - This method is used for all injections of the current sample and will be discarded after the sample is complete. All method parameters are saved with each separation
  - If you would like to save the modified method for later use, select SAVE AS before you exit the Method Editor and create a unique name for the new method.

**Sample Position** - This field indicates where the sample for this separation is located. If the sample size is too large for a single vial, create a second line in the queue and access the second vial.

**Sample Volume** – Enter the amount of sample to be separated. Typically this amount is slightly greater than the amount of sample to ensure all of the sample gets purified. This information is used in conjunction with the NUMBER OF INJECTIONS column to calculate the volume of each injection.

**Number of Injections** – Used in conjunction with the SAMPLE VOLUMES column to determine the size of each injection.

**Start Tube** - Allows you to optimize rack usage by sharing

- 3. Once the queue is complete, select the RUN tab and press the PLAY button. Alternatively, you can select the "≡" menu on the first unfinished run and select the RUN option.
- 4. While a separation is in process, the QUEUE screen can be accessed to add samples to the queue. Samples that have been completed cannot be edited. They can be viewed, however, by touching the file name corresponding the that run.
- 5. During the separation, completed fraction racks can be removed when full and replaced with empty racks to allow continuous separations. If a single rack containing fractions is removed and then replaced into the rack position, PeakTrak will display a prompt to determine if the rack contains empty tubes. If you respond that the tubes are empty, PeakTrak will consider this rack available for future separations. Otherwise, PeakTrak will continue to mark this rack as full of samples and not available for frac-

tion use. This feature allows you to remove a rack to obtain a sample for verification while leaving the rack in the instrument for convenience and later removal.

### ✓ Note

The number of rows in the queue is limited to 28. Additional samples can be added by deleting completed rows from the queue. If there are more rows than can be displayed on screen, up and down arrows are displayed at the top and bottom of the screen that allow access to hidden rows.

6. Once the entire queue has completed, the RESULT screen is displayed with the last separation. To easily view previous separations, select the separation on the QUEUE screen or select FILE > OPEN to view previous separations.

If a sample had multiple injections, the run sequence would be displayed within the FILE > OPEN dialogue under a single name preceded by a "+" symbol. This symbol indicates there are multiple injections with the same base file name. Names are appended with "-I(#)" to signify the numerical order of the separation.

For example:

- If you have a single injection run, it can be named A01.
- For multiple injections, the first injection can be named A03-I1, the second will be A03-I2, and the third will be named A03-I3.

## 3.2.7 AutoSampler Injection Techniques

This section outlines the operating procedures for the AutoSampler Module.



The sequence of operation show below is valid for software version 4.1.185 and newer.

The AutoSampler supports two different injection protocols. The default technique injects samples and washes the sample probe using the wash station solvent. The alternative bracketed sample injection minimizes the risk of sample crash by placing a small amount of a user selected solvent on each end of the sample fluid. All sample injection steps are listed below for reference.

Default Injection Sequence

- 1. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any left-over fluid from a previous separation and fills the loop with the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
- 2. The sample probe is placed in the wash station.
- 3. About 1.5 mL of the solvent is pulled from the wash station through the probe to remove air from the probe line.

- 4. The sample probe is lifted from the wash station and is moved up and down slightly. When the sample probe is dipped in the sample or solvent, some liquid sticks to the outside of the probe when it is raised. Therefore, the probe is moved up and down over the fluid source to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
- 5. A small amount of air (0.05 mL) is drawn into the sample probe to minimize the mixing of the wash solvent and the soon to be loaded sample. This also minimizes the potential of the sample coming out of solution.
- 6. The sample probe is moved to the sample container.
- 7. A portion of the programmed injection amount is aspirated into the sample probe displacing most of the wash solvent in the sample probe. This compensates for the volume of the sample probe. Since the loop is still in the separation position, the wash solvent is sent to waste.
- 8. The loop is switched to the load position.
- 9. The remaining portion of the programmed injection volume is aspirated into the sample probe and loop. Once the programmed amount of sample is aspirated into the probe, the probe is lifted out of the sample vial.
- 10. The probe is moved up and down over the sample tube to shake off any excess liquid to prevent the contamination of other samples or collection tubes.
- 11. The probe is dipped into the wash station to rinse the exterior of the probe to prevent sample residue from drying on the exterior of the probe.
- 12. Air is drawn into the probe to eliminate any remaining solvent in the probe.
- 13. The sample is now loaded and the separation begins.
- 14. The next injection of the sample is accomplished by repeating the process above.

