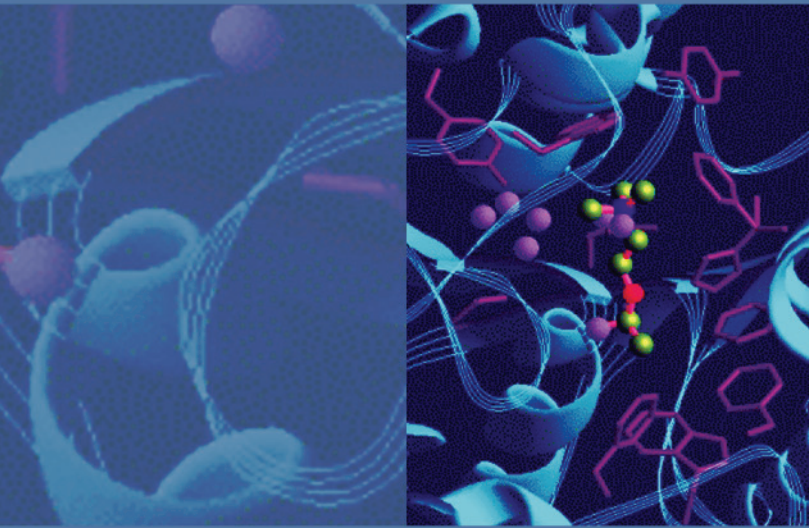




Thermo PAL

for the PAL Autosampler

User Guide

XCALI-97169 Revision C

April 2010

DOCUMENTATION
SURVEY

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Firmware Version: 2.4.0 for the PAL autosampler

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Preface

The CTC PAL™ autosampler manufactured by CTC Analytics is supplied by Thermo Fisher Scientific as part of a solution for high-throughput LC/MS applications. Thermo Pal, one of the device drivers provided on the LC Devices software CD, is the device driver that enables Xcalibur control of the autosampler.

This user guide provides you with information on how to connect the Thermo PAL autosampler to other devices in the LC/MS system and how to operate it from the Xcalibur™ data system. This manual is provided in electronic format only. You can access this manual by choosing the following from the computer desktop:

Start > All Programs > Thermo Instruments > Manuals > LC Devices > CTC > Thermo PAL

To provide us with comments about this document, click the link below. Thank you in advance for your help.



Related Documentation

The Thermo PAL autosampler includes Help and this manual as a PDF file:

- *Thermo PAL Autosampler User Guide*

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:



CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

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❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

❖ **To suggest changes to documentation or to Help**

- Fill out a reader survey online at
http://www.surveymonkey.com/s.aspx?sm=R7gKOvhLXn3NTkpK2BefHQ_3d_3d.
- Send an e-mail message to the Technical Publications Editor at
techpubs-lcms@thermofisher.com.

Getting Connected

The Thermo PAL is a high-throughput autosampler manufactured by CTC Analytics and supplied by Thermo Fisher Scientific. For high-throughput LC/MS applications, use the PAL, a Thermo Scientific or an Agilent liquid chromatography (LC) pump, and a Thermo Scientific MS detector.

This chapter describes the contact closure connections between the PAL, one or two Thermo Scientific LC pumps or an Agilent pump, a Thermo Scientific MS detector, and the Xcalibur data system computer. For information on setting up the PAL, refer to the *CTC Analytics PAL System User Manual*.



CAUTION Follow all of the recommendations given in the Safety Information section of the *CTC Analytics PAL System User Manual*. The Safety Information section includes information on the PAL electrical hazards, lithium battery, and safety labels.

Contents

- [Ordering Information](#)
- [Setting Up an LC/MS System with the Autosampler](#)
- [Connecting the Contact Closure Cables](#)
- [Connecting the PAL to the Data System Computer](#)

Ordering Information

The Thermo PAL options that Thermo Fisher Scientific supplies include the serial communication cable that you use to connect the Thermo PAL to the data system computer and the contact closure cable that you use to connect the Thermo PAL to a Thermo Scientific LC pump and a Thermo Scientific MS detector.

Table 1 lists the Thermo PAL options that you can order from Thermo Fisher Scientific.

Table 1. Thermo PAL ordering information

Part number	Description
OPTON 13008	HTS PAL
OPTON 13009	HTC PAL
OPTON 13010	HTC PAL with the 98 × 2 mL tray holder

Table 2 lists the contact closure cables that you use to connect an Agilent pump to a Thermo Scientific MS detector and an autosampler. You can order the DB15 × 15 wire cable from Agilent or Thermo Fisher Scientific.

Table 2. Contact closure cables used to connect an Agilent pump

Part number	Supplier	Description
CBL 7890	CTC Analytics	PAL Interface Cable for APG Remote DB15 connector × DB9 connector cable
00012-27716	Thermo Fisher Scientific	DB15 connector × 15 wire cable
G1103-61611	Agilent	DB15 connector × 15 wire cable

Setting Up an LC/MS System with the Autosampler

Typical pentop setups include placing the autosampler on top of the MS detector, on top of the two pumps in a dual-pump setup, and to the left of the MS detector. Ensure that the pentop area for the autosampler is level, and provide adequate space behind the autosampler for system cables.

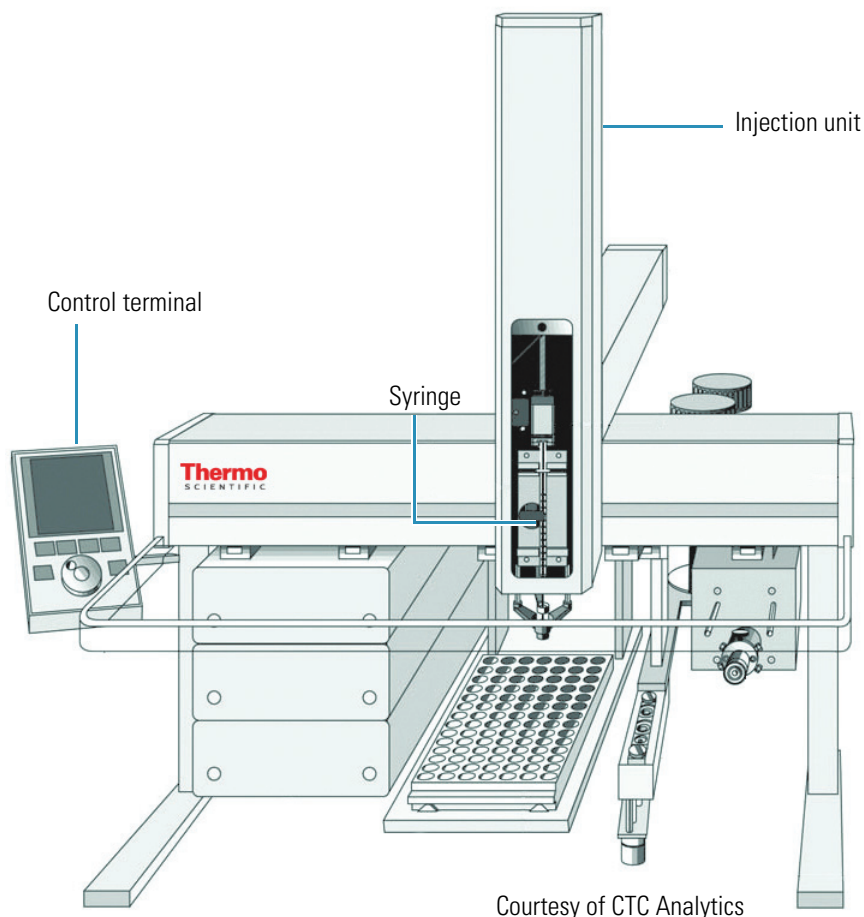


CAUTION To prevent damage to the injection unit during operation, place the autosampler on a level surface.

For information on assembling the PAL and connecting the solvent lines, refer to the *CTC Analytics PAL System User Manual* supplied in hard copy format with the autosampler. During the installation of a Thermo Scientific MS detector, a Thermo Fisher Scientific field service engineer connects the LC system to the MS detector.

Figure 1 shows a front view of the Thermo PAL. During installation, a Thermo Fisher Scientific field service engineer connects the solvent lines between the injection valve and the LC pump and between the injection valve and the Thermo Scientific mass spectrometer.

Figure 1. PAL autosampler



Connecting the Contact Closure Cables

During an injection cycle, the PAL sends and receives electrical signals. To start an injection cycle, the autosampler must receive an electrical signal from the LC pump. When the autosampler completes an injection cycle, it sends an electrical signal to the LC pump. For more information on interfacing the PAL with other devices, refer to the *CTC Analytics PAL System User Manual* (Interfacing the PAL to Other Devices section).

This section describes how to hardwire one or two LC pumps and a Thermo Scientific MS detector to the PAL for contact closure.

To make the contact closure connections, follow the procedure for your LC/MS system:

- [Making Contact Closure with Thermo Scientific Devices](#)

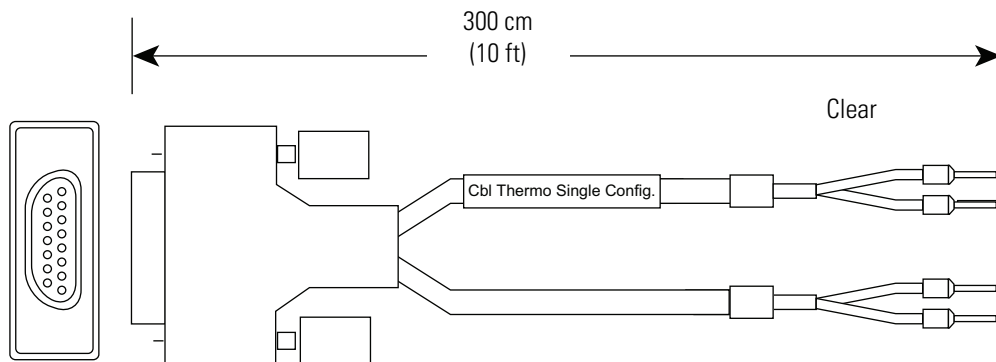
- [Connecting the Start Pump to the Sample Pump in a Dual Pump System](#)
- [Dual-pump setup with two Accela Pumps](#)

Making Contact Closure with Thermo Scientific Devices

The accessory kit supplied by Thermo Fisher Scientific contains the contact closure cables that you need to connect the PAL to an LC/MS system with one or two Thermo Scientific LC pumps and a Thermo Scientific MS detector

[Figure 2](#) shows the Thermo Single Configuration cable assembly that you use to connect one LC pump and a Thermo Scientific MS detector to the autosampler. The cable assembly consists of a DB15 connector with two 2-pin legs. One leg connects to the LC pump, and the other leg connects to the MS detector. During the installation of the LC/MS system, a Thermo Fisher Scientific field service engineer uses the appropriate mini comb icon connectors to complete the contact closure cable assembly, and then connects the assembly to the appropriate pins on the LC pump or LC pumps and the MS detector.

Figure 2. Contact closure cable for LC/MS with one Thermo Scientific LC Pump



❖ To connect the contact closure cable to the LC pump and the MS detector

1. Plug the DB15 connector of the contact closure cable into the 15-pin receptacle on the back of the autosampler.

The receptacle label depends on the autosampler model (see [Table 3](#)).

Table 3. PAL contact closure receptacle

PAL model	Receptacle label
HTC, LC	Interface
HTS	Interface 1

2. Using an 8-pin combicon connector, connect one leg of the contact closure cable to the appropriate pins on the back panel of the Thermo Scientific LC pump ([Table 4](#)).

Table 4. Contact closure cable connection to Thermo Scientific pump

Pump type	Clear wire	Black wire
Surveyor LC Pump Plus	PROG. START (pin 4)	GND
Surveyor MS Pump Plus	GRAD START (pin 8)	GND
Accela Pump	GRAD START (pin 4)	GND

- Using a 2-pin combicon connector, connect the other leg of the contact closure cable to the Start in pins of the Thermo Scientific MS detector.

Figure 3 and Figure 4 show the contact closure connection between the Thermo PAL and a Thermo Scientific LC pump and the contact closure connection between the autosampler and a Thermo Scientific MS detector. Figure 3 is for firmware 2.5.2 and Figure 4 is for firmware 4.1.0.

Figure 3. Contact closure connections to a Thermo Scientific LC pump and an MS detector (firmware 2.5.2)

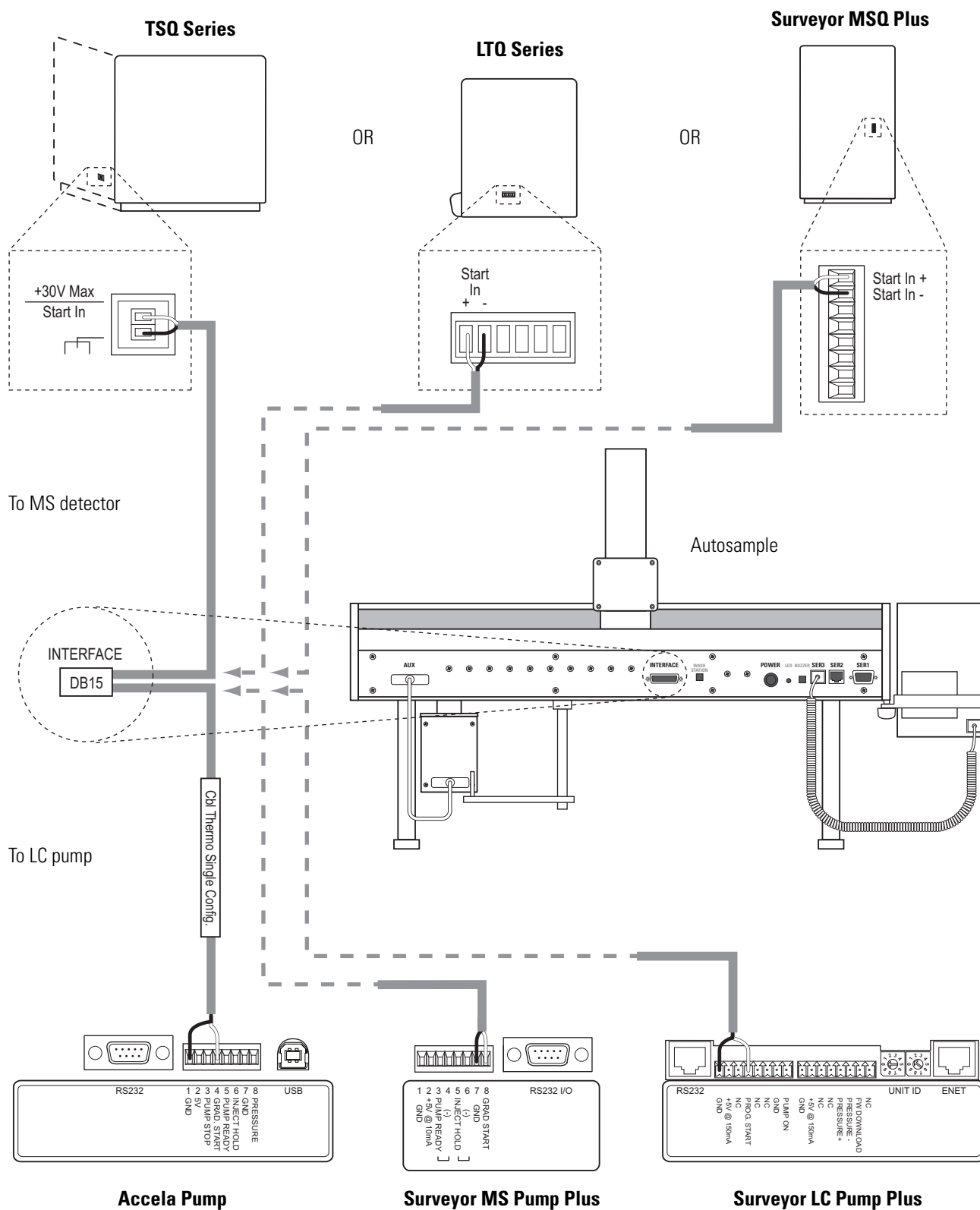
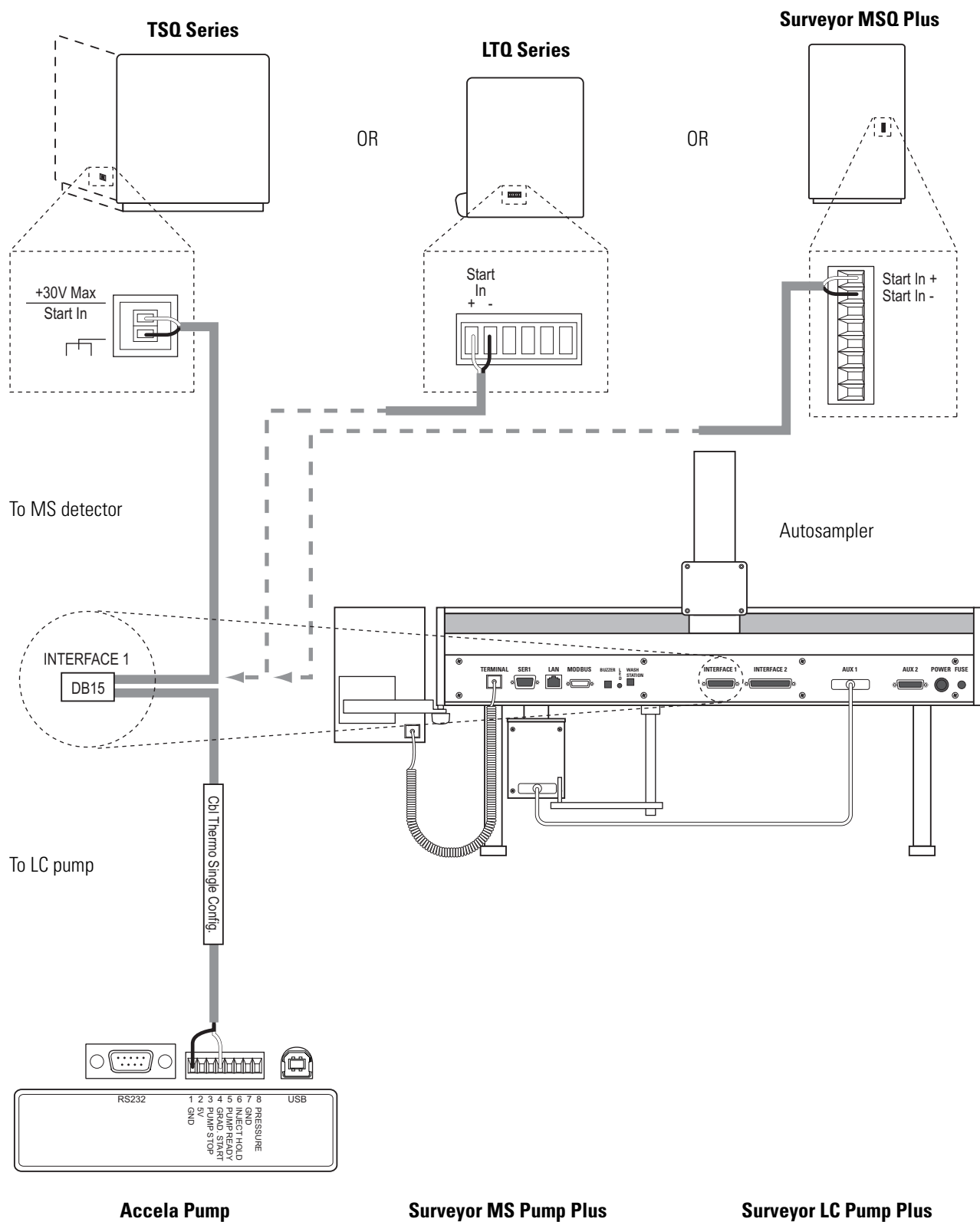


Figure 4. Contact closure connections to a Thermo Scientific LC pump and an MS detector (firmware 4.1.2)



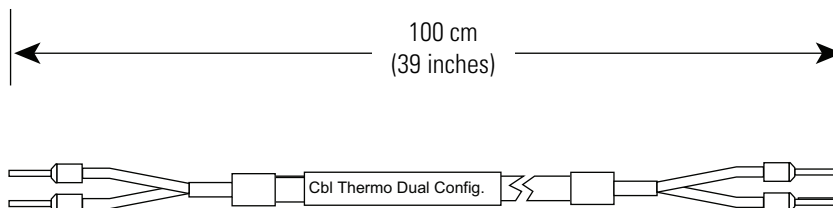
Connecting the Start Pump to the Sample Pump in a Dual Pump System

In a dual-pump system, the start pump triggers the sample pump.

To connect the start pump to the sample pump, you can use the contact closure cable that CTC Analytics supplies, or you can use one insulated electrical wire to connect the ground pins and one insulated electrical wire to connect the GRAD START or PROG. START pins.

Figure 5 shows the contact closure cable that you can order from CTC Analytics.

Figure 5. Contact closure cable (supplied by CTC Analytics) used to connect two LC pumps



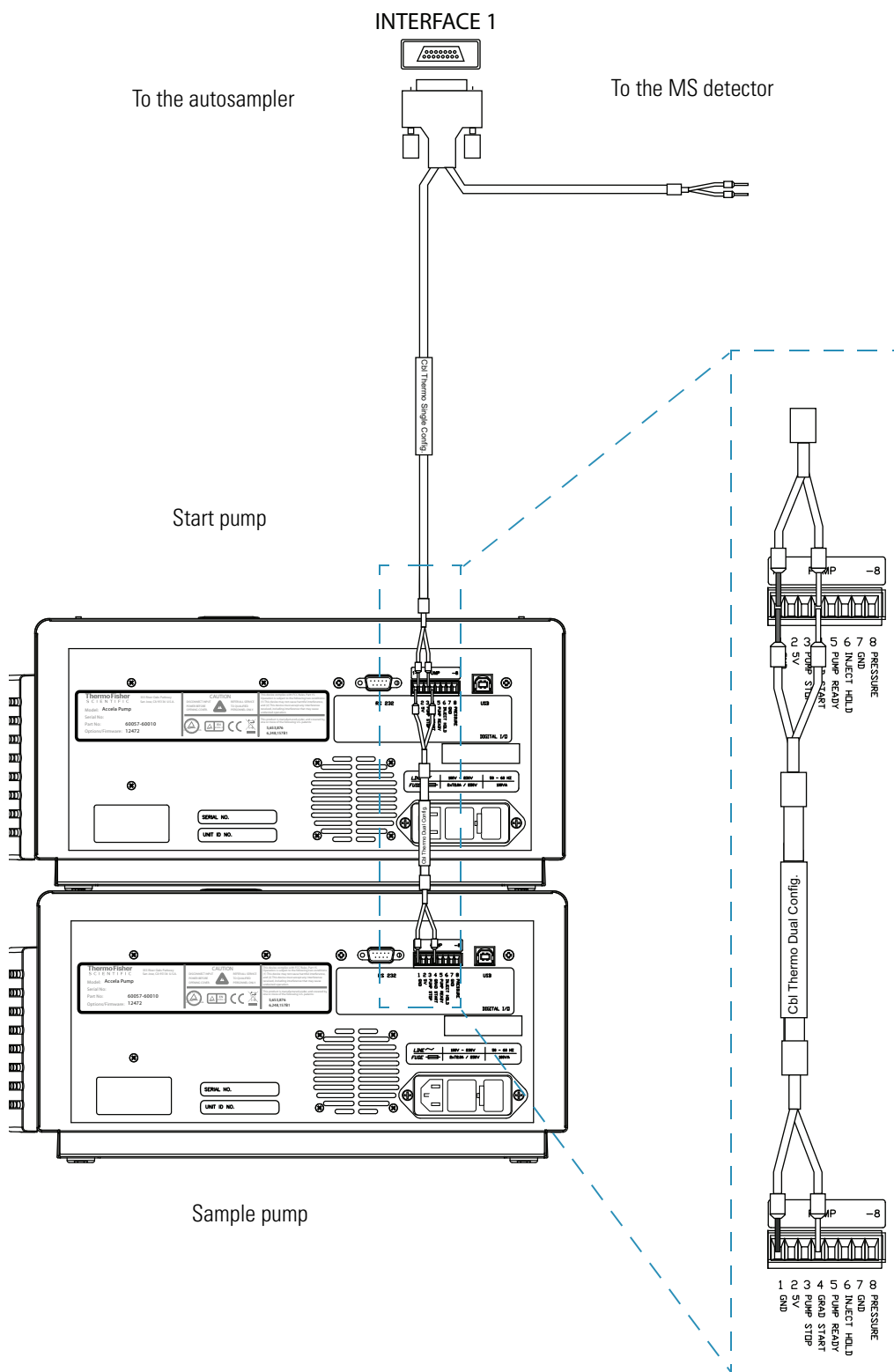
❖ **To make contact closure for a dual- pump system**

1. Connect the DB15 connector of the Thermo Single Config cable to the INTERFACE 1 or INTERFACE receptacle on the back panel of the Thermo PAL.
2. Connect one of the two pin legs of Thermo Single Config cable to the start pump.
3. Connect the other two pin leg of the Thermo Single Config cable to the Thermo Scientific MS detector.
4. Connect the start pump to the sample pump:
 - Connect the start pump ground pin to the sample pump ground pin.
 - Connect the Grad Start or Prog. Start pin on the start pump to the Grad Start or Prog. Start on the sample pump.

Table 4 on page 5 lists the pin assignments of the Accela Pump, Surveyor MS Pump Plus, and the Surveyor LC Pump Plus.

Figure 6 shows two Accela Pumps connected together for contact closure.

Figure 6. Dual-pump setup with two Accela Pumps



Making Contact Closure with the Autosampler, an Agilent Pump, and an MS Detector

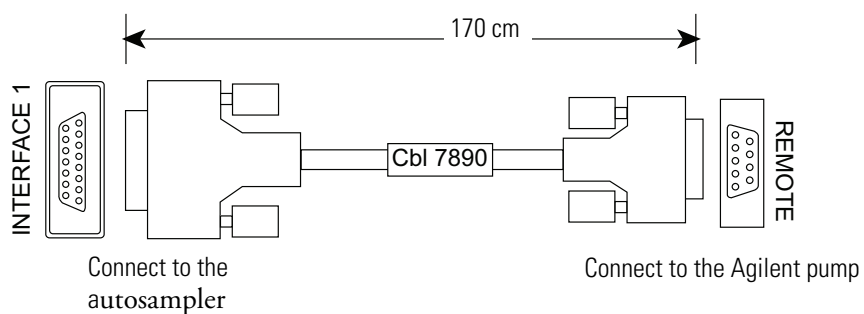
To make contact closure between the autosampler and an Agilent pump and between an Agilent pump and a Thermo Scientific MS detector, you must have the following cables:

- PAL interface cable (supplied by CTC or Agilent) for an Agilent pump (see [Figure 7](#))
- External contact trigger cable supplied by Thermo Fisher Scientific (see [Figure 8](#))

❖ To make contact closure between the autosampler, an Agilent Pump, and a Thermo Scientific MS detector

1. Using the PAL interface cable (see [Figure 7](#)), connect the Agilent pump to the CTC PAL:
 - Connect the DB15 connector to the INTERFACE (HTC) or INTERFACE 1 (HTS) receptacle on the back panel of the PAL (see [Figure 9](#) and [Figure 10](#)).
 - Connect the DB9 connector to the REMOTE receptacle on the back panel of the Agilent pump (see [Figure 9](#) and [Figure 10](#)).

Figure 7. PAL interface cable for an Agilent pump (supplied by CTC Analytics)



2. Using the external contact trigger cable (see [Figure 8](#)), connect the Agilent pump to the MS detector:
 - Connect the DB15 connector to the RELAY CONTACTS receptacle on the back panel of the Agilent pump (see [Figure 9](#) and [Figure 10](#)).

For information on installing the external contact board, refer to the *Agilent Getting Connected Guide* (1100 Series or 1200 Series) that is provided as a PDF with LC Devices.

- Connect the white wire to the Start In pin and the brown wire to ground on the MS detector (see [Figure 9](#) and [Figure 10](#)). Secure the remaining 13 wires to the cable with duct tape.

Figure 8. External contact trigger cable (supplied by Thermo Fisher Scientific)

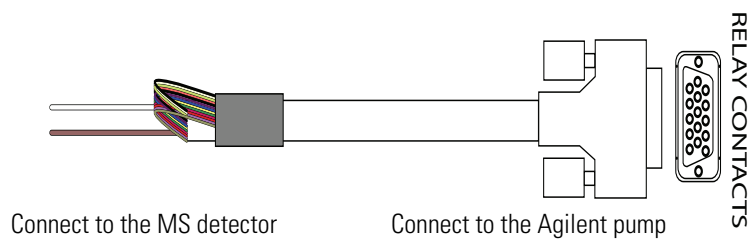


Figure 9. Contact closure with an Agilent pump, the autosampler, and a Thermo Scientific MS detector (firmware 2.5.2)

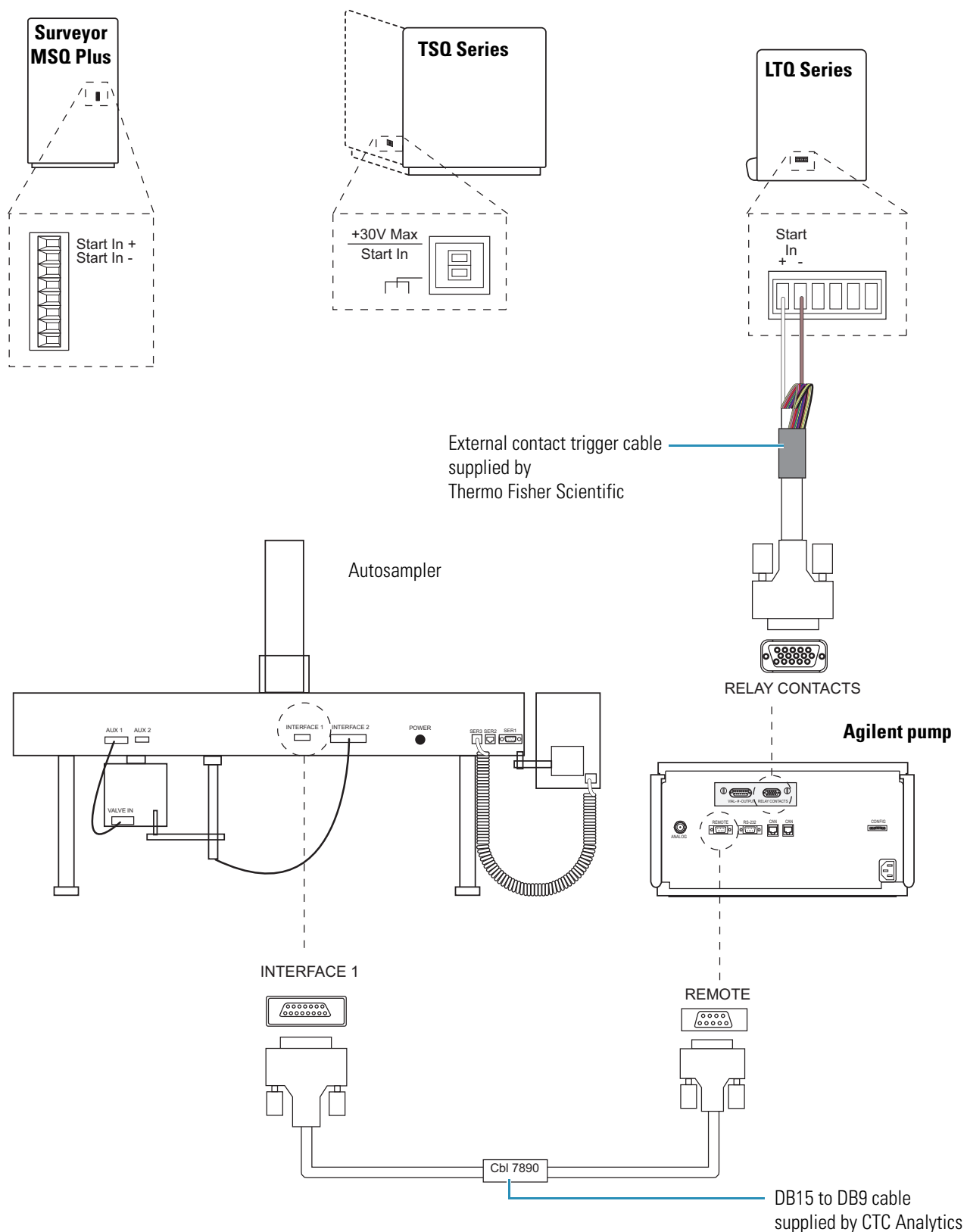
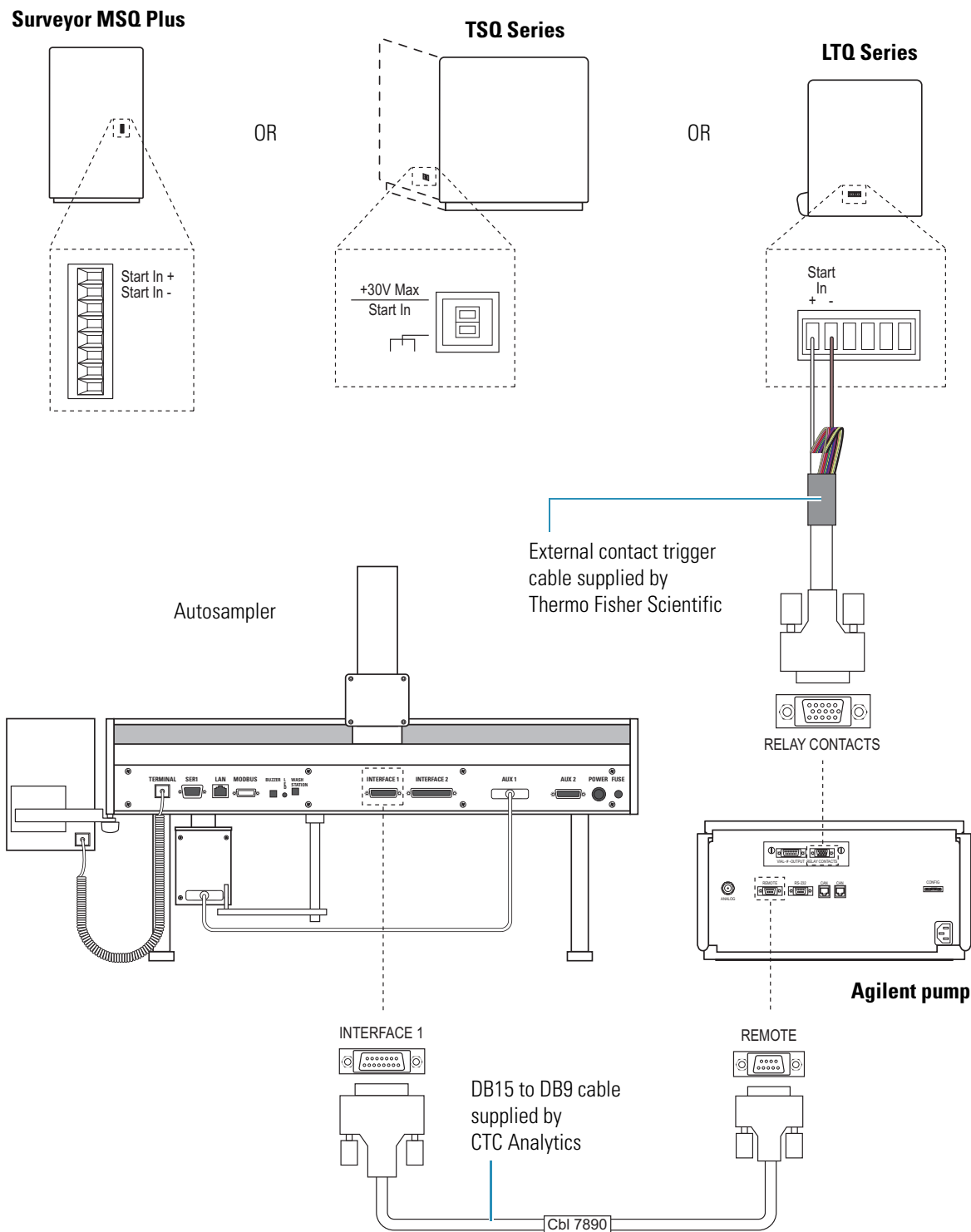


Figure 10. Contact closure with an Agilent pump, the autosampler, and a Thermo Scientific MS detector (firmware 4.1.2)



Connecting the PAL to the Data System Computer

❖ To connect the PAL to the data system computer

Using the serial cable that is supplied with the autosampler, connect the SER 1 port on the back panel of the autosampler to the serial port on the back panel of the data system computer (see [Figure 11](#) and [Figure 12](#)).

