

HP 1100 Series  
Combinatorial  
Chemistry  
Analysis System

Guidebook for Installation  
and Familiarization

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Hewlett-Packard GmbH  
Chemical Analysis Group Europe  
Hewlett-Packard-Strasse 8  
D-76337 Waldbronn  
Germany

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HP 1100 Series Combinatorial Chemistry Analysis  
System

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Guidebook for Installation and  
Familiarization

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# About This Manual

This manual describes

- the hardware and software installation of the HP 1100 Series combinatorial chemistry analysis system including products INB22000 and INB22001,
- the familiarization with the combinatorial chemistry analysis software, and
- the system check-out.

The purpose of the manual is to give the service engineer an overview about the tasks that have to be accomplished before and during the installation.

The user of this handbook should be familiar with the following:

- setting parameters on the HPLC equipment,
- system control, data acquisition and data evaluation using the HPLC ChemStation.

## **Additional Information:**

Additional information can be retrieved from the product documentation that comes with the Gilson products.

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## **Introduction**

This chapter gives an overview of the HP 1100 Series Combinatorial Chemistry Analysis System.

## Introduction

The HP 1100 Series combinatorial chemistry analysis system is a fully automated analysis solution designed to speed up the process of drug discovery.

The system combines the HP 1100 Series modules and systems for liquid chromatography with a Gilson sampling injector, HP ChemStation and a dedicated software to provide a single point of control for sample analysis, optional fractionation, data trackability and data management.

The software runs within the Microsoft Windows 95 and NT environments and fulfills the special requirements of combinatorial chemistry analysis with the use of multiple 96-well plates for sampling and fraction collection, along with color-coded graphical sample and fraction tracking and data evaluation in a single screen.

The software has two modes.

- 1** In the sampler mode, samples can be injected from each of the 96-well plates and analyzed. The sampler mode is specifically designed for high-throughput analysis.
- 2** In the sampler/fraction collector mode, the sampler can take samples from each of the 96-well plates and collect fractions into other 96-well plates.

The software supports the following Gilson products

- the Gilson 233 XL sampling injector for sampling and fractionation
- the Gilson 215 liquid handler for sampling only

## Introduction

### Introduction

The HP 1100 Series combinatorial chemistry analysis system comprises the HP 1100 HPLC system with the HP ChemStation and a Gilson injection system. The recommended configuration of the system is:

- Quaternary pump or binary pump with degasser
- Thermostatted column compartment
- Diode-array detector (with standard flow cell)
- Autosampler (optional)
- LC/MSD (optional)
- HP ChemStation
- Gilson 233 XL sampling injector for sampling and fraction collection (optional) or
- Gilson 215 liquid handler for sampling
- Micro-plate sampling software to control the Gilson 233 XL and 215 from the HP ChemStation
- Interface module to allow sample fraction collection with a Gilson 233 XL

Introduction  
**Introduction**

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## **Site-Prep Information**

This chapter summarizes the tasks to be performed prior to the installation.

---

# Site-Prep Information

The steps below will ensure that the installation of the combinatorial chemistry analysis system products takes place smoothly.

- 1** Check that the order includes the HP 1100 system and/or the combinatorial chemistry analysis system products INB22000/22001. If yes, then proceed with step 2.
- 2** Check with the customer that the Gilson autosampler has already been installed, with the options required for Combinatorial Chemistry— see ordering guide 5965-8811E.

If yes, then proceed with step 3.

If no, then the Gilson autosampler must be installed by the Gilson representative first.

- 3** Ensure that a check-out column, e.g. cartridge column Hypersil ODS 125 x 4 mm (79926OD-564, holder 5021-1845) and the standard isocratic check-out sample (01080-68704) are available.

---

**NOTE**

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If the HP 1100 and/or LC ChemStation has to be installed, refer to the Site-Prep documents of those products.

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## **Pre-requisites**

This chapter describes the tasks that must be completed prior to the installation.

## Pre-requisites

The tasks listed below must be completed prior to starting the installation:

- Gilson Autosampler installed by Gilson representative.
- HP 1100 and HP ChemStation installed according to their installation documentation.
- Check-out cartridge column Hypersil ODS 125 x 4 mm (79926OD-564, holder 5021-1845) and the standard isocratic check-out sample (01080-68704) are available.
- **With order INB22000 (Gilson 233 XL and 215), the following parts are included:**
  - 3 capillaries (G1312-67305, 60 cm long, 0.17 mm i.d.) are available.
  - Control cable (HCP-CBLE-CS) from the ChemStation to the Gilson sampler (RS-232C).
  - Remote start cable (HCP-CBLE-RS) from the HP 1100 detector to the Gilson sampler.
  - Software for Windows 95/NT ChemStation on 4 HD diskettes.
  - Operating Manual
- **With order INB22001 (Gilson 233 XL only), the following parts are included:**
  - Interface Module with real time clock option.
  - Power Supply (Western Europe, UK, or US).
  - Terminal cable (HCP-CBLE-TM) from the ChemStation to Interface Module.
  - Analog cable (HCP-CBLE-AN) from the HP 1100 detector to the Gilson sampler.
  - Control cable (HCP-CBLE-CO) from the Interface Module to the Gilson sampler (RS-232C).

---

## **Hardware Installation of the Gilson 233 XL Autosampler**

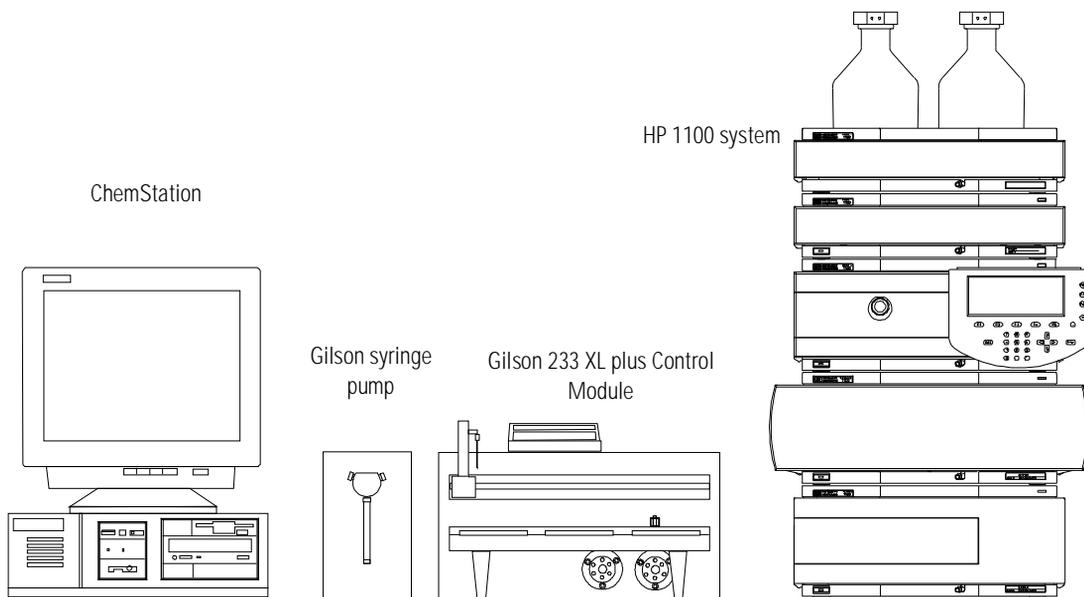
This chapter describes the installation of the combinatorial chemistry analysis system hardware.

## Hardware Installation of the Gilson 233 XL Autosampler

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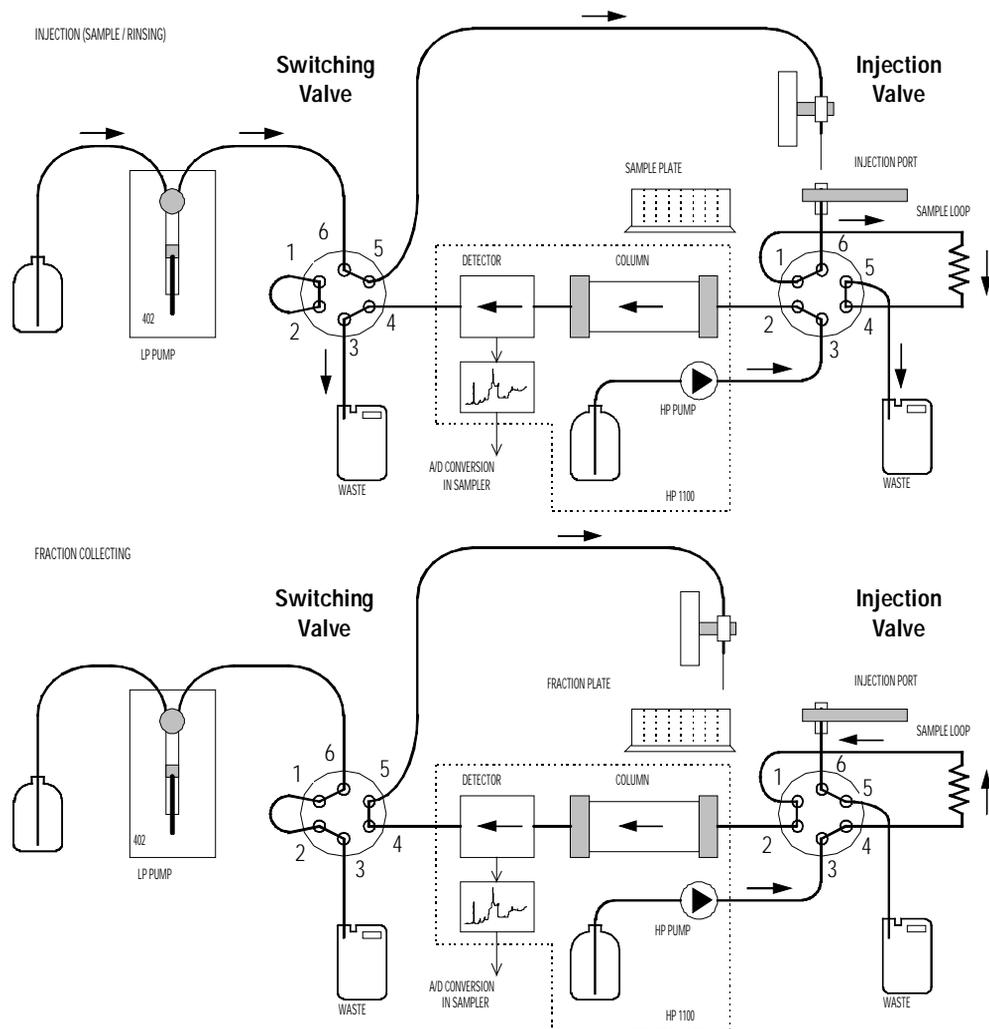
## Arranging the Instruments on the Bench

The figure below shows the recommended arrangement on the bench for the HP 1100 and the Gilson 233 XL Sampling Injector for the sampling and sampling/fraction collection mode. The Gilson 402 syringe pump may be placed on the 233 XL to save space.



## Hydraulic Flow Path

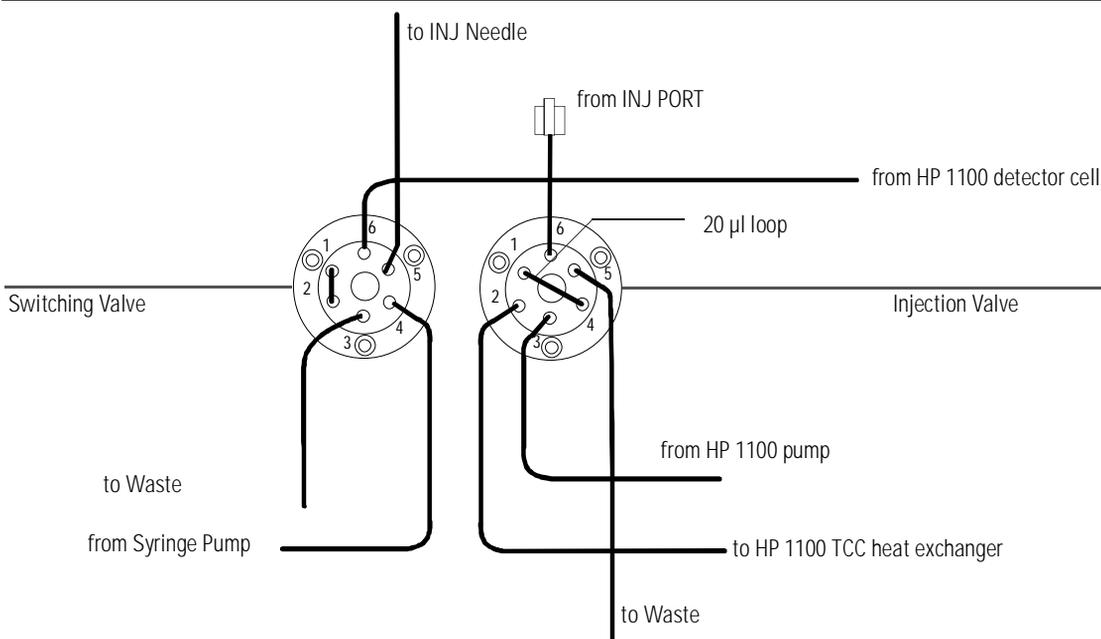
The schematic below shows the hydraulic flow path for both the sampler and sampler/fraction collector modes. A schematic of the valve connections is shown on page 23 in more detail.



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## Valve Connections at the Gilson 233 XL Sampling Injector

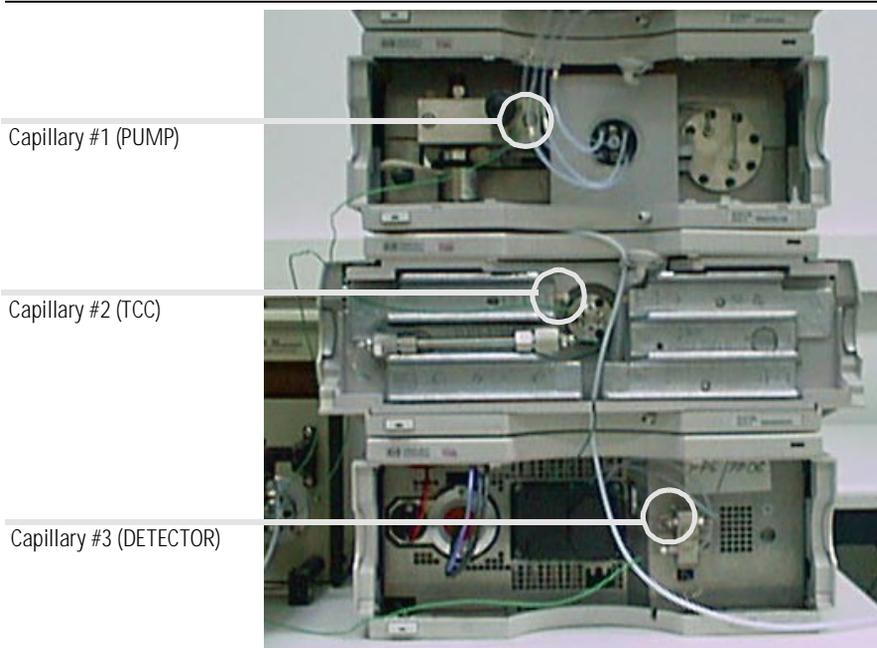
The Gilson 233 XL is equipped with two valves. The right hand valve is the injection valve, which is used to inject the sample. The left hand valve is the switching valve, which is used for fraction collection. The schematic below shows the connections at the injection valve and the switching valve in more detail.



---

## Connecting Capillaries to the HP 1100

- o Use the three capillaries (60 cm, 0.17 mm i.d.) from the accessories of the INB22000.
- o Connect capillary #1 to the HP 1100 pump outlet. If the capillary from the pump to HP 1100 thermostatted column compartment (TCC) heat exchanger was already installed, remove this capillary.
- o Connect capillary #2 to the heat exchanger inlet.
- o Connect capillary #3 to the outlet of the HP 1100 detector flow cell at the union holder. If the capillary flow cell outlet to waste was already installed, remove this capillary.

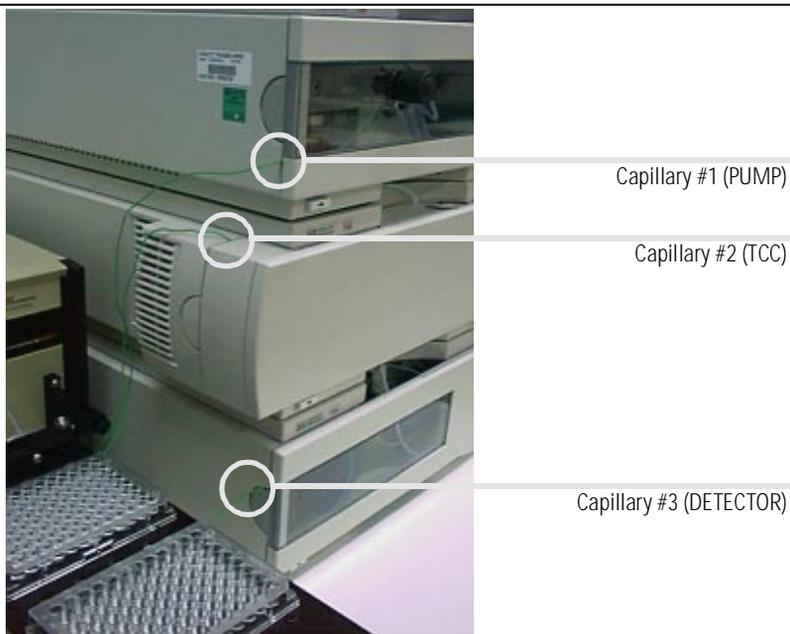


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## Routing Capillaries through the Cover

The front covers of the HP 1100 modules have openings at the side for routing capillaries on the shortest way out of the instrument while the cover is closed.

- o Route all three capillaries to the left of each module so that they fit through the openings of the covers.



