1.0 Purpose

This procedure provides guidelines for the use of Agilent OpenLab Chromatography Data System for HPLC instrument control, data processing, and reporting.

2.0 Scope

This procedure applies to all chromatographic data acquired using OpenLab Chromatography Data System in the Quality Control laboratory at Ion Labs.

3.0 Responsibility

3.1 It is the responsibility of QC Laboratory analysts to follow this procedure.

3.2 QC Laboratory Management and/or Analytical Development personnel are responsible for ensuring analysts follow the guidelines set forth herein.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development personnel to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **CDS** – Chromatography Data System

4.3 **GC** – Gas Chromatography

4.4 **HPLC** – High Performance Liquid Chromatography

5.0 References

5.1 D-808-F1, Form, HPLC/ GC Analyst and Data Reviewer Checklist

5.2 D-807, SOP, HPLC Operation Maintenance and Qualification

5.3 D-801, SOP, Agilent 7890 GC Operation Maintenance and Qualification

5.4 D-903, SOP, Conversion Factors Used in Analytical Determinations and New Product Formulation

6.0 General Documentation and Reporting Guidelines

6.1 The following parameters should be recorded in the laboratory notebook:

6.1.1 Instrument Ion Number

6.1.2 Instrument Cal Due Date

6.1.3 Column identifier

6.1.4 Sequence Identification

6.2 HPLC/ GC Data Review Checklist

6.2.1 Each sequence generated from the use of HPLC and/ or GC should include Form D-808-F1 HPLC/ GC Analyst and Reviewer Checklist.

6.2.1.1 In the event a sequence was rejected for any reason (e.g. system suit failure), this checklist is still required to be completed, listing the rationale for the rejection.

6.2.2 This checklist is completed by both the analyst and the data reviewer to ensure all essential information was captured and is present in the data packet.

6.2.3 Additionally, both the analyst and the data reviewer use the checklist to show assessment of all method suitability requirements.

7.0 Data Acquisition

7.1 If not already started, start the OpenLab Control Panel from the Windows start menu by selecting Agilent Technologies → Control Panel. Alternatively, click the Control Panel shortcut on the desktop.

