# **MassLynx NT Inlet Control Guide**

Version 4.0

Waters Part No - 715000399

Micromass Part No - 6666678

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## MassLynx NT Inlet Control Guide

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# Chapter 1 Controlling Inlet Systems and Autosamplers

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### Introduction

Mass spectrometers are usually used in conjunction with an inlet system such as a liquid chromatograph (LC) or a gas chromatograph (GC). MassLynx can control this equipment during data acquisition to provide complete control of an experiment. Autosamplers will often be used to automate the running of samples.

#### Inlets

There is a wide range of Inlets available with MassLynx, these are selected using the Inlet Configuration wizard (see page 1-9).

The method used to control the inlet system is set up before you start to acquire any data and is saved on disk for use by the acquisition system. Different methods can be saved, accessible by name in the usual manner. You must supply the name of the inlet method that you wish to use when you start an acquisition by entering it into the 'Inlet' field in either the Single or Multiple sample start editors (these are covered in the next section).

**N.B.** Make sure that any changes that you make to an inlet program are saved to disk before you start an acquisition. This is done by selecting the Save option on the File menu of the inlet editor or by pressing the toolbar button. If you do not save the parameters then the previous

ones will be used as MassLynx reads the parameters from disk, not from the editor, when it starts to acquire. Iconising the display does **not** save the parameters but you will be given the option to save any changes that you have made if you actually close the editor.

**Note.** For older Transputer based machines select **Configure, Select Interface, GC or LC system** (ACE) from the Acquisition Control Panel to enable the Inlet Configuration wizard. The list of inlet options that appears in the Select Interface dialog reflects the inlet systems, which were selected when MassLynx was installed. To change an Inlet for other non ACE systems MassLynx will need to be re-installed to gain access to the control software for the new inlet system. These inlets will not be available to Non-Transputer based Instruments e.g. CE Instruments (Chapter 3).

**Note.** For Transputer based instruments acquisitions that use an inlet system, can only be started from the Instrument Control Panel and cannot be started from the tune page.

#### Autosamplers

An autosampler can only be used with the Sample List Editor on the MassLynx top level screen.

The rules regarding the saving of parameters for inlet editors apply to autosampler editors as well.

## The Inlet Editor

The Inlet Editor program is an integral part of the MassLynx software suite, which is primarily for the editing of control parameters for a HPLC or GC system. In addition, it also enables direct control of these instruments, by enabling the user to download these parameters to the instrument and carry out other actions appropriate to the specific instrument.

The Inlet Editor supports a large number of instrument configurations. An instrument configuration is set up when MassLynx is installed but alternative configurations can be selected from within the editor itself. A configuration generally consists of a HPLC or GC (i.e., an Inlet), an Autosampler and a Detector (either UV or PDA). However, it is also possible to have a dual detector configuration using a SAT/IN analogue input box. Corresponding to these components the Inlet Editor provides three main windows (four for a dual detector set-up) referred to as views. There is also a status view for displaying information concerning the actual instruments. Only one

view can be displayed at any one time, which is selected by menu, toolbar button or Shortcut bar menu icon. Each main view may consist of one or more sub-views or pages, which are selected by a tab, and it is on these pages that the user enters method parameters (unless it is a status view). Alternatively, if multiple-inlet support is enabled then up to 8 pumps and 8 detectors can be configured to run alongside a single Autosampler.

A particular instrument control software implementation provides the views (and pages) discussed in the previous paragraph. These views are described later on this manual in the chapters for the relevant instruments.

In summary the Inlet Editor is used to

- View the status of the current system.
- Define the GC or LC, autosampler and detector methods.
- Change instrument configuration
- Control pumps and lamps and run methods.

Click on the Inlet Editor button, shown below, on the MassLynx Shortcut bar,



or select Methods, Inlet from the Acquisition Control Panel menu bar.

👼 Untitled.gil - Inlet Method 📃 🗌 🗙							
File View Tools LC Gilson Pump Gilson Sampler Gilson Tools Help							
🗅 😂 🖬 🍊 🍋	🏣 🛵   🚑 🤗   💷	🏶   🍸 🖄	<b>a</b>				
70	Status						
Status	Indicators	_LC			Gradient		1
	<ul> <li>Running</li> </ul>	🝈 Run Tim	ie (mins)	0.000	-	100.0 %	
	O Pump On	🐴 Flow Ra	te (ml\min) :	0.000	₿	0.0 %	
Inec		Pressure	e (bar) :	0.0	Č	0.0 %	
<b>E</b>	<ul> <li>Inject Lycle</li> </ul>	Column	Left (°C):	0.0	6	0.0 %	
Autosampler	兽 Ready	Column	Right (°C):	0.0	PDA De	tector	]
	🔵 ок	Sample Sample	Temp (°C) :	0.0	Mode: Scan:	Not Installed 0	
Detector							
For Help, press F1							

Figure 1.1 The Inlet Editor

### **MassLynx Options**

The Inlet Editor requires some settings to be selected in the MassLynx Options dialog, which is invoked from the MassLynx Shortcut bar.

Options	×
Multi-probe	
Multi-probe Source	
4 Prob <u>e</u> s 🗖 Pos/Neg Switchir	ng 🛛
- Dual Source	
Lock Spray	
Inlets Allow Random Bottle Locations in Sample List	
Multi-Inlet Capability	
4 Number of Parallel Inlets	
Sample Prep	
OK	Cancel

Figure 1.2 MassLynx Options Dialog

The following options are relevant to the Inlet Editor: -

Allow Random Bottle Access	This switches the capability to have non-sequential vial references in a sample list when on a MUX system.		
Multi-Inlet Capability	This puts the inlet system into multi inlet mode and allows the user to select a number of parallel inlets.		
Number of Parallel Inlets	A number of parallel inlets from 2-8.		
Sample Prep	Ticking this allows multiple inlets to be used on a non-MUX system using a prep file.		

# The Inlet Editor Toolbar

The toolbar is displayed across the top of the application window, below the menu bar. The toolbar provides quick mouse access to many tools used in the control software, the buttons have the following actions.

The exact appearance of the toolbar will vary depending upon the installed configuration. Details of specific toolbar buttons are given in the Instrument Guides in later chapters.



# The Short Cut bar



#### Figure 1.3 The Inlet Editor Short Cut bar

The Short Cut bar allows for quick and easy access to the System Status Page and the setup pages for the pump, autosampler and detector.

# The System Status Page

Indicators				Gradient
<ul> <li>Running</li> </ul>	Ö	Run Time (mins)	0.000	% 0.0
O Pump On	Č3	Flow Rate (ml\min) :	0.000	6 0.0 %
	Ø	Pressure (bar) :	0.0	0.0 %
<ul> <li>Inject Cycle</li> </ul>		Column Left (°C) :	0.0	6 0.0 %
🖲 Ready		Column Right (°C) :	0.0	PDA Detector
🖲 ок	1	Sample Temp (°C) :	0.0	Mode : Not Installed Scan : 0

Figure 1.4 System Status page

The System Status page displays information about the state of the machine being controlled. This page can be accessed within the Inlet Editor by selecting **View**, **Status** from the menu bar, by

pressing the distance of the status of the s

**Note**: This changes for a GC, see HP6890 later in the manual. The Waters Cap LC also has a different System Status page see the Waters Cap LC System Status Page later in this manual.

Indicators	The Running, Pump On and Injector Cycle indicators at the left-hand side of the screen give you information on the current status of the LC system. The OK and Ready Indicators become illuminated in red if the LC System has an error. You can then click on the red indicators to give you more information on the cause of the malfunction.
Run Time	Displays how long the method has been running.
Flow Rate	This is the current flow rate as returned by the instrument.
Pressure	Displays the current pressure in the instrument.
Column Left and Column Right	Displays the current temperature of the left and right columns. These will be grayed out if column heaters are not installed.
Sample Temp	Displays the current temperature of the sample. This will be grayed out if a sample heater is not installed.
Gradient	Displays the solvent percentages at which the LC System is currently operating.
PDA Detector	When acquiring diode array data the Diode Array Status displays the number of scans currently acquired.

## **Multi Inlet Status View**



#### Figure 1.5 Multi Inlet Status page

The Multi Inlet Status view appears on tabbed pages. The first page of which represents the overall system status. The other pages are functionally identical to the page described in "The System Status Page" and display the status for each pump connected, on a 1 to 1 relationship. There is a page (Status 1, Status 2 etc) for each pump that is configured.

#### Indicators

Colored yellow when a method is running on any of the instruments, otherwise colored gray.
Colored yellow when flow is on in any of the LC systems, otherwise colored gray.
Colored yellow when the Autosampler is carrying out an injection or is performing some other operation such as washing the needle. Otherwise colored gray.
Colored red if the system is not ready, otherwise colored yellow.
Colored red if there is an error any all of the connected systems or there has been a communication error on any of the systems, otherwise colored green. If there is a communication error all other indicators are colored gray.

#### **Pump Status**

These represent each connected LC system's OK indicator. These duplicate the OK indicators on each of the status pages

#### Saving and Loading LC Parameter Files

The Current LC parameters can be saved to disk by choosing **Save** or **Save As** from the Inlet Editor **File** menu.

A set of previously saved LC parameters can be recalled from disk by choosing **Open** from the Inlet Editor **File** menu.

#### To Print an LC Method Report

Choose **Print** from the Inlet Editor **File** menu or press the <sup>SSE</sup> toolbar button. Press **OK** to print a report detailing the parameters used in the current LC Method.

#### To Download Parameters to the LC System

To download the parameters to the LC system, press the button or choose Load Method from the LC menu.

The status bar will indicate the progress of downloading the parameters. Once values have been downloaded you can start the pump running with the initial conditions.

#### To Run the Pump with Initial Conditions

Select **Pump On** from the **LC** menu or click on the button. The pump will begin running with its initial conditions.

#### To Turn on the Lamp

Select Lamp On from the LC menu or click on the **button**.

#### To Begin a Gradient Method or Start an Injection

You can run a single injection with the Autosampler by selecting **Run Method** from the **LC** menu as soon as the menu item is enabled (it is disabled when the system is running a method). If a method is already running in the LC System it will not be possible to start a new method (either inject or run gradient only) until the previous method has stopped.

Selecting **Run Method (No Injection)** from the **LC** menu starts the gradient (if entered) to allow manual injections.

# **Inlet Configuration**

The Inlet Configuration dialog (Figure 1.6) shows the current setup of Inlets, Autosamplers and detectors.

Multiple Inlets are only shown if Multi-Inlet Capability has been selected in the Mass Lynx Options dialog.

Inlet Configuration				X	
	Inlet	Autosampler	Detector 1	Detector 2	
	Waters 1525 Waters 1525 Waters 1525 Waters 1525	Not Configured	Not Configured Not Configured Not Configured Not Configured	Not Configured Not Configured Not Configured Not Configured	
	Selected Configuration Information				
	Pump Address = 2 AutoSampler not configured Detector not configured				
Inlet				Configure	
	Click a line in the list above to select an inlet channel. Click "Configure" to configure currently selected inlet channel.				
Click "Events & Trigger", to	set triggering metho	d and events.	vents & Triggering	Finish	

### Adding Pumps, Autosamplers and Detectors

Figure 1.6 Instrument Configuration dialog

- To access the dialog select **Tools, Instrument Configuration** from the Inlet Editor menu.
- To change the current setup, highlight a line in the list and select Configure to invoke the **Inlet Configuration Wizard** (Figure 1.7).

The Wizard dynamically restricts the choices available, based upon the selections made. To add an Inlet, Autosampler and Detector: -

- 1. On the Configuration Wizard Welcome page follow the on screen instructions. Click Next
- 2. Select the required pump from the Select Pump dialog (Figure 1.8). Click Next.
- 3. Select the required autosampler from the Select Autosampler dialog (this is similar in appearance to the Select Pump dialog). This dialog will show only the autosamplers that are compatible with the chosen pump. **Click Next.**
- 4. Select the required detector from the Select Detector dialog. This dialog will show only the detectors that are compatible with the chosen pump and autosampler. **Click Next.**
- Depending on the configuration selected the HPIB Communication (page 1-12) or GPIB Communication (page 1-12) dialogs are invoked. After filling out the values, if needed Click Next.
- 6. The Configuration Successful dialog is displayed. **Click Finish**, this returns to the Inlet Configuration dialog



Figure 1.7 The Inlet Configuration Wizard

	×
Select Pump Choose a chromatography device.	
Choose a chromatography device, and then press Next to continue.	
None         Waters2695         Waters515         Waters1525         Waters2600         Waters2795         Waters2795         WatersCapLC         HP1090         HP1050         HP1100         Jasco900         Jasco1500         HP6890         Gilson         Shimadzu	
< <u>B</u> ack <u>N</u> ext > Car	ncel

Figure 1.8 Inlet Configuration Wizard: Select Pump dialog

#### **HPIB** Communication

				×
<b>Config</b> u Configur	u <b>re HPIB</b> e HPIB communications.			-]
С	onfigure HPIB communica	ations, and then pre	ess Next to continue.	
	Device Addresses			1
	HP1100	Inlet 22 💌	C C Connection	
	HP1100	AutoSampler 22 ▼	0	
	HP1100 UV	Detector	·	
				J
		< <u>B</u> ack	<u>N</u> ext >	Cancel

Figure 1.9 HPIB Communication dialog

When an Instrument configuration has been selected on the Instrument tab default device addresses are written to this dialog. Values can be changed if required. For the HP1100 DAD detector the PC Connection should be set to HP100 DAD.

#### **GPIB** Communication

When an Instrument configuration has been selected on the Instrument tab default IDs are written to this dialog. These may need changing and will be defined on setup by a Micromass engineer. For more information consult the relevant instrument instruction manual.

Inlet Config	uration Wizard			×
Configur Configure	Configure GPIB Configure GPIB communications.			
Cor	nfigure GPIB comm	nunications, and ther	n press Next to continue	
	Instruments	Model	ID	
	<u>P</u> ump: '	Waters 2790	2 🔻	
	<u>A</u> utosampler:	None GPIB device	3 💌	
	Detector	Waleis 2407 UV	*	
	GPIB			
	<u>C</u> or	ntrol Board ID	0 💌	
	1/0	<u>T</u> imeout	10 s 💌	
		< <u>B</u> ack	< <u>N</u> ext >	Cancel

Figure 1.10 GPIB Communication dialog

# **Events and Triggering Wizard**

This wizard is for configuring input and output events and triggering methods for certain pumps and detectors.

- 1. To invoke the Events and Triggering Wizard select the **Events & Triggering** button on the Instrument Configuration dialog, the Events and Triggering Welcome Page is displayed.**Click Next**.
- 2. The Events page (Figure 1.11) is displayed and can be used to configure input and output events such as triggering an acquisition on a Mass Spectrometer.Click Next

Note. The Events page is only available on non Transputer instruments.

Events and T	riggering Wiz	ard			×
Choose Ex Choose inpu	<b>vents</b> it/output events				
Selec	t input/output e	vents, and ther	i press Next to	continue.	
	Event In				
	۵	<b>2</b>	<b>1</b> 3	<b>4</b>	
	Event Out				
	<b>1</b>	<b>2</b>	<b>1</b> 3	□ 4	
		<	<u>B</u> ack	<u>N</u> ext >	Cancel

Figure 1.11 Events and Triggering: Events Page

**Input Events** When acquiring from the sample list, the acquisition will not commence until a contact closure is seen on the specified port.

**Output Events** These events are sent at the end of an acquisition from the sample list.

ents and Triggering Wizard	×
Choose Triggering Method Choose software or contact closure triggering.	-
Choose pump and/or detector trigger method, and then press Next to	o continue.
Pump Trigger by Contact Closure Trigger by Software	
Detector     Trigger by Contact Closure     Trigger by Software	
<back next=""></back>	Cancel

Figure 1.12 Events and Triggering: Triggering page

3. The Triggering page (Figure 1.12) is displayed and allows for triggering to selected by contact closure or by software. **Click Next** 

**Note.** Triggering allows the definition of how the LC or detector run is triggered, whether by contact closure or software.

4. The end page is displayed. Either click **Finish** to accept the configuration or **Back** to change it.

# **Preparation Methods**

When it is a requirement to run several pumps on a single spray system, this facility allows for the creation of an "Umbrella" method that will tie the various inlet methods for the pumps together. It is this method that is selected when running from the sample list

### **New Prep Method**

If **Sample Prep** has been selected in the MassLynx Options dialog (page 1-5), the Prep Options menu will be available on the Inlet Editor Menu Bar. Selecting **Prep options, New Prep Method** will invoke the **Prep Wizard** (Figure 1.13)

Prep Wizard	×
	This wizard will assist you in creating a new Prep method. Please ensure that you have created any inlet method files that you require before proceeding.
	Name this method.
Inlet Configuration	Press Next to continue
< <u>B</u> ack	Next > Cancel Help

Figure 1.13 Prep Wizard: New Prep Method page

Enter the name of the Method in the Edit box and Click next.

The next page (Figure 1.14) allows the required method files to be put into the method. Choose the method from the drop down box, for each pump configuration.

Click Finish to close the Prep Wizard and return to the Inlet Editor.

### **Edit Prep Method**

Existing Preparation Methods can be edited by selecting **Prep Options, Edit Prep Method.** This Invokes the **Edit Method Page** (Figure 1.15).

Choose the method from the drop down box and Click Next.

The Method Editor page (Figure 1.14) is invoked with the currently selected methods showing in the drop down boxes. These methods can now be changed.

Prep Wizard : Method Editor	×
	Choose an inlet method file for each of the following configurations:
1. Default	Pump: Shimadzu; Autosampler: External; Detector: None
2. Default	Pump: Waters 1525; Detector: None
3. Default	Pump: Waters 1525; Detector: None
4. Default	Pump: Waters 1525; Detector: None
1	
< <u>B</u> ack	Finish Cancel Help

Figure 1.14 Prep Wizard: Method Editor page



Figure 1.15 Prep Wizard: Edit Method page

### **View Prep Method**

To view a Preparation Method select **Prep Options, View Prep Method,** this works in a similar fashion to the Edit Prep Method option but Method Editor Page (Figure 1.14) has all the drop down boxes disabled.

# **Chapter 2 Waters Systems**

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## Introduction

This chapter describes the function of Waters Inlet Systems under MassLynx Version 4.0.

The following Inlets are not included, but their use under MassLynx is covered in the specific Waters User Guides for that instrument.

- Waters 2767 Sample Manager and Collector
- Waters 2747 Sample Manager and Collector
- Waters 2525 Pump
- Waters 2488 IEEE Detector

### Installing Waters Control Software

Copies of the drivers for the Waters 2487 IEEE, 2488 IEEE and 2525 can be found on the MassLynx Installation CD. Each instrument has its own setup program in a folder on the MassLynx CD. This is installed automatically when the instrument is first selected.

Should it be necessary to install the software manually, find the Waters 2487 IEEE, 2488 IEEE and 2525 Pump folders on the MassLynx CD and double click on **Setup.exe** to instigate the installation routine for that instrument.

Follow the on screen instructions.

Selecting any of the instruments from the Inlet Editor Toolbar will now invoke the Waters Control Software rather than MassLynx. Example screen dumps are shown below and on page 2-24 for the Waters 2487 IEEE Detector

Note: These Instruments cannot be accessed from the short cut bar.

Modify Instrument Method (Un	titled.w24)			_ 🗆 🗙
Wavelength Mode:				ок
λ 💿 Single	<b>λλ</b> Ο Dual			Cancel
Wavelength A Wavelength B	Events			
Wavelength: S	Sampling Rate:	Data Mode: AbsorbanceA	<b>_</b>	
Bun Time: 5 minutes		Noise Filter Type: None	Time Constant:           0         seconds	
Auto Zero: Dn Wavelength Change On Inject Start or Auto Zero Event In or A	uto Zero Kev Press			
Description:				

Figure 2.1 Waters 2488 UV Detector; Waters Control Software Interface.

📩 Modify II	nst	rument	Method (C	:\MassLyna	<\Microbel	.ynx.PRO\A	cqudb	\Default	.bgm)	×
Chromat	og	raphic	Pump (2	2525)			I	Run Time	: 1.00	min
M	M	obile Pha Solvent A: © B: ©	se Events Selections A1 C A2 B1 C B2	Analog Solven A: A B: B	t Names I	2	-] -]	Pressure Low: High:	Limits 0 psi 6000 psi	
			Seal Was	sh Period: 5.	0 min	utes				
	G	iradien	t							
			Time (min)	Flow (mL/min)	%A	%В	Curve			
		1	Initial	1.00	100.0	0.0	Initial			
		2								
		3		·						
		5								
		6						<b>T</b>		
			1	:		?		· •		
								OK	Cance	el

Figure 2.2 Waters 2525 Pump; Waters Control Software Interface

# Waters 600 Pump

The Waters 600 Pump pages can be accessed by selecting View, Waters600 Pump from the Inlet

Editor menu bar, selecting Inlet from the short cut bar or by pressing the toolbar button.

### Waters 600 Initial Conditions

Solvents	Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must not exceed 100%.
Pump A	This is the remainder percentage after the solvent percentages have been set for the other pumps.
Pump B, C and D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Solvent Name	Enter the name of the solvent that will be delivered through the corresponding Pump.
Flow Rate	This is the total flow rate of the solvent channels according to how you have configured the instrument.
Pressures	Enter the upper and lower limits of the pressure within the solvent delivery system (SDS) if the pressure falls outside of this range the SDS switches off.

- Solvents Pump <u>A</u> % 100 □ Pump <u>B</u> % 0 □ Pump <u>C</u> % 0 □ Pump <u>D</u> % 0 <u>F</u> low Rate (ml/min)	Solvent Name Solvent A Solvent B Solvent C Solvent D	Pressures         High Limit (psi)       4000         Low Limit (psi)       0         Column Temperature       0         Set (°C)       0         High Limit (°C)       25         Timings       1
--	--	--

Figure 2.3 Initial Conditions page

ColumnIf the instrument has an oven present then the column temperature can be set to<br/>a specified temperature in degrees centigrade. Enter the temperature to heat the<br/>column to in the Set box and a High Limit. If the temperature exceeds the<br/>High Limit then the system will shut down. If the software has been configured<br/>to operate without a column oven then these boxes will be greyed out.

**Run Time** Enter the time in minutes that the method will run from the point of injection.

- Gradient Entry - Time( mins )	1.00	<u>G</u> radient 1	l'able			+-	X
<u>B</u> % <u>C</u> % D% Flo <u>w</u> (ml/min) <u>C</u> urve	0 0 1.00 6	Time	8%	C%	D%	Flow	Curve

### Waters 600 Gradient Page

Figure 2.4 Gradient page

This page allows a gradient to be entered and edited. To operate in isocratic mode ensure that the timetable is empty.

To enable the **B%**, **C%** and/or **D%** boxes check the relevant boxes on the **Initial Conditions** page.

To add a gradient, enter a time and percentage in the relevant boxes and press the toolbar button. **Note:** The first entry must have a time of 0.

To delete a single gradient, click on a time in the list and press the 📧 toolbar button.

To delete all entries press the *toolbar* button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed in the edit boxes to the left of the timetable, and can be altered as appropriate. Once changed press

to re-enter the values into the timetable. If, however, you modify the time value such that it

does not correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

Flow Enter the flow rate for the solvent delivery system.

**Curve** This sets the rate at which the solvent is to change to the new proportions and/or flow rates. See the Waters 600 Operator's Guide for a list of values.

### Waters 600 Initial Events Page

Waters 600	
Initial Conditions Gradient Initial Events Programmed Events	
Outputs	
Switch 1 Switch 2 Switch 3 Switch 4	
No Change	

Figure 2.5 Initial Events page

This page allows the initial state of switches 1 to 4 to be defined. Select from the drop down lists On, Off or No Change.

### Waters 600 Programmed Events Page

This page allows pump events to be entered and edited.

To add an event, type in a time, select an event from the drop down list box, select an action or enter a value and press the  $+ \blacksquare$  toolbar button.

To delete a single event, click a time in the list and press the 📧 toolbar button.

To delete all entries press the itoolbar button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in

the edit boxes above the timetable, and can be altered as appropriate. Once changed press  $+ \pm$  to re-enter the values into the timetable. If, however, you modify the time value such that it does not

correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

Waters 600		
Initial Conditions Gradient Initial	Events Programmed Event	8
<u>Iime:</u> <u>Event:</u>	Event Ta <u>b</u> le	· <b>引</b> 尾风
0.00 • Switch 1	Time Event	Function Value
0 <u>0</u> n	0.00 Switch 1 1.00 Switch 2	Off Pulse 1.00
⊙ Off		
© <u>P</u> ulse0		
	1	

Figure 2.6 Programmed Events page

## Waters 2690/2695 Autosampler

To control the Waters 2690/2695 autosampler and pump from the keypad rather than the MassLynx software the **Inlet** must be configured as **None**.

### Waters 2690/2695 Toolbar

Two additional toolbar buttons are available for the Waters 2690/2695.





Change Mode. Switch to local or remote mode.

### Waters 2690/2695 Autosampler Initial Conditions Page

This page is used to set parameters specific to the Sampler, to access it select View, Waters

**2690/2695 Autosampler,** select Autosampler from the short cut bar or press the toolbar button.

Sample Heater	If the sample heater is installed, enter the temperature that the sample should be to be heated or cooled to.
Temperature	Range: 4.0 to 40.0 °C.
Sample Heater	Enter the maximum deviation in sample temperature allowed. If this is exceeded the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red.
Temperature Limit	Range: $\pm 1.0$ to $\pm 20.0$ °C.
Injection Volume	Enter the volume of sample to be injected, in microlitres. Range: 0 to 2000 $\mu$ l. <b>Note:</b> If you are running from the Sample List, the injection volume in the sample list entry overrides the value entered here.

Waters 2695 Autosampler			
Autosampler Initial Conditions Autosampler Pur	ge )		
Sampler Initial Conditions			
Sample Heater	Injection Parameters		
Iemperature (°C)			
Temperature Limit (+/- *C) 20	Needle Depth (mm) 0 Draw Speed (µl/sec) Slow 💌		
Dptional Sample Loop	⊻ial Number 1		

Figure 2.7 Autosampler Initial Conditions page

Needle Depth	This adjusts the depth of the needle tip to accommodate for sedimented samples or non-standard vials. A value of 0 corresponds to the bottom of the vial. Range: 0.0 to 20.0 mm in 0.1mm increments.				
Draw Speed	This determines the rate in microlitres per second at which sample is extracted into the autosampler needle. It should be set according to the viscosity of the sample. Select one of <b>Fast</b> , <b>Normal</b> or <b>Slow</b> from the dropdown list box. The table below shows the draw rate for each selection using a 250 $\mu$ l syringe.				
	Selection		Draw Rate for a 250 µl Syringe		
-------------------------	---	----	--	--	--
	Fast		5.0 µl/sec		
	Norm	al	2.5 µl/sec		
	Slow		1.0 µl/sec		
Optional Sample Loop	le To inject sa sample loo check this		ample volumes greater than 100 microlitres an additional p can be installed (in series with the existing sample loop), box if an additional sample loop is used.		
Vial number	The vial to from the M overrides th		inject from. <b>Note:</b> If a multisample acquisition is being run lassLynx Sample List, the Bottle # entry in the sample list he value entered here.		

## Waters 2690/2695 Autosampler Purge Page

This page is used to set the Autosampler purge volume, to access it click on the Autosampler Purge tab.

Loop Volumes	Enter the number of times the loop should be filled to purge the sample
	loop and syringe of traces of the previous sample. When set to a value
	greater than zero, this action is performed after every injection.

Waters 2695 Autosampler	
Autosampler Initial Conditions Autosampler Purge	
- Autosampler Purge	
Loop <u>Vo</u> lumes 0	

Figure 2.8 Autosampler Purge page

# Waters 2690/2695 Pump

**Note:** To control the Waters 2690/2695 autosampler and pump from the keypad rather than the MassLynx software the **Inlet** must be configured as **None**.

The Waters Pump pages can be accessed by selecting **View**, **Waters 2690/2695 Pump** from the Inlet Editor menu, selecting Inlet from the short cut bar or by pressing the toolbar button.

# Waters 2690/2695 Solvents and Flows Page

Solvents	Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must equal 100%. Solvent Names entered here will be displayed on the Pump Gradient page.
Pump A	This displays the remainder percentage after the solvent percentages have been set for the other enabled pumps.
Pump B, C and D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Run Time	This value is set to the time in minutes that the method will run from the point of injection.
Degasser	This value is set to the time in minutes that the method will run from the point of injection.
Flow	This is the total flow rate for the system.
Flow Ramp	Enter the time (in minutes) for the solvent delivery system to reach the maximum system flow rate (10 ml/min). Recommended minimum setting: 0.5 min.

Waters 269	95 LC			
Solvents and Flo	ws Column Set	up   Pump Gradie	nt Pump Events	
- Solvents-				
Pu	mpA 🕺	100	Solvent Name Solvent A	_
E Pu	mp <u>B</u> %	0	Solvent B	
🗖 Pu	mp <u>C</u> %	0	Solvent C	
🗖 Pu	mp <u>D</u> %	0	Solvent D	
🗖 Aft	er running gradier	nt hold flow at las	setting in gradient table	
Run <u>T</u> i Degas	me (mins) ser Norm	1 nal 💌	Flow Flo <u>w</u> (ml/min) Flow <u>B</u> amp (mins)	0.2

Figure 2.9 Solvents and Flows page

# Waters 2690/2695 Column Setup Page

Waters 2695 LC	
Solvents and Flows Column Setup Pump Gradient	Pump Events
Column Heater	Pressures
Temperature (°C) 20	Low P <u>r</u> essure (Bar)
Temperature Limit (+/- °C) 20	High Pressure (Bar) 300
<u>P</u> re-column Volume (μl)	0
L	

Figure 2.10 Column Setup page

Column Heater Temperature	Enter the target operating temperature for the optional column heater. This value must be at least 5 °C above ambient. Range: 20 to 60 °C.
Column Heater Temperature Limit	This is the maximum deviation in column temperature allowed. If this is exceeded the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red. Range: $\pm 1$ to $\pm 20$ °C.
Low Pressure and High Pressure	Enter values as required. If the pressure falls outside these limits the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red. Low Pressure Range: 0 to 310 bar. Low Pressure Range: 0 to 345 bar.
Pre-column Volume	Enter the volume of solvent to pump through the column before an injection. Range: 1 to 10000 $\mu$ l.

## Waters 2690/2695 Pump Gradient Page

Vaters 2695	LC Column Setur	Pump Gradient Bump Events	
Solvents and Flows		Gradient Table	+8 <b>4</b>
<u>T</u> ime (mins)		]	
Solvent A	% 100	Time A B C D	Flow Curve
Solvent B	% 0	0.00 100.0 0.0 0.0 0.0	1.00 1
Solvent C	% 0		
Solvent D	% 0		
Flo <u>w</u> (ml/min)	1		
C <u>u</u> rve	1		

Figure 2.11 Pump Gradient page

This page allows a gradient to be entered and edited. If you wish to operate in isocratic mode then you should enter parameters on the Solvents and Flows page and ensure that the timetable is empty.

Time (mins)	Specifies the time at which the specified conditions (%A to %D, Flow, and Curve) for the row should take effect. Make sure you set Time for the first row to 0.00, to establish initial conditions for the gradient run. The range for rows other than row 1: 0.01 to 999.99 minutes
Solvent A %– Solvent D%	Specifies the percentage of solvent flow from each reservoir. For each row, the total of all solvents must equal 100%. Range: 0 to 100%.
Flow (ml/min)	Specifies the total flow rate for the solvent delivery system. Range: 1 to 10 ml/min.