After the completion of the final injection of a sample, the system washes the probe in the following sequence:

- 1. The inject valve is moved to bypass to prevent contamination of the loop during the cleaning process.
- 2. Air is drawn into the probe to eliminate any remaining solvent in the probe.
- 3. The probe is placed into the wash station. The wash station pump flushes wash fluid over the exterior of the probe while 10 mL of wash fluid is drawn into the probe to wash the interior flow path. The probe syringe pump uses half strokes to improve the rinsing of any tiny amounts of compound that may be present due to the wash process. Once again, the probe is moved up and down over the wash station to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.

#### Bracketed Sample Injection Sequence

- 4. Air is drawn into the probe to eliminate the strong wash solvent from the sample flow path.
- 1. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any left-over fluid from a previous separation and fills the loop with the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
- 2. The sample probe is placed into the wash station
- 3. About 1.5 mL of the solvent is pulled from the wash station through the probe to remove air from the probe line. This minimizes injection volume errors due to air in the probe. Since the loop is still in the column flow path, this aspirated fluid bypasses the loop and is sent to waste
- 4. The sample probe is lifted from the wash station and is moved up and down slightly. When the sample probe is dipped in the sample or solvent, some liquid sticks to the outside of the probe when it is raised. Therefore, the probe is moved up and down over the fluid source to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
- 5. A small amount of air (0.05 mL) is drawn into the sample probe to minimize the mixing of the wash solvent and the bracket solvent.
- 6. The probe moves to the bracket solvent sample station (just to the left of the wash station). 0.05 mL of the bracket solvent is aspirated into the probe.
- 7. The probe is raised into the air and an additional 0.05 mL of air is aspirated into the probe.
- 8. The sample probe is moved to the sample container.
- 9. A portion of the programmed injection amount is aspirated into the sample probe displacing most of the wash solvent remaining in the sample probe. This compensates for the volume of the sample probe. Since the loop is still in the separation position, the wash solvent is sent to waste.
- 10. The loop is switched to the load position.
- 11. The programmed injection volume is aspirated into the sample loop. Once the programmed amount of sample is aspirated into the probe, the probe is lifted and a small amount of air is drawn into the sample probe. This minimizes mixing of the sample with the bracketing solvent which is aspirated next.
- 12. The probe is dipped in the wash solvent to rinse the exterior of the probe, then raised and shaken. This minimizes sample contamination of the bracket solvent.
- 13. The probe moves to the bracket solvent sample container. 0.05 mL of the bracket solvent is aspirated into the probe.

- 14. The probe is raised into the air and the fluid in the loop is aspirated into the loop along with ~.05 mL of air to ensure that all off the sample and bracket solvent is loaded.
- 15. The loop is switched to the separate position.
- 16. The sample is now loaded and the separation begins.
- 17. The next injection of the same sample is accomplished by repeating the above process.

After the completion of the final injection of a sample, the system washes the probe with the following sequence:

- 1. The inject valve is moved to bypass to prevent contamination of the loop during the cleaning process.
- 2. Air is drawn into the probe to eliminate any remaining solvent in the probe.
- 3. The probe is placed into the wash station. The wash station pump flushes wash fluid over the exterior of the probe while 10 mL of wash fluid is drawn into the probe to wash the interior flow path. The probe syringe pump uses half strokes to improve the rinsing of any tiny amounts of compound that may be present due to the wash process.
- 4. Once again, the probe is moved up and down over the wash station to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
- 5. Air is drawn into the probe to eliminate the strong wash solvent from the sample flow path.

## **Automation**

## Section 4 The AutoInjector Module

## 4.1 AutoInjector Module Installation

### **✓** Note

Combi*Flash* EZ Prep software must be version 3.1.0 or higher to support the automation capabilities.

Installation

The following instructions are for installing an AutoInjector Module without an AutoSampler Module or a Column Selector Module. Follow the instructions in Section 3-1 if an AutoSampler is also being added to the system or Section 2-1 if only a Column Selector Module is being added.

The AutoInjector Module may be mounted in two different places: on top of the Column Selector Module or the EZ Prep standard prep column mount (if the Column Selector Module is not installed), or onto the AutoSampler Module (Section 3-1).

Additionally, USB and fluid connections change depending on other modules installed or being installed.

- 1. Turn off the EZ Prep.
- 2. Remove the top cover of the EZ Prep column mount by grasping and pulling straight up (Figure 4-1).



