

Introduction to Agilent Chem Station

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Overview

- Things to consider
- Instrument familiarization
- Software Familiarization
- How to set up an analytic run

Things to consider

- What type of analysis do you need?
 - Analytic: for crude and purified peptide QC
 - Semi-preparative: for peptide purification
- Each analysis has different instrument parameters that must be addressed prior to loading your sample
- Every solvent that is used in the Agilent system must be HPLC grade

Instrument overview

Important HPLC-MS Components

- Solvent degasser & cabinet
- Quad pump
- Thermostat column component
- Dual loop auto sampler
- Diode array detector
- Active splitter
- Fraction Collector
- Quadrupole MS

Quaternary Pump

- Drives the main solvent flow of the HPLC system
- Solvents Bottles
 - Preparative
 - Bottle A: Water with 0.1% TFA
 - 2 liter bottle
 - Bottle B: Acetonitrile with 0.08% TFA
 - 2 liter bottle
 - Analytic
 - Bottle C: Water with 0.1% FA
 - 1 liter bottle
 - Bottle D: Acetonitrile with 0.1% FA
- Maximum Pump Limits
 - 10 mL per minute flow rate
 - 400 bar pressure

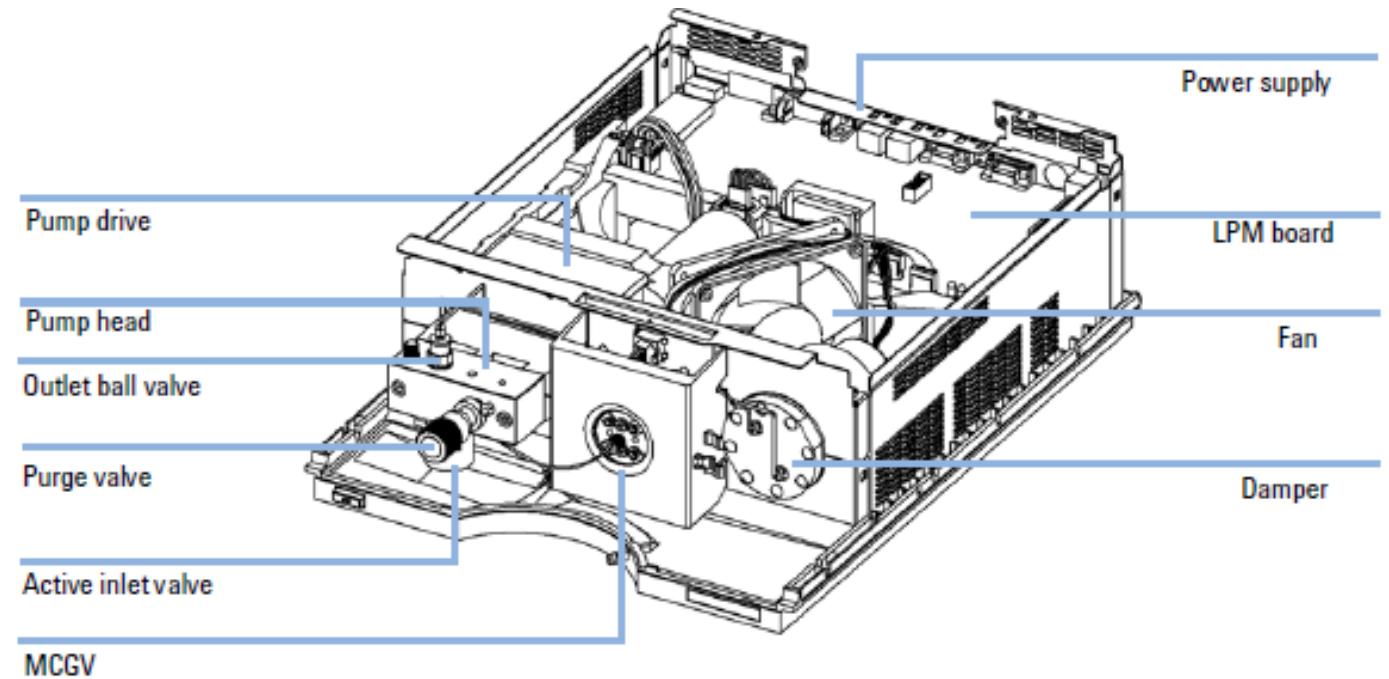


Figure 1 Overview of the Quaternary Pump

Dual Loop Auto Sampler

- Solenoid Valve
- Metering Device
- Peristaltic Pump
- 10-Port Valve
 - 2 Sample Loops
 - Top Sample Loop-5000 μ L
 - Bottom Sample Loop-50 μ L
- Maximum Pump Limits
 - 400 bar pressure