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## Removing the Gilson 233 XL Microplate Holder

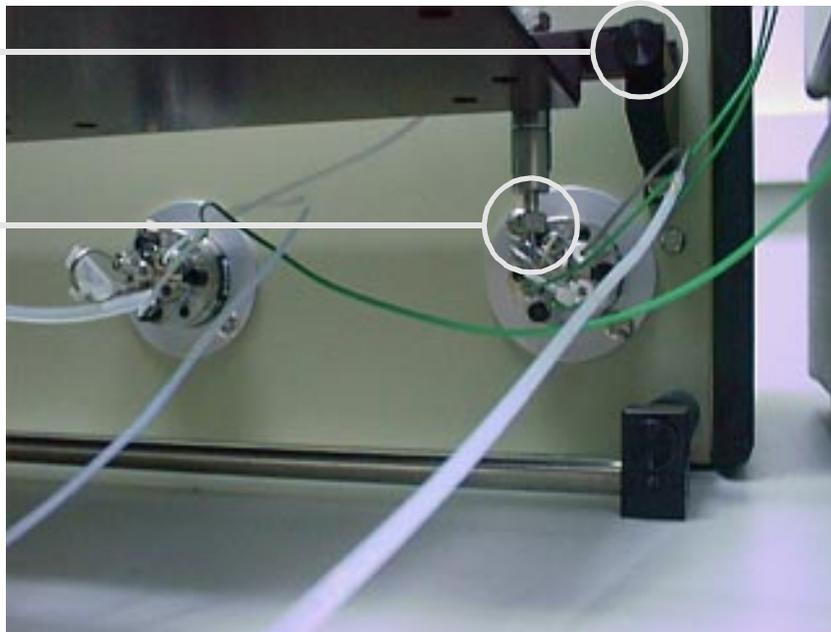
For easier installation of the capillaries at the valves of the Gilson 233 XL sampling injector the microplate holder should be removed.

- Disconnect the injection port (right valve port 6 injection valve).
- Unscrew the two thumb screws (one is shown below, the other is on the left side of the tray).
- Remove the holder and place it somewhere safe.

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Thumb screw, 2nd screw is  
on the left tray side

Injection port

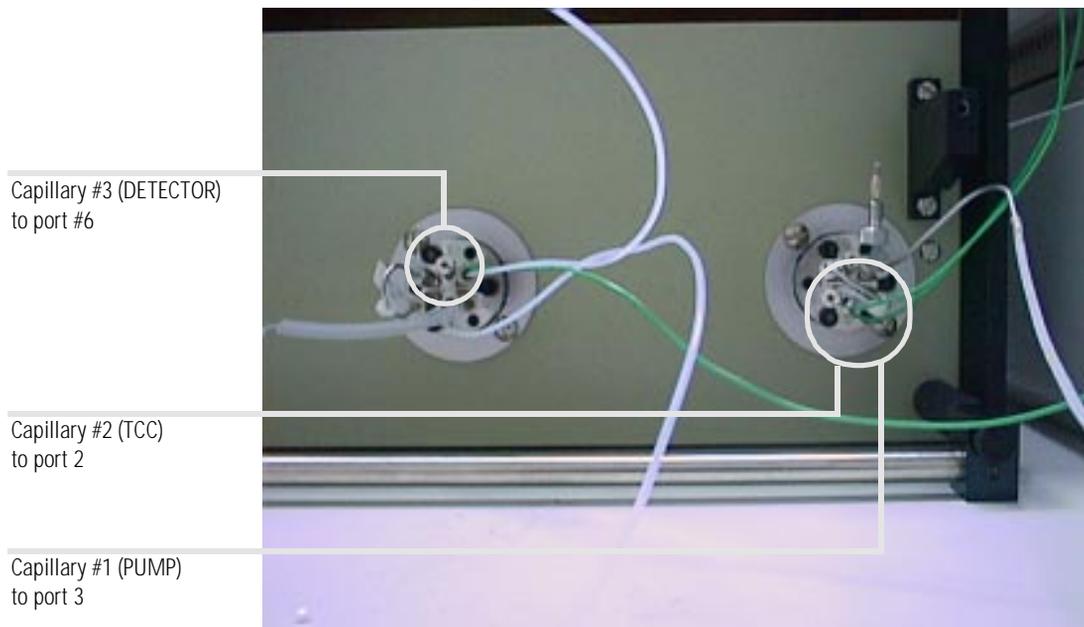


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## Connect the HP 1100 Capillaries to the Gilson 233 XL Valves

Next the connections to the injection valve (right valve) and the switching valve (left valve) have to be made. Refer to the hydraulic connection diagram [on page 22](#) and the valve connection diagram [on page 23](#).

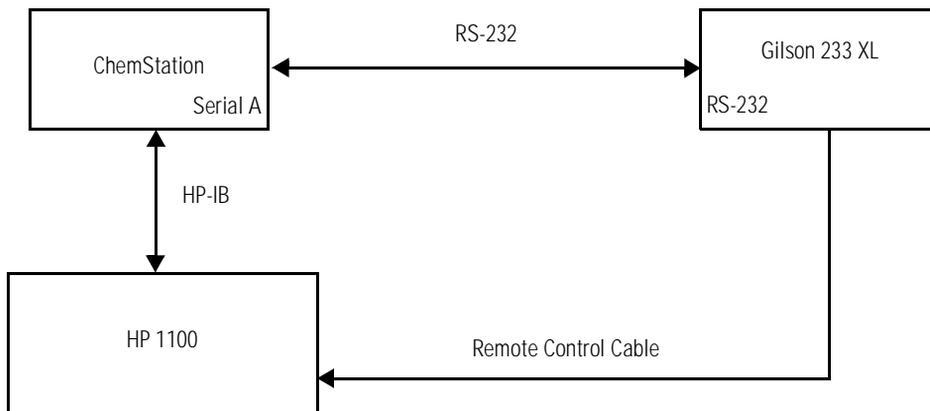
- Connect capillary #1 (PUMP) to port 3 of the injection valve.
- Connect capillary #2 (TCC) to port 2 of the injection valve.
- Connect capillary #3 (DETECTOR) to port 6 of the switching valve.



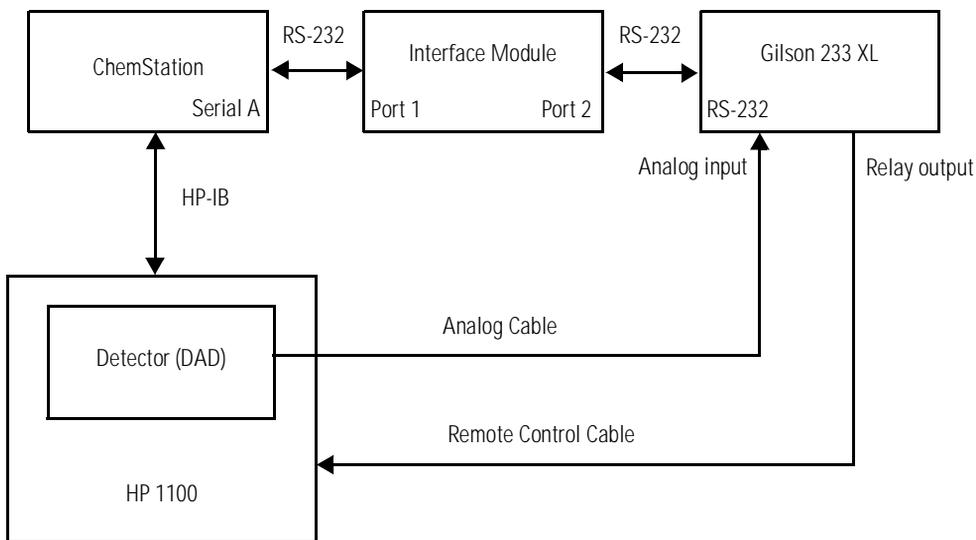
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## Electrical Connections with Gilson 233 XL

### Sampler Mode



### Sampler and Fraction Collection Mode

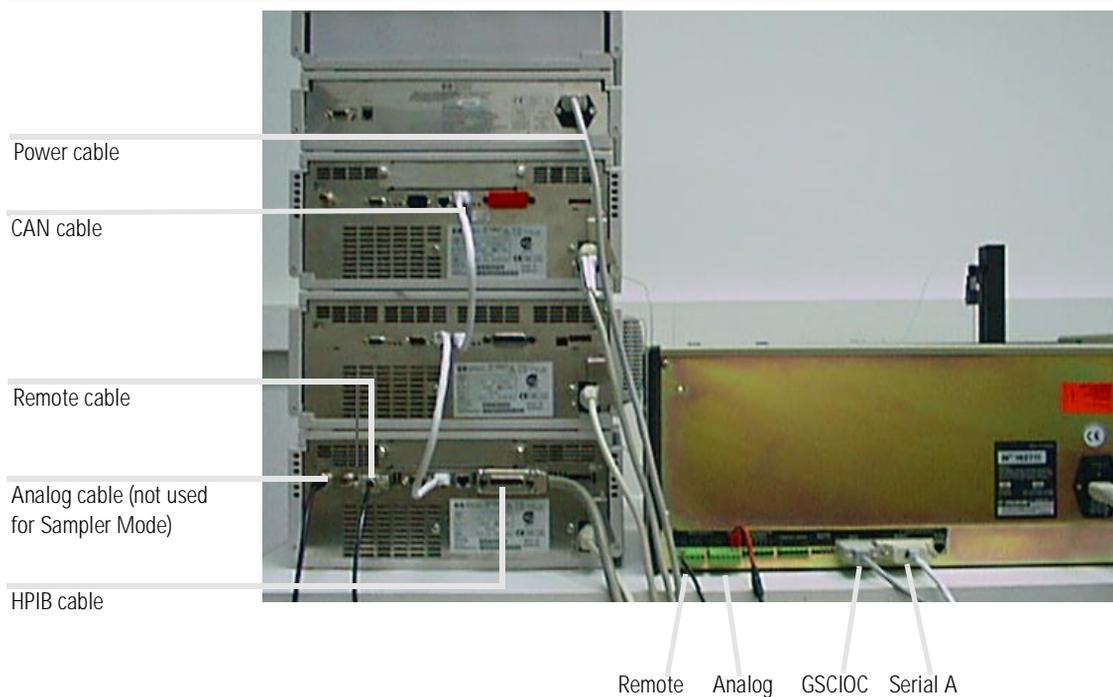


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## Electrical Connections at the HP 1100

Now make the electrical connections between the HP 1100, the Gilson 233 XL injector, the Gilson 402 Syringe Pump and the HP ChemStation.

- o Connect the CAN cables between each HP 1100 module.
- o Connect the power cables to each HP 1100 module.
- o Connect the HP-IB cable to the HP 1100 detector and the HP ChemStation.



## Electrical Connections at the Gilson 233 XL (Sampler Mode)

- Connect the power cable to Gilson 233 XL.
- Connect the power cable to the Gilson 402 Syringe Pump.
- Connect the Gilson 402 Syringe Pump to the 233 XL with the GSIOC cable.
- Connect the direct control cable (HCP-CBLE-CS) from a free COMx on the HP ChemStation to the RS-232C receptacle of the Gilson 233 XL autosampler.

---

### NOTE

This COMx must also be set in the CombiChrom software.

- Connect the remote start cable (HCP-CBLE-RS) from the HP 1100 detector to the Gilson 233 XL autosampler.

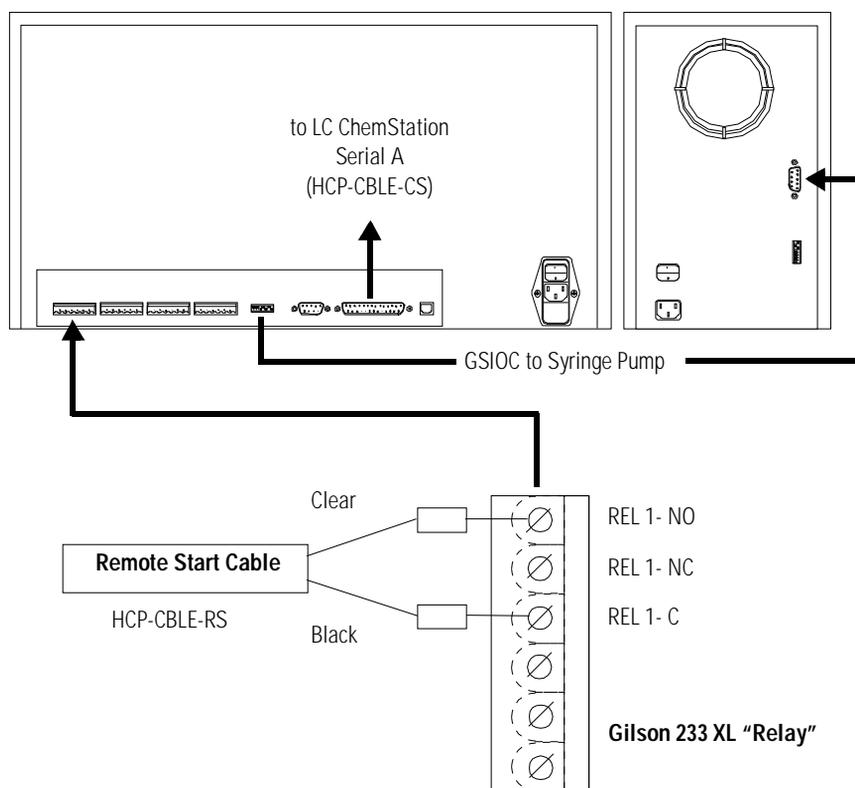


GSIOC

HPIB

Serial A

Hardware Installation of the Gilson 233 XL Autosampler  
Electrical Connections at the Gilson 233 XL (Sampler Mode)



## **Electrical Connections at the Gilson 233 XL (Fractionation Mode)**

- o Set the power switch of the Interface Module in the OFF position (down) and connect the power adapter.

---

**NOTE**

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Check for proper mains rating.

- o Connect the terminal cable (HCP-CBLE-TM) from a free COMx on the ChemStation to the PORT-1 of the Interface Module.

---

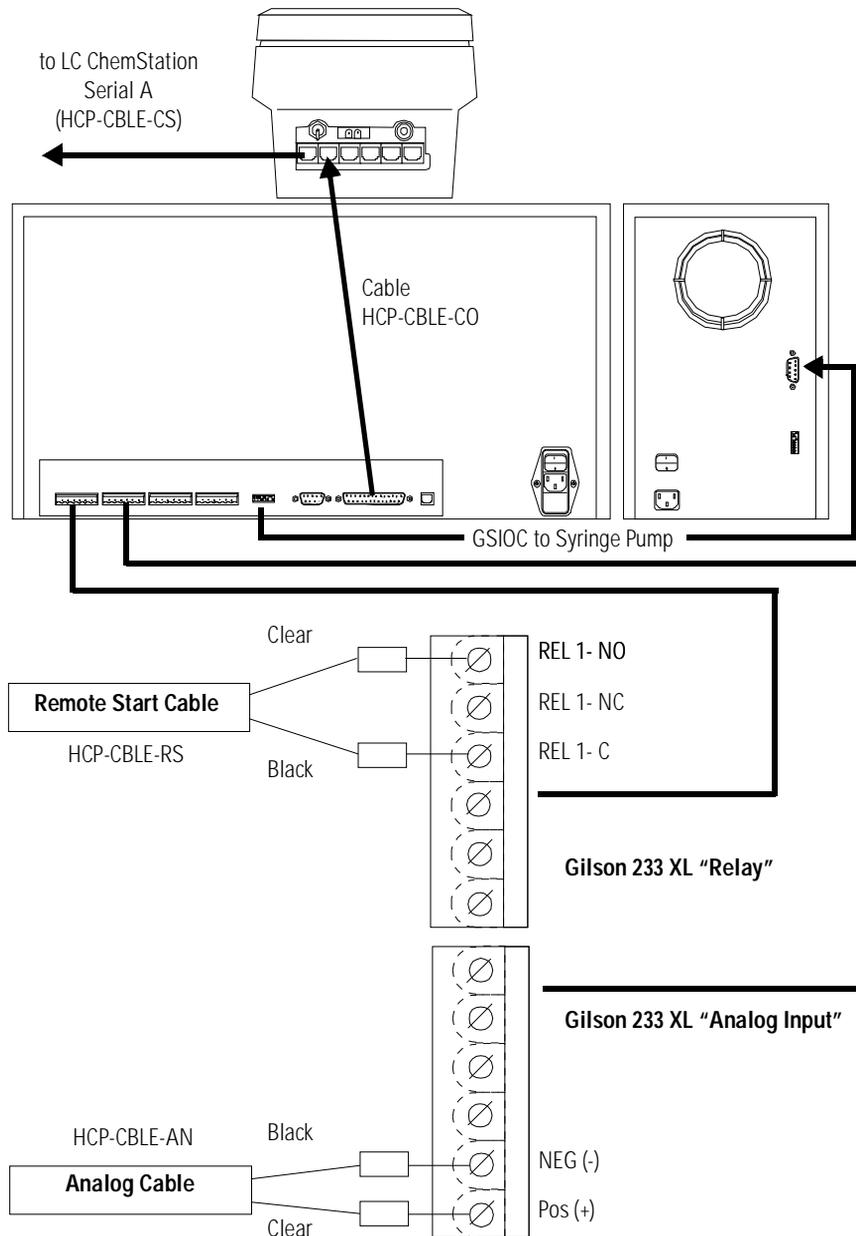
**NOTE**

---

This COMx must also be set in the CombiChrom software.

- o Connect the control cable (HCP-CBLE-CO) from PORT-2 of the Interface Module to the RS-232C receptacle of the Gilson 233 XL autosampler.
- o Connect the analog cable (HCP-CBLE-AN) from the HP 1100 detector to the Gilson 233 XL autosampler.
- o Connect the remote start cable (HCP-CBLE-RS) from the HP 1100 detector to the Gilson 233 XL autosampler.

