



1. Turn instrument on.
2. Set pumps to equilibrate at desired injection percent.
3. Set rack location.
4. Navigate to Main -> realtime batch->file tab-> open batch-> pick your name

Realtime Analysis (PREP_HPLC-System Administrator) - [Batch Editor - Untitled (LC)]

File Edit View Batch Tools Window Help

Realtime Batch - gretchen2.lcb

Analysis	Val#	Tray Name	Sample Name	Sample ID	Sample Type	Method File	Data File	Level#	Inj. Volume	Report Output	Report Format File	Data Comment
1			gg-2aat-test2	gg-2aat-test2	0:Unknown	ZAT_Test2.lcm	gg-2aat-test2.lcd	0	10		ions\System\DEFAULT.lar	

Folder: c:\Lab Solutions\Data\Project 1

Editor	Val#	Tray Name	Sample Name	Sample ID	Sample Type	Method File	Data File	Level#	Inj. Volume	Report Output	Report Format File	Data Comment
1					0:Unknown			0	10			

5

6

LC Running
LHS Running
PDA Running

Item	Value	Setting	Units
Rack#(Fraction Collec	6	6	
Tube#(Fraction Collec	21	21	
Pump System A Mode	Binary gradient		
Pump A Pressure	55		bar
Pump B Pressure	55		bar
Pump System A Flow	15.00	15.00	mL/min
Pump System A B Con	21.1	20.0	%
Pump System B Flow			mL/min
Pump A Degassing Un	Not Connected		kPa
Pump B Degassing Un	Not Connected		kPa

Message	Sub Message	Date	Time	Code	User Name	Application Name	Instrument Name	
The LC instrument was operated at the data acquisition window.	LC The user changed Pump Pump A+B B Conc from 50.0 % to 20.0 %.	10/20/2021	11:39:52 AM	0x59e5	System Administrator	Analysis Service	PREP_HPLC	DESKTOP-N
Start Batch Processing (Data Acquisition).	C:\Lab Solutions\Data\Project 1\gretchen2.lcb ; #1	10/20/2021	11:46:47 AM	0x2400	System Administrator	Analysis Service	PREP_HPLC	DESKTOP-N
Start Data Acquisition.	#1:gg-2aat-test2.lcd - 2021/10/20 11:47:44	10/20/2021	11:47:44 AM	0x243e	System Administrator	Analysis Service	PREP_HPLC	DESKTOP-N

C: 406GB Free NUM
11:58 AM
10/20/2021

5. Insert file information.

6. Make sure to select your name-> data folder-> name sample to ensure your data saves to your folder. Highlight only desired line you wish to run. Select queue batch run when ready. Insert name of file to save when prompted. Approve shutdown file "ok".

Realtime Analysis (PREP_HPLC-System Administrator) - [Data Acquisition - 2AT_Test2.lcm(Read only), gg-2aat-test2.lcd]

File Edit View Method Instrument Acquisition Data Tools Window Help

Realtime Batch - gretchen2.lcb

Folder: C:\LabSolutions\Data\Project1

Analysis	Val#	Tray Name	Sample Name	Sample ID	Sample Type	Method File	Data File	Level#	Inj. Volume	Report Output	Report Format File	Data Comment
1			gg-2aat-test2	gg-2aat-test2	0.Unknown	2AT_Test2.lcm	gg-2aat-test2.lcd	0	10		ions\System\DEFAULT.lsr	

Main Acquisition Instrument Parameters Start Single Run Stop Quick Batch Snapshot Data Analysis Batch Editor Realtime Batch

LCRunning LHSRunning PDARunning

Sample Name : gg-2aat-test2
Sample ID : gg-2aat-test2
Data Comment :

LC PDA ALL

LC Running Time: 8.32 / 91.00 min AD Ch1: -24mV

Max Intensity : 4
Time : 19.367 Inten. : 0.000
B.Conc

LC Running LHS Running PDA Running

Item	Value	Setting	Units
Rack#(Fraction Collec	6	6	
Tube#(Fraction Collect	21	21	
Pump System A Mode	Binary gradient		
Pump A Pressure	55		bar
Pump B Pressure	55		bar
Pump System A Flow	15.00	15.00	mL/min
Pump System A B Con	20.1	20.0	%
Pump System B Flow			mL/min
Pump A Degassing Lh	Not Connected		kPa
Pump B Degassing Lh	Not Connected		kPa

End Time: 91.00 min Time Program Method File: 2AT_Test2.lcm

Mobile Phase Settings... Data Processing Parameters...