Figure 11. Data system connection (firmware 2.5.2)

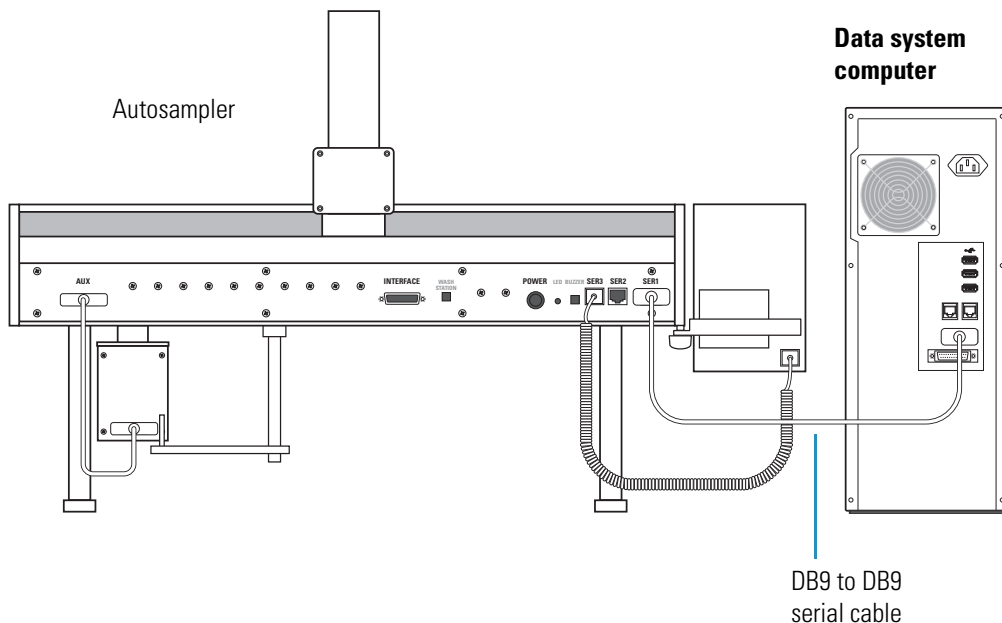
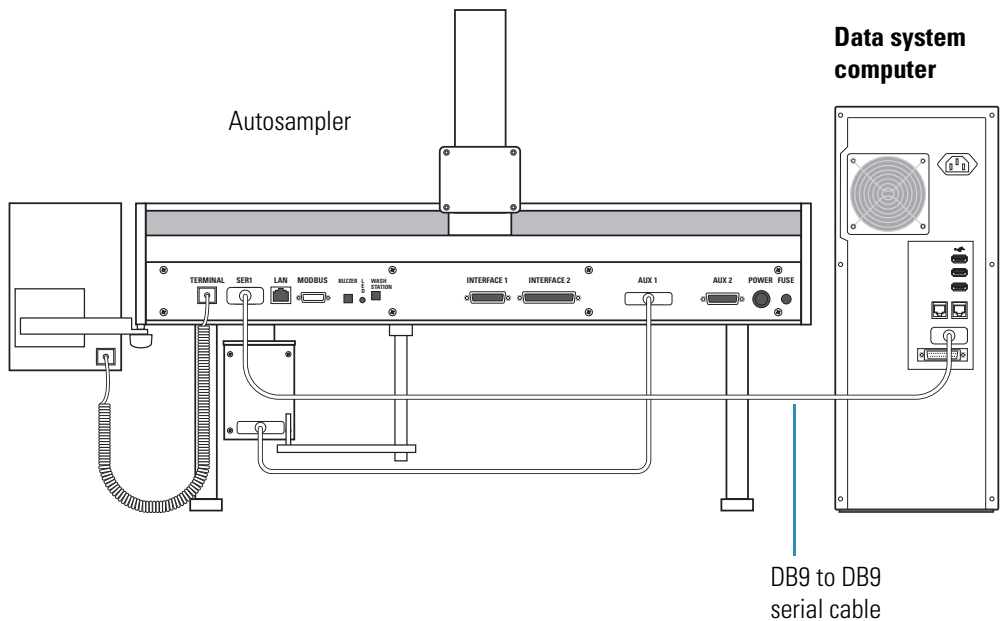


Figure 12. Data system connection (firmware 4.1.2)



Getting Started

This chapter describes how to add the autosampler to the Xcalibur instrument configuration, how to specify the available tray types from the PAL control terminal, and how to specify the injection parameters for the autosampler from the Xcalibur data system.

Contents

- [Adding the Autosampler to the Xcalibur Instrument Configuration](#)
- [Using the Autosampler Control Terminal](#)
- [Specifying the Instrument Method Parameters for the Autosampler](#)

Adding the Autosampler to the Xcalibur Instrument Configuration

The Thermo Pal device driver provides control of the autosampler from the Xcalibur data system. To control the autosampler from Xcalibur, add the autosampler to the Xcalibur instrument configuration and specify the data system COM port that the autosampler is connected to.

Specifying the Configuration Options

This section provides information about how to specify configuration options for the autosampler.

❖ To specify the configuration options for the autosampler

1. Open the Instrument Configuration window from the computer desktop:

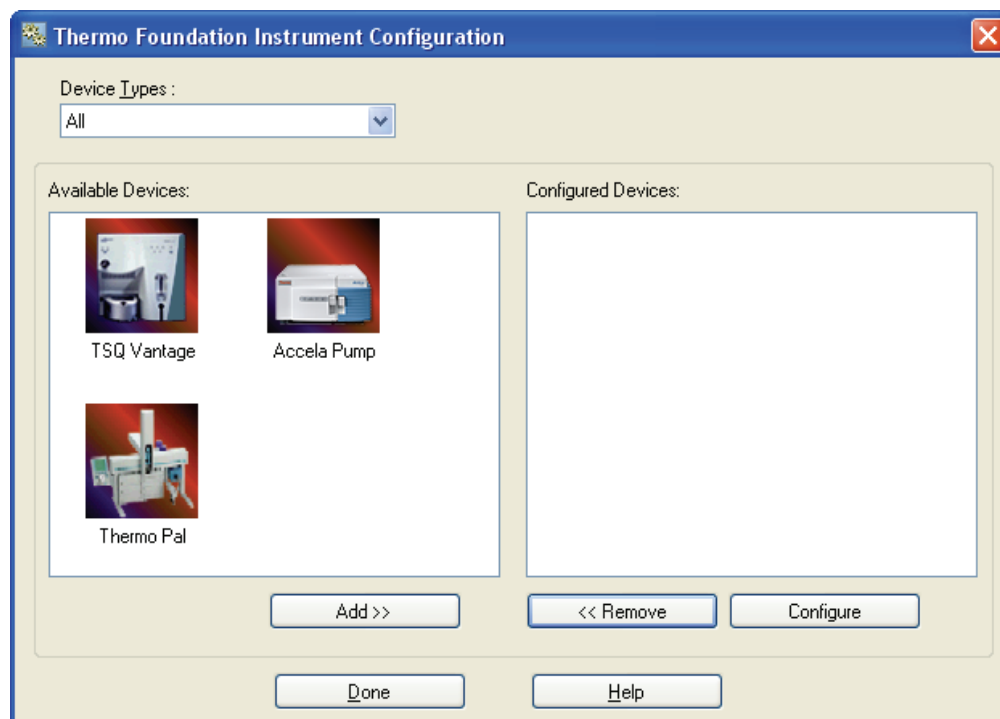
For Xcalibur 2.1.0 or higher, choose **Start > All Programs > Thermo Foundation 1.0 > Instrument Configuration**.

The Unloading Device Drivers dialog box appears, followed by the Thermo Foundation Instrument Configuration window (see [Figure 13](#)).

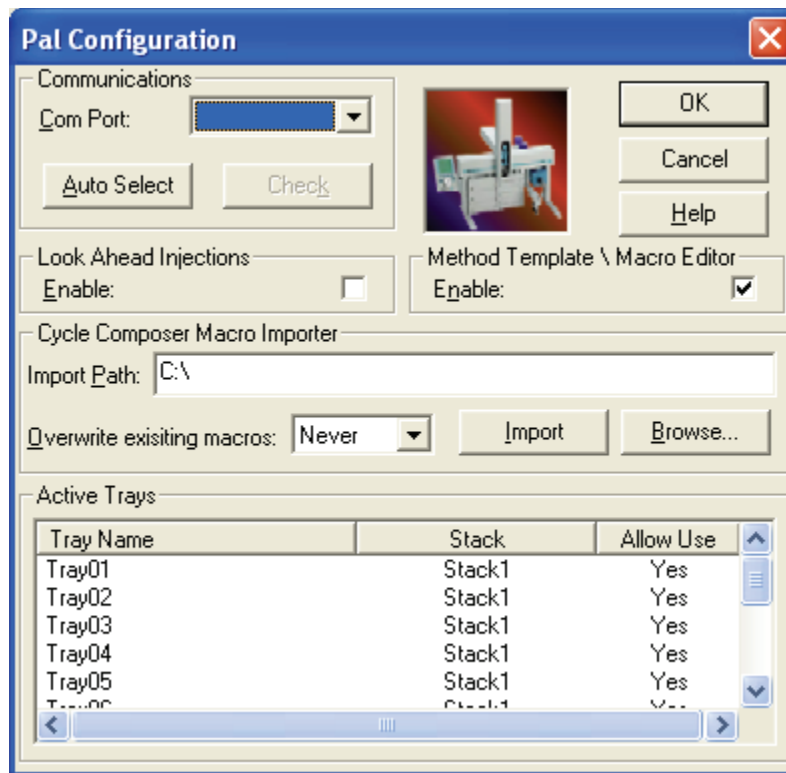
2 Getting Started

Adding the Autosampler to the Xcalibur Instrument Configuration

Figure 13. Thermo Foundation Instrument Configuration window



2. In the Device Types list, select **All**.
3. In the Available Devices list, double-click autosampler
A copy of the Thermo Pal icon appears in the Configured Devices list.
4. In the Configured Devices list, double-click  (Thermo Pal).
The PAL Configuration dialog box appears (see [Figure 14](#)).

Figure 14. Pal Configuration dialog box

5. Select the data system computer Com port that the autosampler is connected to:
 - a. In the Com Port list, select the Com port that the autosampler is connected to. Or, click **Auto Select**.

Tip The autoselect feature works only if the autosampler is connected to the data system computer and turned on.

- b. If you have already connected the autosampler to the data system computer, click **Check** to verify the connection.
6. To shorten the time between injections, select the **Enable** check box under Look Ahead Injections. When you enable look ahead injections, the autosampler sets up for the next injection during the current run. The set up for the next injection involves carrying out the steps up to, but not including, the injection.

Note Do not modify or delete a sequence while the data system is running with the Look Ahead Injections enabled. Use the stop function to finish the current injection before you modify or delete a sequence.

7. To make the Method Template and Macro Editor dialog boxes available in the Instrument Setup window, select the **Enable** check box under Method Template\Macro Editor.

8. In the Cycle Composer Macro Importer area, import the macros that you plan to use to create injection methods:
 - a. In the Import Path box, type the location where the macros reside. Or, click **Browse** to locate the directory that contains the appropriate macros.
 - b. In the Overwrite existing macros list, make one of the following selections:
 - **Never.** The autosampler does not import the specified macro if it has the same filename as an existing macro.
 - **Ask.** The autosampler asks you if you want to overwrite existing macros.
 - **Always.** Imported macros overwrite existing macros.
 - c. Click **Import** to import the macros.
 9. In the Active Trays area, select the trays that you want to use. You can use trays that have a Yes in the Allow Use column. Clicking the tray name clears the Allow Use cell and removes the tray from the list of available trays in the Xcalibur data system.
- IMPORTANT** Use the autosampler control terminal to specify the tray information. You can change the tray information when you are controlling the autosampler from the Xcalibur data system, but these changes are temporary. When you close and reopen the Xcalibur data system, the tray information resets to the autosampler control terminal settings.
10. To accept the configuration settings and close the Pal Configuration dialog box, click **OK**.
 11. Configure the other instruments of your LC/MS system, such as the LC pump and the mass spectrometer.
 12. In the (Thermo Foundation) Instrument Configuration window, click **Done**.

PAL Configuration Parameters

Use the PAL Configuration dialog box to select the PC Com port where the autosampler is connected, specify Macros to import, and select sample trays or stacks.

Table 5. PAL Configuration dialog box parameters (Sheet 1 of 2)

Parameter	Description
Communications	
Com Port	Select the computer port where you plug the autosampler communication cable.
Auto Select	Instructs the system to automatically determine the computer port connected to the autosampler through the autosampler communication cable.

Table 5. PAL Configuration dialog box parameters (Sheet 2 of 2)

Parameter	Description
Check	Instructs the system to verify the computer port connected to the autosampler through the autosampler communication cable.
Look Ahead Injections	
Enable	Selecting this check box enables Look Ahead Injections. When Look Ahead Injections is enabled, the autosampler sets up for the next injection during the current run. The set up for the next injection involves carrying out the steps up to, but not including, the injection.
Method Template\Macro Editor	
Enable	Select this check box when you want the Method Template and Macro Editor to be accessed via Instrument Set Up. Ensuring the check box is clear prevents access to the Method Template and the Macro Editor.
Cycle Composer Macro Importer	
Import Path	Enter the path to the Cycle Macro to be imported.
Overwrite existing macros	Choices are Never, Ask, Always. Uses chosen action to determine if existing macros are to be overwritten when a new macro is imported.
Import	Import the chosen macro.
Browse	Search through files and folders to find a macro.
Active Trays	
Tray Name	Hardware configured tray name.
Stack	Stack location of a given Tray Name.
Allow Use	Click to switch between YES and blank. YES means that a given tray IS accessible to the PAL system for obtaining samples (use is allowed). A blank in this row means that the tray is NOT accessible to the PAL system for obtaining samples (use is NOT allowed).
Communications	
Com Port	Select the computer port where you plug the autosampler communication cable.
Auto Select	Instructs the system to automatically determine the computer port where you plug the autosampler communication cable.

Firmware Versions Supported

For Thermo PAL autosampler, the only supported firmware versions are 2.5.2 and 4.1.2.

Using the Autosampler Control Terminal

For general information on using the autosampler control terminal, refer to the *CTC PAL System User Manual*.

To select the sample trays and to change the default pulse time setting to 4.0 seconds, follow these procedures:

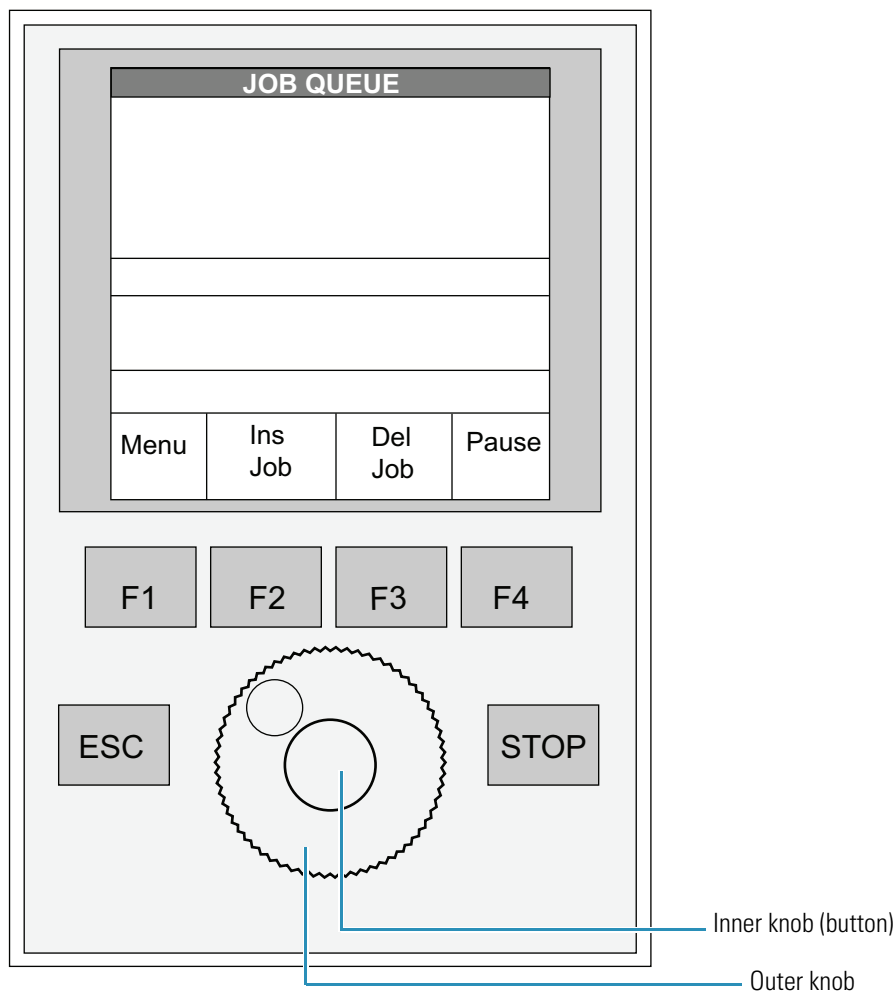
- [Selecting the Tray Type](#)
- [Changing the Pulse Time Setting](#)

Selecting the Tray Type

❖ To select the tray type

1. In the Job Queue screen, select the Menu screen by pressing the F1 key (see [Figure 15](#)).

Figure 15. Autosampler control terminal with the Job Queue screen displayed



2. Open the Tray screen as follows:
 - a. In the Menu screen, rotate the outer knob to move the cursor bar (highlight) to Setup.
 - b. Press the inner knob (button) to select Setup.
 - c. Rotate the outer knob to move the cursor bar to Objects.
 - d. Press the inner knob (button) to select Objects.
 - e. Rotate the outer knob to move the cursor bar to Trays.
 - f. Press the inner knob (button) to select the Trays screen.

The Trays screen lists the available trays.

3. Select a tray as follows:
 - a. Rotate the outer knob to move the cursor bar to the Tray to be selected.
 - b. Select the Tray by pushing the inner knob (button).

The screen now shows the Tray Type.

- c. Make the Tray Type active by pushing the inner knob (button).

The Tray Type is highlighted.

- d. Rotate the outer knob to select the tray type. Select NONE if no tray is present (rotate the outer knob until NONE appears as the Tray Type).
 - e. Push the inner knob (button) to select this tray type.
4. Repeat [step 3](#) for all the trays to be used.
5. Return to the Job Queue screen by pushing the F4 key (Home).

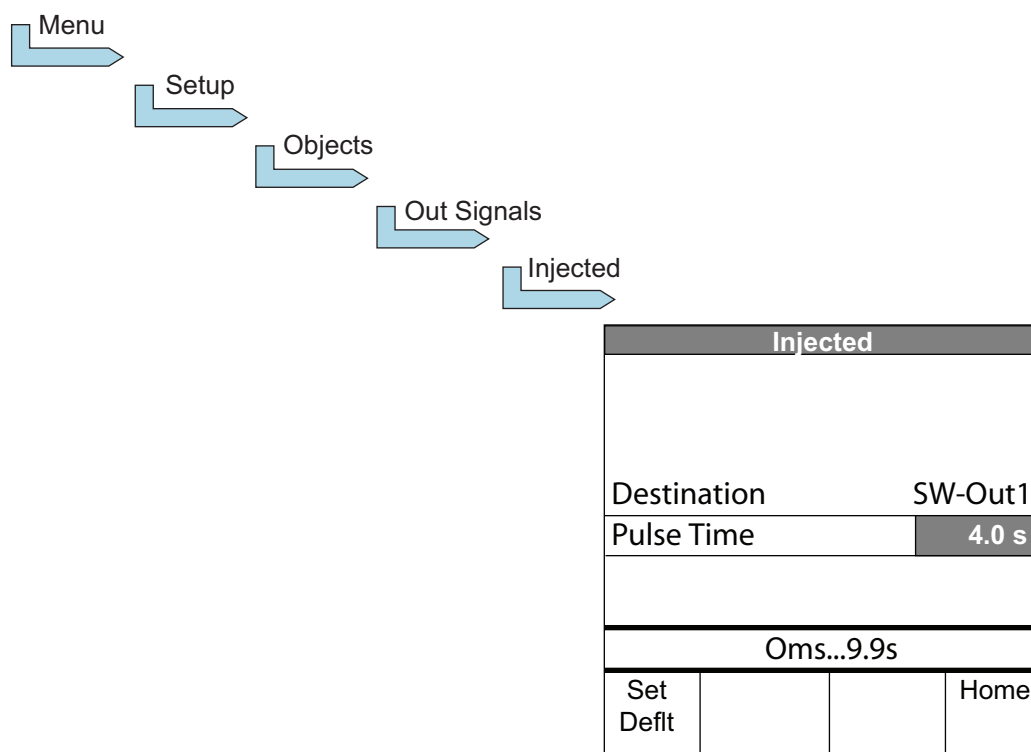
Changing the Pulse Time Setting

To control LC systems with a Thermo Scientific LC pump and an autosampler from the Xcalibur data system, you must change the default pulse time setting for the autosampler from 2 seconds to 4 seconds. The pulse time is the length of time that the autosampler sends an output signal after it makes an injection. The LC pump receives this output signal through the contact closure cable.

IMPORTANT If your LC system contains a Thermo Scientific LC pump and an autosampler, you must change the autosampler's default pulse time setting. If you leave the default pulse time set to 2 seconds, the status of the LC pump remains at waiting for contact closure after you make an injection.

Change the pulse time setting to 4.0 seconds from the Injected screen (see [Figure 16](#)).

Figure 16. Menu path to Injector screen



❖ **To change the pulse time setting**

1. Open the Injected screen:
 - a. In the Job Queue screen, select the Menu screen by pressing the F1 key (see [Figure 15](#)).
 - b. Rotate the outer knob to move the cursor bar (highlight) to Setup, and then press the inner knob (button). The Setup screen appears.
 - c. Rotate the outer knob to move the cursor bar (highlight) to Objects, and then press the inner knob (button). The Objects screen appears.
 - d. Rotate the outer knob to move the cursor bar (highlight) to Out Signals, and then press the inner knob (button). The Out Signals screen appears.
 - e. Rotate the outer knob to move the cursor bar (highlight) to Injected, and then press the inner knob (button). The Injected screen appears (see [Figure 16](#)).
2. Rotate the outer knob to move the cursor bar (highlight) to Pulse time, and then press the inner knob (button). The time cell is highlighted.
3. Rotate the out knob to change the pulse time to 4.0 seconds, and then press the inner knob (button) to set this selection as the default.
4. Return to the Job Queue screen by pushing the F4 key (Home).

Specifying the Instrument Method Parameters for the Autosampler

To inject a sample set automatically, you must create an instrument method that contains the chromatographic conditions and mass spectrometer data acquisition settings, and then create an acquisition sequence that specifies the instrument method and vial location for each run. This section describes how to specify the basic instrument method parameters for the autosampler. For information about creating acquisition sequences, refer to the Sequence Setup view Help.

Creating an Instrument Method

This section provides information about how to create an instrument method.

❖ To specify the instrument method parameters for the autosampler

1. Open Xcalibur from the computer desktop:

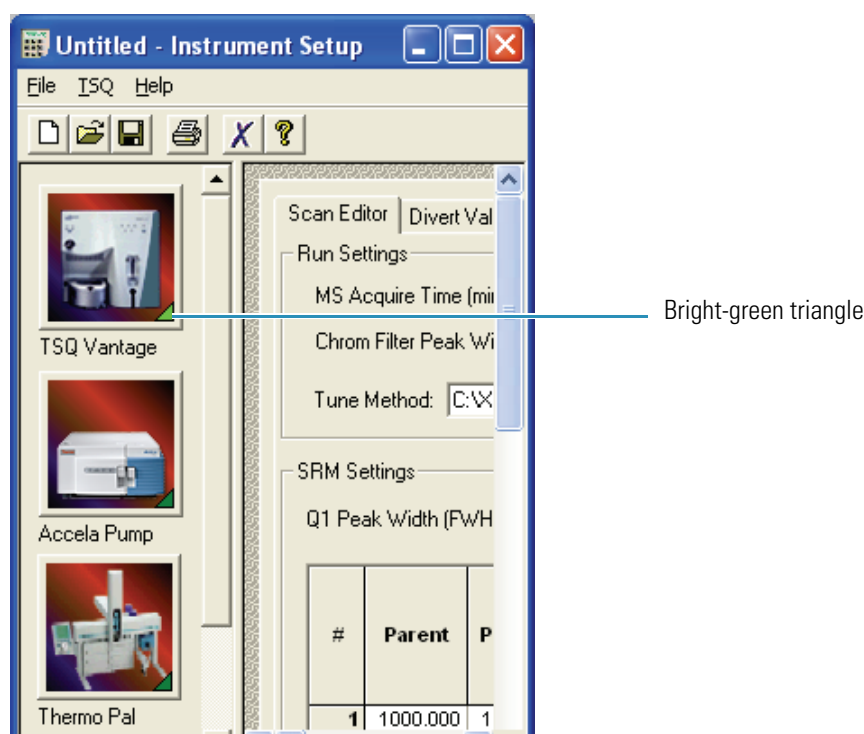
For Xcalibur version 2.1.0 or higher, choose **Start > All Programs > Thermo Xcalibur > Xcalibur**.

The Roadmap Home Page appears.

2. Click  (Instrument Setup).

The Instrument Setup window appears with the configured devices displayed in the view bar (see Figure 17). By default, the view for first device is open. A bright-green triangle in the lower-right corner of the device icon indicates that the view for the device is open.

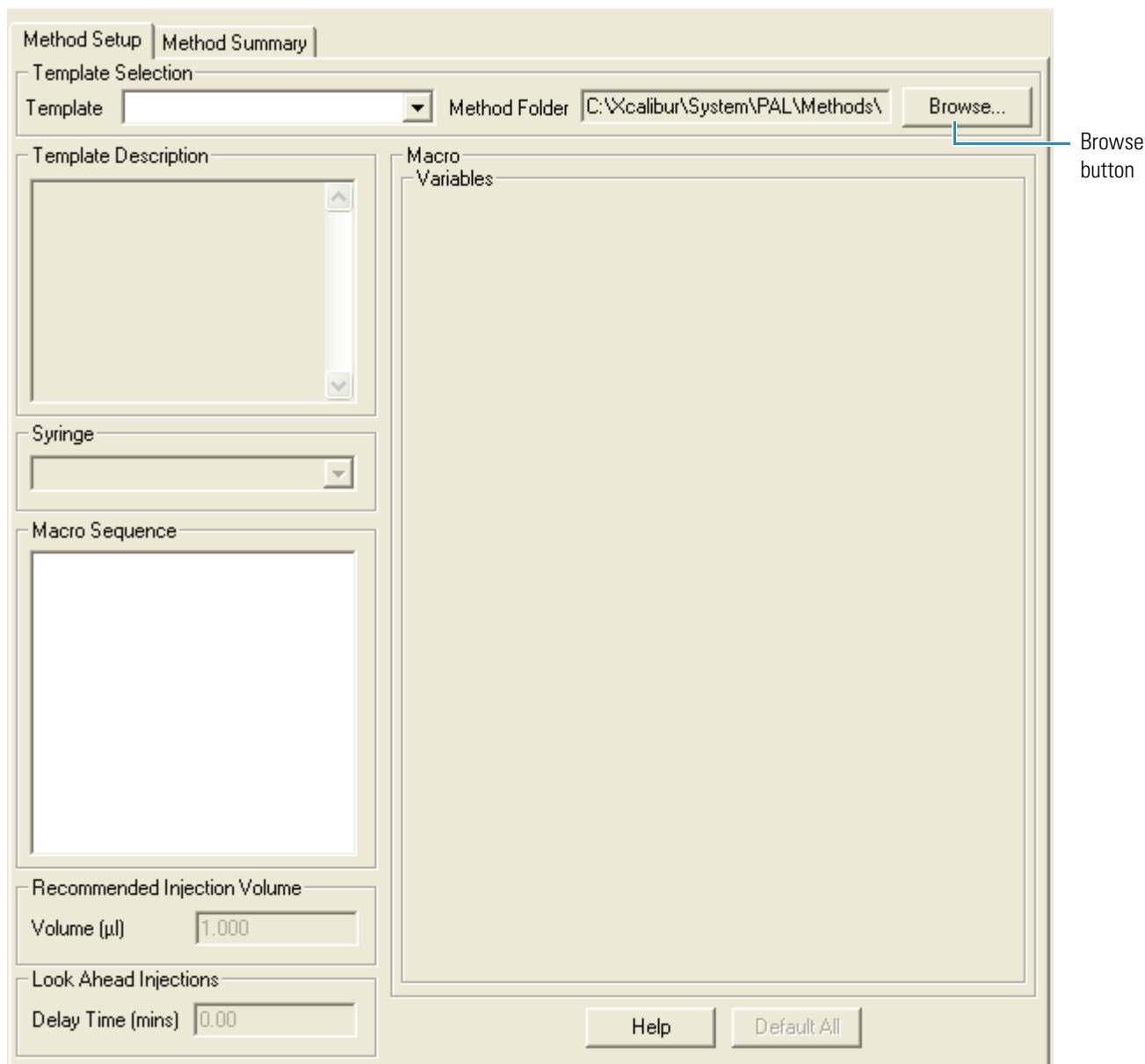
Figure 17. Instrument Setup window



3. In the view bar, click  (Thermo Pal).

The Method Setup page for the autosampler appears (see [Figure 18](#)).