## Hardware Installation of the Gilson 233 XL Autosampler Electrical Connections at the Gilson 233 XL (Fractionation Mode)



Hardware Installation of the Gilson 233 XL Autosampler  
Electrical Connections at the Gilson 233 XL (Fractionation Mode)

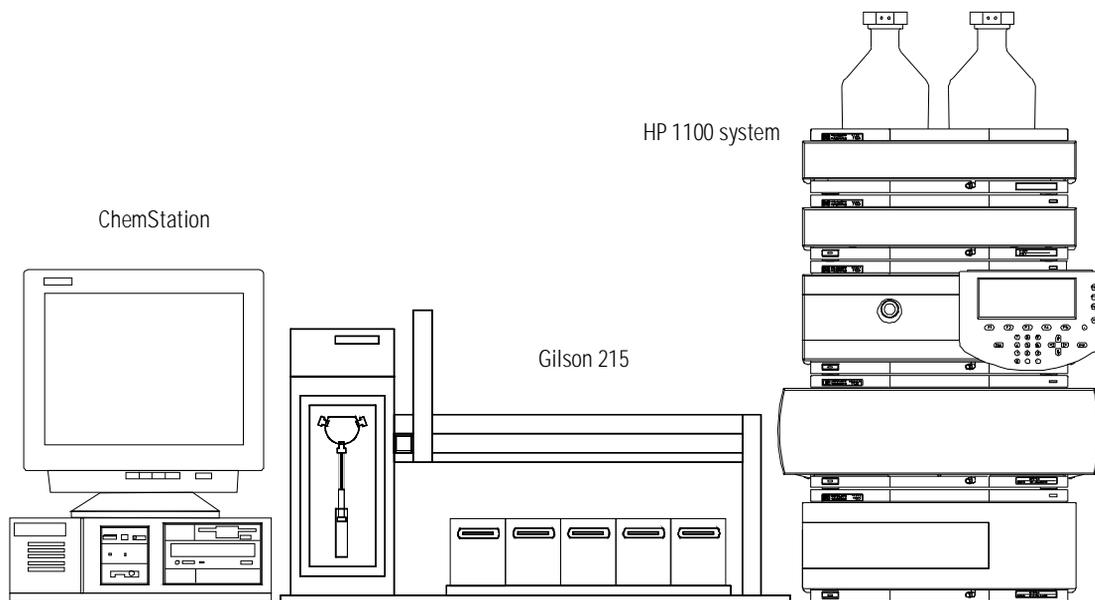
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## **Hardware Installation of the Gilson 215 Liquid Handler**

This chapter describes the installation of the combinatorial chemistry analysis system hardware.

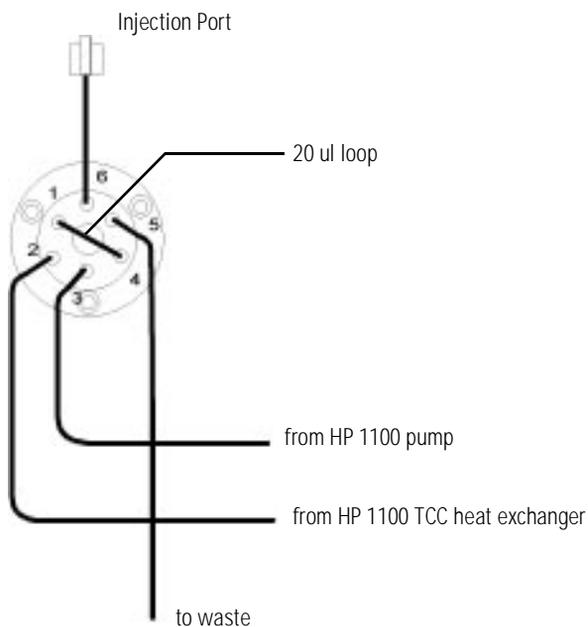
## Arranging the Instruments on the Bench

The figure below shows the recommended arrangement on the bench for the HP 1100 and the Gilson 215 Liquid Handler for the sampling mode.



## Valve Connections at the Gilson 215 Liquid Handler

The Gilson 215 is equipped with an 819 valve actuator which is used to inject the sample. The schematic below shows the connections at the injection valve in more detail.



## **Connecting Capillaries to the HP 1100**

Refer to “Connecting Capillaries to the HP 1100” on page 24 and “Routing Capillaries through the Cover” on page 25.

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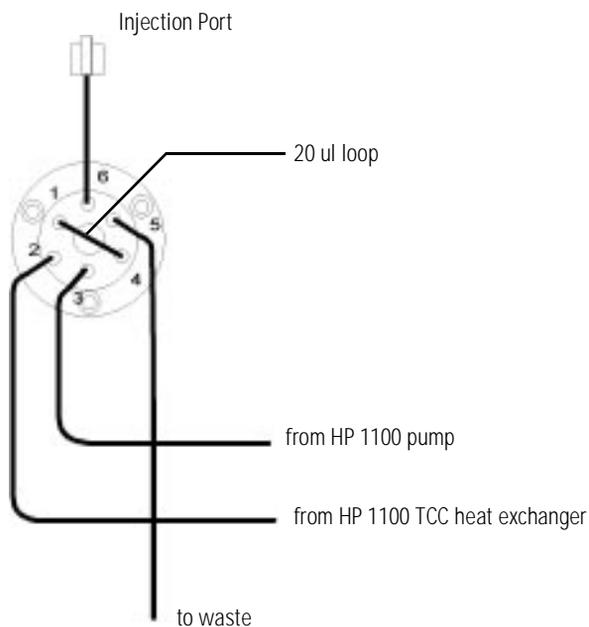
**NOTE**

For the 215 Liquid Handler only two capillaries are used (#1 and #2).

## Connect HP 1100 Capillaries to the Gilson 819 Valve Actuator

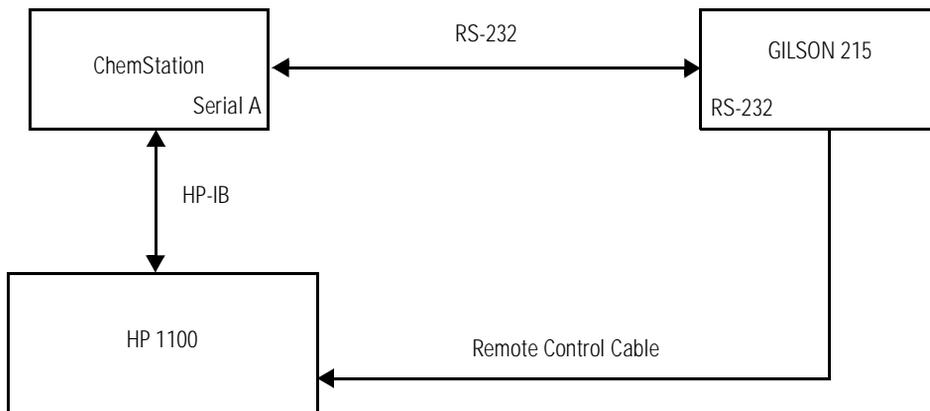
Next the connections to the injection valve have to be made.

- o Connect capillary #1 (PUMP) to port 3 of the injection valve.
- o Connect capillary #2 (TCC) to port 2 of the injection valve.



## Electrical Connections with Gilson 215

### Sampler Mode



## **Electrical Connections at the HP 1100**

Now make the electrical connections between the HP 1100, the Gilson 215 injector and the HP ChemStation.

- o Connect the CAN cables between each HP 1100 module.
- o Connect the power cables to each HP 1100 module.
- o Connect the HP-IB cable to the HP 1100 detector and the HP ChemStation.

Refer to “Electrical Connections at the HP 1100” on page 29.

## Electrical Connections at the Gilson 215 Liquid Handler

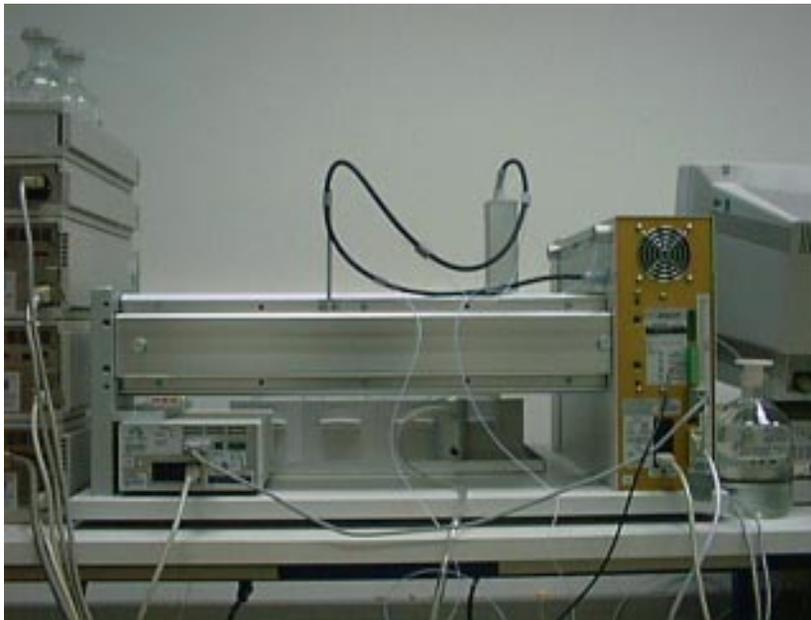
- Connect the power cable to Gilson 215 injector.
- Connect the power cable to the Gilson 819 Valve Actuator.
- Connect the Gilson 215 injector to the 819 Valve Actuator with the GSIOC cable.
- Connect the direct control cable (HCP-CBLE-CS) from a free COMx on the HP ChemStation to the RS-232C receptacle of the Gilson 215 injector.

---

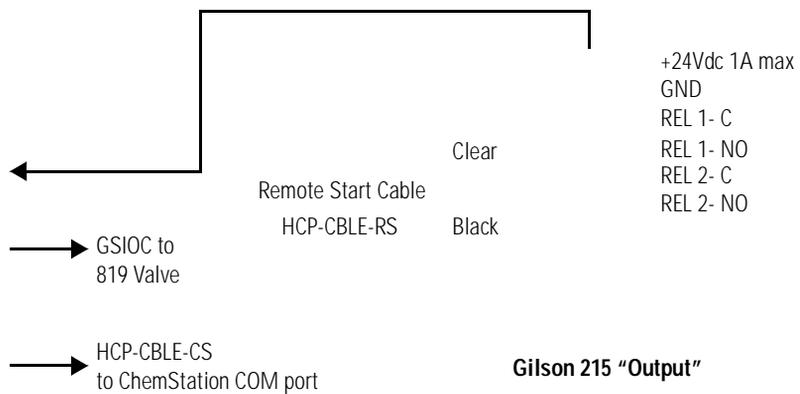
### NOTE

This COMx must also be set in the CombiChrom software.

- Connect the remote start cable (HCP-CBLE-RS) from the HP 1100 detector to the Gilson 215 injector.



Hardware Installation of the Gilson 215 Liquid Handler  
Electrical Connections at the Gilson 215 Liquid Handler



Hardware Installation of the Gilson 215 Liquid Handler  
**Electrical Connections at the Gilson 215 Liquid Handler**

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## **CombiChrom Software Installation**

This chapter describes the installation of the combinatorial chemistry analysis system software.

## **Pre-requisites**

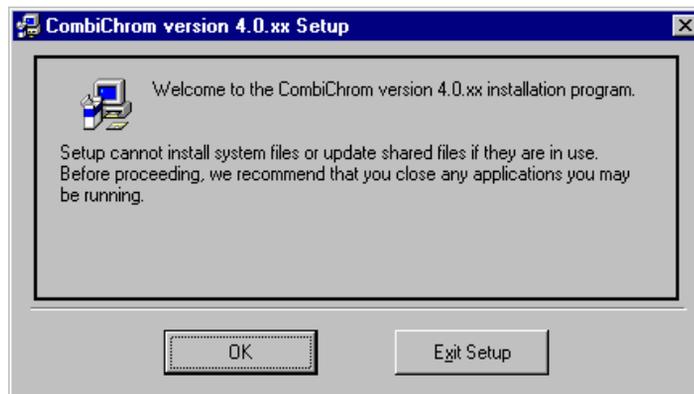
The tasks listed below must be completed prior to starting the software installation:

- o All hardware must have been installed (HP 1100, INB22000/22001, Gilson autosampler).
- o All electrical connections must have been made.
- o HP ChemStation software A.05.XX must be loaded to the PC and ChemStation should be operational.
- o Turn down (off?) the ChemStation.

## CombiChrom Software Installation

The CombiChrom software is distributed on four High Density disks. This section describes the step-by-step software installation.

- 1 Check that you have a complete set of distribution disks—numbered from one through four.
- 2 Start Windows 95 / Windows NT 4.0 and close all running application programs.
- 3 Insert disk 1 into the floppy drive (A:).
- 4 With the mouse pointer positioned over MY COMPUTER, click on the right mouse button and start EXPLORE. Select the '3½-Floppy', and the contents of drive a should appear.
- 5 Double click on A:\setup.exe. Setup will start and the following window will appear:



- 6 Accept OK to start the installation of CombiChrom 4.0.xx.

CombiChrom Software Installation  
CombiChrom Software Installation

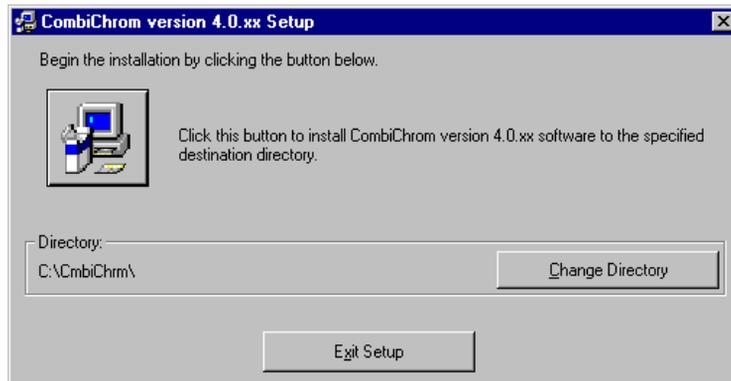
- 7 The software default directory is c:\cmbichrm. To accept this click the button indicated, or click to Change Directory.

---

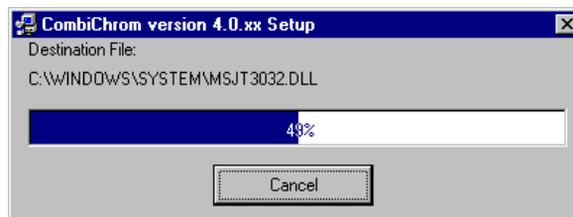
**NOTE**

---

If you wish to change the directory, the name of the install directory must not be longer than 8 characters, and needs to be a valid file name.



- 8 Setup starts copying files from the setup-disks to the destination directory. The progress of this operation is displayed in the bar as shown below.



- 9 Setup will prompt you to insert the next installation disk (2..4). Follow these instructions until installation is completed.



## Start-up Sequence

### Sampler Mode

- It is now assumed that the hardware and software installation have been successfully completed.
- 1 Start-up the HP 1100 system.
  - 2 Turn on the Gilson autosampler and the Gilson syringe pump.
  - 3 Start the HP ChemStation

---

#### NOTE

---

The HP Chemstation must be turned on after the Gilson autosampler and syringe pump.

“CombiChrom” should appear In the item menu of the menu bar.

### Sampler and Fractionation Mode

- It is assumed that the hardware and software installation have been successfully completed.
- 1 Start-up the HP 1100 system.
  - 2 Turn on the Gilson autosampler, Gilson syringe pump and Interface Module. The Interface Module should display firmware revision 6.23 and must be in TERMINAL Mode.
  - 3 Start the HP ChemStation.

In the menu bar the item “CombiChrom” must appear.

- 4 Select this item to start CombiChrom.

When the Interface Module has cycled power before this step, a progress bar should appear in CombiChrom. This indicates the Interface Module is downloading its control program (BASIC). After completion the Interface Module starts initializing the sampler and syringe pump.

---

## Changing between Modes

By default, CombiChrom is configured to Sampler only mode. This mode needs the direct control cable (HCP-CBLE-CS) to work properly. Also the COM1 serial port of the HP ChemStation is used as default setting. The Sampler only mode does not allow fraction collection.

---

## Upgrading to Fractionation Mode

To enable the collection of fractions, the Interface Module must be present. With the Interface Module, the Sampler only mode remains available. To use CombiChrom with the Interface Module the following steps need to be completed:

- 1 Start CombiChrom from the menu item in the HP ChemStation and select Configuration, then System. From this menu select “sampler / fractionator mode” and accept by clicking on OK. CombiChrom must be restarted to enter the sampler / fractionator mode. Also the Interface Module must be installed as described in the previous section. CombiChrom communicates to the autosampler using the Interface Module in both modes.
- 2 CombiChrom can now operate in the two different modes. Select the required mode using the “Sampler Method” tab.

---

### NOTE

Changing the mode here does not require CombiChrom to be restarted!

**CombiChrom Software Installation**  
**Upgrading to Fractionation Mode**

---

## **CombiChrom Software Familiarization**

This chapter describes the familiarization of the HP 1100 Series combinatorial chemistry analysis software.

## **Overview**

This chapter describes the CombiChrom software, and includes

- starting CombiChrom from the Method & Run Control screen of the HP ChemStation.
- description of the Menu Bar items
- description of the Tool Bar items, and a
- description of the TAB screens.

## Start CombiChrom from the Method & Run Control Screen

The standard HP ChemStation for LC has been extended with the addition of a dedicated software which runs within the Microsoft® Windows 95 and NT environments.

The software can be accessed from the Method & Run Control screen of the HP ChemStation (Figure 1).

**Figure 1**                      **The Method & Run Control Screen**



---

## Description of the Menu Bar Items

### Navigation Overview

**Table 1**

**Navigation Overview**

File	Configuration	Sampler	Help
New Study	System	Switch Valve On	Contents
Open Study	Sampler Type	Injection Valve On	About CombiChrom
Save Study	Gilson 233 XL	Syringe Pump Rinse	
Delete Study	Gilson 215	Stroke Volume	
Delete Sampler Method	Communication	Rinse Flow	
Import	Com 1 - Com 4	Reset Sampler	
Export	Sampler Only Mode		
Print	Sampler/Fractionator		
Exit	Injector Constants		
	Plate Format		
	Dead Volume		
	Syringe Volume		
	Enable Pop Up Plot		

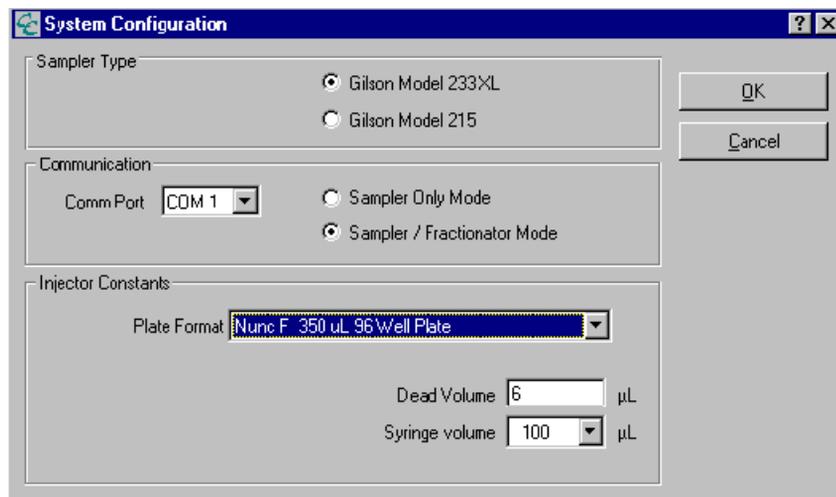
### Configuration

Once the CombiChrom software is running, the user needs to configure the system. This is done by selecting "Configuration" from the main screen. Figure 2 shows the options available to the user in this menu. The user has a choice to use the system as a sampler, or as a sampler and fraction collector, and this is done by selecting a sampler type and either "Sampler Only Mode" (Gilson 233 XL and 215) or "Sampler/Fractionator Mode" (Gilson 233 XL only).