7.2 In the bottom left corner of the Control Panel, select Instruments.

7.3 On the left side under Instruments, select the desired instrument.

7.4 Select the desired Project near the center/top of the screen.

7.5 Click Launch on the right side of the screen.

7.6 The data acquisition window opens.

7.7 To create a HPLC data acquisition method:

7.7.1 Click the Method Icon  at the top of the screen.

7.7.2 Click the New Icon  at the top left of the main window.

7.7.3 Under Instrument Setup → Pump, set the following to match the analyte specific method:

7.7.3.1 Flow rate.

7.7.3.2 Initial solvent makeup.

7.7.3.3 Gradient timetable.

7.7.3.4 Stoptime.

7.7.4 Under Instrument Setup → Sampler:

7.7.4.1 Set the injection volume to match the analyte specific method.

7.7.4.2 Set stoptime to "As Pump/No Limit".

7.7.5 Under Instrument Setup → Column Comp:

7.7.5.1 Set column temperature to match the method.

7.7.5.2 Set Enable Analysis when temperature is within ± 0.8 °C.

7.7.5.3 Set stoptime to "As Pump/Injector".

7.7.6 Under Instrument Setup → DAD:

7.7.6.1 Set the wavelength for each channel required by the analyte specific method.

7.7.6.2 Set the bandwidth for each channel to 4 nm (or that required by the analyte specific method).

7.7.6.3 If using a reference, set the reference wavelength(s) and bandwidth(s).

7.7.6.4 Set the Peakwidth to an appropriate value (e.g. HPLC = 2.5 Hz, UPLC = 10 Hz).

7.7.6.5 Set Stoptime to "As Pump/Injector".

7.7.6.6 Under Spectrum, set Store to "All", set the Range to match the analyte specific method, set Step to 2.0 nm.

7.7.6.7 Set margin for negative absorbance to 100 mAU.

7.7.6.8 Set the slit width to 4 nm.

7.7.6.9 Under Autobalance, select Prerun.

7.7.6.10 Under Lamps on required for acquisition: select UV lamp and Vis lamp.

7.8 To create a new GC data acquisition method:

7.8.1 Click the Method Icon  at the top of the screen.

7.8.2 Click the New Icon  at the top left of the main window.

7.8.3 On the left side, select Instrument Setup → Agilent 7890B.

7.8.4 Under Inlets → SSL – Front or Back (depending on which inlet you are using):

7.8.4.1 Select the Heater check box, and set the inlet temperature to match the method.

7.8.4.2 Select the Pressure check box. Do not change the setpoint.

7.8.4.3 Select the Septum Purge Flow check box, and set the septum purge flow to match the method. If not specified in the method, use a septum purge flow of 3 mL/min.

7.8.4.4 Set the Inlet Mode to Split or Splitless to match the method.

7.8.4.5 If using split injection, set the Split Ratio.

7.8.4.6 Ensure that the Gas Save checkbox is not selected.

7.8.5 Under Inlets → SSL – Front or Back (for the inlet you are **NOT USING**):

7.8.5.1 Ensure that the Heater, Pressure, and Septum Purge Flow check boxes are not selected.

7.8.6 Under Configuration → Columns:

7.8.6.1 Select Line 1.

7.8.6.2 Click Catalog, and enter the column length, diameter, film thickness, column type, maximum temperature, maximum program temperature, minimum temperature, and description. Then click OK.

7.8.6.3 Select the Inlet that the column will be connected to.

7.8.6.4 Select the Outlet that the column will be connected to.

7.8.6.5 Select Heated By: Oven.

7.8.7 At the left side, select Columns:

7.8.7.1 Select column #1.

7.8.7.2 Under Control Mode, ensure the On checkbox is selected.

7.8.7.3 Set the control mode to match the method: Constant Pressure, Ramped Pressure, Constant Flow, or Ramped Flow.

7.8.7.4 Set the Flow (or Pressure depending on control mode) to match the method.

7.8.7.5 If using ramped flow or ramped pressure, set the time program to match the method.

7.8.8 At the left side, select Oven:

7.8.8.1 Ensure the Oven Temp On checkbox is selected.

7.8.8.2 Under the Oven Temp On checkbox, enter the initial oven temperature specified in the method.

7.8.8.3 Enter the Equilibration Time specified in the method, or use a default of 1 min.

7.8.8.4 Set the maximum oven temperature to 20 °C above the maximum temperature of the method.

7.8.8.5 Adjust the oven temperature program to match the method.

7.8.8.6 Set Post Run to 100 °C.

7.8.8.7 Set Post Run Time to 0 min.

7.8.9 At the left side, select Detectors → FID – Back:

7.8.9.1 Ensure that the Heater, Air Flow, Fuel Flow, Makeup Flow, and Flame check boxes are selected.

7.8.9.2 Adjust the Heater, Air Flow, Fuel Flow, and Makeup Flow settings to match the method.

7.8.9.3 Set the Carrier Gas Flow Correction to "Column + Makeup = Constant".

7.8.10 At the left side, select Aux Heaters:

7.8.10.1 If using headspace injection, ensure the checkbox is selected and set the temperature to match the transfer line temperature specified in the method.

7.8.11 At the left side, select Signals:

7.8.11.1 Set the Signal Source to Back Signal (FID).

7.8.11.2 Set the Data Rate to a value that is appropriate for the peak width. The data system should generate at least 10 points across the width of the narrowest peak.

7.8.11.3 The check boxes for Zero and Save should be selected.

7.8.12 At the left side, select Readiness:

7.8.12.1 Ensure that only the components being used are selected.

7.8.13 Under Instrument Setup, select Agilent 7697A.

7.8.14 Select Temperatures. If using headspace injection, ensure all boxes are checked and the temperatures match the method. Otherwise, ensure all boxes are not checked.

