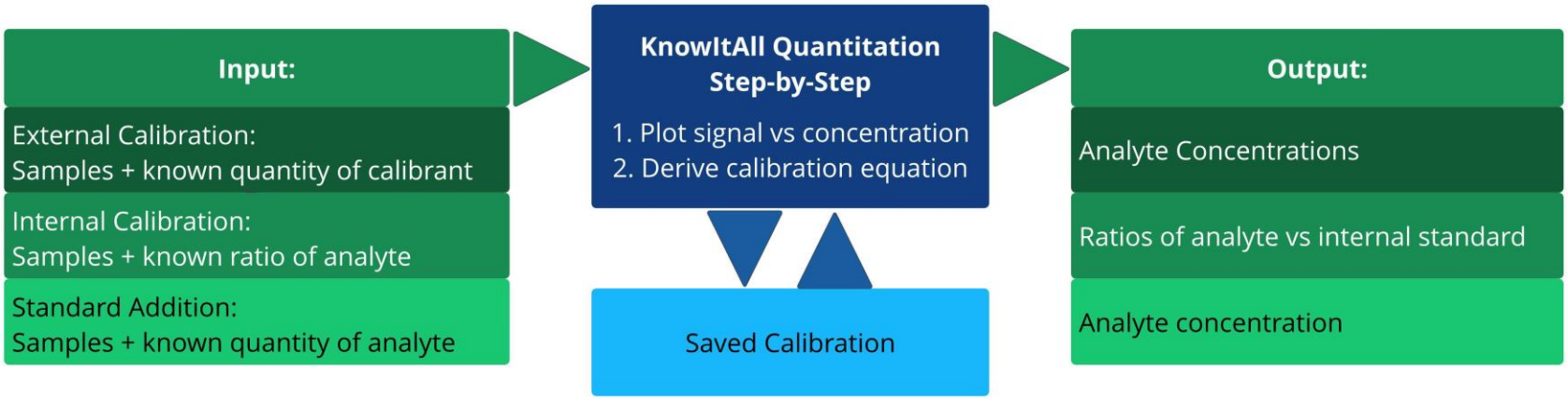
KnowItAll Informatics Training

Quantitation

WILEY

Quantitation Workflow



External Calibration Quantitation

Perform External Calibration Quantitation

Purpose

These exercises demonstrate how to perform external calibration quantitation using KnowItAll Quantitation software.

Objectives

This exercise will teach you:

- How to create external calibration
- How to perform quantitation

Background

Wiley's KnowItAll Quantitation application performs accurate quantitation over comprehensive types of analytical data.

Training Files Used in This Lesson

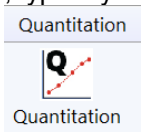
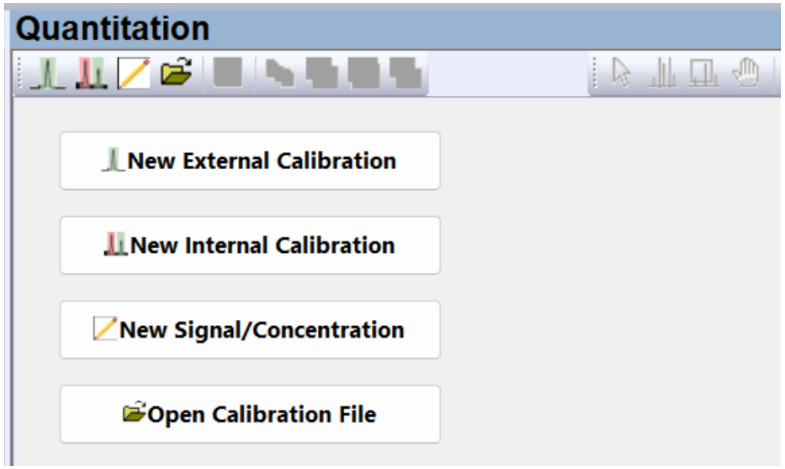
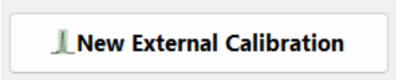
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

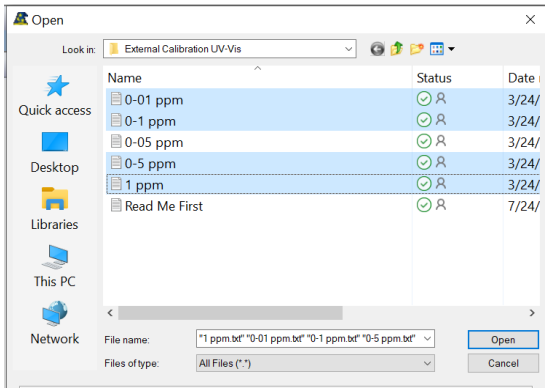
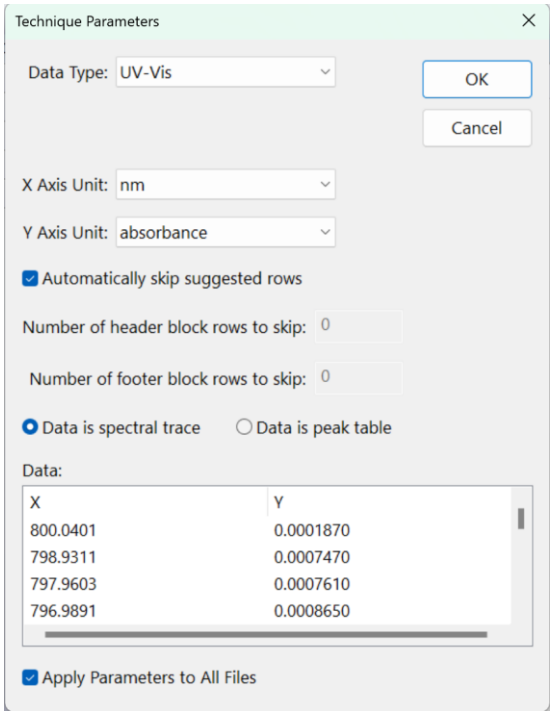
- External Calibration UV-Vis
- External Calibration IR

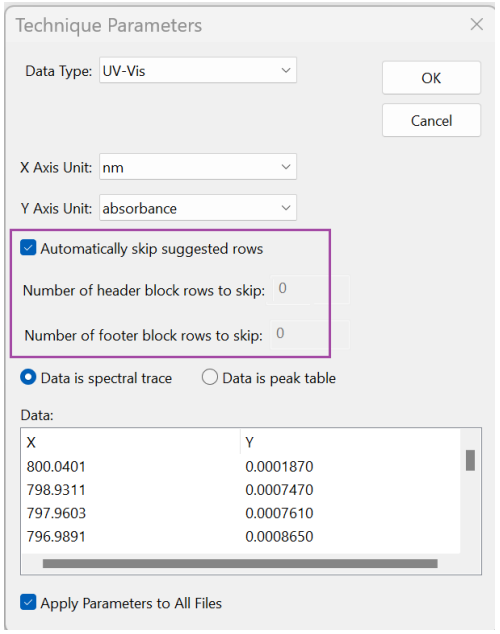
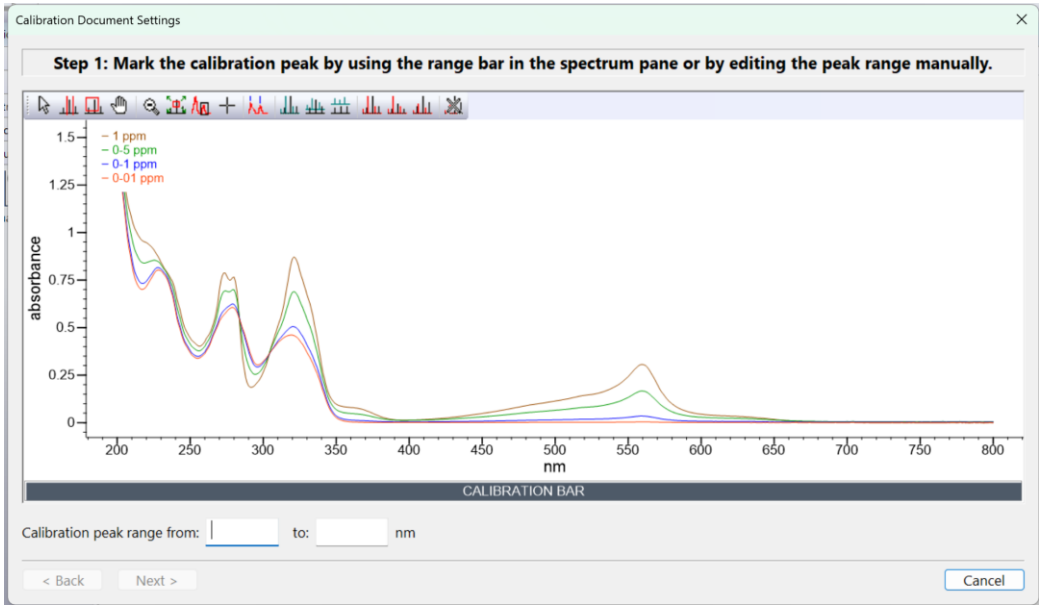
KnowItAll Applications Used

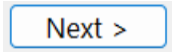
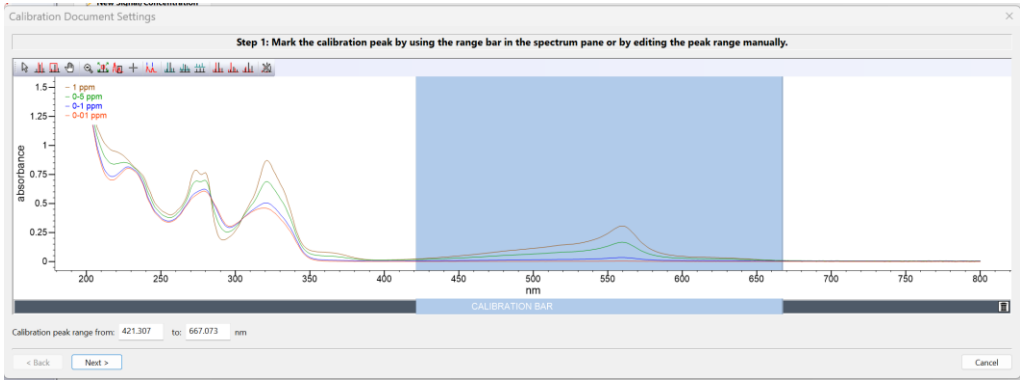
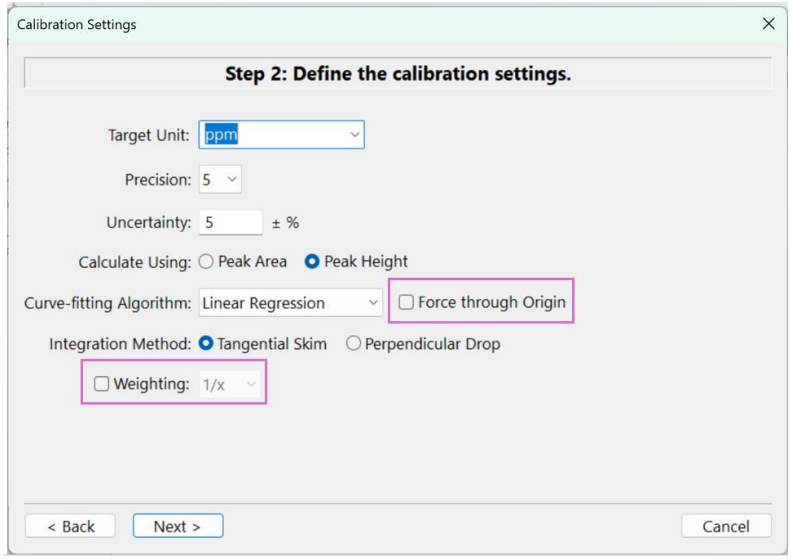
- Quantitation

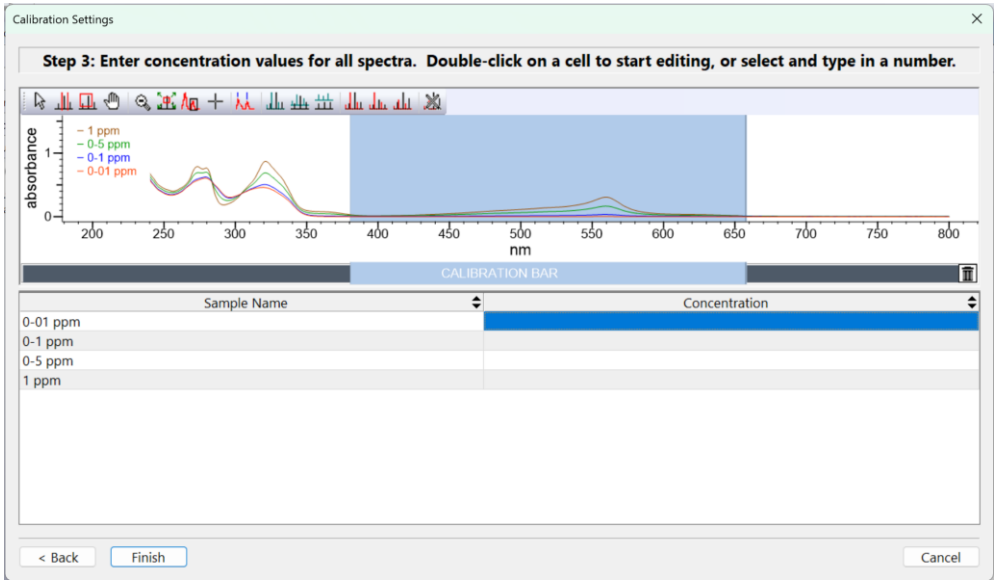
UV-Vis

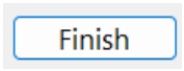
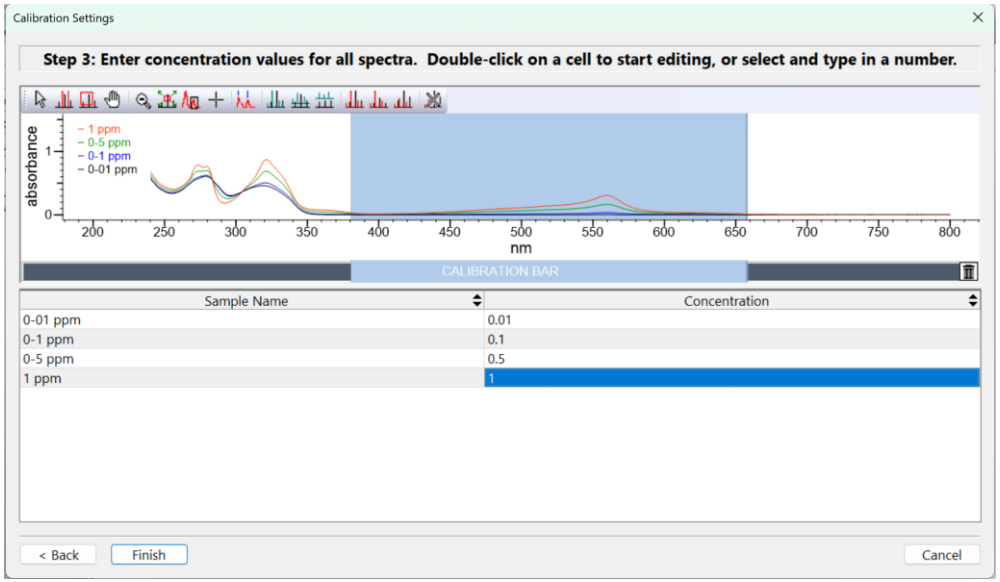
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group. 	**Quantitation** application is displayed: 
2	Click **New External Calibration** button. 	**KnowItAll** prompts user to open calibrant files.