Figure 18. Method Setup page for the autosampler

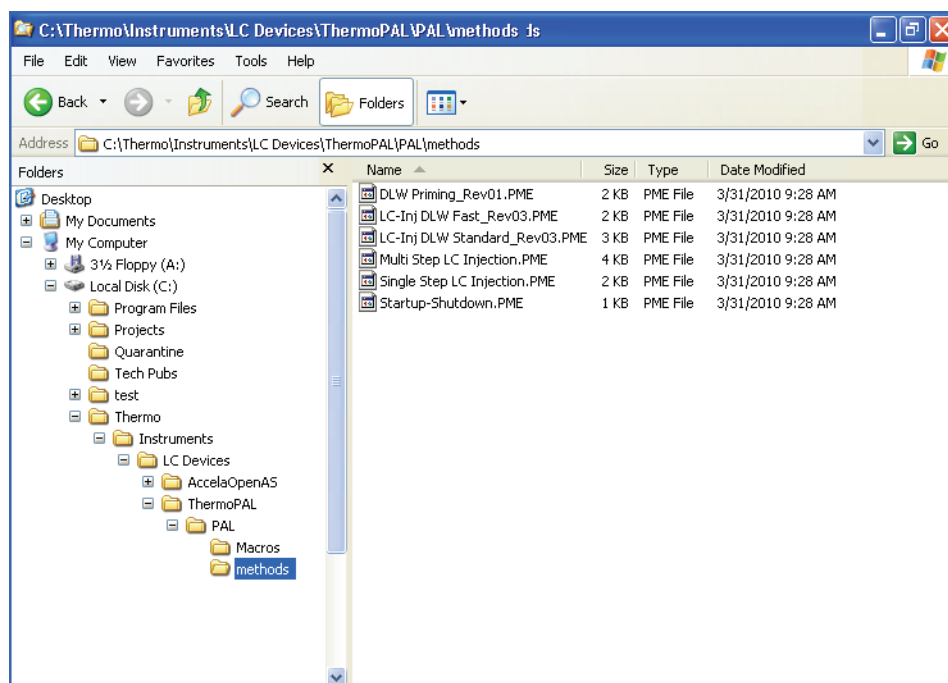


4. Under Template Selection, click **Browse** and browse to the folder that contains the autosampler methods (*.pme).

For a standard installation, the folder that contains the autosampler method is located in the following directory (see [Figure 19](#)):

C:\Thermo\Instruments\LC Devices\ThermoPAL\PAL\methods

Figure 19. Location of Thermo PAL autosampler templates



5. In the Template list, select a Template that represents the task that you want to do.

The autosampler comes with three templates: Single Step LC Injection.pme, Multi Step LC Injection.pme, and Startup-Shutdown.pme.

For high-throughput applications, use Single Step LC Injection.pme. If your applications require more pre- and post-injection cleaning steps, use Multi Step LC Injection.pme. The Startup-Shutdown.pme template is reserved for future use.

In addition to these templates, you can create your own custom templates (see [“Using the Template Editor to Create Custom Templates”](#) on page 33). When you store a custom template in the same folder as the standard templates, the template appears in the Template list.

[Figure 20](#) shows the macro sequence and the macro variables for the Single Step LC Injection template.

Figure 20. Method Setup page with LC-Inj template

Method Setup | Method Summary

Template Selection

Template: C:\Xcalibur\System\PAL\Methods\Single Step LC Injection.pme New Template

Template Description

Duplication of PAL local LC-Inj cycle.
This is only a sample method for short runs. For analytical work the method parameters must be set according to the specific needs.

Syringe

100ul

Macro Sequence

LC-Inj

Recommended Injection Volume

Volume (µl) 10.000

Look Ahead Injections

Delay Time (mins) 0.00

Macro "LC-Inj", 1 of 1

Variables

Air Volume (µl)	0
Pre Clean with Solvent 1	0
Pre Clean with Solvent 2	0
Pre Clean with Sample	0
Filling Speed (µl/s)	50
Filling Strokes	1
Inject to	LC Vlv1
Injection Speed (µl/s)	100
Pre Inject Delay (ms)	500
Post Inject Delay (ms)	500
Post Clean with Solvent 1	1
Post Clean with Solvent 2	0
Valve Clean with Solvent 1	1
Valve Clean with Solvent 2	1

Help Default All

Macro sequence for the Single Step LC Injection template

Variables list

When you select a template, the macros used by the template appear in the Macro Sequence area. When you click on a macro listed in the Macro Sequence area, the variables used by the Macro appear in the Variables area.

- In Variables area, keep the parameters set to the defaults or type the appropriate values for your application.

7. In the Syringe list, select the size of syringe that is installed in the autosampler.

The default variable settings for the Single Step LC Injection template and the default setting of 10 µL in the Recommended Injection Volume box are suitable for a 100 µL syringe. Changing the syringe size can change the allowable ranges for the recommended injection volume and the variables specified in the LC-Inj macro.

IMPORTANT Make sure that your syringe selection matches the size of the syringe that is installed in the autosampler.

- If the autosampler is set up to recognize the installed syringe size, an error message appears when you download methods that specify a different syringe size.
- If the autosampler is not set up to recognize the installed syringe and the specified syringe size does not match the actual syringe size, the autosampler does not inject the specified injection volume.

8. Under Recommended Injection Volume, in the Volume (µL) box, type the volume of sample that you want the autosampler to inject.

The allowable injection volume range is based on the syringe size.

IMPORTANT For partial loop injections (variable volume), make sure that the recommended injection volume is less than half the sample loop size.

9. Under Look Ahead Injections, if the Delay Time (mins) box is available, type the amount of time that you want the autosampler to wait after it completes the current injection before it starts the next injection.

During a look ahead injection, the autosampler aspirates the sample for the next injection and waits until the end of the current run to inject the sample into the valve. When you add a delay time, the autosampler does not begin the next injection cycle immediately after completing the current injection cycle. If you enter a delay time longer than the method run time, the autosampler begins the next injection at the end of the current run. This means that adding a long delay time does not add additional run time to the method run time, but it does cancel the effect of using the Look Ahead Injection feature.

Note The Look Ahead Injections feature is available if you selected the Enable check box in the Look Ahead Injections area of the PAL Configuration dialog box.

10. Specify the acquisition parameters for the other devices of your LC or LC/MS instrument.

11. Save the method:

- a. From the Instrument Setup window menu bar, choose **File > Save As**.

The Save As dialog box appears.

- b. Select an appropriate file location for the method.
- c. In the File name box, type an appropriate name for the method.

- d. Click **Save**. The File Summary Information dialog box appears.
- e. (Optional) In the Comment box, type additional information about the method.
- f. Click **OK**.

Xcalibur (or other Thermo Scientific data acquisition application such as LCquan) stores the method as a *.meth file in the specified file location.

Instrument Setup Parameters

Use the Method Setup page to specify instrument method parameters for the autosampler.

Table 6. Method Setup page parameters (Sheet 1 of 2)

Parameter	Description
Template Selection	
Template	This box shows the names of the default method templates. After you select a template, the Template box changes to a pane. This pane shows the path to the chosen method template file.
Method Folder	This pane shows the path to the default template folder location before a default method template is chosen. After a default method template is chosen (via the Template box) the Method Folder label disappears, leaving the Template label next to a pane that shows the path to the chosen template.
Browse	The Browse button can be seen next to the Method Folder pane before the method template is chosen in the Template box. Click the browse soft button to browse through the computer file system for the method template of your choice. When you select a method template, the window changes to show the path to the chosen template and the Browse button changes to the New Template button.
New Template	This soft button appears after a method template is chosen (in the Template box). Selecting this button brings back the Template box, Method Folder pane, and Browse button. These are used to find a new method template.
Template Description	A description of the template chosen in the Template Selection portion of the Method Setup page.
Syringe	This list contains the allowed syringe volumes. Select the appropriate syringe volume.
Macro Sequence	Displays the sequence of macros that make up the method chosen in the Template window. Select a macro name to be displayed in the Macro area; its variables also appear in the Variables area. (See table continuation.)

Table 6. Method Setup page parameters (Sheet 2 of 2)

Parameter	Description
Recommended Injection Volume	
Volume (µl)	1) When you select an instrument method for use to generate a new sequence (on the Xcalibur Home Page), the injection volume is set to what is entered in this box. 2) When "From AS" (From Auto Sampler) is specified in the LCquan injection volume sequence cell, the CTC PAL autosampler uses the volume in this box when you submit the LCquan sequence for acquisition.
Look Ahead Injections	
Delay Time (mins)	Enter the Delay Time (in minutes) between injections when using the Look Ahead feature. You enable the Look Ahead Injections option from the PAL Configuration dialog box. When you enable this option, the next Look Ahead Injection is delayed the amount of time entered in this box. The delay time countdown begins at the completion of any post-injection steps. This prevents a sample from being held in the injection syringe for the duration of the current run. If the entered Delay Time is too long, or the current run ends prematurely, the entered Delay Time is canceled and the next injection commences in the normal manner.
Macro	The Macro area shows the name of the macro selected in the Macro Sequence pane. The sequence number (<i>x</i>) of the selected macro out of the total number of macros (<i>y</i>) in the Macro Sequence is also displayed next to the name of the selected macro (Macro " <i>name</i> ", <i>x</i> of <i>y</i>). This area also lists the Variables associated with the macro chosen in the Macro Sequence pane.
Variables	The variable parameters used by the selected macro are displayed in this portion of the Macro area. Variables with specific allowed values (discrete variables) are displayed in lists. Continuous variables are displayed in panes. The allowed values of the discrete variables may be chosen from those presented in the list for the variable. The allowed values for continuous variables are displayed when you hover the cursor over the respective pane for the variable. The desired value for the continuous variable may be typed in its pane.
Default All	This button changes the variables, used in the selected Macro Sequence, to their default values.

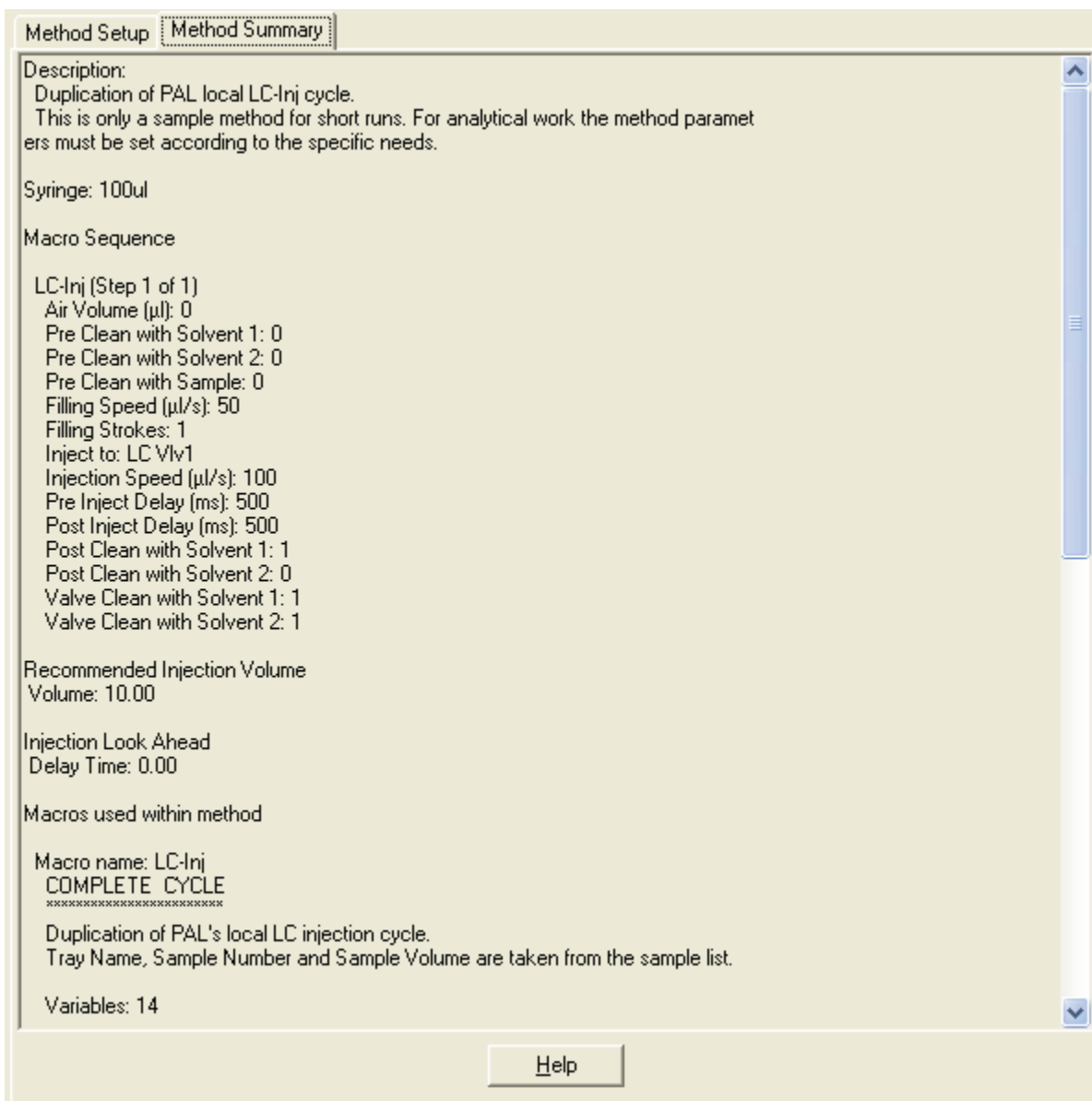
Viewing the Method Summary

❖ **To view a summary of your autosampler portion of an instrument method**

1. Open the Instrument Setup view for the autosampler (see “[Specifying the Instrument Method Parameters for the Autosampler](#)” on [page 24](#)).
2. Choose **File > Open** and select the instrument method that you want to review.
3. Click the **Method Summary** tab.

The Method Summary page appears with a summary of the autosampler parameters for the active instrument method (see [Figure 21](#)).

Figure 21. Method Summary page



Method Summary

Use the Method Summary page to view the details of the Macro Sequence that makes up a autosampler Method or Template.

The contents of the Method Summary/Template Summary page is read-only

Creating Custom Templates and Macros

This chapter describes how to create custom templates and macros.

Contents

- [Using the Template Editor to Create Custom Templates](#)
- [Testing a Custom Template](#)
- [Viewing the Template Summary](#)
- [Standard Macros](#)
- [Using the Macro Editor to Create Custom Macros](#)
- [Defining Variables](#)

Using the Template Editor to Create Custom Templates

Three standard templates are provided with the autosampler. To create a custom template, use the Template Editor dialog box.

Creating a Custom Template

This section provides information about how to create a custom template.

❖ To create a custom template

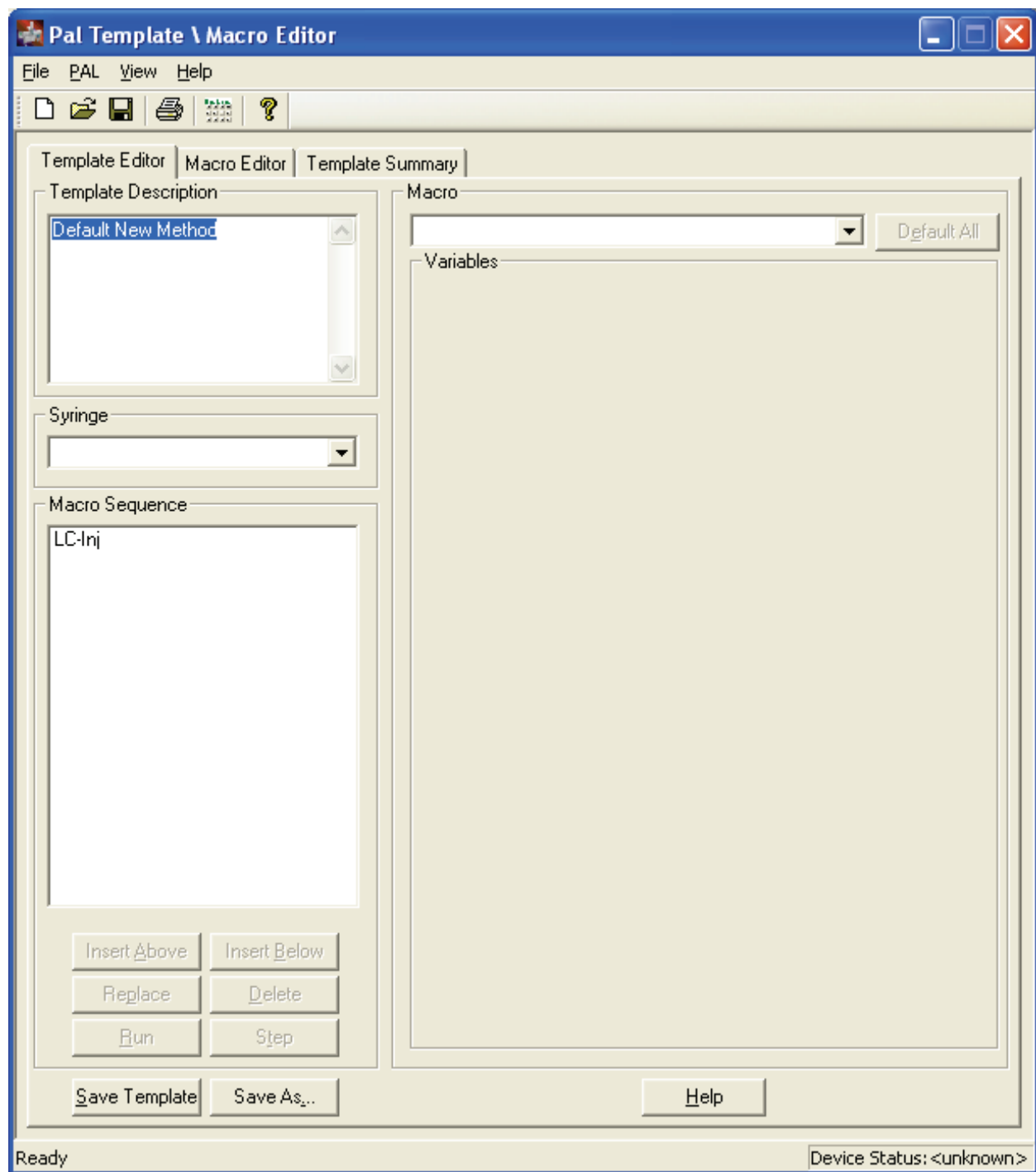
1. Open the Template Editor page:
 - a. Open the Instrument Setup view for the autosampler (see [“Specifying the Instrument Method Parameters for the Autosampler”](#) on [page 24](#)).
 - b. From the menu bar, choose **PAL Auto Sampler > Template \ Macro Editor**.

The Template Editor page of the Pal Template \ Macro Editor window appears (see [Figure 22](#)).

3 Creating Custom Templates and Macros

Using the Template Editor to Create Custom Templates

Figure 22. Template Editor page of the Pal Template \ Macro Editor window



2. (Optional) In the Template Description box, type a description of your custom template.
3. In the Syringe list, select the appropriate syringe size for the parameters to be specified in the template.
4. To add macros to the template:
 - a. Under Macro, select a macro from the list.
The Insert Above and Insert Below buttons become available
 - b. Click **Insert Above** or **Insert Below**.
The selected macro appears in the Macro Sequence list.
5. To delete macros from the Macro Sequence list:
 - a. Select the macro that you want to delete from the Macro Sequence list.
 - b. Click **Delete**.
6. To replace a macro in the Macro Sequence list:
 - a. Under Macro, select a macro from the list.
 - b. Select the macro that you want to replace in the Macro Sequence list.
 - c. Click **Replace**.
7. To save the template:
 - a. Click **Save As**.
The Save As dialog box appears.
 - b. Select a file location.
 - c. In the File name box, type an appropriate name.
 - d. Click **Save**.
The autosampler saves the template with the file extension *.pme.

Template Editor Page

Use the Template Editor page of the Pal Template \ Macro Editor window to make a new template (a sequence of macros). Each macro in the sequence is made up of variables. You may also edit these variables on this page.

Table 7. Template Editor page parameters (Sheet 1 of 3)

Parameter	Description
Template Description	Enter the method description in this box.
Syringe	Select the syringe volume from this list.

Table 7. Template Editor page parameters (Sheet 2 of 3)

Parameter	Description
Macro Sequence	This box displays the macros chosen for the method being made in the Method Template Editor. The macros in this sequence are selected from the Macro list (see below). Double-clicking a macro in the Macro Sequence box displays it in the Macro list as described later in this table, along with the variables that make up the macro (in the Variables area below the Macro list).
Insert Above	A Macro in the Macro list (see below) is placed above the macro selected in the Macro Sequence box (highlighted in blue).
Insert Below	A Macro in the Macro list (see below) is placed below the macro selected in the Macro Sequence box (highlighted in blue).
Replace	Replaces the macro (highlighted in blue) in the Macro Sequence box with the macro that is in the Macro list (see explanation below).
Delete	Deletes the macro that is selected (highlighted in blue) in the Macro Sequence box.
Run	Automatically executes the macros that constitute the Method (Macro Sequence).
Step	Executes the Method (Macro Sequence) in a stepwise manner.
Macro	Individual PAL macros are chosen in this list. When you select a macro, it is highlighted in blue. Displayed at the top of the Macro list is the sequence number (<i>x</i>) of the chosen macro and the total number of macros (<i>y</i>) in the Macro Sequence pane ("Macro <i>x</i> of <i>y</i> "). The chosen macro may be placed in the Macro Sequence box (see explanation above) by using the Insert Above, Insert Below, or Replace buttons as appropriate (see corresponding explanations above).
Default All	Changes all of the Method Template Editor variables to their default values.

Table 7. Template Editor page parameters (Sheet 3 of 3)

Parameter	Description
Variables	The variables that make up the macro in the Macro list are displayed in this area. Variables with specific allowed values (discrete variables) are displayed in lists. Continuous variables are displayed in boxes. You may choose the allowed values of discrete variables from those presented in the list for the variable. The allowed values for continuous variables are displayed when you hover the cursor over the respective box for the variable. The desired value for the continuous variable is then typed in its box.
Save Template	Saves the Method Template under the current file name (overwrites the current file).
Save As	Saves the Method Template under a new file name or in a new location that you type in the Save As dialog box.

Testing a Custom Template

This section provides information about testing a custom template.

Using the Template Editor dialog to Test a Custom Template

You can test a custom template from the Template Editor dialog box.

❖ To test your new template step by step

1. If the Template Editor dialog box is closed, open it.
2. Open the template that you want to review by choosing **File > Open Method Template....**

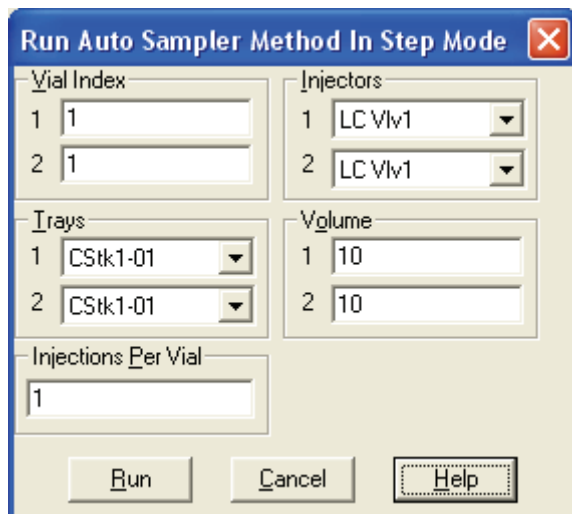
The default path to open a template is as follows:

C:\Thermo\Instruments\LC Devices\ThermoPAL\PAL\methods

3. Click **Step**.

The Run Auto Sampler Method In Step Mode appears (see [Figure 23](#)).

Figure 23. Run Auto Sampler Method In Step Mode dialog box



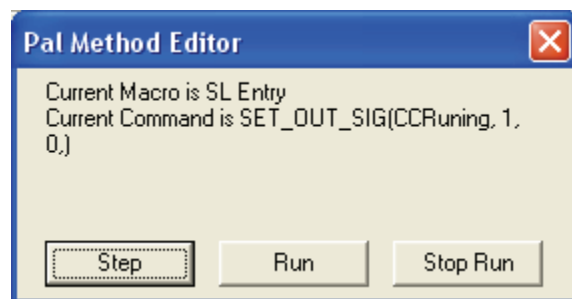
4. Make the appropriate entries and selections:

- Under Trays, select the appropriate trays.
- Under Vial Index, type an appropriate vial location for this template you are testing.
- Under Volume, type the volume that you want to inject.
- In the Injection Per Vial box, type the number of injections that you want to make from each vial.

5. Click **Run**.

The autosampler performs the actions specified in the template step-by-step. At the completion of each step, The autosampler pauses the injection sequence and prompts you with the Pal Method Editor dialog box (see [Figure 24](#)).

Figure 24. Pal Method Editor dialog box



6. Do one of the following:

- To continue stepping through the template, click **Step**.

- To run the remaining portion of the template, click **Run**.
- To stop the run, click **Stop Run**.

❖ **To run the complete sequence of macros in the template without pausing**

1. If the Template Editor dialog box is closed, open it.
2. If you closed the custom template, choose **File > Template > Open**.
3. Click **Run**.

The Run Auto Sampler Method dialog box appears (see [Figure 25](#)).

Figure 25. Run Auto Sampler Method dialog box

4. Make the appropriate entries and selections.
5. Click **Run**.

The autosampler executes the macros listed in the template.

Run Auto Sampler Method Dialog Box

Use the Run Auto Sampler Method dialog box to enter the Methods information required for the PAL to carry out autosampling.

Table 8. Run Auto Sampler Method dialog box parameters (Sheet 1 of 2)

Parameter	Description
Vial Index	
1	In this box type the vial index to be used with the method (SL.index).
2	In this box type the vial index to be used with the method (SL.index2).

Table 8. Run Auto Sampler Method dialog box parameters (Sheet 2 of 2)

Parameter	Description
Trays	
1	From this list select the tray to be used with the method (SL.tray).
2	From this list select the tray to be used with the method (SL.tray2).
Injectors	
1	From this list select the injector to be used with the method (SL.injector).
2	From this list select the second injector to be used with the method (SL.injector2).
Volume	
1	In this box type the injection volume to be used with the method (SL.volume).
2	In this box type the second injection volume to be used with the method (SL.volume2).
Injectations Per Vial	Type the number of replicate injections to be made from each vial.
Run	Run the selected method.
Cancel	Cancel any changes and close the page.

Viewing the Template Summary

- ❖ **To view a summary of a template**

1. Open the PAL Template \ Macro Editor window (see “Using the Template Editor to Create Custom Templates” on page 33).
2. Open the template that you want to review by choosing **File > Open Method Template....**

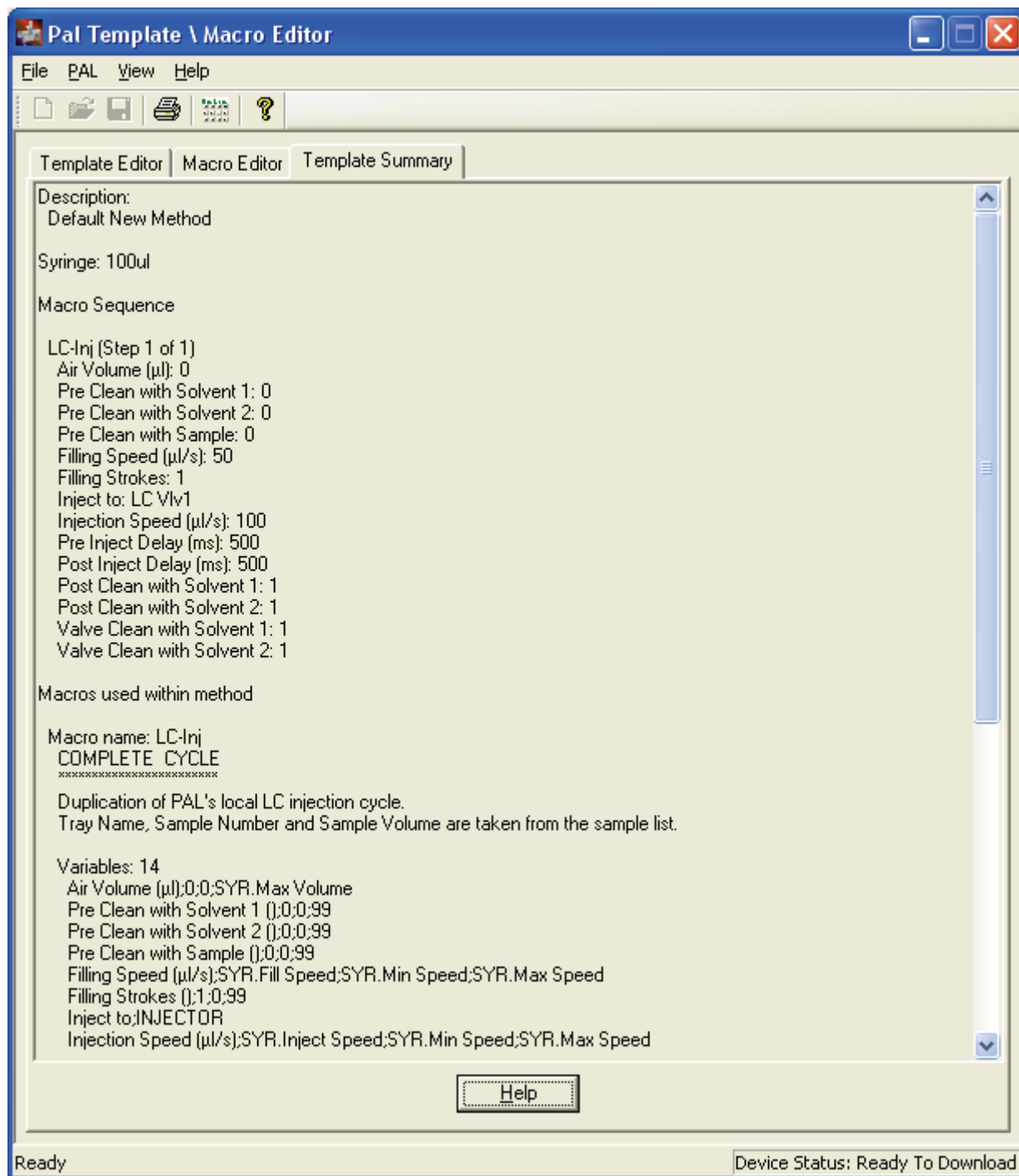
The default path to open a template is as follows:

C:\Thermo\Instruments\LC Devices\ThermoPAL\PAL\methods

3. Click the **Template Summary** tab.

The Template Summary page appears with a summary of the active template (see Figure 26).