Curve This sets the rate at which the solvent is to change to the new proportions and/or flow rates. Curves are specified by number, available choices: 1 to 11 are shown in the table below and Curve profiles are shown in Figure 2.12.

Curve Number	Effect
1	Immediately goes to specified conditions
2 to 5	Convex
6	Linear
7 to 10	Concave
11	Maintains start condition until next step



Figure 2.12 Curve Profiles

#### Waters 2690/2695 Gradient Table Operation

To add a gradient, enter values in the relevant boxes and press the toolbar button. You can add up to 15 rows to the table. **Note**: The first entry must have a time of 0.

To delete a single gradient, click a time in the list and press the 📧 toolbar button.

To delete all entries press the *integrate toolbar button*. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does

not correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

## Waters 2690/2695 Pump Events Page

This page allows pump events to be entered and edited.

Use the Event Table to program up to 16 events (both external and internal). The external events are triggered by four contact closures (relays) through output terminals (S1–S4) on the 2690/2695 Separations Module. The internal events are used to control the sample compartment temperature

and column heater temperature, and to prime and flush the 2690/2695 Separations Module. Events can be triggered more than once and multiple events can be triggered simultaneously.

Waters 2695 LC					
Solvents and Flows Column Setup	Pump G Event <u>T</u>	radient Pump Event	s   +Ę		
0.00 Switch 1					
C <u>O</u> n C <u>O</u> ff	Time 10.00	Event Switch 1	Action No Change	Value	
C T <u>ogg</u> le C <u>P</u> ulse <u>W</u> idth (min) 0.01					
<u>N</u> o Change					
	1				

Figure 2.13 2690/2695 Pump Events page

- TimeEnter the time at which the event should start. Event rows are sorted<br/>automatically by time. Note: Different events can be programmed to occur at<br/>the same time. Range: 0.00 to 999.99 min.EventEnter the type of event signal required: one of the four TTL-level output<br/>switches (S1–S4), or one of the internal events (column heater temperature,<br/>sample compartment temperature or flush/prime). Available choices are shown<br/>below:-
- Switch 1 to Switch 4 Corresponds to terminal strip positions S1 to S4 on the rear of the 2690/2695 module. Activating a Switch event triggers a contact closure for controlling an external device. After selecting a switch event, set a state for the switch by selecting On, Off, Toggle, Pulse Width or No Change. This state appears in the Action column of the table (refer to Switch States, below). Note: If Pulse is selected for a switch state, the duration of the pulse must be entered in the Width (min) field.
- Set Temperature (Column or Sample) Specifies the temperature of an optional column heater, or an optional sample compartment heater/cooler. After selecting this event, select Column or Sample and enter the required Temperature in °C. Note: When a Column Temperature event occurs, the temperature of the column heater changes from the value set in the Heaters and Pressures page to the value set for the event. When the event times out, the temperature changes back to the Heaters and Pressures page value. Column range: 20 to 60 °C. Sample range: 4 to 40 °C.
- Flush/Prime Specifies a flush/prime operation for the 2690/2695 module. Use this event only when creating Inlet Pre-run and Inlet Post-run methods. These methods will use the solvent percentages and the run time from the Solvents and Flows page but will use the Flow value entered on this page. Note: The Time field is not accessible when you select a Flush/Prime event.

#### **Switch States**

• **On** – Turns on a contact closure that triggers an external or internal event. With this function, the contact closure remains closed until an off function is sent.

- Off Turns off the contact closure for the event. With this function, the contact closure is broken.
- **Pulse** Transmits a single On/Off pulse. The contact closure is maintained for the number of seconds defined in the Value column. Range: 0.01 to 10.00 sec.
- **Toggle** Changes the current state of the switch.
- No Change Leaves the switch in its current state.

#### Waters 2690/2695 Event Table Operation

To add an event, enter a time, select an event from the drop down list box, select an action and press the toolbar button. Up to 16 events can be programmed.

To delete a single event, click a time in the list and press the 📧 toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in

the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does not

correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

# Waters 996 PDA Detector

This page is used to set parameters specific to the UV detector, to access it select View,

Waters996 PDA Detector, select Detector from the short cut bar or press the <sup>1</sup> toolbar button.

### Waters 996 PDA Page

Start Wavelength	Enter the wavelength at which to start acquiring data.
End Wavelength	Enter the wavelength at which to stop acquiring data.
	The range with Resolution set to $1.2$ is 190.0 nm to 800.0 nm. The range at all other Resolution settings is $190.0 + (\text{Resolution/2})$ to $800.0$ (Resolution/2).
Resolution	Enter the number of diodes that are averaged together as a single spectral data point. To differentiate closely related spectra and obtain greater spectral resolution, use a small resolution number. Be aware, however, that a small resolution value generates more data points and therefore requires more disk space than a large resolution value. Find a resolution value just small enough to identify spectral features. Range: 1.2 to 24.0 nm in multiples of 1.2
Sampling Rate	Select the number of Spectra to be acquired per second, from the dropdown list box. For good integration and quantitation, acquire 15 to 20 spectra across a peak.

Auto Exposure

Check this box to enable the detector optics to calculate the optimum exposure time needed to recharge the diodes, based on the lamp energy, the lamp spectrum and the selected wavelength range. **Tip:** Enable Auto Exposure for most routine analyses.

Waters996 PDA	
PDA Channel 1 Channel 2	
Data Acquisition	
Start Wavelength (nm) 210	End Wavelength (nm) 400
Resolution (nm) 1.2	Sampling R <u>a</u> te (spectra/sec)
Instrument Control	
Auto E <u>x</u> posure	Stop Time (mins) 5
☑ Interpolate 656 nm	Filter Response 1
Exposure <u>T</u> ime (ms) 15	🔽 Save To <u>D</u> isk

Figure 2.14 UV Detector Configuration page

Interpolate	Check this box to instruct the detector to ignore the signal from the photodiode at 656 nm and interpolate a value from the adjacent diodes. This prevents over-saturation at 656 nm (Balmer line for deuterium). Only applicable if the <b>Auto Exposure</b> option has been selected.
	If this box is not checked the detector reports the signal from the photodiode at 656 nm, this is only necessary if you are working with compounds that absorb in the 656 nm range.
	<b>Note</b> : If this parameter is unchecked, the deuterium lamp high emission line at 656 nm may cause spectral artifacts and autoexposure errors.
Exposure Time	The exposure time is the time that the photodiodes are exposed to light before they are read. To set a different Exposure Time, ensure that the Auto Exposure box is not checked and enter the required time in milli seconds. Range: 11.00 to 500.00 ms.
Stop Time	To specify a different Acquisition Stop Time enter the time in minutes when the PDA should stop scanning.
Filter Response	Enter the response time for filtering acquired data. The filter is an enhanced rolling average filter applied to absorbance data from the PDA detector before the data is sent to MassLynx. The filter reduces high-frequency noise across the entire wavelength range specified for the acquisition. High values decrease peak response. Available choices: 0, 1, 2 and 3.

Save to Disk Check this box to save the Photo Diode Array data to the raw datafile. If this data is not required for further processing then uncheck the box, the data is not saved to disk thus reducing the size of the file.

## Waters 996 Channel Detector Configuration Pages

The Channel 1 and Channel 2 pages contain the same information. Select the page relevant to the channel required, by clicking on the tab.

Waters996 PDA		
PDA Channel 1 Channel 2		
Analog Channel 1		
Output Mode Off	•	Filter Type Hamming 💌
Wavelength (nm)	254	Filter Response (s)
Ratio Denominator Wavelength (nm)	254	Offset (AU)
Bandwidth (nm)	2.4	Threshold (AU) 0.1

Figure 2.15 Channel 1 Detector Configuration page

Output Mode Select one of:-

- Off no analog output signal.
- Absorbance Output is in absorbance units at the wavelength specified.
   Note: Ratio Denominator Wavelength and Threshold parameters are not accessible when Absorbance mode is selected.
- Ratio Output represents the ratio of absorbances at two wavelengths. The numerator wavelength is specified by the Wavelength parameter, and the denominator wavelength is specified by the Ratio Denominator Wavelength parameter (see below).

# WavelengthEnter the output wavelength to monitor. In Ratio mode, the absorbance at the<br/>Wavelength is used to calculate ratio in the formula:<br/>Ratio = Absorbance at Wavelength/Absorbance at Ratio Denominator<br/>Wavelength<br/>Wavelength must be within the wavelength range specified by the Start<br/>Wavelength and End Wavelength parameters on the PDA page

Range when Resolution is set to 1.2: Start Wavelength to End Wavelength. Range at all other Resolution settings: Start Wavelength + (Bandwidth/2) to End Wavelength – (Bandwidth/2). Default: 254 nm.

Ratio Denominator Wavelength	Enter the denominator wavelength (in nanometers) for the analog output channel. Ratio Denominator Wavelength must be within the wavelength range specified by the Start Wavelength and End Wavelength parameters on the 996 PDA page.
Bandwidth	Enter the spectral bandwidth of the analog output channel. The range is 1.2 to 24.0 nm.
Filter Type	Select <b>Hamming</b> or <b>Single Pole</b> from the dropdown list box. The Hamming filter is designed to create the same degree of peak-height degradation as the Single Pole filter for the same response time, but enhances filtering of high-frequency noise.
Filter Response	Enter the response time for the filter. The range is 0 to 5 seconds.
Offset	If required enter an offset to the analog output channel. The range is $-0.2$ to 2.0 AU.
Threshold	Enter a threshold above which the ratio (Wavelength / Ratio Denominator Wavelength) must be to be valid data. The range is $-0.1$ to 2.0 AU.
	<b>Note:</b> If no ratio is plotted one or both channels are below the current Threshold and a lower Threshold value should be entered.

# Waters 486 UV Detector

This page is used to set parameters specific to the UV detector, to access it select **View**, **Waters486 UV Detector** or press the **L** toolbar button.

Waters	UV Detector					
486 UV D	etector					
	486 UV Detector-					
	Wavelength (nm)	254	<u>P</u> olarity	+ 💌		
	<u>S</u> ensitivity (AU)	2	∐ime Filter (s)	1		

Figure 2.16 486 UV Detector Configuration page

**Wavelength**  $(\mu)$  Enter the wavelength to monitor.

**Polarity** Select the polarity of the output signal from the drop down list box.

Sensitivity Enter the required sensitivity of the output signal. (AUFS)

**Time Filter** Enter the response time for filtering acquired data.

A full description of all the parameters in this editor is given in the *Waters 486 Instruction Manual*.

# Waters 2487 UV Detector

(seconds)

This page is used to set parameters specific to the Waters 2487 UV detector, to access it select

View, Waters2487 UV Detector or press the Loolbar button.

## 2487 Single Wavelength Absorbance Detector

The 2487 detector can be used as a single or dual wavelength detector. To use as a single wavelength detector select **Single Wavelength** from the **Waters2487 UV** menu. A tick mark will appear next to the name if single wavelength is selected and the **2487 Channel B** parameters are grayed out.

Waters	Waters UV Detector				
2487 Dual	Absorbance Detector				
24	487 Channel A	254	– 2487 Channel B – – – – – – – – – – – – – – – – – –	254	
	<u>S</u> ensitivity (AUFS)	2	S <u>e</u> nsitivity (AUFS)	2	
	<u>⊺</u> ime Filter (s)	1	Time Filter (s)	1	
	<u>P</u> olarity	+ 💌	P <u>o</u> larity	+ -	
					l
					J

Figure 2.17 2487 UV Detector Configuration page (Dual Wavelength)

Wavelength (µ)	Enter the wavelength to monitor.
Sensitivity (AUFS)	Enter the required sensitivity of the output signal.
Time Filter (seconds)	Enter the response time for filtering acquired data.
Polarity	Select the polarity of the output signal from the drop down list box.

A full description of all the parameters in this editor is given in the *Waters 2487 Instruction Manual*.

## 2487 Dual Wavelength Absorbance Detector

The 2487 detector can be used as a single or dual wavelength detector. To use as a dual wavelength detector ensure that the **Single Wavelength** option on the **Waters2487UV** menu is not selected. If a tick mark appears next to the name then single wavelength is selected, selecting the option again will return the detector to dual wavelength mode and both channel parameters will be available.

The parameters are the same as for single wavelength mode.

# Waters 2487 IEEE Detector

The 2487 IEEE Detector should be selected if the detector is connected via a GPIB card in the back of the PC.

The 2487 IEEE Detector is used to collect binary data, through the IEEE interface, rather than through the analog interface.

**Note.** Waters control software needs to be installed before using this instrument (see Installing Waters Control Software page 2-7).

This page is used to set parameters specific to the Waters 2487 IEEE detector, to access it select

Waters2487 IEEE Detector from the View menu or press the <sup>1</sup> toolbar button.

The 2487 detector can be used as a single or dual wavelength detector. Check Single or Dual under **Wavelength Mode** 

Modify 2487 Instrument Method (C:\MassLynx\Diverse.	PRO\Acqudb\Default.w24)	_ 🗆 🗙
Wavelength Mode: λ ⓒ Single λλ ◯ Dual		OK
Channel A Channel B Analog Out A Analog Out B Event	5	
Wavelength:       Sampling Rate:         Image: minutes       Image: minutes         Auto Zero:       Image: minutes         Image: minutes       Image: minutes <th>Data Mode: AbsorbanceA  Noise Filter Type: Time Constant: Hamming AU</th> <th></th>	Data Mode: AbsorbanceA  Noise Filter Type: Time Constant: Hamming AU	
Description: A description/purpose		

Figure 2.18 2487 IEEE Instrument Method Dialog

A full description of all the parameters in this editor is given in the *Waters 2487 Instruction Manual*.

# Waters SAT/IN PDA Detector

This page is used to set parameters specific to the PDA detector, to access it select View,

WatersSATIN PDA Detector, Select Detector from the short cut bar or press the toolbar button.

Wat	ers SATIN		
SAT	ZIN		
	Channel 1	SAT/IN Channel 1	Sampling Rate (scans / sec)
	Channel 2 🔽	SAT/IN Channel 2	
	<u></u>		

Figure 2.19 SAT/IN Configuration page

Channel 1 and Channel 2	Check the box(es) for the required channels.
Sampling Rate	Select the number of Spectra to be acquired per second, from the dropdown list box. For good integration and quantitation, acquire 15 to 20 spectra across a peak.

#### Notes

Data collected through a Waters SAT/IN PDA Detector is shown as analog data in the acquired data files, this is the same as analog data coming in through the analog inputs from the back of an MS instrument.

The Waters SAT/IN PDA Detector can be used to collect analog data or the MS analog inputs can be used. Do not try to collect analog data with the SAT/IN and the MS analog inputs at the same time. Collecting analog data from both sources will result in unpredictable behavior.

SAT/IN analog data will only be collected in a system configured with a spectral data source. An MS detector and/or a PDA detector must also be used to successfully collect SAT/IN data.

Negative data is not supported by the Waters SAT/IN but can be avoided by applying an appropriate offset in the connected Detector.

# Waters 2700 Autosampler

These pages are used to set parameters specific to the autosampler, to access them select View,

Waters2700 Autosampler, select autosampler from the short cut bar or press the toolbar button.

# Waters 2700 Injection Configuration

Waters 2700 Autosampler
Injection Configuration       Dilutor Configuration       Wash Parameters       Sampler Configuration         Sampler Inject Parameters       Inject       Inject       Injection Type       Full Loop       Loop Volume (μl)       Image: Separation Air Gap (μl)       Image: Separation Air G

Figure 2.20 Waters 2700 Sampler Injection Configuration Page

Injection Type	Select from Full or Partial Loop.
Loop Volume (µl)	Enter the volume of the sample loop in microlitres
Injection Volume (μl)	Only enabled for Partial Loop. Enter the volume of the sample to inject into the loop for single sample acquisitions. For samples acquired via a sample list this is overridden by the value in the sample list. If the Injection Volume is equal to the Loop Volume then twice the Injection Volume is drawn to ensure that the loop is full.
Separation Air Gap (µl)	Enter the volume of air to draw before the sample.
Loop Overfill	Enabled only for Full Loop inject mode.
Vial Reference	Enter the position of the vial to use for single sample acquisitions. For samples acquired via a sample list this is overridden by the value in the sample list.

# Waters 2700 Dilutor Configuration

Syringe Size	Select the size of the syringe installed from the drop down list box.
Aspiration Speed	Enter a value for the speed at which to draw the sample into the needle (the pump will be on its downward journey). Range: 1 to 32, with 1 being the fastest.
Dispense Speed	Enter a value for the speed at which to eject the sample from the needle (the pump will be on its upward journey). Range: 1 to 32, with 1 being the fastest.

Waters 2700 A	utosampler			
Injection Configuration	Dilutor Configuration	Wash Parameters	Sampler Configuration	
Dilutor Configuration	on			
			<b>.</b>	
S	yringe Size	Aspiration Speed	Dispense Speed	
1	00 μΙ 💌	4	16	
	The higher the value	e, the slower the spe	ed.	

Figure 2.21 Waters 2700 Sampler Dilutor Configuration Page

## Waters 2700 Wash Parameters

Waters 2700 Autosampler
Injection Configuration       Dilutor Configuration       Wash Parameters       Sampler Configuration         Wash Configuration       Needle Rinse       Injection Port Flush         Rinse Volume (µl)       50       Flush Volume (µl)       50         Mini Wash Prime       10       Flush Speed       10

Figure 2.22 Waters 2700 Sampler Wash Parameters Configuration Page

Needle Rinse Volume (µl)	Enter the volume of mobile phase required to wash the needle after an injection. A value of zero will result in no wash. If the needle rinse volume is greater than $800\mu$ l then the mini-wash pump is used instead.
Wash Time (seconds)	Enter the time for which the mini-wash pump is activated during a mini- wash prime. Mini-wash prime is activated from the <b>Waters2700</b> menu.
Injection Port Flush Volume (µl)	Enter the volume of mobile phase required to flush the inject port after the sample has been injected. A value of zero will result in no port flush.

Injection PortEnter the speed at which the flush volume is dispensed. Range: 1 to 32, with<br/>1 being the fastest.

## Waters 2700 Sample Configuration

Waters 2700	) Autosampler	
Injection Configuration   Dilutor Configuration   Wash Parameters   Sampler Configuration		
	Current Layout	
	Six Position 384	
	384 Well Plate 384 Well Plate 384 Well Plate	
	384 Well Plate 384 Well Plate 384 Well Plate	

Figure 2.23 Waters 2700 Sampler Configuration Page

**Current Layout** This shows the currently selected rack configuration. To change the current layout select a new one from the drop down list box.

## Waters 2700 Bed Layout

Bed layouts are created, deleted or amended from this dialog. To display the Bed Layout Editor dialog, select **Waters2700, Bed Layout**.

Bed Layout		×
Select/Create Bed Layout Modify Be	ed Layout	
Bed Layouts: Four Position Deep Microtiter Four Position Deep Microtiter Four Position Shallow Microtite One Eppendorf100 Two Micro Six Position Microtiter Two Microtiter One Eppendorf Two Position Eppendorf 100	Rows In Columns:	
OK Cancel	Apply Help	

Figure 2.24 Bed Layout Dialog

#### To Create A New Bed Layout (Waters 2700)

- 1. Highlight a bed layout similar to the one you want to create and press the *button* to create a new layout. The layout appears in the **Bed Layouts** list as the same name with a 1 at the end, e.g. Six Position Microtiter1.
- To change the name of the layout, type the new name into the Bed Layouts text box and press the button. The name is updated in the Bed Layouts list box.

New bed layouts are saved to the MassLynx **Racks** directory.

#### To Delete A Bed Layout (Waters 2700)

1. Highlight the bed layout to delete and press the button. A dialog box will ask you to confirm the deletion. Press the **OK** button to delete the bed layout. **Note:** The bed layout which is selected as the current bed layout on the Sampler Configuration page cannot be deleted.

#### Other Bed Layout Options (Waters 2700)

- 1. To change the number of rows in the current column, type the new number into the Rows box and press the button.
- 2. To append a new column, press the button.
- 3. To delete the current column press the 🔀 button.
- 4. To insert a column, click on the column before which you want to insert and press the button. **Note:** The column inserted will have the same number of rows as the column highlighted.



Figure 2.25 Modify Bed Layout Dialog

#### Modify Bed Layout (Waters 2700)

If the plate position or type needs changing select the Modify Bed Layout tab.

Click on one of the code plates to display the Plate Position and Type dialog.

Plate Position and Type	×
Plates: 24 Well 1500uL Plate 24 Well 2mL Plate 384 Well 80uL Plate <b>384 Well Plate</b> 48 Well 2mL Plate 48 Well 500uL Plate 96 Deep Well Plate	Cancel
	1/10th mm
Top left vial X position from probe home.	2270
Top left vial Y position from probe home.	0
Vial bottom from probe home.	570
🔲 OpenLynx plate login	

Figure 2.26 Plate Position and Type dialog

This dialog allows you to select a new plate from a list of possible options, and change its actual position on the bed. Measurements for plate positions are always taken from the top left corner of each plate. The X value is the measurement from the vial position in the top left corner of the plate to the home position. The Y value is the measurement from the vial position in the top left corner of the plate to the home position.

Vial bottom from probe home	This is the distance the needle must travel downwards to reach the bottom of the well.
OpenLynx plate login	If this box is checked and <b>Use current MassLynx autosampler bed</b> <b>layout</b> is checked in the OpenLynx Manager program, then the plate at this position can only be used for plate login on the OpenLynx Login program.

Pressing the button will move the needle to the top left vial position defined by the X, Y and Vial bottom from probe home positions. If the needle is not above the top left vial then the plate will need moving or the X and Y values will need changing.

Pressing the button will take the needle to the bottom right vial position (The software will calculate this from the plate type and the X and Y positions defined). This is used to test that the plate will fit on the autosampler, if it does not then an error message is displayed. The plate will need changing or moving or the X and Y values will need changing.

## Waters 2700 Fixed Positions

This dialog allows the positions of the Injector Port, Cleaner Stations and the Waste station to be defined. It is accessed by selecting **Waters2700, Edit Fixed Positions**.

Fixed Position	×
Fixed Positions	
Injector Port Top 620 X Y Z 17 640	
Cleaner Station Shallow X Y Z 3935 1305 350	
Waste Station           X         Y         Z           3935         1415         350	
Cleaner Station Deep X Y Z 3935 1520 350	
All values represent 1/10th mm	
Home arm	
OK Cancel Help	

Figure 2.27 Fixed Position Dialog

The X and Y values are the distance the needle must travel from the Home position to the required station.

The **Z** value is the distance the needle must travel downwards to reach the required station.

To test that the values entered are valid press the button. The needle will travel to the position specified. To return the needle to the Home position, press the button.

When all values are correct press the **OK** button.

## Waters 2700 Plate Generator

To display the Plate Generator dialog, select Plate Generator from the Waters2700 menu.

Plate Name	The name of the plate that is currently being edited.
Rows	The number of vials in a row and the distance between each center.
Columns	The number of vials in a column and the distance between each center.

<b>≝<sup>™</sup>PlateGenerator</b>	
Plate	
Plate Name 384 Well Plate	Vial Reference Horizontal O A B C
Number Of Vials Vial Spacing	• 1 2 3 Vertical • A B C • C 1 2 3
Rows         16         45           Columns         24         45	Referencing     XY       Priority     Image: Horizontal first
Offsets 1/10 mm Row 0 Every 2nd Row	Plate Size     Top Left Vial Offset       X     1265     1/10 mm       Y     845     1/10 mm       Z     140     1/10 mm
Column 0 Every 2nd Column	Depth 110 Diameter 40
Ready	NUM

Figure 2.28 Plate Generator

Offsets	Allows alternate vial rows or columns to be offset.
	<b>Note:</b> Entering a positive value will shift even numbered rows to the right and negative values will shift even numbered rows to the left.
Vial Reference	Allows the user to select the way that the vial rows and columns are referenced, e.g. whether the rows are alphabetical or numerical.
Referencing	This has three options
	• XY which references the vials A1, B1 etc.
	• Sequential Discontinuous which numbers the vials 1, 2, 3 across a row, left to right, and then starts the next row from the left again.
	• Sequential Continuous which numbers the vials 1, 2, 3 across a row, left to right, then continues number the next row, right to left etc.
	If the Waters 2700 autosampler is used with OpenLynx then the vial referencing must be set to either sequential continuous or sequential discontinuous.

Priority	Check the <b>Horizontal First</b> box if samples are to be acquired horizontally across the plate.
	If Referencing = X,Y, Horizontal = Letter, Vertical = Number and Horizontal Priority is checked, this will result in samples being acquired in the order A1, A2, A3. If the Horizontal Priority box is not checked samples will be acquired in the order 1A, 1B, 1C etc.
	If Referencing = sequential continuous or discontinuous and Horizontal Priority is checked, this will result in samples being acquired from row 1 then row 2. If the Horizontal Priority box is not checked samples will be acquired from column 1 then column 2 etc.
Plate Size	The size of the plate to its outside edges.
Top Left Vial Offset	The measurement to the center of the first vial from the top left corner of the plate
Vial	The depth and diameter values are used for display only. They appear in the description for a single shot login on the OpenLynx Login screen.

#### **Creating and Deleting Waters 2700 Plates**

To create a new plate, press the button. A new default plate is displayed, change the **Plate** 

Name, enter the appropriate values and press the save button or select Save Plate from the Plate menu. New plates are saved to the MassLynx Plates directory.

To copy a plate, page through the list of saved plates using the **1** and **1** toolbar buttons. The **Previous Plate** and **Next Plate** options on the **Plate** menu perform the same operation. When the required plate is displayed change the **Plate Name**, enter the appropriate values and press the save

button or select **Save Plate** from the **Plate** menu. New plates are saved to the MassLynx **Plates** directory.

To delete a plate select the plate, by typing the name in the **Plate Name** box or by paging through as above, and press the delete **vert** button or choose **Delete Plate** from the **Plate** menu.

**Note:** All of the spacings and the **vial section** are stored in 0.1mm units.

**Note:** When defining a custom plate for use with a multi-injector the plate is required to be compatible with the position of the 8 needles of the autosampler.

- The Plate must have eight columns.
- The position of the vials should allow all eight needles to enter a separate vial.
- There should be no odd or even offsets for any of the vial positions.

**Note:** If the Plate currently selected on the Sample Configuration page is changed here, then **Reset Injector** should be selected from the **LC** menu to reset communications.

## Waters 2700 Menu

Prime Syringe	This option is used to remove air from the syringe and any tubing connected to it. It repeatedly draws the mobile phase into the needle and flushes it out until the toolbar button is pressed, or <b>Stop Method</b> is selected from the <b>LC</b> menu, on the Inlet Editor. <b>Note:</b> Before Prime Syringe is selected the toolbar button appears as and the menu as <b>Run Method</b> .
Change Syringe	Selecting this option moves the needle to a position where it can be removed and replaced. When the syringe has been changed, Prime Syringe should then be selected to get the needle into a state ready for injection.
Prime Mini-Wash	This option moves the needle to the waste position and pumps the mobile phase through it for the <b>Wash Time</b> defined on the <b>Wash Parameters</b> page.

# Waters 2790/2795 Autosampler

**Note:** To control the Waters 2790/2795 autosampler and pump from the keypad rather than the MassLynx software the **Inlet** must be configured as **None**. See Configuring the Inlet System in the Acquisition Control Panel chapter.

These pages are used to set parameters specific to the Sampler, to access them select View,

Waters2790/2795 Autosampler, select autosampler from the short cut bar or press the toolbar button.

## Waters 2790/2795 Injection Parameters Page

Figure 2.29 Injection Parameters page

Inject Type	Select Sequential or Parallel from the drop down list box.
	<b>Sequential</b> – Sample aspiration occurs at the start of each injection cycle, after completion of the previous injection.
	<b>Parallel</b> – Sample aspiration and loop fill occur concurrently with other separation method functions for higher throughput.
Fill Mode	Select Full Loop or Partial Loop from the drop down list box.
	<b>Full Loop</b> – The autosampler draws in the loop volume, the overfill factor number of times, to ensure that the loop is full.
	<b>Partial Loop</b> – The autosampler will draw in the volume specified in the sample list and center it in the loop.
Aspirate Air Pre- sample	Enter the volume of air to be drawn into the needle before the sample, to separate it from the previous sample. Range: 0 to half the loop size.
Aspirate Air Post- sample	Enter the volume of air to be drawn into the needle after the sample, to separate it from the next sample. Range: 0 to half the loop size.
Loop Off-line Time	For Parallel Injection mode, enter the time in minutes when the injector valve is switched back from the inject position to the load position for the next sample to be preloaded into the sample loop. Range 0.00 to the Run Time defined on the Pump Mobile Phase page, in minutes.
<b>Overfill Factor</b>	For full loop mode enter the number of times to draw the loop volume into the loop to ensure that it is full. Range 1.0 to 20.

# Waters 2790/2795 Autosampler Parameters Page

Waters 2795 Autosampler		
Injection Parameters       Autosampler Parameter         Sample Temperature       20         Limit (+/- °C)       20         Sampler Configuration       20         Loop Size (μL)       50         Syringe Size (μL)       500	Sample Parameters         Draw Depth (mm)         Draw Speed         Normal         Custom Speed (uL/sec)         Seek Well Bottom         Check Plate Height	

#### Figure 2.30 Autosampler Parameters page

Sample Temperature Set	If the sample heater is installed, enter the temperature to heat or cool the sample to. Range: 4.0 to 40.0 °C.	
Sample Temperature Limit	This is the maximum deviation in sample temperature allowed. If this is exceeded the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red. Range: $\pm 1.0$ to $\pm 20.0$ °C.	
Loop Size (µl)	This is a display only field showing the volume of the sample loop installed.	
Syringe Size	This is a display only field showing the size of the syringe installed.	
Draw Depth	Adjusts the depth of the needle tip to accommodate for sedimented samples or non-standard vials. A value of 0 corresponds to the bottom of the vial. Range: 0.0 to 20.0 mm.	
Draw Speed	This determines the rate in microlitres per second at which sample is extracted into the autosampler needle. This should be set according to the viscosity of the sample. Select one of <b>Fast</b> , <b>Normal</b> or <b>Slow</b> from the dropdown list box. The table below shows the draw rate for each selection using a 250 $\mu$ l syringe.	
Selection	Draw Rate for a 250 µl Syringe	
<b>Selection</b> Fast	Draw Rate for a 250 μl Syringe 5.0 μl/sec	
<b>Selection</b> Fast Normal	Draw Rate for a 250 μl Syringe 5.0 μl/sec 2.5 μl/sec	
Selection Fast Normal Slow	Draw Rate for a 250 μl Syringe         5.0 μl/sec         2.5 μl/sec         1.0 μl/sec	
Selection Fast Normal Slow Custom	Draw Rate for a 250 μl Syringe 5.0 μl/sec 2.5 μl/sec 1.0 μl/sec Value entered in the Custom Speed box.	
Selection Fast Normal Slow Custom Seek Well Bottom	Draw Rate for a 250 μl Syringe         5.0 μl/sec         2.5 μl/sec         1.0 μl/sec         Value entered in the Custom Speed box.         If this box is checked then, for the first well on a plate, the needle will automatically seek the bottom of the well before drawing the sample. The depth of the well will be saved by the software and used as the depth for all other wells on the plate. This will be repeated for the first well on each plate. Note: If a value has been entered in the Draw Depth field then this operation will not be performed.	

# Waters 2790/2795 Wash Parameters Page

Wash Frequency	Select the wash frequency from the drop down list box.
	• None Do not perform a wash.
	• <b>Inject</b> Perform a wash after each injection.
	• Well Perform a wash after all samples have been taken from the current well.