Figure 4-1 Removing the top cover of the EZ Prep column mount

3. Remove the Luer fitting from the EZ Prep inject valve (Figure 4-2).



Figure 4-2 Removing the Luer fitting from the EZ Prep inject valve

4. Remove the waste line from the EZ Prep inject valve. Remove the check valve from this line and set it aside for later use. Discard the waste line (Figure 4-3).



Figure 4-3 Removing the waste line from the EZ Prep inject valve

5. Install the AutoInjector Module by placing it on top of the prep column mount and pressing downwards. The tab on the bottom of the AutoInjector Module snaps into the receptacle on the top of the prep column mount (Figure 4-4).



Figure 4-4 AutoInjector Module attached to the EZ Prep column mount

- 6. Connect the USB cable from the AutoInjector Module to the USB port on the rear of the EZ Prep. If you have a CombiFlash Rf+ PurIon™ Mass Detector attached to the EZ Prep, obtain a USB hub to connect both modules.
- 7. Connect the included power supply from the AutoInjector Module to a suitable power connection. Place the power supply in a location that minimizes the possibility of a chemical spill damaging the power supply.
- 8. Locate the supplied waste tubing and fitting package (PN 60-5234-653).
- 9. Unscrew the attached plastic fitting in this fitting package until the plastic fitting is almost completely detached from the steel fitting.
- 10. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 4-5 Waste tubing fitting attached to the EZ Prep inject valve

- 11. Turn the plastic fitting until it is attached hand tight to the valve.
- 12. Turn the steel fitting  $^1\!/_2$  turn tighter. If it is difficult to do so, loosen the plastic fitting slightly (Figure 4-5).
- 13. Remove the plastic fitting while leaving the steel fitting attached (Figure 4-6).



Figure 4-6 Waste fitting with the plastic fitting removed

14. Attach the supplied tubing from this package to the fitting just installed. Tighten the fitting finger tight (Figure 4-7).



Figure 4-7 Waste fitting with tubing attached

15. Connect the opposite end of this tubing to the left port of the AutoInjector Module (Figure 4-8).



Figure 4-8 Connection of the waste tubing from the EZ Prep inject valve to the left AutoInjector Module port

16. Connect the supplied waste line (PN 60-5234-654) to the right port of the AutoInjector Module (Figure 4-9).



Figure 4-9 Waste line connected to the right AutoInjector Module port

17. Connect the check valve removed in Step 4 to the waste line from the AutoInjector Module. Be sure that the arrow on the check valve points away from the AutoInjector Module (Figure 4-10).



Figure 4-10 Check valve connected to the waste line of the AutoInjector Module

- 18. Connect another length of tubing to the open end of the check valve and either run it to the waste container or tee into another line running to the waste container.
- 19. Locate the supplied sample probe tubing and fitting package (PN 60-5234-657).
- 20. Unscrew the attached plastic fitting in this package until the plastic fitting is almost completely detached from the steel fitting.
- 21. Attach the steel fitting in this package to the EZ Prep inject valve waste port. This steel fitting should be hand tight.



Figure 4-11 Sample tube fitting attached to the EZ Prep inject valve

- 22. Turn the plastic fitting until it is attached hand tight to the valve.
- 23. Turn the steel fitting  $^1\!/_2$  turn tighter. If it is difficult to do so, loosen the plastic fitting slightly (Figure 4-11).
- 24. Remove the plastic fitting while leaving the steel fitting attached (Figure 4-12).



Figure 4-12 Sample probe tube fitting with the plastic fitting removed

25. Attach the supplied tubing from this package to the steel fitting by screwing the new plastic fitting into the steel fitting. Tighten the steel fitting finger tight with the wrench supplied in the package (Figure 4-13).



Figure 4-13 Sample tube fitting with sample tubing attached

Figures 4-14 and 4-15 show completely assembled AutoInjector setups with and without and AutoSampler.

26. Turn on the EZ Prep, or cycle power if the EZ Prep is already turned on.

Before using the AutoInjector Module for the first time, the EZ Prep should be configured for the proper injection loop volume.

- 1. Go to Tools > Configuration. The Configuration window opens. Select the PREP HPLC tab.
- 2. Enter the LOOP VOLUME (Figure 4-16).

### ✓ Note

Figure 4-16 depicts what is shown when a Column Selector Module is installed. If it is not installed, the CONFIGURATION window may appear differently.