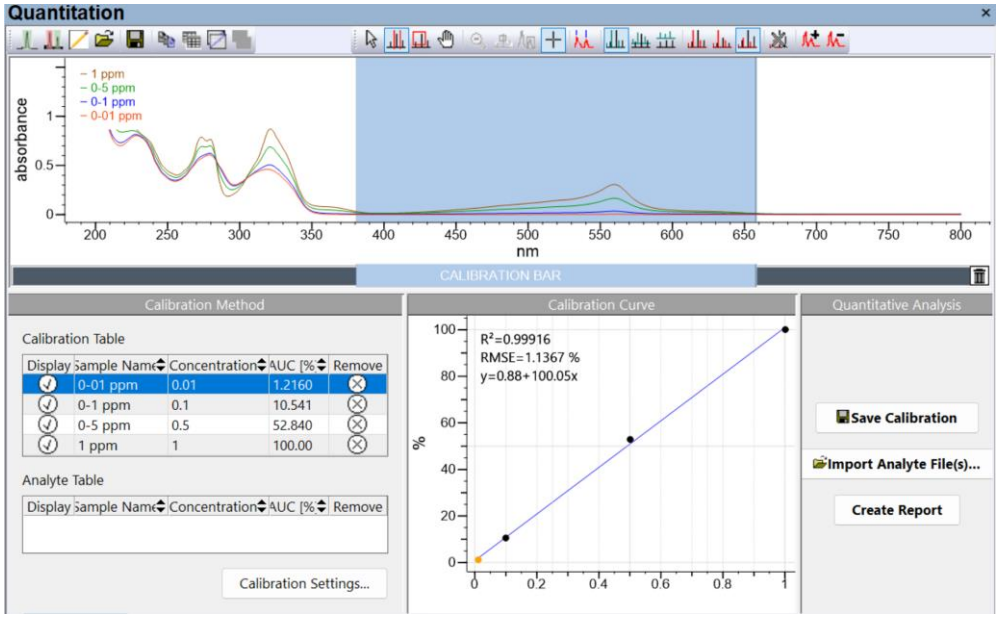
	Action	Result
3	Navigate to the "**C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis**" folder. Select 4 sample files and leave one out to be the unknown sample (*e.g.*, as shown in image below). Click **Open.** 	The **Technique Parameters** dialog is launched: 

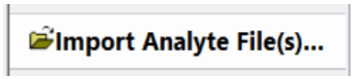
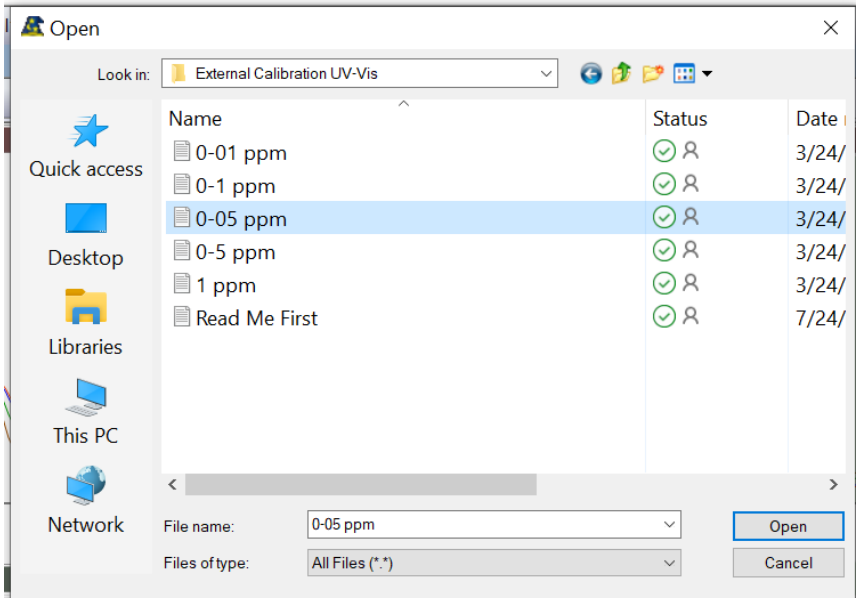
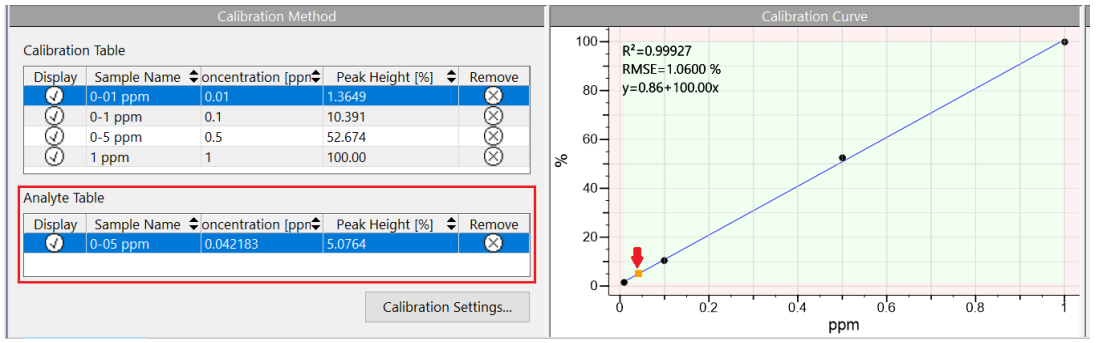
	Action	Result
4	In **Technique Parameters** prompt window: • Set **Data Type** to **UV-Vis**. • Check **Apply Parameters to All Files**. Click **OK**.  *Note*: The options highlighted above are added to skip lines which are not spectral *x,y* coordinates.	The **Calibration Document Settings** popout window appears, displaying the selected UV-Vis files. The **Step** is identified as “Step 1”: 

	Action	Result
5	Select the region around the peak at 560 nm by clicking down the left mouse button on the **CALIBRATION BAR** and dragging the button over the peak region, *e.g.,* from ~ 425 nm to ~ 650 nm. Release the mouse button at the end of the selection. Click **Next >** button. 	The selected region is displayed in blue coloration:  Upon clicking **Next** button, “Step 2” loads in the popup window.
6	In the popup window, define calibration settings as shown in the image • Target Unit: **ppm** • Calculate Using: **Peak Height** Remaining parameters can retain the default selection. *Note*: New options (purple boxed) are added for additional control of data.	

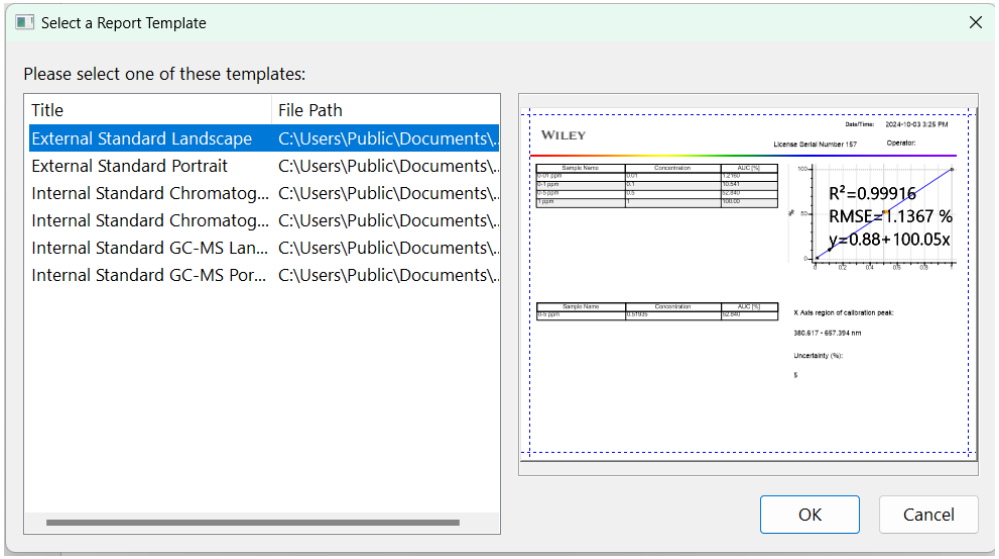
	Action	Result
7	Click **Next >** button.	Upon clicking **Next** button, “Step 3” loads in the popup window: 

	Action	Result
8	In the popup window, enter concentrations in the right column based on the file names: • **File**: 0-01 ppm, **Concentration**: 0.01 ppm • **File**: 0-1 ppm, **Concentration**: 0.1 ppm • **File**: 0-5 ppm, **Concentration**: 0.5 ppm • **File**: 1 ppm, **Concentration**: 1 ppm Click **Finish** button. 	The manually entered **Concentration** values are shown in the table below. Upon clicking **Finish**, the dialog closes and the spectra display in **Quantitation** application. 

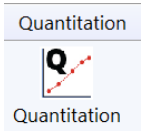
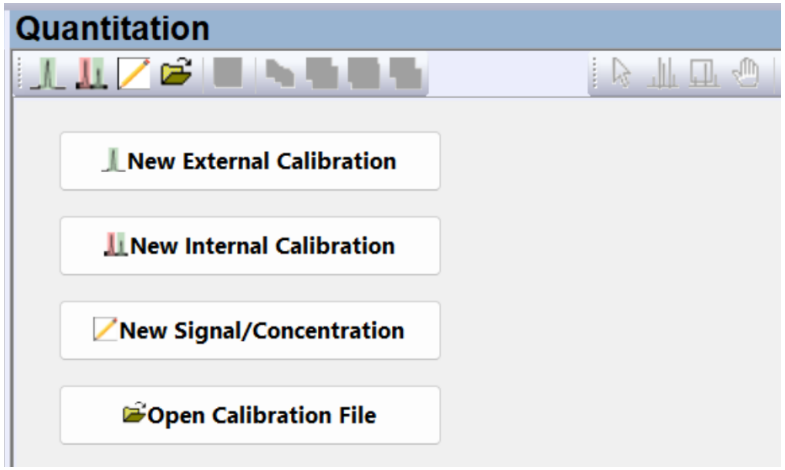
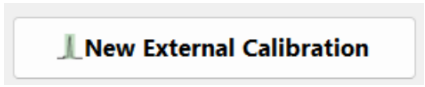
	Action	Result
9	Analyze the results in **Quantitation** application.	• Statistics are reported in the **Calibration Curve**. • The lower the value for **RMSE** (**Root Mean Squared Error)**, the better the curve fitting is. • The closer the **R^2** (**Coefficient of Determination**) is to 1**,** the better the curve fitting is. • The **Calibration Settings** button launches the **Calibration Settings** popup window, which allows for resetting the calibration parameters. • The calibration can be saved for future use or file sharing by clicking the **Save Calibration** button in the **Quantitative Analysis** panel. 

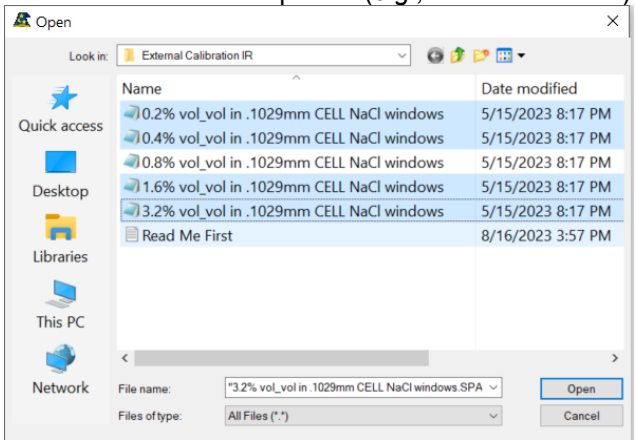
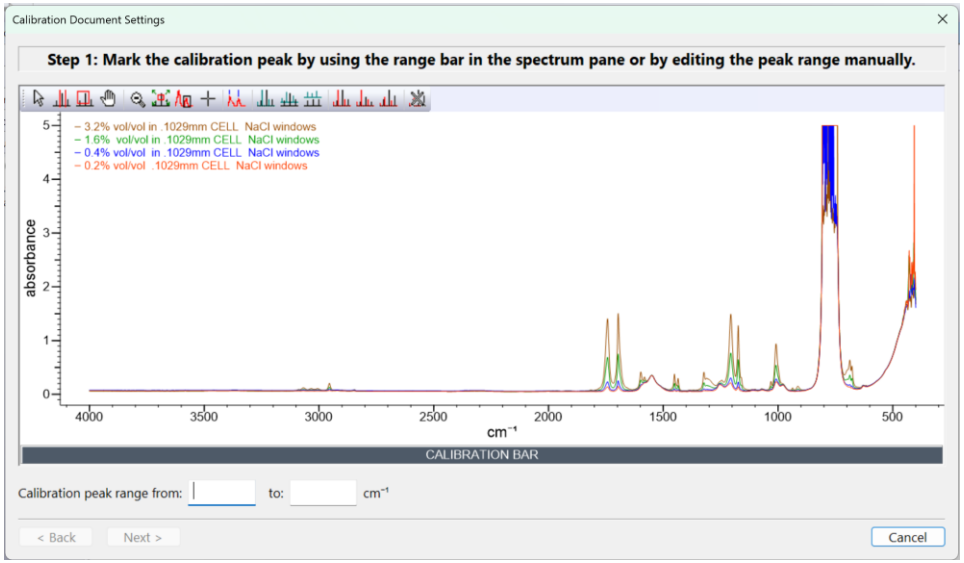
	Action	Result
10	Click the **Import Analyte File(s)** button.  Navigate to and select the file that was excluded in Step 3 “0-05 ppm”, located “**C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis**”. Click **Open.** On the **Technique Parameters** dialog window, select **Data Type** to be **UV-Vis.** Click **OK.**	The selected file in the **Open** file dialog window is shown. Upon clicking **OK** on the **Technique Parameters** dialog, the imported file opens in the previous calibration window. 
11	Analyze the results of the unknown calculation by viewing the **Analyte Table**, selected in red in the image on the right.	The concentration of the unknown is calculated and marked. 