CombiChrom Software Familiarization  
Description of the Menu Bar Items

Ensure the correct port is selected to match the cable to the Gilson sampler.

**Figure 2** System Configuration



The software has two modes. In the sampler mode, which is specifically designed for high-throughput analysis, samples can be injected from each of the 96-well plates. In the sampler/fraction collector mode, the sampler can take samples from each of the 96-well plates and collect fractions into other 96-well plates.

Next, the user needs to select the type of plate that is to be used—either microtiter plates or deep well plates (see Figure 2 “Plate Format”). This screen also enables selection of the syringe size for the Gilson 402 syringe pump and of the 215 dilutor (default size: 100 µl), which is useful when injecting higher sample volumes. In this case the user needs to change to a higher volume sample loop (default 20 µl) of the Gilson autosampler.

The dead volume is used for fraction collection and is the volume of the Teflon tubing which connects the detector cell outlet with the switching valve of the Gilson 233 XL (default volume = 2 µl).

---

**NOTE**

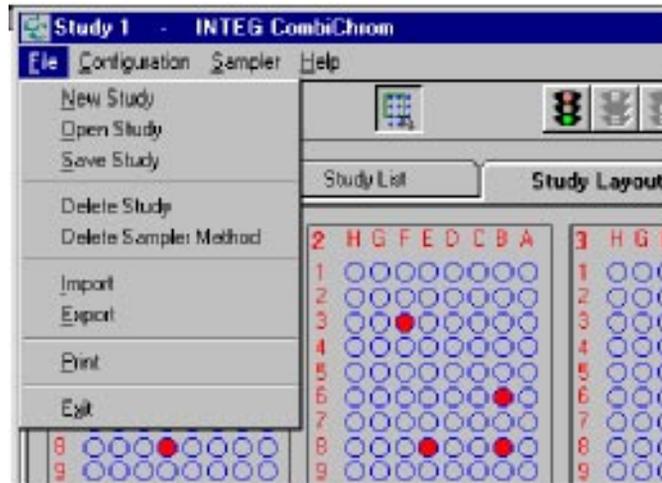
---

System configuration is only necessary the first time the system is used, and is not necessary at other times unless changes are required, e.g. to select other types of well plates.

### File

Once configured the user has the choice of opening a new study or an existing study by selecting "File" then "New Study" or "File" then "Open Study" (Figure 3).

**Figure 3** Access to Studies, Import/export and Print of Data



CombiChrom Software Familiarization  
Description of the Menu Bar Items

If a new study is requested, then the user is prompted for a study name (Figure 4).

**Figure 4**                      **Create a New Study**

Alternatively you can open an existing study (Figure 5).

**Figure 5**                      **Open an Existing Study**

The “Save Study” is used to write a modified study to disk.  
The user can also delete Studies and Sampler Methods.

---

**NOTE**

Data in CSV format can be imported from external applications, like Microsoft Excel, to create sample lists. Data from Fraction lists can be exported to CSV files to be processed by other applications. Sample and fraction data can also be printed (see “Print Sample/Fraction List” on page 118).

### Sampler

This menu gives the option of flushing the tubings of the Gilson samplers with solvent or to replace the solvent by another one using the 402 syringe pump of the 233 XL or the diluter of the 215. The software provides several maintenance functions for controlling the Gilson autosampler. These functions are invoked from the sampler menu (Figure 6).

Figure 6

### Menu Bar Sampler

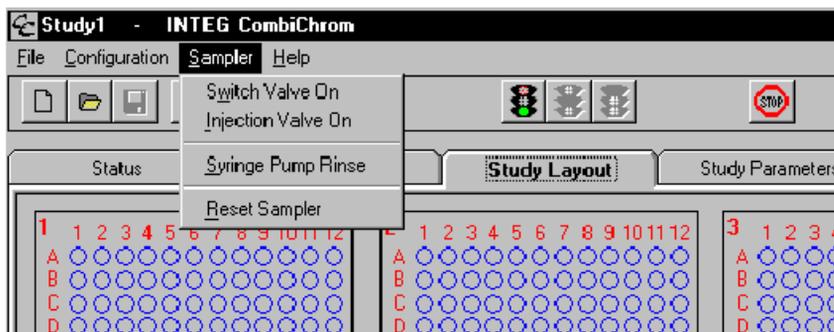
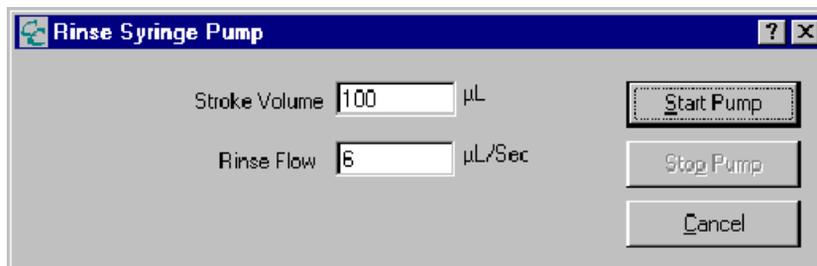


Figure 7 shows the screen to select the conditions for the Gilson 402 syringe pump and 215 diluter for tubing rinse.

Figure 7

### Rinse of the Sampler Tubings



The stroke volume cannot be higher than the syringe volume installed and configured in the Configuration screen. Rinse flow can be adjusted depending on the viscosity of the solvent.

### Help

The system is equipped with a detailed help mode which offers advice relating to all aspects of the software. This can be accessed either by selecting the help menu from the menu bar (Figure 8) or by clicking on the question mark symbol in the right corner of the tool bar and placing it on the section for which help is required (see next section).

Figure 8

### Help Information



---

## Description of the Tool Bar Items

Figure 9

Tool Bar



By clicking on one of the tools several functions can be invoked. The following table explains the functions of each tool:

---

	New Study	Creates a new Study.
	Open Study	Loads existing Studies.
	Save Study	Saves the current study to the disk.
	Print	The Samples and Fractions are printed according to the Sample and Fraction List.
	View Mode	Button up is the <b>view mode</b> . Single wells can be clicked on with the mouse pointer and the relation between samples and fractions are made visible. Sample information is presented on the Status bar. (bottom of CombiChrom Form).
	Select Mode	Button down is the <b>select mode</b> . This mode is used to add to or remove samples from the Study
	Start Sequence	This button starts the sequence. You have the following choices: <ul style="list-style-type: none"><li>• to run whole sequence</li><li>• to run partial sequence</li><li>• to resume paused sequence</li><li>• to run fraction sequence</li><li>• to re-analyze whole sequence</li><li>• to re-analyze partial sequence</li><li>• to resume paused ReAnalyse</li></ul>
	Pause Sequence	This pauses the sequence after the current sample analysis has finished.

## CombiChrom Software Familiarization

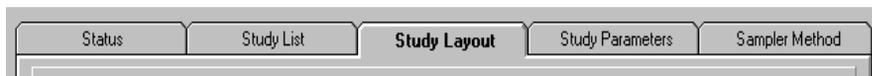
### Description of the Tool Bar Items

---

	Stop Sequence	This stops the sequence after the current sample analysis has finished.
	Abort	Aborts all activities.
	Help	With the Help Button you invoke context sensitive help. To get context sensitive help: click this button (with the left mouse button), move the cursor over the item to be queried, and click the left mouse button again.

---

## Description of the Tab Screens



### Navigation Overview

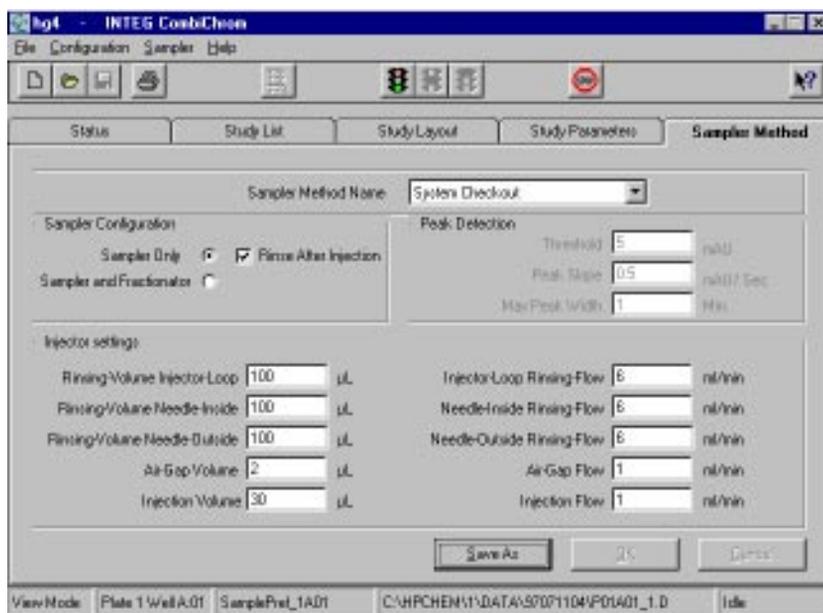
**Table 2** Navigation Overview

Status	Study List	Study Layout	Study Parameters	Sampler Method
Status	Sample	Add Samples to Study	Method Selection	Sampler Method Name
ChemStation	Partial Sequence	Remove Samples	ChemStation Method	Sampler Configuration
Sampler	Location	Undo Selection	Study Layout	Sampler Only
Current Sequence	Sample Name		# of Sample plates	Sampler/Fractionator
Sample	Data File Name	Add Fractions to Study	# Fraction plates	Peak Detection
Study	# Injection	Remove Fractions	Sample Name Format	Threshold
Sample Name	Method	Undo Selection	Name Prefix+Coordinates	Peak Slope
Well Position	Fractions		Sample Plate ID	Max. Peak Width
Data File	Sample Name		# Injections	Injector Settings
	Sample Location		Sequence	Rinse Volume/Speed
	Peak #		Shut Down the instrument	Needle Inside/Outside
	Fraction Location		Operator	Airgap Volume
			Auto Sub Dir	Injection Volume
			SubdirReport Options	

## Sampler Method screen

Figure 10

### Sampler Method Screen



### Sampler Method Name

This field shows the name of the current (active) Sampler Method.

By clicking the 'Drop Down' Button the user can choose from a list with available Sampler Methods.

### Sampler Configuration

The user can choose whether to have "Sampler Only" (233 XL and 215) or "Sampler and Fractionator" (233 XL) (Figure 10).

In the sampler mode, "Rinse after Injection" can be selected. The needle and injection port are rinsed immediately after injection of the sample which saves time especially when analyzing samples without column (flow injection analyses).

### **Sampler Only (233 XL and 215)**

Select this mode if the sampler is to be used for sampling only. In this setting, the peak parameter settings are disabled.

### **Sampler and Fractionation (233 XL only)**

Select this mode if you want to collect fractions. In this setting, the peak parameter settings are enabled.

### **Rinse after injection**

When 'unchecked' the sampler rinses the needle and injection port before each injection.

When 'checked' the sampler rinses the injection port and needle right after each injection.

Before the first sample of the sequence is injected, the port and needle are rinsed.

## **Setting the Peak Detection Criteria using 233 XL in Sample/Fractionator Mode**

To detect the peak-start and peak-end, three parameters are entered in the sampler defaults area which define the criteria. These are Threshold, Peak Slope and Max Peak Width. The analog output signal of the detector is continuously monitored by the Interface Module. The peak-start and peak-end times are stored in the Interface Module. These times are sent to the HP ChemStation when the run has ended. Each new detected peak is collected in a new well. Multiple wells can be filled from the same peak.

### **Peak threshold**

This value is relevant for the peak-start and peak-stop detection. For the peak-start criterion, the peak signal must exceed the threshold before the peak is detected. The peak-stop criterion is met when the peak signal drops below the threshold.

### **Peak slope**

The Interface Module monitors the slope of the peak signal by calculating the first derivative out of the last five measured analog values of the peak. The peak-start criterion is met when the peak signal exceeds the threshold AND the slope is equal or greater than the peak slope setting.

### **Peak width**

Sometimes the baseline of the chromatogram exceeds the threshold value. In this situation the peak-stop criterion is never met. To eliminate this problem, the peak width parameter is introduced. The peak width is defined from the peak-start detection in minutes. If the current peak width exceeds the peak width value, the peak is considered to have reached the end.

### **Peak-Start criterion**

Signal  $\geq$  Peak Threshold AND Signal Slope  $>$  Slope Setting

### **Peak-Stop criterion**

Signal  $<$  Threshold OR Peak Width  $>$  Peakwidth Setting

## **Injector Settings**

### **Optimizing Performance**

This chapter describes how to optimize the autosampler to achieve best chromatographic results.

Use the following guidelines to minimize sample consumption, to eliminate cross-contamination and to maximize injection volume precision, without sacrificing analysis time.

Some of the parameters are entered during the configuration process. The software offers default values according to the type and configuration of your system. Other parameters are submitted with the tasks, as you enter your Method Program.

### **Automated Needle Wash**

In some instances, sample carryover may become significant when using small injection volumes or injecting a sample of low concentration immediately after a sample of high concentration. The automated needle wash moves the needle into the rinsing station and the injection port between injections, reducing the carryover to a minimum.

The automated needle wash can be programmed either as "Rinse After Injection". In this case the needle is washed immediately after sample injection to reduce cycle time. If this mode is not selected the needle and the injection port are washed before the next sample is drawn from a well. The main contribution to carryover comes from sample on the outside of the

## Description of the Tab Screens

needle. By washing the needle after drawing sample, the sample is removed from the surface of the needle immediately. When the injection valve switches to main pass, all of the sample in the sample loop and needle is flushed onto the column. This combination of needle wash and flow through design reduces carry over to an absolute minimum.

### Draw and Eject Speed

- Sample and Solvents
- The speed of the draw and eject cycles of the syringe can be adjusted for sample and solvent viscosity. When using viscous samples or solvents, it may be beneficial to reduce the speed of the syringe in order to improve injection volume precision.

The speed at which the syringe draws sample out of the well may have an influence on the injection volume precision when using viscous samples. If the draw speed is too high, air bubbles may form in the sample plug, affecting precision. The default draw speed is 1 ml/min. This speed is suitable for the majority of applications, however, when using viscous samples, set the draw speed < 1 ml/min for optimum results.

- Air Gap Volume

An air gap is drawn into the needle before a sample is drawn from a well. This ensures that the sample is separated from the liquid in the transfer tubing. The best compromise for the air gap volume is that it should create an air gap about 10 mm in length inside the transfer tubing. Therefore you should enter: 3  $\mu$ l for 0.5 mm id tubing, 6  $\mu$ l for 0.8 mm id tubing, 38  $\mu$ l for 2 mm tubing.

- Calibrated Tubing Volume

The calibrated tubing connects the injection port to the Rheodyne injection valve. The default setting for the factory installed calibrated tubing is 4  $\mu$ l. If you change the size of the calibrated tubing, determine the volume of the new calibrated tubing and enter this value using the configuration menus.

## **Injection Techniques - Loop Filling Modes**

There are three possible modes of filling the loop when performing an injection: Total, Partial, and Centered.

The sample loop can be either completely or partially filled. This depends on the amount of sample you have.

### **Completely Filling the Sample Loop**

This is the conventional method in which an excess of sample is used to fill the sample loop completely. The volume of the loop determines the injection volume.

### **Partially Filling the Sample Loop**

Use this method when only small quantities of sample are available. In this method the syringe determines the injection volume.

### **How Much Sample is Actually Injected**

When the sample loop is completely filled, the amount of sample injected is equal to the volume of the sample loop plus the volume of the valve passages (1 in rotor and 2 in stator). This means that the actual amount of sample injected will be different to the nominal value designated to your sample loop. However, since both standards and samples are analyzed using the same loop, you rarely need to know the absolute volume of the loop.

If you do need to know the actual volume of a sample loop, we recommend you calibrate it fitted to the valve, so that you also take the valve passages into account.

### **How much sample do I need?**

To completely fill the sample loop an excess of sample is required. this is about 2 to 3 loop volumes of sample to achieve 95% of the maximum loop volume (the remainder is residual mobile phase in the sample loop). Determine the optimum number of loop volumes experimentally for your particular application.

When you partially fill the sample loop, do not inject more than half of the sample volume. As you inject your sample into the loop, the sample mixes with the solvent already in the loop and some of the sample could be lost through port 6 if you try to inject too much.

The Loop filling mode you select depends on the amount of sample available and the accuracy you require. If you have a large volume of sample, use the

## Description of the Tab Screens

Total Loop mode. If you have a very limited quantity, use the Centered Loop mode.

When injecting using either the Total Loop or Partial Loop modes, it is important to note that although air is aspirated before the sample, the air gap remains in the needle and does not enter the injection valve components, it is ejected into the drain when the needle is rinsed.

## Injector Parameters

### Rinsing-Volume Injector Loop

This is the volume used to rinse the injector port. The dimensions are one 1 microliter (default = 100).

---

**NOTE**

---

The rinsing volume cannot be greater than the syringe volume.

### Injector-Loop Rinsing Flow

This is the flow speed to rinse the injector loop (default=6). The dimensions are ml/min. The minimum and maximum rinsing flow depends on the syringe size.

### Rinsing-Volume Needle-Inside

This is the volume used to rinse the injector needle inside. The dimensions are microliter (default = 100).

---

**NOTE**

---

The rinsing volume cannot be greater than the syringe volume.

### Needle Inside-Rinsing Flow

This is the flow speed to rinse the injector needle inside (default=6). The dimensions are ml/min. The minimum and maximum rinsing flow depends on the syringe size (default = 6).

### Rinsing-Volume Needle-Outside

This is the volume used to rinse the injector needle outside. The dimensions are microliter (default = 100).

---

**NOTE**

---

The rinsing volume cannot be greater than the syringe volume.

### **Needle Outside-Rinsing Flow**

This is the flow speed to rinse the injector needle outside (default=6). The dimensions are ml/min. The minimum and maximum rinsing flow depends on the syringe size.