Figure 4-14 AutoInjector mounted on the EZ Prep



 $Figure~4-15\,Auto Sampler~installed~with~an~Auto Injector\\mounted~on~the~EZ~Prep$ 

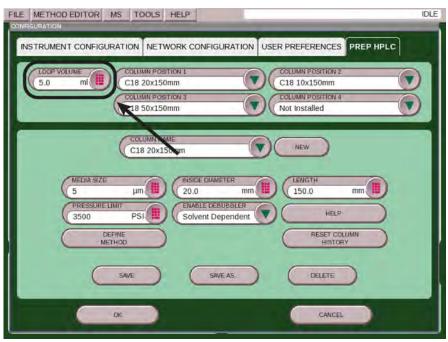


Figure 4-16 Configuring the injection loop volume

For best results, the loop should generally be 50% filled for maximum compound recovery. If the parameters entered for the individual injection exceed a 50% loop fill, a warning message recommending a change in the parameters appears. This advice can be ignored at your discretion.

## 4.2 AutoInjector Module Operation

#### 4.2.1 Operation

To use the AutoInjector Module, follow the steps below:

- 1. Place the sample probe into your sample container.
- 2. Press PLAY to start a separation as you would for an individual separation.
- 3. If you want to automatically perform multiple injections (or perform a single injection using the AutoInjector Module), select the "Multiple Injections" from the SAMPLE LOADING list (Figure 4-17). This is the default selection if an AutoInjector Module is present.

### ✓ Note

If you want to manually inject your sample without using the AutoInjector Module, replace the sample probe fitting with the Luer injection port.

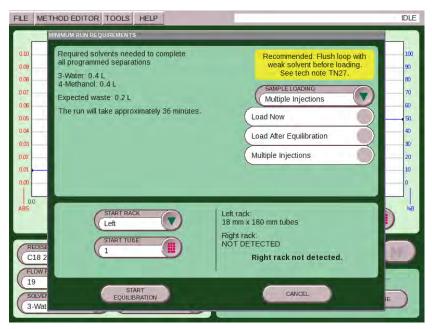


Figure 4-17 Selecting for multiple injections

4. In the dialogue box, enter the TOTAL SAMPLE volume and the NUMBER OF INJECTIONS (Figure 4-18).

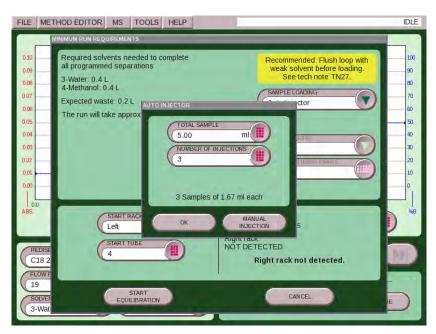


Figure 4-18 Selecting the sample volume and number of injections

PeakTrak will divide the total volume by the number of injections to determine the injection volume for each separation. If this volume exceeds 50% of the loop volume, a warning message recommending a change in the parameters will appear. This advice can be ignored at your discretion. PeakTrak will perform exactly as programmed. It will also compensate for the volume of tubing used for the supplied sample probe. If the sample probe is ever lost or damaged, it should be replaced by the AutoInjector Needle Assembly (PN 60-5234-657).

During the programmed separation sequence, any changes made to the method will be included on any of the remaining injections. This includes automatic changes such as using the peak hold feature or automatic run length extension. For the most reliable operation, it may be best to disable these automatic features. Mid-separation flow rate changes will occur at the beginning of the next separation. Be aware that changes of flow rate will not automatically affect the overall run length.

5. After the last injection is performed, a sample probe wash window is shown to prompt you to wash the probe with a strong solvent followed by a weak solvent. Perform these duties as requested.

### ✓ Note

A weak solvent is used following the usage of the strong solvent to inhibit poor separation due to excessive strong solvent in the probe tubing. It is important to perform Step 5 to ensure there is no carryover into the next separation.

- 6. At the completion of the sequence, the final separation results are displayed. To easily view previous separations, press REWIND to return to the HOME screen.
- 7. Select FILE > OPEN to view previous separations.

If a sample had multiple injections, the run sequence is displayed under a single name preceded by a "+" symbol. This symbol indicates there are multiple injections with the same base file name. Names are appended with "-I(#)" to signify the numerical order of the separation. For example:

- If you have a single injection run, it can be named A01.
- For multiple injections, the first injection can be named A03-I1, the second can be A03-I2, and the third can be named A03-I3.
- 8. Select the file you want to display.
- 9. The remaining injections can be immediately viewed by pressing the left or right arrows at the lower left and right of the RUN FILE viewer window (Figure 4-19). This function can also be used to immediately view files before or after the currently viewed file even if they are not part of the same injection sequence.