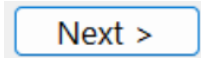
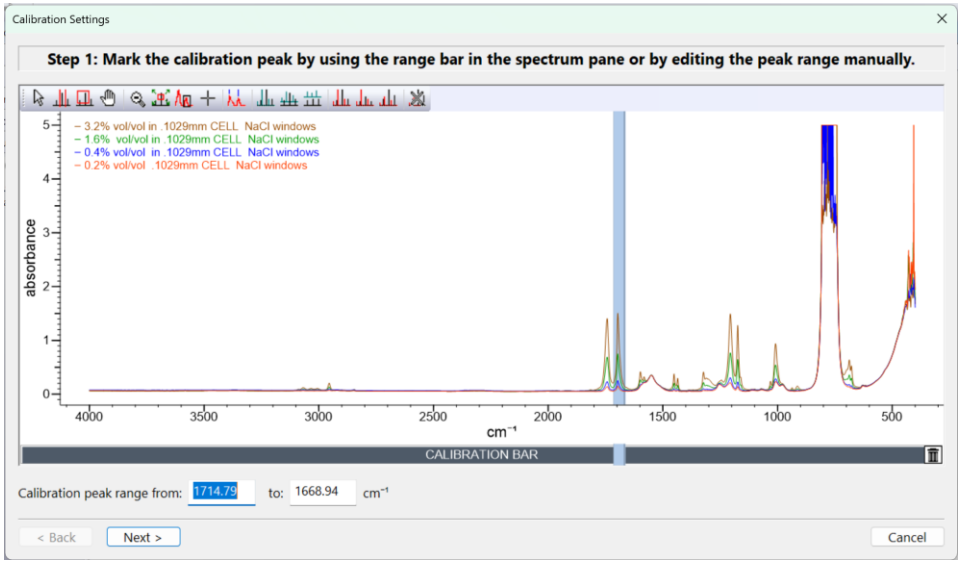
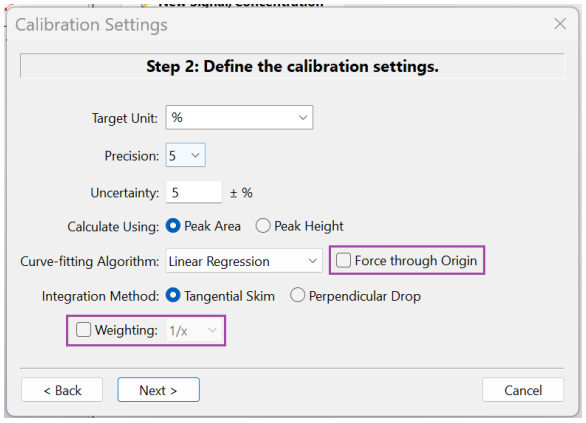
WILEY

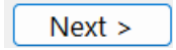
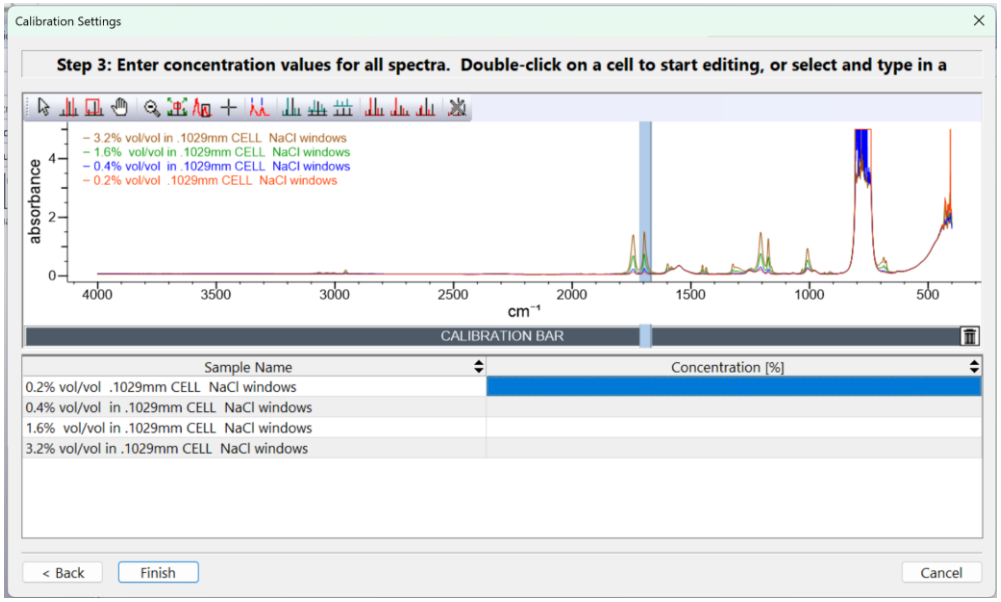
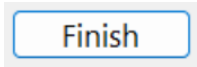
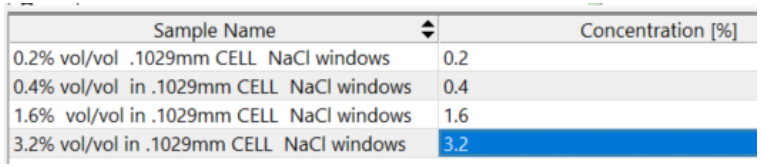
	Action	Result
12	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools.  On the **Select a Report Template** dialog, retain the selection of “External Standard Landscape”. Click **OK** on the dialog window to create the report in the selected template. *Note*: If a template is used for the first time, the user has to execute the following steps before transfer data to **ReportIt** application: • Choose **File > Edit Report Templates**. • Click **Add** button. • Navigate to the template files located **“C:\Users\Public\Documents\Wiley\KnowItAll\Report Templates\Quantitation”**. • Highlight to select all of the templates in the folder and click **Open**.	Upon clicking to generate the report, the **Report Templates** dialog window appears which prompts the user to choose the desired template for selection.  Upon clicking **OK** on the **Select a Report Template** dialog window, the report is generated in **ReportIt** application.

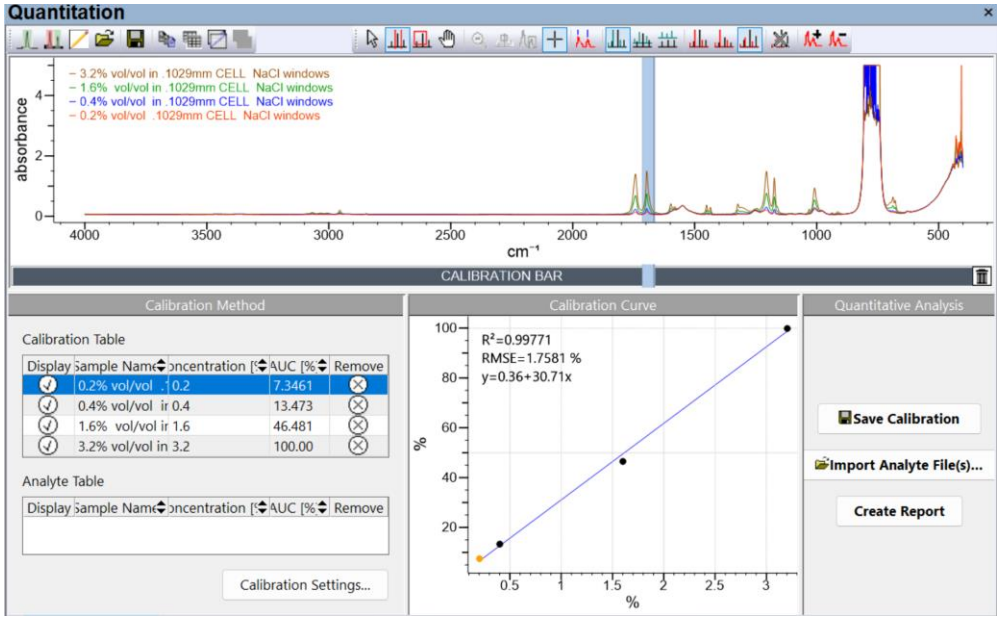
IR

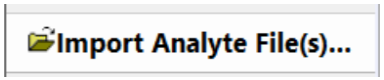
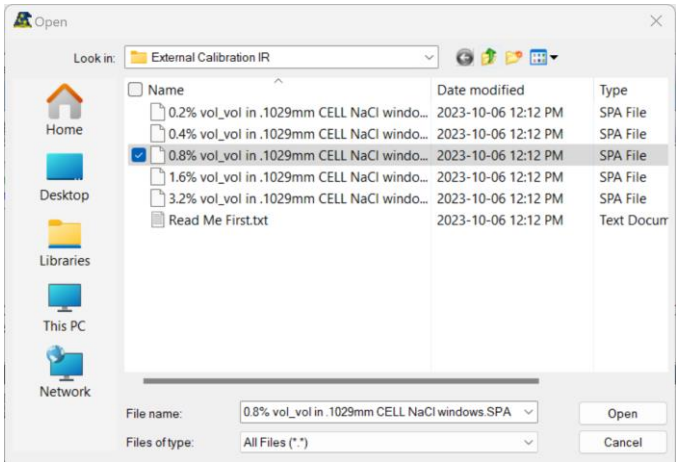
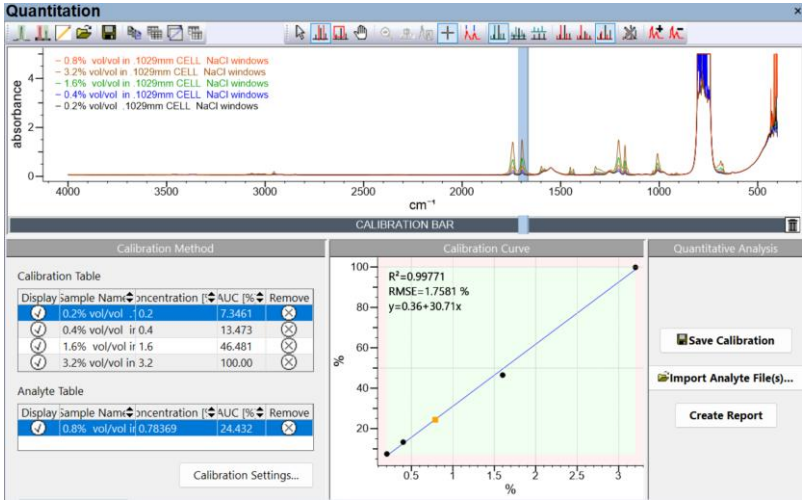
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.  *Note:* If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.	**Quantitation** application is displayed: 
2	Click **New External Calibration** button.  	**KnowItAll** prompts user to open calibrant files.

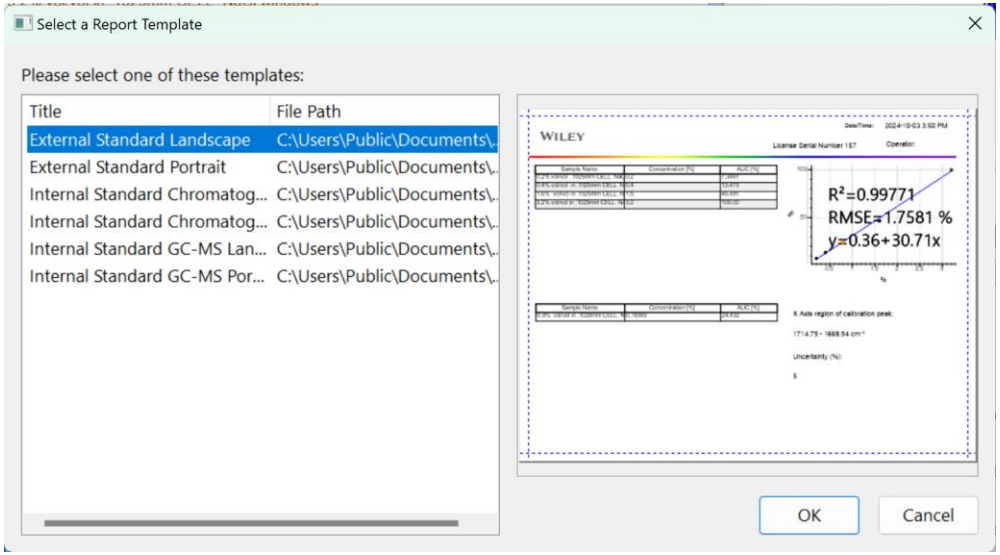
	Action	Result
3	Navigate to the "**C:\Users\Public\Documents\Wiley\KnowItAll\ Samples\Quantitation\External Calibration IR**" folder. Select sample files and leave the 0.8% file deselected to be the unknown sample file (*e.g.,* as shown below).  Click **Open.**	The **Calibration Document Settings** popout window appears, displaying the selected IR files. The **Step** is identified as "Step 1": 

	Action	Result
4	Select peak region around the peak at 1696 cm^{-1} by clicking down the left mouse button on the **CALIBRATION BAR** and dragging the button over the region, *e.g.,* from ~1720 to ~1650 cm^{-1}. *Note:* In IR quantitation, one should avoid using the strongest peak. Click **Next >** button. 	The selected region is displayed in blue coloration. Upon clicking **Next** button, "Step 2" loads in the popup window. 
5	In the following window, define calibration settings: • **Target Unit**: % Remaining parameters can retain the default selection. *Note*: New options (purple boxed) are added for additional control of data.	