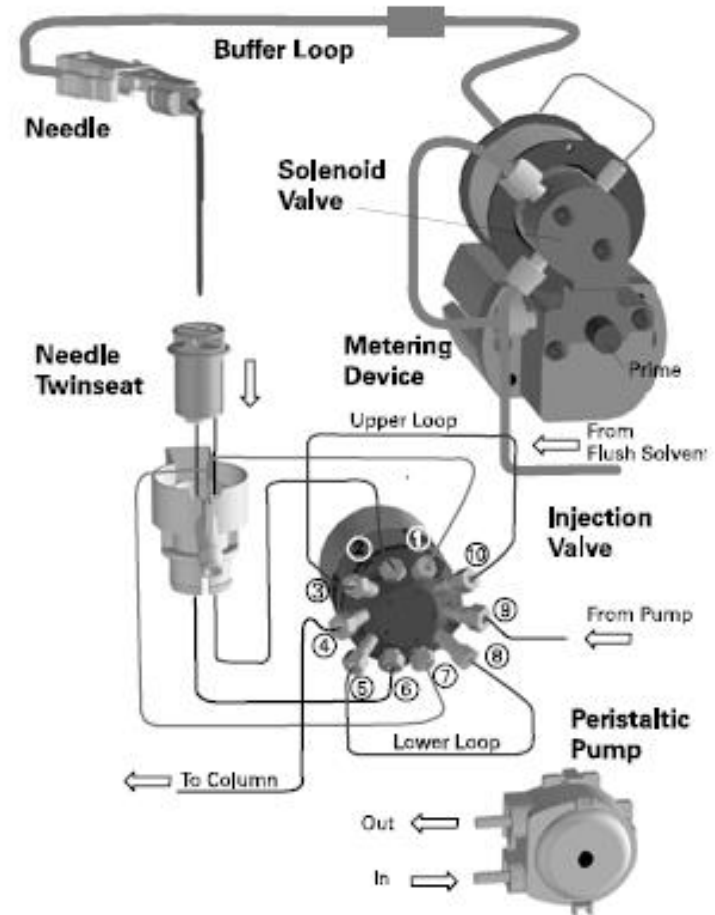
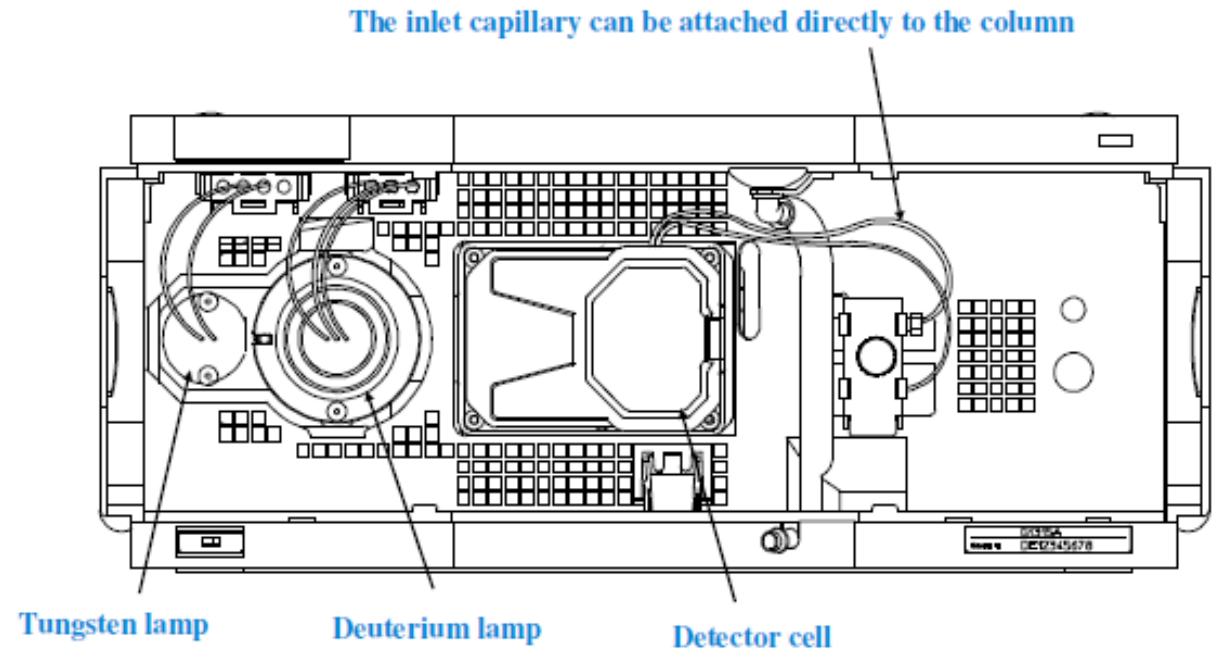