### **Air Gap Volume**

This is the volume of the air gap used to separate the sample from the rinse liquid for injection. The dimensions are microliter (default = 2).

---

**NOTE**

---

The sum injection volume and air gap volume cannot be greater than the syringe volume.

### **Air Gap Flow**

This is the flow speed to create the Air Gap. The dimensions are ml/min (default=1). The minimum and maximum rinsing flow depends on the syringe volume.

### **Injection Volume**

This is the injection volume of the sample. The dimensions are microliter (default=30).

---

**NOTE**

---

The sum injection volume and air gap volume cannot be greater than the syringe volume.

### **Injection Flow**

This is the flow speed to draw and eject the injection volume. The dimensions are ml/min (default=1). The minimum and maximum flow speed depends on the syringe size.

## **The CANCEL, OK and SAVE AS Buttons**

### **CANCEL Button**

Cancel discards all the changes made to the Sampler Method screen and restores the original values.

### **OK Button**

The OK button saves the settings in the Sampler Method screen to the current Sampler method.

Before the method is saved, the parameters are checked for validity.

### **Save As Button**

This button lets the user save the current Sampler parameters under a different name.

## Study Parameters Screen

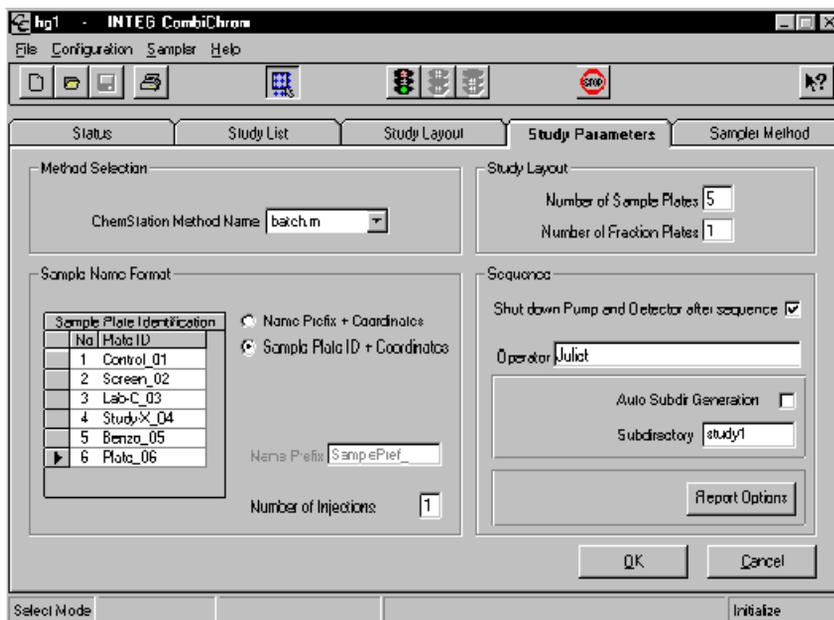
This user interface allows selection of the study parameters (Figure 11). The user can select

- the HP ChemStation method for analysis;
- the number of sampling and fraction plates for the Study Layout;
- the Sample Name Format of the plate names—either “Name Prefix and Coordinates”, or “Sample Plate ID and Coordinates” (these can either be typed in or entered using a barcode reader placed between the keyboard and PC) and the number of injections;
- the sequence parameters, including operator name; and
- the report options (for more details refer to “Reporting” on page 121 in chapter “How To Do”).

The Study parameters are stored with the current study.

Figure 11

### Study Parameters Screen



## **ChemStation Method Name**

Enter or select here the name of the ChemStation Method you want to use for data acquisition and processing.

From now on this method will be used for all subsequent samples added to the Study. The method name for existing samples does not change, unless you remove them and add them again to the Study. To use different methods in a study select a new method, and add the desired sample to the study or select the ChemStation method in the Study List - Samples (for more details refer to “Changing a Method for a Sample” on page 117 in chapter “How To Do”).

## **Study Layout**

- Number of Sample Plates defines the number of plates used for samples. (six plates for 233 XL and ten plates for 215).
- Number of Fraction Plates defines the number of plates reserved for fractions (maximum of 5 plates for 233 XL only).

## **Number of Injections**

Enter here the number of injections for the samples. This must be a positive number ranging from 1 to 9.

This number of injection will be used for all subsequent samples added to the Study. The number of injections for existing samples does not change, unless you remove them and add them again to the Study.

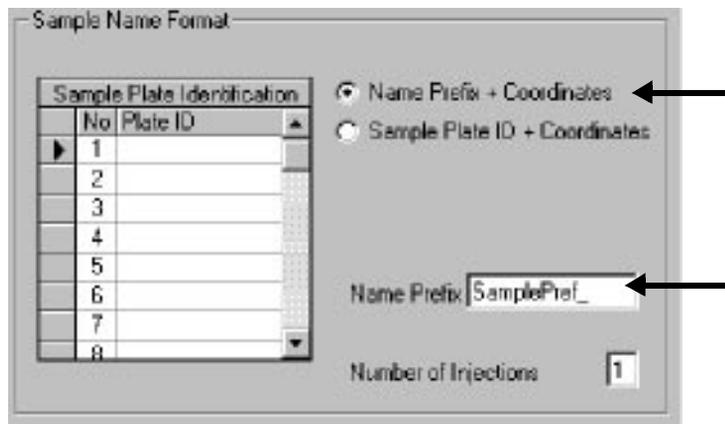
## **Sample Name Format**

The user can select one of two options for sample naming:

- **Name Prefix + coordinates**

Figure 12

Name Prefix + Coordinates



In this mode the format for the sample name is:

<prefix><well position>.

The prefix will be the text that you entered in the 'Name Prefix' box, and the coordinates (well position) for this sample. An example for a sample name is:

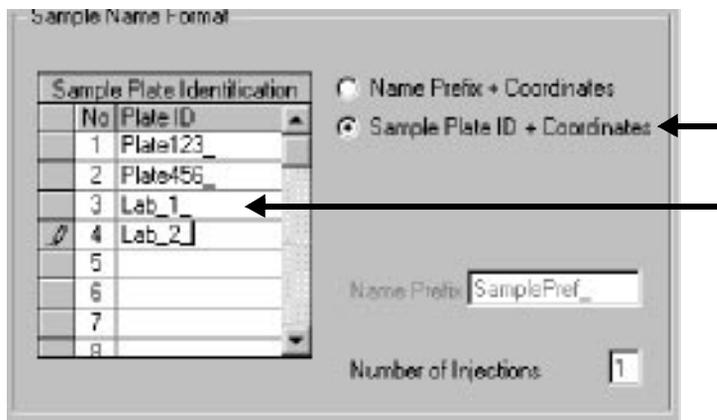
Sample\_3\_1G05

Where 'Sample\_3\_' is the prefix and 1G05 the coordinate (1 = plate, G05 well position).

- **Sample Plate ID + coordinates**

Figure 13

**Sample Plate ID + Coordinates**



In this mode the format for the sample name is:

<Plate ID><well position within plate>.

The Plate ID text can be entered in the table, for each (sample) plate, either by keyboard or by using a bar code reader. The well position is the coordinate of the sample inside this plate. An example for an sample name is:

Lab\_2\_G05

Where 'Lab\_2\_' is the Plate ID and 'G03' the coordinate on this plate.

**Operator Name**

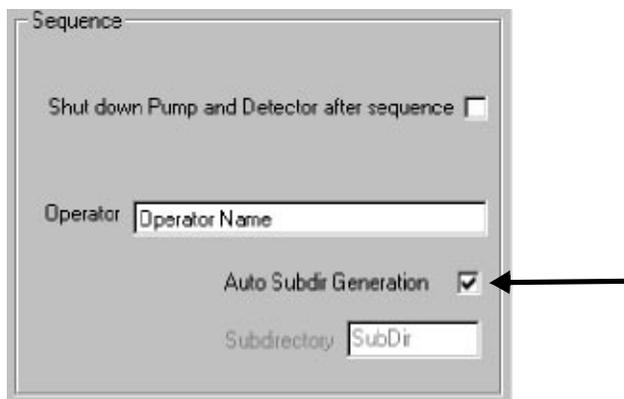
In this field you can enter the name of the operator.

This field is used to identify the operator on the ChemStation report.

### Auto Subdir Generation

Figure 14

#### Auto Subdir Generation checked



The image shows a dialog box titled "Sequence". It contains several controls: a checkbox for "Shut down Pump and Detector after sequence" which is unchecked; a text input field for "Operator" containing "Operator Name"; a checked checkbox for "Auto Subdir Generation" with a black arrow pointing to it; and a text input field for "Subdirectory" containing "SubDir".

When the Auto Subdir Generation box is checked the Subdirectory name is automatically generated. The format of the Subdirectory name is:

yyymmddnn

---

where yy is the last 2 digits of the year.

mm is the month.

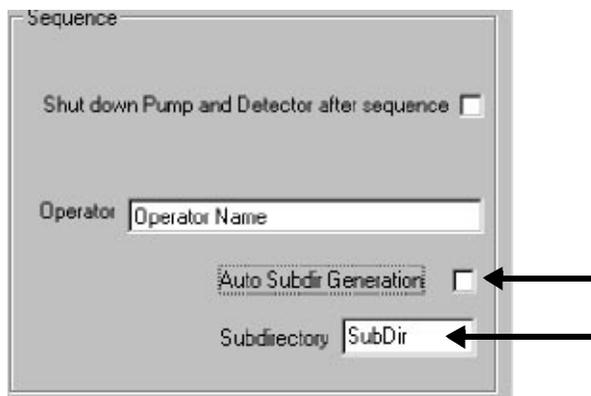
dd is the day.

nn is a number, nn starts at 1 and is incremented each time a Sub directory is created on the same date.

---

Figure 15

**Auto Subdir Generation unchecked**



When this box is unchecked the text entered in the 'Subdirectory box is used for sub directory name.

Under the sub directory name, you must enter a valid file name.

To shut down the instrument after the last run check Shut Down box.

**CANCEL Button**

Cancel discards all the changes made to the Study Parameter screen and restores the original values.

**OK Button**

With the OK button the user accepts the settings in the Study Parameter Screen, and the parameters are checked for validity.

---

**NOTE**

---

By clicking OK the settings in the Study Parameter screen are checked and made actual, but they are not saved yet.

To save the Study Parameters use the 'Save Study' button from the tool bar or the Save study function in the File menu.

## Study Layout Screen (233 XL and 215)

In the Study Layout a sequence can be built by selecting samples from this graphical user interface (Figure 16). This can be done either randomly, by row and column, or by dragging.

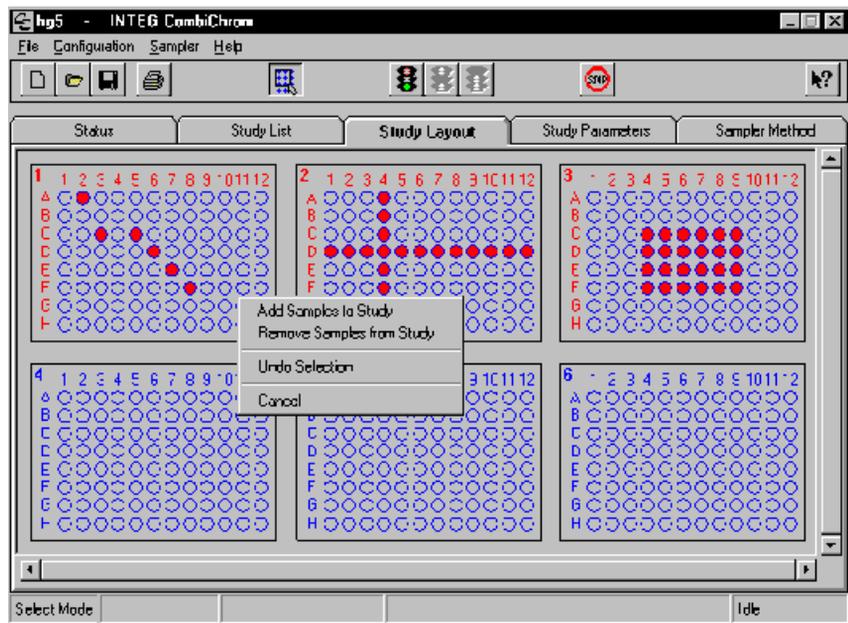
The selected samples can be added or removed from the study using the selection menu, which is activated by clicking once on the right mouse button. Once selected the sample color changes to red.

This screen also shows the number of plates that are being used for samples (red) and for fraction collection (blue).

You can also inject from fraction wells. The added fraction well will turn to blue (for details refer to “Injecting from Fraction Wells” on page 113 in chapter “How To Do”).

Figure 16

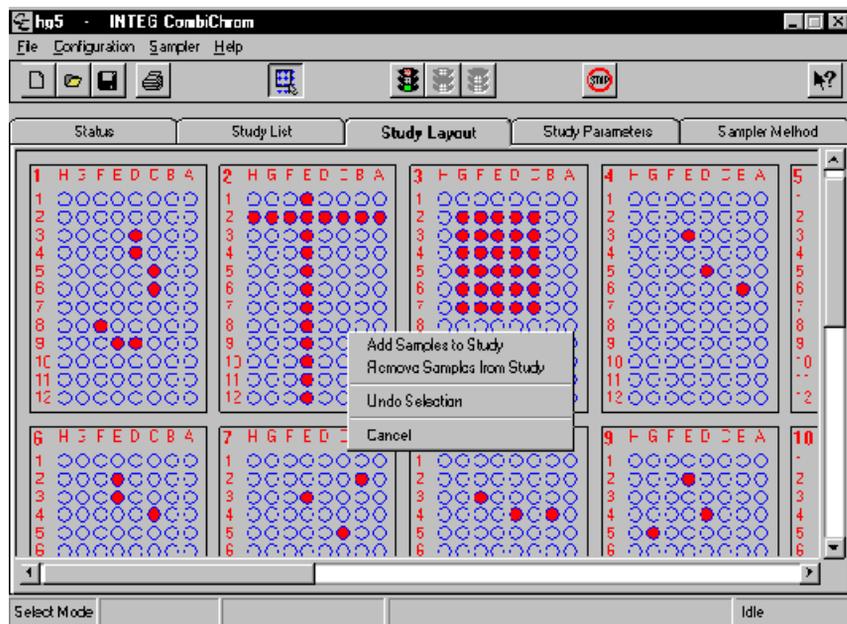
Study Layout Screen 233 XL



CombiChrom Software Familiarization  
Study Layout Screen (233 XL and 215)

Figure 17

Study Layout Screen 215



A sample can be added to a study in the following ways:

- **Randomly**

By clicking on individual sample wells with the left mouse button:

- **By row and column**

By clicking with the left mouse button on the label or letters on top and at the left side of the plate:

By clicking on the numbers on the top a whole column is selected.

By clicking on the label on the left a whole row is selected.

- **By dragging**

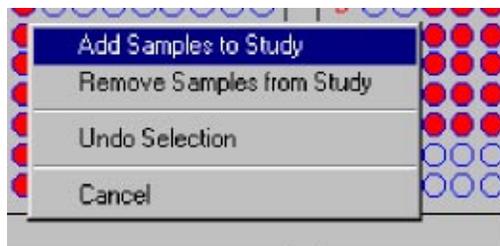
Drawing a rectangle around sample wells by holding down the left mouse button and dragging the rectangle over the wells.

Selected samples are indicated by a white rim around the well.

Once a selection has been made by one (or more) of the above mentioned procedures, the selected samples can be added to, or removed from the Study, by clicking the right mouse button, which will bring up a selection menu.

Figure 18

**Pop Up Menu**



## Sample—Fraction/Fraction—Sample Tracking

In the Study Layout screen, samples and fractions are differentiated with the use of color. Alternating colors are used to distinguish the fractions from each other.

---

Color Coding	Well Type	Description
	Empty well	Blue circle with gray background
	Sample well	Blue circle, red filled
	Selected well	White circle
	Fraction well	Green filled blue circle. The Fraction wells are alternating dark and light green to indicate which wells are from the same fraction.
	Indicated well	To indicate the relation between samples and the corresponding fractions, a white dot appears inside the sample and fraction well.

---

To view samples, fractions relations and the corresponding chromatogram, the Group Select button in the toolbar (this must be in the **Up** mode—view mode). Single wells can be clicked and the relation between samples and fractions (if available) is made visible. In addition, **Enable Pop Up Plot** in the file menu **Configuration** has to be activated.

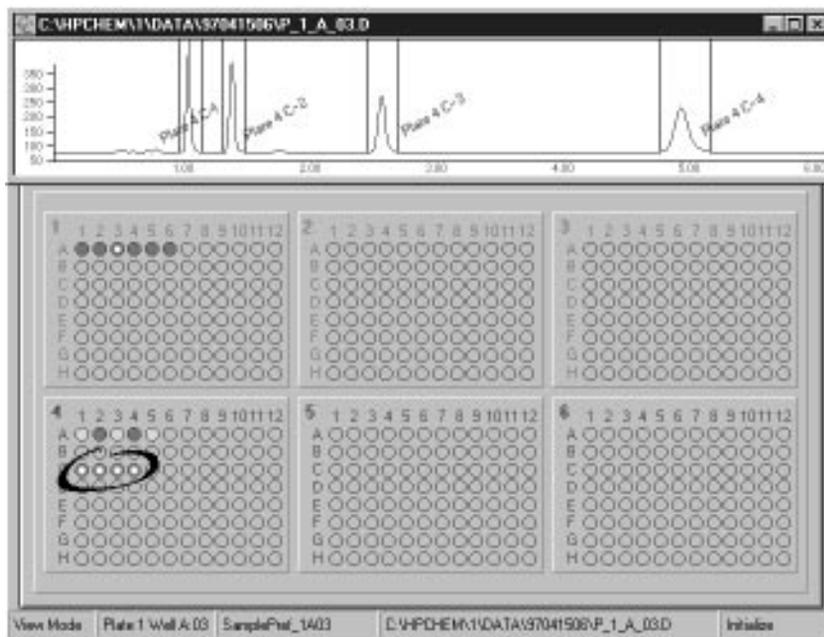
When a sample is selected the status bar shows the coordinates, sample name and data file name of the selected sample.

When fractions from the selected sample exist they will be indicated by a white dot inside the well (Figure 19).

CombiChrom Software Familiarization  
Sample—Fraction/Fraction—Sample Tracking

When a fraction is selected the status bar shows the coordinates of the fraction and the coordinates, sample name and data file name of the sample that the selected fraction originates from. The sample will also be indicated by the addition of a white dot inside the well (Figure 19).