7.8.15 If using headspace, select Times. Set the times to match the method.

7.8.16 If using headspace, select Vial and Loop. Set the parameters to match the method.

7.8.17 If using headspace, select Advanced Functions. Select Single Extraction and Vent Vial Pressure After Extraction. If settings for Purge Flow and Purge Time are specified in the method, set Post Injection Purge to Custom, and change the settings for Purge flow and Purge time to match the method. If no settings for Purge Flow and Purge Time are specified in the method, set Post Injection Purge to Default.

7.8.18 If using headspace, select Sequence Actions. Set the actions to the desired values.

7.9 For HPLC, purge the mobile phase channels that will be used by the method:

7.10 Purge the HPLC system using the sequence of solvents recommended for the column required by the analyte specific method or as outlined in D-807 HPLC Operation Maintenance and Qualification

7.10.1 Click the Method Icon  at the top of the screen.

7.10.2 At the left side of the screen under Instrument Setup, click Pump.

7.10.3 Ensure that the pump purge valve is in the correct position as required by the desired operation (closed = flow through column, open = flow to waste).

7.10.4 Set the desired flow rate and solvent composition.

7.10.5 Click send to instrument  .

7.10.6 Click the Status Icon  at the top of the screen.

7.10.7 In the Instrument Status window, hover over the Pump section and click the green On button to initiate flow. To stop flow, click the red Off button.

7.10.8 Repeat steps 7.10.1 to 7.10.7 for each of the solvents recommended for the column required by the analyte specific method or as outlined in D-807 HPLC Operation Maintenance and Qualification.

7.11 For GC, Install the column specified in the method by following the directions outlined in D-801 GC Operation Maintenance and Qualification.

7.12 Open the data acquisition method:

7.12.1 Click the Method Icon at the top of the screen.

7.12.2 Click Open Acquisition Method at the upper left side of the screen.

7.12.3 Select the desired method, click Open.

7.13 Click send to instrument to download the method to the instrument.

7.14 Click the Status Icon at the top of the screen.

7.15 For HPLC, hover over each instrument module (pump, column oven, detector) and click on the green On button. The instrument begins equilibrating at initial conditions.

7.16 To monitor the baseline, click the Status Icon at the top of the screen. If the baseline is not visible, click Online Signals at the top of the screen.

7.17 When a stable baseline is achieved, the run may be started.

7.18 Creating and Run a Sequence

7.18.1 Click on the Sequence Icon at the top of the screen.

7.18.2 For each injection, enter the vial number, acquisition method, and sample name.

7.18.3 Use the Add, Insert, and Delete icons to add or delete rows as required.

7.18.4 Enter the desired name for the result set in the Result Name box at the bottom left.

7.18.5 To start the run, click Run in the bottom right corner to begin the run.

7.18.6 After the sequence has been started, it may be paused or aborted if necessary:

7.18.6.1 Click on the Status Icon.

7.18.6.2 If the Run Queue is not visible, click Run Queue at the top of the screen.

7.18.6.3 In the Run Queue window, select the running sequence.

7.18.6.4 Click Pause or Stop run at the top of the Run Queue window.

7.18.7 After the sequence has been started, it may be altered if necessary:

7.18.7.1 Click on the Sequence Icon  at the top of the screen.

7.18.7.2 Just above the sequence table and to the left, click on Edit.

7.18.7.3 Make any necessary changes.

7.18.7.4 Click Update at the bottom of the screen.

8.0 Data Processing

8.1 Open the Sequence:

8.1.1 If not already started, start the OpenLabs Control Panel from the Windows start menu by selecting Agilent Technologies → Control Panel. Alternatively, click the Control Panel shortcut on the desktop.