Figure 3 Plumbing Diagram of the Dual Loop Autosampler

Diode array detector

- Components to know
 - Lamps
 - Tungsten (vis-range)
 - Deuterium (UV-range)
 - Flow cell
 - Maximum pressure is 400 bar
- Measurements
 - 214nm
 - 280nm

Front View of the MWD or DAD



Active Splitter

- Active splitter controlled by stand alone software
- 4-Port valve
 - Maximum Pressure of 80 bar
 - See Fig. 1
 - HPLC stream comes from DAD output
 - Isochratic pump drives the make up flow

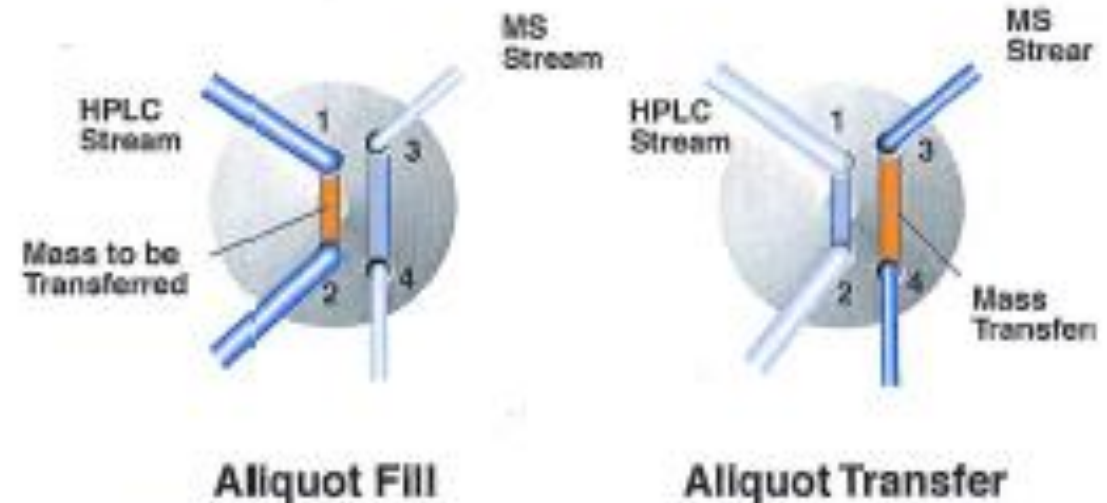


Fig.1. Schematic flow path and transfer action of the MRA.

