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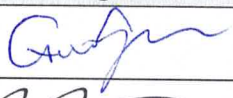
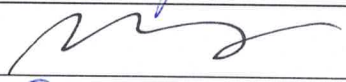
Owner	**Sijin Guo**	**20Mar2025**	
Operation Management	**Baozhong Zhao**	**20Mar2025**	
Quality Assurance	**Xibo Li**	**20Mar2025**	

Changes from previous version:

Section	Summary of Changes	Change Control Number
ALL	1. New document	

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1. PURPOSE

The purpose of this document is to describe the procedure for spectral and quantitative analysis of an analyte (or analytes) using the Thermo-Scientific Evolution One UV-Vis Spectrophotometer.

2. SCOPE

This SOP encompasses the principles, use, maintenance, and troubleshooting of the Thermo-Scientific Evolution One UV-Vis Spectrophotometer, as well as use and operation of the Insight™ Software and Peltier Control and Cooling Unit (PCCU1).

3. INTERNAL REFERENCES

Document ID	Title

4. EXTERNAL REFERENCES

Document ID	Title
Evolution One Series Quick Guide	Evolution One Series- Getting Started (©2021 Thermo Fisher Scientific Inc.- Revision A)
Evolution One Series User Manual	Uv-Vis Instruments Evolution One Series User Guide (©2021 Thermo Fisher Scientific Inc. – Revision A)
Insight™ Pro Software Guide	Insight™ Pro Software
PCCU1 Operating Manual	Thermo Scientific Operating Manual PCCU1 Peltier Control and Cooling Unit
ICH Q7 (API)	Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients
ICH Q9	Quality Risk Management
ICH Q10	Pharmaceutical Quality System

5. RESPONSIBILITIES

Job Function and/or Department	Responsibility
Operational Employees	Employees who are trained in the operation of the Evolution One UV/Vis spectrophotometer are responsible for adhering to this procedure.
Operational Management	Responsible for training operational employees on the operation of the Evolution One UV/Vis spectrophotometer and ensuring

6. DEFINITION

Term	Definition
Evolution One	Spectrophotometer system for measurement of absorbance.
Fixed Application	Measures light through the sample at one or more wavelengths.
Insight™	Software used in the analysis and interpretation of data from the Evolution One
Live Display Application	Used for quick measurements and simplified data collections in Fixed or Scan mode.
Peltier Control and Cooling Unit (PCCU1)	Accessory the provides thermoelectric heating and cooling through the cell block for temperature control and sample monitoring from 5 to 100°c (with the 8-cell accessory).
Quantitative (Quant) Application	Used to set up and perform quantitative analyses of sample data.
Quartz cuvettes	Transparent container containing a solution of the compound being analysed in a transparent solvent.
Rate Application	Used to make time based kinetic measurements.
Scan Application	Measures light that passes through the sample over a range of wavelengths.
SOP	Abbreviation for Standard Operating Procedure provides detailed high-level direction on performing a specific task.

7. PROCEDURE

7.1. Sample Preparation and Disposition

7.1.1. At least 200 µL of solution (sample) will be added to 10 mm path length quartz cuvettes. Add a magnetic stirrer if agitation is required. Cuvettes will then be placed in either the single cell holder or the linear 8-cell holder.

7.1.2. Following analysis, samples will be stored until acceptable to discard as hazardous waste.

7.2. Operation of the Evolution with Insight™ Software

7.2.1. Power on and log into the associated PC with Insight™ software. User names and passwords are case sensitive.

7.2.2. Turn on the power switch on the back of the spectrophotometer. Wait for the system to initialize. The power indicator on the keypad stops blinking when initialization is complete.