	Action	Result
6	Click **Next >** button. 	Upon clicking **Next** button, “Step 3” loads in the popup window: 
7	Enter concentrations in the popup window based on the numbers in the sample names (*e.g.,* as shown in image on the righthand side). Click **Finish** button. 	The entered concentration values are shown, representing the concentration in %. Upon clicking **Finish**, the popup window is closed and the IR files load in **Quantitation** application. 

	Action	Result
8	Analyze the quantitation results in the application.	• Statistics are reported in the **Calibration Curve**. • The lower the value for **RMSE** (**Root Mean Squared Error)**, the better the curve fitting is. • The closer the **R^2** (**Coefficient of Determination**) is to 1**,** the better the curve fitting is. • The **Calibration Settings** button launches the **Calibration Settings** popup window, which allows for resetting the calibration parameters. • The calibration can be saved for future use or file sharing by clicking the **Save Calibration** button in the **Quantitative Analysis** panel. 

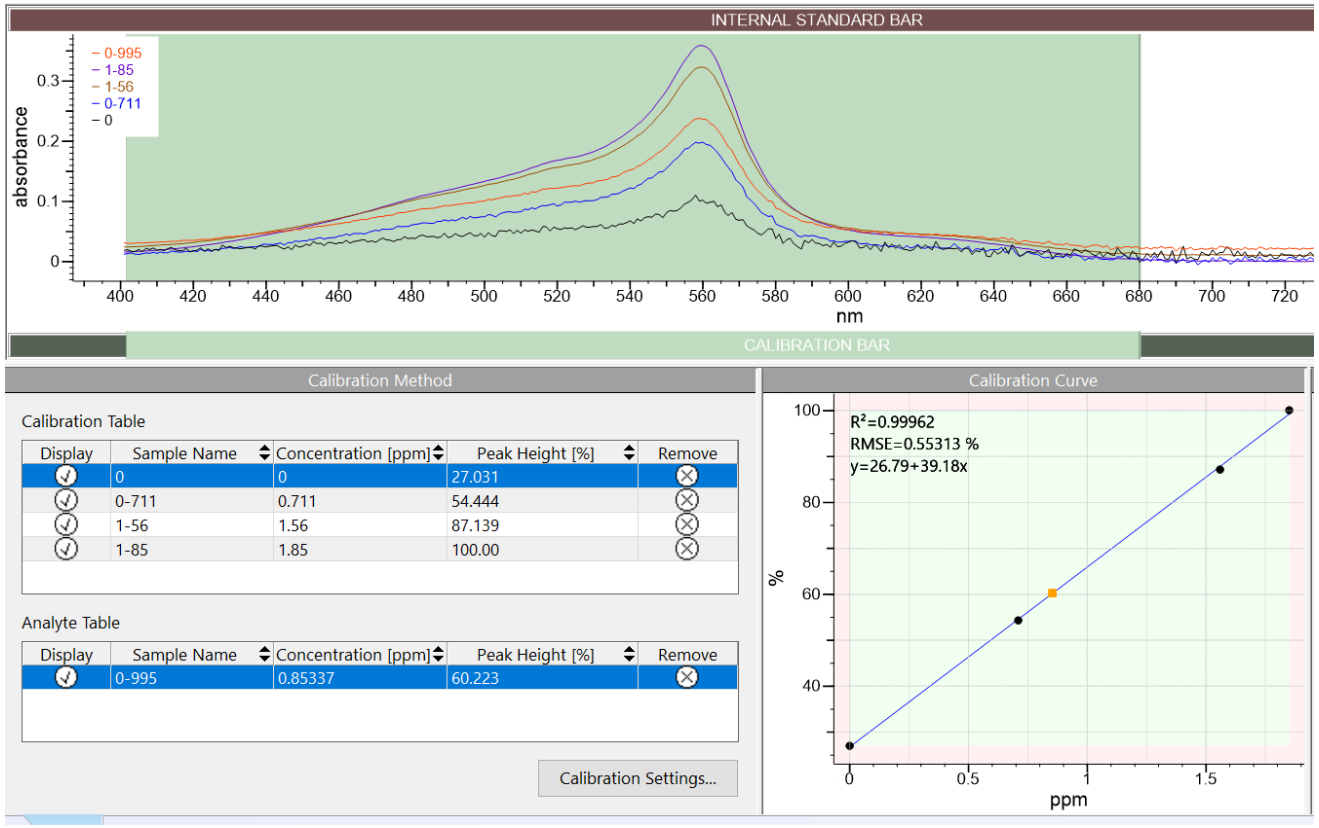
	Action	Result
9	Click the **Import Analyte File(s)** button in the **Quantitative Analysis** panel.  Navigate to and select the file that was excluded in Step 3 “0.8%”, located “**C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR**”. Click **Open.**	The **Open** file dialog window is shown. Upon clicking **OK**, the imported file opens in the previous calibration window. 
10	Analyze the results of the unknown calculation by viewing the **Analyte Table** located in the **Calibration Method** panel.	The concentration of the unknown is calculated and marked. 

	Action	Result
11	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. **Create Report** On the **Select a Report Template** dialog window, retain selection “External Standard Lanscape” and click **OK** to create the report in the selected template. *Note*: If a template is used for the first time, the user has to execute the following steps before transfer data to **ReportIt** application: • Choose **File > Edit Report Templates**. • Click **Add** button. • Navigate to the template files located “C:\Users\Public\Documents\Wiley\KnowItAll\Report Templates\Quantitation”. Highlight to select all of the templates in the folder, and click **Open**.	Upon clicking to generate the report, the **Report Templates** dialog window appears which prompts the user to choose the desired template for selection.  Upon clicking **OK** on the **Select a Report Template** dialog window, the report is generated in **ReportIt** application.

Standard Addition Quantitation

Perform Standard Addition Quantitation

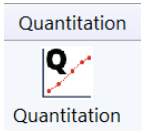
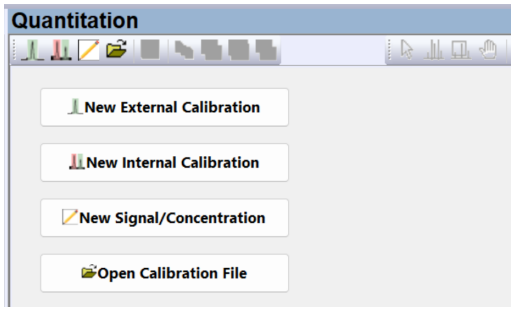
This screenshot shows a Standard Addition result, where when the added concentration is 0, the Y-axis value of 26.79 is the signal (due to iron in this case) in the original unknown sample:

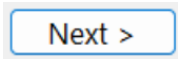
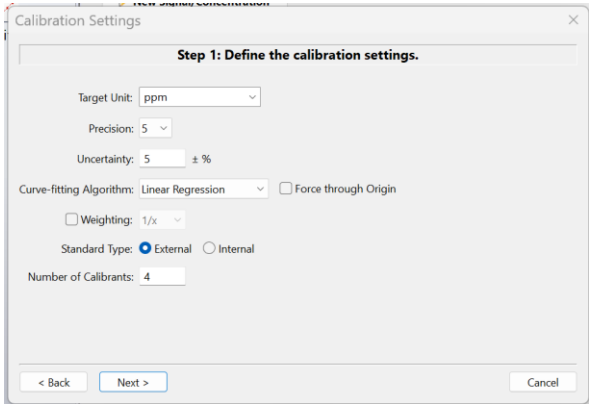
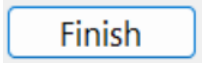
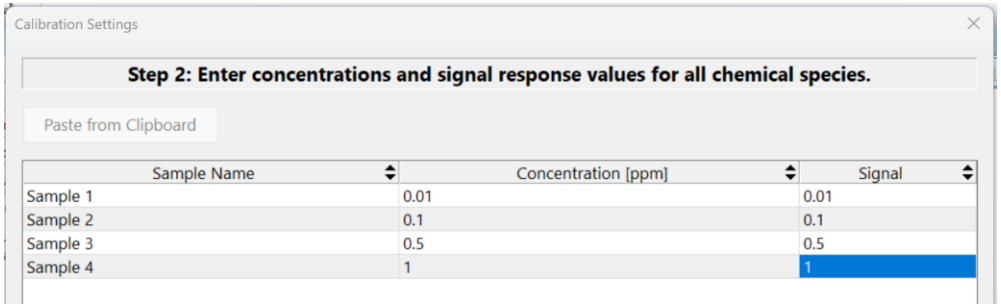


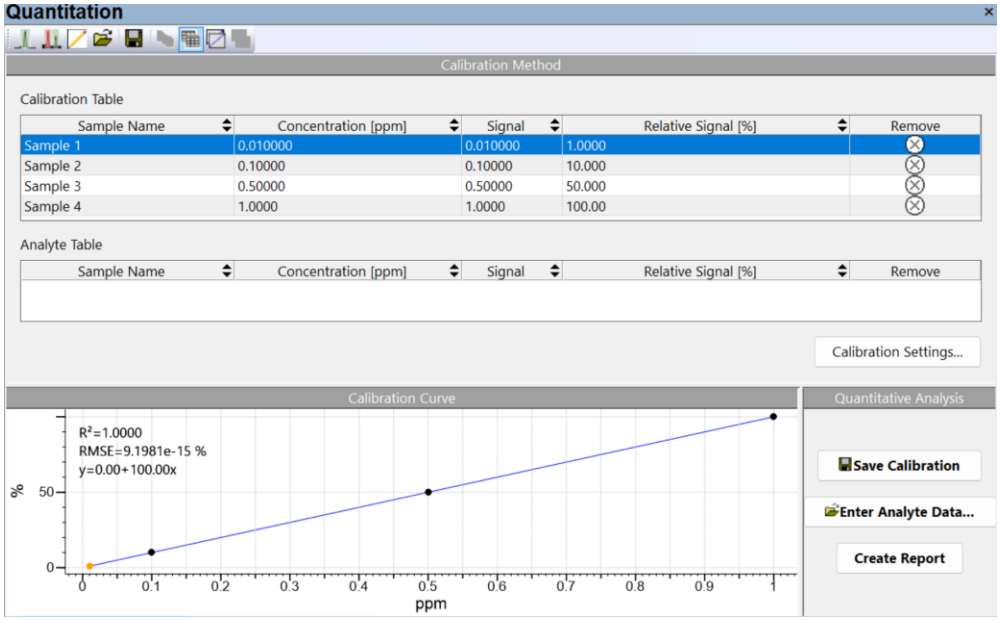
Directly Enter Signal Concentration Data for Quantitation

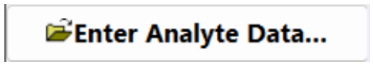
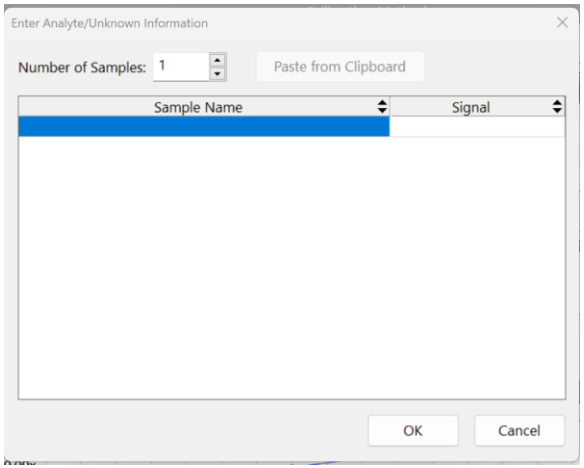
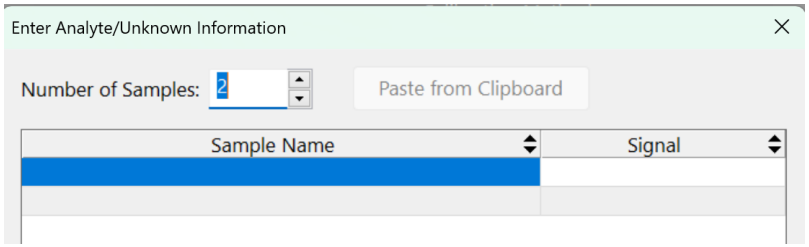
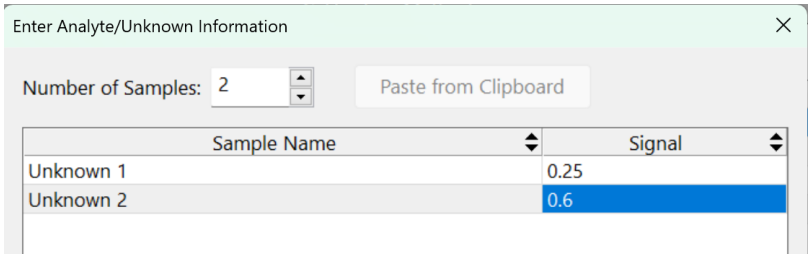
Directly Enter Signal Concentration Pairs to Create Calibration

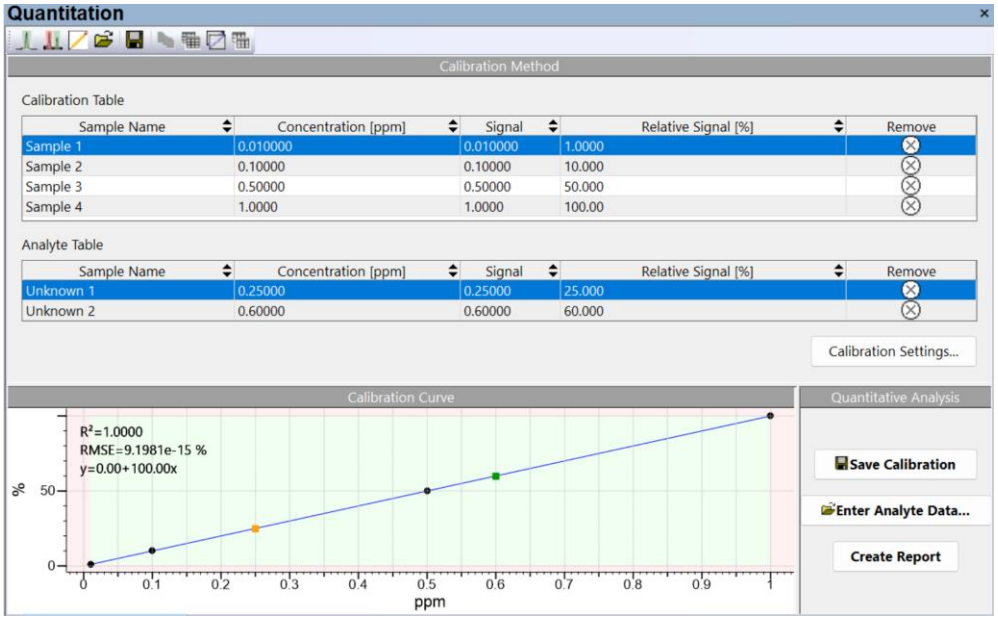
This is a new feature in **KnowItAll 2025**, which can be used to manually enter calibration values by importing calibrant and unknown information from reports.