Waters 2795 Autosampler
Injection Parameters Autosampler Parameters Wash Parameters
Wash Solvent Wash Erequency Inject Image Inject Port (s) 6 Needle Exterior (s) 15 Wash Cycles 1 Purge Solvent Replacement Volume (µL) 600.0
Wash <u>S</u> equence

Figure 2.31 Wash parameters page

Inject Port	Enter the time in seconds to wash the interior of the needle for.
Needle Exterior	Enter the time in seconds to wash the exterior of the needle for. Range: 0 to 99 seconds.
Wash Cycles	Enter the number of times the Inject Port and Needle Exterior washes are to be performed. Range: 0 to 10.
Replacement Volume	Enter the volume of wash solvent to leave in the needle after the wash/flush operation has been performed. This volume is then drawn through the waste valve and dispensed into the sample line through the needle. Range: 0 to 9999 $\mu$ l.
Wash Sequence	Choose from Wash – Purge or Purge- Wash - Purge

# Waters 2790/2795 Pump

**Note:** To control the Waters 2790/2795 autosampler and pump from the keypad rather than the MassLynx software the **Inlet** must be configured as **None** 

The Waters Pump pages can be accessed by selecting View, Waters2790/2795 Pump from the

menu bar, selecting Inlet from the short cut bar or by pressing the toolbar button.

Solvents Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must equal 100%. Solvent Names entered here will be displayed on the Gradient page.
 Pump A This displays the remainder percentage after the solvent percentages have been set for the other enabled pumps.
 Pump B, C and D These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.

Flow	Enter the total initial flow rate of the system. Range: 0.000 to 10.000 ml/min.
Ramp	Enter the time (in minutes) for the solvent delivery system to reach the maximum system flow rate (10 ml/min). This limits the rate of change of the flow rate to protect the column from potentially damaging sudden changes in pressure. Range: 0.01 to 30 minutes. Recommended minimum setting: 0.5 min.

# Waters 2790/2795 Mobile Phase Page

Waters 2795 LC			
Mobile Phase	Column   Rap	id Equilibration   1/0   Gradient	Events Method Type
Solvents-	x 100.0 x 0.0 x 0.0 x 0.0 x 0.0	Solvent Name Solvent A Solvent B Solvent C Solvent D	Pressures <u>H</u> igh Limit (Bar) 300 <u>L</u> ow Limit (Bar) 0 Degasser Stroke Length Auto I
Elow (mL/n	nin) 0.200	Ramp Time to reach 10mL/min (mins) 2.00	Run <u>T</u> ime (mins) 1.00

Figure 2.32 Mobile Phase page

Low Pressure Limit and High Pressure Limit	Enter values as required. If the pressure falls outside these limits the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red. Low Pressure Range: 0 to 310 bar. High Pressure Range: 0 to 345 bar.	
Degasser	Select one of Off, Normal or Continuous from the drop down list box.	
	• <b>Off</b> The degasser is always off.	
	• Normal The degasser cycles on and off.	
	• <b>Continuous</b> The degasser is always on.	
Stroke Length	This sets the volume of solvent delivered for each piston stroke. Select the required option from the drop down list box. If Auto is selected then the volume is automatically adjusted to provide optimal performance for the selected solvent flow rate, otherwise the volume selected will be used.	
Run Time	Enter the time in minutes that the method will run from the point of injection. <b>Note:</b> Run time is for the solvent delivery system only. Detectors have independent run times. The MS method (Scan Function Editor) run time must be greater than all other run times.	

# Waters 2790/2795 Column Page

Waters 2795 L	.C
Mobile Phase Column	Rapid Equilibration   1/0   Gradient   Events   Method Type
	Column
	Position Column 1
	Equilibration Time (mins) 0.00
	<u>S</u> et (*C) 20
	Limit (+/- °C) 20
l	

Figure 2.33 Column page

Position	This field allows the column to be selected for the method. The options available will depend on the column setup on the Waters 2790/2795 Separations Module.
	If only one column is installed then this box will display <b>Column 1</b> and cannot be changed. For other configurations this box will allow the selection of a column (between 1 and 6 depending on configuration) or No change from the drop down list box. Selecting a numbered column will use this column for the method, selecting No Change will use the column defined in the last method used to acquire a sample. See the <i>Waters 2790/2795 Separations Module Operator's Guide</i> for more information on the column selection valve.
Equilibrium Time	Enter the time required to reach equilibrium (i.e. run in initial conditions), before performing an injection, after a column change. Range: 0.00 to 999.99 minutes.
Temperature Set	Enter the target operating temperature for the optional column heater. This value must be at least 5 °C above ambient. Range: 20 to 60 °C.
Temperature Limit	Enter the maximum deviation in column temperature allowed. If this is exceeded the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red. Range: $\pm 1$ to $\pm 20$ °C.

# Waters 2790/2795 Rapid Equilibration Page

Path	Select the path to be used for flushing solvent during rapid equilibration from the drop down list box. <b>Waste</b> , <b>Off</b> or <b>Column 1</b> to <b>Column 6</b> depending on the instrument configuration.
Flow	Enter the system equilibration flow rate. Range: 0.00 to 10.00 ml/min.

Time	Enter the length of time (in minutes) to equilibrate. Range 0.00 to 999.99 minutes.
Re-equilibration Time	Enter the time that column should be maintained at initial flow/composition conditions after completion of a gradient run. This delay is imposed on a per injection basis if defined.
Pre-column Volume	Enter the volume of solvent to pump through the column between the time the gradient starts and the time of injection. Range: 0.0 to 10000.0 $\mu$ l

Waters 2795 LC	
Mobile Phase Column Rapid Equilibration 1/0 Gradient Events Method Type	
System         Path         Elow (mL/min)         Lime (mins)         Column         Re-equilibration Time (mins)         0.000         Pre-column ⊻olume (μL)	

Figure 2.34 Rapid Equilibration page

## Waters 2790/2795 I/O Page

Switch Initial<br/>ConditionsSelect the state that switches 1 to 4 should be in initially, from the drop<br/>down list box. At the beginning of each injection cycle each switch returns<br/>to the state defined here. Available choices:

- **On** Turns on a contact closure that triggers an external or internal event. With this function, the contact closure remains closed until an Off function is sent.
- **Off** Turns off the contact closure for the event. With this function, the contact closure is broken.
- **Pulse** Transmits a single On/Off pulse. The contact closure is maintained for the number of minutes set in the Pulse Width field on the Events page. Range: 0.01 to 100.00 sec.
- **Toggle** Changes the current state of the switch.
- No Change Leaves the switch in its current state.

# Chart OutputSelect Flow Rate, System Pressure, %A, %B, %C, %D, ColumnSettingTemperature or Sample Temperature from the drop down list box.

The Analog output signals are sent through the terminals on the back of the 2790/2795, to an optional analog device such as a strip chart recorder. If, for example, System Pressure is selected the recorder will chart the system pressure while the method is being run.

Waters 2795 LC	
Waters 2795 LC          Mobile Phase       Column       Rapid Equilibration       1/0       Gradient         Switch Initial Conditions       Switch 1       Switch 2       Switch 3         On       Off       Pulse       Image: Chart Output         Setting       Flow Rapid	nt Events Method Type

Figure 2.35 I/O page

# Waters 2790/2795 Gradient Page

		Vaters 2795 LC			
obile Phase 🖡 Colu	umn   Rapid E	Equilibration 1/0 Gradient Events Method Type			
<u>I</u> ime (mins)	0.00	Gradient Table + 星尾月			
Solvent A	A% 100.0	Time A B C D Flow Curve			
Solvent B	<u>B</u> % 0.0				
Solvent C	<u>C</u> % 0.0				
Solvent D	<u>D</u> % 0.0				
<u>F</u> low (mL/min)	1.000				
C <u>u</u> rve	1				
<u>F</u> low (mL/min) C <u>u</u> rve	1.000				

Figure 2.36 Gradient page

This page allows a gradient to be entered and edited. If you wish to operate in isocratic mode then enter parameters on the Mobile Phase page and ensure that the timetable is empty.

Time (m	ins)	Specifies the time at which the specified conditions (%A to %D, Flow, and Curve) for the row should take effect. Make sure the Time for the first row is set to 0.00, to establish initial conditions for the gradient run. The range for rows other than row 1 is 0.01 to 999.99 minutes.			
Solvent . Solvent .	A % - D%	Specif the tot	ties the percentage of solvent flow from each reservoir. For each row al of all solvents must equal 100%. Range: 0 to 100%.		
Flow (ml/min) Spe 10 r		Specif 10 ml/	ifies the total flow rate for the solvent delivery system. Range: 1 to l/min.		
Curve		Note: perfor Mobil at the the ini the va This s and/or 11.(se	If column equilibration, rapid equilibration or wet prime are med then the flow rate will return to the value defined on the e Phase page. If they are not performed then the flow rate will stay value defined for the last entry in the Gradient Table. To return to tial flow rate an entry must be added to the end of the table setting lue to that defined on the Mobile Phase page. ets the rate at which the solvent is to change to the new proportions flow rates. Curves are specified by number. Available choices: 1 to e also Figure 2.12 Curve Profiles		
	Curve Num	ber	Effect		
	1		Immediately goes to specified conditions		
	2 to 5		Convex		
	6		Linear		
	7 to 10		Concave		
	11		Maintains start condition until next step		

#### Waters 2790/2795 Gradient Table Operation

To add a gradient, enter values in the relevant boxes and press the toolbar button. Up to 15 rows can be added to the table. **Note:** The first entry must have a time of 0.

To delete a single gradient click a time in the list and press the **k** toolbar button.

To delete all entries press the itoolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does not correspond to any existing entry in the timetable, pressing will result in a new entry being.

# Waters 2790/2795 Events Page

Use the Event Table to program up to 16 events (both external and internal). The external events are triggered by four contact closures (relays) through output terminals (S1–S4) on the 2790/2795 Separations Module. The internal events are used to control the sample compartment temperature,

column heater temperature and column change. Events can be triggered more than once and multiple events can be triggered simultaneously.

Waters 2795 LC					
Mobile Phase Column Rapid Equ	uilibration	I/O Gradient	Events   Methor	d Type   B 📌 E 🔏	
0.00 Switch 1 O On O Off O Toggle O Pulse Width (min) 0.01 O No Change	Time 0.00	Event Switch 1	Action No Change	Value	

Figure 2.37 Events page

Time	Enter the time at which the event starts. Event rows are sorted automatically by time. <b>Note:</b> Different events can be programmed to occur at the same time. Range 0.00 to the Run Time defined on the Mobile Phase page, in minutes.
Event	Enter the type of event signal: one of the four TTL-level output switches (S1-S4), or one of the internal events (column heater temperature, sample compartment or column change).
	• Switch 1 to Switch 4 Corresponds to terminal strip positions S1 to S4 on the rear of the 2790/2795 module. Activating a Switch event triggers a contact closure for controlling an external device. After selecting a switch event, set a state for the switch by selecting On, Off, Toggle, Pulse Width or No Change. This state appears in the Action column of the table (see Switch States, below). Note: If Pulse is selected the duration of the pulse must be entered in the Width (min) field.
	• Set Temperature (Column or Sample) Specifies the temperature of an optional column heater, or an optional sample compartment heater/cooler. After selecting this event, select Column or Sample and enter the required Temperature in °C. Note: When a Column Temperature event occurs, the temperature of the column heater changes from the value set in the Column page to the value set for the event. When the event times out, the temperature changes back to the Column page value. Column range: 20 to 60 °C. Sample range: 4 to 40 °C.
	• <b>Column Change</b> Specifies a column change operation for the 2790/2795 module, as describe on the Column page.

- On Turns on a contact closure that triggers an external or internal event. With this function, the contact closure remains closed until an Off function is sent.
  - **Off** Turns off the contact closure for the event. With this function, the contact closure is broken.
  - **Toggle** Changes the current state of the switch.
  - **Pulse** Transmits a single On/Off pulse. The contact closure is maintained for the number of seconds that defined in the Value column. Range: 0.01 to 10.00 sec.
  - No Change Leaves the switch in its current state.

#### Waters 2790/2795 Event Table Operation

To add an event, type in a time, select an event from the drop down list box, select an action and press the toolbar button. Up to 16 events can be programmed.

To delete a single event click a time in the list and press the 📧 toolbar button.

To delete all entries press the itoolbar button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in

the edit boxes and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does not correspond to any existing entry in the timetable, pressing will result in a new entry.

## Waters 2790/2795 Method Type Page

Figure 2.38 Method Type page

This page is used for creating normal, pre and post run methods. First create a **Normal** method and save the file, then create any pre or post run methods (saving them under different names). These methods can then be defined in the Inlet Pre run and Inlet Post run columns of the Sample List.

Method Type	Select the type of method to create. The <b>Parameters</b> section will be updated to show the parameters required for the selected method.
Normal	Creates a normal method. No extra parameters need to be defined.

For all other method types see the Waters 2790/2795 Menu, on page 2-45 for details of the parameters required.

## Waters 2790/2795 Menu

#### Waters 2790/2795 Wet Prime



Figure 2.39 Wet Prime dialog

Wet PrimeA wet prime should be performed when changing the solvent in the system<br/>to flush out the previous solvent. The new solvent is pumped through the<br/>tubing and the Prime port of the Inject valve to waste.

Enter the **Flow** rate, **Time** and the **Percentage of solvents** to use then press the **Prime** button. Waters recommend that the wet prime is started using the solvent with the lowest viscosity to help purge air from the lines, especially if the in-line vacuum degasser is installed.

**Note:** If the solvent lines are dry then a dry prime must be performed before a wet prime. See the *Waters 2790/2795 Separation Module Operator's Guide* for more information on performing a dry prime.

#### Waters 2790/2795 Equilibrate

Equilibrate	×
Time (mins)	1.0
OK I	Cancel

Figure 2.40 Equilibrate dialog

Equilibrate Equilibrates the system using the parameters defined on the Mobile Phase page.

Enter the **Time** to equilibrate the system for and press **OK**. The time needed to equilibrate the system will depend on environmental and application-specific factors.

#### Waters 2790/2795 Condition Column

Condition Column	×
Time (mins)	1.0
0K	Cancel

Figure 2.41 Condition Column dialog

**Condition Column** Runs solvent through the column without injecting samples or running the Events table. Solvent is delivered to the Column defined on the Column page, using the Gradient Table defined on the Gradient page.

Enter the **Time** in minutes to condition the column for and press **OK**. Ensure that the time is equal to or greater than the Time of the last entry in the Gradient Table (defined on the Gradient page) plus the Re-equilibration Time (defined on the Rapid Equilibration page).

#### Waters 2790/2795 Wash Needle

₩ash Needle	×
Cycles Volume (μL)	1.0
OK	Cancel

Figure 2.42 Wash Needle dialog

Wash NeedleWashes the inject port, and both the interior and exterior of the needle<br/>with wash solvent, and then fills the needle with fresh solvent.

Enter the number of wash **Cycles** to perform and the **Volume** of wash solvent to use then press **OK**. Waters recommend a volume of  $600 \ \mu$ l.

#### Waters 2790/2795 Refresh Syringe

Refresh Syringe refills the syringe with fresh, degassed, purge solvent.

Enter the number of **Cycles** and the replacement **Volume** and press **OK**. Waters recommend 12 Cycles and a volume of  $600 \mu l$ .

Refresh Syringe	×
Cycles Volume (μL)	1 1.0
(OK)	Cancel

Figure 2.43 Refresh Syringe dialog

## Waters 2790/2795 Plate Generator

To display the Plate Generator dialog, select Plate Generator from the Waters2790/2795 menu.

PlateGenerator	
Plate Name Code 201 Plate	Vial Reference Horizontal C A B C C 1 2 3
Number Of Vials         Vial Spacing 1/10 mm           Rows         8         90           Columns         12         90	Vertical © A B C © 1 2 3 Referencing XY Priority T Horizontal first
Offsets 1/10 mm Row 0 Every 2nd Row	Top Left Vial Offset           X         1275         1/10 mm         X         135         1/10 mm           Y         855         1/10 mm         Y         110         1/10 mm           Z         0         1/10 mm         Y         110         1/10 mm
Column 0 Every 2nd Column	Vial Depth 1065 Diameter 70

Figure 2.44 790/2795 Plate Generator

Plate Name	The name of the plate that is currently being edited.
Rows	The number of vials in a row and the distance between each center.
Columns	The number of vials in a column and the distance between each center.
Offsets	Allows alternate vial rows or columns to be offset. <b>Note:</b> Entering a positive value will shift even numbered rows to the right and negative values will shift even numbered rows to the left.
Vial Reference	Allows the user to select the way that the vial rows and columns are referenced, e.g. whether the rows are alphabetical or numerical.
Horizontal	Sets the horizontal axis of the plate as either alphabetic (ABC) or numeric (123), when using XY referencing.

Vertical	Sets the vertical axis of the plate as either alphabetic (ABC) or numeric (123), when using XY referencing.
Referencing	This has three options
	• XY which references the vials A1, B1 etc.
	• Sequential Discontinuous which numbers the vials 1, 2, 3 across a row, left to right, and then starts the next row from the left again.
	• Sequential Continuous which numbers the vials 1, 2, 3 across a row, left to right, then continues number the next row, right to left etc.
	If the Waters 2790/2795 autosampler is used with OpenLynx then the vial referencing must be set to either sequential continuous or sequential discontinuous.
Priority	Check the <b>Horizontal First</b> box if samples are to be acquired horizontally across the plate.
	If Referencing = X,Y, Horizontal = Letter, Vertical = Number and Horizontal Priority is checked, this will result in samples being acquired in the order A1, A2, A3. If the Horizontal Priority box is not checked samples will be acquired in the order 1A, 1B, 1C etc.
	If Referencing = sequential continuous or discontinuous and Horizontal Priority is checked, this will result in samples being acquired from row 1 then row 2. If the Horizontal Priority box is not checked samples will be acquired from column 1, then column 2 etc.
Plate Size	The size of the plate to its outside edges.
Top Left Vial Offset	The measurement to the center of the first vial from the top left corner of the plate.
Vial	The depth and diameter values are used for display only. They appear in the description for a single shot login on the OpenLynx Login screen.

#### **Creating and Deleting Waters 2790/2795 Plates**

To create a new plate, press the Debutton. A new default plate is displayed, change the Pla	te
Name, enter the appropriate values and press the save 🔟 button or select Plate, Save Plat	e.
New plates are saved to the MassLynx <b>Plates</b> directory.	
To copy a plate, page through the list of saved plates using the <b>Previous Plate</b> and <b>Next Plate</b> options on the <b>Plate</b> menu perform the same operation. Whe required plate is displayed change the <b>Plate Name</b> , enter the appropriate values and press the	s. The n the e save
button or select Plate, Save Plate. New plates are saved to the MassLynx Plates direct	ory.
To delete a plate select the plate, by typing the name in the <b>Plate Name</b> box or by paging thr	ough

as above, and press the delete **Lee** button or choose **Plate**, **Delete Plate**.

Note: All of the spacings and the vial section are stored in 0.1 mm units.
**Note:** When defining a custom plate for use with a multi-injector the plate is required to be compatible with the position of the 8 needles of the autosampler.

- The Plate must have eight columns.
- The position of the vials should allow all eight needles to enter a separate vial.
- There should be no odd or even offsets for any of the vial positions.

**Note:** If the Plate currently selected on the Sample Configuration page is changed here, then **Reset Injector** should be selected from the **LC** menu to reset communications.

Note: All of the spacings and the vial section are stored in 0.1 mm units.

#### **Vial Referencing Examples**

The following tables show four examples of vial referencing for a simplified  $4 \times 3$  vial plate.

Havirontals 122	4	3	2	1	
Vertical: ABC	4,A	3,A	2,A	1,A	A
Referencing: XY	4,B	3,B	2,B	1,B	В
Priority: Horizontal First Checked	4,C	3,C	2,C	1,C	С

	1	2	3	4	
Α	A,1	A,2	A,3	A,4	Horizontal: 123 Vertical: ABC
В	B,1	В,2	В,3	В,4	Referencing: XY
С	C,1	C,2	С,3	C,4	Priority: Horizontal First NOT Checked

	1	2	3	4	
Α	1	2	3	4	Horizontal: N/A Vertical: N/A
В	5	6	7	8	Referencing: Sequential Discontinuous
С	9	10	11	12	Priority: Horizontal First Checked

	1	2	3	4	Hard-and h N/A
					Horizontai: N/A
Α	1	6	7	12	Vertical: N/A
В	2	5	8	11	Referencing: Sequential Continuous
С	3	4	9	10	Priority: Horizontal First NOT Checked

### Waters 2790/2795 Bed Layout

Use the Bed Layout Editor to define the type, number, and location of the well plates on the 2790/2795 plate carrier. To access the Bed Layout Editor, select **Bed Layout** from the **Waters2790/2795** menu.

Bed Layout 🛛 🗙
Select/Create Bed Layout Modify Bed Layout
Bed Layouts:
Four Position 96 Well 700uL Plate1
Four Position 384 Well 400uL Plate
Four Position 96 Well 700uL Plate1
Current Layout:
Four Position 96 Well 700uL Plate1
OK Cancel Help

Figure 2.45 2790/2795 Bed Layout dialog

Bed LayoutsLists the available Bed Layouts.

**Current Layout** Specifies the bed layout currently in use.

#### To Delete A Bed Layout (Waters 2790/2795)

Highlight the bed layout to delete and press the button. A dialog box will ask you to confirm the deletion. Press the **OK** button to delete the bed layout.

Note: You cannot delete the bed layout, which is selected as the Current Layout.

#### To Create A New Bed Layout (Waters 2790/2795)

Highlight a bed layout similar to the one you want to create and press the button to create a new layout. The layout appears in the **Bed Layouts** list as the same name with a 1 at the end, for example Six Position Microtiter1.

1.

- To change the name of the layout, type the new name into the Bed Layouts text box and press the button. The name is updated in the Bed Layouts list box.
- 3. If the plate position or type needs changing select the **Modify Bed Layout** tab.

Note: New bed layouts are saved to the MassLynx Racks directory.

#### To Modify a Bed Layout (Waters 2790/2795)

Use the Modify Bed Layout page to modify an existing bed layout. To access the Modify Bed Layout page, click the **Modify Bed Layout** tab. The Modify Bed Layout page shows a graphical representation of the selected bed layout. There are four plate positions in the 2790/2795.

ed Layout X Select/Create Bed Layout Modify Bed Layout				
Four Position 9	16 Well 700uL	Plate		
96 Well 700uL Plate	96 Well 700uL Plate	96 Well 700uL Plate	96 Well 700uL Plate	
	OK	Cance	el H	lelp

Figure 2.46 2790/2795 Modify Bed Layout dialog

Click the plate that you want to change to display the Plate Position and Type dialog.

Plate Position and Type Plates:	
48 Well 2mL Plate 48 Well 500uL Plate 96 Well 1mL Plate 96 Well 2mL Plate 96 Well 300uL Plate 96 Well 300uL Plate 96 Well 700uL Plate ▼	Cancel
🔲 OpenLynx plate login	

Figure 2.47 2790/2795 Plate Position and Type dialog

This dialog allows you to select a new plate from a list of possible options, and change its actual position on the bed. Select the plate type you want to use in the bed layout, then click **OK**.

OpenLynx plate login If this box is checked and Use current MassLynx autosampler bed layout is checked in the OpenLynx Manager program, then the plate at this position can only be used for plate login on the OpenLynx Login program.

# Waters CapLC System Status Pages

The System Status pages display information about the state of the machine being controlled. These pages can be accessed within the Inlet Editor by selecting **View**, **Status**, selecting Status

from the short cut bar or by pressing the <sup>C</sup> toolbar button.

### Waters CapLC Solvent Status Page

🚓 Default.clc, Default	🛪 Default.clc, Default.wat - Inlet Method					
<u>File View T</u> ools Cap	le <u>V</u> iew <u>T</u> ools Cap LC <u>C</u> ontrol Cap LC Tools <u>H</u> elp					
🗅 📂 🖬 🍊 🍋	n 🐜 🖊 🧳 🤗   💷   🧇   🎒					
70	Waters CapLC Status					
	Solvent Status Valve Status PDA Status					
Status						
	Indicators CapLC Ternary Mode					
	Running  Time (mins): 0.00  100 %					
Inlet	Pump On Solution Flow (ul/min): 0.000 0.0 %					
<b>E</b>	Inject Cycle     Pressure (psi): 0     C     0.0 %					
Autosampler	C Ready					
	OK     Column (*C): 0.0     Sample (*C): 0.0					
Detector	General Status: Status Unknown					
For Help, press E1	,					

#### Figure 2.48 Solvent Status page

Indicators	The Running, Pump On and Injector Cycle indicators at the left of the screen give information on the current status of the LC system. The OK and Ready Indicators become illuminated in red if the LC System has an error. Click on the red indicators to display more information on the cause of the malfunction.		
Time	This displays how long the method has been running		
Flow	This displays the current flow rate as returned by the instrument.		
Pressure	This displays the current pressure in the instrument.		
	To the right of the Time, Flow and Pressure fields is a display of the solvent percentages at which the LC System is currently operating.		
Column	This displays the current temperature of the column.		

**Sample Temp** This displays the current temperature of the sample.

### Waters CapLC Valve Status Page

Wa	Naters CapLC Status					
Sol	Solvent Status Valve Status PDA Status					
	Valve Status					
	<b>6</b> 5	Solvent Select Valve:	A1			
	Y	Stream Select Valve:	Position One			
	$\odot$	Vent Valve:	System			
	6	Injection Valve:	Injecting			

Figure 2.49 Valve Status page

This page shows what position the Valves are currently set to, if installed.

See the Waters Cap LC Users' Guide for details of the valves.

### Waters CapLC PDA Status Page

Waters CapLC Stat	ıs	
Solvent Status Valve Status	PDA Status	
Detector Status-		
	Current State: Not Instal	lled
	Scan Number: 0	

Figure 2.50 PDA Status page

**Current State** 

This displays the current state of the PDA Detector

**Scan Number** When acquiring diode array data this displays the number of scans currently acquired.

# Waters CapLC Pump

The Waters Pump pages can be accessed by selecting **View**, **Waters CapLC Pump** or by pressing the toolbar button.

### Waters CapLC Initial Conditions Page

Waters CapLC	
Initial Conditions Gradient Initial Events Timed Events	Chart Recording Method Type
Solvents     Solvent Name       Image: Solvent Select A1     Solvent A1       Image: Solvent Select A2     Solvent A2	Pressures Low P <u>r</u> essure (psi) 0.0 <u>H</u> igh Pressure (psi) 5000.
C Solvent Select A3 Solvent A3	Column
Solvent C	Temperature (°C) 20.0
Flo <u>w</u> (ul/min) 1.000 Run <u>T</u> ime (mins) 5.00	Range(+/-°C) 20.0 Name Column

Figure 2.51 Initial Conditions page

Solvent Select A1 to A3	Select the solvent to deliver through Pump A.		
Solvent B and C	Enter the percentage of solvent flow from pump B and/or C (if installed).		
Solvent Name	Enter the name of the solvent in the corresponding solvent reservoir.		
Flow	Enter the total flow rate for the system in $\mu$ l/min.		
Run Time	Enter the length of time (in minutes) until the next injection occurs.		
	<b>Note:</b> Run time is for the pump and autosampler only. Detectors have independent run times. The MS method run time must be greater than all other run time (see The Function List Editor chapter for details).		
	<b>Note:</b> If you are running a gradient or setting timed events, make sure you set the initial conditions Run Time to a value greater than or equal to the greatest Time value in the Gradient or Timed Events Table.		
Low Pressure	Enter the low-pressure limit for the system. If the system pressure falls below this limit, the flow stops and the LC Status error light turns red. Range: 0 to 4500 psi.		

High Pressure	Enter the high-pressure limit for the system. If the system pressure exceeds this limit, the flow stops and the LC Status error light turns red. Range: 0 to 5000 psi.
Temperature	Enter the target operating temperature for the optional column heater. This value must be at least 5 °C above ambient. Range: 20 to 60 °C. Minimum setting: 5 °C above ambient.
Range	Enter the maximum allowable temperature deviation from the value set for the column heater temperature. If the column heater temperature deviates beyond the specified range, the run stops and the LC Status error light turns red. Range: $\pm 0.0$ to $\pm 10.0$ °C.
Name .	Enter the name of the installed column

### Waters CapLC Gradient Page

Gradient Entry	0.00	<u>G</u> radient Ta	ble		+:	8/28/28
<u>Tune(unite)</u>		Time	B%	C%	Flow	Curve
<u>B</u> % C C% C Flo <u>w</u> (ul/min) 1. Cur <u>v</u> e	000	0.00	0.0	0.0	1.000	6
<u>-</u> -						

Figure 2.52 CapLC Gradient page

Use the Gradient Table to define conditions for a gradient run. For each row in the Gradient Table, define the percent composition of up to four solvents that are to be delivered at the desired flow rate for the specified time. Enter the number of the gradient curve required. This defines how changes to solvent percentages and flow rates take place over the elapsed time of each gradient segment (the time that elapses between the start time of one row and the start time of the next row).

**Note:** For an isocratic run, set the solvent percentages, run time and flow on the Initial Conditions page. Do **not** add any rows to the Gradient Table.

#### Waters CapLC Gradient Table Parameters

Time (mins)Specifies when the conditions (%A-%D, Flow, and Curve) for the row<br/>take effect. Make sure the Time for the first row is set to 0.00 to establish<br/>initial conditions for the gradient run. Range for rows other than row 1:<br/>0.01 to 999.99 minutes

B% and C% S		Spec row,	pecifies the percentage of solvent flow from each reservoir. For each ow, the total of all solvents must equal 100%. Range: 0 to 100%			
		Note calcu	e: Percent flow for reservoir A is not displayed. Percent A is alated as: $100\% - (B\% + C\%)$			
Flow (µl/	min)	Spec	ifies the total flow rate for the system.			
Curve		Spec time For 1 Prof	tifies the rate of change of solvent composition and flow rate over , based on the curve number and the length of the gradient segment. more information, see Gradient Curves, below and Figure 2.12 Curve iles.			
	Curve Numb	oer	Effect			
	1		Immediately goes to specified conditions			
	2 to 5		Convex			
	6		Linear			
	7 to 10		Concave			
	11		Maintains start condition until next step			

#### Waters CapLC Gradient Table Operation

To add a gradient, enter a time, percentage, flow rate and curve number in the relevant boxes and press the toolbar button. **Note:** The first entry must have a time of 0.

To delete a single gradient click a time in the list and press the 📧 toolbar button.

To delete all entries press the itoolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does

not correspond to any existing entry in the timetable, pressing + will result in a new entry being created.

### Waters CapLC Initial Events Page

Use the Initial Events page to set the initial condition of the two contact-closure output switches, the initial position of the stream select valve, and the initial position of the optional diverter valve.

**Note:** To change the settings of these switches and valves during a run, use the Timed Events Table.

Switch 1 and 2	Select the initial state of contact-closure Switch 1 and 2 from the drop down list boxes.
Stream Select	Select the initial state of the stream select valve (Position 1 or 2) from the drop down list box.

**Diverter** Select the initial position of the optional diverter valve (System or Vent) from the drop down list box.

۷	Vaters CapLC				
	Initial Conditions   Gradient	Initial Events Tir	ned Events 🗍	Chart Recording Metho	od Type
	Events				
		Switch 1	Off	-	
		Switch 2	Off	_ _	
		<u>S</u> tream Select	Position 1	•	
		⊻ent Valve	System	•	
					, 

Figure 2.53 CapLC Initial Events page

### Waters CapLC Timed Events Page

<u>Time:</u> <u>Event:</u> 0.00 ▼ Switch 1	Event ]	[able	+4	* *
Switch T	Time	Event	Action	Value
© <u>0</u> n © Off © Toggle © <u>P</u> ulse <u>W</u> idth (min) 0.01		SYRCELL	no change	

Figure 2.54 CapLC Timed Events page

Use the Event Table to program up to 16 events (both external and internal). The external events are triggered by four contact closures (relays) through output terminals (S1–S4) on the 2790/2795 Separations Module. The internal events are used to control the sample compartment temperature, column heater temperature, and to prime and flush the 2790/2795 Separations Module. Events can be triggered more than once and multiple events can be triggered simultaneously.