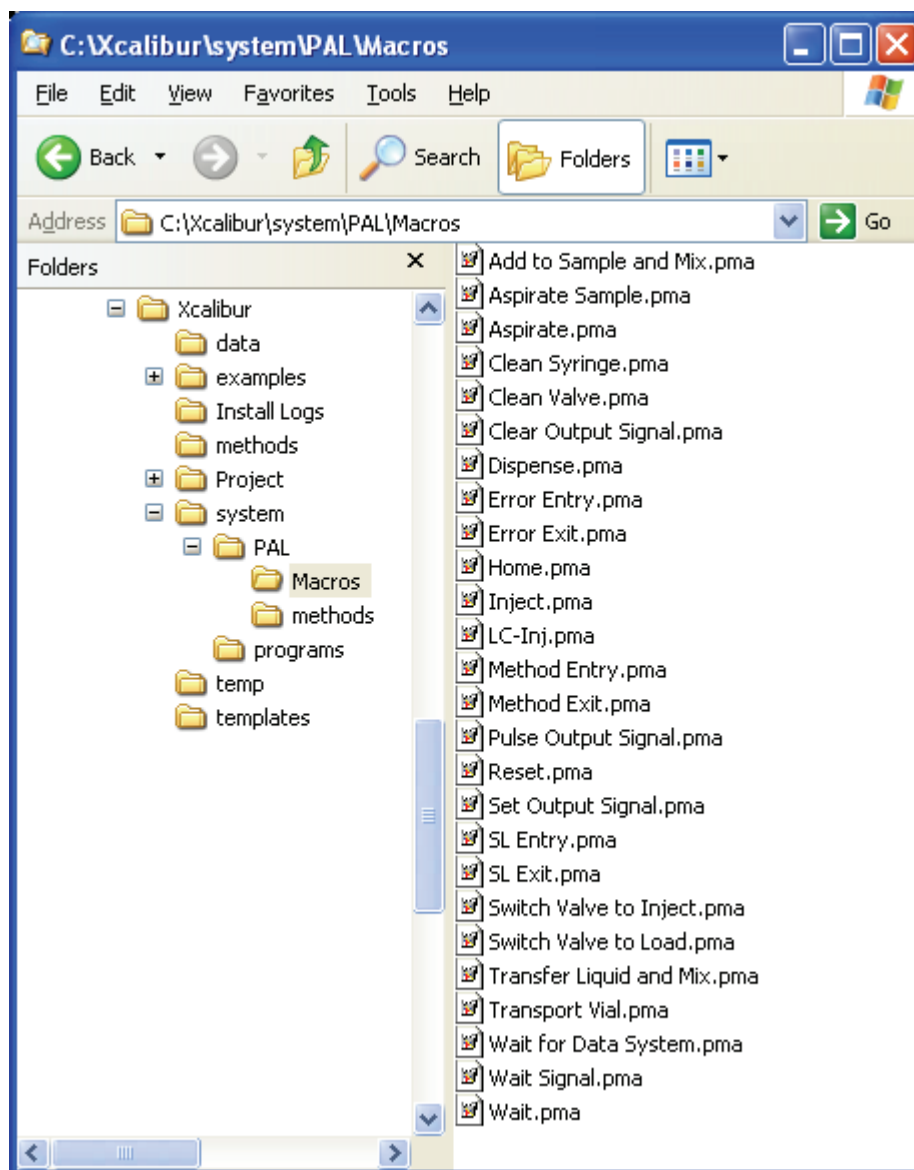
Figure 26. Template Summary page



Standard Macros

Figure 27 shows the macros that are provided with a standard installation.

Figure 27. Standard macros



Using the Macro Editor to Create Custom Macros

Macros are the building blocks that you use to create templates.

Creating a Custom Macro

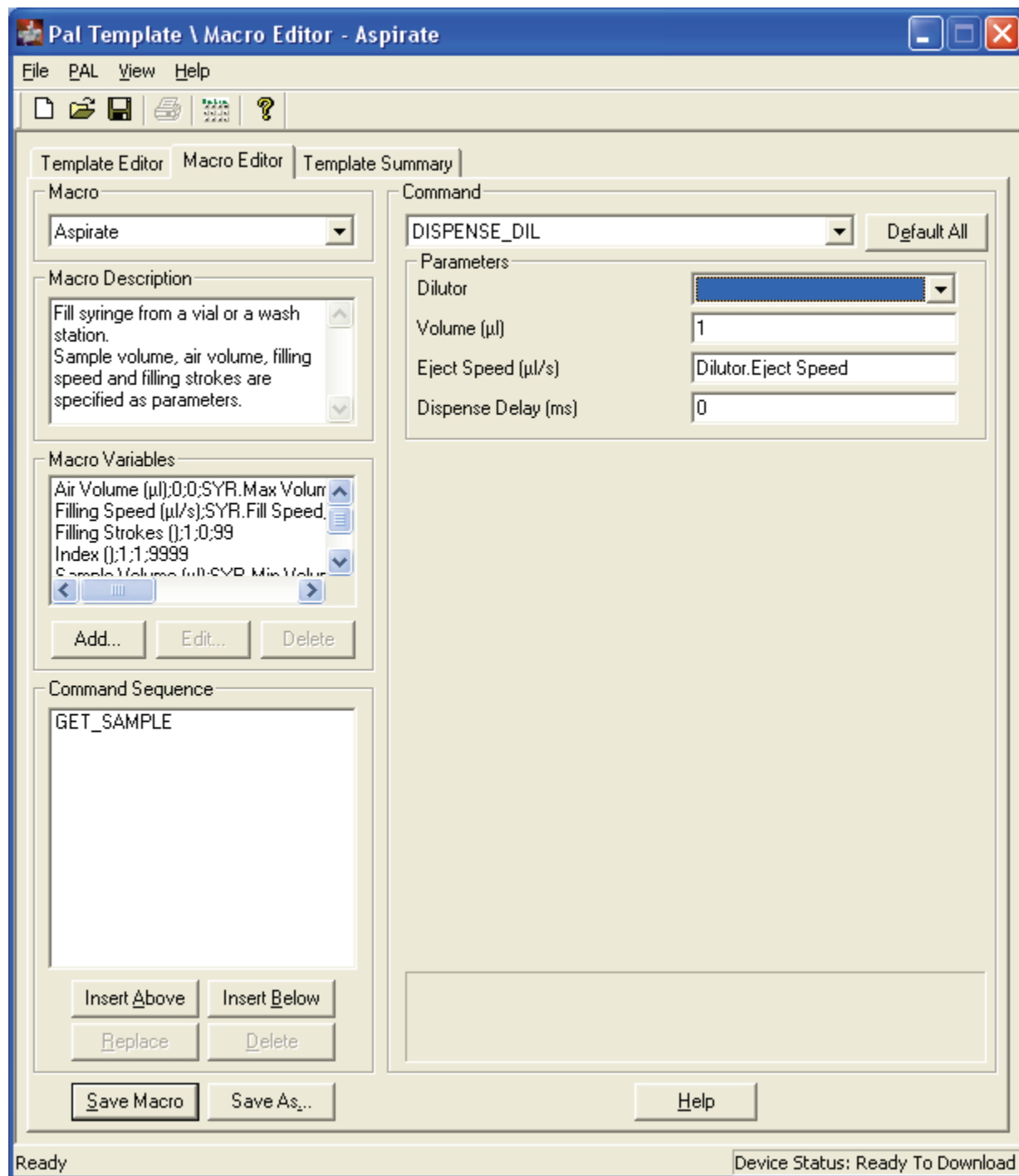
This section provides information about how to create a custom macro.

❖ To create a custom macro

1. Open the Macro Editor dialog box:
 - a. Open the Instrument Setup view for the autosampler.
 - b. From the menu bar, choose **PAL Auto Sampler > Template \ Macro Editor**.
 - c. Click the **Macro Editor** tab.

The Macro Editor page of the Pal Template \ Macro Editor window appears (see [Figure 28](#)).

Figure 28. Macro Editor page of the Template \ Macro Editor window



2. Under Macro, select a macro from the list.

The description of the selected macro appears under Macro Description and a list of macro variables appears under Macro Variables.

3. To add a variable to the macro, click **Add**.

The Variable Definition dialog box appears.

4. Define the variable (see “[Defining Variables](#)” on [page 48](#)).

5. To save the macro:

- a. Click **Save As**.

The Save As dialog box appears.

- b. Select a file location.
- c. In the File name box, type an appropriate name.
- d. Click **Save**.

The autosampler saves the macro with the file extension *.pma.

PAL Template - Macro Editor Page

Use the Macro Editor page of the Pal Template \ Macro Editor window to create custom macros.

Table 9. Macro Editor page parameters (Sheet 1 of 3)

Parameter	Description
Macro	This list displays existing autosampler macros. Select the macro that you want to edit.
Macro Description	A description of the macro selected in the Macro list.
Macro Variables	Variables used by the macro selected in the Macro list.
Add	Adds a new variable to the macro variables. The new variable is defined in an Edit Parameter dialog box that opens when you click Add. The Edit Parameter dialog box has a check box for the Parameter Type (numeric or object). If you select a numeric Parameter Type, you see a pane in which you enter the new parameter name, a list for the parameter units, and panes in which you enter the default value for the parameter along with the lower and upper limits for the parameter. If you select an object Parameter Type, you see a check list of objects that might be represented by the parameter.

Table 9. Macro Editor page parameters (Sheet 2 of 3)

Parameter	Description
Edit	Edits an existing macro variable. The editing is carried out in an Edit Parameter dialog box that opens when you click Edit. The Edit Parameter dialog box has a check box for the Parameter Type (numeric or object). If you select a numeric Parameter Type, you see a pane in which you enter the new parameter name, a list for the parameter units, and panes in which you enter the default value for the parameter along with the lower and upper limits for the parameter. If you choose an object Parameter Type, you see a check list of objects that may be represented by the parameter.
Delete	Deletes the selected Macro Variable.
Command Sequence	The sequence of commands that make up the macro selected in the Macro list. Double-clicking one of the commands highlights it in blue and causes it to be displayed in the Command area. In the Command area the command parameters are shown in both panes (continuous variables) and lists (discrete variables).
Insert Above	The command shown in the Command area is inserted above the command selected (highlighted in blue) in the Command Sequence pane.
Insert Below	The command shown in the Command area is inserted below the command selected (highlighted in blue) in the Command Sequence pane.
Replace	The command selected (highlighted in blue) in the Command Sequence pane is replaced by the command shown in the Command area.
Delete	Deletes the command selected (highlighted in blue) in the Command Sequence pane.

Table 9. Macro Editor page parameters (Sheet 3 of 3)

Parameter	Description
Command	Individual autosampler commands are displayed in a list. These individual commands make up the command sequence of an autosampler macro. Select the command by rolling the cursor over the command (no click is needed), highlighting each command in blue. Clicking the command displays it in this list (highlighted in blue). Simultaneously, the command parameters are displayed in the Parameters area below the Command list. At the bottom of this area (below the Parameters area) is a pane that describes the function of the selected command.
Parameters	The Parameters area displays the command parameters used by the autosampler command that you select in the Command list. Parameters with specific allowed values (discrete parameters) are displayed in lists. Continuous parameters are displayed in panes. The allowed values of discrete parameters may be chosen from those presented in the list for the parameter. The allowed values for continuous parameters are displayed when you place the cursor over the respective pane for the parameter. The desired value for the continuous parameter may be typed in its pane. You can view other allowed continuous parameter values in a shortcut menu. This menu is activated by right-clicking the continuous parameter box of interest. The parameter you select from the shortcut menu appears in the box.
Command Description	A description of the command displayed in the Command list.
Save Macro	Saves the macro (command sequence) under the current filename (overwrites the current file).
Save As	Saves the macro (command sequence) under a new filename, in a new location, or with both a new filename and location that you type in the Save As dialog box.

Defining Variables

Variables are the building blocks that you use to create macros.

Creating Custom Variables

This section provides information about how to create custom variables.

❖ To create custom variables

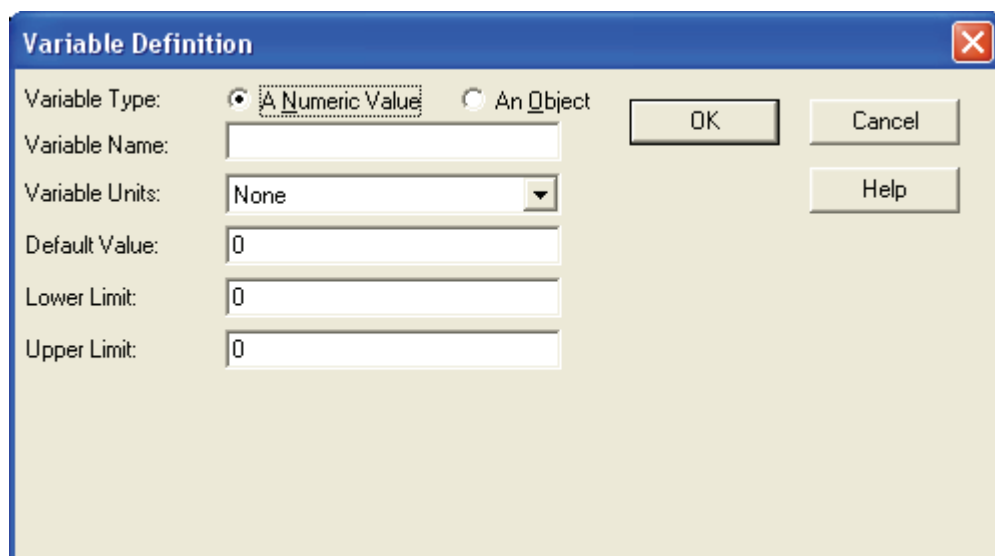
1. Open the Macro Editor dialog box:
 - a. Open the Instrument Setup view for the PAL autosampler (see [“Specifying the Instrument Method Parameters for the Autosampler”](#) on page 24).
 - b. From the menu bar, choose **PAL Auto Sampler > Template \ Macro Editor**.
 - c. Click the **Macro Editor** tab.

The Macro Editor page of the Pal Template \ Macro Editor window appears (see [Figure 28](#)).

2. Click **Add**.

The Variable Definition dialog box appears (see [Figure 29](#)).

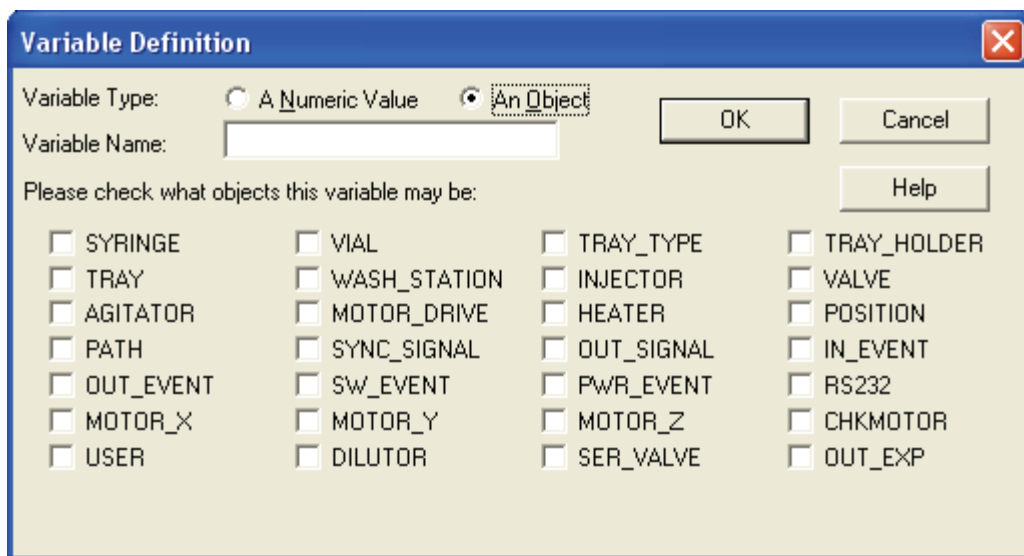
Figure 29. Variable Definition dialog box

The image shows a 'Variable Definition' dialog box with a blue title bar and a close button (X) in the top right corner. The dialog contains several input fields and radio buttons. The 'Variable Type' section has two radio buttons: 'A Numeric Value' (which is selected) and 'An Object'. Below this are five text input fields: 'Variable Name', 'Variable Units' (with a dropdown arrow), 'Default Value', 'Lower Limit', and 'Upper Limit'. All five input fields currently contain the value '0'. On the right side of the dialog, there are three buttons: 'OK', 'Cancel', and 'Help'.

3. Select a variable type:
 - To create a variable that is a numeric value, select the **A Number Value** option. Then go to [step 4](#).
 - To create an object variable, select the **An Object** option. A list of objects appears. Go to [step 5](#).

4. To complete the definition of a numeric variable:
 - a. In the Variable Name box, type an appropriate name.
 - b. In the Variable Units list, select a unit of measure.
 - c. In the Default Value box, type a default value for the variable.
 - d. In the Lower Limit box, type a lower limit for the variable.
 - e. In the Upper Limit box, type an upper limit for the variable.
 - f. Click **OK** to save the variable and close the Variable Definition dialog box.
5. To complete the definition of an object variable:
 - a. In the Variable Name box, type an appropriate name.
 - b. Select the appropriate objects (see [Figure 30](#)).
 - c. Click **OK** to save the variable and close the Variable Definition dialog box.

Figure 30. Object variable selections



The image shows a 'Variable Definition' dialog box with a blue title bar and a red close button. It contains two radio buttons for 'Variable Type': 'A Numeric Value' and 'An Object'. The 'An Object' option is selected. Below this is a text field for 'Variable Name'. To the right of the text field are 'OK' and 'Cancel' buttons. Below the text field is a section titled 'Please check what objects this variable may be:' followed by a 'Help' button. This section contains a grid of 20 checkboxes, each followed by an object name: SYRINGE, VIAL, TRAY_TYPE, TRAY HOLDER, TRAY, WASH_STATION, INJECTOR, VALVE, AGITATOR, MOTOR_DRIVE, HEATER, POSITION, PATH, SYNC_SIGNAL, OUT_SIGNAL, IN_EVENT, OUT_EVENT, SW_EVENT, PWR_EVENT, RS232, MOTOR_X, MOTOR_Y, MOTOR_Z, CHKMOTOR, USER, DILUTOR, SER_VALVE, and OUT_EXP.

Variable Definition			
Variable Type:		☐ A Numeric Value	☒ An Object
Variable Name:			
		OK	Cancel
Please check what objects this variable may be:		Help	
☐ SYRINGE	☐ VIAL	☐ TRAY_TYPE	☐ TRAY HOLDER
☐ TRAY	☐ WASH_STATION	☐ INJECTOR	☐ VALVE
☐ AGITATOR	☐ MOTOR_DRIVE	☐ HEATER	☐ POSITION
☐ PATH	☐ SYNC_SIGNAL	☐ OUT_SIGNAL	☐ IN_EVENT
☐ OUT_EVENT	☐ SW_EVENT	☐ PWR_EVENT	☐ RS232
☐ MOTOR_X	☐ MOTOR_Y	☐ MOTOR_Z	☐ CHKMOTOR
☐ USER	☐ DILUTOR	☐ SER_VALVE	☐ OUT_EXP

Variable Definition Dialog Box

Use the Variable Definition dialog box to specify the details of a numerical or object Macro Variable that you want to add or edit in the Macro Variables box.

Table 10. Variable Definition dialog box

Parameter	Description
Variable Type	
A Numeric Value	Select this if the parameter is a numeric value. If you are editing an existing numerical parameter, this box is marked with a black dot.
An Object	Select this if the parameter is an object. If you are editing an existing object parameter, this box is marked with a black dot.
Variable Name	If you are editing an existing variable, this box shows the name of the variable. Type your choice for the name of the variable if you are adding a new one.
OK	Accepts the new Variable Definition or accept the edits to an existing Variable Definition.
Cancel	Deletes changes made in the Variable Definition dialog box.
Numeric Value Variable Type	
Variable Units	If you are editing an existing variable, this list shows the units for the selected variable. If you are adding a new variable, you may enter units appropriate for that variable. When you click the list arrow, you can select the appropriate units from the options in the list.
Default Value	The nominal or center value of the variable.
Lower Limit	The lowest acceptable value of the variable.
Upper Limit	The highest acceptable value of the variable.
Object Variable Type	
Please check what objects this variable may be	Select one or more objects that are described by the variable.

Using the Direct Controls to Operate the Autosampler

This chapter describes how to operate the autosampler from the Direct Control dialog box.

Contents

- [Opening the Direct Control Dialog Box](#)
- [Making Single Injections from the Tune Window](#)
- [Changing the Tray Configuration](#)
- [Specifying the Location of the Direct Control Methods](#)
- [Wrapping Direct Control Injections with Additional Macros](#)

Opening the Direct Control Dialog Box

The direct control pages for the autosampler are available from the Instrument Setup window and the Tune window.

❖ To open the Direct Control dialog box from the Instrument Setup window

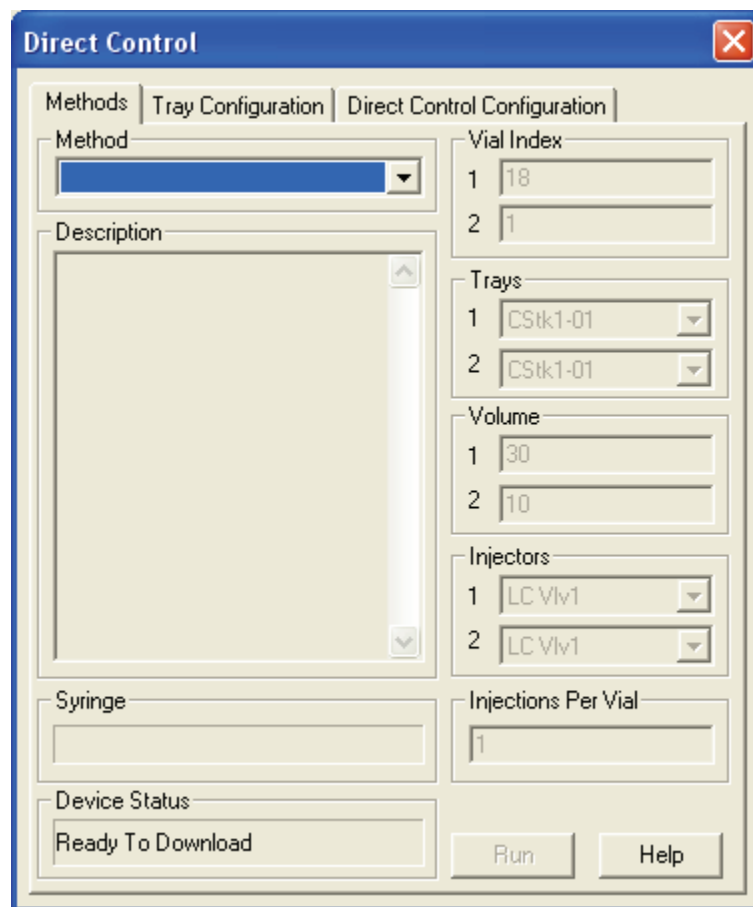
1. In the view bar, click  (Thermo Pal).
2. The Instrument Setup view for the autosampler appears.
3. From the menu bar, choose **PAL Auto Sampler > Direct Control**.

The Direct Control dialog box appears with the Methods page displayed (see [Figure 31](#)).

4 Using the Direct Controls to Operate the Autosampler

Opening the Direct Control Dialog Box

Figure 31. Direct Control dialog box



❖ To open the Inlet Direct Control dialog box from the Tune window

1. From the menu bar, choose **Setup > Inlet Direct Control**.
2. Click the **Thermo Pal** tab.

The Inlet Direct Control dialog box for the autosampler appears with the Methods page displayed. The Inlet Direct Control dialog box has the same functionality as the Direct Control dialog box shown in [Figure 31](#).

Making Single Injections from the Tune Window

You can make single injections from the Tune window.

❖ **To make single injections from the Tune window**

1. Open the Tune window for your mass spectrometer.
2. Open the Inlet Direct Control dialog box (see [“Opening the Direct Control Dialog Box”](#) on [page 51](#)).
3. Specify the location of the methods that you want to use (see [“Direct Control - Tray Configuration Page”](#) on [page 57](#)).
4. Specify the tray types that are loaded in the autosampler L tray holders (see [“Changing the Tray Configuration”](#) on [page 56](#)).
5. Click the Methods tab.

The Methods page appears. The Syringe area lists the size of the configured syringe. The Device Status area lists the status of the autosampler.

6. In the Method list, select a method.

A description of the method appears in the Description box (see [Figure 32](#)). The method list depends on the file locations specified on the Direct Control Configuration page.

Figure 32. Methods page of the Inlet Direct Control dialog box

The screenshot shows the 'Inlet Direct Control' dialog box with the 'Methods' tab selected. The 'Thermo Pal' and 'Accela Pump' tabs are also visible at the top. The 'Methods' section includes a dropdown for 'Method' (set to 'Latanoprost') and a text area for 'Description' containing a sample method note. To the right, 'Vial Index' has two input fields (18 and 1), 'Trays' has two dropdowns (both 'CStk1-01'), and 'Volume' has two input fields (30 and 10). Below these, 'Injectors' has two dropdowns (both 'LC Vlv1'). At the bottom left, 'Syringe' is set to '100ul' and 'Device Status' is 'Ready To Download'. 'Run', 'Help', and 'Close' buttons are at the bottom right.

7. Make the following selections and entries:
 - Under Vial Index, type the location of the sample vial.
 - Under Trays, select the tray where the sample vial is located.
 - Under Volume, type the volume that you want the autosampler to inject.
8. Click the tab for the configured LC pump and start the solvent flow from the LC pump.

Note Thermo PAL only downloads the method parameters for the autosampler. It ignores the method parameters for the LC pump and the MS detector.

9. Return to the Methods page for the Thermo Pal, and click **Run**.

Direct Control - Methods Page

The Methods page of the Inlet Direct Control dialog box has the following parameters:

Table 11. Methods page parameters

Parameter	Description
Method	Use this list to select a method file.
Description	This box contains a description of the selected method.
Syringe	This box displays the configured syringe.
Device Status	This box displays the status of the PAL. Status states
Vial Index	
1	In this box type the vial index to be used with the method (SL.index).
2	In this box type the vial index to be used with the method (SL.index2).
Trays	
1	From this list select the tray to be used with the method (SL.tray).
2	From this list select the tray to be used with the method (SL.tray2).
Volume	
1	In this box type the injection volume to be used with the method (SL.volume).
2	In this box type the second injection volume to be used with the method (SL.volume2).
Injectors	
1	From this list select the injector to be used with the method (SL.injector).
2	From this list select the second injector to be used with the method (SL.injector2).
Injectons Per Vial	Type the number of replicate injections to be made from each vial.
Run	Runs the selected method.

Changing the Tray Configuration

Use the Tray Configuration dialog box to specify the tray types to be used for direct control injections while you have the Direct Control dialog box open. The autosampler uses this tray information instead of the tray information specified in the autosampler Control Terminal.

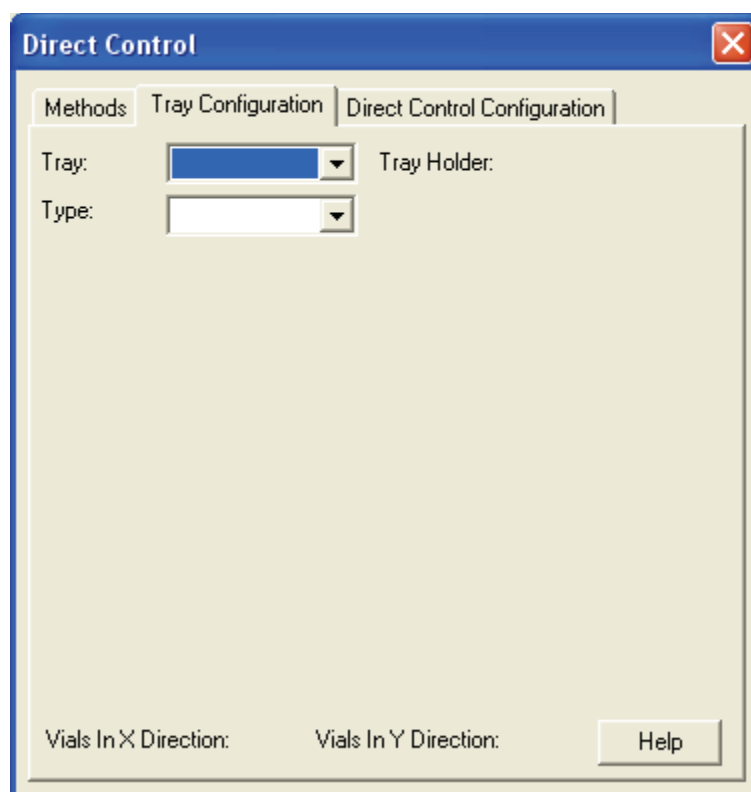
IMPORTANT The autosampler does not store the tray configuration that you specify on the Tray Configuration page of the Direct Control dialog box. When you close the dialog box, the tray configuration resets to the autosampler Control Terminal settings.

❖ To change the tray configuration

1. Open the Direct Control dialog box (see “Opening the Direct Control Dialog Box” on page 51).
2. Click the **Tray Configuration** tab.

The Tray Configuration page appears (see Figure 33).

Figure 33. Tray Configuration page



3. In the Tray list, select the tray that you want to modify.
4. In the Type list, select the tray type.

Direct Control - Tray Configuration Page

Use the Tray Configuration page in the Direct Control dialog box to enter the Tray Configuration information required for the autosampler to carry out autosampling.

Table 12. Tray Configuration page parameters

Parameter	Description
Tray	From this list, select the tray location in the three drawer (deep drawer) configuration: tray 1 & 2=drawer #1, tray 3 & 4=drawer #2, tray 5 & 6=drawer #3. Up to six drawers and twelve trays are possible with the shallow drawer configuration.
Type	From this list, select the tray type.
Tray Holder	Stack 1 and Stack 2 are the allowed values. Tray holders can hold one or more trays. A Stack is a tray holder that is designed to hold microplates. The autosampler firmware determines the type and number.
Vials in X Direction, Vials in Y Direction	Determined by choice of tray type.

Specifying the Location of the Direct Control Methods

Use the Direct Control Configuration dialog box to specify the location of the methods that you can open in the Methods page of the Direct Control dialog box.

❖ To specify the method folders

1. Open the Direct Control dialog box (see [“Opening the Direct Control Dialog Box”](#) on page 51).
2. Click the **Direct Control Configuration** tab.

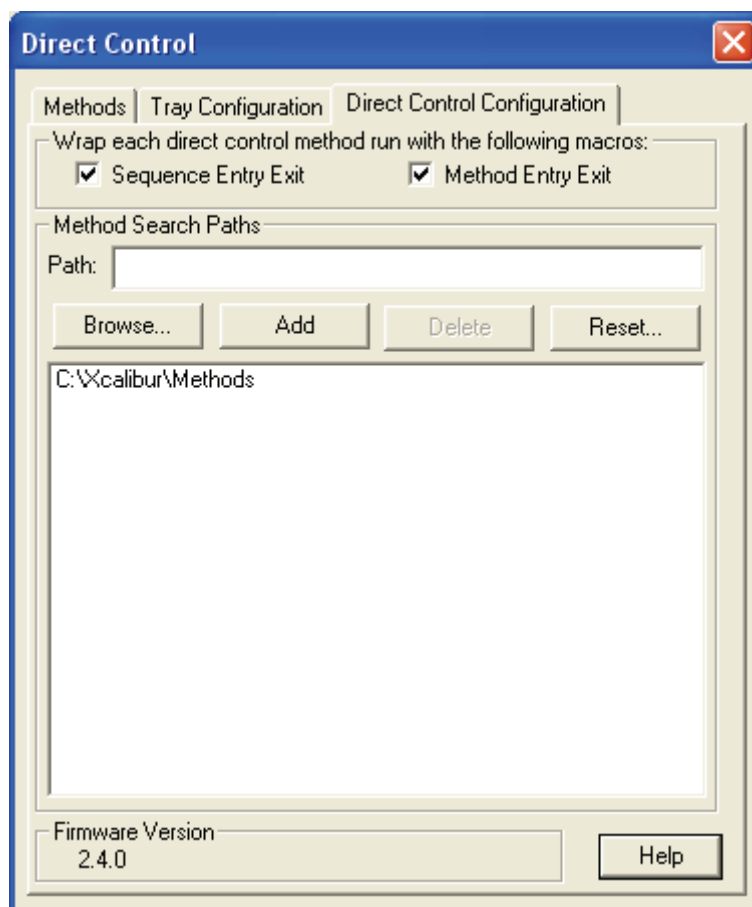
The Direct Control Configuration page appears (see [Figure 34](#)). By default, the methods that appear in the Method list on the Method page are located in the following directory:

C:\Xcalibur\Methods

4 Using the Direct Controls to Operate the Autosampler

Specifying the Location of the Direct Control Methods

Figure 34. Direct Control Configuration page



3. To add additional file locations:
 - a. In the Path box, type the folder location, or click **Browse** and browse to the appropriate folder to select it.
 - b. Click **Add**.