	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.  *Note:* If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.	**Quantitation** application is displayed: 
2	Click **New Signal/Concentration** button: 	The **Calibration Settings** dialog window is launched on “Step 1”.

	Action	Result
3	Define the following parameters, as shown in the image on righthand side. • **Target Unit**: ppm • **Number of Calibrants**: 4 Remaining parameters can retain the default settings. Click **Next >** button to continue. 	The selected **Calibration Settings** are displayed. Upon clicking **Next**, Step 2 settings appear in the **Calibration Settings** dialog window. 
4	Enter calibration concentration-signal pairs: • **Sample Name**: 1, **Concentration**: 0.01 ppm, **Signal**: 0.01 • **Sample Name**: 2, **Concentration**: 0.1 ppm, **Signal**: 0.1 • **Sample Name**: 3, **Concentration**: 0.5 ppm, **Signal**: 0.5 • **Sample Name**: 4, **Concentration**: 1 ppm, **Signal**: 1 Tip: You can use the Tab button to move to the next field. Click **Finish** button. 	The **Calibration Settings** are displayed. • **Sample Name** represents the name of the sample file that was measured. • **Concentration** is the concentration of the calibrant sample. • **Signal** is the measured concentration of the sample.  Upon clicking **Finish**, the dialog window closes.

	Action	Result
5	Analyze the manual calibration results.	A calibration equation is created. 

	Action	Result
6	To add an unknown sample for quantitation, click **Enter Analyte Data** button found in the **Quantitative Analysis** panel. 	The **Enter Analyte/Unknown Information** dialog window is launched: 
7	In the **Number of Samples** cell, enter “2” as the value.	Two rows appear in the **Samples Table**: 
8	Enter the following information regarding the unknown samples in the **Samples Table**: • **Sample Name**: Unknown 1, **Signal**: 0.25 • **Sample Name**: Unknown 2, **Signal**: 0.6	The imported unknown information is displayed in the **Samples Table**: 

	Action	Result
9	Click **OK** on the **Enter Analyte/Unknown Information** dialog window. Analyze the calibration results in application.	The unknown samples are quantified according to the calibration equation: 
10	As described in previous sections, a report can be generated by clicking **Create Report** button. 	

Internal Standard Calibration Quantitation

Perform Internal Standard Calibration Quantitation

Purpose

These exercises demonstrate how to perform internal standard calibration quantitation using KnowItAll Quantitation software.

Objectives

This exercise will teach you:

- How to create internal standard calibration
- How to perform quantitation

Background

Wiley's KnowItAll Quantitation application performs accurate quantitation over comprehensive types of analytical data.

Training Files Used in This Lesson

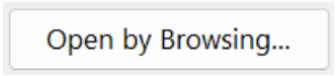
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

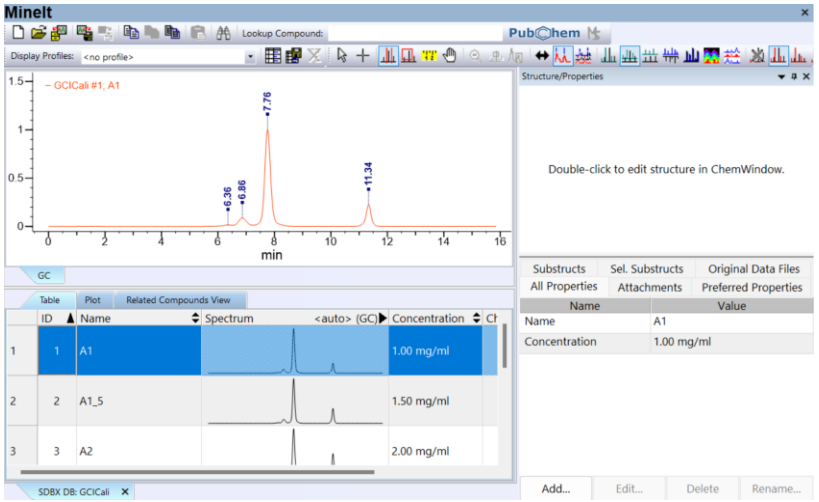
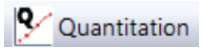
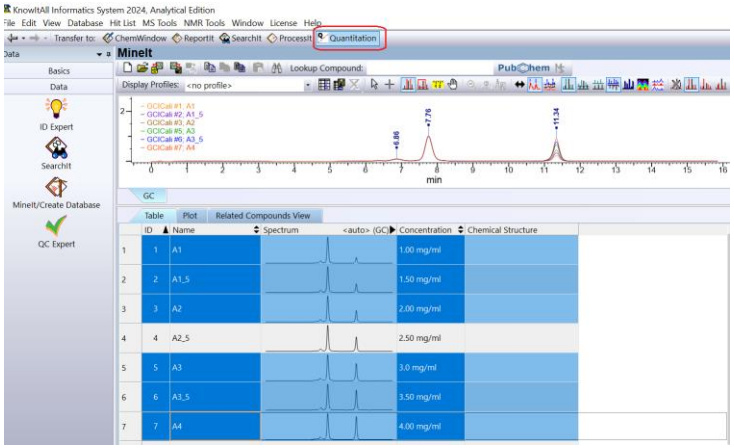
- Internal Calibration Chromatogram

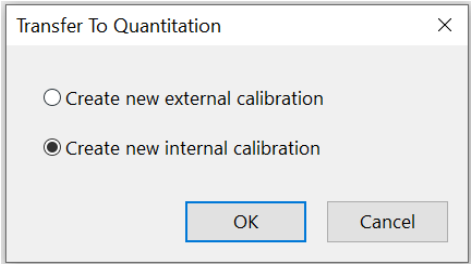
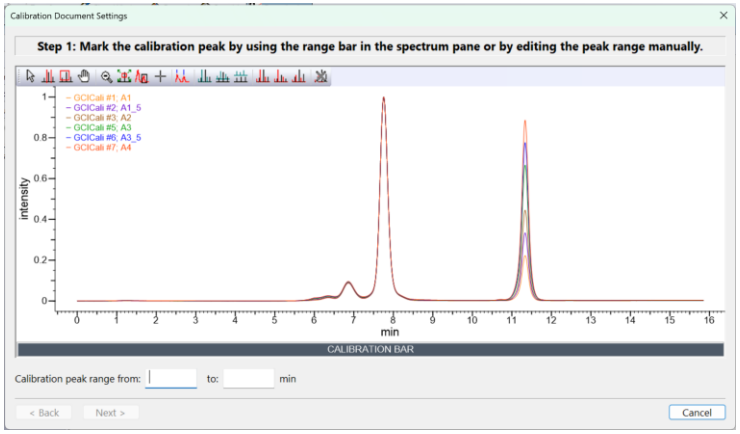
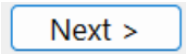
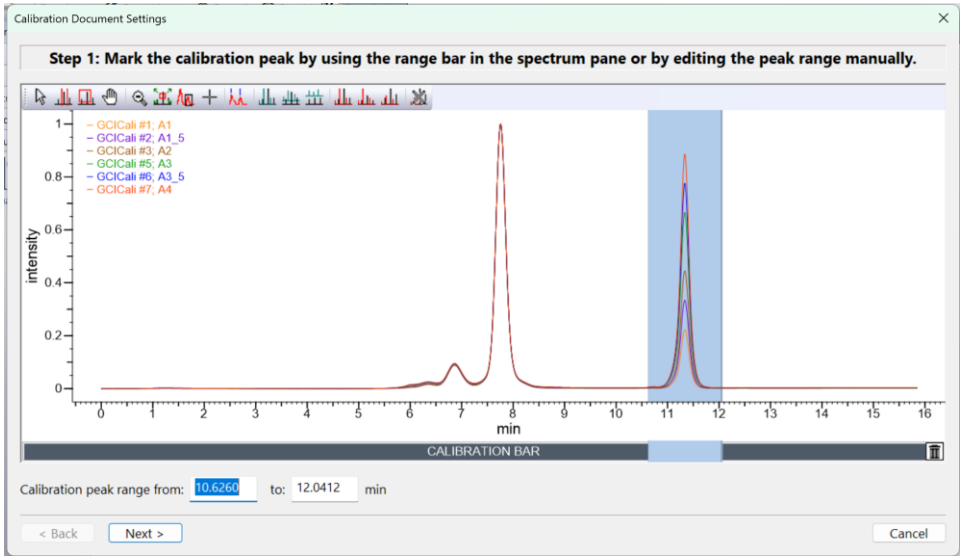
KnowItAll Applications Used

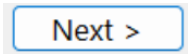
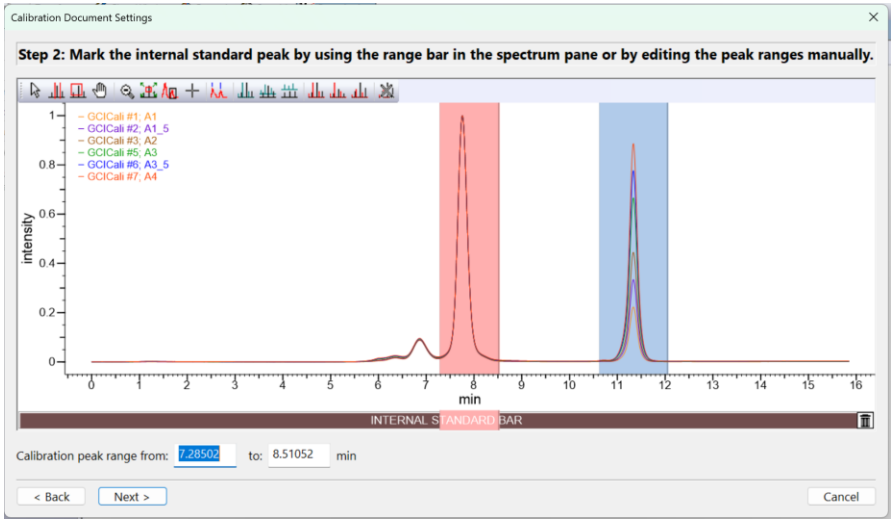
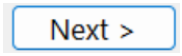
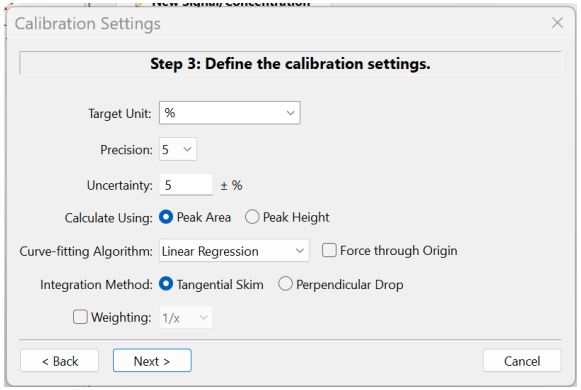
- Quantitation