Fraction Collector

- Components to know
 - Needle & carrier assembly
 - Trays
 - 126
 - 205

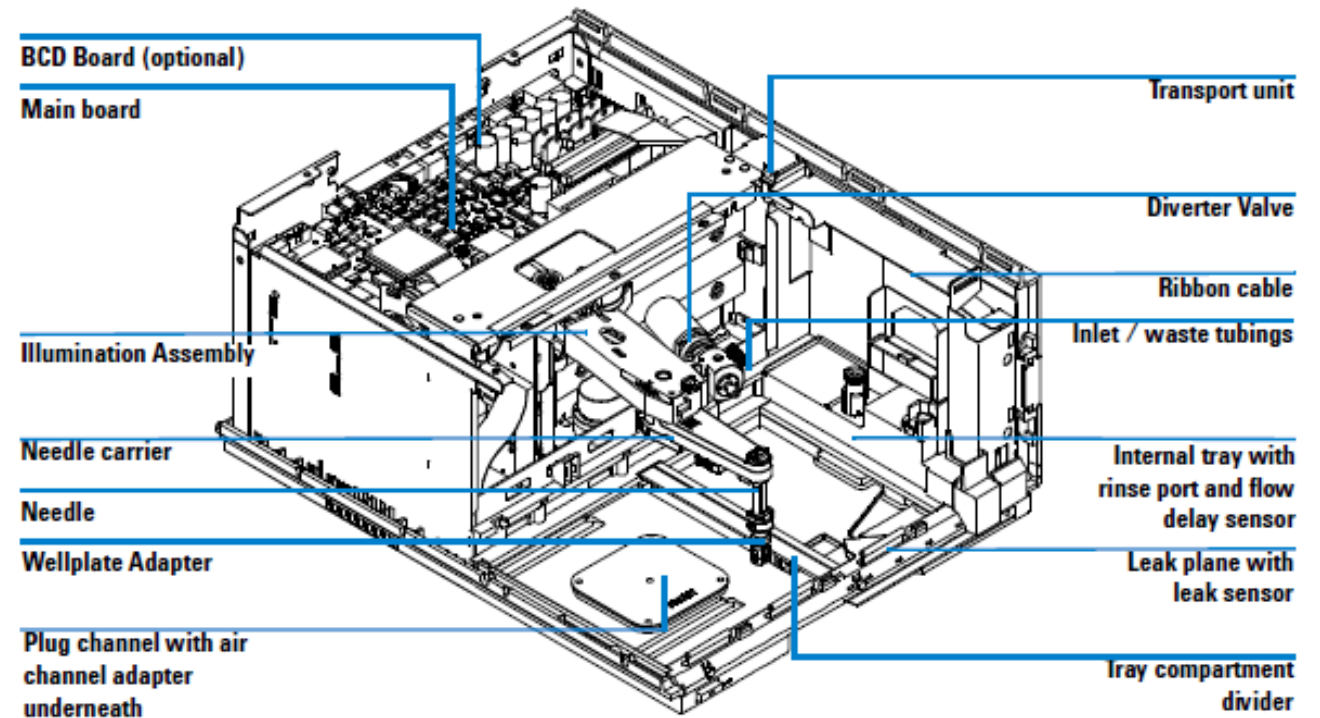


Figure 40 Main Assemblies (Example displays the analytical-scale module G1364C. The preparative-scale fraction collector G1364B contains a different needle)

6130A Single Quadrupole MS

- Multimode ionization source can operate in different mode (APCI, ESI or simultaneously)—We only use the ESI source
 - Signal acquisition
 - Can acquire 2 signals during a run
 - Scan modes
 - Positive
 - Negative

Chem Station Software Familiarization

Chem Station

Modules

- There are two Chem station modules on the desktop
 - Online: this module controls the instruments and can be used for data processing
 - Offline: this module can only be used for data analysis and method review
 - Note: Both modules can be used simultaneously but the online chem station module must be the first chem station software open.
 - Both modules can view, create and modify methods

Things to consider

- A method comprises all the parameters necessary to perform the acquisition and data analysis of one sample
- The instrument\acquisition parameters control the instruments
- The data analysis parameters define how the data will be processed

Getting Started

- The Familiarization Tool provided by Agilent technologies has a step-by-step process of creating a new method
 - The familiarization tools can be found on Dropbox, as a CD-ROM and on the HPLC computer
 - Path: Parker Lab Shared Files\CURRENT LAB MEMBERS\Minervo\Minervo\1200 Series Infinity LCMS\Manuals\Familiarization Tools

The screenshot displays the 'ChemStation Edition - LC Methods' software familiarization tool. The interface is divided into a left-hand navigation pane and a main content area. The navigation pane includes a 'Contents' section with a tree view showing the following items: 'Working with Methods', 'How to navigate this module', 'Objectives', 'What is a method?', 'Creating a New Method', 'Specifying Column Information', 'Specifying Sample Information', 'Setting the Run Time Checklist', 'Running a Single Sample', and 'Summary and Assessment'. The main content area features a purple header bar with the text 'Software eFamiliarization: OpenLAB CDS ChemStation Edition'. Below this, the title 'Working with Methods' is centered above a photograph of two scientists in a laboratory setting. A large blue gear icon is overlaid on the photograph. Below the image, a purple text box states: 'This module takes approximately 30 minutes to complete.' At the bottom of the interface, there is a search bar on the left, the Agilent Technologies logo in the center, and a 'NEXT >' button on the right. The version information '10/9/13 Version 1.1' is displayed in the bottom right corner.

Creating a Method

- Load the current default method, “DEF_LC.M”
- Follow the step-by-step instructions
- You can “Save As” from an existing method to avoid making a new method

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Agilent Technologies
Software Familiarization

ChemStation Edition - LC Methods

File RunControl Instrument Method Sequence View Abort Help

Methods DEF_LC.M Sequences DEF_LC.S

Ready/Reprocess Data Mode

Method and Run Control

C:\CHEM32\1\METHODS

- BATCH.M
- DEF_LC.M
- DEMOCAL1.M
- DEMOCAL2.M
- DGALSTST.M
- DGCALAS.M
- DGCALQ1.M
- DGCALQ2.M
- DGCALQ3.M
- DGCALQ4.M
- DGCALQ5.M
- DGCALQ6.M
- DGCALPS.M
- DGCPTST.M
- DGCPTST.M

Methods Sequence templates

Method and Run Control

Data Analysis

Review

Report Layout

Verification (OQ/PV)

Search...

Instrument Control Easy Sequence Easy Sequence Setup

Single Sample Pause Resume Stop DEF_LC.M

HIP Sampler Quat. Pump Column Comp. DAD

Offline Offline Offline Offline

mL/min

On Off

Data Analysis

Single Sample

Location	Sample Name	Sample Info	Datafile	Data Dir.
				C:856 GB C:\data\

Sampling D...

Vial Symbol Legend

< PREV NEXT >

Editing Methods

- Once a method parameter has been changed, there will be a yellow asterisk indicating a change
- Ensure to save your method in the desire location
 - Under: chem32>1>methods>your name
 - Name your method accordingly
 - What analysis are you doing?
 - Always, state what changed in the method in the “comment for audit trial” pop-out