#### Waters CapLC Event Table Parameters

Time	Enter the time (after injection) at which the event starts. Event rows are sorted automatically by time. <b>Note:</b> Different events can be programmed to occur at the same time. Range: 0.00 to 999.99 min.		
Event	Select the type of event signal: one of the two contact-closure output switches (Switch1or Switch 2), or one of the internal events (Set temperature, Stream Select or Vent Valve). Choose from these event types to program up to 16 events. <b>Note:</b> The same event can be programmed more than once. Available choices:		
	• Switch 1 and 2 Corresponds to terminal strip positions S1 and S2 on the rear of the unit. Activating a Switch event triggers a contact closure for controlling an external device. Select a switch event and a state for the switch (On, Off, Toggle, Pulse or No Change). This state appears in the Action column of the table (see Switch States, below). Note: If Pulse is selected for a "switch state" the duration of the pulse must be entered in the Width (min) field.		
	• Set Temperature Specifies the temperature of an optional column heater. If Set Temperature is selected for a switch state the temperature in (°C) must be entered in the Column Temperature field. Note: When this event occurs, the temperature of the column heater changes from the value set on the Initial Conditions page to the value set for the event. When the event times out, the temperature returns to the value on the Initial Conditions page value.		
	• Stream Select (1 or 2) Specifies the position of the stream select valve.		
	• Vent Valve (System or Vent) Specifies the position of the vent valve.		
Waters CapLC Switch States			
_			

# • **On** – Turns on a contact closure that triggers an external or internal event. With this function, the contact closure remains closed until an Off function is sent.

- **Off** Turns off the contact closure for the event. With this function, the contact closure is broken.
- **Toggle** Changes the current state of the switch.
- **Pulse** Transmits a single On/Off pulse. The contact closure is maintained for the time entered in the **Width** box. Range: 0.01 to 100.00 minutes.
- No Change Leaves the switch in its current state.

### Waters CapLC Event Table Operation

To add an event, enter a time, event, action and value in the relevant boxes and press the toolbar button. Note the first entry must have a time of 0.

To delete a single event click a time in the list and press the **k** toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in the edit boxes, and can be altered as appropriate. Once changed press  $\mathbf{+}\mathbf{E}$  to re-enter the values into the timetable. If, however, you modify the time value such that it does not correspond to any existing entry in the timetable, pressing  $\mathbf{+}\mathbf{E}$  will result in a new entry being created.

### Waters CapLC Chart Recording Page

Waters CapLC
Initial Conditions Gradient Initial Events Timed Events Chart Recording Method Type
Chart Recording
Chart Record 1 Chart Record 2 None  None
Flow Pressure Percent A Percent B
Percent L

Figure 2.55 CapLC Chart Recording page

Use the Chart Recording page to select up to two analog signals to be output to an external device such as an integrator or strip-chart recorder. Select one of the following the signals to output, from the drop down list box.

- None
- Flow
- Pressure
- Percent A, B, or C
- Column temperature

**Note:** To record a signal, you need to connect each external device to the appropriate Chart Out terminal pair on the rear of the unit. Refer to the Waters CapLC System Installation and Maintenance Guide for installation and specification details.

### Waters CapLC Method Type Page

Specifies the type of method to create. Select one of:-

Normal Method	The Method Type used for standard injections. Ensure that the Method Type is set to <b>Normal</b> unless you are performing one of the procedures listed below.
Column Condition Method	Runs solvent through the column without injecting samples or running the Events table. Solvent is delivered using the gradient table specified in the Gradient page.

Equilibrate MethodDelivers solvents and maintains solvent parameters using the values<br/>defined on the Initial Conditions page.Wet PrimeReplaces solvent in the tubing with fresh solvent from the reservoirs<br/>through the Prime port of the inject valve to waste. Use a Wet Prime<br/>Method when changing the solvents in the system. Check the boxes for<br/>the solvent lines to prime, and the number of loop volumes to use.Waters recommends starting the wet prime using the solvent with the<br/>lowest viscosity to help purge air from the lines, especially if the in-line<br/>vacuum degasser is installed.

**Note:** If the solvent lines in the CapLC are dry, you must perform the dry prime procedure before performing a wet prime.

- Method Type			
O <u>N</u> ormal Meth	od 🗵 🖻 🖻	in Event Table	
O <u>C</u> olumn Cond	lition Method		
○ <u>E</u> quilibrate M	ethod		
Wet Prime	☐ Solvent <u>A</u> ☐ Solvent <u>B</u>	<u>N</u> umber of Volumes	4
	☐ Solvent <u>C</u>		

Figure 2.56 CapLC Method Type page

# Waters CapLC Autosampler

These pages are used to set parameters specific to the Autosampler, to access them select **View**, **WatersCapLC Autosampler** or press the toolbar button.

### Waters CapLC Autosampler Page

Draw Height	Adjusts the depth of the needle tip to accommodate for sedimented samples. A value of 0 corresponds to the top of the plate carrier. Range: 0 to 40 mm.
Draw Speed	Select the draw rate of the syringe from the drop down list box. The different rates accommodate for samples of varying viscosity. The rate for each selection is dependent on the size of the installed syringe. The table below shows the draw rates for each selection.

Waters CapLC Auto	psampler		
AutoSampler Mix Method			
- Injection Parameters		Wash	
Draw <u>H</u> eight(mm)		Wash Volume (ul)	10.0
Draw Speed	Normal 💌		
Injection <u>T</u> ype	Partial Loop 💌		
<u>F</u> lush Volume (ul)	10.0	Temperatures	
Head Space Pressure		Sample Tempe <u>r</u> ature (*C)	20.0
🗖 Air Space Segment		Temperature Limit (°C)	20.0
High Efficiency Mode			

Figure 2.57 CapLC Autosampler page

	Draw Rate for 25 µl Syringe	Draw Rate for 100 µl Syringe	Draw Rate for 250 µl Syringe	Draw Rate for 500 µl Syringe
Fast	94 µl/min	375 µl/min	940 µl/min	1875 µl/min
Normal	63 µl/min	250 µl/min	625 µl/min	1250 µl/min
Slow	32 µl/min	375 µl/min	315 µl/min	625 µl/min

**Injection Type** Select one of the following injection types from the drop down list box:

**Full Loop** – The sample loop is completely filled.

**Partial Loop** – The sample loop is partially filled with the volume defined in the Sample List. The value in the Sample List must not exceed the Full Loop volume.

 $\mu$ l Pickup – The sample loop is filled with only the amount of sample to be injected (resulting in no sample loss). Sample is transported into the loop by transport liquid (mobile phase) from the transport vial.

**Manual** – Specifies that the manual injector is used (the autosampler is disabled). Switching the manual injector to the Inject position initiates any programmed gradients and/or timed events.

- **Flush Volume** Enter the volume (in microliters) of sample taken from a vial before the loop is filled with sample. This flushes out previous samples.
- Head SpaceIf this box is checked the prepuncturing needle will put approximately 0.5Pressurebar of pressure on the sample to stop formation of air or vapour bubbles.<br/>Enable this parameter only when using sample vials with air-tight caps.

Air Space Segment	If this box is checked an air segment is added to the front of the flush volume to minimize dilution and bandspreading and reduce the amount of flush volume required. In Full and Partial Loop modes, the air segment is flushed to waste; in $\mu$ l Pickup mode, the air segment is injected. Disable this parameter if the air segment causes problems in $\mu$ l Pickup mode.
High Efficiency Mode	If this box is checked the sample loop will be taken out of the flow stream after the sample has been flushed, but before the gradient front reaches the injection valve.
Wash Volume	Enter the volume (in microliters) of wash solvent used to clean the needle and buffer tubing.
Sample Temperature	Enter the target operating temperature for the optional sample heater/cooler Range: 4.0 to 40.0 $^{\circ}$ C.
Temperature Limit	Enter the maximum allowable temperature deviation from the value set for the Sample Temperature. If the sample temperature deviates beyond the specified range, the LC Status error light turns red and the run stops. Range: $\pm 1.0$ to $\pm 20.0$ °C.

## Waters CapLC Mix Method Page

Mix Delay	Enter the delay time before mixing, in minutes. A value of 0 corresponds to the top of the plate carrier. Range: 0 to 99.9 mins.
Mix Cycles	Enter the number of times to perform the Mix operation.
<b>Reagent Position 1</b>	Enter the position of the first reagent to mix.
Volume Reagent 1	Enter the volume of reagent 1 to mix.
<b>Reagent Position 2</b>	Enter the position of the second reagent to mix.
Volume Reagent 2	Enter the volume of reagent 2 to mix.

### Waters CapLC Autosampler

AutoSampler Mix Method	
Mix	Reagents Reagent A Position
<u>M</u> ix Delay(mins) 0.00 Mix <u>C</u> ycles 1	Reagent A Volume(ul) 1.00 Reagent B Position 0 Reagent B Volume(ul) 1.00

Figure 2.58 CapLC Mix Method page

### Waters CapLC Bed Layout

Bed Layout	
Select/Create Bed Layout Modify Bed Layout	
Bed Layouts: 7 Position Microtiter 96 Well Plate 7 Position Microtiter 96 Well Plate	Rows In Columns: Col. 1: 1 Col. 2: 1 Col. 2: 1 Col. 3: 1 Col. 4: 1 Col. 5: 1 Col. 5: 1 Col. 6: 1 Col. 7: 1
Current Layout: 7 Position Microtiter 96 Well Plate	
ОК	Cancel Help

Use the Bed Layout Editor to define the type, number, and location of the well plates on the CapLC plate loader. To access the Bed Layout Editor, select **Cap LC Tools, Bed Layout**.

Figure 2.59 CapLC Bed Layout dialog

Bed Layouts Lists the available Bed Layouts.

**Current Layout** Specifies the bed layout currently in use.

#### To Delete A Bed Layout (Waters CapLC)

Highlight the bed layout to delete and press the button. A dialog box will ask you to confirm the deletion. Press the **OK** button to delete the bed layout.

Note: You cannot delete the bed layout that is selected as the Current Layout.

#### To Create A New Bed Layout (Waters CapLC)

- 1. Highlight a bed layout similar to the one you want to create and press the button. The layout appears in the **Bed Layouts** list as the same name with a 1 at the end, for example Six Position Microtiter1.
- To change the name of the layout, type the new name into the Bed Layouts text box and press the button. The name is updated in the Bed Layouts list box.
- 3. If the plate position or type needs changing select the Modify Bed Layout tab.

Note: New bed layouts are saved to the MassLynx Racks directory.

#### To Modify a Bed Layout (Waters CapLC)

Use the Modify Bed Layout page to modify an existing bed layout. To access the Modify Bed Layout page, click the **Modify Bed Layout** tab. The Modify Bed Layout page shows a graphical representation of the selected bed layout.

Bed Layout
Select/Create Bed Layout Modify Bed Layout
7 Position Microtiter 96 Well Plate
licrotitu 96 Well Well Well Well Well Well
OK Cancel Help

Figure 2.60 CapLC Modify Bed Layout dialog

Click on the plate that you want to change to display the Plate Position and Type dialog.

Plate Position and Type	×
Plates: 96 Well 2mL Plate 96 Well 300uL Plate 96 Well 350uL Plate 96 Well 700uL Plate Microtiter 384 Well Plate Microtiter 96 Deep Well Plate Microtiter 96 Well Plate	Cancel

Figure 2.61 CapLC Plate Position and Type dialog

This dialog allows you to select a new plate from a list of possible options, and change its actual position on the bed. Select the plate type to use in the bed layout, then click **OK**.

OpenLynx If this box is checked and Use current MassLynx autosampler bed layout is checked in the OpenLynx Manager program, then the plate at this position can only be used for plate login on the OpenLynx Login program.

#### Other Bed Layout Options (Waters CapLC)

- 1. To append a new column, press the **button**.
- 2. To delete the current column press the 📧 button.

3. To insert a column, click on the column before which you want to insert and press the button. Note: The column inserted will have the same number of rows as the column highlighted.

Note: The number of rows in a column cannot be changed and so the button is grayed out.

### Waters CapLC Plate Generator

To display the Plate Generator dialog, select Cap LC Tools, Plate Generator.

Plate Name	The name of the plate that is currently being edited.
Rows	The number of vials in a row and the distance between each center
Columns	The number of vials in a column and the distance between each center.
Offsets	Allows alternate vial rows or columns to be offset. <b>Note:</b> Entering a positive value will shift even numbered rows to the right and negative values will shift even numbered rows to the left.
Vial Reference	Allows the user to select the way that the vial rows and columns are referenced, e.g. whether the rows are alphabetical or numerical.
Horizontal	Sets the horizontal axis of the plate as either alphabetic (ABC) or numeric (123), when using XY referencing. Default: numeric.
Vertical	Sets the vertical axis of the plate as either alphabetic (ABC) or numeric

Sets the vertical axis of the plate as either alphabetic (ABC) or numeric (123), when using XY referencing. Default: alphabetic.

<b>PlateGenerator</b>	
Plate	
Plate Name 24 Well 1500uL Plate	Vial Reference Horizontal O A B C O 1 2 3
Number Of Vials Vial Spacing 1/10 mm	Vertical © A B C © 1 2 3
Columns 6 198	Priority V Horizontal first
Offsets 1/10 mm	Plate Size         Top Left Vial Offset           ×         1265         1/10 mm           Y         845         1/10 mm
Row U Every 2nd Row Column 0 Every 2nd Column	Z         338         1/10 mm         Y         112         1/10 mm           Vial
Ready	NUM

Figure 2.62 CapLC Plate Generator

Referencing	This has three options
	• XY which references the vials A1, B1 etc.
	• Sequential Discontinuous which numbers the vials 1, 2, 3 across a row, left to right, and then starts the next row from the left again.
	• Sequential Continuous which numbers the vials 1, 2, 3 across a row, left to right, then continues number the next row, right to left etc.
	If the Waters CapLC autosampler is used with OpenLynx then the vial referencing must be set to either sequential continuous or sequential discontinuous.
Priority	Check the <b>Horizontal First</b> box if samples are to be acquired horizontally across the plate.
	If Referencing = X,Y and Horizontal First is checked, then the horizontal value be read first when referencing a vial $(1,A)$ . If Horizontal First is not selected, then the vertical value be read first when referencing a vial $(A,1)$ . Default: Horizontal First selected.
	If Referencing = Sequential Continuous or Discontinuous and Horizontal First is checked, then vials will be numbered horizontally. This will result in samples being acquired from row 1 then row 2. If Horizontal First is not checked, then vials will be numbered vertically. This will result in samples being acquired from column 1 then column 2 etc.
	Default: Horizontal First selected.
Plate Size	The size of the plate to its outside edges.
Top Left Vial Offset	The measurement to the center of the first vial from the top left corner of the plate.
Vial	The depth and diameter values are used for display only. They appear in the description for a single shot login on the OpenLynx Login screen.

### **Creating and Deleting Waters CapLC Plates**

To create a new plate, press the button. A new default plate is displayed, change the **Plate** 

Name, enter the appropriate values and press the save button or select Save Plate from the Plate menu. New plates are saved to the MassLynx Plates directory.

To copy a plate, page through the list of saved plates using the and toolbar buttons. The **Previous Plate** and **Next Plate** options on the **Plate** menu perform the same operation. When the required plate is displayed change the **Plate Name**, enter the appropriate values and press the save

button or select **Save Plate** from the **Plate** menu. New plates are saved to the MassLynx **Plates** directory.

To delete a plate select the plate, by typing the name in the **Plate Name** box or by paging through as above, and press the delete **vertex** button or choose **Delete Plate** from the **Plate** menu.

Note: All of the spacings and the vial section are stored in 0.1 mm units.

**Note:** When defining a custom plate for use with a multi-injector the plate is required to be compatible with the position of the 8 needles of the autosampler.

- The Plate must have eight columns.
- The position of the vials should allow all eight needles to enter a separate vial.
- There should be no odd or even offsets for any of the vial positions.

**Note:** If the Plate currently selected on the Sample Configuration page is changed here, then **Reset Injector** should be selected from the **LC** menu to reset communications.

#### **Vial Referencing Examples**

The following tables show four examples of vial referencing for a simplified  $4 \times 3$  vial plate.

Howizontal, 193	4	3	2	1	
Horizontai: 125	4.4	2.4	2.4	1 4	
Vertical: ABC	4,A	3,A	2,A	I,A	Α
Referencing: XY	4,B	3,B	2,B	1,B	B
Priority: Horizontal First Che	4,C	3,C	2,C	1,C	С

	1	2	3	4	Havirontale 122
Α	A,1	A,2	A,3	A,4	Vertical: ABC
В	B,1	В,2	В,3	B,4	Referencing: XY
С	C,1	C,2	C,3	С,4	Priority: Horizontal First NOT Checked

	1	2	3	4	
					Horizontal: N/A
Α	1	2	3	4	Vertical: N/A
В	5	6	7	8	Referencing: Sequential Discontinuous
С	9	10	11	12	Priority: Horizontal First Checked

	1	2	3	4	Horizontal N/A
Α	1	6	7	12	Vertical: N/A
В	2	5	8	11	Referencing: Sequential Continuous
С	3	4	9	10	Priority: Horizontal First NOT Checked

### Waters CapLC Plate Feeder

Plate Loader	×
Sample Plate	
Unloading Lift Unload Done	Loading Lift Load/Unload Done
	se

To display the Plate Loader dialog, select Plate Loader from the CapLC Tools menu.

Figure 2.63 CapLC Plate Feeder

The Plate Loader dialog is used when a plate needs to be changed.

## Waters CapLC PDA Detector

This page is used to set parameters specific to the UV detector, to access it select **View**, **WatersCapLC PDA Detector** or press the **L** toolbar button.

Start Wavelength	Enter the wavelength at which to start acquiring data.			
	Range (with Resolution set to 1.2): 190.0 nm to 800.0 nm. Range (at all other Resolution settings): 190.0 + (Resolution/2) to 800.0 – (Resolution/2). Default: 200 nm.			
End Wavelength	Enter the wavelength at which to stop acquiring data.			
	Range with Resolution set to 1.2: Start Wavelength to $800.0 \text{ nm}$ . Range at all other Resolution settings: Start Wavelength + Resolution to $800.0 \text{ nm}$ - Resolution/2.			
Resolution	Enter the number of diodes to be averaged together as a single spectral data point. To differentiate closely related spectra and obtain greater spectral resolution, use a small resolution number. Be aware, however, that a small resolution value generates more data points and therefore requires more disk space than a large resolution value. Find a resolution value just small enough to identify spectral features. Range: 1.2 to 24.0 nm in multiples of 1.2.			
Sampling Rate	Select the acquisition rate in spectra per second from the drop down list box. For good integration and quantitation, acquire 15 to 20 spectra across a peak.			

Wate	Waters CapLC PDA						
PDA	Channel 1 Channel 2						
	Data Acquisition	End Wavelength (nm) Sampling Rate	400				
	Resolution (nm) 1.2	(spectra/sec)					
	Instrument Control						
	Auto E <u>x</u> posure	St <u>o</u> p Time (mins)	5				
	🔽 Interpolate 656 nm	Filter Response	1				
	Exposure <u>T</u> ime (ms) 15	🔽 Save To <u>D</u> isk					

Figure 2.64 CapLC PDA Detector Configuration page

Auto Exposure	Check this box to enable the detector optics to calculate the optimum exposure time needed to recharge the diodes based on the lamp energy, the lamp spectrum and the selected wavelength range.
	Tip: Enable Auto Exposure for most routine analyses.
Interpolate	Check this box to instruct the detector to ignore the signal from the photodiode at 656 nm and to interpolate a value from the adjacent diodes. This prevents over-saturation at 656 nm (Balmer line for deuterium).
	If this box is not checked the detector reports the signal from the photodiode at 656 nm. Disable this parameter only if you are working with compounds that absorb in the 656 nm range.
	<b>Note:</b> If this parameter is unchecked, the deuterium lamp high emission line at 656 nm may cause spectral artifacts and autoexposure errors.
Exposure Time	Enter the length of time in milliseconds that the photodiodes are exposed to light before they are read. This parameter is not accessible if Auto Exposure is checked. Range: 11.00 to 500.00 ms.
Stop Time	Enter the time, in minutes after injection, when the PDA will stop scanning. <i>This value is independent of the instrument method run time</i> .
Filter Response	Enter the response time (in seconds) for filtering acquired data. The filter is an enhanced rolling average filter applied to absorbance data from the PDA detector before the data is sent to the MassLynx software. The filter reduces high-frequency noise across the entire wavelength range specified for the acquisition. High values decrease peak response. Range: 0 to 3.
Save to Disk	Check this box to save the Photo Diode Array data to the raw datafile. If this data is not required for further processing then uncheck the box, the data is not saved to disk thus reducing the size of the file.

### Waters CapLC Channel Detector Configuration Pages

The Channel 1 and Channel 2 pages contain the same information. Select the page relevant to the channel required, by clicking on the tab.

Waters CapLC PDA							
PDA Channel 1 Channel 2							
Analog Channel 1							
Output Mode Off	•	Filter Type Hamming 💌					
Wavelength (nm)	254	Filter Response (s)					
Ratio Denominator Wavelength (nm)	254	Offset (AU)					
Bandwidth (nm)	2.4	Threshold (AU) 0.1					
L							

Figure 2.65 CapLC Channel 1 Detector Configuration page

<b>Output Mode</b>	Select one of:			
	<b>Off</b> – no analog output signal.			
	<b>Absorbance</b> – Output represents absorbance at the wavelength specified by the Wavelength parameter (see below).			
	<b>Note: Ratio Denominator Wavelength</b> and <b>Threshold</b> parameters are not accessible when Absorbance mode is selected.			
	<b>Ratio</b> – Output represents the ratio of absorbances at two wavelengths. The numerator wavelength is specified by the Wavelength parameter, and the denominator wavelength is specified by the Ratio Denominator Wavelength parameter (see below).			
Wavelength	Enter the output wavelength. In Ratio mode, the absorbance at the Wavelength is used to calculate ratio in the formula:			
	Ratio = Absorbance at Wavelength/Absorbance at Ratio Denominator Wavelength			
	Wavelength must be within the wavelength range specified by the Start Wavelength and End Wavelength parameters on the PDA page.			
	Range when Resolution is set to 1.2: Start Wavelength to End Wavelength. Range at all other Resolution settings: Start Wavelength + (Bandwidth/2) to End Wavelength – (Bandwidth/2).			

Ratio Denominator Wavelength	Enter the denominator wavelength (in nanometers) for the analog output channel. Ratio Denominator Wavelength must be within the wavelength range specified by the Start Wavelength and End Wavelength parameters in the 996 PDA page.
Bandwidth	Enter the spectral bandwidth of the analog output channel. Range: 1.2 to 24.0 nm in multiples of 1.2.
Filter Type	Select the filter type (Hamming or Single Pole) from the drop down list box for use on the analog output channel. The Hamming filter is designed to create the same degree of peak-height degradation as the Single Pole filter for the same response time, but enhances filtering of high-frequency noise.
Filter Response	Enter the response time in seconds for the Filter Type specified above. Range: 0 to 5 seconds.
Offset	If required enter an offset to the analog output channel. Range: $-0.2$ to 2.0 AU.
Threshold	Enter a threshold above which the ratio (Wavelength / Ratio Denominator Wavelength) must be to be valid data. The range is $-0.1$ to 2.0 AU.
	<b>Note:</b> If no ratio is plotted (one or both channels are below the current Threshold), enter a lower Threshold value.

# Waters 515 and 1525 Pumps

The Waters 515 and 1525 Pump pages can be accessed by selecting **View**, **Waters 515 Pump** or **Waters 1525 Pump**, selecting Inlet from the short cut bar or by pressing the toolbar button.

### Waters 515/1525 Initial Conditions Page

Solvent A	Enter the name of the solvent that will be delivered through Pump A.		
Solvent B	Enter the name of the solvent that will be delivered through Pump B and enter the percentage of the solvent flow from Pump B.		
Flow (ml/min)	Enter the total flow rate for the solvent delivery system. Range: 1 to 10 ml/min.		
Run Time	Enter the time in minutes that the method will run, from the point of injection.		
	<b>Note:</b> If you are running a gradient or setting timed events, make sure the <b>Run Time</b> value is greater than, or equal to the greatest <b>Time</b> value, specified on the Gradient or Timed Events pages.		
Low Pressure	Enter the low pressure limit for the system. If the pressure falls below this limit, the solvent flow will stop and the LC status light will turn red.		
High Pressure	Enter the high pressure limit for the system. If the pressure exceeds this limit, the solvent flow will stop and the LC status light will turn red.		

Waters 1525	
Solvents	Pressures
Solvent A	Low P <u>r</u> essure (psi) 5000
Solvent B         0.0 %           Flow (ml/min)         1.000         Run Time (mins)         5.00	

Figure 2.66 Waters 1525 Initial Conditions page.

#### Waters 515

Waters 515	
Initial Conditions Gradient Initial Events Timed Eve	ents Method Type
Solvents	Auxiliary Pump Solvent
Solvent A	Name Column
Solvent B 0.0 %	
Flo <u>w</u> (ml/min) 1.000 Run <u>T</u> ime (mins) 5.00	Pressures Low P <u>r</u> essure (psi) 0.0 <u>H</u> igh Pressure (psi) 5000.

Figure 2.67 Waters 515 Initial Conditions page.

If a Waters 515 Pump was selected, the Auxiliary Pump Solvent options are displayed:

Flow (ml/min)Enter the total flow rate for the auxiliary solvent delivery system.<br/>Range: 1 to 10 ml/min.NameEnter the name of the solvent that will be delivered through the auxiliary pump.

#### Waters 515/1525 Gradient Page

Waters 1525					
Initial Conditions Gradient Initia	al Events   Time	ed Events   M	ethod Type		
Gradient Entry				<b>→문 </b> √문  ∑8	
Time( mins ) 0.00	Time	B%	Flow		
<u>B</u> % 0.00					
Flo <u>w</u> (ml/min) 1.000					
Cur <u>v</u> e 6					
	1				

Figure 2.68 Waters 515/1525 Gradient page

Use the Gradient Table to define conditions for a gradient run. For each row in the Gradient Table, you need to define the % composition of up to two solvents that are to be delivered at the desired flow rate for the specified **Time**.

**Note:** For an isocratic run, set the solvent percentages, run time and flow, on the Initial Conditions page. Do not add any rows to the Gradient Table.

To add a gradient, enter a time and percentage in the relevant boxes and press the toolbar button. **Note:** The first entry must have a time of 0.

To delete a single gradient, click with left mouse button on a time in the list and press the key toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed in the edit boxes to the left of the timetable, and can be altered as appropriate. Once changed press

to re-enter the values into the timetable. If, however, you modify the time value such that it

does not correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

Flow Enter the flow rate for the solvent delivery system.

**Curve** Enter the number of the gradient curve required. This sets the rate at which the solvent is to change to the new proportions and/or flow rates. See the Waters Operator's Guide for a list of values.

### Waters 515/1525 Initial Events Pages

The external events are triggered by four contact closures (relays) through output terminals, which are located at the back of the instrument.

#### Waters 515

Waters 515				
Initial Conditions Gradient	Initial Events	Timed Events Me	thod Type	
Belay 1 Off	<b>_</b>	Event 1	Off	
Relay 2 Off		Event 2	Off 💌	
		Event 3	Off	
		Event 4	Off 💌	

Figure 2.69 Water 515 Initial Events page

Relays 1 and 2	From the drop down list box, select <b>ON</b> or <b>Off</b> to activate or deactivate the relay.
Events 1 to 4	From the drop down list box, select <b>ON</b> or <b>Off</b> to activate or deactivate the event.

#### Waters 1525

Waters 1525				
Initial Conditions Gradient Initial Events Timed Events Method Type				
		0.4		
	Helay 1			
	Relay 2	Off 📃		
	Relay 3	Off 🗨		
	Relay 4	Off		

Figure 2.70 Waters 1525 Initial Events Page

**Relays 1 to 4** From the drop down list box, select **ON** or **Off** to activate or deactivate the relay.

### Waters 515/1525 Timed Events Page

Waters 515		
Initial Conditions   Gradient   Initial E	vents Timed Events Metho	od Type
<u>I</u> ime: <u>E</u> vent:		<b>+뮘 ≭</b> ≭
Kelay I	Time Event	Action
● <u>O</u> n © Off		
	J	

Figure 2.71 Waters 515/1525 Timed Events page

Use the Event Table to program up to 16 events (both external and internal). Events can be triggered more than once and multiple events can be triggered simultaneously.

Time	Enter the time (after injection) at which the event starts. Event rows are sorted automatically by time.
	<b>Note:</b> Different events can be programmed to occur at the same time. Range: 0.00 to 999.99 min.
Event	Select an Event or Relay from the drop down list box.
Switch States	<b>On</b> Turns on a contact closure that triggers an external or internal event. With this function, the contact closure remains closed until an Off function is sent.
	<b>Off</b> Turns off the contact closure for the event. With this function, the contact closure is broken.

#### **Event Table Operation**

To add an event, enter a time, event, action and value in the relevant boxes and press the  $\mathbf{1}$  toolbar button. Note the first entry must have a time of 0.

To delete a single event, click a time in the list and press the 📧 toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in the edit boxes, and can be altered as appropriate. Once changed press +1 to re-enter the values into the timetable. If, however, you modify the time value such that it does not correspond to any existing entry in the timetable pressing +1 will result in a new entry being created.

### Waters 515/1525 Method Type Page

Method Type	Specifies the type of method to create.
Normal Column Method	The Method Type used for standard injections. Ensure that the Method Type is set to <b>Normal</b> unless you are performing one of the <b>procedures</b> listed below.
Condition Method	Runs solvent through the column without injecting samples or running the Events table. Solvent is delivered using the gradient table specified in the Gradient page.
Equilibrate Method	Delivers solvents and maintains solvent parameters using the values defined on the Initial Conditions page.
Run Event Table	Check this box to run the table of timed events during the method. This option is only active if the <b>Normal</b> or <b>Column Condition Methods</b> are selected.

### Waters 515

Initial Conditions Gradient Initial Events Timed Events Method Type	
Method Type	
C Column Condition Method	
○ <u>E</u> quilibrate Method	

Figure 2.72 Waters 515/1525 Method Type page

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## **CE Instruments GC8000 Gas Chromatograph**

**Note.** CE Instruments are now only available on Transputer based instruments. Newer Non-Transputer based instruments no longer support these instruments.

On a CE Instruments GC8000, MassLynx can control the oven temperature, the injector zone temperatures, the valve times, the dump valve and 4 external event times.

#### To change GC Parameters

1. Choose **Set up Inlet** from the Acquisition Control Panel Instrument menu.

- or -

Double click on the picture of the GC on the Acquisition Control Panel to display the GC8000 inlet editor shown below.

- 2. Make any changes to the parameters. **Note:** The oven temperature ramp can be modified either by using the keyboard to enter times, temperatures and rates, or by dragging the small red handles on the graph with the mouse.
- 3. Save the method using either Save or Save As from the File menu.



Figure 3.1 GC8000 Inlet Editor

The time and temperature range of the oven temperature ramp can be controlled using the buttons displayed to the right of the ramp. Clicking on will increase the range shown on the time axis. Clicking on will increase the range shown on the temperature axis. Clicking on alters the display ranges so that the oven temperature display fills the graph.

A full description of all the parameters in this editor is given in the *GC8000 Series Instruction Manual*.