The new folder location appears in the box below the row of buttons.
4. To delete file locations:
 - a. Select the file location in the box below the row of buttons.
 - b. Click **Delete**.
5. To reset the location of the methods files to the default folder, click **Reset**.

Direct Control - Direct Control Configuration Page

Use the Direct Control Configuration page in the Direct Control dialog box to enter the Direct Control Configuration information required for the autosampler to carry out autosampling.

Table 13. Direct Control Configuration page parameters

Parameter	Description
Wrap each direct control method run with the following macros	
Sequence Entry Exit	Use this check box to wrap methods with the sequence list entry and exit macros (SLEntry and SLExit).
Method Entry Exit	Use this check box to wrap methods with the method entry and exit macros (Method Entry and Method Exit).
Method Search Paths	
Path	Use this box to specify the extra path you want added to the method search routine.
Browse	Use this button to search for a path.
Add	Use this button to add the current path to those searched.
Delete	Use this button to delete the currently selected path.
Reset	Use this button to remove all of the user added paths.
text box	This text box displays the directories that the autosampler searches for methods.
Firmware Version	Specifies the firmware version downloaded from the autosampler.

Wrapping Direct Control Injections with Additional Macros

Use the Direct Control Configuration dialog box to specify whether the autosampler is to run the SL Entry and SL Exit, the Method Entry and Method Exit, or all of these macros before and after an injection.

❖ To specify the use of the SL Entry and SL Exit macros

Select the **Sequence Entry Exit** check box.

❖ To specify the use of the Method Entry and Method Exit macros

Select the **Method Entry Exit** check box.

Using Dynamic Load and Wash (DLW)

This chapter describes how to use the Dynamic Load and Wash (DLW) option, which includes how to install the Cycle Composer Macros or ICC Cycles, how to operate the DLW, and a step-by-step illustration of the DLW cycles.

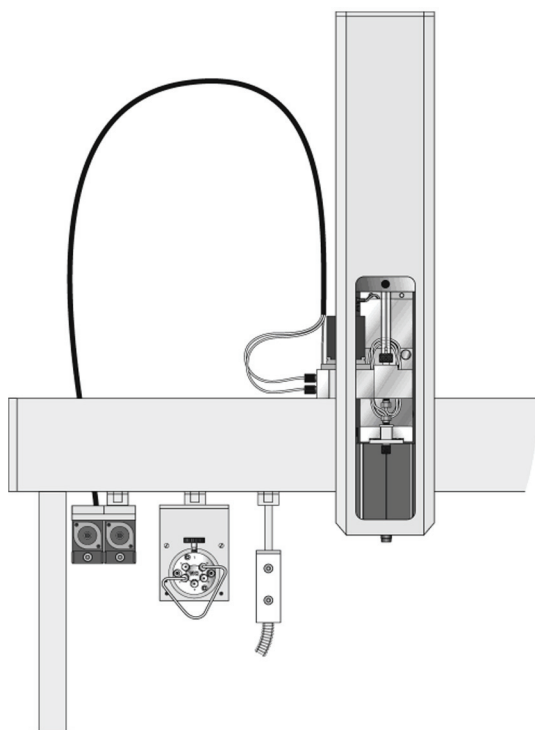
Contents

- [Overview](#)
- [Installing Cycle Composer Macros or ICC Cycles](#)
- [Operating Dynamic Load and Wash \(DLW\)](#)
- [DLW Cycle Step-by-Step](#)

Overview

This section contains an overview of the Dynamic Load and Wash (DLW) option.

Figure 35. Dynamic Load and Wash (DLW)



The DLW option represents a new wash station concept that combines an injection cycle with wash steps. The linked combination of the two steps, which are usually separate, minimizes cycle time and carryover.

There are two characterizing features for the DLW option:

- The sample solution never contacts the syringe itself; it is held between the holding loop.
- Wash solvents are pumped from back to front into the DLW system to intensely flush all critical parts that are in contact with the sample.

The DLW option consists of two self-priming micro pumps (mounted on a dedicated bracket), and the wetted parts are Ryton PPS and Kalrez (FFPM). The pump IN ports are connected to the wash solvent bottles and the OUT ports are connected to the DLW manifold, which is part of the assembly of the dedicated DLW syringe holder. A holding loop separates the syringe and the DLW actuator to avoid sample from contacting these parts.

The syringe and holding loop are preloaded with wash solvent #1 at the start. The sample is picked up and remains separated from wash solvent #1 by an air gap. After loading the loop and injection, wash solvent #1 is pushed into the system, followed directly by wash solvent #2 to flush the critical valve paths.

The DLW syringe assembly is moved to the wash station for further cleaning steps and for preparing the Syringe and holding loop for the next cycle.

For further details, refer to “DLW Cycle Step-by-Step” on [page 73](#)

Installing Cycle Composer Macros or ICC Cycles

You can only operate the autosampler DLW option in combination with PAL control software, such as Thermo PAL driver for Xcalibur or Cycle Editor, for ICC interpretation in another data handling system software.

For software control, three macros or three cycles are provided.

The first of three macros covers the initial and daily priming of the solvent lines and covers a complete injection cycle, a second macro is used for Standard speed with optimized washing possibilities, and the last for a Fast cycle for optimized throughput and less focus on carryover. See [Table 14](#).

For detailed descriptions of all macros (or cycles), see [Table 14](#), [Table 15](#), and [Table 16](#).

Table 14. The DLW macro definitions

Macro name	Macro description
Priming Accela Open	For initial and daily routine priming of the solvent lines and DLW manifold. The Clean Time for both wash solvents is a variable for the user to define the intensity of washing.
Standard Injection Accela Open	Standard injection cycle using all possibilities of the DLW option. The injection valve inlet port and the needle are washed with both wash solvents (inside and out). You can add an extra Stator Wash for intensive washing of the injection valve (valve toggle).
Fast Injection Accela Open	Tuned for speed and high-throughput application. It differs from the Standard DLW macro in that some steps are left out to shorten the cycle time.

Installing the Cycle Composer Macros or ICC Cycles

The autosampler is shipped with a CD-ROM containing various cycles for the DLW option. Macros for Xcalibur are installed by the LC Devices 2.4 installer.

Copy these macros to your Thermo PAL driver method folder or corresponding folder for application within the integrated system.

❖ To copy cycles to your Cycle Composer

1. Navigate in Windows Explorer to the Thermo PAL driver folder. The usual folder location is as follows:

C:\Thermo\Instruments\LC Devices\ThermoPAL\PAL
2. If you wish to add the DLW option macros to an existing method folder, copy the macros (*.pma) and the methods (*.pme) files from the DLW Option folder on the CD-ROM to the method folder.
3. If the PAL system is integrated in a data system software that controls the PAL using the Cycle Editor for PAL ICC interpretation (for example, Analyst, ChemStation, Empower, EZChrom, MassLynx, Xcalibur), an ICC Cycle is used and not the Cycle Composer Macro, the cycle extension is *.cyx.

Note You can convert a Cycle Composer macro to a cycle (extension *.cyx) by using the Cycle Editor software. Conversion is available starting with Cycle Editor version 1.4.0.4.

Note The provided macros are written for standard injection valve drives, which are controlled and activated through the AUX interface.

General Considerations

You must establish the duration of the wash steps for each configuration and application. Consider factors such as the viscosity and surface tension of the individual wash solvent composition and the backpressure of the system.

Be aware that a higher backpressure builds up if the valve bore size (standard valve bore 0.25 mm) or the installed loop internal diameter is lower. Standard loop internal diameter (ID) for Thermo-defined loops with a volume of 5, 10, and 20 µL is 0.25 mm. The loop with 2 µL content volume has an ID of 0.125 mm.

Keep the tubing internal diameters of the tubing in line with the valve dimensions, loop ID, and flow rate. For details, see “Tips & Hints for HPLC Technique.”

Priming the Solvent Lines, Wash1 and Wash2

This macro is used at installation to prime the entire system. Set the wash time to approximately 120 seconds for each solvent.

After installation, for best results prime the system before activating the first run. For daily preparation of the system, the wash time can be much shorter: approximately. 20 seconds. The goal is an entire liquid system free of any air bubbles.

Macro Name: Priming Accela Open AS_Rev01

Table 15. Macro Priming Accela Open AS (Sheet 1 of 2)

Macro description	Macro variable
The injection unit moves to the DLW Wash Station, position Wash1.	Clean Time solvent 1 Eject Speed DLW Syringe
The injection unit moves to the DLW Wash Station, position Wash2.	Clean Time solvent 1 Eject Speed DLW Syringe
The DLW system is rinsed with Wash Solvent 1 in position Waste.	

Table 15. Macro Priming Accela Open AS (Sheet 2 of 2)

Macro description	Macro variable
The injection valve is cleaned first with the content of DLW syringe (Wash Solvent 1), followed by Wash Solvent 2 and finally, the last wash to prepare the system for injection cycle, rinsed with Wash Solvent 1 again. Remark: The Atom Rinse Inj is new, available starting with FW 4.1.x. The DLW actuator/solenoid is activated; the Wash Solvent (pump), the Needle Gap and the Rinse Time are selectable.	Needle Gap is a parameter from Rinse Inj Atom. The variable in this macro is: Needle Gap Valve Clean. The function of this parameter is to raise the needle in the injection port very little to allow rinsing around the needle tip. The pressure of the spring-loaded balls in the DLW Syringe Holder assembly is released by moving approx 3 mm up (default). This leaves a gap, between the needle tip and the valve bottom, of approximately. 1 mm to enable a flush at this contact point.
A Repeat-End loop enables adding an extra rinsing step, valve toggle.	Stator Wash: Counter 0 = disable valve toggle steps Counter 1 = enable valve toggle steps.
If the counter is set to 1, follow the described steps below. If the counter is set to 0, the macro finishes at this point.	
The injection moves to the injection valve. The valve is switched to the Active position.	
The valve is rinsed with Wash Solvent 2, followed by Wash Solvent 1	Stator Wash Time Solvent 2 Stator Wash Time Solvent 1 Remark: The loop is filled with the last rinse with Wash Solvent 1. Verify the composition of Wash Solvent1. The solvent should have a lower elution power than the solvent gradient starting conditions or sample solvent composition. This is important for partial loop filling.
The injection valve is switched back to the Standby position.	
End of macro DLW Priming.	

Standard DLW Injection Cycle

Macro Name: Standard Injection Accela Open_Rev03

Table 16. Macro Standard Injection Accela Open (Sheet 1 of 3)

Macro description	Macro variable
The PAL system waits first for the Sync Signal Ready before the injection cycle is started.	Remark: Sync Signal setting Start
The injection valve is brought in a defined Standby position.	Inject to Standby
The Rear air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed
The sum of Rear-, Sample List-, and Front-Volume is aspirated into the Holding Loop.	Front Volume Rear Volume (SL.volume)
The Front air segment is aspirated.	Airgap Volume Filling Speed Pullup Delay
The injection unit moves to the DLW Wash Station, Wash1 position.	
The needle is inserted (dipped) for 1 second to wash the outer needle surface. No plunger movement at this step.	
The injection unit moves to the specified injection valve. The Front- and Airgap-Volume is ejected.	Inject to Front Volume Airgap Volume Injection Speed
The PAL system waits for the data system.	Wait for DS
The injection valve is switched to Active position. The time Pre Inject Delay is awaited.	Inject to Pre Inject Delay
The loop is filled with the sample volume as specified in the sample List.	(SL.volume) Injection Speed
The injection valve is switched to Standby position, the loop content is injected. Timer 1 Delay Stator Wash is started and a Start signal to the HPLC system is sent.	Post Inject Delay Timer 1

Table 16. Macro Standard Injection Accela Open (Sheet 2 of 3)

Macro description	Macro variable
The plunger of the DLW Syringe is pushed down to dispense the Rear Sample and Air Segment to Waste. The Holding Loop is still filled with Wash Solvent 1.	(Syr. Eject Speed)
The DLW Actuator/Solenoid is activated to deliver Wash Solvent 2 into the Holding Loop to clean the injection valve from Port 1 to Port 2.	Wash2 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 2
For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point.	Remark: For a detailed explanation of the Needle Gap Valve Clean, see DLW Priming.
The injection unit is moved to the DLW Wash Station, Wash2 position.	Wash2 (Syr.Eject Speed)
The needle is rinsed inside and out with Wash Solvent 2.	Post Clean Time Solvent2
The injection unit is moved back to the injection valve. The Inlet Port and engraving to waste Port are flushed with Wash Solvent 1 to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1
The injection unit is moved back to the DLW Wash Station, Wash1 position to flush the syringe needle inside and out with Wash Solvent 1.	Wash1 Post Clean Time Solvent 1
This is a preparation step for next injection, and especially important for biofluid samples.	
Cycle end for LC-Inj DLW Standard macro.	
An optional cleaning step is attached to the DLW Standard injection cycle: Stator Wash or valve toggle.	Stator Wash Stator Wash count: 1 = Cleaning step active Stator Wash count: 0 = Cleaning step disabled
A Repeat-End loop can be activated with the Count.	
If Stator Wash is activated, the following steps will be executed.	

Table 16. Macro Standard Injection Accela Open (Sheet 3 of 3)

Macro description	Macro variable
The injection unit is moved to the injection valve. From the last step above, the Holding Loop is filled with Wash Solvent 1.	Inject to Delay Stator Wash (Active)
Timer 1 is awaited to switch the valve (Toggle) into Active position (fill loop).	
The DLW Actuator/Solenoid is activated to deliver Wash Solvent 2 to the Holding Loop and into the valve system.	Inject to Wash2 Stator Wash Time Solvent 2
The first solvent flush arriving at the valve is Wash Solvent 1 parked in the Holding Loop at the beginning, followed by Wash Solvent 2.	
Wash Solvent is changed to Wash Solvent 1.	Inject to Wash1 Stator Wash Time Solvent 1
The injection valve is switched back to the Standby position.	Inject to (Standby)

Fast DLW Injection Cycle

The Fast injection cycle differs from the Standard cycle as follows:

- The needle is not dipped in the Wash station Wash1 after sample pickup and before it moves to the injection valve.
- The wash steps after injection are reduced to Valve Clean with Wash Solvent 1 and Wash Solvent 2. The DLW needle is flushed in the DLW Wash Station with Wash Solvent 1 only.
- Stator Wash (valve toggle) is not available.

Macro Name: Fast Injection Accela Open_Rev03

Table 17. Macro Fast Injection Accela Open (Sheet 1 of 3)

Macro description	Macro variable
The PAL system waits first for the Sync Signal Ready before the injection cycle is started.	Remark: Sync Signal setting Start
The injection valve is brought to a defined position: Standby.	Inject to Standby
The Rear air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed

Table 17. Macro Fast Injection Accela Open (Sheet 2 of 3)

Macro description	Macro variable
The sum of Rear-, Sample List-, and Front-Volume is aspirated into the Holding Loop.	Front Volume Rear Volume (SL.volume) Airgap Volume
The Front air segment is aspirated.	Filling Speed Pullup Delay
The injection unit moves to the specified injection valve.	Inject to Front Volume
The Front- and Airgap-Volume is ejected to Waste.	Airgap Volume Injection Speed
The PAL system waits for the data system	Wait for DS
The injection valve is switched to Active position.	Inject to
The Pre Inject Delay time is awaited.	Pre Injection Delay
The loop is filled with the sample volume as specified in the sample list.	(SL.volume)
The injection valve is switched to Standby position, the loop content is injected.	Injection Speed Post Inject Delay
The plunger of the DLW Syringe is pushed down to dispense the Rear Sample and Air Segment to Waste. The Holding Loop is still filled with Wash Solvent 1.	Injection Speed
The DLW Actuator/Solenoid is activated to deliver Wash Solvent 2 into the Holding Loop to clean the injection valve from Port 1 to Port 2.	Inject to Wash2 Needle Gap Valve Clean Valve Clean Time Solvent 2
For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point.	Remark: For complete details on the Needle Gap Valve Clean, see DLW Priming.
Wash Solvent 1 follows to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1

Table 17. Macro Fast Injection Accela Open (Sheet 3 of 3)

Macro description	Macro variable
The injection unit is moved back to the DLW Wash station, Wash1 position to flush the syringe needle inside and out with Wash Solvent 1.	Wash1 Post Clean Time Solvent 1
This is a preparation step for the next injection, and is especially important for biofluid samples.	
Cycle end for LC-Inj DLW Fast macro.	

Operating Dynamic Load and Wash (DLW)

This section describes how to operate the Dynamic Load and Wash (DLW) option.

- [Priming the Solvent Lines](#)
- [Location of Solvent and Waste Bottles](#)
- [DLW Pumps](#)

Priming the Solvent Lines

Note For trouble-free DLW operation, make sure the two solvent lines are free of air bubbles at all times. If the solvent lines are being connected for the first time or during a solvent change, you must prime the solvent lines properly until air bubbles are no longer visible. Solvent degassing is recommended.

In order to make the initial and daily priming efficient and controllable, the Open Autosampler comes with a Cycle Composer macro, or ICC cycle.

❖ To prime the solvent lines

1. Load the macros and methods into the folder.
2. Start the corresponding macro for initial or daily priming.
3. Check the solvent lines and prime until air bubbles are no longer visible.
4. Press F4 for Home.

Location of Solvent and Waste Bottles

The DLW option contains self-priming membrane pumps. The solvent bottles can be placed either in the fast wash station holder or on the lab bench.

You must place the Waste bottle must be placed > 30 cm (in.) below the injection valve. Make sure that the waste liquid can flow into the waste bottle without restriction. Place the waste tubing above the level of the liquid. Ideally, the tube is fixed at the neck of the waste bottle.

Note Use good lab practice to avoid contaminating the wash solvents and the wash bottles. Avoid biological growth in pure water by either replacing it regularly or adding a small percentage of organic solvents, such as methanol or acetonitrile. Certain buffer solutions can decompose at room temperature when exposed to light. Filtering the wash solvents before filling the bottle, especially if salt buffers are used, is mandatory to avoid any clogging of the solvent paths.

DLW Pumps

From the control point of view, the DLW pumps respond in the same manner as the fast wash station. Power-out signals activate the pumps. Because the electric current setting for the DLW is different, the corresponding PAL Firmware Objects must be loaded for the DLW wash station type.

The wetted parts in the pump are made from the following materials:

- Membrane: Kalrez (FFPM)
- Body, valves: Ryton PPS

The pumps are self priming with a suction lift of up to 3 m water column.

DLW Actuator/Solenoid

The DLW Actuator/Solenoid has the function of separating and completely shutting-off the lines in the direction of the syringe (sample loading) or the wash solvent lines.

After opening the DLW Actuator/Solenoid for the wash solvent lines, you can pump the desired wash solvent into the system by activating the corresponding DLW pump.

Figure 67 illustrates this functionality.

The wetted parts in the DLW Actuator Solenoid are made from the following materials:

- Solenoid body: PEEK
- Seal material: FFKM (Simriz)

Note PEEK exhibits excellent chemical resistance to most of the chemicals used. However, the following solvents are not recommended for use with PEEK: DMSO, THF, methylene chloride (dichloromethane), nitric acid, or sulfuric acid. For more details refer to the compatibility tables provided by the manufacturer of PEEK material or components.

Note Current applied from the actuator control PCB to the actuator/solenoid activates a green LED. This activation does not indicate that the solenoid opens or closes.

Figure 36. DLW Manifold and Actuator/Solenoid

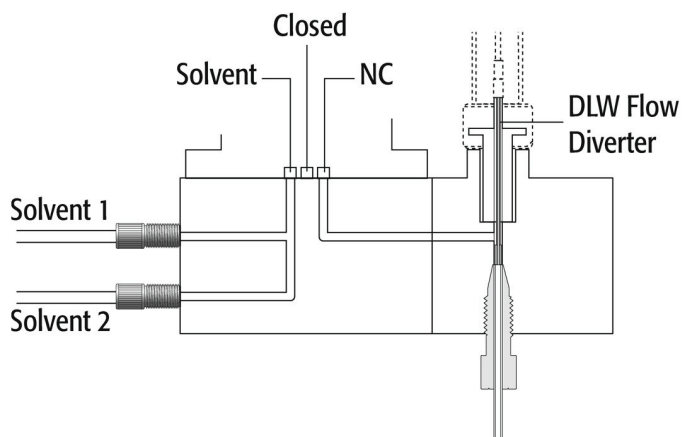
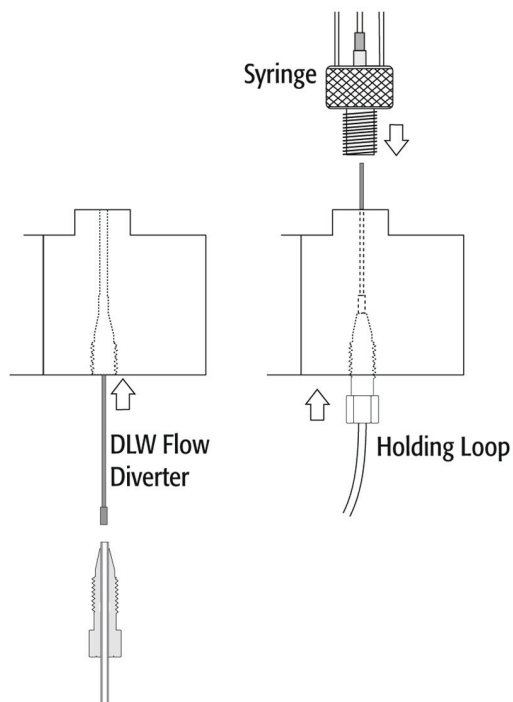


Figure 37. Inserting DLW Flow Diverter



DLW Cycle Step-by-Step

This section provides illustrations to demonstrate a step-by-step DLW cycle.

- [Cycle for Standard Injection](#)
- [Additional Valve Toggle Step to DLW Standard Cycle](#)
- [Cycle for Fast Injection](#)

Cycle for Standard Injection

[Figure 38](#) to [Figure 51](#) illustrates a step-by-step cycle for the standard injection.

Figure 38. Standard: Cycle start

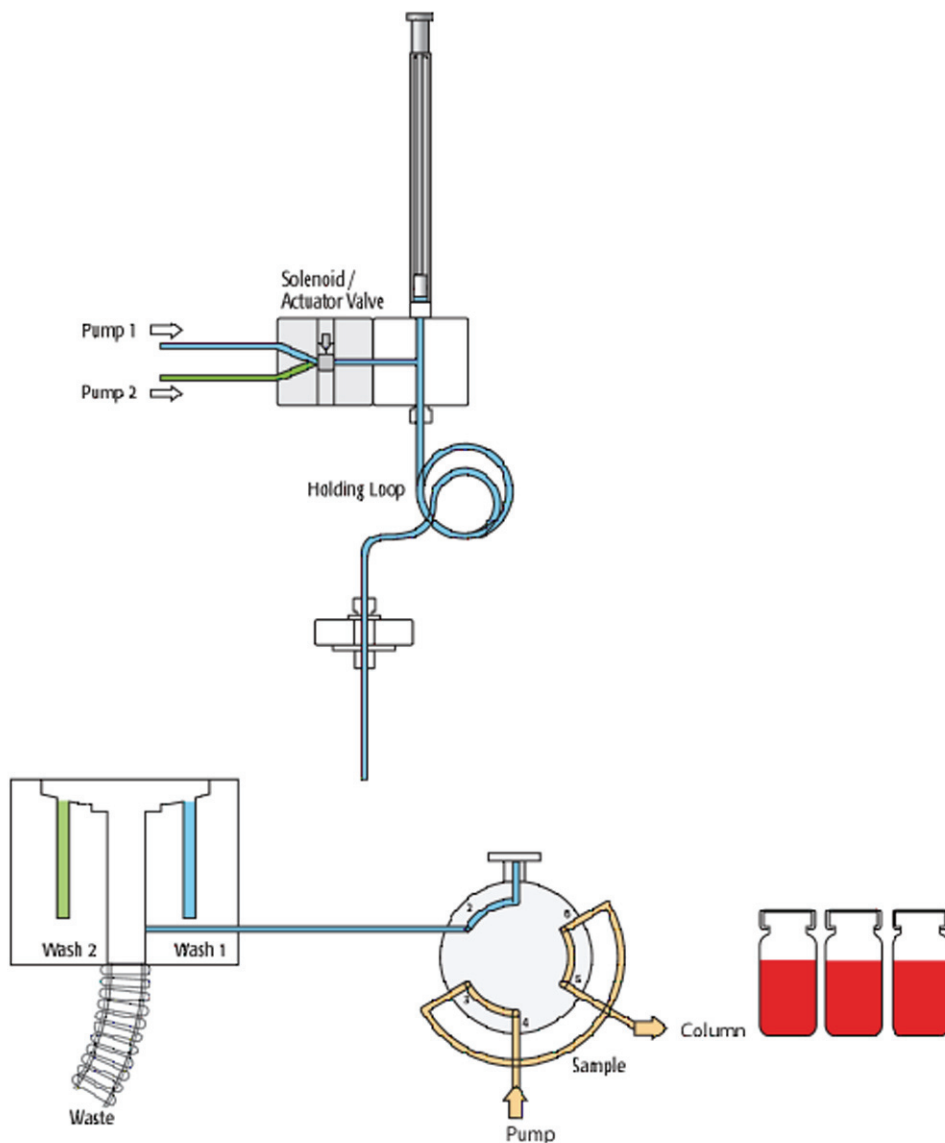


Figure 39. Standard: Step 1 – A spirate rear air segment

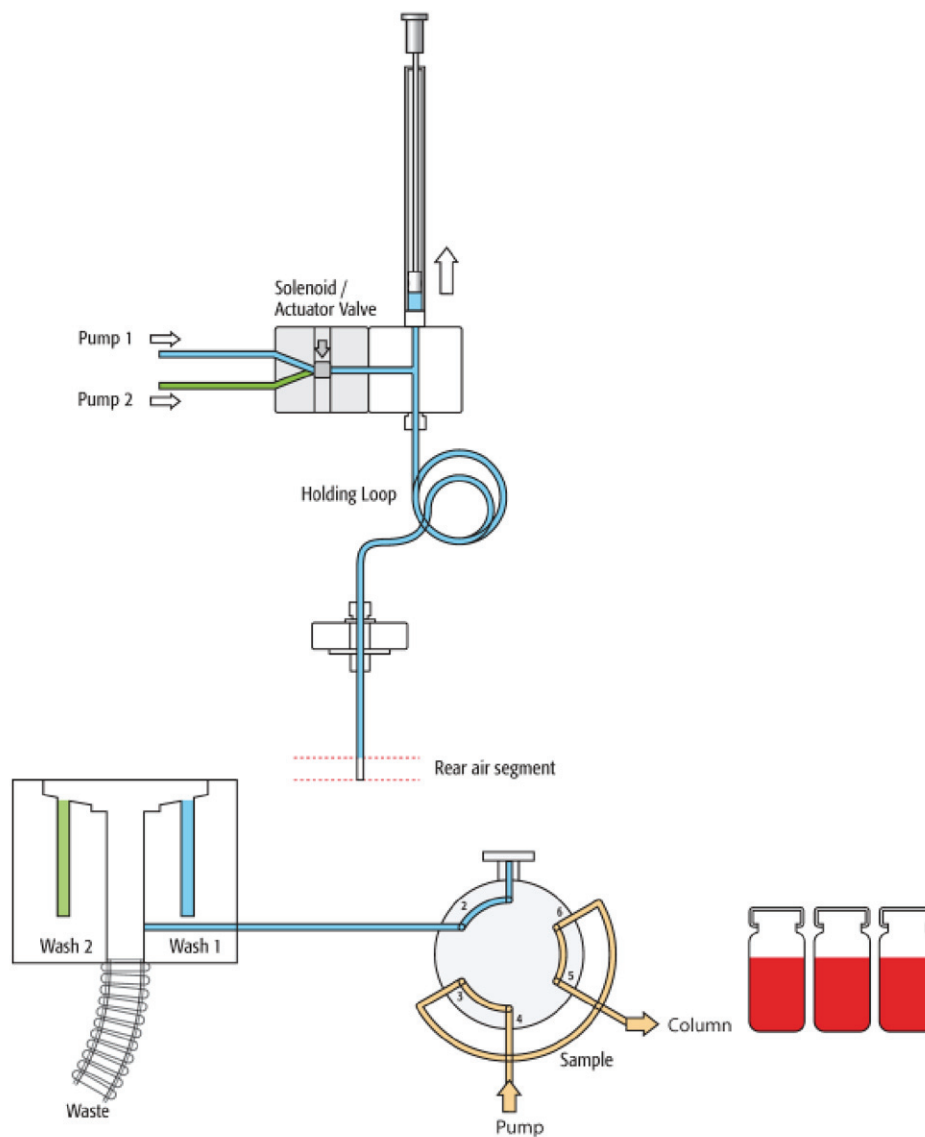


Figure 40. Standard: Step 2 – Get sample, aspirate rear, inject, and front volume