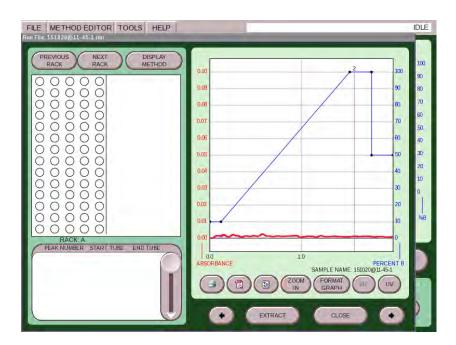


Figure 4-19 Viewing injections

## **4.2.2** Explanation of Operating Procedures

This section outlines the operating procedures for the AutoInjector Module.

- 1. The user is prompted to place the sample probe into the sample container before the separation begins.
- 2. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any left-over fluid from a previous separation and fills the loop with the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
- 3. Near the end of the equilibration, the inject valve moves to the sample load position.
- 4. The AutoInjector Module aspirates enough sample to fill the loop with the programmed amount and to compensate for the volume of the sample probe.
- 5. After the equilibration has completed, the injection valve moves to the separation position and the separation process continues.
- 6. After the first separation has completed, the next separation begins with the equilibration as described above (from Step 2). During this process, the AutoInjector Module continues to aspirate the programmed injection volume.
- 7. When the sample is aspirated for the final injection of the sequence, the total volume of all the sample injections matches the programmed amount.
- If the sample container held less sample than programmed, the final injection aspirates a small

amount of air. Small amounts of air will not damage the HPLC column.

- If the sample container held more sample than programmed, there may be some sample remaining in the probe after the final injection. For this reason, programming the injection sequence with about 0.5 mL more sample volume than the actual sample amount provided in the sample container is recommended. This ensures that all of your sample is processed.
- If some sample remains in the sample probe, it can be recovered by loosening the sample probe fitting at the injection valve to allow the sample to drain back into the sample container.

After completion of the final injection, the system washes the sample probe to prevent sample carryover during the next separation. To perform the wash, the system prompts you to follow the proper wash steps. This is the wash sequence:

- 1. The system prompts you to place the probe in a strong wash solvent. This solvent should be capable of completely washing the sample from the probe.
- 2. 10 mL of strong solvent is aspirated through the probe to wash away any remaining sample from the probe.
- 3. The system prompts you to place the probe in a weak wash solvent.
- 4. 10 mL of weak solvent is aspirated through the probe to wash away any remaining strong solvent from the probe so that it cannot interfere with future separations.

## Automation

## Appendix A Specifications

# A.1 AutoInjector Module Specifications

Dimensions	5.0 x 1.9 x 6.3 inches
Dimensions	5.0 x 1.9 x 6.3 inches
	12.7 x 4.8 x 16.0 cm
Weight	4 lb
	1.8 kg
Temperature	20 - 40 °C
Maximum Humidity	95% relative humidity
Input Voltage	100 - 240 VAC; 50/60 Hz
Amps	2
Disconnect Device	Line cord
Electrical Safety	per EN 61010-1
Pollution Degree	2
Installation	Category II
Maximum Altitude	2,000 m
Sample Size	0.1 mL minimum

# A.2 AutoSampler Module Specifications

Dimensions	18.9 x 14.0 x 19.3 inches
	48.0 x 35.6 x 49.0 cm
Weight	31 lbs
	14.1 kg
Temperature	20 - 40 °C
Maximum Humidity	95% relative humidity
Input Voltage	100 - 240 VAC; 50/60 Hz
Amps	2
Disconnect Device	Line cord
Electrical Safety	per EN 61010-1
Pollution Degree	2
Installation	Category II
Maximum Altitude	2,000 m
Sample Size	0.1 mL minimum

### A.3 Column Selector Module Specifications

Dimensions (vertical setup)	14.5 x 4.6 x 16.1 inches
	36.8 x 11.7 x 40.9 cm
Dimensions (horizontal setup)	4.6 x 14.5 x 16.1inches
	11.7 x 36.8 x 40.9 cm
Weight	12.9 lb
	5.9 kg
Temperature	20 - 40 °C
Maximum Humidity	95% relative humidity
Input Voltage	100 - 240 VAC; 50/60 Hz
Amps	2
Disconnect Device	Line cord
Electrical Safety	per EN 61010-1
Pollution Degree	2
Installation	Category II
Maximum Altitude	2,000 m