#### **Changing Valve Event Times**

Timed events such as Purge and Split times can be included in the GC method. These are programmed using the GC 8000 Valve Control editor shown below.

GC 8000 Valve Co	ntrol	×	C
Valve Times (Mins)		ок	
<u>P</u> urge	0.00	Cancel	
<u>S</u> plit	0.00		
Secondary <u>C</u> ool	0.00		

Figure 3.2 GC8000 Valve Editor

To display this dialog select **Valve Timetable** from the **Valves** menu. The events parameters are stored to disk when the GC parameters are saved, not when this dialog is closed so ensure that parameters are saved before starting an acquisition.

#### To control the GC8000 DPFC option

MassLynx can control the DPFC option on the GC8000 for Quattro II and Platform instruments.

1. Select **Configuration** from the GC8000 editor **DPFC** menu.

DPFC Configura	×	
Left Channel <u>R</u> ight Channel	Manual 💌 Manual 💌	OK Cancel

Figure 3.3 DPFC Configuration dialog

- 2. Configure the left and right channels as required. Each channel can be set to flow, pressure or off.
- 3. Select Left Channel or Right Channel from the GC8000 editor DPFC menu to load either the Flow Ramp or Pressure Ramp Editor depending how the channel is configured.

DPFC R	ght Chan	nel Contr	ol			×
	CFlow Ramp					
	Time (Mins)	Flow (ml/Min	Rate (ml/Min-2)		ল	Cancel
S <u>B</u>		1.0	-	15		
<u>s</u> s		1.0	-		١	
☑ 1	2.0	10.0	0.6	ml/Min	•	
☑ 2	2.0	15.0	10.0			
	2.0	20.0	10.0			
	2.0	25.0	10.0	0-1 Mins		
<u>□</u> <u>5</u>	2.0	30.0		0 5 10		

Figure 3.4 DPFC Flow Ramp Editor

DPFC Left	Channe	l Contra	d			×
	<sup>p</sup> ressure R	amp				
	Time (Mins)	Press (kPa)	Rate (kPa/Min)		53	Cancel
S <u>B</u>		3.0	-	15		
<u>s</u> s		3.0	-		٢	
₩1	2.0	10.0	0.6		•	
<u>₹</u> 2	2.0	15.0	10.0			
<u> </u>	2.0	20.0	10.0			
<u> </u>	2.0	25.0	10.0			
<u> </u>	2.0	30.0		0 2 4 6 8 10 12		

Figure 3.5 DPFC Pressure Ramp Editor

4. Make any changes required and press **OK** to exit and save changes.

# **CE Instruments AS800 Auto Injector**

The CE Instruments AS800 Auto Injector can be used with the CE Instruments GC8000 gas chromatograph. The autosampler is programmed from MassLynx using the A200S editor. It is programmed in exactly the same way as the A200S system described above.

A full description of all the parameters in this editor is given in the AS800 Autosampler Instruction Manual.

🔔 AS800 Setup - DEFAU	LT.ATS	_ 🗆 🗙
<u>F</u> ile <u>H</u> elp		
-Injector Control	Washes	
Sample ⊻olume (μl) [1	Before Injection	1
<u>A</u> ir Volume (μl)	0 After Inje <u>c</u> tion	1
Injection Delay (s)	0	
Pull-Out Delay (s)	0 Pull-Ups	
	<u>N</u> umber	1
	Vo <u>l</u> ume (μl)	0.0
	<u>D</u> elay (s)	1.0

Figure 3.6 AS800 Auto Injector Editor

# Chapter 4 Gilson Systems

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# **Gilson Autosamplers**

## Introduction

#### **Supported Models**

The Gilson Software can be used to control any of these models:

Gilson 215	Gilson 231XL
Gilson 232XL	Gilson 233XL
Gilson 222XL	Gilson Quad-Z 215

The Gilson 232XL and 233XL also require the Gilson 402 Dilutor. The Gilson 401C Dilutor is also supported although this has now been discontinued by Gilson.

The Gilson 215 has a dilutor built in, but it does require a Gilson 819 Valve Actuator.

#### Setting up

The first time MassLynx is run with a Gilson Autosampler, it needs to know which autosampler is being used. The following dialog is displayed.

GilsonDefaults		
Autosampler O <u>G</u> ilson 215	Dilutor O No Dilutor	O No Injector
O Gjison 231XL	O <u>4</u> 01 C	C Gilson <u>8</u> 19
O Gilson 232XL	C 402 Single Dilutor	Gilson 8 <u>4</u> 9
◯ Gil <u>s</u> on 233XL	C 402 Twin Dilutor (Tee)	🔿 Gilson 88 <u>9</u>
O Gils <u>o</u> n 222XL	C 402 Duel Dilutor	
Gilson Quad-Z 215     Gilson Quad-Quad-Quad-Quad-Quad-Quad-Quad-Quad-	€ 444 <u>Q</u> uad Dilutor	ОК

Figure 4.1 Gilson Defaults dialog

Select the installed Autosampler, Dilutor and Injector and press **OK**. **Note:** This need only be done the first time you use MassLynx or if the type of autosampler is changed.

Selecting a particular autosampler grays out unsupported combinations of modulus.

Valid combinations of modules are listed in the Table 4-1 below.

Autosampler	Dilutor	Injector
Gilson 215	No Dilutor	No Injector
		Gilson 819
		Gilson 889
Gilson 231XL or	No Dilutor	No Injector
Gilson 232XL or	401 C	
Gilson 233XL	402 Single Dilutor	
	402 Twin Dilutor	
	402 Duel Dilutor	
Gilson 222XL	No Dilutor	No Injector
Gilson Quad-Z 215	444 Quad Dilutor	Gilson 849
		Gilson 889

Table 4-1Valid Combinations of Gilson Modules

Once completed if a Gilson 215 or a Gilson Quad-Z 215 has been selected the following dialog is invoked:



Figure 4.2 Prompt dialog for Travel Height and Needle Height

Clicking Ok invokes the first property page of the Advanced Configuration dialog box described on 4-21. The travel and needle height values for the autosampler can be entered, although the defaults should be fine for most circumstances.

**Note: -** for the Quad-Z the travel height detected during the homing phase of the autosampler is used instead of the user-defined value if they are different.

## The Gilson Toolbar

The Gilson toolbar has two extra buttons on it, which are:

Prime the Dilutor.



T

Generate Custom Racks.

## **Gilson Configuration Pages**

These pages contain information that is used to configure the autosampler. To access them press

the to button, select Autosampler from the short cut bar or select View, Gilson AutoSampler.

## The Gilson Task List

This page is used to build up a set of tasks into a method that is then used to perform the injection. The available tasks are contained in the **Task** drop down list box and the parameters displayed will depend on which task is selected.

For the Quad-Z 215 all options relating to left/right syringes and injector ports are not applicable and are grayed out.

Gilson Autosampler         Task List       Dilutor Configuration       Sampler Configuration         Task:       Inject Partial Loop         Aspiration Flow Rate (ml/min)       1.50         Displacement Flow (ml/min)       0.30         Value Fluch Volume (ul)       0	Multi-Injector Parameters Task List →
<ul> <li>Left Syringe</li> <li>Eight Syringe</li> <li>Left Eort</li> <li>Right Port</li> </ul>	

Table 4-2 Task List page

#### Adding and Deleting Tasks (Gilson)

To add a task select it from the **Task** drop down list box, set the parameters and press the add **button**. The task will be added to the end of the list.

To delete a single task, select it from the **Task List** and press the delete button.

To clear all of the tasks in the Task List press the clear all 🔀 button.

#### Modifying Tasks (Gilson)

To modify a task select it from the **Task List**, change the required parameters and press the add **tell** button.

#### Moving and Copying Tasks (Gilson)

To move a task, select it with the mouse, hold the mouse button down and move the task to the required position.

To copy a task follow the procedure for moving tasks but hold the **CTRL** key down when moving the mouse.

## The Individual Tasks (Gilson)

This section contains tables listing the individual parameters for each task.

#### **Inject Partial Loop**

This takes in the required values to perform a partial loop injection. In this method the injection volume plus an additional flush volume is aspirated. The Injection volume is entered in the sample list. The flush volume is defined in the Gilson Dilutor Configuration Page.

Parameter	Description
Aspiration Flow Rate	The sample is drawn into the needle at this flow rate.
Displacement Flow	The sample is injected into the loop at this flow rate.
Valve Flush Volume.	This is the volume of solvent that is flushed through the valve after the injection. It is displaced at the Displacement flow.

Table 4-3 Inject partial loop parameters

Detailed below is a list of the steps that occur during the inject partial loop task.

#### Steps:

- 1. Move to vial
- 2. Aspirate air gap if required
- 3. Move Z arm down to vial depth
- 4. Aspirate injection volume + injection flush volume
- 5. Move Z arm back to travel height
- 6. Move to injection port
- 7. Move to injection depth
- 8. Switch valve to inject position
- 9. Dispense injection flush volume
- 10. Switch valve to load
- 11. Dispense injection volume
- 12. Switch valve to inject
- 13. Pulse output contact
- 14. Dispense air gap

15. Rinse valve if a flush volume has been specified

Note: The Left and right valve become enabled when using a Gilson 233XL.

#### **Inject Total Loop**

This is visually identical to inject partial to **inject partial loop**. The difference being it does not make use of a flush volume, i.e. only the sample volume is injected. This function is now implemented for all Gilson autosamplers.

#### **Rinse Injection Port**

This task is used to rinse the injection port with solvent from the reservoir.

Parameter	Description
Rinsing Volume	The volume of solvent that is rinsed through the needle.
Displacement Flow	The solvent is injected into the valve at this flow rate.

Table 4-4	Rinse	Injection	Port	parameters
-----------	-------	-----------	------	------------

Note: The Left and right valve become enabled when using a Gilson 233XL.

#### **Rinse Inside Needle**

This task is used to rinse the inside of the needle with solvent from the reservoir.

Parameter	Description
Rinsing Volume	This is the volume of solvent that is to be rinsed through the needle.
Displacement Flow	The solvent is rinsed through the needle at this flow rate.
Rinse Station	This is the rinse station at which you would like the rinse to take place. If this is set to auto then the nearest rinse station is chosen.

#### Table 4-5 Rinse Inside Needle parameters

#### **Rinse Outside Needle**

This task is used to rinse the outside of the needle with solvent from the reservoir.

Parameter	Description
Rinsing Volume	This is the volume of solvent that is to be rinsed through the needle.
Displacement Flow	The solvent is rinsed through the needle at this flow rate.
Depth	This is set to the depth that the needle should move to for rinsing.
Rinse Station	This is the rinse station at which you would like the rinse to take place. If this is set to auto then the nearest rinse station is chosen.

#### Table 4-6 Rinse Outside Needle parameters

Because this task dispenses the solvent through the inside of the needle it also acts as a rinse inside needle.

Note: Rinsing the Outside of the needle is not available on the Gilson215

#### **Set Electrical Contact**

📒 Untitled.g	jil - InletEditor		
<u>F</u> ile ⊻iew <u>I</u>	ools <u>L</u> C <u>G</u> ilson Sa	ampler <u>G</u> ilson Tools	<u>H</u> elp
0 🖻 日	🛎 <mark>.?</mark> 🚳 🎚	<u>k</u> 27 E	1 D T N ?
Task List	Dilutor Configuration	Sampler Configuration	n   Multi-Injector Parameters
Task:	Set Electrical Con	tact	Task List →문 +- ► ★ ★
	<u>C</u> ontact Number	1	
	State	● <u>O</u> pen ● <u>C</u> lose ● <u>P</u> ulse	
For Help, press	F1		Gilson Autosampler 11:36

Figure 4.3 Task List: Set Electrical Contact

This task is used when you would like to set one of the output contacts.

Note. Different autosamplers have different numbers of contacts

Parameter	Description
Contact Number	This is the number of the contact that you would like to set.
State	This is the state that you want to set. Open, Close or Pulse.

Figure 4.4 Set Electrical Contact parameters

#### Wait For Contact

This task is used when you need to wait for a contact state.

Parameter	Description
Contact Number	This is the number of the contact that you would like to set.
State	This is the state that you want to set. Open, Close or Pulse.

#### Wait For Time

This task is used if you need the machine to wait for a specified time.

Parameter	Description
Time	The time that you wish to wait for.

## **Gilson Dilutor Configuration Page**

This page is used to set parameters specific to the Dilutor (Table 4-6). As already mentioned (in The Gilson Task List page 4-6) when using a Quad-Z 215 there are no left/right syringe options, so there is just an option for **All Syringes**.

Task List     Dilutor Configuration     Sampler Configuration     Multi-Inject       Syringe Volumes	ctor Parameters Flows ( ml/min ) <u>B</u> eservoir Aspiration 2.00 <u>P</u> rime Displacement
Hight         All Syringes         Inlet Tubing volume (μl)         7         Sample Flush volume (μl)         10	2.00 ume (μl) 7 olume (μl) 10

Figure 4.7 Dilutor Configuration page

Syringe Volumes	The size of the currently installed Left and Right syringes.
Reservoir Aspiration Flow	This is the flow rate at which solvent is drawn from the reservoir (the default value is usually sufficient).
Prime Displacement Flow	This is the flow rate at which the solvent is displaced during the prime dilutor (the default value is usually sufficient).
Inlet Tubing volume	This is the volume of the tubing between the injection port and the rheodyne valve. It is the volume of air used to push the sample through into the injection loop.
Sample Flush volume	This is the amount of sample that is drawn with the injection valve. This amount is then injected before the valve switches to fill the tube between the valve and the injection port. Any excess will go to waste.
	<b>Note:</b> When using the Gilson 402 Dilutor, the Left and Right syringe radio buttons are enabled allowing you to use the left and right syringes. This is not yet available.

## **Gilson Sampler Configuration Page**

This page is used to set parameters specific to the Sampler. For the Quad-Z 215 the **Rinse Station** controls are grayed out.

Gilson Autosan	npler		
Task List Dilutor Configuration Sampler Configuration Multi-Injector Parameters			
Current Layout			Rinse Station
Code 205 Bed La	yout	-	
Code 205 Plate Code 205 Plate	Code 205 Plate Code 205 Plate	Code 205 Plate	Left Present
Code Code 205 205 Plate Plate	Code 205 Plate Code 205 Plate	Code 205 Plate	Image: Market Mar Market Market Ma Market Market Marke

Figure 4.8 Sampler Configuration page

Current Layout	Select the required layout from the drop down list box. A picture of the selected bed layout is displayed below the name.
Rinse Stations Present	Check the boxes if the rinse stations are present. Left and right are as you look at the machine.

Note: When using the Gilson 215 the right rinse station will be disabled.

## **Gilson Multi-Injector Parameters Page**

This page is used to set parameters specific to the Gilson Multi-Injector System. This is an autosampler device that injects up to 8 samples simultaneously. The Multi-Injector itself consists of a Gilson 215 autosampler in conjunction with a Gilson 889 multi-valve injector. The device has eight needles connected to the robot arm and a syringe for each needle. The geometry of the autosampler is designed so that the 8 needles will enter 8 separate wells of a microtitre plate. The 8 samples are picked up at the same time and then deposited into 8 separate injection loops. The valves connected to these loops can then be controlled individually so that each of the samples can be sent to the mass spectrometer at any specified times.

To enable these parameters the following configuration must be defined.

- 1. Select Advanced Configuration from the Gilson Sampler menu.
- 2. On the Hardware tab select Gilson 215 from the Sampler drop down list box.
- 3. On the Hardware tab select Gilson 889 from the Valve drop down list box.
- 4. On the **Hardware** tab select **All samples to same file** from the **Multi-Injector Mode** drop down list box.

- 5. On the Hardware tab check the Enable Inject Ahead box.
- 6. Press OK.

Gilson Autosampler	
Task List Dilutor Configuration Sampler Configuration Multi-Injector P	arameters
All Samples To Single File Parameters	
<u>S</u> can Time per Sample (secs): 0	
Delay(secs):	
Length(secs):	

Figure 4.9 Multi-Injector Parameters page

Scan Time per Sample	This defines the analysis time spent on each sample.
Delay	This is the time for the sample to travel from the injection port to the mass spectrometer.
Length	This is the amount (in seconds) of the chromatogram that is used to create the data files for each of the samples

### **Gilson Multi-Injector Processing**

A complete row of 8 samples are collected simultaneously and placed in the injection ports. The injection valve for the first sample is switched to inject and a contact closure is used to signal the mass spectrometer to initiate scanning. Each subsequent sample is then injected every T seconds, where T is the *Scan Time Per Sample* defined on the multi-injector parameters page. Once all 8 samples have been injected the next set of samples are loaded in the injection loops. The first of these samples is injected after a time T from the last sample of the previous row.

The scanning time defined in the Mass Spectrometer method should be long enough so that all the samples can be injected and scanned. This time has to be calculated by the user.

Data for all samples is written to one file, the BatchDataFile, see Figure 4.10. Each peak within this chromatogram relates to a separate sample.



The file name of the BatchDataFile is based either on the sample list name or on the OpenLynx Job ID. A prefix of BATCH\_ is added to this name so that it can be recognised as a BatchDataFile. E.g. for a sample list called *Test.SPL*, the name of the BatchDataFile will be *BATCH Test.RAW*.

Once the scanning for the BatchDataFile is complete, ChroSplit.exe is used to create individual data files for each sample in the batch. ChroSplit.exe should be defined in the Acquire Process column for the first sample in the sample list or on the OpenLynx Setup Acquisition Process page.

ChroSplit reads the BatchDataFile.RAW and splits it into individual files based on the values defined on the Multi-Injector Parameters page. It ignores the first part of the file that is the **Delay**. The rest of the file is split into Scan Time per Sample sections and the scans acquired from the start of each of these sections for the defined **Length** are copied into the individual data files.



Figure 4.11 Multi-Injector Batch File

Some of the scans between any two samples will contain some mixing of samples (e.g. Region B in Figure 4.11) or not contain data relating to either sample (e.g. Region A in Figure 4.11). The data in these regions is not required, therefore only data in Length region is copied.

Once all data files have been created by ChroSplit the acquisition will proceed with any processing defined in the Process column for the first sample in the batch. For all subsequent samples in the batch no data is acquired only the processes are initiated.

ChroSplit currently writes two data files per sample. The first has a re-normalized retention time so that the retention time of the first scan for each sample is set to 0. This allows analysis of the masses to proceed via loop injection, which simply searches for masses contained within the scan at a user-defined retention time. The other file written retains its original retention time and is used to assess the accuracy of the ChroSplit procedure. This second data file has the prefix "CHECK" added to the .RAW filename.



Figure 4.12 The results of using ChroSpilt.exe. Upper shows the Batch file, middle the renormalized files and lower the check files

#### **Problems with ChroSplit**

If ChroSplit fails to copy the BatchDataFile scans correctly (due to an incorrect delay being defined or experimental faults, e.g. a change in pumping speed) then the delay and length parameters can be changed and the BatchDataFile reprocessed.

To do this the values in the timefile.tfl need to be changed. This file contains the time each sample reaches the mass spectrometer and is stored in the BatchDataFile.RAW directory. Also written to this file are the sample number, sample location for each sample and the multi-injector length and delay parameters.

		[timefile]		
	Mas	terRawFile=Batch_	XXXXX	
		Delay=4.5		
		Length=15		
[Sam	ple] [Loc	ation] [Injection Ti	me] [Sample ID]	
1	"1,A"	0.195375	"Not used"	
2	"1,B"	26.472376	"Not used"	
3	"1,C"	46.591373	"Not used"	
4	"1,D"	66.700378	"Not used"	

Figure 4.13 Example timefile.tfl

- 1. Open the **timefile.tfl** using a text editor, change the **Delay** or **Length** as required and save the file.
- 2. Open the Sample List and change the Process for the first sample to ChroSplit.exe.
- 3. Press the **button to display the Start Sample List Run** dialog.

- 4. Ensure that only the **Auto Process Samples** box is checked, the **Run From Sample** = 1 and the **Run To Sample** = 1, then press **OK**.
- 5. This will split the BatchDataFile into the individual \*.RAW files.
- 6. On the Sample List change the Process back to the original *processname* and repeat step 3.
- 7. Ensure that only the **Auto Process Samples** box is checked, the **Run From Sample** = 1 and the **Run To Sample** = *last sample in the list*, then press **OK**.

The data file for each sample will be recreated overwriting the previous files.

See the OpenLynx Users Guide, Introduction chapter for details on importing OpenLynx Batch files into the Sample List.

## **Gilson Advanced Options**

#### **Gilson Tray Options**

To display the Tray Options dialog, select **Options** from the **Gilson Tools** menu on the Inlet Editor.

Options	×
Tray Options	]
	Colours
	Not selected vial
_	Background 📃 🗸
	Display Gridreference on tray Gridreference text
	OK Cancel Help

Figure 4.14 Tray Options dialog

Colours	Select the colour to display the <b>Selected vial</b> , <b>Non selected vial</b> and <b>Background</b> from the appropriate drop down list box.
Display Gridreference on tray	Check this box to display the grid reference on the tray
Gridreference text	From the drop down list box, select the colour in which you want the grid reference text to be displayed.

#### **Gilson Plate Generator**

-

To display the	Custom Rack	Generator dialog	, select Gilson	n Tools, Plate	Generator or p	ress the
膨 button.						

RackGenerator					_ 🗆 X
<u>Rack View Tools H</u> elp					
1 2 3 4 5 6 7 8 9 10 11 12 A 6 6 7 8 9 10 11 12 B 6 6 7 8 9 10 11 12	Rack Name			Origin Top Left Bottom Left	C Top Right C Bottom Right
C* E* * * * * * * * * * * * * *		Number Of Vials	Vial Spacing	Grid Reference Horizontal : C A C 1	B C 2 3
G* * * * * * * * * * * * * * *	Columns	12	90	Vertical :	B C 2 3
	Vial 1/10mm	Diameter 70	Depth 1065	Referencing: XY	
	Offsets			Priority : 🔲 Ho	orizontal first
	Column 0	Add offset to 1/10mm © Odd Add offset to 1/10mm © Odd	which columns ? O Even which raws ? O Even	Plate Size           X         1275           Y         855           1/10mm	Top Left Vial Offset × 135 <sub>V10mm</sub> Y 110 <sub>V10mm</sub>
Ready					Scale : 90%

Figure 4.15 Custom Rack Generator

Rack Name	The name of the plate that is currently being edited.
Origin	The corner of the rack that the vial grid referencing starts from.
Rows	The number of vials in a row and the distance between each center.
Columns	The number of vials in a column and the distance between each center.
Vial	Diameter does not affect any parameters apart from how the tray looks in the Rack Generator. Depth affects how deep the needle travels into each vial when sampling. Decreasing the depth value will make the needle travel down further into the vial.
	This control is very important. An incorrect setting could send the needle through the bed and bend it.
Grid Reference	Allows the user to select the way that the vial rows and columns are referenced, e.g. whether the rows are alphabetical or numerical.
Referencing	This has three options
	• XY which references the vials A1, B1 etc.
	• Sequential Discontinuous which numbers the vials 1, 2, 3 across a row, left to right, and then starts the next row from the left again.
	• Sequential Continuous which numbers the vials 1, 2, 3 across a row, left to right, then continues number the next row, right to left etc.
	If the Gilson autosampler is used with OpenLynx then the vial referencing must be set to either sequential continuous or sequential discontinuous.

Priority	Check the <b>Horizontal First</b> box if samples are to be acquired horizontally across the plate.
	If Referencing = X,Y, Horizontal = Letter, Vertical = Number and Horizontal Priority is checked, this will result in samples being acquired in the order A1, A2, A3. If the Horizontal Priority box is not checked samples will be acquired in the order 1A, 1B, 1C etc.
	If Referencing = sequential continuous or discontinuous and Horizontal Priority is checked, this will result in samples being acquired from row 1 then row 2. If the Horizontal Priority box is not checked samples will be acquired from column 1 then column 2 etc.
Offsets	Allows alternate vial rows or columns to be offset.
Plate Size	The size of the plate to its outside edges.
Top Left Vial Offset	The measurement to the center of the first vial from the top left corner of the plate.

#### **Creating and Deleting Plates (Gilson)**

To create a new rack, press the unit button. A new default rack is displayed, change the **Rack** 

Name, enter the appropriate values and press the save button or select Save Current Rack from the Rack menu. New racks are saved to the MassLynx Plates directory.

To copy a custom rack, page through the list of saved custom racks using the **I** and **I** toolbar buttons. The **Previous Rack** and **Next Rack** options on the **Rack** menu perform the same operation. When the required rack is displayed change the **Rack Name**, enter the appropriate

values and press the save button or select **Save Current Rack** from the **Rack** menu. New racks are saved to the MassLynx **Plates** directory.

To delete a custom rack select the rack to delete, by typing the name in the Rack Name box or by

paging through as above, and press the delete **button** or choose **Delete Current Rack** from the **Rack** menu.

Note: All of the spacings and the vial section are stored in 0.1mm units.

**Note:** When defining a custom plate for use with a multi-injector the plate is required to be compatible with the position of the 8 needles of the autosampler.

- The Plate must have eight columns.
- The position of the vials should allow all eight needles to enter a separate vial.
- There should be no odd or even offsets for any of the vial positions.

#### **Default Plate Settings (Gilson)**

Selecting **Default Settings for New Rack** from the **Tools** menu displays the **Default Settings** dialog. This dialog allows the default settings used when creating a new rack to be defined. Field descriptions are the same as above.

#### **Rotating and Scaling Plates (Gilson)**

Selecting Rotate Rack from the View menu will rotate a rack by 90 degrees. For example:





Selecting Scale Rack from the View menu displays the following dialog.

Percer	ntage - 10		 					 	 	 2	200
90		· · ·	 	ı ı	ļ	1.		 	 	 	- -
				Cl	ose		]	 	 	 	

Figure 4.1 Scale Rack dialog

Move the slider or enter a new value to change the size of the rack as displayed in the Plate Generator dialog.

### The Gilson Bed Layout Editor

To display the Bed Layout Editor dialog (Figure 4.16), select Gilson Tools, Bed Layout Editor.

#### To Create a New Bed Layout (Gilson)

- 1. Highlight a bed layout similar to the one you want to create and press the button to create a new layout. The layout appears in the **Bed Layouts** list as the same name with a 1 at the end, e.g. Code 201 Bed Layout1.
- To change the name of the layout, type the new name into the Bed Layouts text box and press the button. The name is updated in the Bed Layouts list box.

New bed layouts are saved to the MassLynx **Racks** directory.

#### To Delete a Bed Layout (Gilson)

Highlight a bed layout you wish to delete and press the button. A dialog box will ask you to confirm the deletion. Press the **OK** button to delete the bed layout.

Bed Layout	<b>X</b>
Bed Layouts: Code 201 Bed Layout Code 205 Bed Layout Code 207 Bed Layout Code 207 Bed Layout	Rows In Columns: 2 Rows Col. 1: 2 Col. 2: 2 Col. 3: 2 Col. 4: 2 Col. 5: 2
OK Cancel	Apply Help

Figure 4.16 Gilson Bed Layout Dialog

#### To Create a New Bed Layout (Gilson)

- 1. Highlight a bed layout similar to the one you want to create and press the button to create a new layout. The layout appears in the **Bed Layouts** list as the same name with a 1 at the end, e.g. Code 201 Bed Layout1.
- To change the name of the layout, type the new name into the Bed Layouts text box and press the button. The name is updated in the Bed Layouts list box.

New bed layouts are saved to the MassLynx Racks directory.

#### To Delete a Bed Layout (Gilson)

1. Highlight a bed layout you wish to delete and press the button. A dialog box will ask you to confirm the deletion. Press the **OK** button to delete the bed layout.

#### Modifying the Number of Rows and Columns (Gilson)

To change the number of rows in the current column, type the new number into the **Rows** box and press the **button**.

To append a new column, j	press the	button.
---------------------------	-----------	---------

To delete the current column press the button.

To insert a column, click on the column before which you want to insert and press the button. **Note:** The column inserted will have the same number of rows as the column highlighted.

## Modify Bed Layout (Gilson)

Bed Layout					×
Select/Crea	ite Bed Layo	out Modify	Bed Layout		
Code 201 B	Bed Layout				
Code 201 Plate					
Code 201 Plate					
ОК	Ca	ancel	Apply	Help	

If the plate position or type needs changing, select the Modify Bed Layout tab.

Figure 4.17 Modify Bed Layout dialog

Click on one of the code plates to display the Plate Position and Type dialog.

Plate Position and Type	×
Plates: Code 201 Plate Code 205 Plate Code 207 Plate	OK Cancel
Rotated: Top Left X from previous top left or home:	123
Top Left Y from previous top left or home:	498
🔽 OpenLynx plate login	

Figure 4.18 Plate Position and Type dialog

This dialog allows you to select a new plate from a list of possible options, and change its actual position on the bed. Measurements for plate positions are always taken from the top left corner of each plate. The X value is the measurement from the currently selected plate to the plate immediately to the left. The Y value is the measurement from the currently selected plate to the plate to the plate immediately above. If there is no plate, to the left or above, then measurements are taken from the Home position, which is where the needle sits when not in use.

Rotated

Check this box if the plate is rotated.

OpenLynx plate login If this box is checked and Use current MassLynx autosampler bed layout is checked in the OpenLynx Manager program, then the plate at this position can only be used for plate login on the OpenLynx Login program.

## Adjusting The Arm Height on a Gilson 215 / Quad-Z 215

The first time the Gilson software configuration pages are accessed the following dialog will be displayed. Type in the **Needle Height** that you have set the Gilson to and press **OK**.

Wanced Co	nfiguration Positions Communications		3
	Model	ID	
Sampler	Gilson 215	• 22	
Dilutor	No External Dilutor	<b>v</b> 22	
Valve	Gilson 819	• 29	
Multi-Inje	ector Mode Single Injection	1	
	_		
I <u>E</u> na	ble Inject Ahead 🛛 🗌 Inje	ctor and Collect	tor
<u>E</u> na	ble Inject Ahead	ctor and Collect	tor
	ble Inject Ahead 🛛 🔄 Inje	Ctor and Collect	tor
	ble Inject Ahead I Inje Initial Contacts Pulse Width (1/10 s )	Ctor and Collect	tor
	ble Inject Ahead I Inje Initial Contacts Pulse Width (1/10 s ) Travel Height (1/10 mm )	111111111 10 1250	tor
	ble Inject Ahead I Inje Initial Contacts Pulse Width (1/10 s ) Travel Height (1/10 mm ) Needle Height (1/10 mm )	111111111 10 1250	tor
	ble Inject Ahead I Inje Initial Contacts Pulse Width (1/10 s ) Travel Height (1/10 mm ) Needle Height (1/10 mm ) Inject Contact <u>1</u>	111111111 10 1250 1250	tor
	ble Inject Ahead I Inje Initial Contacts Pulse Width (1/10 s ) Travel Height (1/10 mm ) Needle Height (1/10 mm ) Inject Contact <u>1</u> Inject Contact <u>2</u>	111111111 10 1250 1250 1250 2	tor

Figure 4.19 Injection Parameters dialog

For the Quad-Z 215 autosampler the Multi-Injector mode is always set to "Single Injection" and grayed out, and the "Enable Inject Ahead", "Injector and Collector", "Inject Contact 1" and "Inject Contact 2" are always grayed out.

## **Other Advanced Options (Gilson)**

The advanced options dialogs can be accessed by selecting **Gilson Sampler**, **Advanced Configuration**.

The parameters on these pages will be set up at installation and should not need changing.