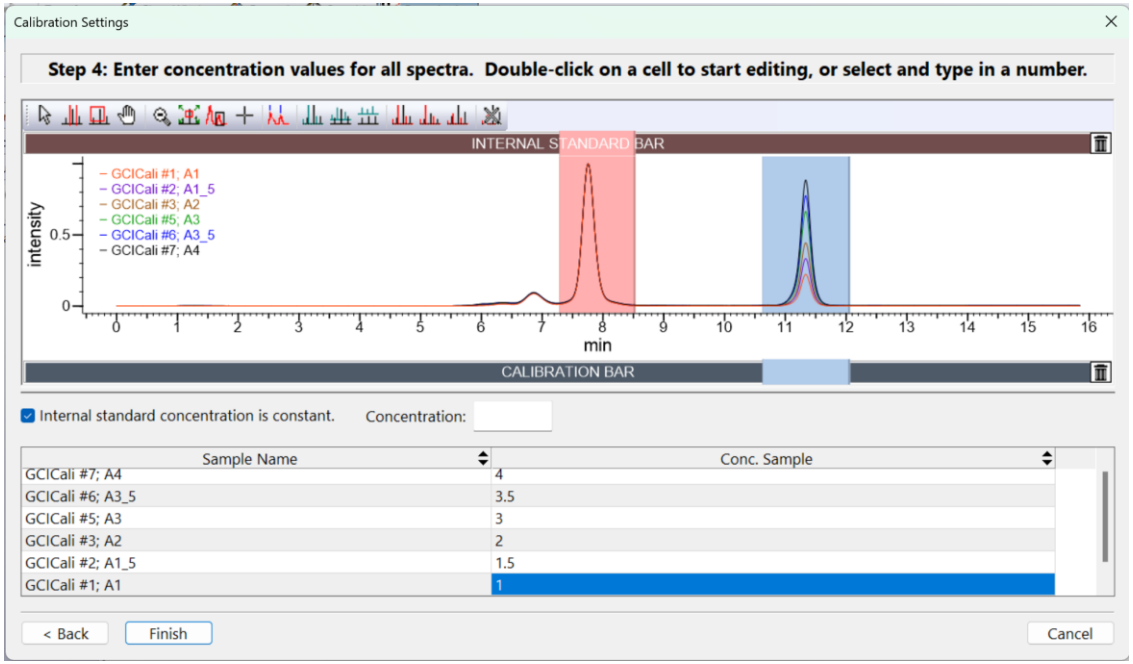
Chromatogram

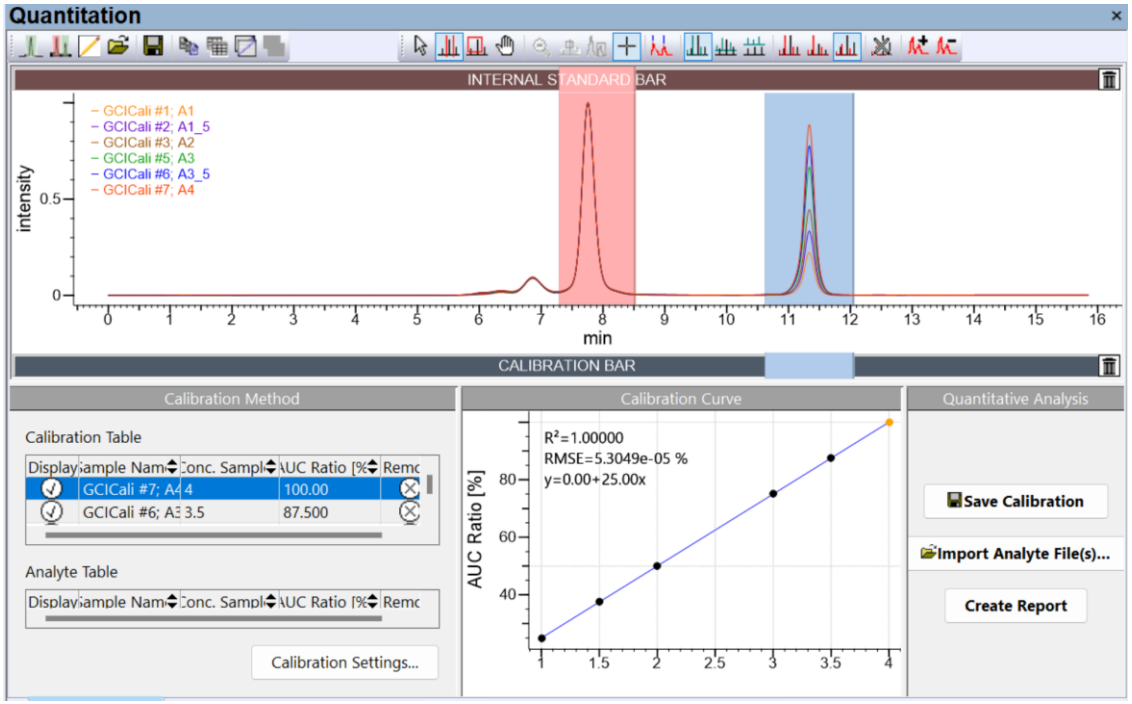
	Action	Result
1	Clear any calibration study presently open in **Quantitation** application by clicking on the X icon () on the top right corner.	
2	Open the **MineIt** application by clicking its icon, typically found in the **Data** group.  Choose **Database > Open.**	The **Select a Database** popup window opens.
3	In the **Select a Database** popup window, click the button **Open by Browsing** button. 	The **Browse for a Database** dialog window opens.

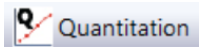
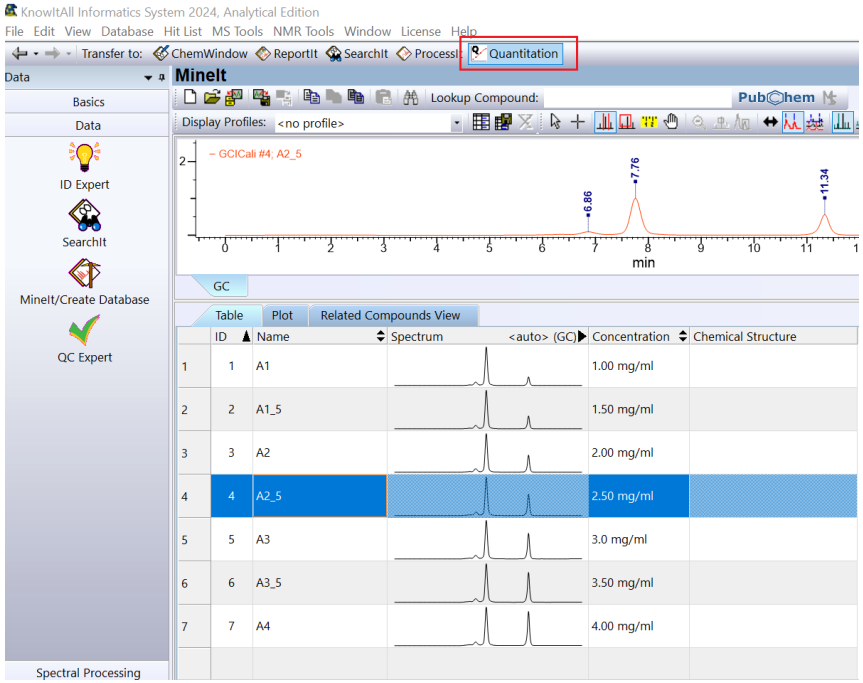
	Action	Result
4	Navigate to the **"C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration Chromatogram"** folder. Select the **Chromatograms For Internal Calibration Demo.sdbx** file. Click **Open.**	The database file opens in **MineIt**: 
5	Hold the CTRL button down on keyboard. Click with left mouse button on all but one record row, which will be the unknown (*e.g.,* A2_5). Then select **Transfer To: Quantitation.** 	The series of selected records is shown in **MineIt** application:  Upon using the **Transfer To** button, **KnowItAll** loads **Quantitation** application with a **Transfer to Quantitation** popup window.

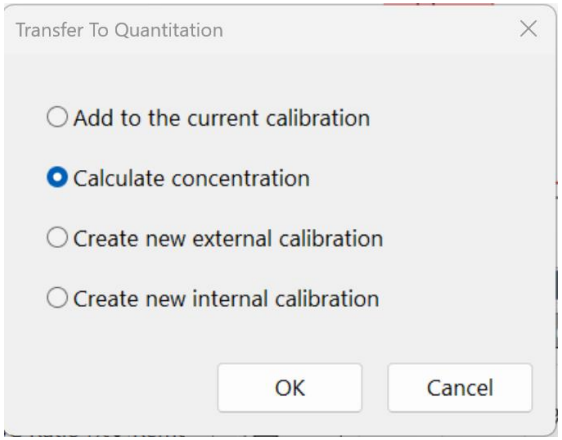
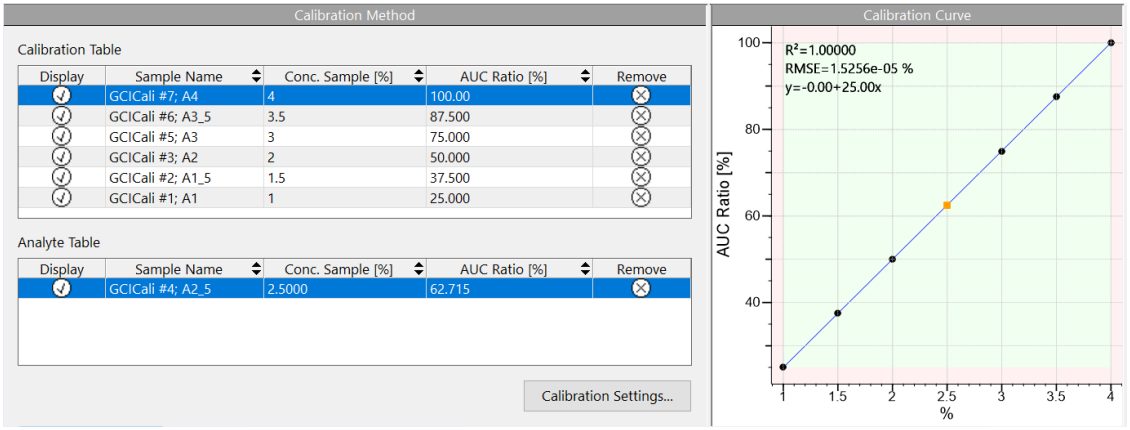
	Action	Result
6	On the **Transfer to Quantitation** popup window, select **Create new internal calibration** at the prompt window. Click **OK.** 	The chromatograms load in the **Calibration Document Settings** popup window: 
7	Select peak region around 11.3 min as the calibrant peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.*, ~ 10.6 – 12 min). Click **Next >** button. 	The selected region is displayed in blue coloration. Upon clicking **Next** button, “Step 2” loads in the popup window. 

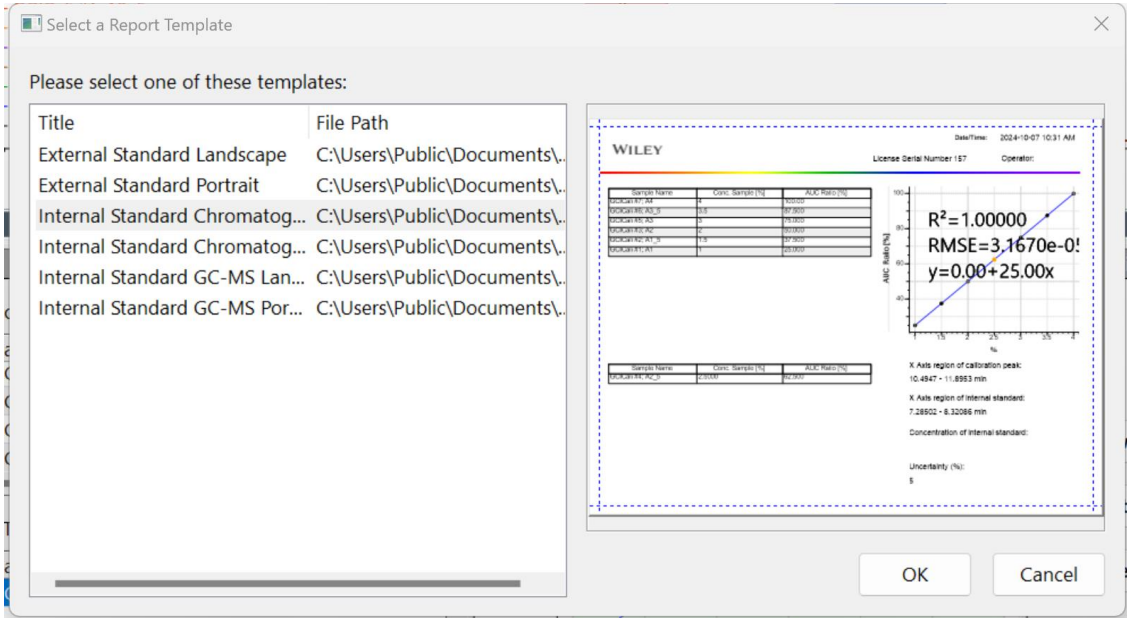
	Action	Result
8	Select peak region around 7.8 min as the internal standard peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.*, ~ 7.3 – 8.5 min). Click **Next >** button. 	The selected region is displayed with red coloration:  Upon clicking **Next** button, "Step 3" loads in the popup window.
9	In the **Calibration Settings** window, define calibration the settings: • **Target Unit**: % Remaining options can retain the default selection. Click **Next >** button. 	The selected calibration settings are shown in the popup window. Upon selecting **Next >**, Step 4 settings load in the popup window. 

	Action	Result
10	Enter concentration ratios in the popup window: • **File**: A4, **Concentration**: 4 • **File**: A3.5, **Concentration**: 3.5 • **File**: A3, **Concentration**: 3 • **File**: A2, **Concentration**: 2 • **File**: A1.5, **Concentration**: 1.5 • **File**: A1, **Concentration**: 1 Ensure that the checkbox **for Internal standard concentration is constant** remains selected. Click the **Finish** button.	The calibration settings are shown in the popup window. Upon clicking **Finish**, the dialog closes and the chromatograms are displayed in **Quantitation** application. 

	Action	Result
11	Analyze the calibration results in **Quantitation** application.	• Statistics are reported in the **Calibration Curve**. • The lower the value for **RMSE** (**Root Mean Squared Error)**, the better the curve fitting is. • The closer the **R^2** (**Coefficient of Determination**) is to 1**,** the better the curve fitting is. • The **Calibration Settings** button launches the **Calibration Settings** popup window, which allows for resetting of calibration parameters. • The calibration can be saved for future use or file sharing by clicking the **Save Calibration** button in the **Quantitative Analysis** panel. 

	Action	Result
12	Return to **MineIt** application. Click to select the file that was left out, *i.e,* **A2_5.** Select **Transfer To: Quantitation.** 	The selected chromatogram is shown in **MineIt** application. Upon using the **Transfer To** button, **KnowItAll** loads **Quantitation** application with a **Transfer to Quantitation** popup window. 

	Action	Result
13	On the **Transfer to Quantitation** popup window, select **Calculate concentration**. Click **OK.**	The selection is shown on the popup window. Upon clicking **OK**, the unknown chromatogram loads in **Quantitation** application. 
14	Analyze the results of the unknown concentration calculation. Review the **Analyte Table** and **Calibration Curve**.	The concentration of the unknown file is calculated in the **Analyte Table**. The unknown concentration is displayed on the **Calibration Curve**. 