7.2.3. Double click the Insight™ desktop icon to start the software.

7.2.4. There are two default views of the Insight Pro window (the Home page): classic view and Bio Methods.

FIGURE 1: INSIGHT PRO HOME WINDOW

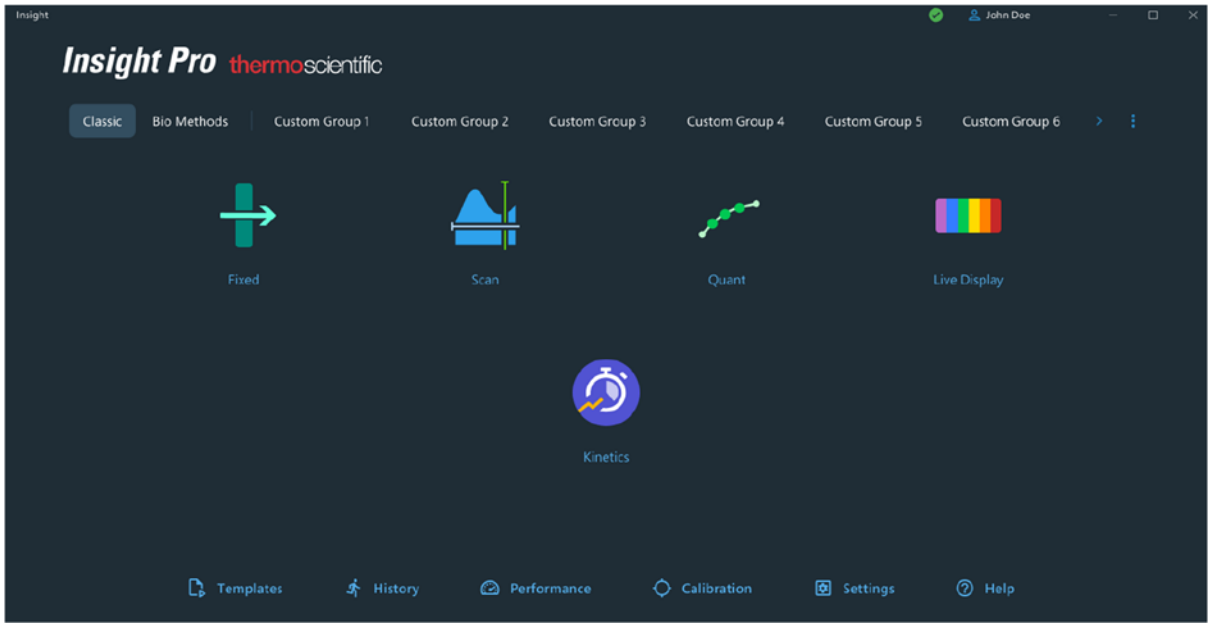


TABLE 1: CLASSIC WINDOW

Button	Description
Fixed	The Fixed application measures the light passing through the sample at one or more wavelengths. To work with this application, click Fixed in the Insight Pro Classic Window.
Scan	The Scan application measures the light that passes through the sample over a range of wavelengths. To work with this application, click Scan in the Insight Pro Classic Window.
Quant	Use the Quant application to set up and perform quantitative analyses of sample data for Fixed or Scan applications. To work with this application, click Quant in the Insight Pro Classic Window.
Live Display	Use the Live Display application for quick measurements and simplified data collections in Fixed or Scan mode. To work with this application, click Live Display in the Insight Pro Classic Window.
Kinetics	Use the Kinetics application to make time- and temperature-based kinetics measurements. To work with this application, click Kinetics in the Insight Pro Classic Window.

TABLE 2: BOTTOM RIBBON

Feature	Description
Template	Experiment parameters can be saved as templates for later use. To use a template, double click it's row in the templates table.

Feature	Description
History	Stores records of experiments you have run. To access the data from a past experiment, double click its row in the history table.
Performance	Run performance verification tests from Thermo Scientific, USP, and EP lists. If the Evolution Pro instrument is used, there are additional performance verification tests available from the JP list.
Calibration	Perform wavelength and accessory calibrations, as well as beam alignment.
Settings	The Settings feature on the Insight Pro window includes Applications, Reports, Preferences, Data, and Formulas & Units. Formulas & Units include Default Formulas, Default Units, and Custom Calibration Equations.

7.3. **General Data Acquisition**

7.3.1. Start the Insight™ software, display the Home screen, and select the **Classic** group before beginning the first task.

7.3.2. Click on an application button such as **Fixed, Scan, Quant, Live Display or Kinetics**, click **Settings**. Update the settings as needed for each application. Refer to sections 7.5, 7.6, 7.7 and 7.8 respectively for each application.

7.3.3. Set the parameters on the tabs in the right pane.

7.3.4. Click **Measure** (a new workbook is created at least once per day of use.)

7.3.5. Follow the instructions that appear, providing information or installing a blank, sample or standard.

7.3.6. The acquired data appears in the right pane.

7.4. **Instrument Performance Verification**

7.4.1. To verify instrument accuracy and reliability, perform a **Performance Verification**. Click on Performance Verification in the Home screen.