#### Hardware Page

Advanced Co	onfiguration		×
Hardware	Positions Communications		_
	Model	_ID	
Sampler	Gilson Quad-Z 215	• 22	
Dilutor	Gilson 444 QuadDilutor	• 22	
Valve	Gilson 849	• 29	
Multi-Inje	ector Mode Single Injection		
🗖 <u>E</u> na	able Inject Ahead 🛛 🗌 Inje	ctor and Collector	
	Initial Contacts	0000	
	Pulse Width (1/10 s )	10	
	Travel Height (1/10 mm )	1250	
	Needle Height (1/10 mm)	1250	
	Inject Contact <u>1</u>	1	
	Inject Contact 2	2	
		. 1	
	OK Ca	ancel Help	

Figure 4.20 Hardware Configuration Dialog

This page defines the type of Gilson AutoSampler, Dilutor and Valve used. It also shows the state of the initial electrical contacts to the AutoSampler and pump, the pulse width and needle parameters.

### **Positions Page**

Advanced Configuration	×
Hardware Positions Communications	
Origin The X origin is taken as the furthest left edge of the white support block around needle 1 at home. The Y origin is taken from the position of needle 1 itself. Injector (0.1 mm) Position of the furthest RIGHT port. This port should be	
Connected to injection valve 1. X 4002 Depth 425 Y 30 Width between ports 90	
Rinse Station ( 0.1 mm ) Position of the furthest LEFT port	
× 832 Depth 500	
Y 37 Width between ports 90	
OK Cancel Help	,

Figure 4.21 Positions Page (Quad-Z 215)

Advanced Configuration	×
Hardware Positions Communications	
Injector(s) ( 0.1 mm ) Left X 2470 Y 117 Z 586	
Right X 2020 Y 117 Z 586	
Rinse Station(s) (0.1 mm) Left Right	
Inside 0 Inside 3930	
Middle 70 Middle 3860	
Outside 140 Outside 3790	
Y - Offset 0 Y - Offset 0	
Depth 400 Depth 400	
OK Cancel He	elp

Figure 4.22Positions Page

The **Positions** page is configured differently, depending on which autosampler has been selected on the **Hardware** page. If the Quad-Z 215 is selected the **Positions** page is configured as in Figure 4.21 for any other autosampler it is configured as in Figure 4.22. This page defines the integer position from the Home position of the needle, i.e. the position of the needle when not in use. It also defines the distances the needle needs to travel to the rinse stations.

#### **Communications Page**

dvanced Configuration		×
Hardware Positions Commun	nications	
		_
Comm Port COM1	Baud Rate O 9600	
Parity 2	Binary Mode 1	
Stop Bits 2	RTS Control 3	
Byte Size 8	End of Data 26	
Timeout 500	Abort on Error	
Enable Parity Checking	Enable Null Stripping	
	7	
OK	Cancel He	lp

Figure 4.23 Communications Page

This page defines the serial line communication between the Gilson and the PC.

# **Gilson Pump**

The Gilson Pump pages can be accessed by selecting **View**, **Gilson Pump**, selecting Inlet from the short cut bar or by pressing the toolbar button.

## **Gilson Solvents and Flows Page**

metable   Events T	imetable   Pres	sures   Mixer	
Solvent Name	Refill Time (ms)	Compress- ability (Mbar) 46	Inlet Pres- sure (bar)
	- Flow Poto -		
1.00	Flow Rate (r	ml/min)	1.00
	metable Events T Solvent Name Solvent A	metable Events Timetable Pres Solvent Name Refill Time (ms) Solvent A 125 Flow Rate 1.00 Flow Rate (n	metable       Events Timetable       Pressures       Mixer         Solvent Name       Refill       Compress- Time (ms)       ability (Mbar)         Solvent A       125       46         125       46       46         Flow Rate         1.00       Elow Rate (ml/min)

Figure 4.24 Solvents and Flows page

Solvents	Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must not exceed 100%.
Pump A	This is the remainder percentage after the solvent percentages have been set for the other pumps.
Pump B, C, D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Solvent Name	Type in the solvent name.
Refill Time	This is the time required for the piston return stroke. Normally it is set to the lowest value (125ms). If cavitation or degassing occurs, then a higher value must be used. The minimum value is 125ms and the maximum 1000ms.
Compressibility	This is used to calculate a flow rate compensation for the compressibility of the solvent. See the Gilson User Guide for suitable values.
Run Time	This is the length of time, in minutes, the pump should run for
Flow Rate	This is the total flow rate of the solvent channels according to how you have configured the instrument.

## **Gilson Gradient Timetable Page**

### Gilson LC Solvents and Flows Gradient Timetable Events Timetable Pressures Mixer Gradient Table ₊₽ 0 Time (mins) D Δ B Flov Time 100 Pump A 2 0 Elow (ml/min)

#### Figure 4.25 Gradient Timetable page

This page allows a gradient to be entered and edited. To operate in isocratic mode ensure that the timetable is empty.

To add a gradient, type in a time, the required percentages and the flow rate, in the relevant boxes and press the +2 toolbar button. Note: The first entry must have a time of 0.

To delete a single gradient click a time in the list and press the 📧 toolbar button.

To delete all entries press the *integrate* toolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does

not correspond to any existing entry in the timetable, pressing + will result in a new entry being created.

## **Gilson Events Timetable Page**

Gilson LC		
Solvents and Flows Gradient Timet	table Events Timetable Pro	essures Mixer
<u>_i</u> ime: <u>E</u> vent: 0 <b>▼</b> Contact 1	Time Event	Action
C <u>D</u> n		
© <u>P</u> ulse		

#### Figure 4.26 Events Timetable page

To add an event, enter a time, select an event from drop down box and press the toolbar button.

To delete a single event, click a time in the list and press the 📧 toolbar button.

To delete all entries press the 🔀 toolbar button.

To modify an event, select the required entry in the timetable. The values will then be displayed in

the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does not

correspond to any existing entry in the timetable, pressing will result in a new entry being created.

## **Gilson Pressures Page**

Gilson LC	
Solvents and Flows Gradient Timetable Events Timetable Pressures Mixer	
Pressure Pressure Limits          Image: Manometric Module       Minimum (Bar)       0         Maximum (Bar)       35	

Figure 4.27 Pressures page

To set pressure limits check the **Manometric Module** box and enter a **Minimum** and **Maximum** pressure.

The maximum pressure limit is determined by the smallest pump head size.

## **Gilson Mixer Page**

This page allows the mixing parameters to be changed for Gilson321/322 pumps. It will configure itself depending on the pumps selected in the Pump Configuration.

Gilson LC	
Solvents and Flows Gradient Timetable Ev	rents Timetable   Pressures   Mixer
Pump B's mixer	Selcted pump's mixing options Use mixer Mixer volume (ml) (For record purposes only) Mixer Mode:  HPM LPM

Figure 4.28 Mixer page

## **Pump Configuration**

Pump Configur	ation				×
Pump					
- Pump Confid	uration				
r amp coring	Pump Type	Mode	Head Position	Pump Head	GSIOCID
C Pump A	Gilson322 💌	SINGLE 💌	Right head	H2 💌	1
C Pump B	Gilson322 💌	SINGLE 💌	Right head	H1 💌	0
• Pump C	Gilson322 💌	SINGLE 💌	Left head	H2 💌	
C Pump D	Gilson305 Gilson306 Gilson207	SINGLE 💌	N/A	<b>_</b>	0
	Gilson307 Gilson321				
	Gilson322		ОК	Cancel	Help

To change the number of pumps used select **Pump Configuration** from the **Gilson Pump** menu.

#### Figure 4.29 Pump Configuration dialog

Select the number of pumps, the pump type, pump head and the GSIOC ID (Gilson Serial Input Output Channel) required.

# **Gilson UV Detector**

The Gilson UV Detector page can be accessed by selecting **View**, **Gilson UV Detector**, selecting Detector from the short cut bar or by pressing the toolbar button. A single page of three editable parameters is displayed.

Gilson LC
UV Detector Wavelength (nm) Sensitivity (AU) D.001 Peak Width GSIOC ID ID for the UV Detector is fixed at 16

Figure 4.30 The Gilson UV Detector page

Wav	elength (nm)	Enter the wavelength for monitoring
Sens	itivity (AU)	Enter the required sensitivity of the output signal.
Peak	x Width	Determines the rate at which the data is acquired. There are approximately eight spectra per peak so a peak width of 0.1 minutes means eight spectra will be acquired every 6 seconds.

# **Chapter 5 Agilent Systems**

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# Hewlett Packard 5890 Gas Chromatograph

Both the HP5890 Series I and Series II can be controlled by MassLynx. For the Series II instrument it is necessary to configure the GC to respond to Series I commands by setting a jumper in the GC. A Micromass installation engineer will have already done this. Before starting to use the GC the software must be configured to reflect the GC equipment in use e.g. the number of injectors and detectors etc.

Note: The HP 5890 has to be selected on installation.

#### To change GC Parameters

1. Choose Configue, Select Interface, GC (TP RS232) from the Acquisition Control Panel.

or

Double click on the picture of the GC on the Acquisition Control Panel to bring up the HP5890 inlet editor shown below.

- 2. Make any changes to the parameters. **Note:** The oven temperature ramp can be modified either by using the keyboard to type in times, temperatures and rates, or by dragging the small red handles on the graph itself using the mouse.
- 3. Save the method using either Save or Save As from the File menu.

E HP5890 - DEFAULT.GCC					
<u>F</u> ile <u>E</u> d	lit <u>H</u> elp				
_ <sup>Oven</sup>	Temperatu	re			
♥ [] ♥ 2 ■ 3 ■ 4 ♥ Eg	Time (mins) 1.00 0.00 0.00 0.00	Temp (°C) 60 240 0	Rate (°C/Min) 15.00 10.00	240 •C •C •C •C •C •C •C •C •C •C •C •C •C	× ↑
	ryogenic	<u>∏</u> <u>D</u> um	p Valve		

Figure 5.1 HP5890 Editor

The time and temperature range of the oven temperature ramp can be controlled using the buttons that appear to the right of the ramp. Clicking on will increase the range shown on the time axis, clicking on will increase the range shown on the temperature axis. Clicking on alters the display ranges so that the oven temperature display fills the graph.

A full description of all the parameters in this editor is given in the *HP5890 Gas Chromatograph Reference Manual.* 

#### To Change GC Configuration (Hewlett Packard 5890 Gas Chromatograph)

- 1. Choose **Configuration** from the HP5890 Edit menu to display the configuration editor shown below.
- 2. Make any changes to the parameters.

3. Press **OK**. The parameters will be saved with the GC method when either **Save** or **Save As** is selected from the HP5890 File menu. Buttons in the HP5890 Edit menu that are not appropriate for the selected configuration will be grayed out.

Configuration			×
Injectors Injector <u>A</u> Injector <u>B</u>	Detectors Detector A Detector B	Signals <u>Sig</u> nal A Signal B	OK Cancel
Max oven temp 350			

Figure 5.2 HP5890 Configuration Editor

The main HP5890 editor is used to set up the GC oven temperature program and to control the dump valve and cryogenic cooling options if fitted.

# Hewlett Packard 7673A Auto Injector

The HP 7673A auto injector can only be used with the HP 5890 gas chromatograph.

🛃 HP7673 Setup - DEFAULT.#	ATS	_ 🗆 ×
<u>F</u> ile <u>H</u> elp		
Injector <u>C</u> ontrol	- Washes	
Sample⊻olume (µl) <b>1.0</b>	Sample <u>C</u> leans	1
Sample Viscosity (µl) 1.0	Solvent <u>A</u> Post-washes	0
Number of Pumps	Solvent <u>B</u> Post-washes	0
<u>O</u> n-Column Injection		

Figure 5.3 HP7673A Auto Injector Editor

**Note:** This dialog only applies if the Dice option is selected at setup, if the Dice option is not selected a "No parameters to set" message is displayed.

#### To Change Autosampler parameters (Hewlett Packard 7673A Auto Injector)

1. Choose Set up Auto Injector from the Acquisition Control Panel Instrument menu

or

Double click on the picture of the auto injector on the Acquisition Control Panel to display the HP7673A editor shown above.

- 2. Make any changes to the parameters.
- 3. Save the method using either Save or Save As from the File menu.

A full description of all the parameters in this editor is given in the *HP7673A Automatic Sampler Operating Manual.* 

An autosampler will usually be used with the multiple sample acquisition page where further information such as bottle number will be entered. Starting an acquisition with an autosampler will be covered in the next section

# **Hewlett Packard HPLC Systems**

The HP1050, HP1090 and HP1100 HPLC systems can be controlled from MassLynx. All three photo diode array (PDA) and UV detectors are supported.

The software can be used, to control the pump during instrument tuning, or acquisition and can be used to provide multi-sample acquisitions. Both isocratic and gradient modes of operation are supported.

The HP1050 is described in the examples below. Differences for the HP1090 and HP1100 are also described. On installing the HP1100 the program will automatically detect whether the G1367A Well Pate Autosampler or one of the older HP 1100 autosamplers (G1313 or G1329 is installed and will configure the Inlet Editor accordingly. For a full description of the HP1100 G1367A, see page 5-12.

## Hewlett Packard Sampler Configuration Page

Select View, HP1050 AutoSampler, HP1090 AutoSampler or HP1100, Autosampler from the short cut bar or press the toolbar button.

HP1050 Autosampler				
Sampler Initial Conditions Ex Injection Parameters - Injection Volume (j Draw Speed (µl/m Eject Speed (µl/m Draw Position (mm <u>S</u> top Time (mins)	I)     [1.0]       I)     [1.0]       n)     200       n)     200       Vial Size     100       0     0.00       Vial Number     1			

Figure 5.4 HP1050 Sampler Initial Conditions Window

Injection Volume	This is the volume in microlitres to inject.	
	<b>Note:</b> If you are running from the Sample List the injection volume in the sample list entry overrides the setting defined here.	
Draw Speed	This determines the rate in microlitres per minute at which sample is extracted into the autosampler needle. This should be set according to the viscosity of your sample.	

Eject Speed	This is the speed in microlitres per minute at which sample is ejected from the needle on injection. Again set this according to the viscosity of the sample. Consult your HP documentation for further information.
	<b>Note:</b> This facility is only available with the HP1050 and HP1100 and will not be visible on an HP1090 system.
Draw Position	This is an offset value in mm from position 0 and determines how far the needle is inserted into your sample. Consult your HP documentation for further information. Again this facility is only available with the HP1050 and HP1100 and will not be visible on an HP1090 system.
Stop Time	This value is set in minutes to be the time that the autosampler method will run after injection. This does not apply to the HP1090 since this has an in-built autosampler.
External Vial Tray	If an external vial tray is used check this box.
Vial number	The vial to inject from.
	<b>Note:</b> If a multisample acquisition is being run from the MassLynx Sample List, the Bottle # entry in the sample list overrides the value defined in the Vial Number box.
Syringe Size	Set this to the size of syringe fitted on the HP1090 LC System. This parameter applies to the HP1090 only and will not be visible in a HP1050 or HP1100 system.
Thermostat On	If the autosampler is fitted with a sample heater then this box will be enabled. Check it to use the sample heater. This parameter applies to the HP1100 only and will not be visible in a HP1050 or HP1090 system.
Sample Temperature	Enter the temperature to heat the sample to. This parameter applies to the HP1100 only and will not be visible in a HP1050 or HP1090 system.

## Hewlett Packard Sampler External Events Page

External events allow control of the external contacts found on the HP1050 and HP1090 LC systems. In addition HP1090 column switching can also be controlled. For the HP1100 a separate contact board must be installed in the pump in order to use this functionality.
HP1050 Autosample	r al Events			
Sampler Initial Conditions Extern	Event Table Time Initial 0.00 0.10	Event Contact 1 Contact 1 Contact 1	→ 문 🔀 🔀 State Off Off Off	

Figure 5.5 HP1050 AutoSampler External Events page

- TimeThe time in minutes at which the contact event should occur.EventThe contact event to be performed.
- -
- State The state determines whether the contact is to be opened or closed.

#### Hewlett Packard Pump Initial Conditions Page

Select the View,HP1050 Pump, HP1090 Pump or HP1100 Pump, Inlet from the short cut bar or press the toolbar button.

HP1050 LC	
Initial Conditions Gradient Extern Solvents Pump A 100.0 %	Pressures Low Pressure (bar)
I         Pump B         0.00         ≈           I         Pump C         0.00         ≈           I         Pump D         0.00         ≈	High Pressure (bar) 400 Column Heater Column Temperature (°C) 10.00 Timings
Flo <u>w</u> (ml/min) 1.000	Stop Time (mins) 5.00

Figure 5.6 HP1050 Pump Initial Conditions page

Solvents Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together

must not exceed 100%.

Pump A	This is the remainder percentage after the solvent percentages have been set for the other pumps.
Pump B, C, D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Flow	This is the total flow rate of the solvent channels according to how you have configured the instrument.
Pressures	These set the upper and lower limits of the pressure within the solvent delivery system (SDS) if the pressure falls outside of this range the SDS switches off.
Column Heater	If the instrument has an oven present then the column temperature can be set to a specified temperature in degrees centigrade. Check the Column Temperature box and enter a temperature. If the software has been configured to operate without a column oven then these boxes will be grayed out. <b>Note:</b> For the HP1100 a temperature should be entered in both the <b>Left</b> and <b>Right</b> boxes.
Stop Time	This value is set to the time in minutes that the method will run from the point of injection.

### Hewlett Packard Pump Gradient Timetable Page

HP1050 LC	xternal Events	
Gradient Entry	Gradient Table	्मार्ग्वा
<u>T</u> ime(mins) 0.00		
<u>B</u> %	Time B% C	X D% Flow Pressure
<u>C</u> % 0.00		
<u>D</u> % 0.00		
Flo <u>w</u> (ml/min) 1.000		
Pressure (bar) 400		

Figure 5.7 HP1050 Pump Gradient page

This page allows a gradient to be entered and edited. To operate in isocratic mode ensure the timetable is empty.

To add a gradient, type in a time and percentage in the relevant boxes and press the +1 toolbar button. Note: The first entry must have a time of 0.

To delete a single gradient, click a time in the list and press the 📧 toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does

not correspond to any existing entry in the timetable pressing 📲 will result in a new entry being created.

The gradient parameters that you can set are as below. **Note:** The number of solvent percentages, which appear in the dialog, depends on which type of gradient was selected in the LC Configuration Window.

Time	The time at which you wish the following parameters to be attained during a method run.
%B	The percentage of solvent B you wish to attain at the given time.
%C	The percentage of solvent C you wish to attain at the given time.
%D	The percentage of solvent D you wish to attain at the given time.
Flow	The required flow in ml/min that you wish to attain at the given time.
Pressure	This is only available on the HP1050 and HP1100 and allows the limiting high pressure (in bars) to be reset at the given time.

### Hewlett Packard Pump External Events Page

External events allow control of the external contacts found on the HP1050 and HP1090 LC systems. In addition HP1090 column switching can also be controlled. For the HP1100 you must have the separate contact board installed in your pump in order to use this functionality.

HP1050 LC				
Initial Conditions Gradient Exte	ernal Events			
Event	Event Table			
<u>T</u> ime( mins ) 0.10	Time	Event	State	
Event Type	Initial 0.00 0.10	Contact 1 Contact 1 Contact 1	Off Off Off	
	,			

Figure 5.8 HP1050 Pump External Events page

The contacts can be set to operate under timed control during a method run, as well as having their initial states set. The contacts will be in the initial state before a method run and will return to this state after a method has completed.

To add events see the earlier section Hewlett Packard Sampler External Events Page5-8.

# Hewlett Packard 1100 G1367A Well Plate Autosampler

The Well Plate Autosampler has a number of additional features compared to older HP1100 autosamplers. These differences take the form of three new property pages, an additional menu and two new dialog boxes.

### **Sampler Initial Conditions**

This page covers the basic variables for an injection and is common to all HP 1100 autosamplers all parameters are described in the" Hewlett Packard Sampler Configuration Page" section on page 5-7.

HP1100 Autosampler				
Sampler Initial Conditions Injector Parameters Needle Wash Configuration				
Injection Parameters	1.0	Vial Tray Size 100		
Injection volume (µi)		Viai 1 jay 312e 100		
<u>D</u> raw Speed (μl/min)	200	Vial Number 1,1:1		
<u>E</u> ject Speed (μl/min)	200	Sample		
Draw Position (mm)	0.00	🗖 Ihermostat On		
<u>S</u> top Time (mins)	5.00	Sample Temperature(°C)		

Figure 5.9 The HP 1100 Well Plate Autosampler: - Sampler Initial Conditions page

The maximum value for the Injection Volume depends on the values of the Seat Capillary Volume and the Syringe Volume (Injector Parameters below).

If the Seat Capillary Volume is greater than the Syringe Volume then the maximum Injection Volume is the Seat Capillary Volume plus the Syringe Volume (the autosampler supports multiple draws in this situation). Otherwise the maximum Injection Volume, is either half of the Loop Capillary volume or the Syringe Volume, whichever is smaller.

#### **Injector Parameters**

HP1100 Autosampler	
Sampler Initial Conditions Injector Parameters	Needle Wash Configuration
Injection Procedure	High Throughput
Standard Injection	Automatic Delay Volume Reduction
C Injection With Needle Wash	Enable Overlapped Injection
C Injector Program Edit	when sample is flushed
	C after 2.00 mins
Volumes	
Syringe Volume (μl)  100.0	- Timinas
Seat Capillary Volume (µl) 2.3	Equilibration Time (s) 2.0
Loop Capillary Volume (µl) 200.0	Sample Flush Out Factor 5.00

Figure 5.10 The HP 1100 Well Plate Autosampler: - Injection Parameters

InjectionSelect from Standard Procedure, Injection With Needle Wash orProcedureInjector Program.

**Volumes** Enter the **Syringe, Seat Capillary** and **Loop Capillary Volumes**. Only valid combinations of syringe, seat capillary and loop capillary volumes are allowed, which in turn specify min/max injection and draw speeds as shown below:

Product	Syringe (µl)	seat capillary (µl)	loop capillary (µl)	min. speed µl/min	max. draw speed µl/min	max. eject speed µl/min
µMonawell (standard)	8	0.3	8	1	20	100
µMonawell (optional)	40	0.3	80	1	250	250
Monawell (standard)	100	2.3	200	10	1000	1000
Monawell (optional)	100	400	200	10	1000	1000
Monawell (optional)	100	1400	200	10	1000	1000
Monawell (optional)	100	5000	200	10	1000	1000
Monawell (optional)	900	2.3	1800	90	1000	1000
Monawell (optional)	900	400	1800	90	1000	1000

High Throughput Enable the Automatic Delay Volume Reduction by checking the box.

**Note:** -The other options in this section box are currently not supported by MassLynx.

Timings

Enter an Equilibration Time and a Sample Flush Out Factor

### **Needle Wash**

Figure 5.11 The HP 1100 Well Plate Autosampler: - Needle Wash page

Flush Port	Select the radio button to flush the port.
In Vial	Check the <b>In Vial</b> radio button to enable the In Vial edit box. Enter the number of the vial to wash.
Wash Time	Enter the time for the wash.
Repeat	Enter the number of time to repeat the wash.

# Configuration

HP1100 Autosampler
Sampler Initial Conditions Injector Parameters Needle Wash Configuration
Configuration Tray Type 100 Tray Template
100 Vials Plate

Figure 5.12 The HP 1100 Well Plate Autosampler: - Configuration page

This page selects and displays a schematic of the current bed layout for use with the method. To change the layout select a new one from the **Tray Type** drop down list.

#### **Bed and Plate Layout**

One extra menu has been added for the Well Plate Autosampler - Beds and Plates.

Select **Beds and Plates, Bed Layout** to invoke the **Bed Layout** dialog. This dialog allows a given bed layout and appropriate plates be selected and saved.

Bed Layout	×
Bed Layout Edit Plates on Layout	
Bed Layouts:	
100 Vial Bed2	Rows In Columns:
100 Vial Bed1	Col. 1: 1
100 Vial Bed2 Two Vial Trays1	
Two Well Plates1	
ОК	Cancel Help

Figure 5.13 The HP 1100 Well Plate Autosampler: - Bed Layout dialog

#### **Bed Layout Page**

This displays all the current bed layouts and allows them to be selected, which can then be edited on the **Edit Plates on Layout page.** 



#### Edit Plates on Layout Page

This page allows individual plates to be selected on the bed layout, by clicking on the schematic.



Figure 5.14 The HP 1100 Well Plate Autosampler: - Edit Plates on Layout

Clicking on a plate will result in a Plate Select dialog box, containing the various plates allowed for the position chosen on that particular bed layout. In the case of the 100 vial plate and the 10 vial plate nothing will happen as these are the only valid plates for their default positions.

Clicking on a well plate will reveal the available choices, which can be configured:

Plate Select	×
Plates: Agilent 384 Well Plate Agilent 96 Capped Well Plate Agilent 96 Deep Well Plate <mark>Agilent 96 Well Plate</mark> No Plate	Cancel
X co-ordinate of the tray (1/10 mm)	
Y co-ordinate of the tray (1/10 mm)	_
🔲 OpenLynx plate login	

Figure 5.15 The HP 1100 Well Plate Autosampler: - Edit Plates on Layout - Plate Select

**Note:** No Plate can now be selected as part of a valid bed layout configuration. Also the OpenLynx Login option is enabled

Clicking on a 15 vial plate or a 40 vial plate will result in the choices shown in Figure 5.16 below:

Plate Select			×
Plates: 15 Vials Plate 40 Vials Plate No Plate		OK Cancel	
X co-ordinate of the tray (1/10 mm)	0		
Y co-ordinate of the tray (1/10 mm)	0		
🔲 OpenLynx plate logir	1		

The valid positions for the plates are summarized below.

Bed Layout	Positions	Valid Plates
One hundred vial tray	(1,1)	One hundred vial plate
Two trays	(1,1) and (2,1)	15 vials plate, 40 vials plate
Two well plates plus 10 vials	(1,1) and (1,2)	Agilent 96 Well Plate, Agilent 96 Deep Well Plate, Agilent 96 Capped Well Plate, Agilent 384 Well Plate. Also any user defined well plate
	(2,1)	10 vial plate

#### **Plate Generator**

Selecting **Bed Layout, Plate Generator** invokes the **Plate Generator** dialog. At present, this only allows the plate information to be viewed and not changed.

Plate Name 10 Vials Plate	Vial Reference Horizontal © A B C
Vial Positions Vial Spacing Number Of Vials 1/10 mm	Vertical © 1 2 3 © A B C
Rows 10 170	Referencing Sequential Discontinuous
Columns 1 0	Priority 🗖 Horizontal first
Offsets	Plate Size First Vial Offset
Row 0 Every 2nd Row 1/10 mm	X 210 1/10 mm X 105 1/10 mm
Column Column Every 2nd Column	Z 300 1/10 mm inc. bottles
First Vial Position	Vial
O Back, left edge O Back, right edge	Depth 110 Volume (μl) 2000.00
Front, left edge C Front, right edge	Diameter 130 Vial Tray

Figure 5.17 The HP 1100 Well Plate Autosampler: - Plate Generator dialog

# The HP Diode Array Detector

### HP1050 PDA Detector

Select the **View**, **HP1050 PDA Detector**, Inlet from the short cut bar from the menu or press the toolbar button.

HP1050 PDA Detector		
DAD Spectrum		
· · · · · · · · · · · · · · · · · · ·		
Range (nm) IO 600 Peak Width (mins)		
<u>I</u> nterval (nm) 2 ▼ 0.100	Autobalance	
✓ Use Pump Stop Time Stop Time (mins)	Save Data to Disk	

Figure 5.18 HP1050 DAD Spectrum Window

Range	Enter the minimum ( <b>Range</b> ) and maximum ( <b>To</b> ) wavelengths in nanometers over which diode array spectral data will be acquired.
Peak Width	Determines the rate at which the data is acquired. There are approximately eight spectra per peak so a peak width of 0.1 minutes means eight spectra will be acquired every 6 seconds.
Interval	This determines the number of spectral data points acquired. For example an interval of 4 nanometers means data points will be acquired at the lower wavelength, the lower wavelength plus 4nm and so on.
Autobalance	Check this box to zero the base line of the diode array detector before each analysis.
Use Pump Stop Time	Check this box to use the <b>Stop Time</b> defined on the Pump Initial Conditions page (see page 5-9).
Stop Time	This option is not enabled if the <b>Use Pump Stop Time</b> box is checked. It determines the time in minutes the diode array method will run. Data will be acquired for this amount of time.
Save Data to Disk	Check this box to store the diode array data to disk. If you do not wish to save the diode array data to disk you should uncheck this box.

### **HP1100 DAD**

The HP1100 DAD dialog has **Pre Autobalance** and **Post Autobalance** in place of **Autobalance**. Check the relevant box to zero the baseline of the diode array detector before or after each analysis.

# HP 1050 UV Detector

Select View, HP1050 UV Detector, Inlet from the short cut bar from the menu or press the toolbar button.

HP1050 UV Detector
UV Detector Response (ms)
Figure 5.19 HP1050 UV Detector Window

Response	Select 250, 1000 or 4000 msec from the drop down list box.
Wavelength	Enter the wavelength in nanometers to be monitored.

### Hewlett Packard 1090 UV Detector

Select View, HP1090 UV Detector, Inlet from the short cut bar from the menu or press the toolbar button.

HP10	090 UV Detector			
UV D	letector			
	Response (ms)			
	Attenuation (AUV-1) 1 ▼	Lana Current	○ <u>N</u> ormal	
	Zero Percent 1	Lamp Current	● <u>H</u> igh	
	Filter Position			

#### Figure 5.20 HP1090 UV Detector Window

**Response** Select one of the values from the drop down list.

Attenuation Select one of the values from the drop down list.

Zero PercentIncrease the value to increase the baseline.Filter PositionSet this to the number of the filter required.Lamp CurrentSet to Normal or High.

#### HP 1100 UV Detector

Select View, HP1100 UV Detector, Inlet from the short cut bar from the menu or press the toolbar button.

HP1100 UV Detector
UV Detector Wavlenegth(nm) Response (ms) 60 Analog Zero Offset (%) 5 Attenuation (mAU) 1000

Figure 5.21 HP1100 UV Detector Window

Wavelength	Set to the wavelength in nanometers to be monitored.
Response	Select one of the values from the drop down list.
Zero Offset	Increase the value to increase the baseline.
Attenuation	Select one of the values from the drop down list.

# The HP6890 GC Control

### **System Status Page**

The System Status page displays information about the state of the machine being controlled. This page can be accessed from the Inlet Editor by selecting **Status** from the **View** menu or by pressing the **I** toolbar button.

Indicators	GC		
<ul> <li>Running</li> </ul>	Ö	Run Time (mins)	0.000
<ul> <li>Inject Cycle</li> </ul>	<u>e</u>	Flow Rate (ml\min) :	0.000
Ready	Ç	Pressure (kPa) :	0.0
о ок		Temperature (°C) :	0.00

Figure 5.22 System Status page

Indicators	The Running and Injector Cycle indicators at the left of the screen give information on the current status of the GC system. The OK and Ready Indicators become illuminated in red if the GC System has an error. Click on the red indicators to display more information on the cause of the error.
Run Time	This displays how long the method has been running.
Flow Rate	This is the current flow rate as returned by the instrument. The current value will be the setpoint when using a flow based mode or the actual flow if in a pressure based mode.
Pressure	This displays the current pressure in the instrument. The current value will be the setpoint when using a pressure based mode or the actual pressure if in a flow based mode.
Temperature	This displays the current oven temperature of the GC.

# HP6890 Sampler Configuration Page

Select the **View**, **HP6890** AutoSampler, Autosampler, from the short cut bar or press the toolbar button.