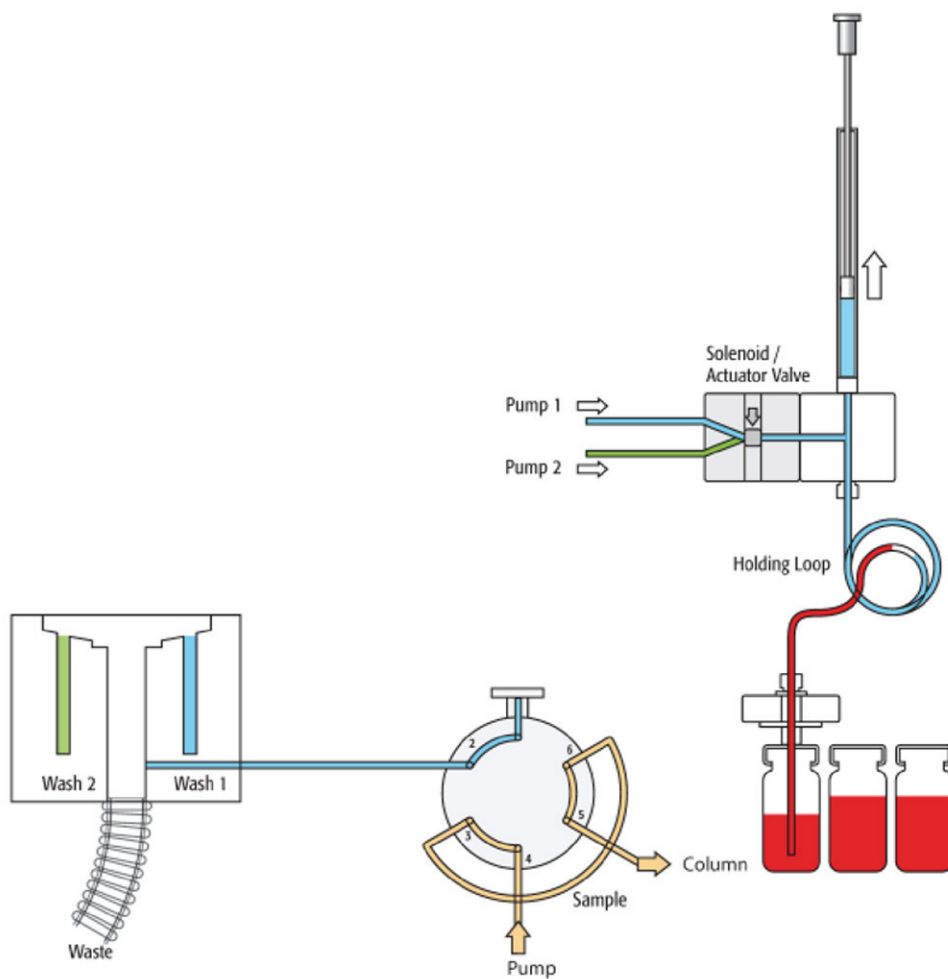


Figure 41. Standard: Steps 3 - 4 – A spirate front air segment

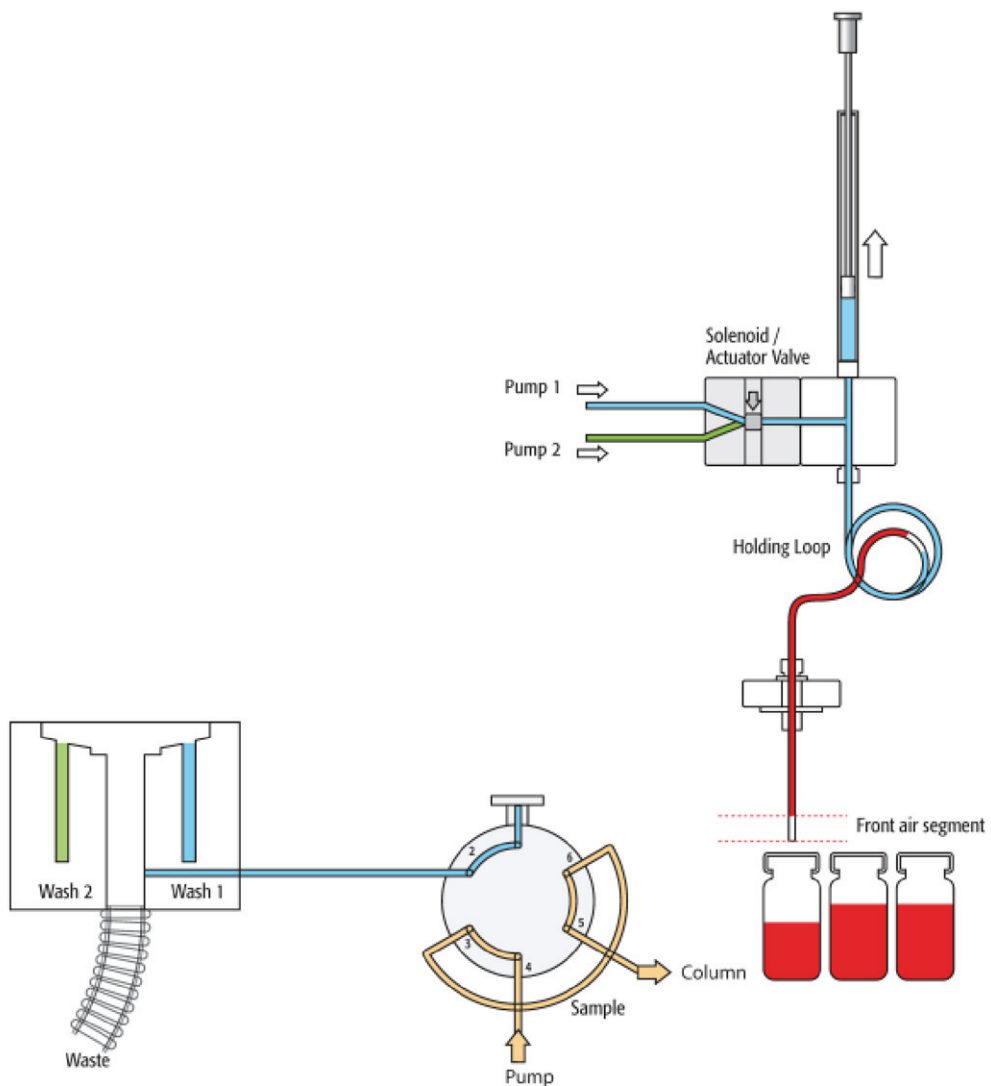
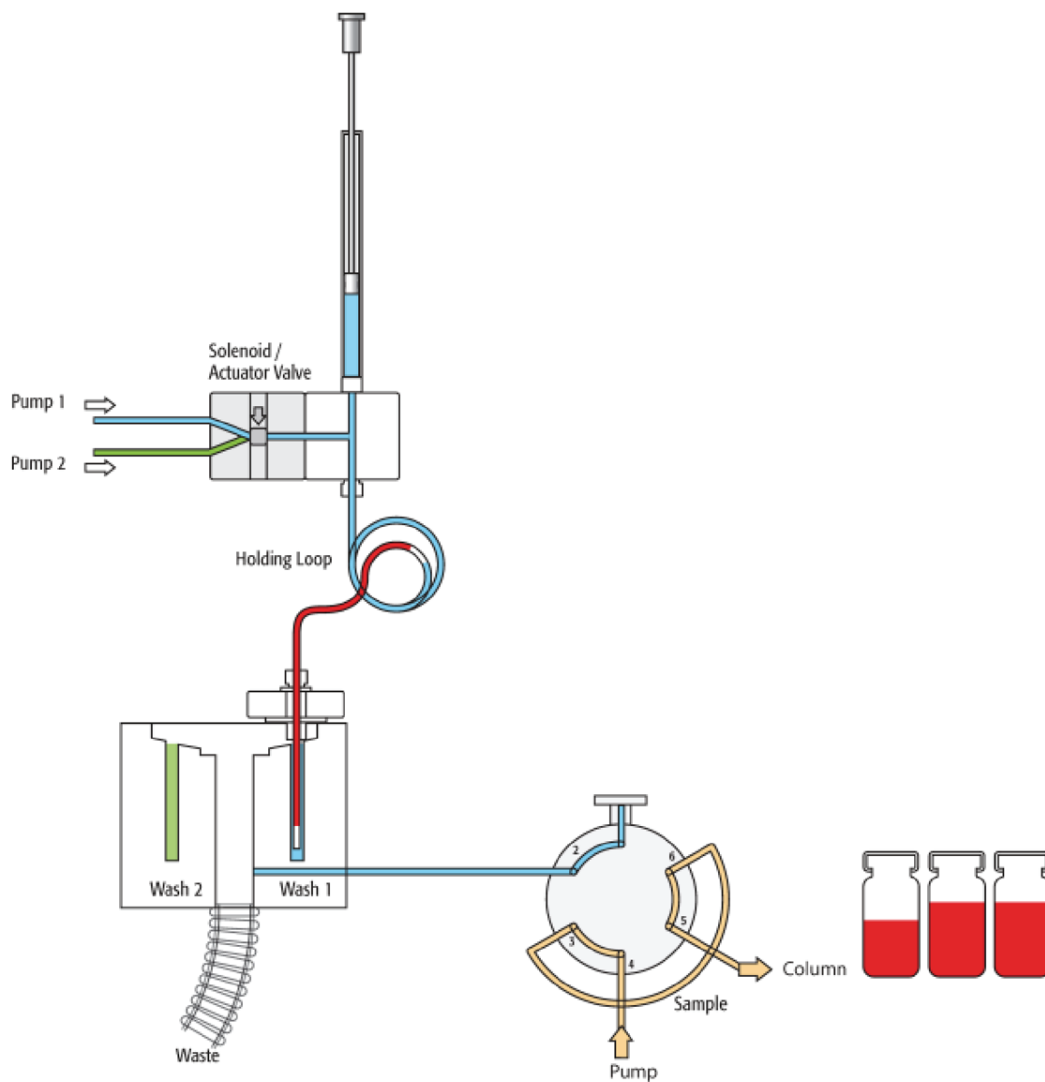


Figure 42. Standard: Steps 5 - 6 – Passive needle clean outside (dip) in wash position 1



5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 43. Standard: Steps 7 - 8 – Dispense front air segment and front sample volume to waste

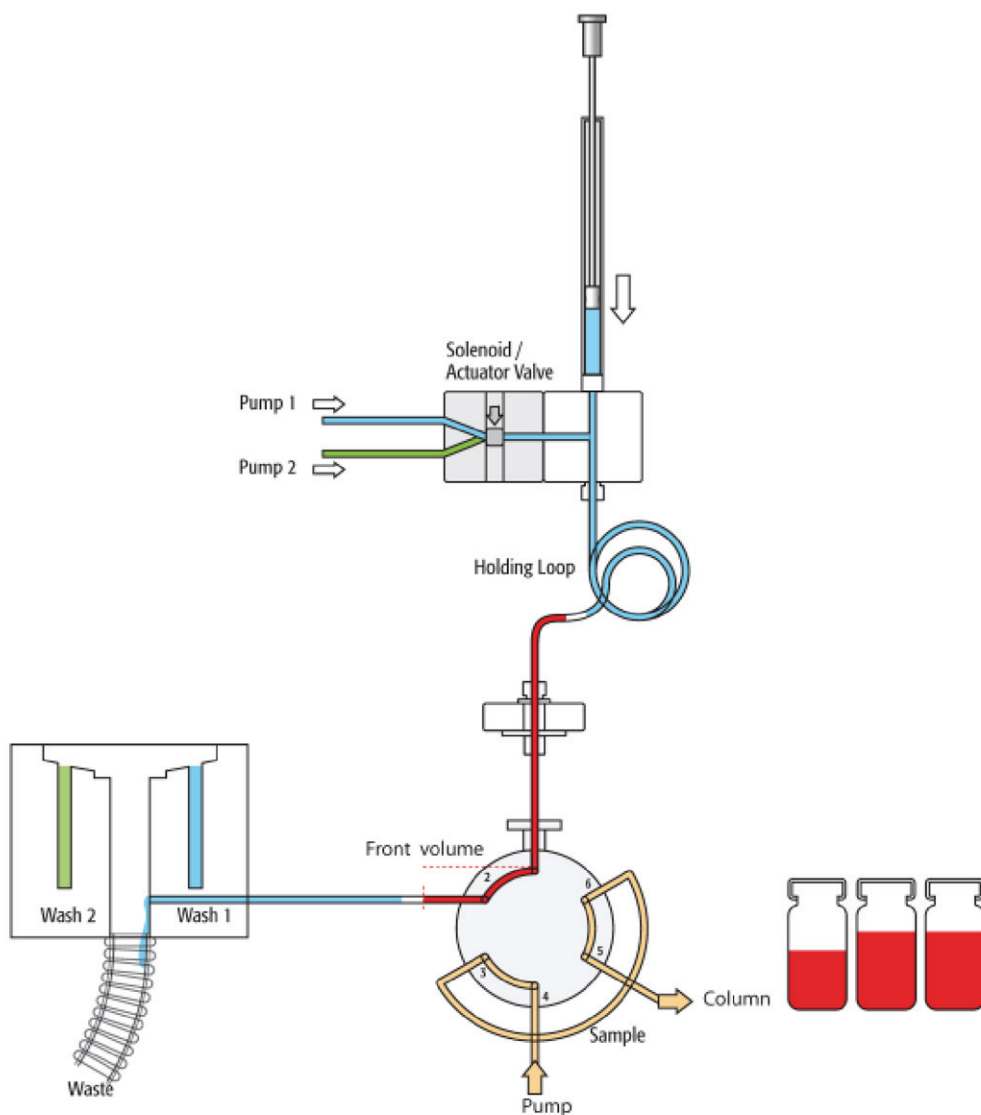


Figure 44. Standard: Steps 9 - 10 – Valve is switched to LOAD position, loop is filled with “Inject Volume”

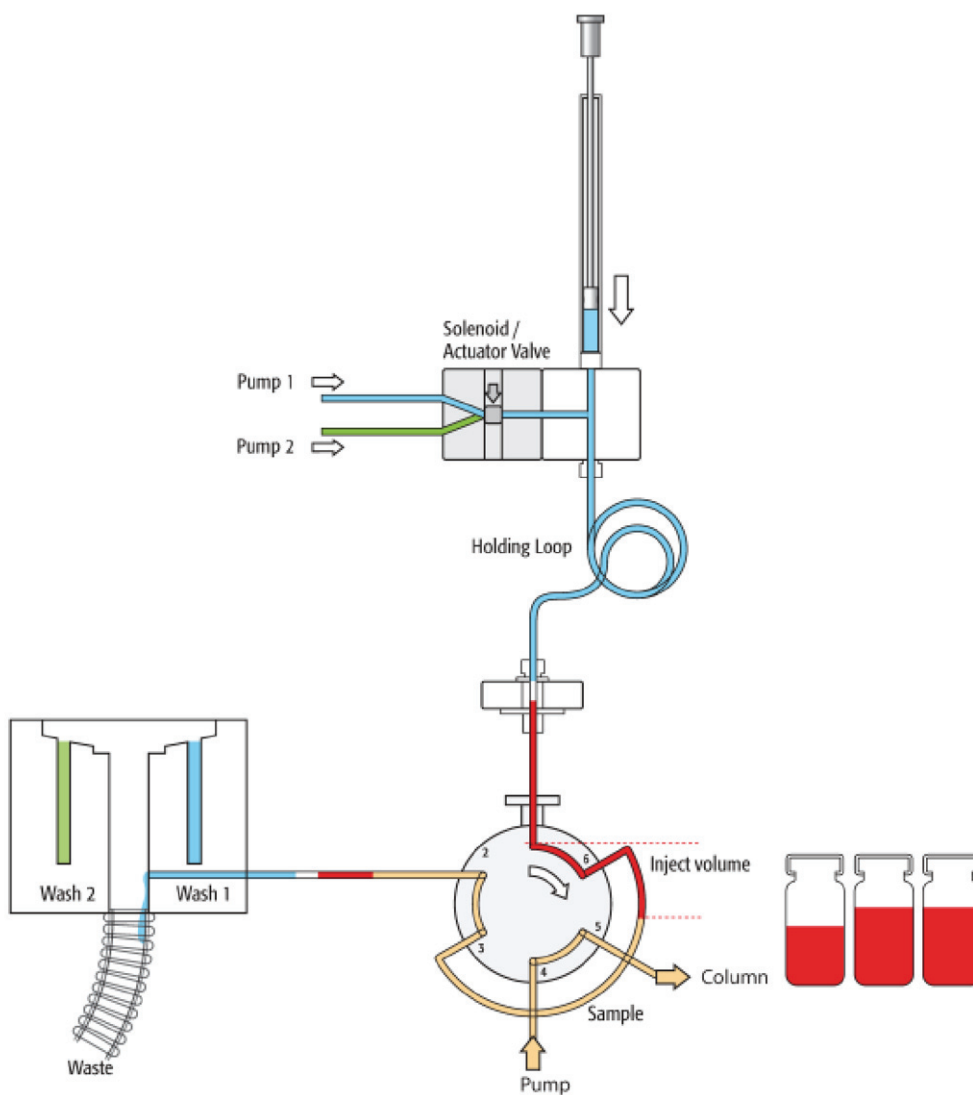


Figure 45. Standard: Step 11— Valve is switched to INJECT position, start chromatographic process

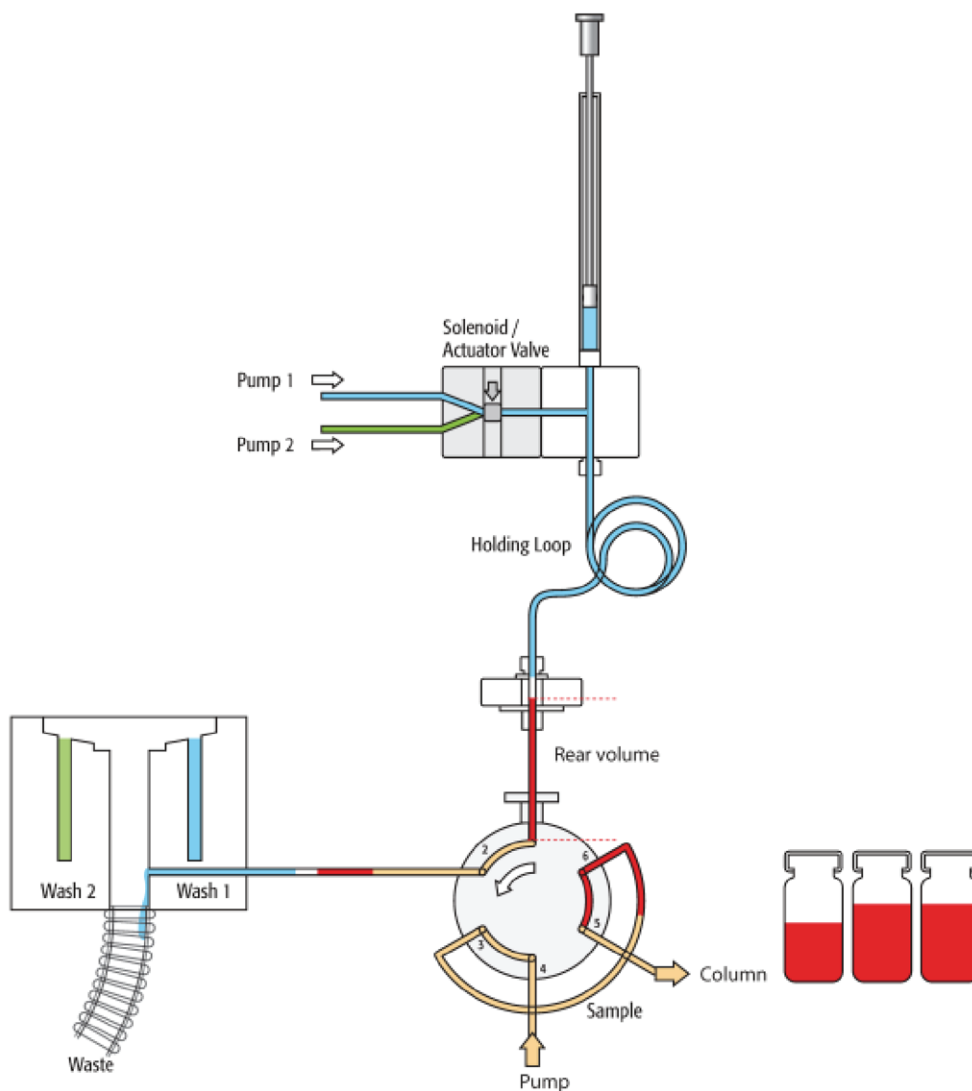
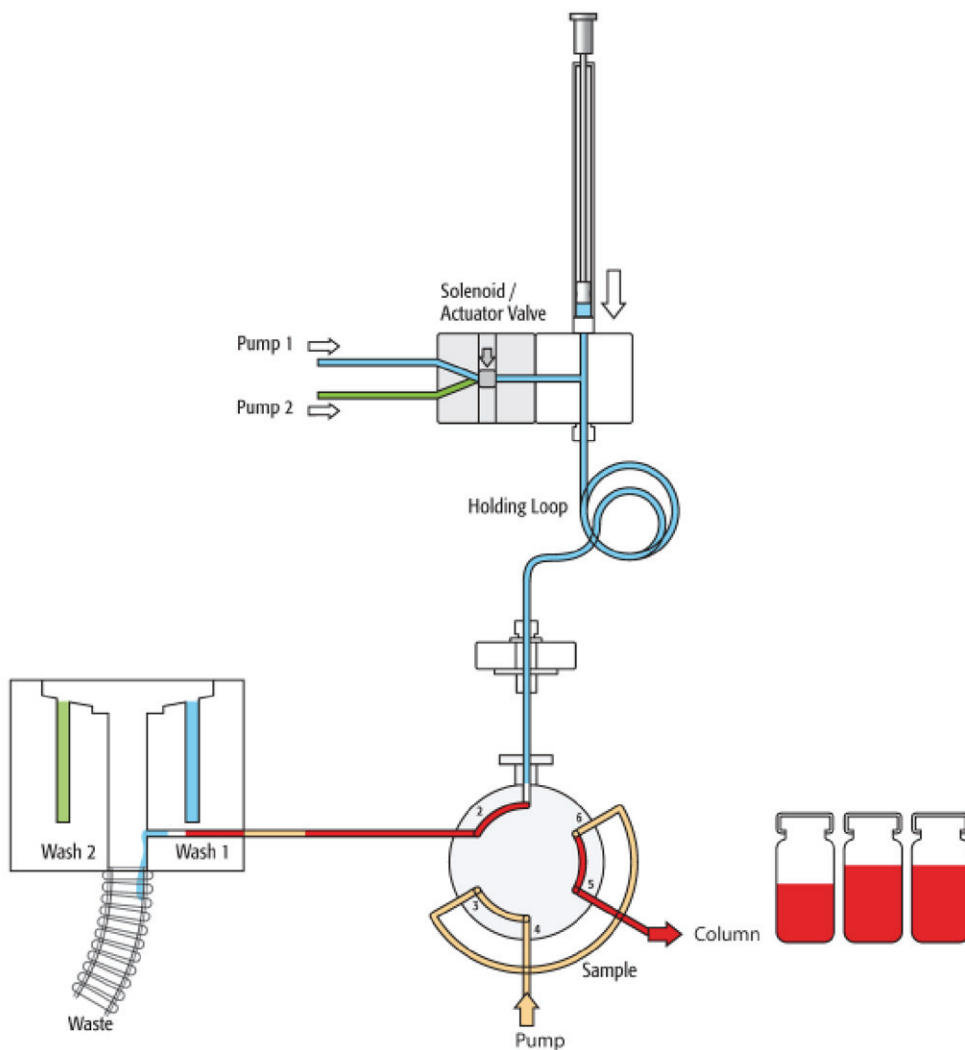


Figure 46. Standard: Step 12 – Rear sample volume and air segment are dispensed to waste



5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 47. Standard: Steps 13 - 14 – Valve clean with Wash Solvent 2

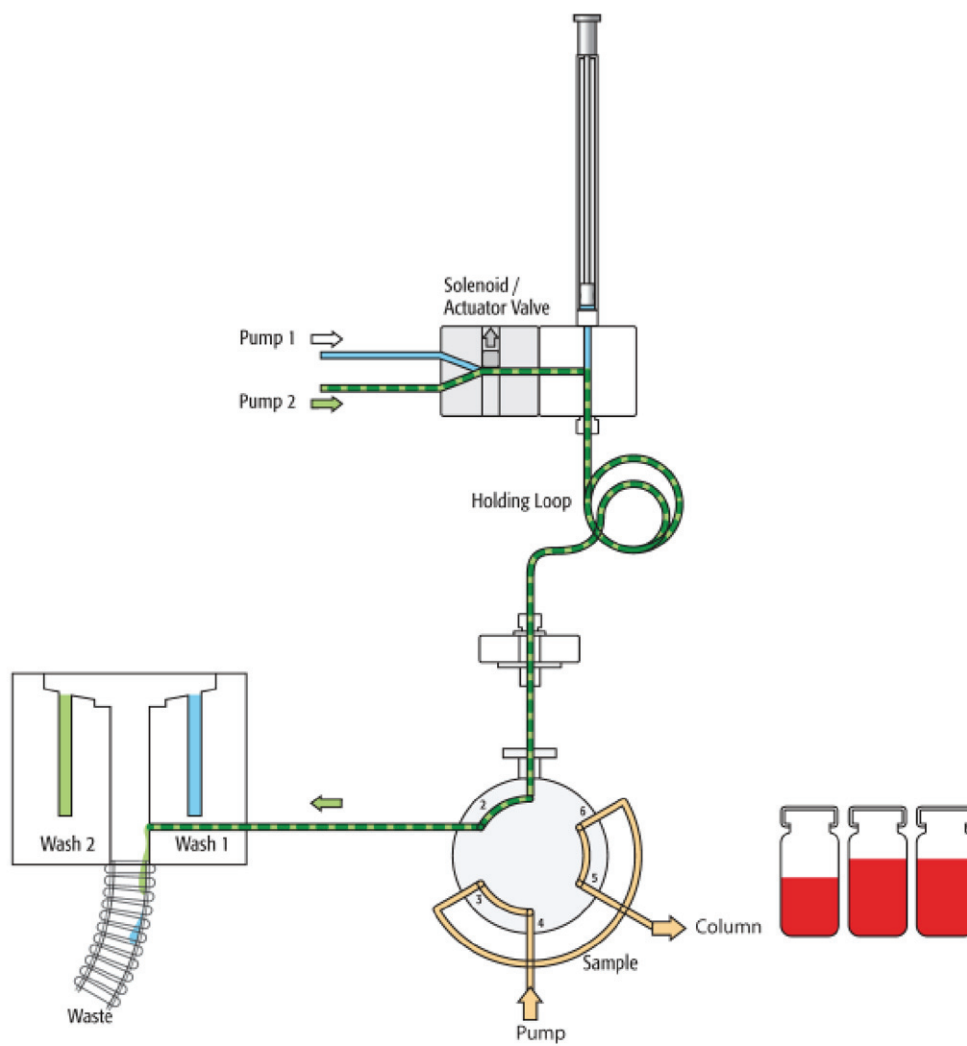


Figure 48. Standard: Steps 15 - 16 – Active syringe needle wash with Wash Solvent 2

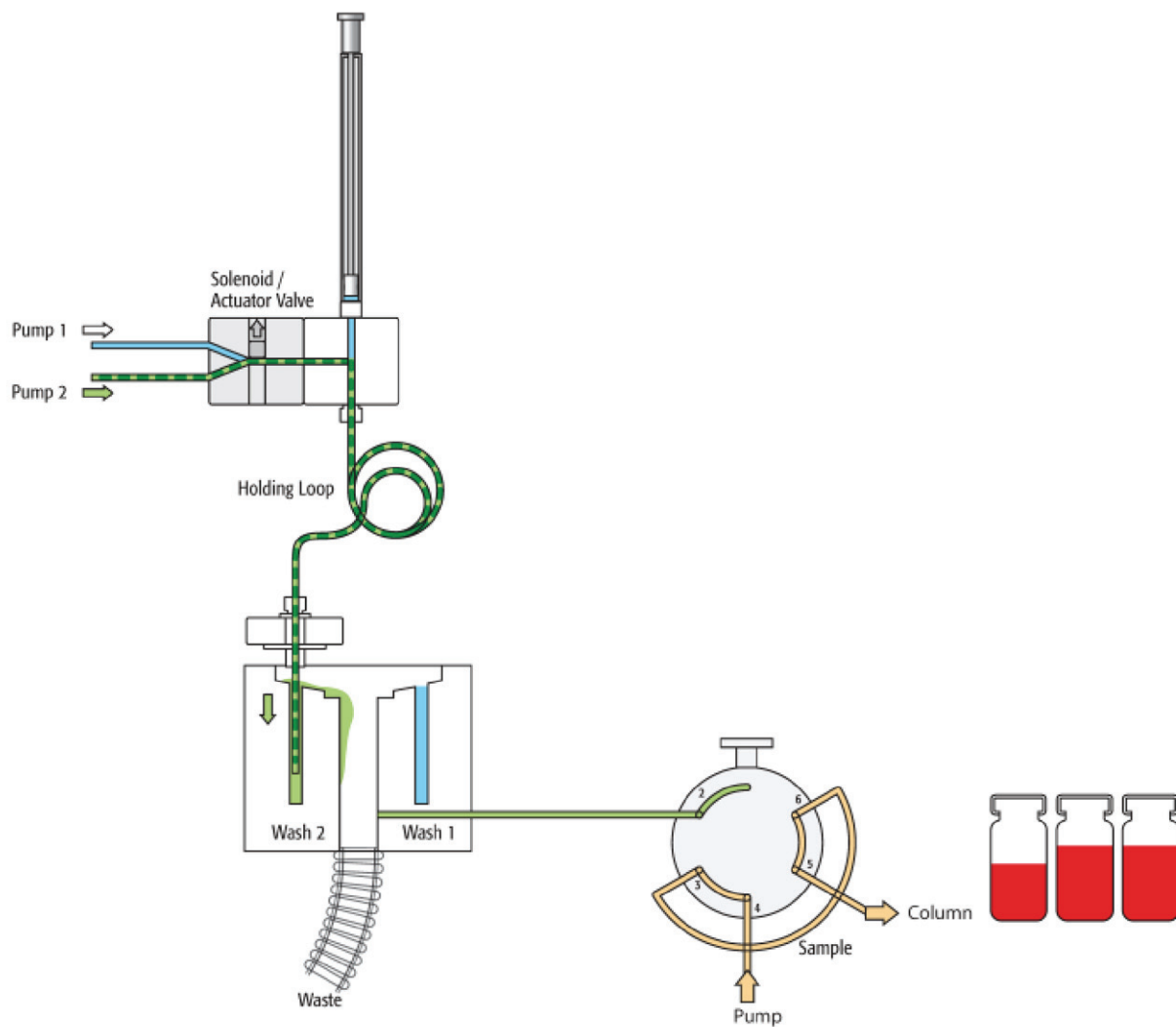


Figure 49. Standard: Steps 17 - 18 – Valve clean with Wash Solvent 1

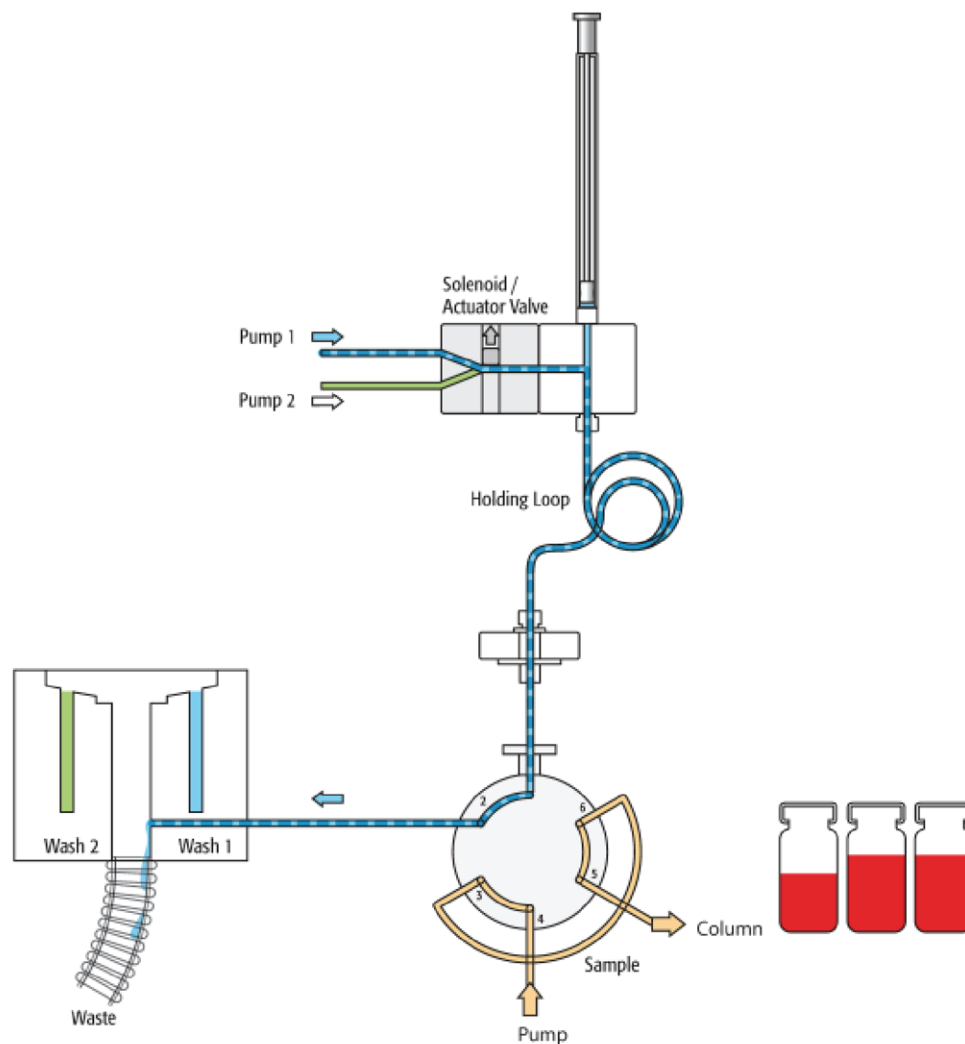


Figure 50. Standard: Steps 19 - 20 – Active syringe needle wash with Wash Solvent 1