8.1.2 Click on Projects in the lower left corner.

8.1.3 In the project list on the left, select the project where your data resides.

8.1.4 At the top, click the Start Data Analysis icon  .

8.1.5 The Data Analysis window opens.

8.2 Setting Up the Processing Method

8.2.1 If the Processing Method has already been created, proceed to Section 8.3.

8.2.2 Click on the Open Method icon  to open one of the default processing methods to use as a template. For HPLC data, select "3D UV Quantitative_DefaultMethod". For GC data, select "GC_LC Quantitative_DefaultMethod".

8.2.3 At the top right, click on Processing Method if it is not already selected.

8.2.4 The Processing Method will be displayed in the Main Window.

8.2.5 Under General → Properties:

8.2.5.1 Select the Global tab.

8.2.5.2 Choose the desired integrator (ChemStation or EZChrom).

8.2.6 Under Integration Events → Standard:

8.2.6.1 Set the Slope Sensitivity (ChemStation) or Threshold (EZChrom). Lower values increase integrator sensitivity (for smaller peaks), while larger values decrease integrator sensitivity (for larger peaks). A value of 1 is a good starting point.

8.2.6.2 Set the Peak Width (ChemStation) or Width (EZChrom) to a value equal to or less than the width at the base of the narrowest peak.

8.2.6.3 Set the Area Reject and Height Reject values (ChemStation) or Minimum Area value (EZChrom). Any peak that is smaller than these values will not be integrated.

8.2.6.4 Signal specific events may be set if desired. For example, if monitoring 210 nm and 254 nm, different values of Slope Sensitivity and Peak Width may be set for each wavelength.

8.2.7 Under Processing Method → Compounds → Identification:

8.2.7.1 For each target analyte, enter the analyte name, associated signal, expected retention time and retention time window (both can be modified later),

8.2.8 Under Processing Method → Compounds → Calibration:

8.2.8.1 Select the General tab and set the following parameters:

8.2.8.1.1 External Standard (most common) or Internal Standard depending on the method type.

8.2.8.1.2 Number of levels is usually 1 unless multiple standard concentrations are being analyzed.

8.2.8.1.3 Curve calculation: From individual calibration points.

8.2.8.1.4 RF definition: Response per amount.

8.2.8.1.5 Normalize to: Not selected.

8.2.8.1.6 Concentration calculation: Amount * Multipliers * Dilution Factor.

8.2.8.1.7 Calculate mass %: Selected.

8.2.9 Under the Calibration → Compound Table tab:

8.2.9.1 If using an internal standard method, select the "Is ISTD" box for the analyte that is the internal standard. Enter 1 for ISTD amount.

8.2.9.2 For all other analytes, set the following parameters:

8.2.9.2.1 Amount unit: Leave blank.

8.2.9.2.2 Concentration unit: Leave blank.

8.2.9.2.3 Response: Area or Height.

8.2.9.2.4 Associated ISTD: If using internal standard, select the associated internal standard.

8.2.9.2.5 Mode: Curve (or Reference if using another analyte as the calibrator RRF).

8.2.9.2.6 Weighting method: None (unless weighting justified by data).

8.2.9.2.7 Manual factor: Leave blank.

8.2.9.2.8 Curve reference: Leave blank (or enter the reference calibrator is using another analyte as the calibrator RRF).

8.2.9.2.9 Ref. correction: 1.0 (or enter the RRF if using another analyte as the calibrator).

8.2.9.2.10 Curve model: Linear (unless other model justified by data).

8.2.9.2.11 Origin: Force (or Ignore if multiple concentrations of standards are used to generate a curve).

8.2.9.2.12 Multiplier: 1.

8.2.9.2.13 Levels: Leave blank.

8.2.10 Under Processing Method → Compounds, select Spectra (HPLC only).

8.2.10.1 On the UV confirmation tab, set the following:

8.2.10.1.1 UV confirmation match factor limit = 900

8.2.10.1.2 Lower wavelength = lower wavelength specified in the method

8.2.10.1.3 Upper wavelength = upper wavelength specified in the method

8.2.10.1.4 Subtract baseline spectra should normally be selected. In rare cases, the spectral match may be improved by turning subtract baseline spectra off.