IP6890 Autosampler
Status and Connectivity Injection Params Syringe Params
Autosampler Has Tray
• Fault
Autosampler Has Barcode Header      Running
Ready     Sample Number
Injector
O Front O Back O Both

Figure 5.23 HP6890 Status and Connectivity page

Fault	A red light indicates that the Autosampler has developed a fault.
Running	A green light indicates that the Autosampler is active. The Autosampler is active if it is moving a vial, rinsing or injecting.
Ready	A green light indicates that the Autosampler is ready to start processing another vial.
Autosampler Has Tray	Check this box if the Autosampler has a tray.
Autosampler Has Barcode Reader	Check this box if the Autosampler has a barcode reader.
Sample Number	Enter the vial number that the injection will be taken from, when the start button is pressed.
Start	Press this button to start an autosampler (and hence GC) run with the currently stored method.
Stop	Press this button to stop the Autosampler. It will not stop a GC run.

# HP6890 Injection Params Page

Injection Volume	Enter the injection volume to be used for a run started using the Start button on the Status and Connectivity page. <b>Note:</b> If the acquisition is started from the Sample List then this value will be overridden by the sample list injection volume.
Tray Temperature	This is used for record purposes only and is the tray temperature at which this method is normally used.
Solvent Washes	These allow a rinse strategy for each sample to be set up. Enter the number of washes of each type in the required boxes.

Status and Connectivity Injection Pa	arams Syringe	Params
Injection Volume 1	(10ths of syringe volume)	Solvent Washes Solvent A Pre-Washes 0 Solvent B Pre-Washes 0
Information Only Tray Temperature	(°C)	Sample Washes 0 Solvent A Washes 0 Solvent B Washes 0

Figure 5.24 HP6890 Injection Parameters page

# HP6890 Syringe Params Page

Sample Pumps	Enter the number of times the syringe will draw in liquid in order to fill it.
Viscosity Delay	Select the length of time that the needle will stay in the vial to ensure that all the sample has been drawn into the syringe, from the drop down list box.
Slow Plunger	For viscous liquids select <b>YES</b> from the drop down list box otherwise select <b>NO</b> .
Allow Sampling Offset	Check this box to enable the Sampling Offset.
Sampling Offset	Enter the distance from the bottom of the vial (in millimeters) that the injection will be taken from. This is to allow samples to be taken from different parts of a multi-phased sample. <b>Note:</b> This box will bot be enabled unless the <b>Allow Sampling Offset</b> box is checked.
Pre Dwell Time and Post Dwell Time	If, for example the sample needs heating before injection, the syringe needle can be held in the GC inlet for <b>Pre Dwell Time</b> and/or <b>Post Dwell Time</b> . Enter the length of time in the relevant box.

HP6890 Autosample <mark>r</mark>	
Status and Connectivity   Injection Params	Syringe Params
	C Allow Sampling Offset
Sample Pumps 0	Sampling Offset 10 (mm)
Viscosity Delay NONE 🔻	Pre Dwell Time 0 (min)
Slow Plunger NO 💌	Post Dwell Time 0 (min)

Figure 5.25 HP6890 Syringe Parameters page

# HP6890 Pump Setup

Select the View, HP6890 Pump, Inlet from the short cut bar or press the toolbar button.

### HP6890 Status Page

HP6890 GC	
Oven Ov Status Colur	en Temp Ramps   Post Run   Front Injector   Back Injector nn 1   Column 1 Pneumatics   Column 2   Column 2 Pneumatics
Prep Run	Run Time 0.00 min Carrier Gas He 🔽
<ul> <li>Running</li> </ul>	Current Temperature 0 °C
O Post Run	Column 1 Current Pressure 0.00 (kPa) (kPa)
<ul> <li>Ready</li> </ul>	Current Flow 0.00 Current Flow 0.00 (ml/min)
Error	
	Load <u>Start</u> Stop

Figure 5.26 HP6890 GC Status page

Prep Run	This will be yellow when the GC is in the Prep Run state. This occurs when the GC is trying to equilibrate before an automatic injection start.
Running	This will be yellow when the GC is in the Running state. This occurs when the GC has started its temperature and/or pressure profiles for a run.

Post Run	This will be yellow when the GC is in the Post Run state
Ready	This will be green when the GC is equilibrated and ready to start a run.
Error	This will be red when the GC is in an Error state or there is a communication problem between the GC and the Host (local PC).
Column 1 and	These boxes indicate the current status of the attached columns.
Column 2	

The **Run Time**, **Current temperature**, **Current Pressure** and **Current Flow** are described in the System Status Page on page 5-21. **Carrier Gas** is described below.

#### HP6890 Column Page

There are two column settings pages, one for each column, both have the same functionality and are identical in appearance.

HP6890 GC			
Oven Oven To	emp Ramps   F	<sup>v</sup> ost Run   Front Injector	Back Injector
Status Column 1	Column 1 Pneu	Imatics   Column 2   C	
Column Dimension	n	Carrier Gas He	essure V
Length	25.00 (m)	Injection	
Internal Diameter	250.00 (μm)	Pressure Mode Constant Pr	
Film Thickness	0.25 (μm)	Injection Port Front	

#### Figure 5.27 HP6890 Column page

Length	Enter the column length in meters.
Internal Diameter	Enter the internal diameter of the column in micrometers.
Film Thickness	Enter the thickness of the column coating in micrometers.
Carrier Gas	Select a carrier gas from the drop down list box.
Injection Pressure Mode	Select Constant Pressure, Ramped Pressure, Constant Flow or Ramped Flow from the drop down list box.
<b>Injection Port</b>	Select Front or Back from the drop down list box.

### **HP6890** Pneumatics Pages

Figure 5.28 HP6890 Column 1 Pneumatics page

This appearance of this page will vary depending on the Injection Pressure Mode selected on the Column page. If Constant Pressure was selected only Initial Pressure and Initial Time are enabled. If Ramped Pressure was selected then the Ramps are enabled as well. If Constant Flow or Ramped Flow was selected, the parameters will be as for the corresponding Pressure page but Pressure will be replaced by Flow.

Initial Pressure/Flow	Enter the pressure/flow required for the Initial state.
Initial Time	Enter the length of time to remain at the initial pressure/flow
Ramps	To enable a Ramp check the relevant Ramp box or enter a non-zero value in the Rate box.
Rate	Enter rate of pressure/flow change for current ramp.
Тетр	Enter final pressure/flow for current ramp.
Time	Enter the length of time to remain at the final pressure/flow of current ramp, before proceeding to the next ramp.

# HP6890 Oven Page

HP6890 GC	
Status Column 1 Column 1 Pneu Oven Oven Temp Ramps F	umatics Column 2 Column 2 Pneumatics Post Run Front Injector Back Injector
Max Oven Temp 450 (°C) Equilibrium Time 0.00 (min)	Enabled  Cryo Uses CO2  Blast Cooling Enabled  Cryo Fault Enabled  Cryo Timeout Enabled  Cryo Timeout Time 0.00 (min)  Ambient Temp 20 (*C)

Figure 5.29 HP6890 Oven page

Max Oven Temp	Enter the maximum oven temperature. Temperatures entered in the ramps on the other pages will not be accepted if they exceed this parameter.
Equilibrium Time	Enter the time to wait at the Initial Temperature (defined on subsequent pages) before the ready signal is displayed.
Cryo cooling parameters	To enable these parameters check the <b>Enabled</b> box.
Cryo Uses CO2	Check this box if the cryo system uses $CO_2$ instead of $N_2$ .
Blast Cooling Enabled	Check this box if the cryo system is to be used above ambient temperatures to speed up cooling of the oven.
Cryo Fault Enabled	Allows a fault to be generated on the GC if the Cryo system has been in continuous operation for more than 16 minutes.
Cryo Timeout Enabled	Allows the cryo system to timeout and switch off the oven when the oven has been left ready at an equilibrated temperature for longer than the time entered in the <b>Cryo Timeout Time</b> box
Ambient Temp	Enter the temperature regarded as normal ambient around the GC.
For a more detailed des	scription of Cryo Fault & Timeout consult your HP6890 manual or site

engineer.

### HP6890 Oven Temp Ramps Page

Oven Oven Temp Ramps Post Run Front Injector Back Inject						
- Initial/Constant Sta	te		-Ramps- Ramp No	Rate (°C/min)	Temp (°C)	Time (min)
Initial Temperature	24 (°(	.)	□1 □2	0.00	0	0.00
Initial Time	0.00 (m	in)		0.00	0	0.00
Run Time	0.00 (m	in)		0.00	0	0.00
				,	·	,

This window allows the oven temperature ramp profile for the GC run to be entered.

Figure 5.30 HP6890 Oven Temperature Ramps page

Initial Temperature	Enter the temperature required for the Initial State.
Initial Time	Enter the length of time to remain at the initial temperature.
Ramps	To enable a Ramp check the relevant <b>Ramp No</b> box or enter a non-zero value in the Rate box.
Rate	Enter rate of temperature change for current ramp.
Тетр	Enter final temperature for current ramp.
Time	Enter the length of time to remain at the final temperature of the current ramp, before proceeding to the next ramp.

When setting Oven Temperature ramps the following points should be remembered: -

- Any ramp will be used to ramp the temperature to the required temperature at the given rate.
- The start time for the ramp will be the final time for the previous ramp.
- Any ramp with a rate of zero will be the last ramp and will void any further ramps.
- For an isothermal run, ramp 1 should be set to zero rate.
- The final time for a ramp is the time at which the settings for that ramp become overtaken by those for the next ramp setting (Figure 5.31)



Figure 5.31 Illustration of Oven Ramp Settings

### HP6890 Post Run Page

Status Column 1 Column 1 I Oven Oven Temp Ramps	Pneumatics Column 2 Column 2 Pneumatics Post Run Front Injector Back Injector
Post Run Enabled	Post Run Parameters Time Temp Pressure (min) (°C) (kPa) 0.00 0 0.00

Figure 5.32 HP6890 Post Run page

This appearance of this page will vary depending on the Injection Pressure Mode selected on the Column page. If Constant Pressure or Ramped Pressure was selected then a Post Run Pressure is required. If Constant Flow or Ramped Flow was selected then a Post Run Flow is required.

Post Run Enabled	Check this box if a Post Run is required.			
Time	Enter the length of time for the post run phase.			
Temp	Enter the temperature for the post run phase.			
Pressure/Flow	Enter the head pressure/flow for the post run phase.			

### HP6890 Front and Back Injector Pages

These two windows allow the type of inlet on the front and back slots to be selected. For all types other than "none" a configuration page is available that will be presented in the front or back inlet.

If none is selected in Injection Port Type there are no parameters to enter.

#### **Cool on Column Inlet**

HP6890	GC							
Status Oven	Column 1	Column 1 Ramps	Pneumatics	Colun Fro	nn 2   nt Injector	Column 2	2 Pneumati 3ack Injecto	cs   pr
Injectio Col Cool	n Port Type None on Column Ramped Tempera	ature		Initial/9	Static Con ctor On	ditions		
	'Splitless			Tem Time	perature e	0	(°C) (min)	
PTV	None			Ramps	Rate (*C/min) 0.00 0.00	FinalTemp (°C)	D Time (min) 0.00 0.00	
				<u> </u>	J0.00	Jo	J0.00	

Figure 5.33 HP6890 Cool on Column Inlet: Ramped Temperature – Front Injector page.

Cool on Column	If <b>Track Oven</b> is selected, there are no parameters to enter.		
	If <b>Ramped Temperature</b> is selected, Figure 5.33 is displayed.		
Thermal Zone On	Check this box to enable the <b>Temperature</b> , <b>Time</b> and <b>Ramps</b> fields.		
	The Initial/Static conditions and Ramps details are used to provide a temperature profile for the inlet independent to the temperature profile of the oven, but the method of use is the same.		
Initial Temperature	Enter the temperature required for the Initial State.		
Initial Time	Enter the length of time to remain at the initial temperature.		
Ramps	To enable a Ramp check the relevant Ramp box or enter a non-zero value in the Rate box.		
Rate	Enter rate of temperature change for current ramp.		
Final Temp	Enter final temperature for current ramp.		
Time	Enter the length of time to remain at the final temperature of the current ramp, before proceeding to the next ramp.		

#### Split/Splitless Inlet

If None is selected, there are no parameters to enter.

If **Split** is selected Figure 5.34 is displayed.

#### Split Mode

HP6890 GC		
Status Column 1 C Oven Oven Temp Rad Injection Port Type © None Cool on Column © None Split/Splitless © Split PTV © None	olumn 1 Pneumatics Column 2 nps Post Run Front In Gas Saver- Enabled Flow Time Operation I Injector Temperatur Split Ratio	Column 2 Pneumatics

Figure 5.34 HP6890 Split Inlet - Front Injector page

Gas Saver Enabled	Check this box to save gas after an injection. The gas Flow and start time parameters are entered into the two edit boxes.
	• The gas saver flow must be at least 15ml/min greater than column flow.
	<b>Note:</b> Auto prep run must be manually set to ON if this box is checked. See the HP6890 manual for details.
Flow	Enter the reduced flow rate.
Time	Enter the length of time to deliver the reduced flow rate for.
Thermal Zone On	Check this box to enable the <b>Temperature</b> and <b>Split Ratio</b> fields.
Temperature	Enter the temperature at which to hold the inlet during the run.
Split Ratio	Enter the split ratio for the inlet flow.
Splitless Mode	

If **Splitless** is selected Figure 5.35 is displayed.

HP6890 GC	;					
Status C Oven	Column 1   Oven Temp	Column 1 Ramps	Pneumatics Post Run	Column 2 Front Injec	Colum tor	n 2 Pneumatics Back Injector
Cool on C Cool on C Split/Split PTV	rt Type column ne tless tless	× •		Gas Saver ✓ Enabled Flow Time Operation ✓ Injector On	15.00	(ml/min) (min)
C Nor	ne	<b>Y</b>		Temperature Purge Time Purge Flow	0 0.00 1.00	(°C) (min) (ml/min)

Figure 5.35 HP6890 Splitless Inlet - Front Injector page

Gas Saver Enabled	<ul> <li>Check this box to save gas after an injection. The gas Flow and start time parameters are entered into the two edit boxes.</li> <li>The gas saver flow must be at least 15ml/min greater than column flow.</li> <li>The gas saver start time should be after the purge time.</li> </ul>		
	<b>Note:</b> Auto prep run must be manually set to ON if this box is checked. See the HP6890 manual for details.		
Flow	Enter the reduced flow rate.		
Time	Enter the length of time to deliver the reduced flow rate for.		
Thermal Zone On	Check this box to enable the <b>Temperature</b> , <b>Purge Time</b> and <b>Purge Flow</b> fields.		
Temperature	Enter the temperature at which to hold the inlet during the run.		
Purge Time	Enter the time at which to open the purge valve.		
Purge Flow	Enter the flow rate to use at the Purge Time.		

#### Pulsed Temperature Vaporization (PTV) Inlet Page

Selecting a PTV mode on the front inlet creates a tabbable page for the PTV configuration. This page is removed should a non-PTV method be chosen.

Note: The back inlet cannot be fitted with a PTV.

There are five modes of operation on this inlet: Split, Splitless, Pulsed Split, Pulsed Splitless, and Solvent Vent. If **None** is selected, there are no parameters to enter.

Figure 5.36 HP6890 PTV Settings – Front Injector Page

PTV Cryo Parameters enabled	Allows the setting of the PTV cooling settings. If cooling is used, then the cryo method must be the same as that used on the oven.	
Quick Cool	Check to enter the reduced flow rate.	
Cryo Fault	Allows a fault to be generated on the GC if the cryo system has been in continuous operation longer than 16 minutes.	
Cryo Timeout	Allows the cryo system to timeout and switch off the oven when the oven has been left ready at an equilibrated temperature for longer than the given time.	
Cryo Method	Select from None, Air CO <sub>2</sub> and N <sub>2</sub> .	
Ambient Temp	Enter the ambient temperature. It has variable minimum values $-160^{\circ}$ C for liquid nitrogen, $-60^{\circ}$ C for liquid Carbon Dioxide and $5^{\circ}$ C for compressed air. If no cooling is selected it will default to $24^{\circ}$ C	
PTV Settings Page		
Min Temp	The minimum temperature for all PTV injection port temperatures is based upon the cooling method. These values are described above for <b>Ambient Temp.</b>	

Temperature	Each method on the PTV can have up to three temperature ramps. Time
Ramps	represents the hold time for the temperature once it has been reached.

#### Split Mode

Split	operation	is si	nilar to	that on	the Sp	plit/S	plitless	Inlet (	page	5-32)
									0.0	/

HP6890 GC	
Status       Column 1       Column 1 Pneu         Oven Temp Ramps       Post Run         Initial/Static Conditions         Remperature       24         (°C)         Time       0.00         Ramps         Rate       FinalTemp	matics Column 2 Column 2 Pneumatics Oven Front Injector Back Injector PTV Settings Gas Saver Enabled Flow 15.00 (ml/min) Time 0.00 (min)
Ramp       (°C/min)       (°C)       (min)         1       0.00       0       0.00         2       0.00       0       0.00         3       0.00       0       0.00	Split Ratio

Figure 5.37 PTV Settings - Split Page

Gas Saver Enabled	Check this box to save gas after an injection. The gas Flow and start time parameters are entered into the two edit boxes.				
	• The gas saver flow must be at least 15ml/min greater than column flow.				
	<b>Note:</b> Auto prep run must be manually set to ON if this box is checked. See the HP6890 manual for details.				
Split Ratio	Enter the split ratio for the inlet flow.				

#### **Splitless Mode**

Splitless operation is similar to that on the Split/Splitless Inlet (page 5-32).

- Purge time and purge flow (Figure 5.38) must be set.
- Three temperature ramps can be set on the inlet

- PTV Splitless	}	
Purge Time	0.00	(min)
Purge Flow	0.00	(ml/min)

Figure 5.38 PTV Splitless parameters

#### **Pulsed Split Mode**

This is similar to Split Mode operation with the addition of pulsed pressure (Figure 5.39).

- Pulsed Split Operation				
Pulsed Pressure	5.00	(kPa)		
Pulsed Time	0.00	(min)		
Split Ratio	0			

Figure 5.39 PTV Pulsed Split Parameters

<b>Pulsed Pressure</b>	Enter the Pulsed Pressure for the inlet valve.	
Pulsed Time	Enter the Pulsed Time for the inlet valve.	

#### **Pulsed Splitless Mode**

This is similar to Splitless Mode but with the addition of pulse pressure and time set points.

- Pulsed Splitless Operation			
Pulsed Pressure	5.00	(kPa)	
Pulsed Time	0.00	(min)	
Purge Time	0.00	(mins)	
Purge Flow	0.00	(ml/min)	

Figure 5.40 PTV Pulsed Splitless Parameters

#### **Solvent Vent Mode**

This mode requires several parameters to be set. Vent pressure and flow must be set, as well as the vent end time. These settings are for venting the solvent from the inlet, to concentrate the analyte. Purge flow and purge time must also be set.

PTV Solvent Vent				
Vent Pressure	Vent Flow V	/ent End Time		
0.00	0.00	0.00		
(kPa)	(ml/min)	(min)		
Purge Flow	Purge Time			
0.00	0.00			
(ml/min)	(min)			

Figure 5.41 PTV Solvent Vent Parameters

#### **HP6890** Communication Parameters

Select **HP6890**, View Comms Settings from the Menu or press the toolbar button to view the current communications settings.

Communication settings should only be changed by an engineer, the Edit Comms Settings and

the  $\square$  toolbar button allow this to be done.

Communication Parameters		
		[OK]
Abort On Error	FALSE	▼ Cancel
Baud Rate	19200	•
Binary Mode	TRUE	7
Byte Size	8	•
Comm Port	COM1	•
Enable Null Stripping	FALSE	<b>V</b>
Enable Parity Checking	FALSE	•
End Of Data	10	(decimal ascii)
Error Char	NULL	7
Parity	NONE	•
RTS Control	DISABLED	•
Stop Bits	ONE	•
Timeout	45000	(ms)

Figure 5.42 HP6890 Communications Parameters dialog

### The HP6890 Toolbar

The HP6890 toolbar has five extra buttons on it, which are:

View current communications settings.
 Edit current communications settings.
 Start and stop method.
 Turn GC on and off.
 Reset Autosampler

# Chapter 6 Jasco Systems

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# Jasco 900 and Jasco 1500 Autosamplers

The Inlet Editor for the Jasco 900 and Jasco 1500 autosamplers is the same. The Jasco 900 is used in the following examples.

#### Jasco Comms Setup

It is possible to connect to the Jasco system using either the LC Net II ISA card or the newer ethernet enabled LC Net II Box. The default setting is to use the LC NetII ISA card. To change the communication setting, open up the Inlet Editor and select the **Comms** menu.

👼 Untitled.j15 - Inlet	👼 Untitled. j15 - Inlet Method 📃 🗖 🗙				
<u>F</u> ile <u>V</u> iew <u>T</u> ools <u>L</u> C	<u>Comms</u> <u>H</u> elp				
🗅 🗳 🖬 🔥 🍋	✓ ISA Net Card ↓	🧇 🍯	3		
	LC-Netll Ethernet				
79					
	- Indicators	-LC			- Gradient
Status		8			π
	O Running	$\Theta$	Run Time (mins)	0.000	🛕 100.0 %
(四)		An			凤
	O Pump On		Flow Rate (ml\min) :	0.000	<b>B</b> 0.0 %
Iniet		0	Pressure (bar) :	0.0	<u>⊼</u> 00 ×
	O Uniect Cucle	Y	riessure (bar).	0.0	
			Column Left (*C) :	0.0	6 0.0 %
Autosampler		E @			
nacosampion	O Ready		Column Right (°C) :	0.0	- PDA Detector
lass.		EU			Mode : Not installed
	🔴 ок		Sample Temp (°C) :	0.0	Scan: 0
Detector					

Figure 6.1 Jasco Comms menu

Selecting the ISA Net Card menu item simply configures the system to use the pre-installed Jasco drivers. If these are not present they must be installed.

Selecting the LC-NetII Ethernet menu item will open up a set of dialog boxes enabling you to configure the IP address of the LC-NetII Box (this must be connected for this operation to work ).

The first dialog allows you to set the IP address.

Set IP Address of LCNetII/AD.			
IP Address	MAC Address	Status	
10.1.52.231	00:c0:28:00:00:34	Ready	
Update	Set Address	Exi	t

Figure 6.2 IP address setup

The second selects which system this IP address refers to.

🔏 IP Address Configur	🗱 IP Address Configuration for HPLC System 📃 🖂 🔀				
System 1	<b>∨</b> use 10.1.52.2	31			
System 2	🗖 use				
IP Address	MAC Address	Status			
10.1.52.231	00:c0:28:00:00:34	Ready			
Update		Save & Exit	Exit		

Figure 6.3 IP address selection

### **Jasco Sampler Initial Conditions Page**

This page is used to set parameters specific to the Sampler, to access it select **View**, **Jasco900 AutoSampler**, **Autosampler** from the short cut bar or press the toolbar button.

Jasco	o Autosampler				
Samp	ler Initial Conditions				
Г	Injection Parameters		-Vial		
	Injection Volume (μl)	1.0			
	<u>F</u> lushes	1	<u>V</u> ial Number	1	
	Analysis Time (mins)	5.00			
	,				

Figure 6.4 Sampler Initial Conditions page

Injection Volume	Enter the volume in microlitres to inject.	
	<b>Note:</b> If a multisample acquisition is being run from the MassLynx Sample List, the injection volume defined in the sample list overrides the value defined here.	
Flushes	Enter the number of times the needle should be flushed between injections.	
Analysis Time	Enter the length of time the run will last.	
Vial number	Enter the number of the vial to inject from.	
	<b>Note:</b> If a multisample acquisition is being run from the MassLynx Sample List, the Bottle # entry in the sample list overrides the value given in the Vial Number entry above.	

# Jasco 900 and Jasco 1500 Pumps

The Inlet Editor for the Jasco 900 and Jasco 1500 pumps is the same. The Jasco 900 is used in the following examples.

The Jasco Pump pages can be accessed by selecting **View**, **Jasco900 Pump**, **Inlet** from the short cut bar or by pressing the toolbar button.

# **Jasco Initial Conditions Page**

Jasco LC		
Initial Conditions Gradient		
Solvents	Mode	Pressures Low P <u>r</u> essure (bar)
5 4 400.00	Isocratic	High Pressure (bar) 400
Pump A 100.0 %	C Binary	Column Heater
	С нр <u>с</u>	Timings
Flo <u>w</u> (ml/min) 1.000	© <u>L</u> PG	Stop Time (mins) 5.00

Figure 6.5 Initial Conditions page

Solvents	Up to three solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must not exceed 100%.
Pump A	This is the remainder percentage after the solvent percentages have been set for the other pumps.
Pump B and C	Check the box for the pump required and enter the percentage of flow to deliver from this pump. To disable the pump, uncheck the box.
Flow	This is the total flow rate of the solvent channels.
Mode	<b>Select</b> Isocratic (One pump), Binary (Two pumps), HPG (High Pressure Gradient) or LPG (Low Pressure Gradient).
Pressures	Enter the upper and lower limits of the pressure within the solvent delivery system (SDS), if the pressure falls outside of this range the SDS switches off.
Column Heater	If the instrument has an oven present then the column temperature can be set to a specified temperature in degrees centigrade. Check the Column Temperature box and enter a temperature. If the software has been configured to operate without a column oven then these boxes will be greyed out.
Stop Time	Enter the time in minutes that the method will run from the point of injection. If a Jasco Autosampler has also been selected, Analysis Time on the autosampler page overrides this value.
**Post Time** 

Enter the time in minutes that the instrument will run in its initial conditions after a method has completed. No further injections can be carried out whilst the system is in postrun thus allowing re-equilibration of the column.

#### **Jasco Gradient Page**

Initial Conditions G	radient					
Gradient Entry	0.00	<u>G</u> radient Tabl	e		+ <b>2</b> × <b>3</b> × <b>3</b>	
<u>I</u> ime( mins )	0.00	Time	B%	Flow	Pressure	
<u>B</u> %	0.00					
Flo <u>w</u> (ml/min) Pressure (bar)	1.000					
<u>r</u> iessuie (bai)	400					

Figure 6.6 Gradient Timetable page

This page allows a gradient to be entered and edited. If isocratic mode was selected on the Initial Conditions page then only relevant fields are displayed and no Gradient can be added to the Gradient Timetable. For other modes the relevant boxes (B% and C%) are enabled.

To add a gradient, enter a time and percentage in the relevant boxes and press the toolbar button. **Note:** The first entry must have a time of 0.

To delete a single gradient, click a time in the list and press the **k** toolbar button.

To delete all entries press the kill toolbar button. This button is only available when there are entries in the timetable.

To modify a gradient select the required entry in the timetable. The values will then be displayed in the edit boxes to the right of the timetable and can be altered as appropriate. Once changed press

to re-enter the values into the timetable. **Note:** If the time is changed and the new time does not correspond with an existing entry in the table, then a new entry will be added. If the new time correspond to an existing entry then the entry at that time will be overwritten.

# Jasco 900 and Jasco 1500 UV Detectors

The Inlet Editor for the Jasco 900 and Jasco 1500 UV detectors is the same. The Jasco 900 is used in the following examples.

This page is used to set parameters specific to the UV detector, to access it select View, Jasco900

UV Detector, Detector from the short cut bar or press the <sup>4</sup> toolbar button.

Jasco UV Detector
UV Detector          Response (ms)       MaxeLength (nm)       254

#### Figure 6.7 UV Detector Configuration page

Response	This can be set to Fast, Standard or Slow depending on the length of time you expect the peak to appear.
Wavelength	Set to the wavelength you want to monitor.

# **Chapter 7 Shimadzu Systems**

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# Shimadzu Autosamplers

### Shimadzu Autosampler Initial Conditions Page

This page is used to set parameters specific to the Sampler, to access it select **View**, **Shimadzu AutoSampler**, select **Autosampler** from the shortcut menu, or press the toolbar button.

Figure 7.1 Autosampler Initial Conditions page

Injection Volume	Enter the volume in microlitres to inject.
	<b>Note:</b> If you are running from the Sample List, the injection volume in the sample list entry overrides the setting used here.
Vial number	The vial to inject from.
	<b>Note:</b> If a multisample acquisition is being run from the MassLynx Sample List, the Bottle # entry in the sample list overrides the value given in the Vial Number entry above.
Rack Select	Select the type of rack required from the drop down list box.
Syringe Volume	Select the size of the currently installed syringe from the drop down list box.
Needle Stroke	Adjusts the depth of the needle tip to accommodate for sedimented samples or non-standard vials.
Rinse Volume	Enter the volume of solvent that is to be rinsed through the needle.
<b>Rinsing Speed</b>	Enter the speed at which the solvent is to be rinsed through the needle.
Sampling Speed	Enter the rate in microlitres per second at which sample is extracted into the autosampler needle. This should be set according to the viscosity of your sample.

Excess Volume	To ensure that the sample is not diluted with the rinse solvent more sample is drawn into the needle than will be injected. Enter the extra volume required.
Cooler Temperature	If the sample cooler is installed, enter the temperature that the sample should be cooled to.
MPT Tray	Select Dual or Single from the drop down list box.

#### To Set-up Communication Parameters (Shimadzu)

Select Shimadzu, Configuration to display the Configuration dialog.

Sh	imadzu X
	Configuration
	Pump Type LC-10ADvp -
	Mode Low-pressure Quaternary Gradient 💌
	Sampler Type SIL-10ADv 💌
	UV Detector SPD-10Avp
	O Deuterium Lamp O Tungsten Lamp
	Column Switching Valves FCV-12AH RV.A RV.B FCV-13AH/FCV-14AH RV.C RV.D
	Communications
	OK Cancel

Figure 7.2 Configuration dialog

These parameters should be defined on setup and should only need changing if the Pump, Autosampler or mode of acquisition is changed. To change a value select a new one from the relevant drop down list box.

Ритр Туре	Choose from the list of Pumps
Mode	Choose from One Pump Isocratic, High Pressure Binary Gradient, High Pressure Ternary Gradient or a Low pressure Quaternary Gradient
Sampler Type	Choose from the list of Samplers
UV Detector	Choose from the list of Detectors.
Column Switching Valves.	Select the models of any column switching that are present and the ports on the options to which they are attached.
Deuterium Lamp	Select either lamp The Tungsten Lamp is available for the SPD-10Avvp

Tungsten Lamp	Pump and the Deuterium Lamp for SPD-10Avp Pump.
Column Switching Valves	Select which column switching valves to use.
Comunications	Select a Com Port and Baud rate

# Shimadzu Pump

The Shimadzu Pump pages can be accessed by selecting **View**, **Shimadzu Pump** on the Inlet Editor selecting Inlet from the short cut bar, or by pressing the toolbar button.

### Shimadzu Initial Conditions Page

Solvents	Soluent Name	Pressures
PumpA% 10	) Solvent A	High Limit (psi) 4000
□ Pump <u>B</u> % □ 0	) Solvent B	Column Temperature
□ Pump <u>C</u> % 0	) Solvent C	0 <u>S</u> et (°C)0
Pump <u>D</u> %	Solvent D	High Limit (°C) 25
Elow Rate (ml/min)	1	<u>B</u> un Time (mins) 1

Figure 7.3 Initial Conditions page

Solvents	Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must not exceed 100%.
Pump A	This is the remainder percentage after the solvent percentages have been set for the other pumps.
Pump B, C and D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Flow Rate	This is the total flow rate of the solvent channels according to how you have configured the instrument.
High Pressure Limit and Low Pressure Limit	Enter values as required. If the pressure falls outside these limits the current acquisition will stop and the LC Status error light, on the MassLynx screen, will turn red.
Column Temperature Set	Enter the temperature to heat the column to. <b>Note:</b> This box will be greyed out if a column heater is not present.