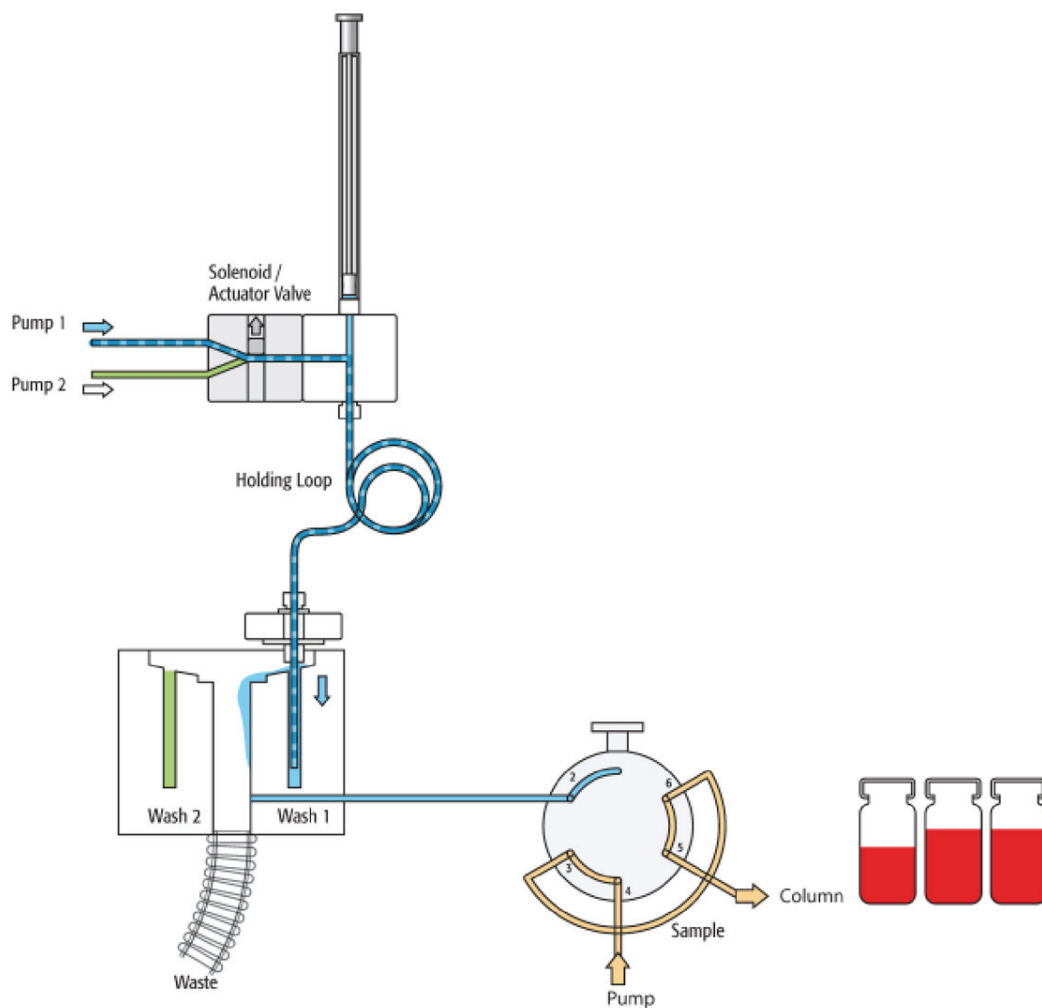
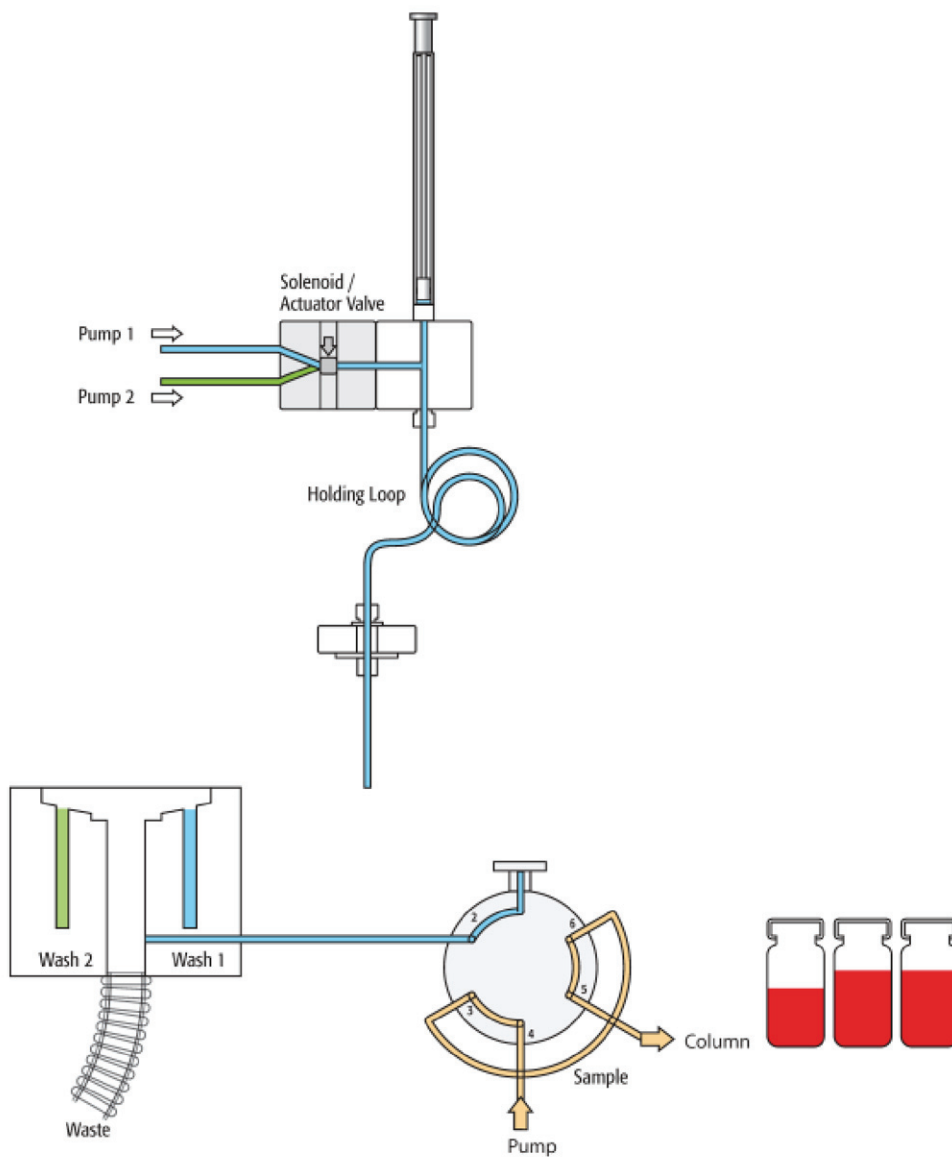


Figure 51. Standard: Cycle end



Additional Valve Toggle Step to DLW Standard Cycle

This section contains information about additional steps that are necessary for a DLW Standard Cycle.

Considerations for Additional Stator Wash Cleaning Step

The DLW Standard Cycle has the built-in option for the user to toggle the injection valve at the end of the chromatographic run before equilibration of the column to the start conditions.

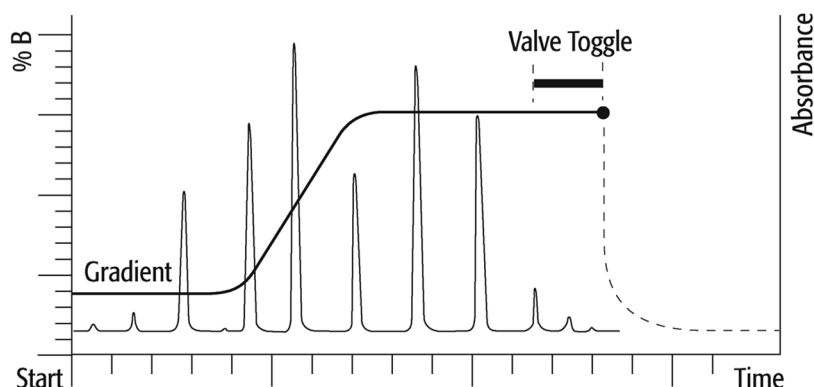
If the method variable “Stator Wash” is set to “1”, the extra cleaning process for the valve, with “Valve Toggle”, is part of the standard cycle.

If the method variable is deactivated (setting “0”), the DLW Standard cycle ends as shown in [Figure 51](#).

The macro (cycle) is written so that the optional valve toggle steps can be executed before re-equilibration of the column. You must synchronize the time to switch the valve with the chromatographic method using the method variable Delay Stator Wash. The two wash solvents are timed by the method variables Stator Wash Time Solvent 1 and Stator Wash Time Solvent 2. After these wash times have elapsed, the valve is switched back to the start position.

[Figure 52](#) illustrates the recommended retention time for Stator Wash or Valve Toggle times.

Figure 52. Timing for Stator Wash Step



From the chromatographic point of view, the optional cleaning step is important to understand. Assuming that the valve stator between ports 1 and 6 (example standard Cheminert valve) is contaminated and cannot be cleaned during the injection process, the valve toggle brings the engraving back between the two ports. Flushing the valve with both wash solvents eliminates remaining sample material located between stator ports 1 and 6.

What points must you consider when you use the Stator Wash or Valve Toggle option?

Observe the rules if biofluid samples are injected. First sample contact should always be with an aqueous solution to avoid protein precipitation. After washing with organic solvent (higher elution power) the system must be flushed again with wash solvent 1.

The first toggle near the end of the chromatographic cycle provides the advantage that the sample loop is already flushed out first with the mobile phase with a solvent of high elution power (assuming gradient application).

5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

The second valve toggle time follows immediately after finishing the second solvent flush. A second switching time cannot be programmed. The waiting time for the second valve toggle should be long enough so that the entire system is flushed out by both wash solvents.

Consider the entire delay volume to determine the second valve switch. The DLW internal volumes are:

Manifold, 90 μ L
Holding Loop, 108 μ L
Syringe Needle Gauge 22, 6.7 μ L
Installed Injection Loop

Total delay volume: 205 μ L + Loop content volume

Do the second valve toggle (back to starting condition) before the system equilibration time has started. The Loop content is ideally a solvent of a low elution power when switched back.

If isocratic chromatography is applied, the remaining contaminants might be washed into the system and can build up higher background noise for the column, the detector, or both over a longer period of time.

Additional Cleaning Step Stator Wash or Valve Toggle Step-by-Step

Figure 53 to Figure 58 illustrate additional cleaning steps for “Stator Wash”.

Stator Wash: End of Standard Injection Cycle

Figure 53. Start for additional cleaning step “Valve Toggle”

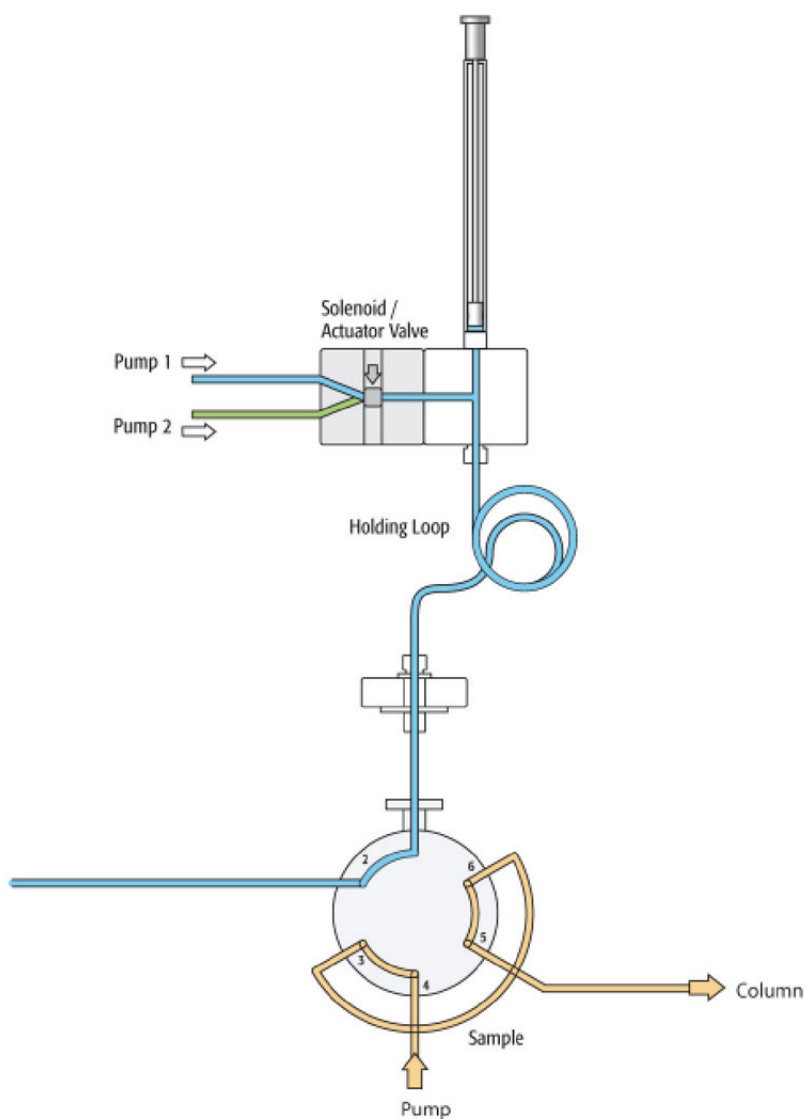


Figure 54. Stator Wash: Step 1 - 2 – Valve switched to Load Position (toggle), valve clean with Wash Solvent 1

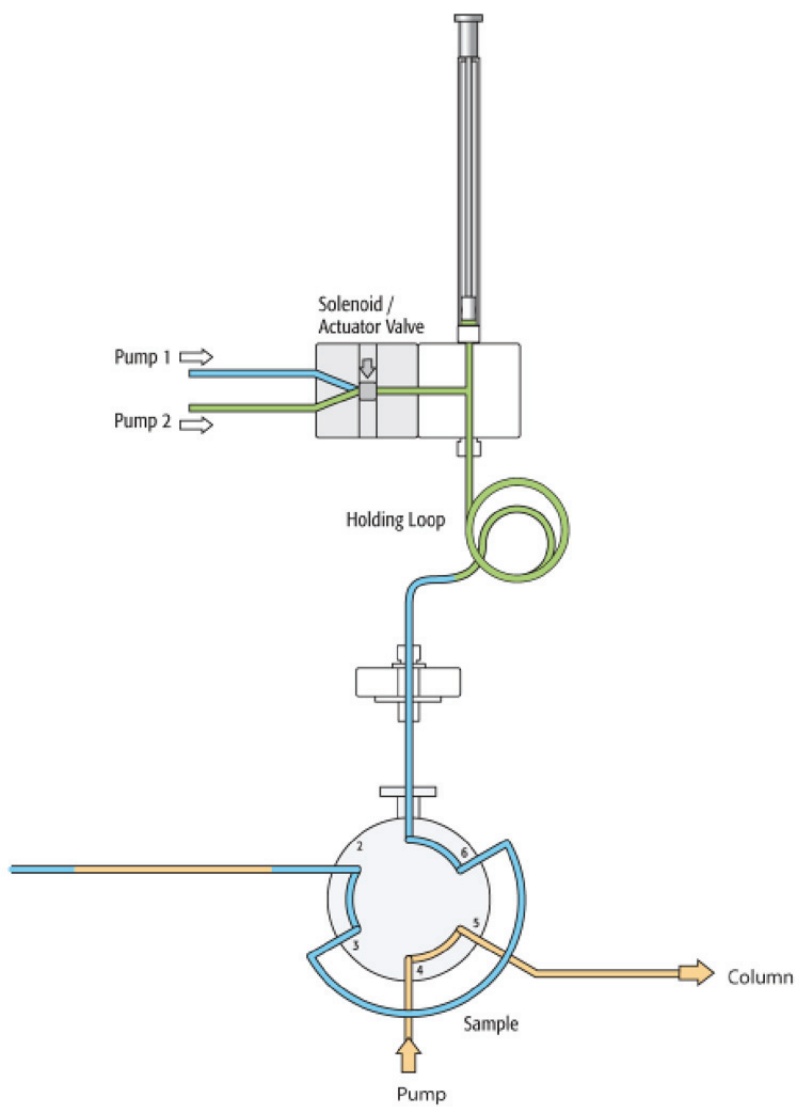


Figure 55. Stator Wash: Step 3 – Valve clean with Wash Solvent 2

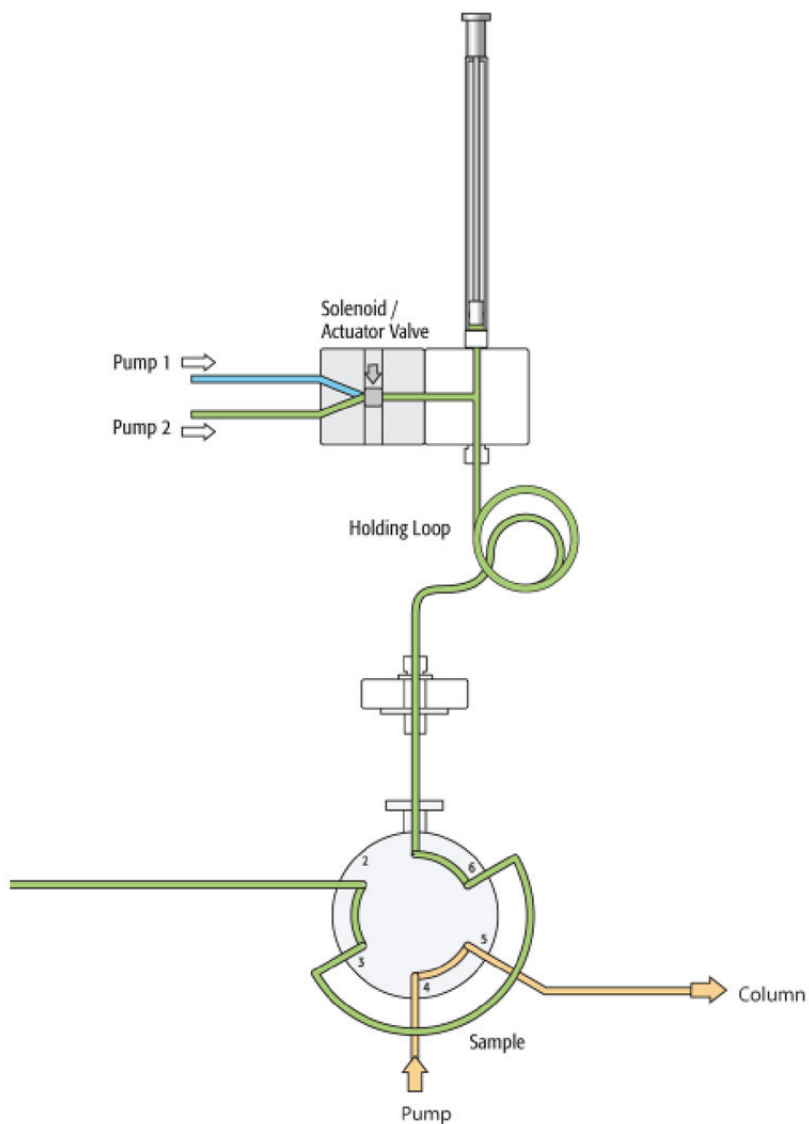


Figure 56. Stator Wash: Step 4 – Wash Solvent 2 dispensed by Wash Solvent 1

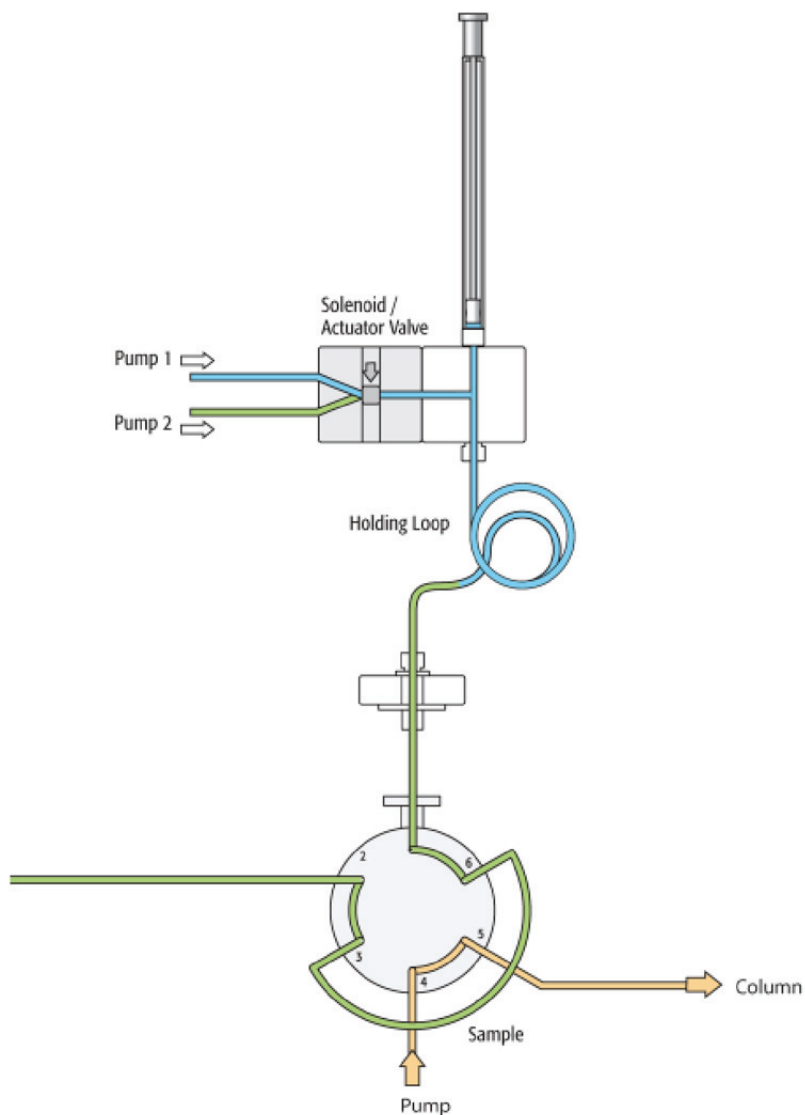


Figure 57. Stator Wash: Step 5 – Second valve clean with Wash Solvent 1

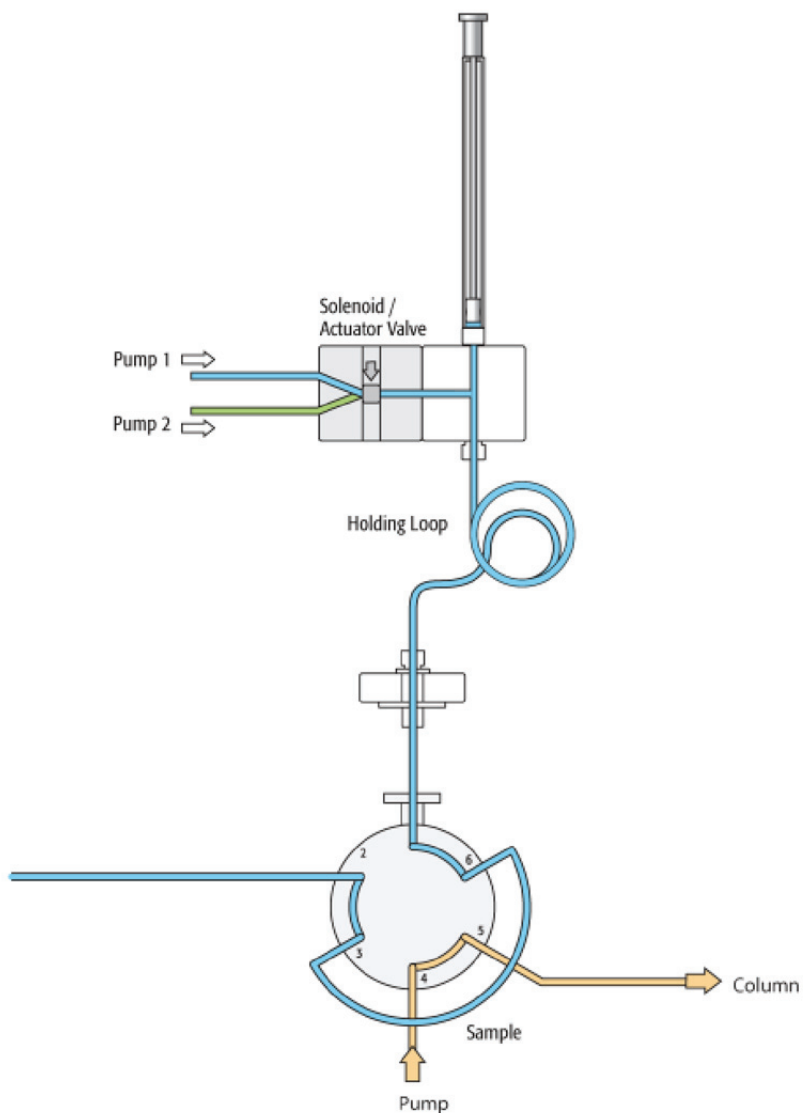
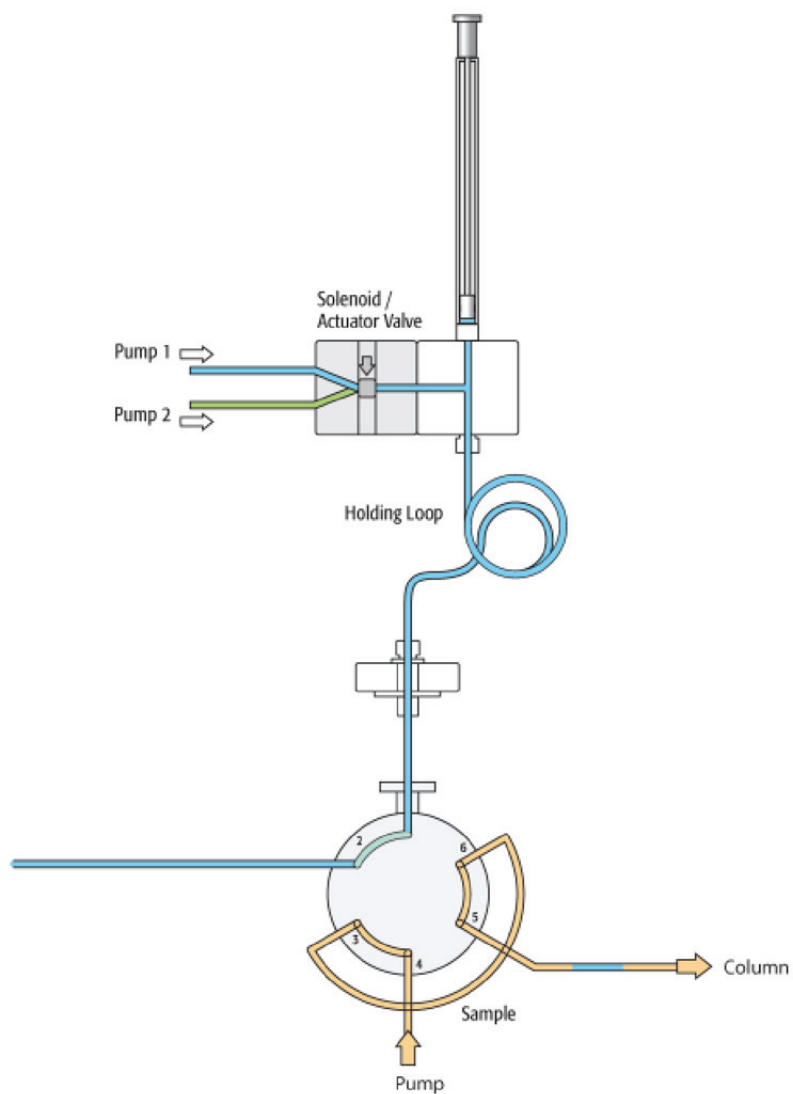


Figure 58. Stator Wash: Step 6 – Valve switched back to Inject Position (toggle)



Cycle for Fast Injection

Figure 59 to Figure 70 illustrate a step-by-step cycle for the Fast Injection.

Figure 59. Fast: Cycle start

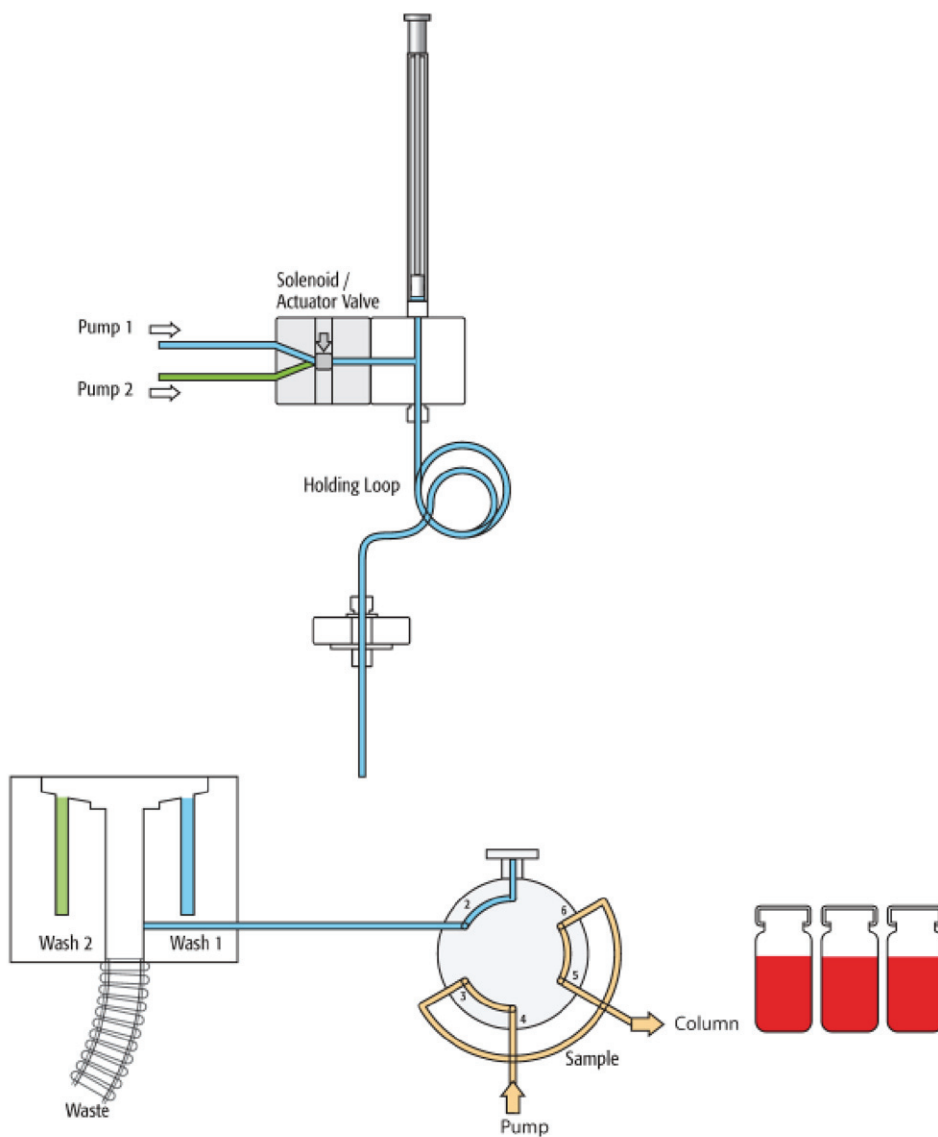


Figure 60. Fast: Step 1 – Aspirate rear air segment

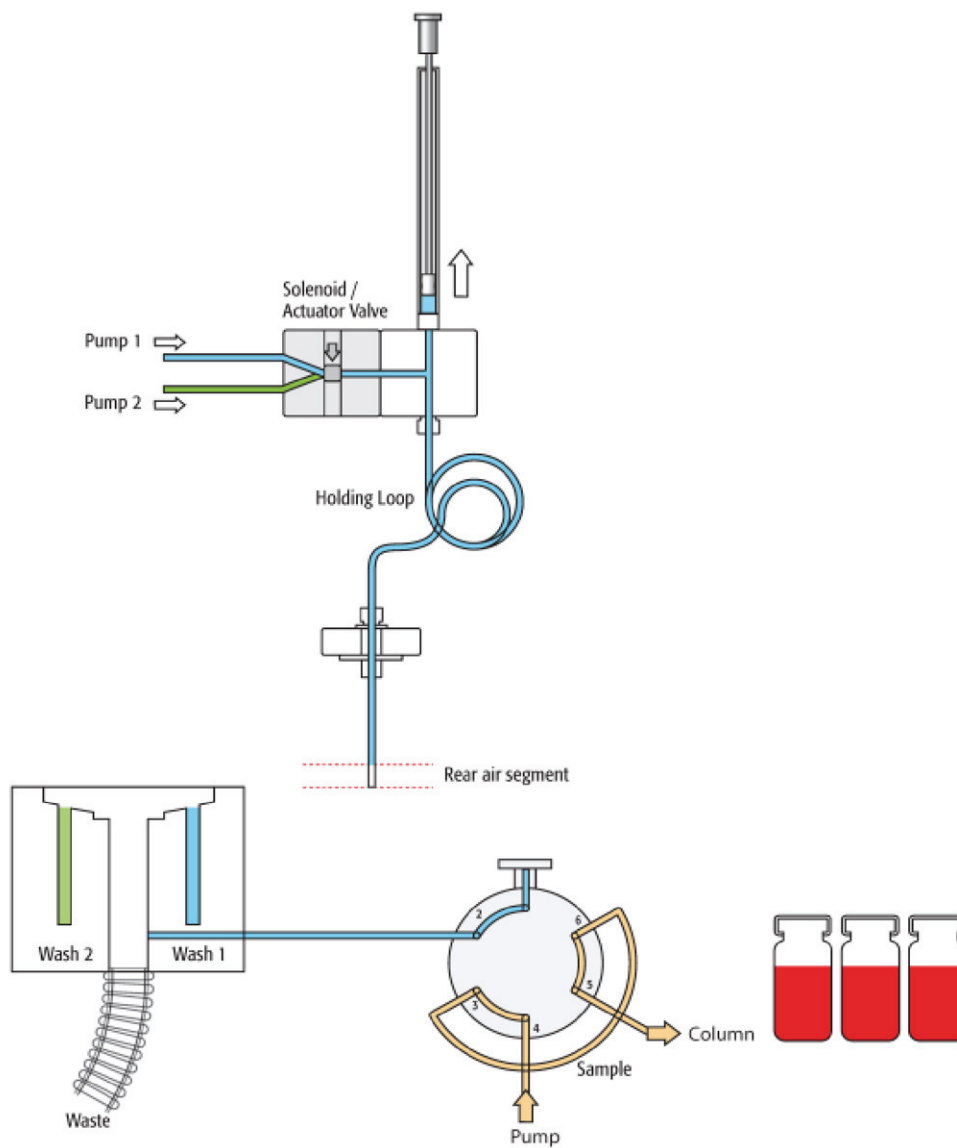
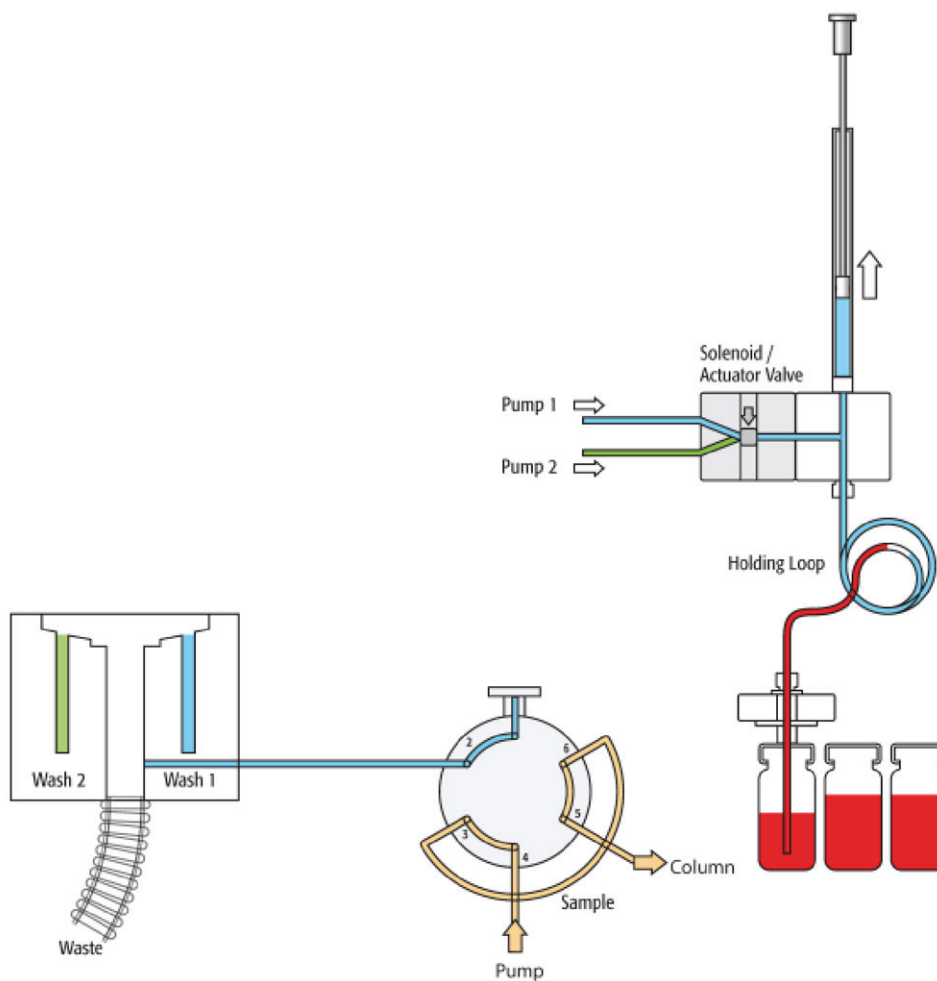