7.4.2. Select the tests to run (some tests require reference materials).

7.4.3. Click **Run**.

7.4.4. Follow the instructions that appear.

7.4.5. Test results are saved and appear in a window from which they can be printed. They can also be accessed later on the PV (Performance Verification) Reports tab in My Data, see section 7.10.

7.5. **Fixed Wavelength Measurements**

7.5.1. From Insight™ Home, click **Fixed** then **Settings**.

7.5.2. Select the **Measurement** tab and set the following features:

7.5.2.1. Enter a method template description in the **Description** box.

7.5.2.2. Select **Calculate additional results** to program result calculations.

7.5.3. Select the **Instrument** tab and set these features:

7.5.3.1. Set **Data Mode** and **Result Mode** to the desired formats for displayed samples data and results.

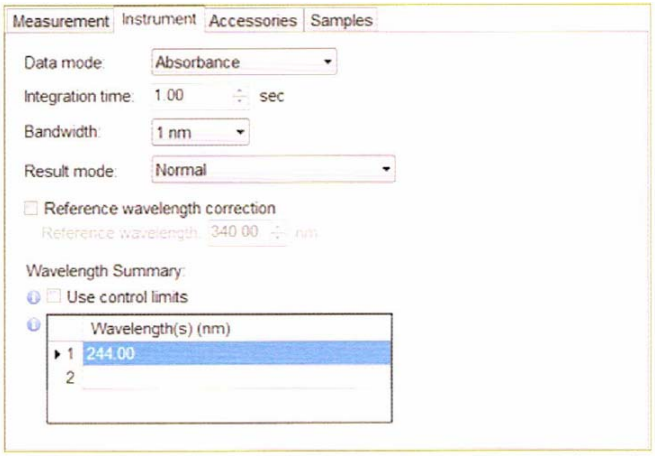
7.5.3.2. Click the first wavelength box in the table, enter a wavelength to measure and press **Enter**. Repeat to add more wavelengths.

7.5.3.3. Set **Integration time** to the duration for data acquisition.

7.5.3.4. Select **Reference wavelength correction** to subtract the absorbance value at a reference wavelength from each measurement. Enter the reference wavelength.

7.5.3.5. Select **Use Control limits** to specify upper and lower measurement limits for each entered wavelength and add control limits lines to the data display.

FIGURE 3: FIXED WAVELENGTH INSTRUMENT SETTINGS SCREEN



7.5.4. Select the **Accessories** tab to edit settings for temperature and stir speed. Refer to section 7.9 for additional information regarding the PCCU1.

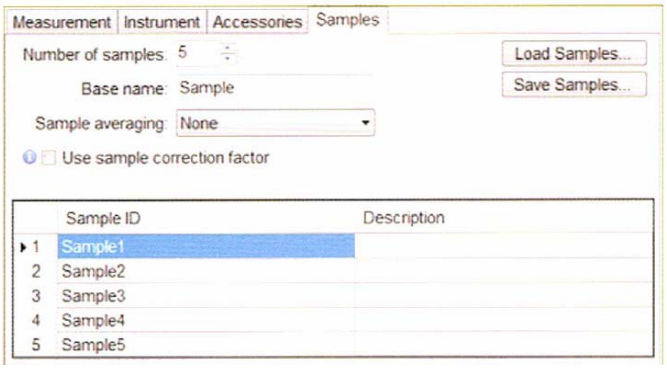
7.5.5. Select the **Samples** tab and set these features:

7.5.5.1. Specify the **Number of Samples** and enter a unique **Base name** for the samples desired.

7.5.5.2. Select a **Sample averaging** option.

7.5.5.3. Select **Use sample correction factor** to apply a correction factor to sample results (i.e., example dilution factors).

FIGURE 4: FIXED WAVELENGTH SAMPLES SETTINGS SCREEN



7.5.6. Choose **File** (menu), then **Save Workbook Settings as Template** to save your method settings as a template for later use.

7.5.7. Click **Measure**, The **Measure Fixed** window is displayed along with the **Loading Guide**.

7.5.8. Place the blank in the light beam, close the cover on the instrument and click **OK** to start the measurement. After the measurement, the **Confirm Sample List** box appears.