Mobile Phase Pump PDA Fraction Collector

Pump A 3.8 L Water Pump ON PDA Lamp PDA Zero Open Close Purge Next tube Clear error

Message	Sub Message	Date	Time	Code	User Name	Application Name	Instrument Name
The LC instrument was operated at the data acquisition window.	LC The user changed Pump Pump A+B B Conc from 50.0 % to 20.0 %.	10/20/2021	11:39:52 AM	0x59e5	System Administrator	Analysis Service	PREP_HPLC
Start Batch Processing (Data Acquisition).	C:\LabSolutions\Data\Project1\gretchen2.lcb : #1	10/20/2021	11:46:47 AM	0x2400	System Administrator	Analysis Service	PREP_HPLC
Start Data Acquisition.	#1:gg-2aat-test2.lcd - 2021/10/20 11:47:44	10/20/2021	11:47:44 AM	0x243e	System Administrator	Analysis Service	PREP_HPLC

C: 406GB Free NUM 11:56 AM 10/20/2021

Collection is automatically triggered when sample is injected.

6. You can adjust what is displayed with right click->display settings (i.e. percent B, 214 nm, 220 nm, pressure, etc)

1*
Building a
method

2

Method Editor (Instrument Parameters)

Normal Advanced End Time: 91.00 min Method: 2AT_Test2.lcm

Data Acquisition LC Time Prog. Pump PDA Controller AutoPurge Fraction Collector

LC Time Program

LC Stop Time: 91.00 min

Apply to All acquisition time

Acquisition Time (PDA)

Sampling: 1.5625 Hz

640 msec

Start Time: 0.00 min

End Time: 91.00 min

Time Constant: Standard 1.280 sec

Max Acquisition Time: 3869.34 min

Item	Value	Setting	Units
Rack#(Fraction Collec	6	6	
Tube#(Fraction Collec	21	21	
Pump System A Mode	Binary gradient		
Pump A Pressure	55		bar
Pump B Pressure	55		bar
Pump System A Flow	15.00	15.00	mL/min
Pump System A B.Con	21.5	20.0	%
Pump System B Flow			mL/min
Pump A Degassing Un	Not Connected		kPa
Pump B Degassing Un	Not Connected		kPa

Message	Sub Message	Date	Time	Code	User Name	Application Name	Instrument Name
The LC instrument was operated at the data acquisition window.	LC The user changed Pump Pump A+B B.Conc from 50.0 % to 20.0 %.	10/20/2021	11:39:52 AM	0x59e5	System Administrator	Analysis Service	PREP_HPLC
Start Batch Processing (Data Acquisition).	C:\Lab Solutions\Data\Project 1\gretchen2.lcb : #1	10/20/2021	11:46:47 AM	0x2400	System Administrator	Analysis Service	PREP_HPLC
Start Data Acquisition.	#1.gg-2aat-test2.lcd - 2021/10/20 11:47:44	10/20/2021	11:47:44 AM	0x243e	System Administrator	Analysis Service	PREP_HPLC

2. File->open method->your folder->methods->Generic Method. Pops up with this edit window.
3. First, adjust total time of run desired (including flush at 95%). Select "apply to all"
4. Navigate to pump tab.