**Figure 19** Sample/fraction information and the corresponding chromatogram in the Study Layout screen



## Status Bar

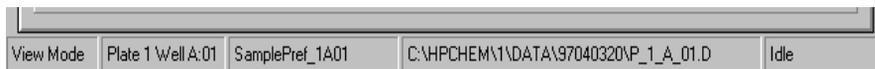
Fraction information is displayed in the chromatogram:

- A vertical green bar indicates the start of the fraction
- A vertical red bar indicates the end of the fraction
- The fraction coordinates are shown in a diagonal label

Reports can be generated with equivalent information.

---

## Status Bar



At the bottom of the form is a status bar. The status bar is divided into four sections:

- 1 Mode: View mode / Select mode
- 2 Location of the currently selected sample well.
- 3 Sample name of the selected well.
- 4 The data file name of the sample.

If a fraction well is selected the status bar shows the fraction origin and the sample origin, name and data file of the sample from where the fraction originated.

## Study List — Samples — screen

Once samples have been added to a study, a sequence is automatically created of all selected samples. This information includes the Location, SampleName, Injection per selected sample and Method used for analysis. Once the run is completed the corresponding Data File Name is added to the study list (Figure 20).

Figure 20

Study List - Samples - screen

PS	Location	SampleName	Data File Name	Inj	Method
P1 A.01	Control_A01	Control_A01	C:\HPCHEM\1\DATA\97041728P_1_A_01.D	1	227c.in
P1 A.02	Control_A02	Control_A02	C:\HPCHEM\1\DATA\97041728P_1_A_02.D	1	227c.in
P1 A.03	Control_A03	Control_A03	C:\HPCHEM\1\DATA\97041728P_1_A_03.D	1	227c.in
P1 B.03	Control_B03	Control_B03	C:\HPCHEM\1\DATA\97041728P_1_B_03.D	1	227c.in
P2 C.02	Screen_C02	Screen_C02	C:\HPCHEM\1\DATA\97041728P_2_C_02.D	1	combi.in
P2 C.03	Screen_C03	Screen_C03	C:\HPCHEM\1\DATA\97041728P_2_C_03.D	1	combi.in
P2 C.04	Screen_C04	Screen_C04	C:\HPCHEM\1\DATA\97041728P_2_C_04.D	1	combi.in
P3 E.06	Lab-CE06	Lab-CE06	C:\HPCHEM\1\DATA\97041728P_3_E_06.D	2	drugn
P3 E.08	Lab-CE08	Lab-CE08	C:\HPCHEM\1\DATA\97041728P_3_E_08.D	2	drugn
P3 F.08	Lab-CF08	Lab-CF08	C:\HPCHEM\1\DATA\97041728P_3_F_08.D	2	drugn
P4 B.02	Study>B02	Study>B02	C:\HPCHEM\1\DATA\97041728P_4_B_02.D	2	aaa.in
P4 B.03	Study>B03	Study>B03	C:\HPCHEM\1\DATA\97041728P_4_B_03.D	2	aaa.in
P4 B.04	Study>B04	Study>B04	C:\HPCHEM\1\DATA\97041728P_4_B_04.D	2	aaa.in
P4 C.05	Study>C05	Study>C05	C:\HPCHEM\1\DATA\97041728P_4_C_05.D	2	aaa.in
P5 B.02	Benzo_B02	Benzo_B02	C:\HPCHEM\1\DATA\97041728P_5_B_02.D	1	335com
P5 B.03	Benzo_B03	Benzo_B03	C:\HPCHEM\1\DATA\97041728P_5_B_03.D	1	335com
P5 B.04	Benzo_B04	Benzo_B04	C:\HPCHEM\1\DATA\97041728P_5_B_04.D	1	335com
P5 B.05	Benzo_B05	Benzo_B05	C:\HPCHEM\1\DATA\97041728P_5_B_05.D	1	335com

From this screen you can also select samples to create a partial sequence. For more details refer to “Creating a Partial Sequence” on page 116 in chapter “How To Do”).

---

## Study List — Fractions — screen

When fractions are collected, the study list shows the Sample Name, Sample Location, the Peak and the Fraction Location (see Figure 21).

Figure 21

Study List - Fractions -Screen

Sample Name	Sample Location	Peak #	Fraction Location
SamplePref_1B02	P1 B.02	1	P4 A.01
			P4 A.02
		2	P4 A.03
		3	P4 A.04
		4	P4 A.05
SamplePref_1C04	P1 C.04	1	P4 A.06
			P4 A.07
		2	P4 A.08
		3	P4 A.09
		4	P4 A.10
SamplePref_1C06	P1 C.06	1	P4 A.11
		2	P4 A.12
		3	P4 B.01
		4	P4 B.02

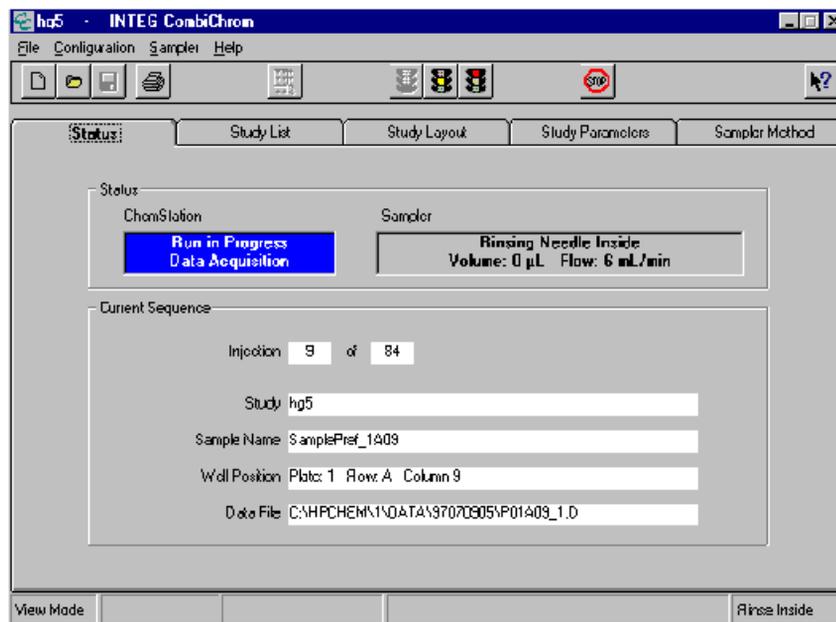
---

## Status Screen

The Status screen (Figure 22) gives information on what is happening with the ChemStation and the samples while the system is running. This includes which sample from the sequence is being analyzed, the study name, the plate name, the well position and the corresponding data file name.

Figure 22

Status Screen



## **Data Structures**

The following section describes the structures of the database tables used in the CombiChrom system.

The CombiChrom database holds the following information:

- System information
- Studies
- Sampler Methods

### **System information**

System information is entered and modified by using the Configuration Screen.

The system information consists of:

- Sampler Type
- Communication parameters
- Syringe size
- Microtiter plate parameters

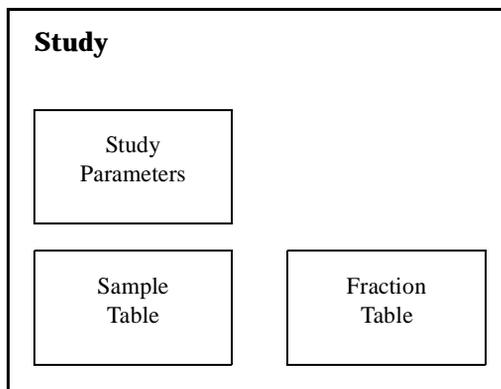
### **Studies**

Studies are created, deleted and saved by means of the menu functions or tool buttons. The active study can be modified via the Study Layout screen and the Study Parameter screen.

The CombiChrom system can store many Studies.

Each study has a unique name.

In a Study the following information is stored:



**Study Parameters:**

- o Number of sample/fraction plates
- o The name of the ChemStation method
- o The name of the Sampler method
- o The Operator name
- o The Sample Name Prefix
- o etc.

**Sample Table:**

- o Sample name
- o Well location of the sample
- o Data file name
- o Number of injections
- o ChemStation Method name

**Fraction table**

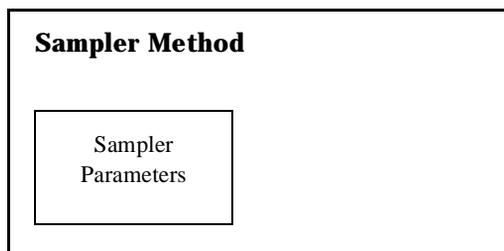
- o Well location of the fraction
- o The name and location of the sample this fraction originates from
- o Peak number

## **Sampler Method**

Sampler Methods are created by using the Save As button in the Sampler Method Screen, and deleted by means of the menu functions. The Sampler Methods can be modified via the Sampler screen.

The CombiChrom system can store many Sampler Methods.

Each Sampler Method has an unique name.



---

## **System Check-out**

This chapter describes the check-out of the HP 1100 Series combinatorial chemistry analysis system.

## Overview

This chapter describes the system check-out of the HP 1100 Series combinatorial chemistry analysis system with the Hewlett-Packard isocratic standard sample using a single injection isocratic analysis:

- What you will need
- Preparing the HPLC system
- Preparing the Gilson autosampler
- Entering settings
- Starting the analysis
- Observing the chromatogram

### What you will need

- o Instruments: HP 1100 Series binary or quaternary pump with on-line degasser, a UV detector, a Gilson 233 XL or 215 autosampler, CombiChrom INB 22000 only or INB 22000 and INB 22001.
- o Column: a 125 mm x 4.0 mm Hypersil ODS, 5  $\mu$ m (Hewlett-Packard part number 7982618-564) or (79926OD-564).
- o Solvents: HPLC grade bidistilled water and acetonitrile.
- o Sample: The Hewlett-Packard isocratic standard sample (Hewlett-Packard part number 01080-68704). This contains 0.15 wt.% dimethylphthalate, 0.1 wt.% diethylphthalate, 0.01 wt% biphenyl and 0.03 wt% o-terphenyl dissolved in methanol.

## Preparing the HPLC system

- 1 Fill the solvent bottles with HPLC-grade bidistilled water (channel A) and acetonitrile (channel B).
- 2 Turn on the detector lamp and pump using the On/Off button in the analysis screen.
- 3 Turn on the degasser by pressing the line-power switch.
- 4 Purge channel A and B of the pump. Enter the following method parameters:

Table 3

---

### Method Parameters

---

Parameter	Value
Flow rate	1.5 ml/min
Channel A (water)	35%
Channel B (acetonitrile)	65%
STOP time	6 minutes
Column temperature	35 °C
Wavelength (DAD)	254/10 nm
Wavelength (VWD)	254 nm

---

- 5 Save this method with the name combi.m.
- 6 Allow the detector at least 15 minutes to provide a stable baseline.
- 7 Place a micro-plate in position 1 on the Gilson autosampler (see plate layout on the CombiChrom Study Layout screen) and fill the contents of a Hewlett-Packard isocratic standard sample ampoule into Plate 1 well A 01.
- 8 Load combi.m from the ChemStation Method & Run Control screen
- 9 Pump the water/acetonitrile (35/65%) mobile phase through the column for 10 minutes at a flow rate of 1.5 ml/min.

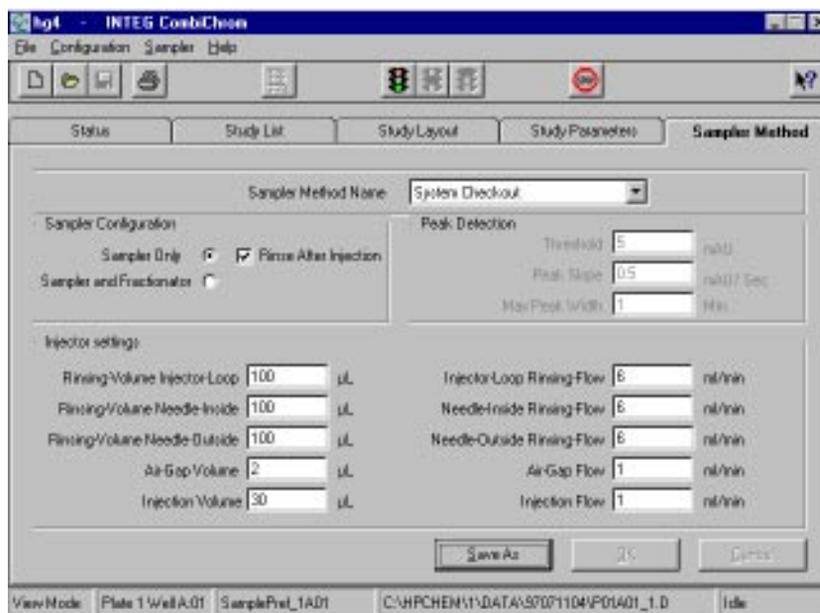
---

## Sampler Method Settings

- 1 Enter the Sampler Method screen and compare settings with Figure 23.
- 2 Press OK

Figure 23

### Sampler Method Screen

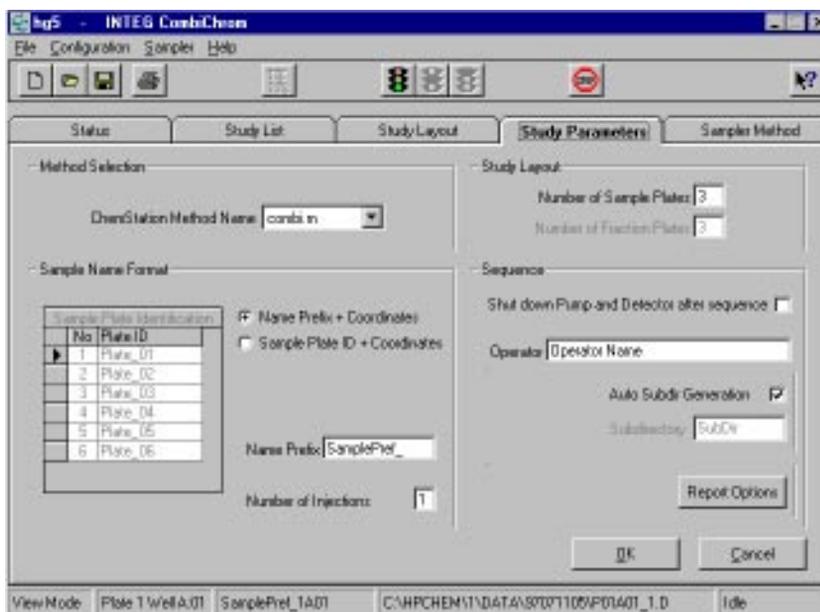


## Study Parameters Settings

- 1 Enter the Study Parameter screen (Figure 24)
- 2 Type in a Name Prefix
- 3 Type in Operator's name
- 4 Press OK

Figure 24

### Study Parameter Screen



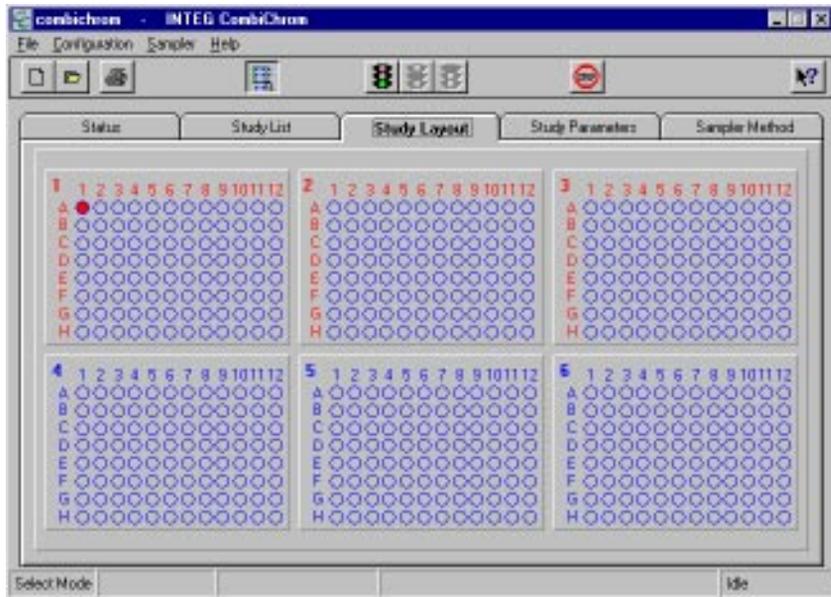
---

## Study Layout

- 1 Enter Study Layout screen (Figure 25)
- 2 Select sample in Plate 1 well A01 by clicking on the sample well with the left mouse button.
- 3 Selected sample is indicated by a white rim around the well. Add the selected sample by clicking the right mouse button, which will bring up a pop menu.

Figure 25

### Study Layout Screen



## **Study List**

- 1** Enter the Study List — Samples — screen (Figure 26).
- 2** Check the sample name you selected. When the Study List sequence is being generated from the selected sample, the sample is named automatically according to the following format:  
  
<prefix><plate number><well position>.

**Figure 26**

**Study List – Samples – screen**

---

## Running the Analysis

### Start the analysis

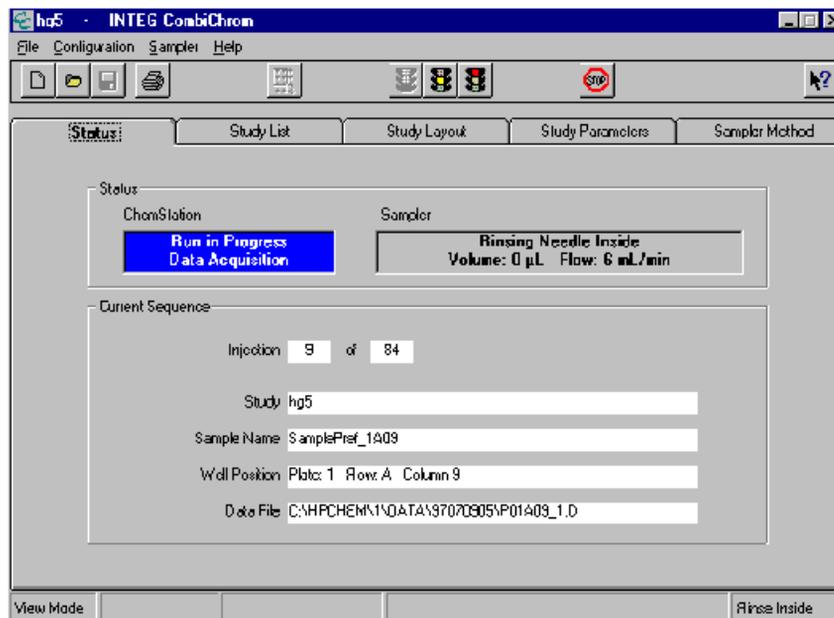
Start the analysis from the toolbar.

