

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
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HPLC SEQUENCE SET UP USING EASY SEQUENCE

Start Instrument

1. If necessary, log into windows. Username: Ocmtox. Password: current password
2. Close Agilent folder that automatically opens.
3. If necessary, start Chemstation: Double click "Instrument 1 offline". Log into Chemstation. Username and password are the same as for the normal user & e-mail.
4. Switch to Data Analysis: On top menu bar select "View" then "2 Data Analysis".
5. On top menu bar select "View" then "Preferences". The Preferences dialogue box opens. Select the "Paths" tab. Ensure that the Sequence Path is "C:\Chem32\1\SEQUENCE\", that the Data Path is "C:\Chem32\1\DATA\" and the Method Path is "C:\Chem32\1\METHODS\".
6. Select the "Sequence" tab. Ensure that the Unique Folder Creation is "ON" and that the Name Pattern is "<SeqN>".
7. Select the "Signal/Review Options" tab. Ensure that "Integrate after load" is checked, but "Load using signal details" and "Integrate and print after load" are not checked. This is to ensure that the sequence will not print reports each time a new data file is loaded. Ensure that "Sequence Method" is checked.
8. Select the "Audit Trail" tab. Ensure that both boxes are unchecked.
9. Select the "Transfer Settings" tab and ensure the following settings:
 - a. Server: <http://ocmeopenlab/>
 - b. Account is 'production'
 - c. Path to "LC\LC4", then "DATA" and then to the month folder
 - d. After Acquisition: is checked
 - e. After Reprocessing: is checked
 - f. After any Data Modification: is unchecked
 - g. Import after Reprocessing: is unchecked
 - h. Always calculate peak performance: is unchecked
 - i. Manage Queue on Connect: is checked
 - j. Cleanup Data on Shutdown: is unchecked

10. After all the settings have been checked, click on “OK”.

Easy Sequence Setup

Note: Set up for calibrators, qc and samples

1. In the Method & Run Control window, click on “Easy Sequence Setup” tab.
2. Click on the “Open Easy Sequence Setup” button. Select the proper Easy Sequence Setup, and then click “open”.
3. In the Easy Sequence Setup tab, click on the “Calibration” tab. Then check that the Calibration Mode is “Cyclic”.
4. In the Sequence Start key, to the left of the Calibration tab, click on each calibrator in turn and check the following:
 - a. Calibrant Name
 - b. Vial Location
 - c. Injection/Vial: normally is 1
 - d. Calibration Level
 - e. Update Response Factor: is “Replace”
 - f. Update Retention Time: is “Replace” for the first Calibrator, then “Average” for the others.
 - g. All “Hide” and “Read Only” boxes are unchecked
 - h. Press “Accept Changes”
5. Then click on the Sequence End tab, to the left of the Calibration tab:
 - a. If this sequence is not the last sequence (i.e. there will be samples of a different matrix after the last sample in this sequence), ensure that the sample name for the last sequence is blank.
 - b. If the sequence is the last sequence of the queue (Shutdown Sequence), ensure that the Sample Name is “Shutdown”, that the Method for the Sample is “Shutdown”, that the Vial location is “blank” and that Injections/Vial is set to 1.
6. In the Easy Sequence Setup tab, click on the “Samples” tab. Then check the following:
 - a. Method: that the Method is correct
 - b. The starting vial location: verified the location of the first sample after the QC blank.
 - c. Number of Samples: Total of QC Blank, Low and High QC and samples.
 - d. Injection Volume: as noted in “Method”
 - e. Sample Name: leave blank

f. Verify Easy Sequence Columns as follows:

<u>Name</u>	<u>Hide</u>	<u>Read Only Value</u>
Inj/Vial	X	1
Sample Amount	X	0
ISTD		(As appropriate for method)
Multiplier	x	1
Dilution		1
Lims ID	x	
Sample Info		

7. At the top of the Easy Sequence Setup Tab, click on the “Extended Parameters” key (just to the left of the Print Icon).
 - a. In the “Sequence Parameters” Tab, ensure that “Parts of Method to run” is set to “According to Runtime Checklist”, that Wait after loading a new method is set as necessary, that the Post Sequence Command Macro is unchecked, that Not Ready Timeout is set to 10 minutes and under the Sequence Comment is entered the sequence and Mobile Phase lot numbers.
 - b. In the “Sequence Output” Tab, ensure that the Print Sequence Summary is unchecked and Individual Reports for Each Run is set as specified in Methods.
 - c. Then click on ‘OK’.
8. Scroll to top of tab and click on “Save Easy Sequence Setup” button.

Easy Sequence Setup

Note: Set up for qc and samples information

1. In The Method & Run Control window, click on the “Easy Sequence tab.
2. Click on the “open Easy Sequence Setup” button. Then select the correct Easy Sequence Setup. Then Click “Open”.
3. Ensure the hat the following information is correct:
 - a. Method
 - b. Data Location
 - c. Data File Name
 - d. Sequence Location
 - e. Sequence Name

- f. Starting Vial Location
 - g. Number of Samples
 - h. Injection Volume: as noted in Method
 - i. Sample Name: (Bank)
4. Click on "Calibration List" tab. Check that the following information is correct:
 - a. Sample Name
 - b. Cal Level
 - c. Update RT: Replace for first calibrator, Average for the rest
 - d. Update RF: is Replace
 - e. ISTD Amount: verify the amount
 - f. INJ Volume: Use Method
 - g. Sample Info: (As appropriate)
 5. Click on "Sample List" tab.
 6. Click on "Fill Samples" tab. The Table will populate. Ensure that the following information entered for each sample is correct
 - a. Sample Name
 - b. ISTD Amount: (As appropriate)
 - c. Dilution: (As appropriate)
 - d. INJ Volume: (As appropriate)
 - e. Sample Info: (As appropriate)
 7. Click on "Save Easy Sequence As" button. Then Fill in the Sequence Name and then click "OK".

Enter Sequence in Queue

1. In the Method & Run Control window, click on the "Sequence Queue" tab.
2. Ensure that the Sequence Queue is paused by pressing the "pause Queue" button (if necessary)
3. In the Method & Run Control window, click on the "Easy Sequence" tab.
4. Scroll down to the bottom and click the "Save and Add to Queue" button.
5. If necessary, in the "Sequence Queue" tab, individual sequence can be edited by selecting the sequence and processing the "Edit Sequence" button (to the right of the print icon).