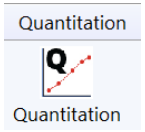
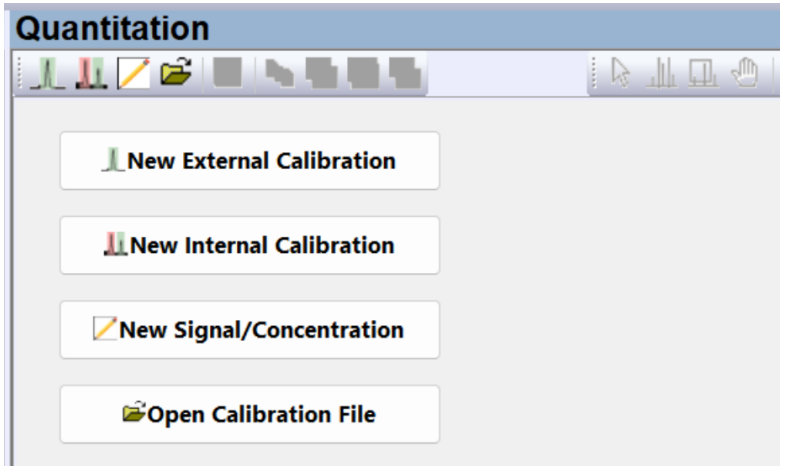
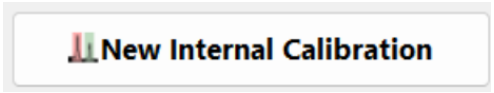
	Action	Result
15	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. **Create Report** On the **Select a Report Template** popup window, select "**Internal Standard Chromatogram Landscape**" report template. Click **OK** popup window to create the report in the selected template.	Upon clicking to generate the report, the **Report Templates** dialog window appears which prompts the user to choose the desired template for selection. Upon clicking **OK** on the **Select a Report Template** dialog window, the report is generated in **ReportIt** application. 

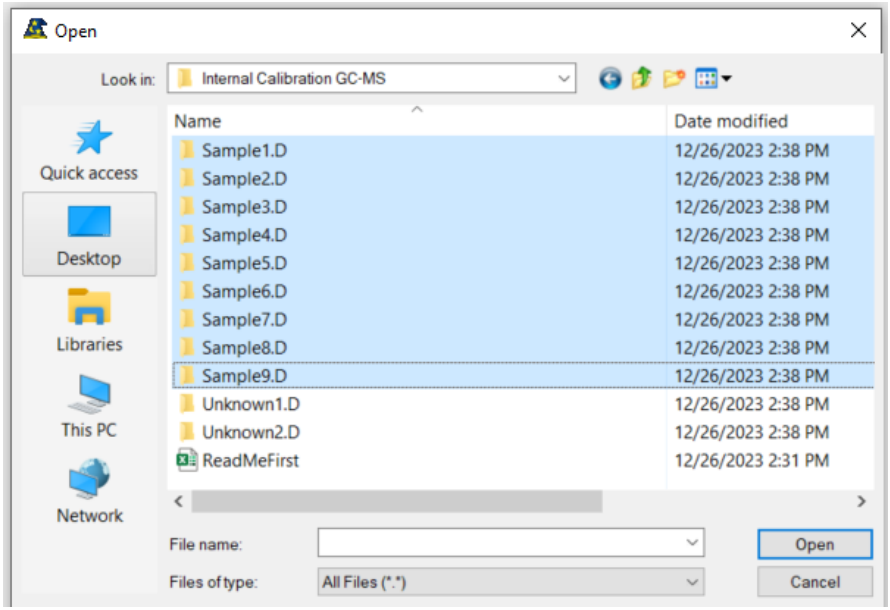
GC-MS

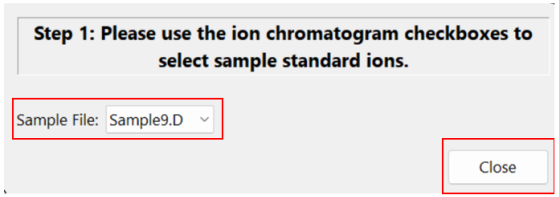
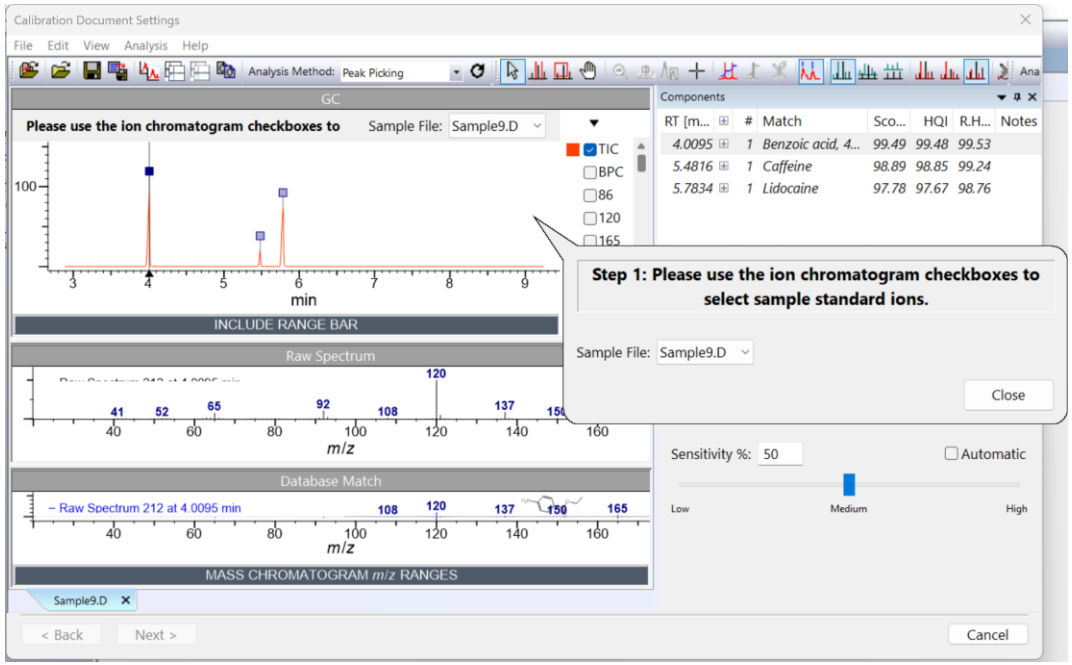
This dataset contains:

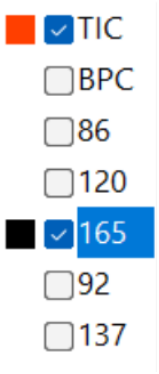
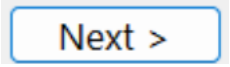
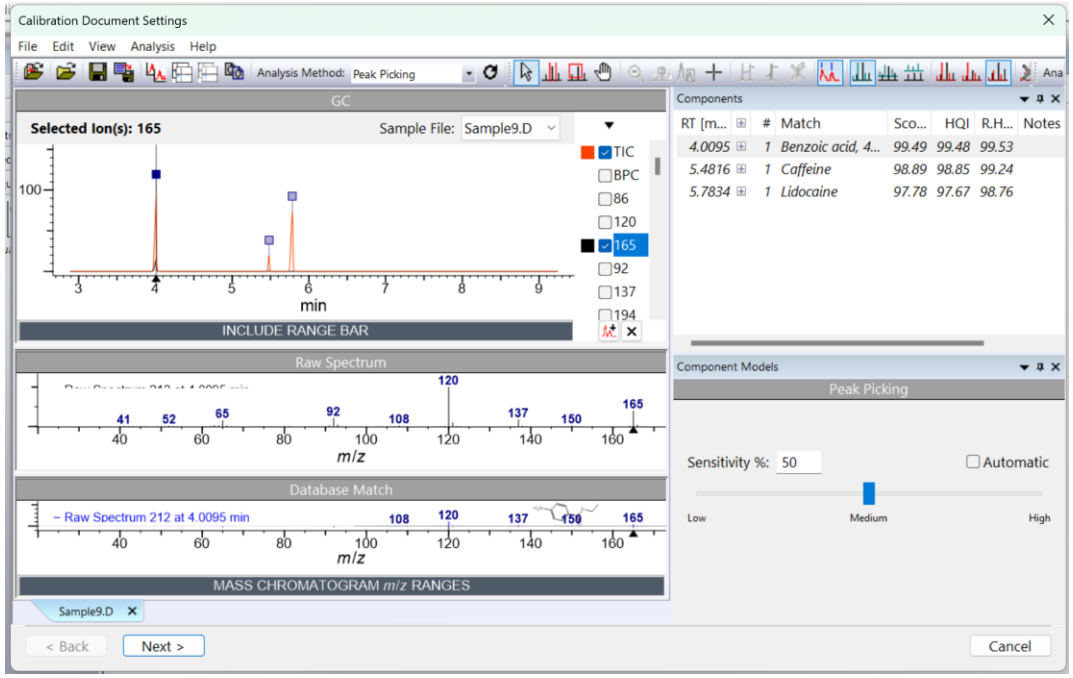
- Two Calibrants:
  - Benzocaine - GC retention time 4.01 min, MS ion to use: 165
  - Lidocaine - GC retention time 5.78 min, MS ion to use: 86 (*Note:* It breaks down in GC, therefore does not have a molecular ion)
- One Internal Standard – Caffeine at 0.7625 mg/mL, GC retention time 5.48 min, MS ion to use: 194
- The dataset to use pick MS ion(s) is Sample 9, representing the calibration sample with the highest concentration.

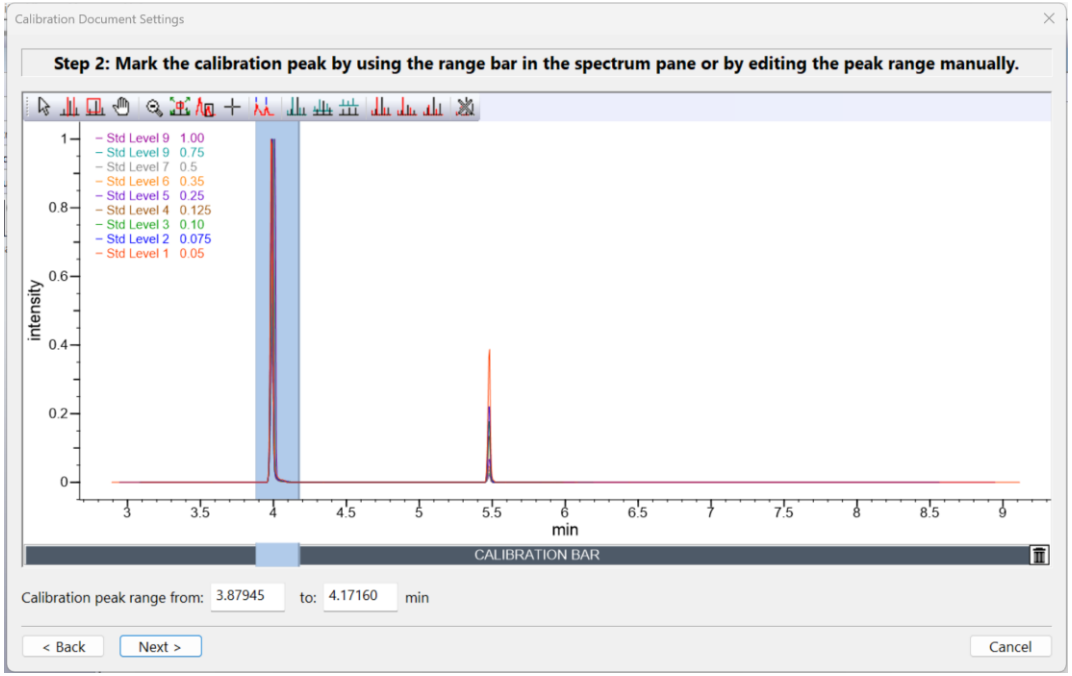
Benzocaine

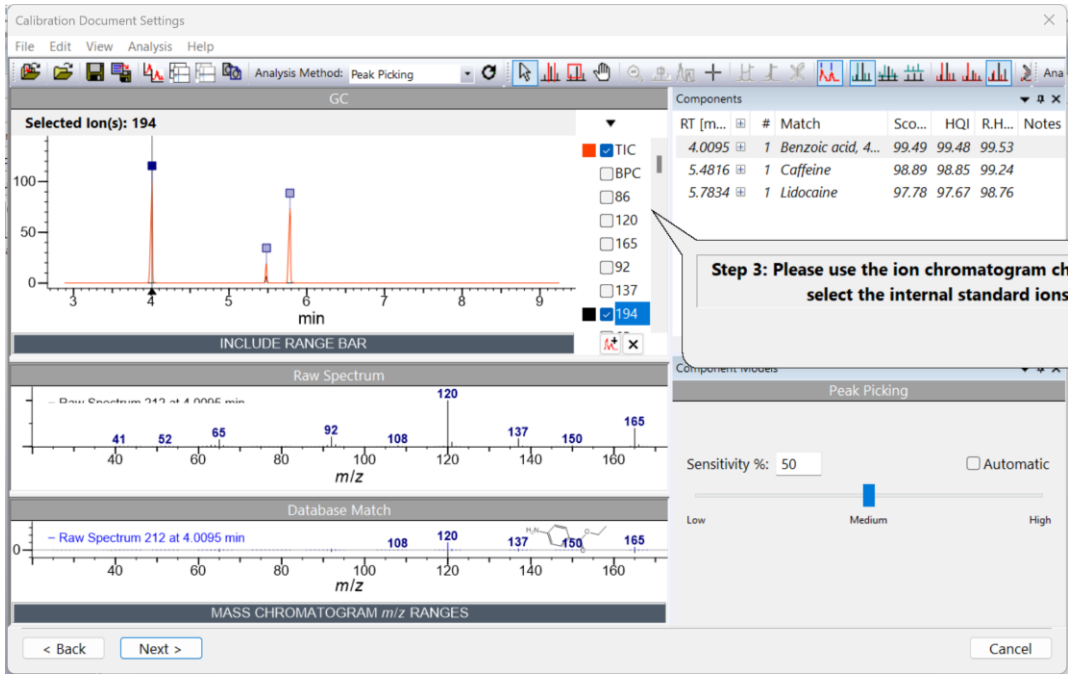
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.  *Note:* If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.	**Quantitation** application is displayed: 
2	Click **New Internal Calibration** button. 	**KnowItAll** prompts user to open calibrant files.