7.5.9. Verify the samples list and click **Continue**. The **Loading Guide** shows the information for the next sample.

7.5.10. Place the sample in the light beam and click **OK**. Repeat for each additional sample. The results appear in the **data display** and the **sample measurements table**.

7.6. **Scan Measurements**

7.6.1. From Insight™ **Home**, click **Scan**, then **Settings**.

7.6.2. Select the **Measurement** tab and set the following features:

7.6.2.1. Enter a method template description in the **Description** box.

7.6.2.2. Select a **Baseline Correction Type**.

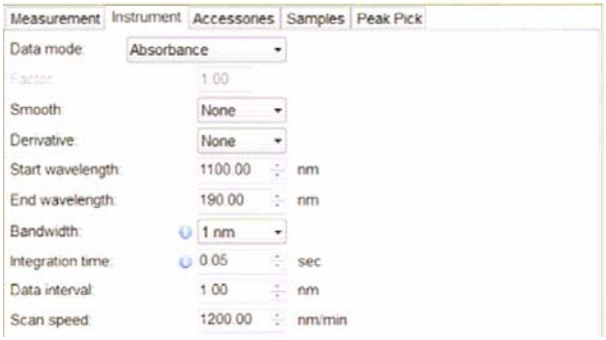
7.6.2.3. Select **Calculate additional results** to program result calculations.

7.6.3. Select the **Instrument** tab and set these features:

7.6.3.1. Select a **Data mode, Smoothing, Derivative**, and **Bandwidth** (if using a variable bandwidth instrument).

7.6.3.2. Select the Start and End wavelengths and the Integration Time, Data Interval and Scan Speed for the measurement.

FIGURE 5: SCAN MEASUREMENTS INSTRUMENT SETTINGS SCREEN



7.6.4. Select the **Accessories** tab to edit settings for temperature and stir speed. Refer to section 7.9 for additional information regarding the PCCU1.

7.6.5. Select the **Samples** tab and set these features:

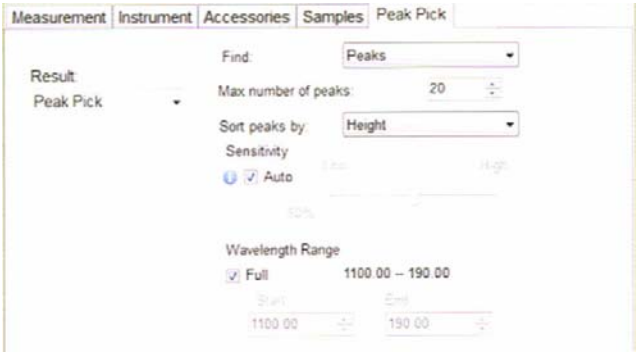
7.6.5.1. Specify the **Number of samples** and enter a unique **Base name** for the samples.

7.6.6. Select the **Peak Pick** tab and set these features:

7.6.6.1. If automatic result analysis is needed, use the **Result** feature to specify the analysis (**Peak Pick** or **Value Level**).

7.6.6.2. Set Peak Pick or Value Level sensitivity and other parameters as needed.

FIGURE 6: SCAN MEASUREMENTS PEAK PICK SETTINGS SCREEN



7.6.7. Choose **File** (menu), then Save **Workbook Settings as Template** to save your method settings as a template for later use.

7.6.8. Click **Measure**. The Measure Scan window and Loading Guide are displayed.

7.6.9. Place the blank in the light beam, close the cover on the instrument and click **OK** to start the measurement. After the measurement, the **Confirm Sample List** box appears.

7.6.10. Verify the sample list and click **Continue**. The **Loading Guide** shows the information for the next sample.

7.6.11. Place the sample in the light beam and click **OK**. Repeat for each additional sample.

7.6.12. To perform a post-measurement analysis, choose **Analyze** (menu), then **Peak Pick** (or **Value Level Crossing**).

7.6.13. To perform post-measurement mathematical functions such as Add or Subtract, select it from the **Math** menu. The results appear in the **data display** and the **sample measurements table**.