Column Temperature High Limit	This is the maximum deviation in column temperature allowed. If this is exceeded the current acquisition will stop and the LC Status error light, or the MassLynx screen, will turn red.
Run Time	Enter the time in minutes that the method will run from the point of injection.

#### Shimadzu Gradient Timetable Page

Figure 7.4 Gradient Timetable page

This page allows a gradient to be entered and edited. To operate in isocratic mode ensure that the timetable is empty.

To add a gradient, enter a time and percentage in the relevant boxes and press the toolbar button. **Note:** The first entry must have a time of 0.

To delete a single gradient click a time in the list and press the 🔀 toolbar button.

To delete all entries press the toolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed

in the edit boxes above the timetable, and can be altered as appropriate. Once changed press to re-enter the values into the timetable. If, however, you modify the time value such that it does

not correspond to any existing entry in the timetable pressing will result in a new entry being created.

**Flow** Enter the flow rate for the solvent delivery system.

**Curve** This sets the rate at which the solvent is to change to the new proportions and/or flow rates. See the Shimadzu Operator's Guide for a list of values.

### Shimadzu Initial Events Page

#### Shimadzu LC Initial Conditions Gradient Initial Events Programmed Events Switch Events Switch 1 Switch 2 Switch 3 Switch 4 Column Switches-Shimadzu FCV-12AH Shimadzu FCV-13AH / FCV-14AH **RV.A Position** -**RV.C** Position 0 • 1 **RV.B** Position 0 • **RV.D** Position 1 •

Figure 7.5 Initial Events page

This page allows the initial state of switches 1 to 4 to be defined.

Switch Events	Check the box(es) for the switches that should have an initial state of off.
	Ensure that Switch 1 and Switch 2 are not selcted if running Contact Closure.
Column Switches	Select the initial positions for column switches from the dropdown box.

### Shimadzu Programmed Events Page

Shimadzu LC		
Initial Conditions   Gradient   Initial	Events Programmed Events	
Ime: Event:	Event Ta <u>b</u> le	* <b>8</b> ×8 ×8
	Time Event	Action
O <u>O</u> n		
⊙ O <u>f</u> f		

Figure 7.6 Programmed Events page

This page allows the state of switches 1 to 4 and column switches A to D to be programmed.

To add an event, enter a time, select an event from the drop down list box, select an action (on or off) for normal switches or select a number from a drop down box for the column switches and press the toolbar button.

To delete a single event, click a time in the list and press the 📧 toolbar button.

To delete all entries press the *toolbar* button. This button is only available when there are entries in the timetable.

To modify an event, select the required entry in the timetable. The values will then be displayed in

the edit boxes above the timetable, and can be altered as appropriate. Once changed press  $\textcircled{\bullet}$  to re-enter the values into the timetable. If, however, you modify the time value such that it does not

correspond to any existing entry in the timetable, pressing will result in a new entry being created.

# Shimadzu UV Detectors

This page is used to set parameters specific to the Sampler, to access it select **View**, **Shimadzu UV Detector**, select **Detector** from the short cut bar, or press the toolbar button.

#### Shimadzu UV Detector Page

Shimadzu UV Detector
Shimadzu UV Detector
Wavelength 1 Wavelength 1 (nm)
Range Signal O/P Range (AUFS) 1

Figure 7.7 Shimadzu UV Detector Page

Wavelength 1	Select between single and dual wavelength mode and enter the
Wavelength 2	wavelength.

**Note.** Depending on the UV detector used the valid range will be different.

Output Mode	Select from Ch2 O/P to Recorder, Ratio to Recorder or Ratio to Integration.
Ratio Range	Select and enter a ratio range. This only available when <b>Ratio to</b> <b>Recorder</b> or <b>Ratio to Integration is selcted.</b>
Ratio Threshold	Select and enter a ratio thrshold. This only available when <b>Ratio to</b> <b>Recorder</b> or <b>Ratio to Integration is selcted.</b>
Polarity	Enter a positive or negative polarity mode. This applies top both wavelengths.
Range	Enter a range in AUFS (Absorbance Unit Full Scale). The valid range is $0.00 - 2.56$ .

# Chapter 8 LC Packings

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### Introduction

The UltiMate HPLC system consists of pumping units, UV detector and an autosampler. Optionally, users can configure the HPLC system with a Switchos. It is a switching unit that consists of two Valco 10 port low dispersion switching valves which allow for the connection of capillary, micro and nano HPLC columns. It is also attached to a high precision solvent loading pump, capable of separate flow rate from the main HPLC pump. There are two types of Famos autosampler, Famos Well Plate and Famos Carousel autosampler.

In addition, a menu is provided for incorporating into the Inlet Editor menu. All other functionality is provided by the Inlet Editor.

There are three separate views in the Inlet Editor, one each for the pump, UV detector and autosampler. Whether these are available or not depends on the configuration chosen in the Inlet Editor (see Chapter 1)

# UltiMate Pump

The Ultimate Pump pages can be accessed by selecting View, Ultimate Pump from the Inlet

Editor menu bar, Inlet from the short cut bar or by pressing the 😐 toolbar button.

#### **Initial Conditions**

UltiMate LC Initial Conditions Gradient Solvents Pump A % 100	Initial Events Solvent Name Solvent A	Pressures High Limit (psi)
□ Pump <u>B</u> % 0 □ Pump <u>C</u> % 0	Solvent B Solvent C	Low Limit (psi) 0 Timings Bun Time (mins) 1
☐ Pump <u>D</u> % 0 Elow Rate (ml/min)	Solvent D	Hold Gradient Hold Active Reduce Flow to (%)

Figure 8.1 Initial Conditions page

- Solvents Up to four solvents will be displayed depending upon the system configuration. The total value of all the solvent percentages added together must not exceed 100%.
- Pump AThis is the remainder percentage after the solvent percentages have been set for<br/>the other pumps.

Pump B, C and D	These can either be enabled or disabled by checking the box next to the individual pumps. The values can be set to the required percentage of flow delivery.
Solvent Name	Enter the name of the solvent that will be delivered through the corresponding Pump.
Flow Rate	This is the total flow rate of the solvent channels according to how you have configured the instrument.
Pressures	Enter the upper and lower limits of the pressure within the solvent delivery system (SDS) if the pressure falls outside of this range the SDS switches off.
Run Time	Enter the time in minutes that the method will run from the point of injection.
Hold Gradient	The Hold Gradient function is used to facilitate better peak separation. If the <b>Hold Active</b> box is checked, a <b>Reduce Flow</b> rate percentage can be entered to allow slower flow and gradient freeze. When a pulse/signal is generated by a mass spec or other scanning apparatus and received via contact closure at the back of the Pump (Start-In slot).

#### Gradient

#### UltiMate LC

Initial Conditions Gradient Initial Event	s	
Gradient Entry <u>I</u> ime( mins ) 1.00 <u>B</u> % <u>C</u> % <u>D</u> % Flow (ml/min) 50 0 0 0 0.3000 1 Off ▼ 2 Pulse ▼ Switchos Controls Loading flow (ml/min) 0.30 3 NoChange ▼ 6 NoChange ▼ 7 NoChange ▼ 8 NoChange ▼	<u>G</u> radient Table	

Figure 8.2 Gradient Page

Note: The available inputs depend on the actual configuration.

This page allows a gradient to be entered and edited. Isocratic Binary, Ternary and Quarternary gradients can be formed when selected in the Initial Events page (page 8-5).

To enable the **B%**, **C%** and/or **D%** boxes check the relevant boxes on the **Initial Conditions** page.

To add a gradient, enter a time and percentage in the relevant boxes and press the **to**lbar button. **Note:** The first entry must have a time of 0.

Enter **Events** from the Ev1 and Ev2 drop down boxes. If Switchos is present further event controls are enabled.

To delete a single gradient, click on a time in the list and press the 📧 toolbar button.

To delete all entries press the itoolbar button. This button is only available when there are entries in the timetable.

To modify a gradient, select the required entry in the timetable. The values will then be displayed in the edit boxes to the left of the timetable, and can be altered as appropriate. Once changed press

to re-enter the values into the timetable. If, however, you modify the time value such that it

does not correspond to any existing entry in the timetable pressing will result in a new entry being created in the timetable.

- **B%** Pump B is not available in Isocratic mode and is grayed out when not enabled on the Initial Conditions page.
- **C%** Pump C is not available in Isocratic or Binary configuration mode and is grayed out when not enabled on the Initial Conditions page.
- **D%** Pump D is not available in Isocratic Binary or Ternary mode and is grayed out when not enabled on the Initial Conditions page.
- Flow Enter the flow rate for the solvent delivery system.
- **Loading Flow** This is only supported when Switchos is present. This flow is for the loading pump below the Switchos valves.

When Switchos is present, four additional events are supported. **Event 3** – Valve A **Event 6** – Valve B **Event 7** – SSV **Event 8** – External Instrument

#### **Initial Events**

This page allows the initial state of Events 1 to 8 to be defined. Select from the drop down lists On, Off, No Change or Pulse.

UltiM	late LC				
Initial	Conditions	Gradient Initial Events	;		
	Event 1	NoChange 💌	Event 2	On 💌	
	Event 3	Off	Event 6	Pulse 💌	
	Event 7	NoChange 💌	Event 8	0n 🔽	

Figure 8.3 Initial Events page

### Configuration

Selecting **UltiMate**, **Configuration** invokes the Configuration dialog allowing the user to change the configuration.

IlltiMate I C Configuration
Gradient Mode Ternary Gradient
Column Type No Column
Column Length 10 cm
Stationary Phase C18, 5µm, 100A
Sampler Type FAMOS Carousel
Switchos Column Switching Unit
Mjcro Mode     C Conventional Mode
Deuterium Lamp
Communication Comm Port
UV Address 47647 Switchos Pump 0
Pump Address 50968 Address
Sampler Device ID 21
OK Cancel

Figure 8.4 UltiMate Configuration dialog

Gradient Mode	Choose from Isocratic, Binary Ternary or Quarternary.
Column Type	Choose from no column, ID 180µm, ID 100µm, ID 75µm, ID50µm.
Column Length	Choose from 5cm, 10cm, 15cm, 20cm, 25cm.
Stationary Phase	Choose from; GPC; C18, 5µm 100A; C18, 3µm, 100A; C18, 5µm, 300A; C18, 3µm, 300A.
Sampler Type	Choose from Famos Well Plate or Famos Carousel.
Mode	Check either Micro or Conventional.
Switchos	Check box to enable Switchos
Lamp	Indicates the Deuterium Lamp
UV Address	This unique instrument ID which can usually be found at the back of the instrument on a sliver label.
Pump Address	This unique instrument ID which can usually be found at the back of the instrument on a sliver label.
Sampler Device ID	This Device identifier can be found on the COMM. Page of the Famos display screen.

# **Famos Autosampler**

### **Autosampler Initial Conditions**

Famos AutoSampler			
Autosampler Initial Conditions Autosampler Method Configuration			
Sampler Initial Conditions			
Injection Volume (μl)	<u>F</u> lush Volume (μl)		
Vial Reference (eg. A,1) A,1	Loop Volume (µl) 5		
Needle <u>H</u> eight (mm) 2	Tubing Volume (µl) 2.4		
Syringe Volume (μl) 25 μl 💌	Syringe Speed Normal		
	Injection Method Partial Loop		

Figure 8.5 Autosampler Initial Conditions page for the Famos Well Plate autosampler

This page is used to set parameters specific to the Sampler, to access it select **View**, **Famos AutoSampler**, **Autosampler** from the short cut bar or press the toolbar button.

Injection Volume	Enter the injection Volume between $0 - 100\mu l$
Flush Volume	Enter the volume of mobile phase required to flush the injector port after the sample has been injected. A value of zero will result in no flush. Enter a value between $0 - 999\mu$ l.
Vial Reference	Enter the position of the vial to use for single sample acquisitions. For samples acquired via a sample list this is over ridden by the value in the sample list.
Loop Volume	Enter the Volume of the Loop between $5 - 1000 \mu$ l.
Needle Height	Enter a Needle Height.
Tubing Volume	Enter a tubing volume between 1µl and 200µl.
Syringe Volume	Select from 25µl, 100µl, 250µl, 500µl and 1000µl.
	If the autosampler is in micro mode, the syringe size will be either $25\mu l$ or $100\mu l$ .
Syringe Speed	Select a Syringe speed from Low, Normal or High.
Rack Select	Select from 48-Vial, 96-Low, 96-Deep and 384 wells.
	If the autosampler is a Famos Carousel, the Rack Select option will disappear and the <b>Tray Configuration</b> option on the <b>UltiMate</b> menu is activated.
Injection Method	Select from µl – pickup, Full Loop or Partial Loop

### Autosampler Method Configuration

Figure 8.6 Autosampler Method Configuration page

Low Dispersion Method	This is only available if Micro mode has been selected in the Pump Configuration (page 8-6). Check the <b>L.D. Injection Method</b> box and enter an <b>L.D Factor</b> .
Tray Cooling	To enable Tray Cooling, check the <b>Tray Cooling</b> box and enter a <b>Tray Temperature.</b>
Transport Vials	Enter the First and Last Transport Vials. If the sampler is of the carousel variety, the format of the Transport Vial will change from numeric to alphanumeric. Both the first and last transport vial have to be on the same vial segment. This option is only available if $\mu l - pickup$ method is selected.
Wash	Check the Wash after injection box and enter a Wash Volume.

### **Tray Configuration**

If the autosampler selected on the Configuration dialog (page 8-6) is a Famos Carousel the Tray Configuration is activated. This is invoked by selecting **UltiMate**, **Tray Configuration**.

Note. No combination of the segment type and number can be the same for any two segments.

FAMOS Carousel	Tray Configu	ration
Segment	Tray type	Segment Tray Number
Segment 1	A	1 💌
Segment 2	В 💌	2 💌
Segment 3	C 💌	3 💌
Segment 4	D 💌	4 💌
Segment 5	A	5 💌
Segment 6	В 💌	6 💌
Segment 7	C 💌	7 💌
Segment 8	D 💌	8 💌
<u>(</u>	<u>o</u> k	<u>C</u> ancel

Figure 8.7 Famos Tray Configuration dialog

### **UV Detector**

This page is used to set parameters specific to the UV Detector, to access it select **View**, **Ultimate UV Detector**, **Detector** from the short cut bar or press the toolbar button.

The UV Detector can operate in single or dual wavelength mode.

UltiMate UV Detector
UltiMate UV Detector
Wavelength 1       Wavelength 2         Wavelength 1 (nm)       254         Analog Out       Max Voltage Signal (V)         Full Signal Range (AU)       1.0
Data <u>I</u> ime Constant (sec)

Figure 8.8 Ultimate UV Detector page

Wavelength 1	Enter the wavelength.
Wavelength 2	Check the box to enable Dual Wavelength mode and enter the wavelength
Full Signal Range	Select from the drop down box, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0.
Max Voltage Signal	Select from the drop down box, 0.2, 1 and 10.
Time Constant	Select from the drop down box, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0.

# Chapter 9 CTC, Cetac and Other Systems

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### **CTC A200S Autosampler**

These pages are used to set parameters specific to the Sampler, to access them select **View**, **CTCA200S AutoSampler** from the menu bar, Autosampler from the short cut bar or press the toolbar button.

#### **CTC A200S Status Page**

CTC200 Autosampler		
CTC A200S Status CTC A200S F	<sup>D</sup> arameters	
兽 Fault	Vial	1
O Running		
Ready		
		Load <u>B</u> un

Figure 9.1 CTC A200S Status page

The Fault, Running and Ready indicators at the left side of the screen give information on the current status of the autosampler.

Indicator	Red	Green
Fault	Fault with the autosampler	No fault
Running	Not running	Running
Ready	Not ready	Ready

**Vial** Enter the number of the vial to take the sample from, for a single injection. **Note:** When samples are acquired from the Sample List the number on the Sample List overrides this value.

Press the Load button to download the parameters to the LC system. Pressing the button or choosing Load Method from the LC or CTC200 menu will perform the same action.

Press the	<u>R</u> un	button to run a single injection. Pressing the button or
choosing	Run Method	from the LC or CTC200 menu will perform the same action.

### **CTC A200S Parameters Page**

CTC200 Autosampler			
CTC A200S Status       CTC A200S Parameters         Sample       Image: Sample Volume (ul)         Sample Volume (ul)       Image: Sample Volume (ul)         Pull-up Volume (ul)       Image: Sample Volume (ul)         Sample Washes       Image: Sample Volume (ul)         Solvent Washes       Image: Solvent Washes         Pre Injection       Image: Sample Volume (ul)         Post Injection       Image: Sample Volume (ul)			

Figure 9.2 CTC A200S Parameters page

Sample Volume	Enter the volume of sample (in microlitres) to inject.
Air Volume	Enter the volume of air (in microlitres) to be drawn into the needle before the sample, to separate it from the previous sample.
Pull-up Volume	Enter the volume of sample (in microlitres) to draw into the needle for a sample wash.
Sample Washes	Enter the number of times to wash the needle with sample.
Solvent Washes Pre Injection	Enter the number of solvent washes to perform using solvent from reservoirs A and/or B, before an injection.
Solvent Washes Post Injection	Enter the number of solvent washes to perform using solvent from reservoirs A and/or B, after an injection.
Pull-up Count	Enter the number of times to pull up the Pull-up volume for a sample wash.
Pull-up Speed	Enter the speed (in microlitres per second) to pull up the Pull-up volume for a sample wash.
Pull-up Delay	Enter the time to wait between each pull up.
Injection Speed	Enter the speed (in microlitres per second) to inject the sample.
Pre Injection Delay	Enter the time to wait (in seconds) between the needle being injected and the plunger being depressed.
Pre Injection Delay	Enter the time to wait (in seconds) for the plunger to be drawn back after an injection.

# **CTC PAL Autosampler**

These pages are used to set parameters specific to the Sampler, to access them select View,

**PAL\_CC AutoSampler** Autosampler from the short cut bar or press the **toolbar** button. When the autosampler parameters are selected the following message is displayed.

Error	×
8	Macro page not enabled
	Cancel

Figure 9.3 Error message

This refers to the Macro Editor, which is not part of the standard Cycle Composer software, see the PAL Cycle Composer User Manual for details. Press the **Cancel** button to proceed.

When the software is installed a series of files are copied to the default.pro/Acqudb directory (\*.pma and \*.pol).

When the autosampler page is selected the software looks for the presence of the PAL autosampler. If one is found information is read from the Latest\_pal.pol file.

To create method editor files when not connected to a PAL copy the Offline\_pal.pol file to the Acqudb directory of the required project. When complete, copy the Method files (\*.ccp) to the Acqudb directory of the required project on the acquisition PC.

### PAL Cycle Composer Method Editor

PAL Cycle Composer offline (C:\MAS	SLYNX\DEFAULT.PRO\Acqudb)	
<u>F</u> ile <u>H</u> elp		
Method Editor		
Method Name	Selected Macro	▼ Default <u>A</u> ll
Method Description	]	
Method Syringe		
Method Macro Sequence		
Insert <u>R</u> eplace <u>D</u> elete		
The Pal is not connected. Software is running offline		

Figure 9.4 Pal Cycle Composer Method Editor dialog

#### To Create a Method (CTC PAL)

1. Select New Method from the File menu. The Choose name of new Method dialog is displayed.

Choose name of new I	lethod		×
Name of new Method	LC method		
	OK	Cancel	

Figure 9.5 Choose name of new Method dialog

Enter the name for the method and press **OK**.

Enter a description of the method in the **Method Description** box.

Select the size of the syringe installed from the Method Syringe drop down list box.

**Note:** The syringe size should be defined before any macros are selected as different default values and ranges are defined for each syringe size. Changing the syringe size after selecting macros could result in the values entered being outside the ranges allowed and so the macro values will have to be adjusted.

Select a macro from the **Selected Macro** drop down list box. The parameters required for the macro are displayed below the selected macro box.

**Note:** Placing the cursor on the macro name will display a short description of the macro function, as in Figure 9.6.



Figure 9.6 Macro description

Placing the cursor on a field will display a description of its valid, as in Figure 9.6.

Pulse Time (ms)	0
	Range: 0 to 9900 Default: 0

Figure 9.7 Macro description

When the parameters for the macro have been entered press the **Insert** button and the macro will be added to end of the list in the **Method Macro Sequence** box.

To replace a macro, click on the macro in the **Method Macro Sequence** box, select the macro to replace it with, define the required values and press the **Replace** button.

To remove a macro from the list, click on the macro in the **Method Macro Sequence** box and press the **Delete** button.

Select **Save Method** or **Save Method As** from the **File** menu. The **Save Method** dialog is displayed with the name defaulted to that entered in step 2. The name can be changed if required, any changes made will be reflected in the **Method Name** in the editor. Press the **OK** button to save the method.

Save Me	thod
Name:	LC method
	OK Cancel

Figure 9.8 Save Method dialog

The method file (\*.ccp) is stored in the Acqudb directory of the current project.

#### To Modify a Method (CTC PAL)

- 1. Select the method required from the **Method Name** drop down list box. The method files (\*.ccp) displayed are those stored in the Acqudb directory of the current project.
- 2. Click on the required macro in the **Method Macro Sequence** list and change the parameters displayed on the right of the dialog.

**Note:** If the syringe size is changed macro values will have to be adjusted as different syringe sizes have different field values.

3. To add a macro, select the macro from the **Selected Macro** drop down list box. The parameters required for the macro are displayed below the selected macro box.

When the parameters for the macro have been entered press the **Insert** button and the macro will be added to end of the list in the **Method Macro Sequence** box.

To replace a macro, click on the macro in the **Method Macro Sequence** box, select the macro to replace it with, define the required values and press the **Replace** button.

To remove a macro from the list, click on the macro in the **Method Macro Sequence** box and press the **Delete** button.

- 4. Select **Save Macro** to save any changes to the macro sequence.
- 5. Select **Save Method** or **Save Method As** from the **File** menu. The **Save Method** dialog is displayed with the name defaulted to that entered in step 2. The name can be changed if required, any changes made will be reflected in the **Method Name** in the editor. Press the **OK** button to save the method.

Save Me	thod
Name:	LC method
	OK Cancel

Figure 9.9 Save Method dialog

6. The method file (\*.ccp) is stored in the Acqudb directory of the current project.

#### To Delete a Method (CTC PAL)

- 1. Select the method required from the **Method Name** drop down list box.
- 2. Select **Delete Method** from the **File** menu and press Yes on the confirmation dialog.

**Note:** Methods can also be deleted using Window Explorer. Method files (\*.ccp) are stored in the Acqudb directory of the current project.

#### Sample List Vial Referencing (CTC PAL)

For the CTC PAL autosampler the default Sample List vial referencing for a 96 well plate in Stack 1 is

Stk1-01:1 to 96 for the first tray in stack 1 Stk1-02:1 to 96 for the second tray in stack 1 etc.

I.e. Stk1-01:1 is entered in the SAMPLE\_LOCATION (Bottle) column of the Sample List. To use the normal MassLynx referencing 1:1, 1:2, 2:1 etc. the trays have to be renamed. See page 9-8 for details.

Note: For OpenLynx the 1:1 tray:vial referencing must be used.

#### Using the PAL CTC Autosampler with OpenLynx

When using the CTC PAL autosampler with OpenLynx the tray names must be defined numerically and in sequence.

**Note:** It is recommended that MassLynx is closed down whilst changes are made to the tray numbering. When MassLynx is restarted the changes will be picked up automatically.

#### To Rename Trays (CTC PAL)

Using the hand held controller, check the order of the tray holders.

Using the hand held controller, number the trays for the tray holders sequentially starting from 1.

E.g. if the tray holders are in the order:

Stack1

THldr1

Then the trays should be numbered starting at 1 for the first tray in stack 1 and continuing sequentially for THdr1 trays (see the example in Table 9-1).

To check that trays are numbered correctly select **OpenLynx Plate Login** from the **Inlet Editor**, **Plate Login** menu.

If the trays are correctly numbered then the dialog will appear as in Figure 9.10. If the numbering is incorrect i.e. trays for THdlr1 are numbered from 1 then the dialog will appear as in Figure 9.11.

Tray Holder	Tray Type	Default Tray Name	OpenLynx Tray Name
Stack1	MT96	Stk-01	1
Stack1	MT96	Stk-02	2
Stack1	MT96	Stk-03	3
Stack1	MT96	Stk-04	4
Stack1	MT96	Stk-05	5
Stack1	MT96	Stk-06	6
Stack1	MT96	Stk-07	7
Stack1	MT96	Stk-08	8
Stack1	MT96	Stk-09	9
Stack1	MT96	Stk-010	10
Stack1	MT96	Stk-011	11
Stack1	MT96	Stk-012	12
THldr1	VT98	THldr-01	13
THldr1	VT78	THdlr-02	14

#### Table 9-1 OpenLynx Tray Naming

OpenLyna	x Plate Login	×
1 2 3 4 5 6 7 8 9	Disabled Disabled Disabled Disabled Disabled Disabled Disabled Disabled	Cancel



OpenLynx	Plate Login	×
13 14 2 3 4 5 6 7	Disabled Disabled Disabled Disabled Disabled Disabled Disabled Disabled Disabled Visabled Disabled Disabled Disabled Visabled Visabled	Cancel

Figure 9.11 OpenLynx Plate Login dialog

#### Using Plates for OpenLynx Plate Login (CTC PAL)

Select OpenLynx Plate Login from the Inlet Editor, Plate Login menu.

If a plate is labelled **Disabled** it can be used for single shot login. If a plate is labelled **Enabled** it can be used for plate login. To change the state double click on the tray number.

### Cetac ASX100 Autosampler

Cetac ASX100 Autosamp	ler
Autosampler Rack definitions	
Sampler Initial Conditions	
Sampling Info	Rinsing
Vial Number	Before sample 🔽
Vial Number Is Not Used During An Automatic Run	Rinse Time 1 s
<u>S</u> tandardTube	Rack Code
Uptake Time 1 s	<u>2</u> 4

Figure 9.12 Cetac ASX 100 Setup dialog

Technical details of this autosampler are to be found in the manual supplied with the Cetac ASX 100 autosampler.

Vial Number	Enter the number of the vial to take the sample from. <b>Note:</b> When samples are acquired from the Sample List the number on the Sample List overrides this value.
Standard Tube	Check this box if standard vials are being used. The rack codes section will be grayed out and vial number can only be 2 to 14.
Uptake Time	Enter the time in seconds for the sample to travel from the sample vial to the Mass Spectrometer.
Before Sample	Check this box to rinse the needle before each sample.
Rinse Time	Enter the time in seconds required to rinse the needle.
Rack Codes	Click on the code required for the rack.

# Cetac ASX500 Autosampler

SX500 Autosample	۲.
ler Rack definitions	
npler Initial Conditions	
Sampling Info	Rack Codes
Vial Number 1 Vial number is not used during an Automatic Run <u>S</u> tandardTube	Back 1       Back 2       Back 3       Back 4         21       C       C       C         24       •       •       •       •         40       C       C       C       C         60       C       C       C       C         90       C       C       C       C
Sipper Depth 150 mm Uptake Time 1 s	Rinsing Before Sample ▼ Rinse Time 1 s
	Isx 500 Autosample

#### Figure 9.13 CetacASX 500 Setup dialog

Technical details of this autosampler are to be found in the manual supplied with the Cetac ASX 500 autosampler.

Vial Number	Enter the number of the vial to take the sample from. <b>Note:</b> When samples are acquired from the Sample List the number on the Sample List overrides this value.
Standard Tube	Check this box if standard tubing is being used. The rack codes section will be grayed out and vial number can only be 1 to 10.
Sipper Depth	Enter the depth in millimeters the needle should travel to.
	<b>Note:</b> The default probe sampling depth is measured from the neck of the tube to the tip of the probe.
Uptake Time	Enter the time in seconds for the sample to travel from the tubing to the Mass Spectrometer
Rack Codes	Click on the code required for each rack.
Rinse Before Sample	Check this box to rinse the needle before each sample.
Rinse Time	Enter the time in seconds required to rinse the needle.

# **Solids Probe**

The temperature of a solids probe can be controlled during an acquisition. To do this you must set a ramp that defines the temperature of the probe tip against retention time. The ramp can have up to 5 'segments' which each have a start temperature, a time for which the probe will be held at that temperature, and a rate at which the probe will be heated to reach the start temperature of the next ramp segment (if there is one).

In addition to these controls, TIC (Total Ion Current) control of the probe is also available. If TIC control is selected then the TIC value is monitored during the acquisition and that information is used to modify the programmed ramp in such a way that the system attempts to keep the TIC at or below the 'Maximum TIC' value. This feature is very useful as it stops samples from being 'burnt off' the probe prematurely.

The actual temperature ramp used can be stored with the data file and a remote contact closure can be used to start the ramp. The **External Contact Start** box should be checked when a robotic probe system is being used.

#### To Change probe control parameters (Solids Probe)

1. Choose Methods, Inlet from the Acquisition Control Panel menu

-or-

Double click on the probe icon on the Acquisition Control Panel to bring up the solids probe editor shown below.

- 2. Make any changes to the parameters.
- 3. Save the method using either Save or Save As from the File menu.



**Figure 9.14 Solids Probe Control Editor** 

#### Programming the Temperature Ramp (Solids Probe)

The probe temperature ramp can be programmed using the keyboard or by dragging the small red handles on the picture of the ramp itself.

#### Using TIC Control (Solids Probe)

To use the TIC control feature you must first check the **TIC Control** box, which will enable the values in the TIC control group to be modified. There are 3 values that you then need to set.

Minimum	Sets the value for the TIC above which the probe ramping is reduced. If the actual TIC seen from the instrument is below this value, the full heating rate, as programmed into the temperature ramp is used to heat the probe. As the TIC rises above this value, the heating rate is linearly adjusted down based on the difference between this minimum value and the maximum value discussed next. For example, a TIC value exactly between the maximum and minimum values would give a 50% rate compared to that requested by the ramp parameters.
Maximum	Sets the value for the TIC at which the probe ramping is suspended. This is done because the system is trying to keep the TIC at this level and further heating would cause it to rise above it. If the TIC reaches this level, heating will not recommence until it falls back down below
Finish	Sets a value for the TIC below which the acquisition of data will terminate. The temperature program will continue however to allow any remaining sample to be burnt off.

### **DCI Probe**

A DCI probe can be controlled in the same way as a solids probe, as described above with the exception that the ramp is programmed for Current rather than temperature and the DCI current can be stored with the data file, not the probe temperature.

# **Thermospray Probe**

A thermospray probe can be controlled in the same way as a solids probe, as described above, with the exception that the thermospray nozzle temperature can be stored instead of the probe temperature.

# RoboProbe

A robotic probe system can be used in conjunction with a CE Instruments A200S auto injector, or a Zymark laboratory robot.

#### **CE Instruments A200S**

If the A200S is used then setting it up is done as described earlier.

#### Zymark Labmate

The Zymark setup editor allows you to select a solvent for your sample.

1. Choose Methods, Inlet from the Acquisition Control Panel menu

or

Double click on the Picture of the auto injector on the Acquisition Control Panel to bring up the Zymark editor shown below.

2. Make any changes to the parameters.

Save the method using either Save or Save As... from the File menu.

🕂 Zymark Setup - I	DEFAULT	.RTS		×
<u>F</u> ile <u>H</u> elp				
	_ Solvent <sup>™</sup>	Туре —		
🗖 Use Solvent	ΘA	С <u>в</u>	<u>0</u> 0	

Figure 9.15 Zymark Solvent Selection Editor

# **Contact Closure**

Contact closure is a common method of providing start/stop control of an external inlet system. Many chromatographs, both LC and GC support contact closure because it is often used to provide control of an integrator unit. MassLynx uses essentially the same method of synchronization for acquiring data, the mass spectrometer's control unit using the start and stop signals produced by the chromatography system to start and stop data acquisition.
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