### Check the System Status

Enter the Status screen (Figure 27).

Figure 27

#### Status Screen



This screen informs you what is happening with the ChemStation and the Gilson autosampler, while the system is running. This includes which sample is being analyzed the study name, the sample name, the well position and the corresponding data file name.

## Observing the Chromatogram

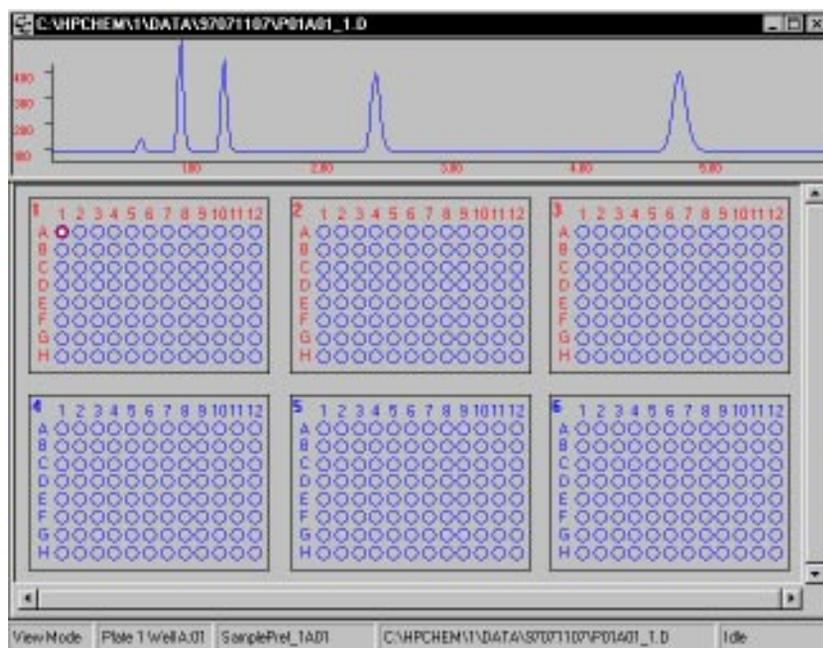
- 1 Select Configuration from the menu bar.
- 2 Activate Enable Pop Up Plot.
- 3 Enter the Study Layout screen.
- 4 Select in the toolbar Group Select in the view mode (up).
- 5 Click on the well containing the sample.

## End of the Analysis

Click on the well containing the sample, the chromatogram is shown (Figure 28).

Figure 28

Study Layout screen with chromatogram of activated sample



The Status bar shows the coordinates, sample name and data file name of the selected sample, the sample will be highlighted and the corresponding chromatogram appears.

System Check-out  
**Running the Analysis**

The exact profile of the chromatogram will depend on the column you have used. Differences in retention times of the peaks in your chromatogram and the one shown in Figure 28 might be a result of variations in the concentration of the sample from batch to batch, the quality of the solvents used and the column temperature.

---

## **How To Do**

This chapter describes the step by step procedures to perform the most common actions in the CombiChrom software.

---

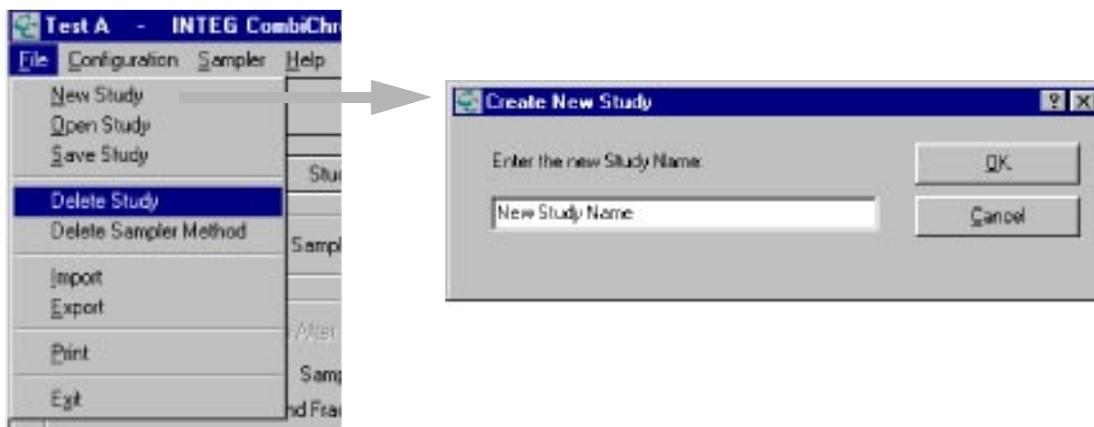
## Creating a new Study

Listed here are the steps needed to create a new Study.

- From the menu bar either choose File - New Study - or click the New Study  button in the Tool Bar.

The Create Study dialog will appear:

Figure 29 Create Study Dialog



### The Create Study Dialog

- Type in a descriptive name for your Study and click OK. Maximum length for a Study name is 30 characters. The Study Layout will now be initialized and will show a number of empty plates as defined by the sampler type: 6 plates for the Gilson 233 or 10 plates for the Gilson 215 sampler. There will be a number of sample plates (indicated by RED labels) and possibly a number of fraction plates (indicated by BLUE labels).

The number of sample and fraction plates is defined in the Study Layout dialog.

- Now you can begin to assign samples to your new study. Before you add samples to your study you may want to change the Sampler Method.

---

## Adding Samples to your Study

Before you add samples to the study you may want to check the settings in the Study Parameter Screen. The system will use these parameters for samples to be added to the study. The parameters are:

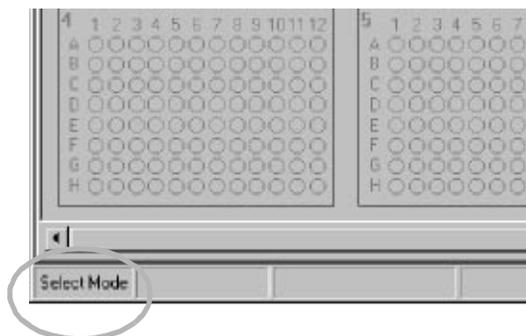
- The Sample Name format
- ChemStation Method Name
- Number of injections

Samples are added to the study in the following manner:

- Verify that the 'Group Select'  button in the tool bar is down.
- You can verify the mode by looking at the left end of the 'Status Bar' (at the bottom of the screen This should read: Select Mode.

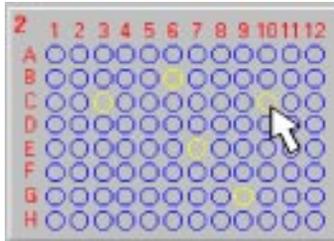
Figure 30

### Verifying the Mode



- Select the samples, you want to add to your study in one of the following ways:
  - Randomly
  - By Dragging
  - Row-Column

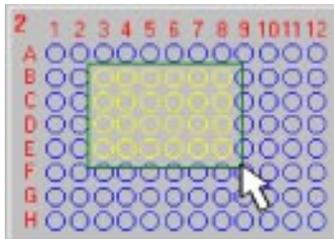
### Randomly



By clicking on each sample-well with the left mouse button.

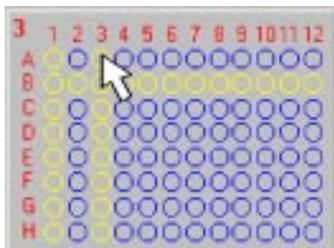
Wells can also be DE-selected by clicking them again.

### Dragging



Drawing a rectangle around sample wells by holding down the left mouse button and dragging the rectangle over the wells.

### Row-Column



By clicking with the left mouse button on the labels on top and at the left side of the plate:

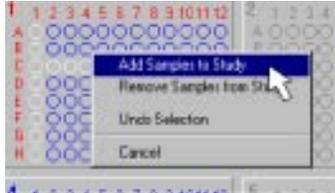
Clicking on the labels on the top selects a whole column

Clicking on the labels on the left selects a whole row.

After you have selected one or more samples, click the right mouse button while the mouse pointer is over one of the sample plates. This shows the following Pop Up menu:

## How To Do Adding Samples to your Study

---



**Add Samples to Study:** The selected wells are added to the Study.

**Remove Samples to Study:** The selected wells are removed from the Study.

**Undo Selection:** The selection is removed.

**Cancel:** The Pop Up window is canceled

- Click the 'Add Samples to Study' from the Pop Up menu.

The selected samples will be added to the sample list in the study. This can be checked in two ways:

- Selected wells are now red colored.
  - Selected wells are added to the Study List - Samples -
-

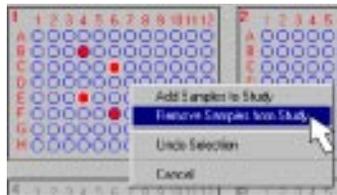
## Removing Samples from the Study

Samples are removed from your Study in the following manner:

- Verify that the 'Group Select'  button in the tool bar is down.
- You can verify the mode by looking at the left end of the 'Status Bar' (at the bottom of the screen This should read: Select Mode, see Figure 30 on page 103.
- Select the samples you wish to remove. See 'Add samples to the Study' for a description of the selection methods.
- After you have selected one ore more samples, click the right mouse button while the mouse pointer is over one of the sample plates. This shows the following Pop Up menu:

---

### Randomly



**Add Samples to Study:** The selected wells are added to the Study.

**Remove Samples to Study:** The selected wells are removed from the Study.

**Undo Selection:** The selection is removed.

**Cancel:** The Pop Up window is canceled.

- 
- Click the 'Remove Samples from Study' from the Pop Up menu.

---

### NOTE

Only samples which have not been analyzed yet can be removed from the study.

---

---

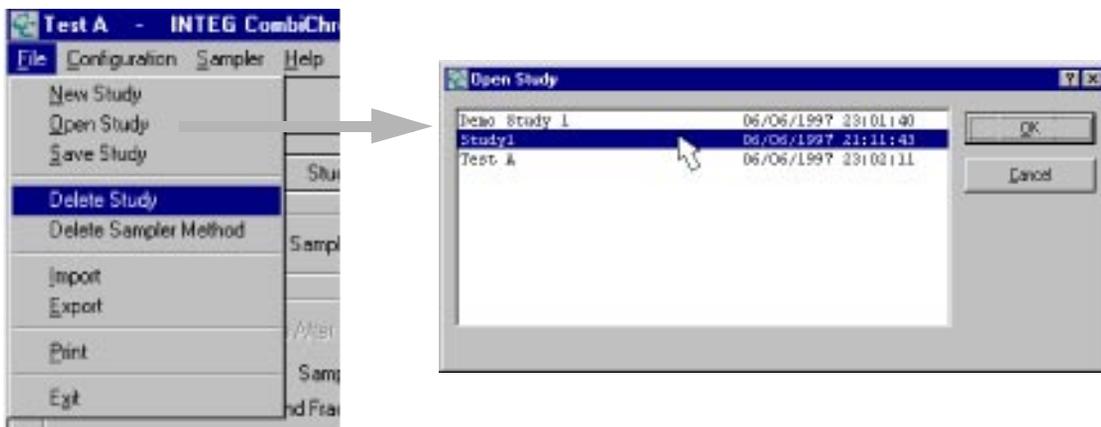
## Opening an existing Study

Listed here are the steps needed to open an existing Study.

- From the menu bar either choose File - New Study - or click the Open Study  button in the Tool Bar.

The Open Study dialog will appear:

Figure 31 Opening an Existing Study



### The Open Study dialog

The Open Study dialog lists all existing studies along with their creation date and time.

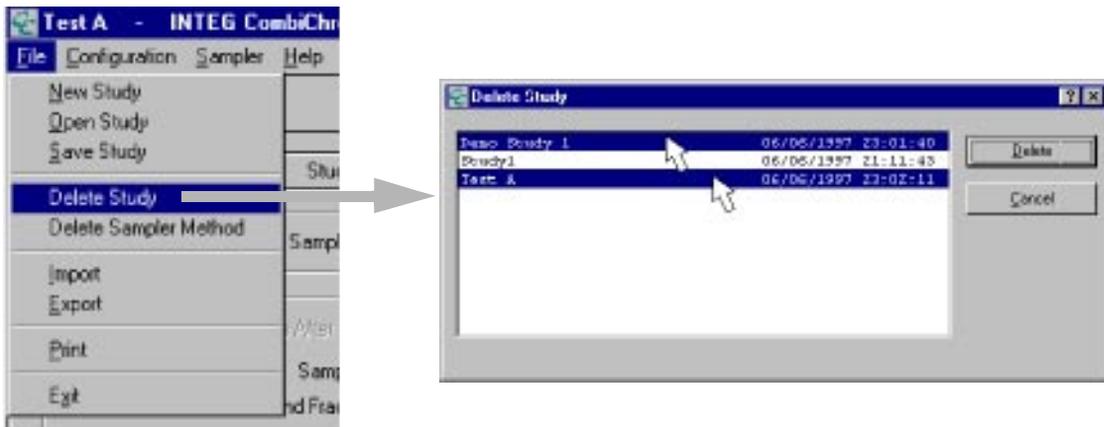
- Click on the study you wish to open and press OK, or double click on the Study name. This will load the selected study and makes it available for examining and/or editing.

---

## Removing Studies from the System

- From the menu bar choose File - Delete Study:  
This will bring up the Delete Study Dialog.

Figure 32 Removing an Existing Study



### The delete Study dialog

- Select one Study to delete by clicking on the name and press OK.
- Multiple Studies can be select by holding the Cntrl-Key and clicking the Study names one by one. After selecting multiple studies click OK to delete the selection.

---

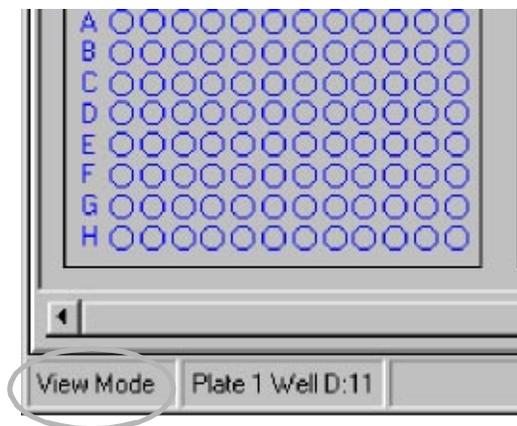
## See Fraction-Sample Relation

To obtain information on the fraction-sample relation follow the next steps:

- Select the view mode:
- Verify that the 'Group Select'  button in the tool bar is up.
- You can verify the mode by looking at the left end of the 'Status Bar' (at the bottom of the screen This should read: View Mode:

Figure 33

### Verifying the Mode

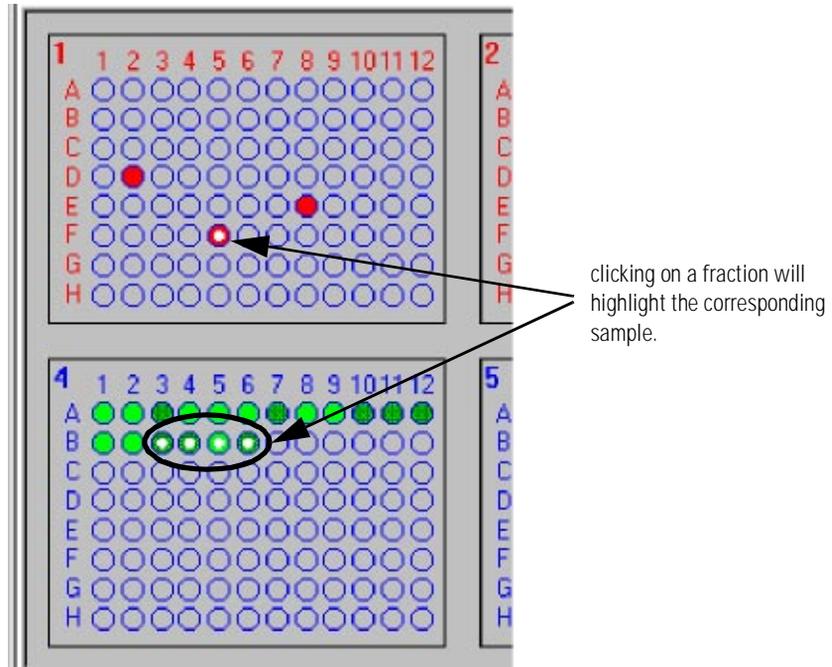


- Click with the left mouse button on the (Red) sample well of interest. The sample well and the fraction well (if any), which were collected from this sample, will be marked with a white dot.
- Clicking on any of the fractions will also show the related sample and fraction wells.

How To Do  
See Fraction-Sample Relation

Figure 34

Fraction-Sample Relation



---

## Interpreting the Fraction Color Coding

The fractions wells are coded with alternating light green and dark green colors.

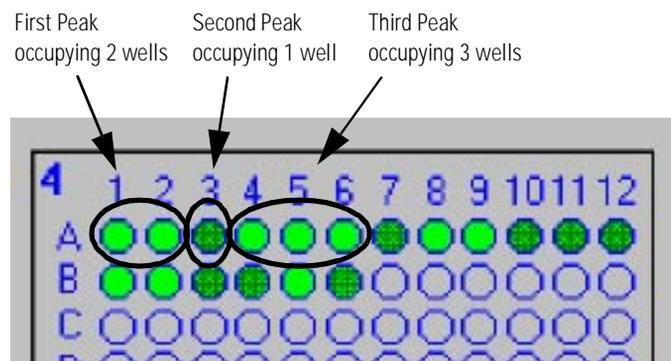
Adjacent wells which belong to the same peak will have the same color, for example light green. The next peak will have wells colored with dark green, and so on.

For example:

From a sample xyz, 3 peaks are collected. The first peak occupies 2 wells A1 and A2, the second peak occupies 1 well A3 and the last peak occupies 3 wells A4, a5 and A6.