Method Editor (Instrument Parameters)

Normal **Advanced** End Time: 91.00 min Method: 2AT_Test2.lcm Download

Data Acquisition LC Time Prog. Pump PDA Controller AutoPurge Fraction Collector

B.GE1 A: LC-20AP B: LC-20AP B.GE

Stop time: 91.00 min

B.Conc A.Conc

Gradient

Advanced Simple

Time	Flow	A.Conc	B.Conc	B.Curve
1	15.00	80.0	20.0	0
2	8.00	15.00	80.0	20.0
3	70.00	15.00	55.0	45.0
4	71.00	15.00	5.0	95.0
5	86.00	15.00	5.0	95.0
6	88.00	15.00	50.0	50.0
7				

Mobile phase settings Gradient start adjustment

Flow: 15.00 mL/min

A.Conc: 80.0 %

B.Conc: 20.0 % B.Curve: 0

Pressure limits

Minimum: 0 bar Maximum: 100 bar

Download and Close Close Help

5. Set starting flow rate (between 10-20 ml/min).

6. Set starting percentage.

7. Navigate to gradient tab. Here select the gradient you desire. Include 95% flush for at least 10 minutes after your gradient.

8. Navigate to fraction collector tab.

9. You can adjust the minimum threshold desired before collection is triggered. You can also adjust the slope. *Option* to load past sample and use previous data to determine desired minimum and slope.
10. Navigate to time program.

Method Editor (Instrument Parameters)

Normal | Advanced | End Time: 91.00 min | Method: 2AT_Test2.lcm | Download

Data Acquisition | LC Time Prog. | Pump | PDA | Controller | AutoPurge | Fraction Collector

Method Parameter

Use Fraction Collector

Fraction Time: 91.00 min

Detector A | Time Program | Other

Perform Waveform Processing in the Lock Section

	Time	Command	Parameter
▶	1	0.01	Lock
	2	15.00	Unlock
	3	15.01	ValveClose
	4	70.00	Lock
	5		
	6		
	7		
	8		
	9		
	10		
	11		
	12		
	13		
	14		
	15		
	16		
	17		
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	27		
	28		
	29		
	30		
	31		
	32		
	33		

Collection Simulation

Data Files: [] [Open...] [Close All] [Setting...]

Tube Volume: 20.0 mL | Flow Rate: 12.0000 mL/min

Fraction Simulation Result

Time Program

[Lock] [Fraction Volume...] [Valve Open] [Delete]

Detector Chromatogram

Peak

[Add] [Move] [Delete] [List...]

[Simulate] [Reflect]

[Download and Close] [Close] [Help]

11. Using time program, you can tell the system when you would like to collect fractions. (It does not like time 0.00. Start with 0.01)

”Lock” indicates that even if your threshold minimum is met, it will not collect. ”Unlock” must be done following lock if you wish to collect. ”Valve close” will remain closed UNTIL your threshold minimum is met.

”Valve open” will collect all.

You can open/close in real time as well.

Real time changes

The screenshot displays a chromatography software interface with several key components:

- Top Panel:** Shows analysis details for 'gg-2aat-test2', including sample ID, method file, and injection volume.
- Instrument Monitor:** Displays real-time parameters for Pump A and Pump B, such as pressure (55 bar) and flow rate (15.00 mL/min).
- Control Panel:** Features buttons for 'Pump ON', 'PDA Lamp', 'PDA Zero', 'Open', 'Close', 'Purge', 'Next tube', and 'Clear error'.
- Tube Status:** A grid showing the status of various tubes (e.g., 7, 8, 9, 6, 5, 8, 17, 20).
- Message Log:** A table at the bottom recording system events, such as 'The LC instrument was operated at the data acquisition window' and 'LC The user changed Pump Pump A+B B Conc from 50.0 % to 20.0 %'.

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13

14

- 12. You can adjust flow rate and percentage in real time but it will NEGATE the gradient you set up. You are now going full manual mode.
- 13. Buttons to open valve/close valve/next tube/clear error.
- 14. General run information.

special notes:

- After selecting open/close valve or next fraction, there is a time delay (~5 s). Do not be alarmed.
- Once the injection loop is opened to load and closed to inject, it will trigger your run. Only do this when you are ready. If you flip it more than once, your run will stop.
- Instrument maxes out pressure at 100 bar. You can monitor your pressure on the far-right panel.
- Make sure you are saving YOUR runs/methods/batch files/data into YOUR folder.
- Change flow collection vessel in the back of the injection loop before injecting.
- Peptides appear to elute a few %B later than the Rainin system (more similar elution percent to the biotage)