8.2.11 Under Processing Method → System Suitability, select Properties and choose the System Suitability parameters that are required by the method:

8.2.11.1 For Tailing, Symmetry Factor, and Resolution, select Column Performance → All peaks. Select the USP box.

8.2.11.2 For Signal to Noise, select Signal to Noise → All peaks, Pharmacopeias → USP, Noise Calculation → P2P, and Noise range → Fixed from Start to End min on current where Start to End is a range in the chromatogram where no peaks elute (noise range).

8.2.12 Under Processing Method → Tools:

8.2.12.1 Select Custom Calculation.

8.2.12.2 Under Linked File, click Browse and select "Calc_Percent_Label_Claim.ccf".

8.2.13 Click the Save Method icon  to save the processing method.

8.3 Setting Up the Injection List and Processing the Data.

8.3.1 Under Data Selection on the left side, select the sequence that contains your data and click Load Data at the top left.

8.3.2 The Data Processing window opens.

8.3.3 If the Create New Processing Method dialog box pops up, click No Method.

8.3.4 At the top, click the Open Method icon  .

8.3.5 Select the desired processing method, and click Open.

8.3.6 On the left side, select all injections in the sequence.

8.3.7 Under Methods on the left side, right-click on the processing method and select "Link selected injections to selected method".

8.3.8 At the top right, select "Injection List" if it is not already selected.

8.3.9 The Injection List will be displayed in the main window.

8.3.10 At the top of the Injection List, change the bracketing mode to Overall.

8.3.11 Change Sample Type to "Cal. Std." for all working standard injections.

8.3.12 For each working standard injection, select the "Compound Amounts" column and enter the standard concentration in µg/mL under "Amount in Sample". The standard concentration calculation should be included in the laboratory notebook. Do not round or truncate the calculated value.

8.3.13 For each of the samples, enter the sample weight in g in the Sample Amount column.

8.3.14 For each of the samples, enter the sample volume in mL in the Dil. Factor 1 column. If multiple dilutions were performed, the dilution factor should account for all dilutions. For example, the sample was initially dissolved in 100 mL to prepare a stock solution. Then, a 2.0 mL aliquot of the stock solution was diluted to 100 mL. In this case, the dilution volume is:

$$Dilution\ Volume = 100\ mL \times \frac{100\ mL}{2\ mL} = 5{,}000\ mL$$

8.3.15 For each of the samples, enter the weight of a single dosage unit in g in the Unit Dose column.

8.3.15.1 For capsules: take the average capsule weight from the weight variation test and subtract the weight of the capsule shell listed in the product profile. Alternatively, combine the fill material from at least 10 capsules, record the weight of the combined fill material, and calculate the average fill weight.

8.3.15.2 For tablets and gummies: record the weight of at least 10 tablets, and calculate the average tablet weight.

8.3.15.3 For liquids, powders, and capsule-in-capsule products: use the unit dose weight listed in the product profile.

8.3.15.4 For raw materials, enter 1.

8.3.16 For each of the samples, click on the Compounds Custom Fields column, and enter the label claim amount in mg for each target analyte in the Label Claim column. For raw materials, enter 1.

8.3.17 For each of the samples, enter any correction factors needed in the Multiplier 1 column. For example, a multiplier is required if the reference standard is a hydrochloride salt and the sample must be reported as the tartrate salt. Common conversion factors are listed in D-903.

8.3.18 At the top, click the Reprocess All icon  .

8.3.19 If the chromatograms window is not open, click on Chromatograms at the top.

8.3.20 Click through each standard or sample on the left side and examine the chromatogram to ensure that it is integrated properly.

8.3.21 If any chromatogram is not integrated properly:

8.3.21.1 If the processing method window is not open, click Processing Method at the top.

8.3.21.2 Adjust the integration events, and reprocess data until all chromatograms are properly integrated.

8.3.21.2.1 Adjustment of the Slope Sensitivity and Peak Width is often sufficient to resolve integration issues. For more advanced integration techniques, consult the manual by choosing Agilent Technologies → OpenLab Help & Learning from the Windows Start Menu. DO NOT USE MANUAL INTEGRATION.