7.7. **Quantitative Measurements**

7.7.1. From Insight™ Home, click **Quant** then **Settings**.

7.7.2. Select the **Type** tab and set these features:

7.7.2.1. Enter a method template description in the **Description** box.

7.7.2.2. Select a **Quant Type** such as **Standard Curve**.

7.7.2.3. Enter 10 mm for the **Path length** of the cuvette(s).

7.7.3. Select the **Measurement** tab and set the available features.

7.7.3.1. For Standard Curve Quant Type enter the **Analysis wavelength**.

7.7.3.1.1. Select a **Correction** and enter baseline correction wavelengths where applicable.

7.7.3.1.2. Enter the **Component Name** and select the unit.

7.7.3.1.3. Select Calculate additional results to program result calculations.

7.7.4. Select **Instrument** tab, set data collection Mode to **Scan** or **Fixed** and set the associated Fixed or Scan parameters. Refer to sections 7.5.2 and 7.6.3 for more information.

7.7.5. Select the **Accessories** tab to edit settings for temperature and stir speed. Refer to section 7.9 for additional information regarding the PCCU1.

7.7.6. Select the **Standards** tab and set these features:

7.7.6.1. Select the **Curve fit type**.

7.7.6.2. Select **Minimum r2** and enter a minimum r2 value for the standard curve.

7.7.6.3. Select a **Standard averaging** option.

7.7.6.4. Enter a name and concentration for the measured component in each standard in the table.

7.7.6.5. Select **Calculate from weight/ volume** to calculate concentrations.

7.7.6.6. Select **Use correction factor** and enter correction factors for the standards.

7.7.7. Select the **Samples** tab and set these features:

7.7.7.1. In the table to edit its information. Specify the **Number of samples** and enter a unique **Base name.**

7.7.7.2. Select a **Sample averaging** option.

7.7.7.3. Select **Use sample correction factor** to apply a correction factor to sample results.

7.7.7.4. Select Use **weight/volume correction** to correct sample concentrations after each measurement according to target values.

7.7.7.5. Select **Use control limits** to specify upper and lower measurement limits for the sample results and add control limit lines to the data display.

7.7.7.6. Select a sample.

7.7.8. Choose **File** (menu), then Save **Workbook Settings as Template** to save your method settings as a template for later use.

7.7.9. Click **Measure**, the **Measure Quant** window is displayed along with the **Loading Guide**.

7.7.10. Place the blank in the light beam, close the instrument cover, and click OK to start the measurement. After the blank measurement is completed, the Confirm standard list window appears. Verify the

standards list and click Continue. The Loading Guide shows the information for the next standard.

7.7.11. Place the standard in the light beam and click **OK**. Repeat for each additional standard. After the standards measurements are completed, standard curve results are displayed.

7.7.12. If the standard curve results are acceptable click **Measure**, the **Confirm sample list** window appears. Verify the sample list and click **Continue**. The **Loading Guide** shows the information for the next sample.

7.7.13. Place the sample in the light beam and click **OK**. Repeat for each additional sample. The sample results appear in the **data display** and the **sample measurements table**.

7.8. **Time-Based Rate Measurements**

7.8.1. From Insight™ Home, click **Rate**, then **Settings**.

7.8.2. Select the **Type** tab and set these features:

7.8.2.1. Enter a method template description in the **Description** box.

7.8.2.2. Select a **Rate Type** such as **Single Wavelength**

7.8.2.3. Set **Mode** to Time or Temperature.

7.8.3. Select the **Measurement** tab and set these features:

7.8.3.1. Enter the **Integration Time** and **Dwell Time**.

7.8.3.2. Select the **Number of Stages** (measurement cycles).

7.8.3.3. Select a **Time Unit**.

7.8.3.4. Enter a **Start Time, End Time**, and **Interval** for each measurement stage.

FIGURE 7: TIME-BASED RATE MEASUREMENT SETTINGS SCREEN

7.8.4. Select the **Instrument** tab and set these features:

7.8.4.1. Select **Data Mode** and **Bandwidth**.

7.8.4.2. Select the **Wavelength box** in the table. Enter the wavelength of interest and press Enter.

7.8.5. Select the **Accessories** tab to edit settings for temperature and stir speed. Refer to section 7.9 for additional information regarding the PCCU1.

7.8.6. Select the **Samples** tab and set these features:

7.8.6.1. Specify the **Number of Samples** and enter a unique **Base name** for the samples if needed.

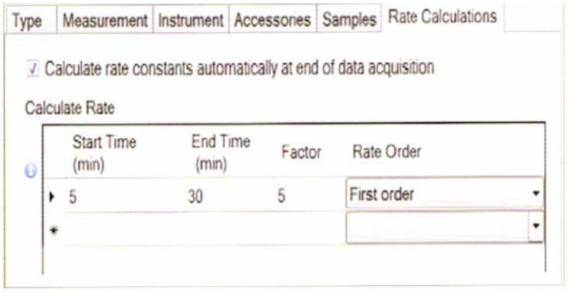
7.8.6.2. Select a sample in the table to edit its information. To add a column, enter a heading in **Set up samples table columns**.