Figure 35

### Interpreting Color Coding

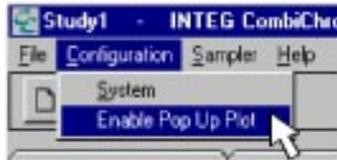


## Enable the Pop Up Chromatogram

To enable the Pop Up chromatogram do the following:  
From the menu bar choose Configuration - Enable Pop Up Plot

Figure 36

### Enable Pop Up Chromatogram



From now on the pop up plot will automatically appear when an analyzed sample well is clicked in view mode.

---

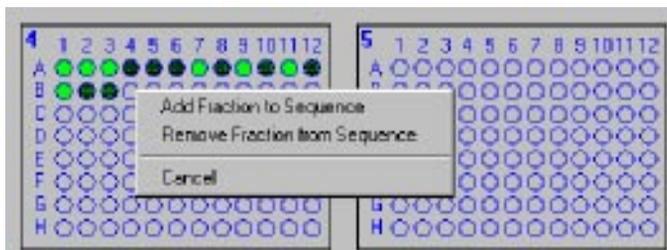
## Injecting from Fraction Wells

It is possible to inject the fluid collected in the fraction wells. Proceed as follows:

- Choose the Select mode:
- Verify that the 'Group Select'  button in the tool bar is down.
- You can verify the mode by looking at the left end of the 'Status Bar' (at the bottom of the screen. This should read: Select Mode, see Figure 30 on page 103.
- Click with the left mouse button on the (green) fraction well of interest. The fraction well will get a white rim around it indicating it has been selected.
- Repeat this for each fraction you want to inject.
- After you have selected one or more fractions, click the right mouse button while the mouse pointer is over one of the fraction plates. This shows the following Pop Up menu:
- Select Add Fraction to Sequence.

Figure 37

Select Add Fraction to Sequence



The added fractions will turn blue.

The fraction is added to the Fraction sequence. The setting in the Study parameters and Sampler method are used to determine the ChemStation method and the Sampler method used for this fraction.

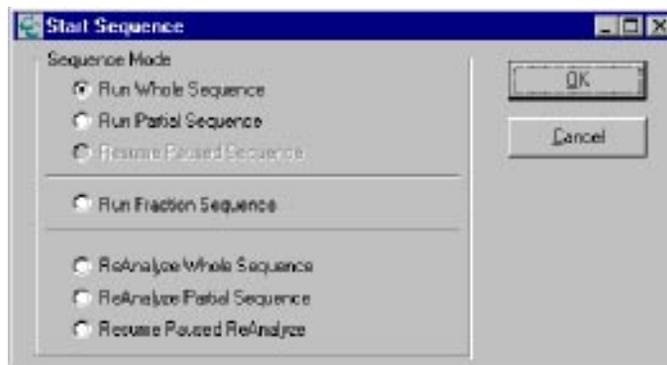
## Starting a Sequence

To start a sequence proceed as follows:

- From the tool bar click the Start Sequence  button:
- The Start Sequence dialog appears:

Figure 38

Start Sequence



From this dialog you have the choice:

- Run Whole Sequence. This will run all samples in the Sample table.
- Run Partial Sequence. This will run all samples which have an '&' in the P.S. column. See 'Creating a partial Sequence how to create a partial sequence.
- Resume Sequence. Select this option to restart a paused Sequence.
- Run Fraction Sequence. Select this option to analyze the selected (blue) fractions.

## Re-analyze a Sequence

To re-analyze samples in a sequence proceed as follows:

- From the tool bar click the Start Sequence button:
- The Start Sequence dialog appears: (see start a sequence).

From this dialog you have the choice:

- ReAnalyze Whole Sequence. This will re-analyze all samples in the Sample table.
- ReAnalyze Partial Sequence. This will re-analyze all samples which have an '&' in the P.S. column. See 'Creating a partial Sequence how to create a partial sequence.
- Resume Paused ReAnalyze. Select this option to restart a paused ReAnalyze.

---

### NOTE

---

The 'Standard Data Analysis' flag in the runtime check list of the ChemStation method must be on to do a (re)-analyze.

## Creating a Partial Sequence

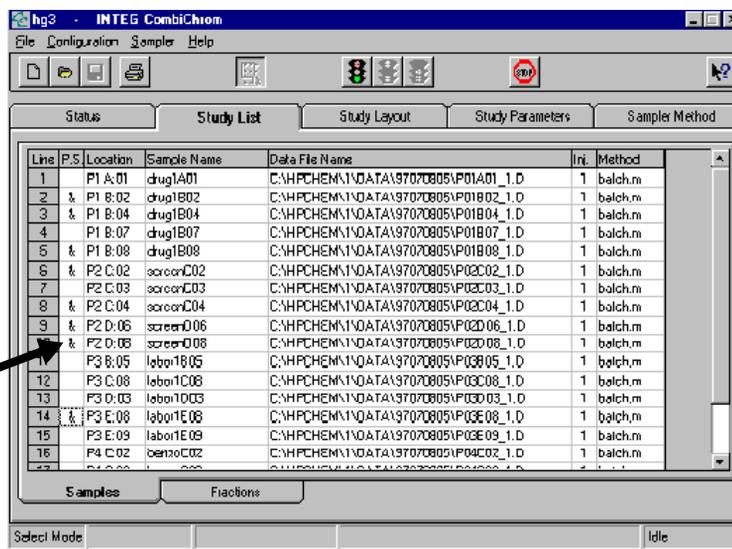
If you want to run only some samples from the study, you can create a partial sequence. To create a partial sequence proceed as follows:

- From the main window click the Study List tab.
- Click the Sample List tab

This will show the sample list:

Figure 39

### Sample List



There are two method to select/deselect a sample from a partial sequence.

- 1 Double click in the 'P.S.' field of the sample you wish to include in the partial sequence.
- 2 Or click once in the 'P.S.' field and use the space bar to select the sample. You can use the up and down arrow keys to move to other samples in the list.

A '&' appears in the 'P.S.' column when a sample is included.

To remove a sample from the partial sequence, repeat the above mentioned sequence. The '&' will disappear.

## Changing a Method for a Sample

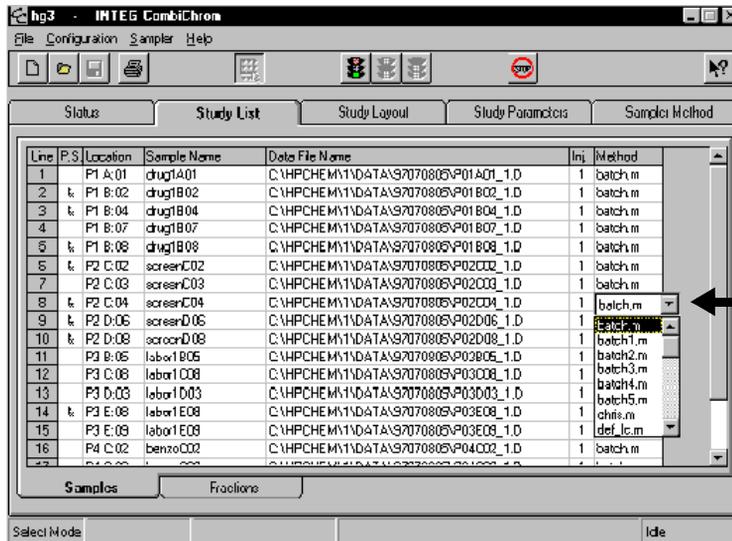
If you wish to change the ChemStation samples for one or more samples individual proceed as follows:

- From the main window click the Study List tab.
- Click the Sample List tab

This will show the sample list:

Figure 40

### Sample List

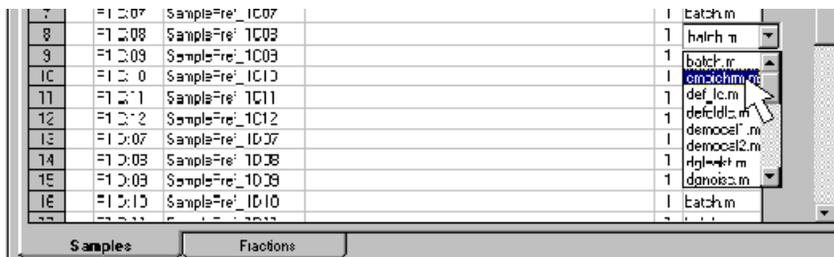


- Click in the 'Method.' field of the sample you wish to change. This will show a drop down list box:

## How To Do Changing a Method for a Sample

Figure 41

### Sample List



- Expand the listbox by clicking on the 'down' arrow. This will show a list of all available ChemStation method for this instrument. Use the scroll bar if not all methods are visible.
- Select the method by clicking on the method name.

### NOTE

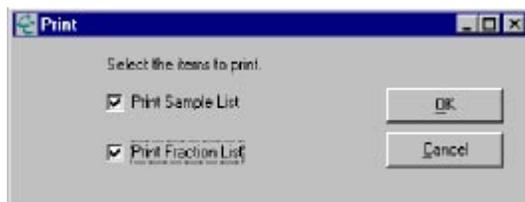
The method list in the drop down box is updated each time you enter the 'Study List' tab. To refresh the list leave the 'Study List' tab by clicking on another tab i.e. 'Study Layout' and then back on the 'Study List' tab. Now the list is most recent.

## Print Sample/Fraction List

To print Sample and/or Fraction List, select File - Print from the menu bar.

Figure 42

### Print Sample/Fraction List



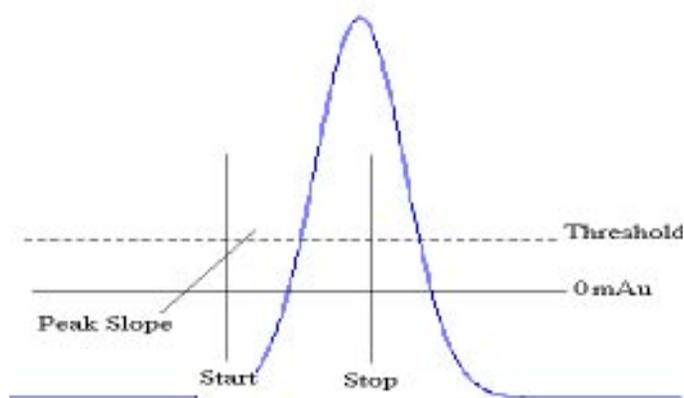
---

## Setting the Peak Detection Criteria

The peak detection is used to control the beginning and ending of the fraction collection. Three parameters determine the duration and moment of collection.

Figure 43

Peak detection criteria



### Peak Threshold

The Peak Threshold value is relevant for the peak-start and peak-stop detection. For the peak-start criterion, the peak signal must exceed the threshold before the peak is detected. The peak-stop criterion is met when the peak signal drops below the threshold.

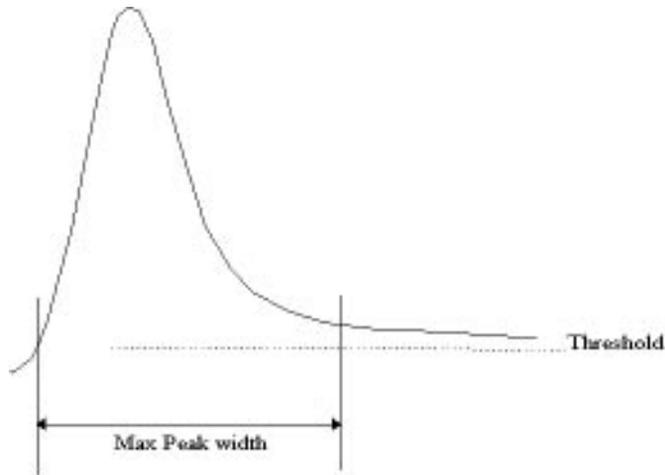
### Peak Slope

The slope of the peak signal is calculated using the first derivative out of the last five measured analog values of the peak. The peak-start criterion is met if the peak signal exceeds the threshold AND the slope is equal or greater than then peak slope setting.

How To Do  
Setting the Peak Detection Criteria

Figure 44

Peak slope



**Peak Width**

In case the signal does not drop below the threshold value, due to drifting base lines, the end criterion would never be met. To eliminate this problem, the peak width parameter is introduced. The peak width is defined from the peak-start detection in minutes. If the current collection time exceeds the peak width value, the collection for this peak is ended.

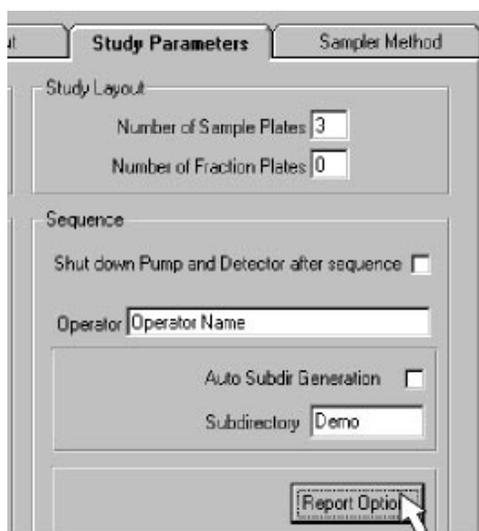
## Reporting

In addition to the report options offered by the ChemStation, CombiChrom adds the following report options:

To set the Report options click on the 'Report Option' button in the 'Study Parameters' screen:

Figure 45

### Select Report Options



This will show the Report Options dialog:

## How To Do Reporting

Figure 46

### Report Options



#### **Enable Multi Plot**

This option enables multiple plots on one page.

The number of plots per page is determined by the 'Plots Horizontal' and 'Plots Vertical' settings.

If, for example, Plots Horizontal is set to 3 and Plots Vertical is set to 4, Each 12 runs a page with the last 12 plots will be printed. At the end of the sequence, or when the sequence is paused or stopped, the remaining plots will be printed. This could result in less than 12 plots for the last page.

---

#### **NOTE**

---

Plots are generated only when the 'Standard Data Analysis' flag is set in the Runtime check list of the ChemStation method.

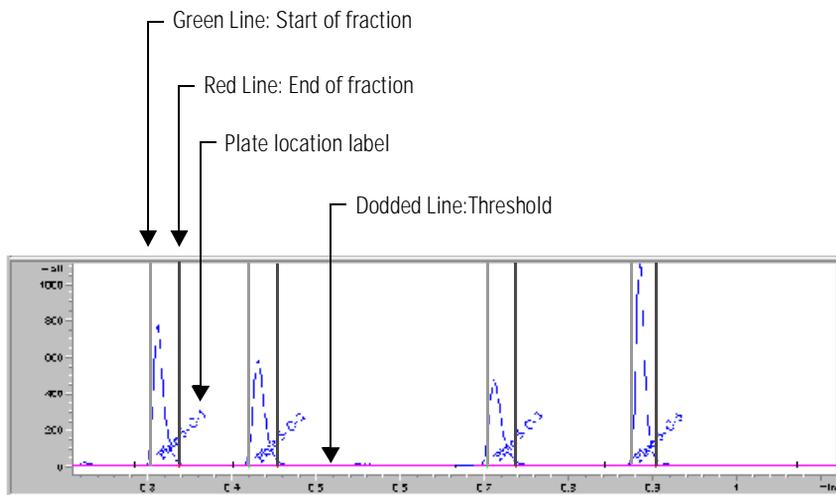
#### **Enable Fraction Info In Plot**

This option puts fraction information in the plot which is generated by the (standard) ChemStation report.

## How To Do Reporting

Figure 47

### Fraction Info in Plot (Example)



---

#### NOTE

---

Fraction information is generated only when the 'Standard Data Analysis' flag is set in the runtime check list of the ChemStation method, and when a report is enabled in the Specify report section from ChemStation.

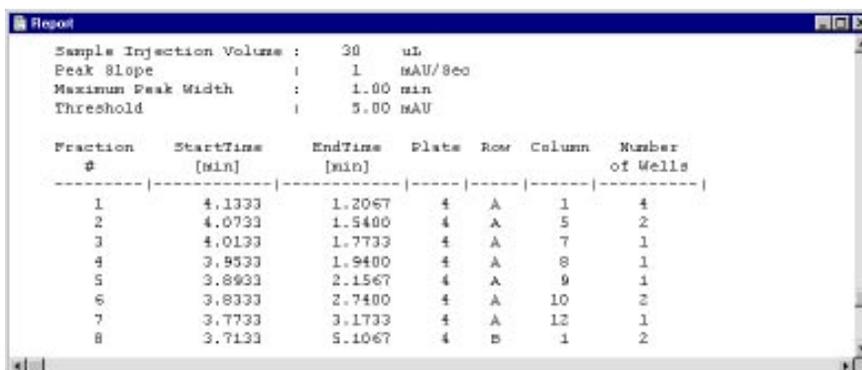
### Enable Fraction Information In Report

This option places Fraction and study information in the (standard) ChemStation report. This information is placed after the plot (if present) and before the actual ChemStation report:

## How To Do Reporting

Figure 48

### Fraction Information In Report (Example)



Sample Injection Volume : 30 uL  
Peak Slope : 1 MAU/Sec  
Maximum Peak Width : 1.00 min  
Threshold : 5.00 MAU

Fraction #	StartTime [min]	EndTime [min]	Plate	Row	Column	Number of Wells
1	4.1333	1.2067	4	A	1	4
2	4.0733	1.5400	4	A	5	2
3	4.0133	1.7733	4	A	7	1
4	3.9533	1.9400	4	A	8	1
5	3.8933	2.1567	4	A	9	1
6	3.8333	2.7400	4	A	10	2
7	3.7733	3.1733	4	A	12	1
8	3.7133	5.1067	4	B	1	2

---

#### NOTE

---

Fraction information is generated only when the 'Standard Data Analysis' flag is set in the runtime check list of the ChemStation method, and when a report is enabled in the Specify report section from ChemStation.

---

# Your Comments Are Welcome

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**1 Please circle Yes or No for each of the following:**

Is it easy to find the information you need when you need it?	Yes	No
Is the information technically accurate?	Yes	No
Are the instructions clear and complete?	Yes	No
Are there enough examples and illustrations?	Yes	No
Are concepts explained clearly?	Yes	No

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**1 = Inadequate. 2 = Adequate. 3 = Superior.**

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Tabs	1	2	3
Glossary	1	2	3
Illustrations	1	2	3
Examples	1	2	3
Readability	1	2	3

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Hewlett-Packard GmbH  
Chemical Analysis Group Europe  
Hewlett-Packard-Strasse 8  
D-76337 Waldbronn  
Germany

Fax (+49) 7243 602 501

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Hewlett-Packard Company  
Publications Department  
2850 Centerville Road  
Wilmington, DE 19808

Fax 302 633 8911

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First edition (Trainings  
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