8.3.22 If all chromatograms have been integrated properly, click Save All Results.

8.4 Creating a Quantitative/Qualitative Results Report

8.4.1 After all integrations are satisfactory and results are saved, click on Reporting at the lower left corner.

8.4.2 On the left side: select the standards/samples to be included in the report, and double click the desired report template. Report templates are designed for either finished product or raw materials (designated by FP or RM).

8.4.3 Filtering Reported Results (if required)

8.4.3.1 At the top, click Editor.

8.4.3.2 Right click the table to be edited, and select Properties.

8.4.3.3 On the left side, select Filtering.

8.4.3.4 To filter by sample type:

8.4.3.4.1 Expression = Sample_Type

8.4.3.4.2 Operation = (or <> for not equals)

8.4.3.4.3 Value = 1 for Cal. Std. or 3 for Sample

8.4.3.5 To filter by sample name or compound name:

8.4.3.5.1 Expression = Sample_Name (or Compound_Name)

8.4.3.5.2 Operation = (or <> for not equals or contains)

8.4.3.5.3 Value = "*STD*" (example for any sample with STD in the name, * is a wildcard)

8.4.3.6 Results can be filtered on multiple parameters. For more information on filtering techniques, consult the manual by choosing Agilent Technologies → OpenLab Help & Learning from the Windows Start Menu.

8.4.3.7 Click on the Save Template icon  at the top of the page.

8.4.4 Click on the Preview Icon  at the top of the page.

8.4.5 Click on the Refresh Preview Icon  at the top of the page.

8.4.6 If the report preview is acceptable, print the report by selecting the Print Icon  at the top of the preview window.

8.5 Creating an Injection Report

8.5.1 On the left side: select all injections in the sequence.

8.5.2 Double click the appropriate injection report which corresponds to the instrument that the sequence was run on.

8.5.3 Click on the Preview Icon  at the top of the page.

8.5.4 Click on the Refresh Preview Icon  at the top of the page.

8.5.5 Print the report by selecting the Print Icon  at the top of the preview window.

8.6 Creating a Chromatogram Report

8.6.1 On the left side: select a blank injection, a standard injection, and the sample injection.

8.6.2 Double click the Chromatogram report.

8.6.3 Click on the Preview Icon  at the top of the page.

8.6.4 Click on the Refresh Preview Icon  at the top of the page.

8.6.5 It may be necessary to change the filtering of the chromatogram to display the chromatogram for the appropriate wavelength.

8.6.5.1 Click Report Editor at the top of the screen.

8.6.5.2 Right click on the chromatogram, and select Properties.

8.6.5.3 Select Filtering.

8.6.5.4 Under Expression, choose Signal_Description.

8.6.5.5 Under Operation, select Contains.

8.6.5.6 Under Value, enter a unique part of the signal description surrounded by asterisks (e.g. *225* or *DAD1A*).

8.6.5.7 Click Refresh Preview.

8.6.6 It may be necessary to change the scaling of the chromatogram so that the peaks are more clearly visible.

8.6.6.1 Click Report Editor at the top of the screen.

8.6.6.2 Right click on the chromatogram, and select Properties.

8.6.6.3 Select Signal Axis.

8.6.6.4 Select Scaling → All signals in given scale.

8.6.6.5 Enter the desired minimum and maximum range of the y-axis.

8.6.7 Print the report by selecting the Print Icon  at the top of the preview window.

9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
~~1~~	~~02/11/20~~ ~~04/11/23~~		N/A	S. Sassman ~~S. Sassman~~
0		New		
01/16/25		Update to reflect current lab practices.	CC-23-0181	M. Maples
2		Add Section 6 General Documentation and Reporting Guidelines and form D-808-F1 Update Section 7.10 and 7.10.8 for clarity Update Section 7.11 to reflect correct method	CC-25-0022	

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