7.8.7. Select the **Rate Calculations** tab and set these features:

7.8.7.1. If you want the software to calculate rate constants automatically, select **Calculate rate constants automatically at end of data acquisition**.

7.8.7.2. For each measurement range, enter the **Start Time** and **End Time** of the rate vector, the rate scaling **Factor**, and the order of the reaction (**Rate Order**).

FIGURE 8: RATE CALCULATIONS SETTING SCREEN



7.8.8. Choose **File** (menu), then **Save Workbook Settings as Template** to save your method settings as a template for later use.

7.8.9. Click **Measure**. The **Measure Rate** window and **Loading Guide** are displayed.

7.8.10. Place the blank in the light beam, close the cover on the instrument and click OK to start the measurement. After the measurement, the Confirm Sample List box appears.

7.8.11. Verify the samples list and click **Continue**. The **Loading Guide** shows the information for the next sample.

7.8.12. Place the sample in the light beam and click **OK**. Repeat for each additional sample. The sample results appear in the **data display** and the **sample measurements table**.

7.8.13. To perform or modify rate calculations post-measurement, choose **Analyze** (menu), then **Modify Rate Curve**.

7.9. **Peltier Control and Cooling Unit (PCCU1)**

7.9.1. When the sample requires to be analyzed at a controlled temperature, be sure the flat cable from the PCCU1 is connected to the cell holder, and not obstructing the path of the light beam.

7.9.2. The PCCU1 must be powered on when Insight™ software is launched in order for it to be recognized by Insight. Restart Insight™ software if the PCCU1 is not recognized.

7.9.3. Define a target temperature via the Insight™ software, from the **Accessory** tab.

7.9.3.1. Select **Start measurement at target temperature**.

7.9.3.2. Enter the **Target temperature** and **Tolerance** in °C.

7.9.3.3. Allow time for equilibration. Select **Wait for equilibration** and enter the duration. The remaining time is displayed during the measurement. If the temperature moves outside the target range, the countdown resets when the temperature is again within range. The Start button becomes active when the temperature remains in the target range for the entire equilibration period.

7.9.3.4. The measurement can be started automatically after equilibration by selecting **Start measurement after equilibration**.

7.9.3.5. When necessary, the stirring speed may also be set with the PCCU1. Place a magnetic stirrer in the cuvette and use the Insight™ software to set the speed.

7.9.3.6. Loss of circulating water in the reservoir occurs over time and will need to be refilled on a regular basis. A **Water level too low** alarm will appear if the water is not refilled. Refill the reservoir with demineralized water per the instructions in the Peltier Control and Cooling Unit Operating Manual.

7.9.3.7. In the event of a temperature or memory failure, turn off the main power switch and then turn it on again. If the problem reoccurs, contact technical support. Refer to section 7.12 for technical support information.

7.10. **Creating a report**

7.10.1. To create a report, open a workbook or measure a sample.

7.10.1.1. To open saved data and the associated application, click on **My Data**.

7.10.1.2. Select the desired workbook file in the list and click **Open**.

7.10.2. Click on **Reports**.

7.10.3. Click the **Samples** tab and select the sample data to include.

7.10.4. Click the **Reportable Data** tab and specify the measurement result columns to include and their order.

7.10.5. Click the **Layout** tab to specify the design of the report.

7.10.6. To preview, print, or export a report, click **Preview**, **Print**, or **Export** respectively.

7.10.7. Results and reports are documented.

7.11. **Maintenance**

7.11.1. Clean the instrument exterior, the sample and reference compartment interior and touchscreen using a lint-free cloth dampened with water and wipe the surface as needed. Dry any wet surfaces.

7.11.2. Document maintenance in an applicable Equipment Logbook.

7.12. **Troubleshooting and Technical Support**

For more information, refer to the Insight™ Help system, from either the Help menu or by pressing F1 on the keyboard from most Insight™ screens. Additionally refer to UV-Vis Instruments Evolution One Series User Guide. For Technical Support, contact Thermo Fisher Scientific (include instrument serial number with request).