Figure 61. Fast: Step 2 – Get sample, aspirate rear, inject, and front volume

5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 62. Fast: Step 3 - 4 – Aspirate front air segment

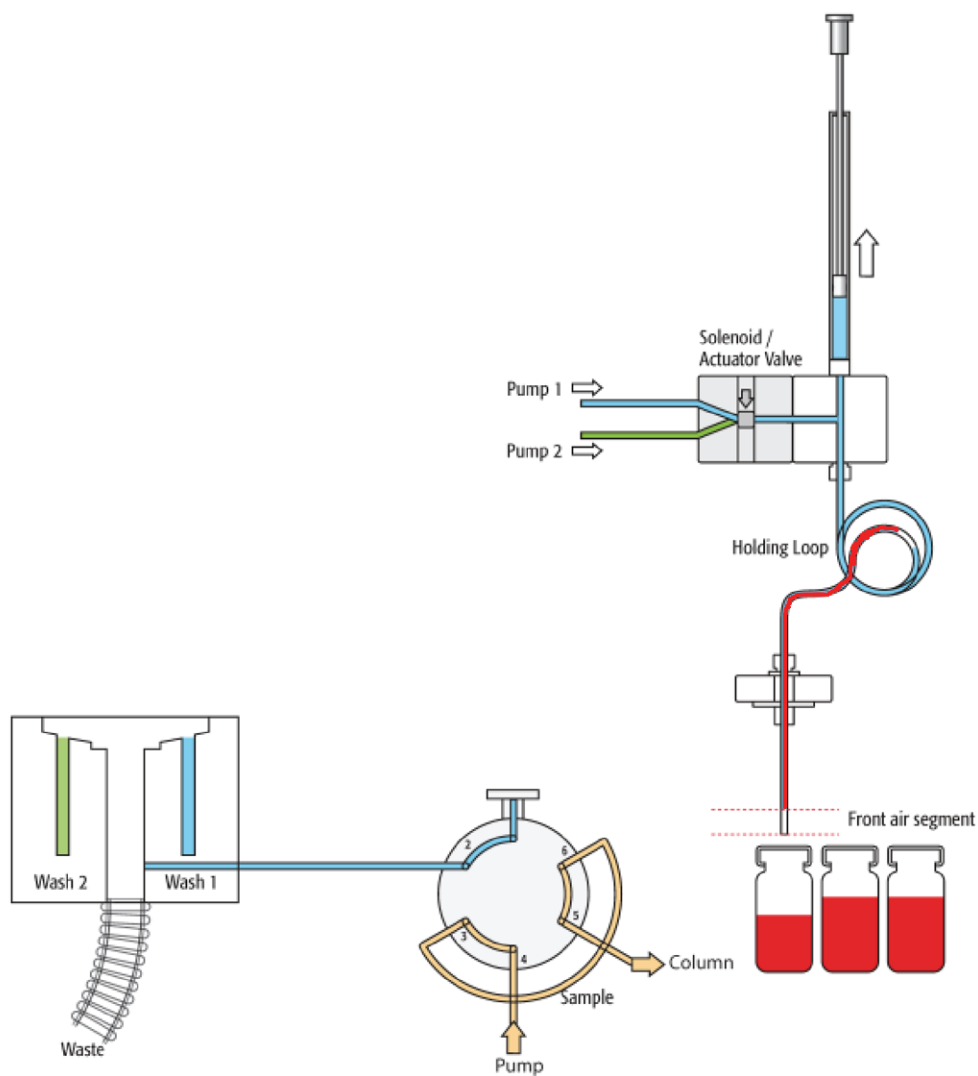
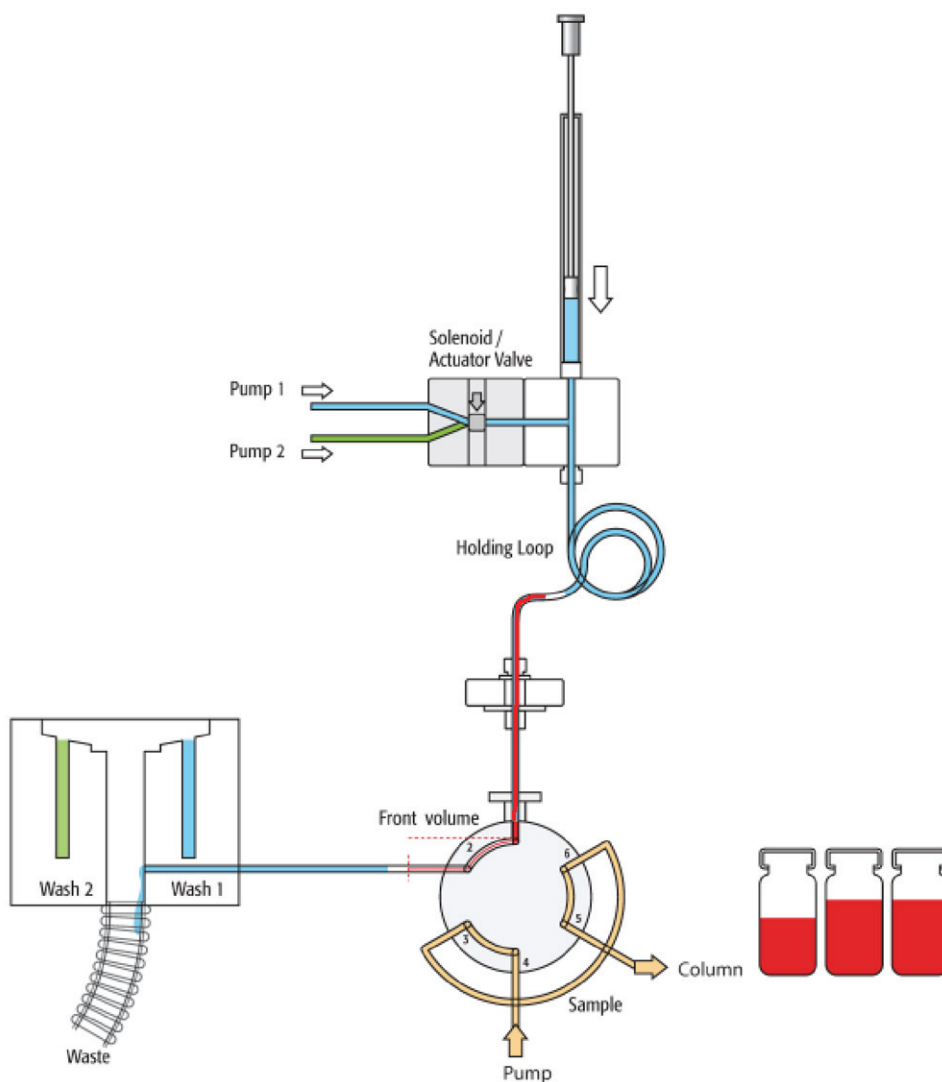


Figure 63. Fast: Steps 5 - 6 – Dispense front air segment and front sample volume to waste

5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 64. Fast: Steps 7 - 8 – Valve is switched to LOAD position, loop is filled with “Inject Volume”

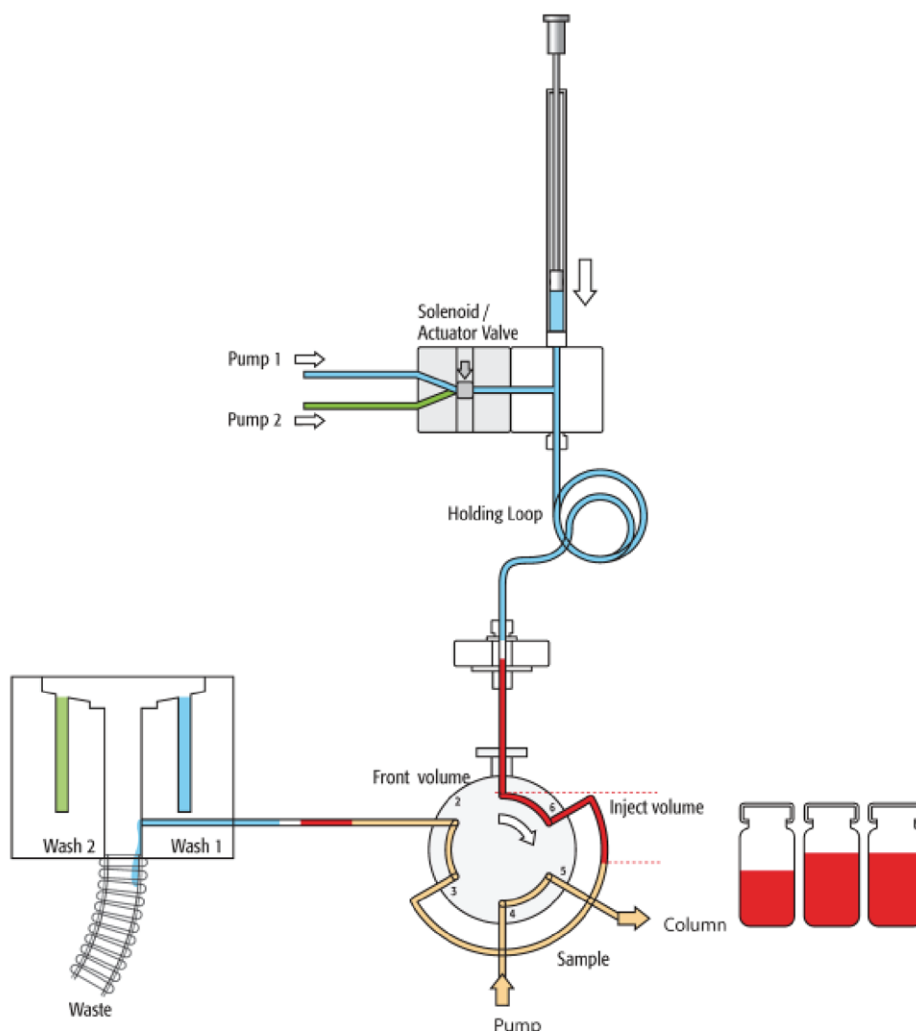
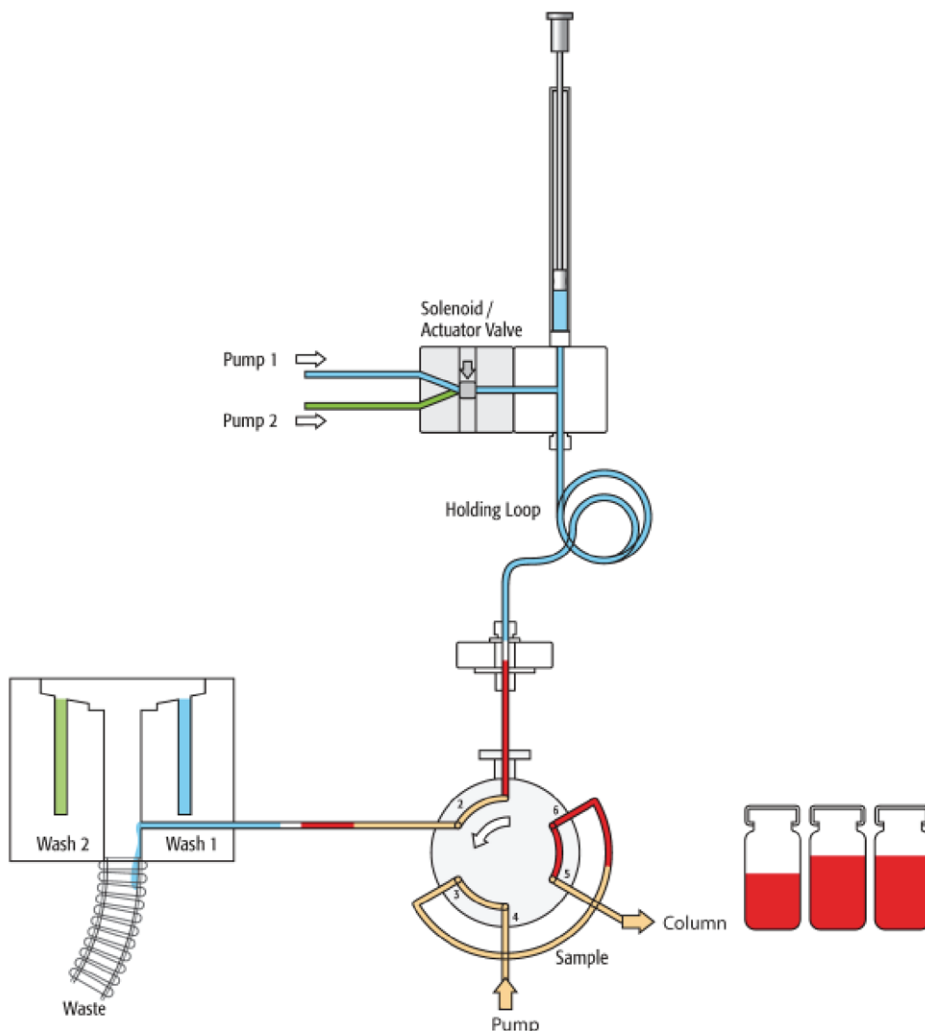


Figure 65. Fast: Step 9 – Valve is switched to INJECT position, start chromatographic process



5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 66. Fast: Step 10 – Rear sample volume and air segment are dispensed to waste

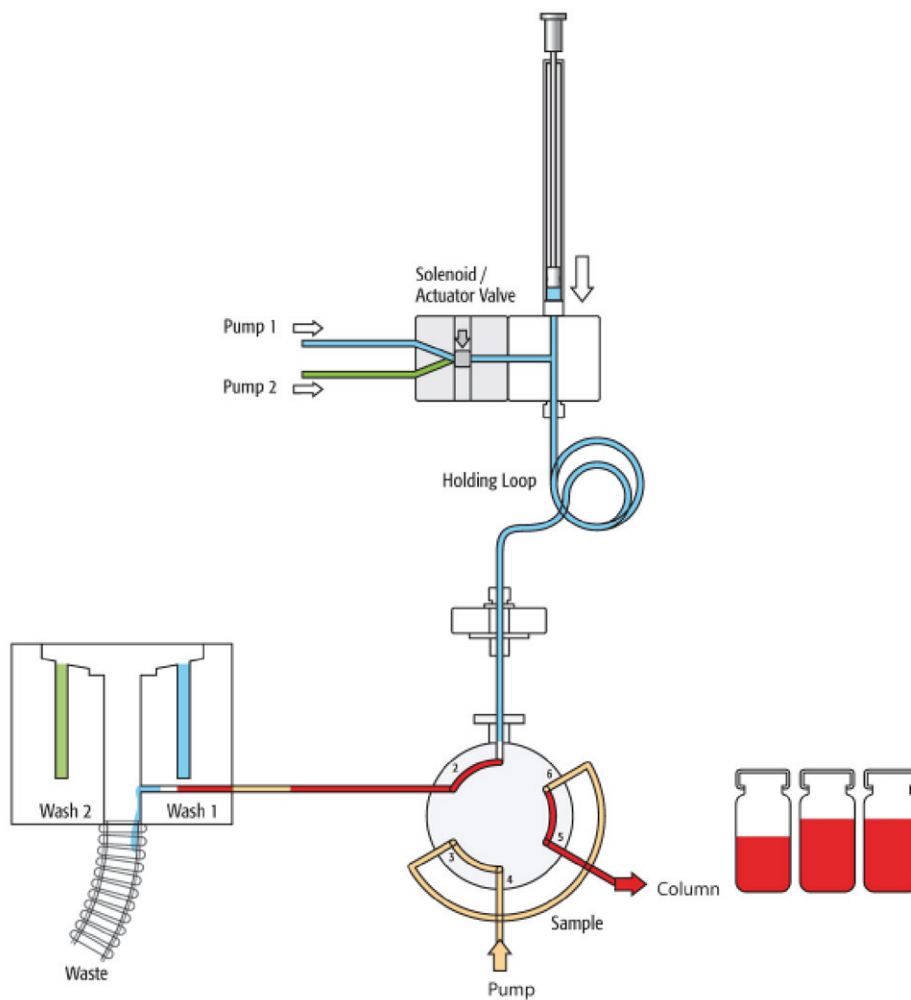
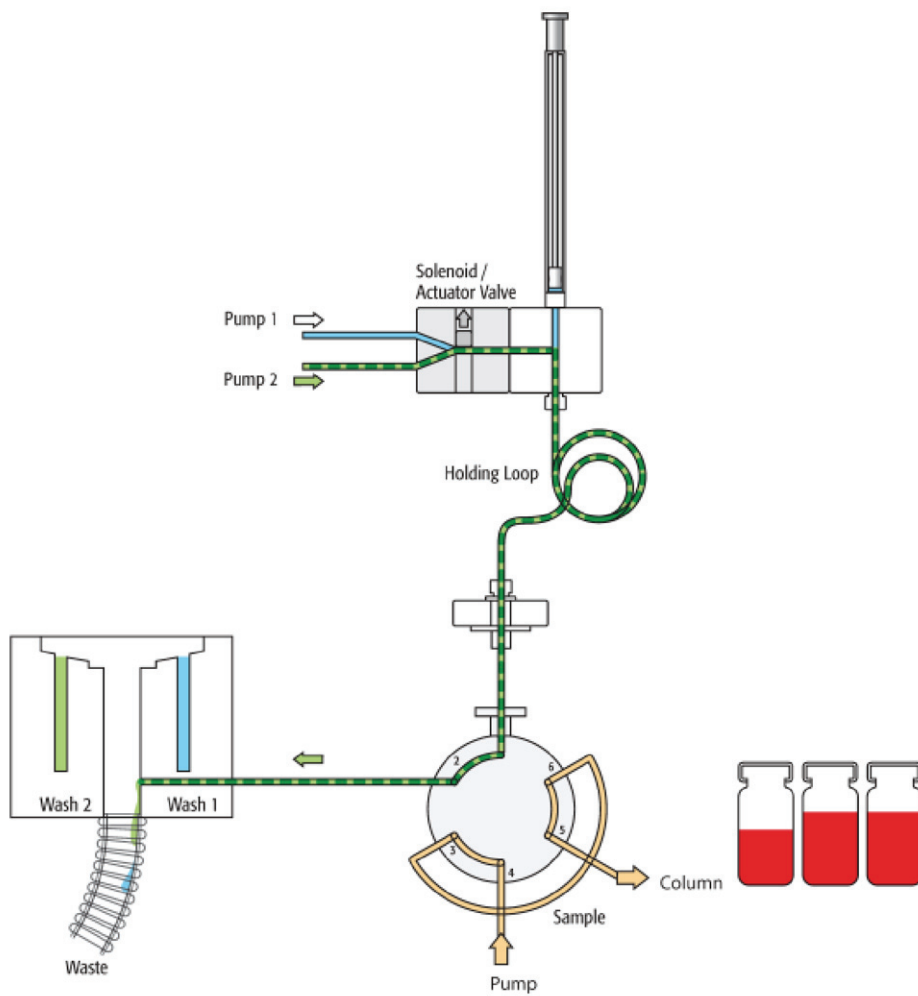


Figure 67. Fast: Steps 11 - 12 – Valve clean with Wash Solvent 2



5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 68. Fast: Steps 13 - 14 – Valve clean with Wash Solvent 1

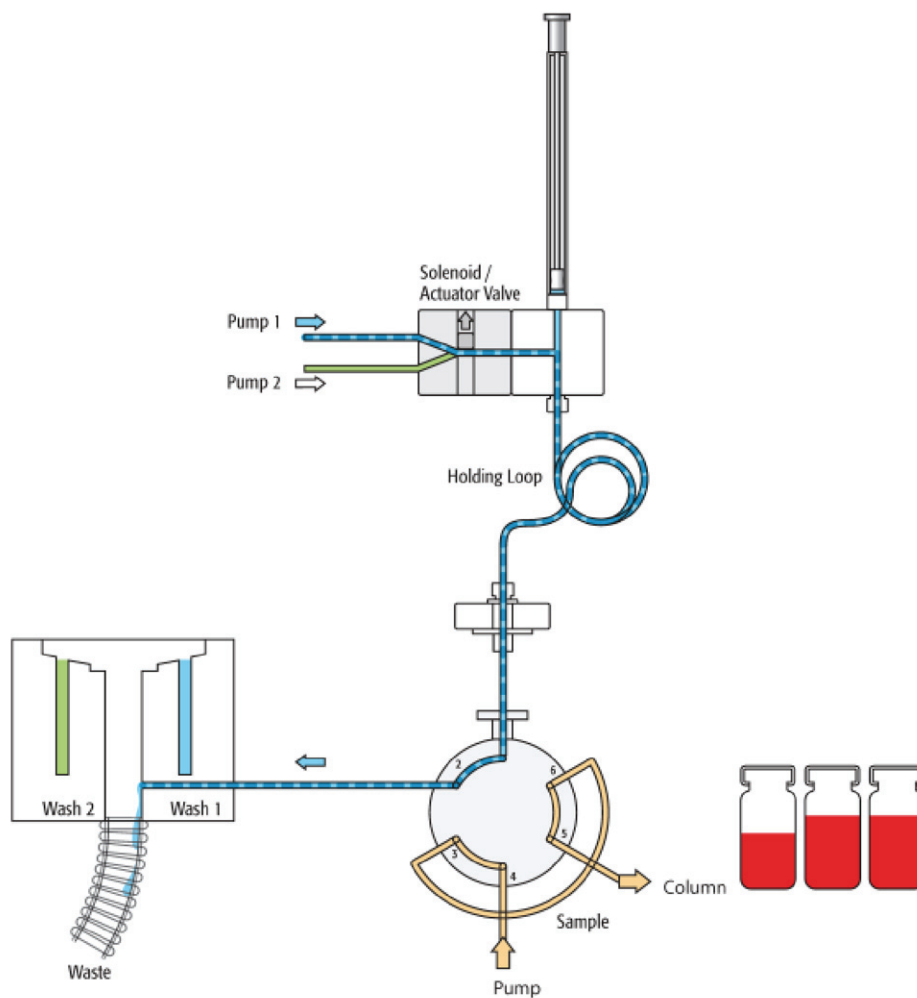
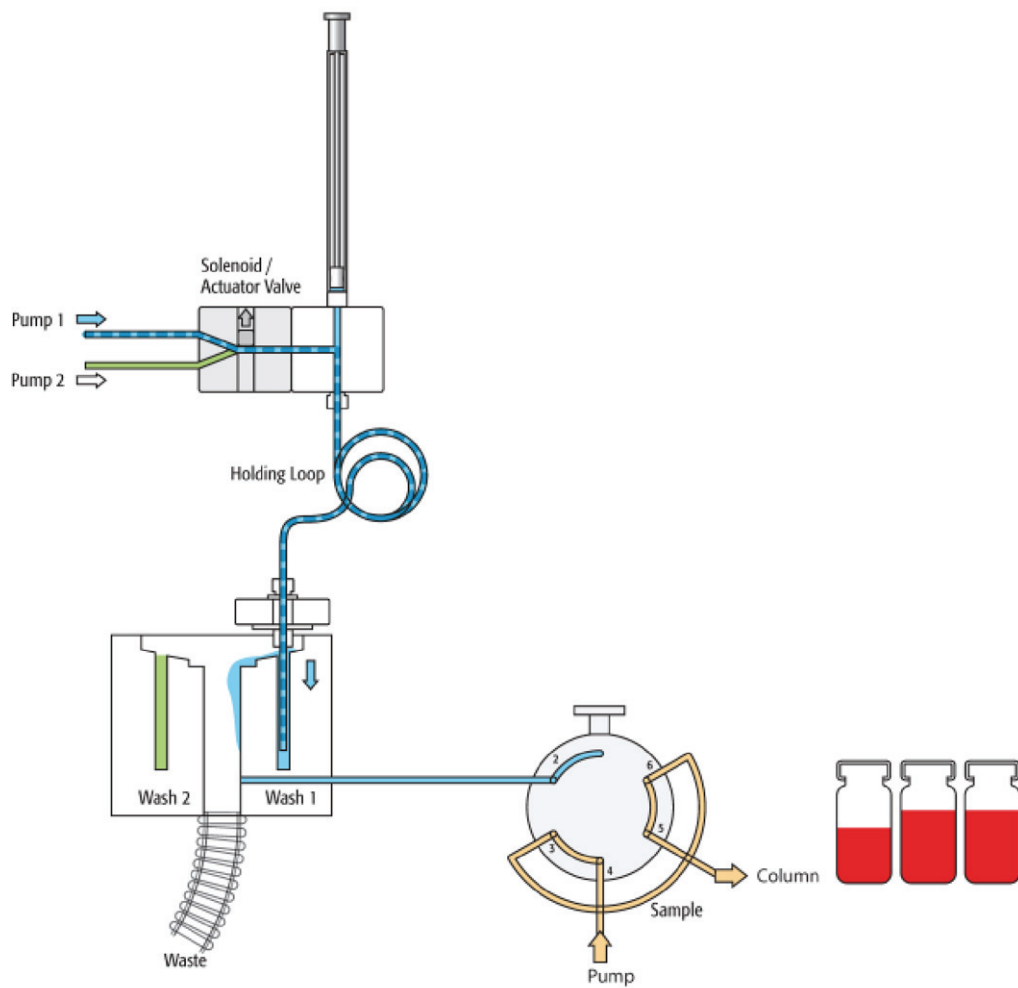


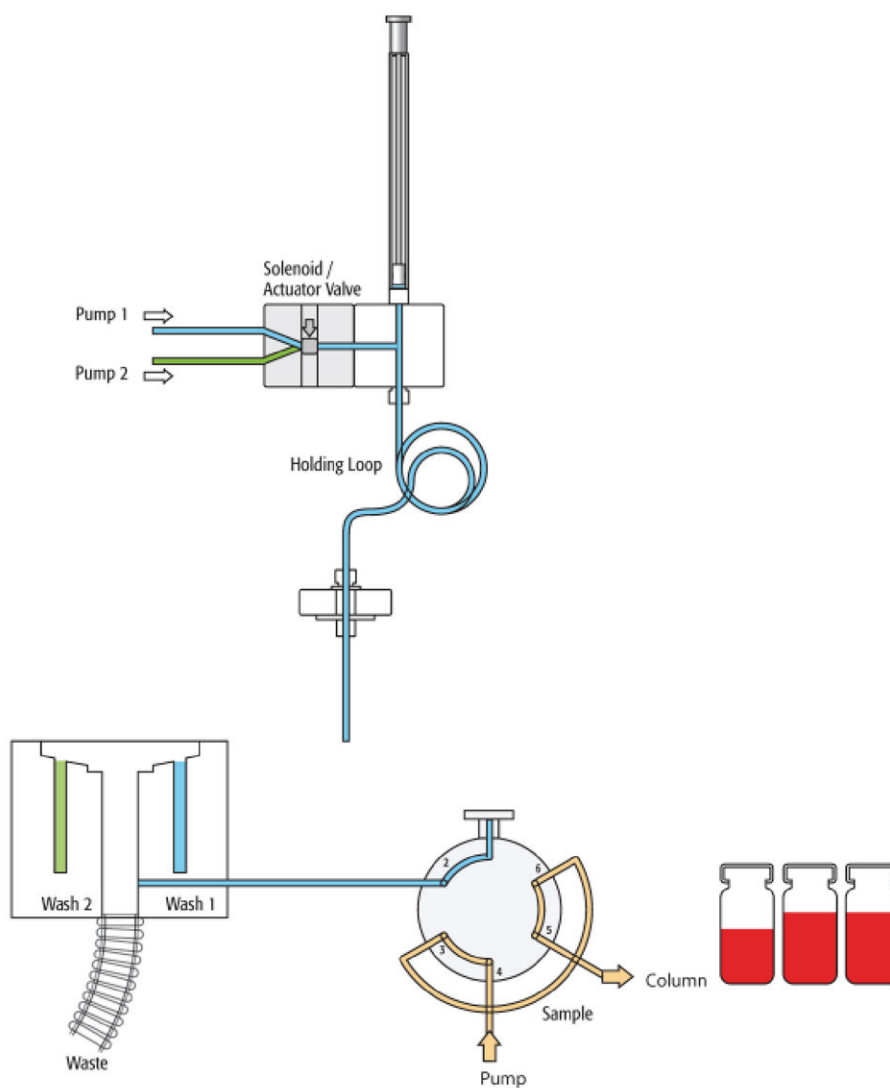
Figure 69. Fast: Step 15 – Active syringe needle wash with Wash Solvent 1



5 Using Dynamic Load and Wash (DLW)

DLW Cycle Step-by-Step

Figure 70. Fast: Cycle end



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