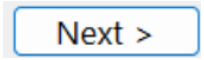
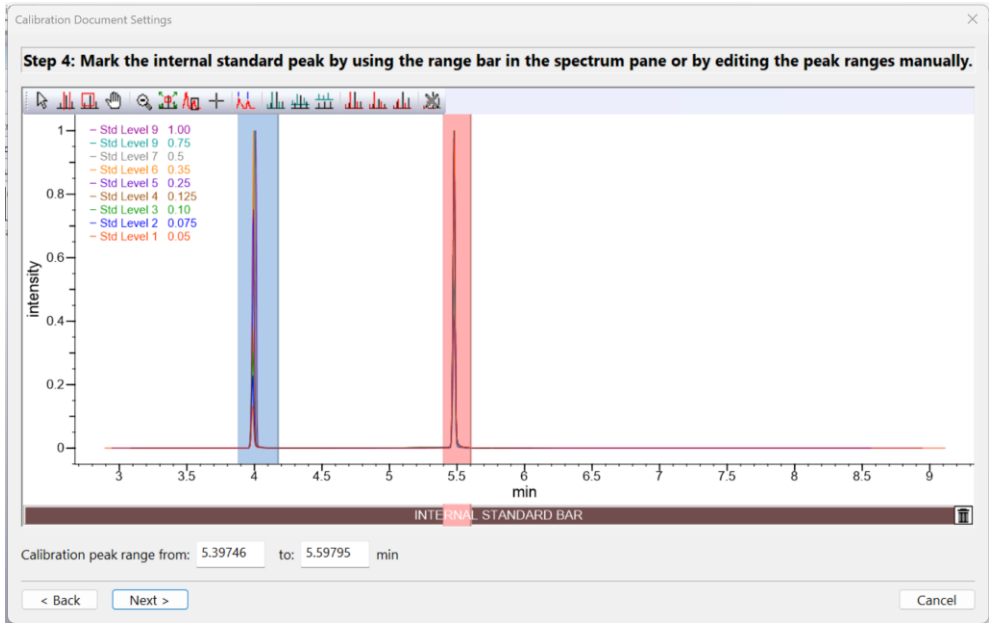
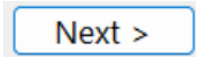
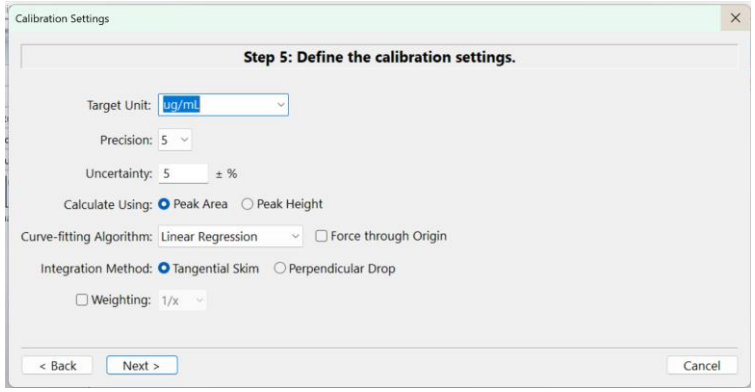
	Action	Result
3	Navigate to **"C:\Users\Public\Documents\Wiley\ KnowItAll\Samples\Quantitation\Internal Calibration GC-MS"** folder. Select all "**Sample_.D**" folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files. Click **Open.**	All sample files are selected in the **Open** popup window. Upon clicking **Open**, the **Calibration Document Settings** popup window appears. 

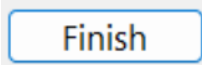
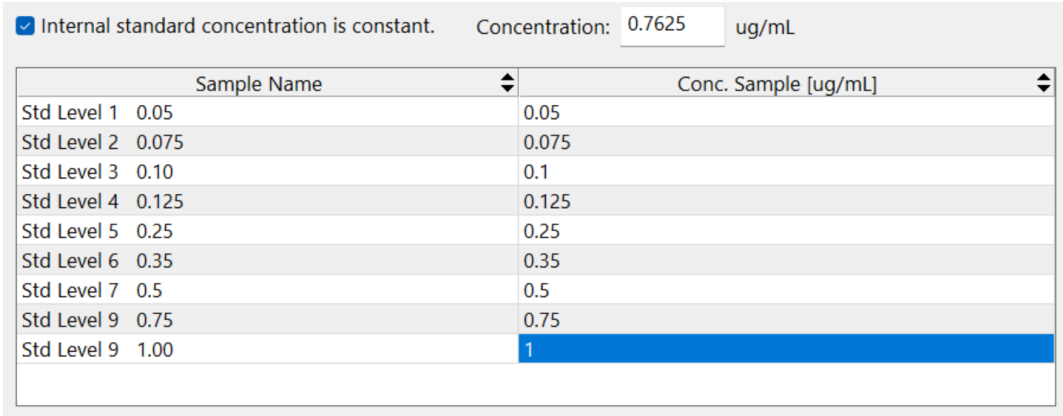
	Action	Result
4	On the **Calibration Document Settings** popup window, use the **Sample File** dropdown menu to choose "**Sample9.D**", which is the calibrant file in which the analyte concentration is the largest. Click **Close** on the bubble window after making the file selection using the dropdown menu (*e.g.,* see highlighted clicks below). 	The chromatogram with the largest concentration, *i.e.,* **Sample9.D.**, is used to select the component peak. 

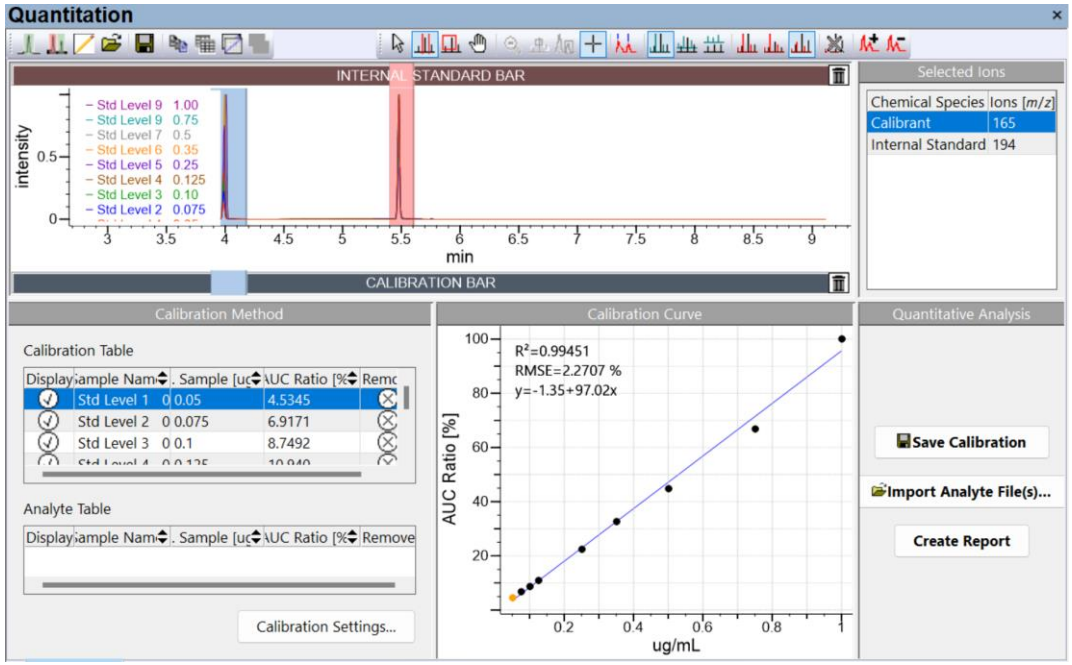
	Action	Result
5	Select a component from the **Raw Spectrum** pan by clicking on the numeric value in the **Mass Chromatogram List Pane**. In this example, the **Component Molecular m/z** to choose is “165”. This represents the TIC component for the peak at 4.01 min.  Click **Next >** button. 	The selected component is highlighted on chromatogram and **Mass Chromatogram List Pane**. Upon clicking **Next**, “Step 2” settings load in the **Calibration Document Settings** popup window. 

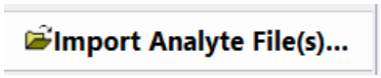
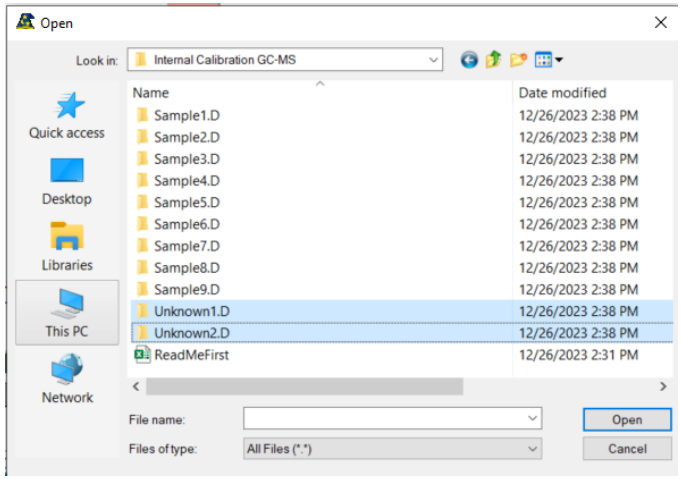
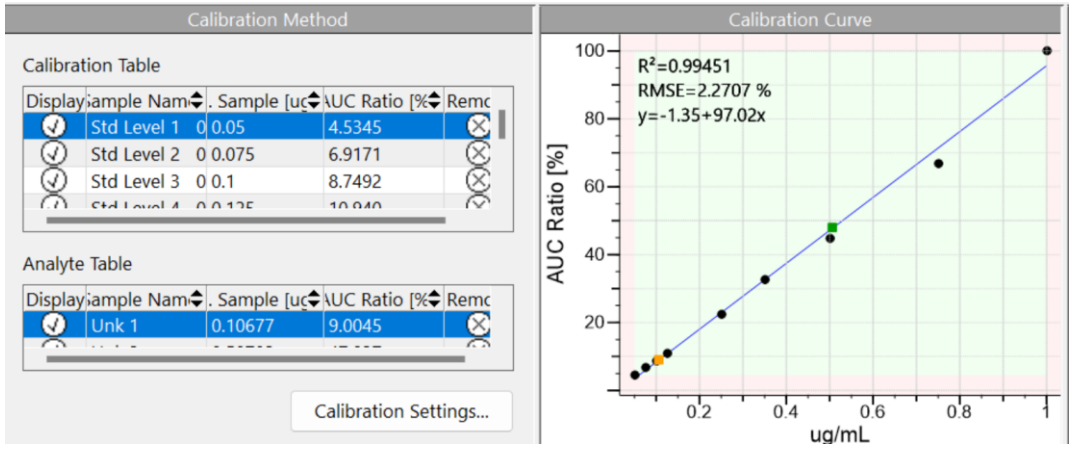
	Action	Result
6	Select peak region around 4.0 min as the calibrant peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.*, ~ 3.9 – 4.2 min). Click **Next >** button. 	The selected region is shaded with blue coloration. Upon clicking **Next >** button, “Step 3” settings load in the **Calibration Document Settings** popup window. 

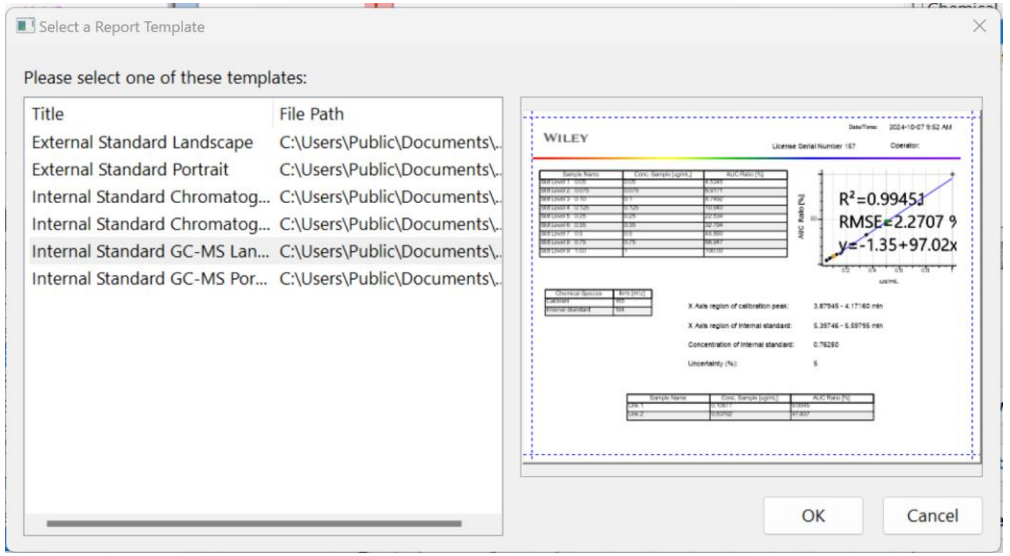
	Action	Result
7	Select a component from the **Raw Spectrum** pan by clicking on the numeric value in the **Mass Chromatogram List Pane**. In this example, the **Component Molecular m/z** to choose is “194”. This represents the TIC internal standard for the peak at 5.48 min. Click **Next >** button. 	The selected component is highlighted on chromatogram and **Mass Chromatogram List Pane**. Upon clicking **Next**, “Step 4” settings load in the **Calibration Document Settings** popup window. 

	Action	Result
8	Select peak region around 5.5 min as the calibrant peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.,* ~ 5.4 – 5.6 min). Click **Next >** button. 	The selected internal standard region is shaded with red coloration. 
9	In the **Calibrations Settings** popup window, define the calibration settings: • **Target Unit:** ug/mL (has to be manually typed in) Remaining parameters can retain the default selection. Click **Next >** button. 	

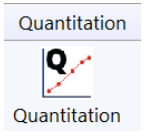
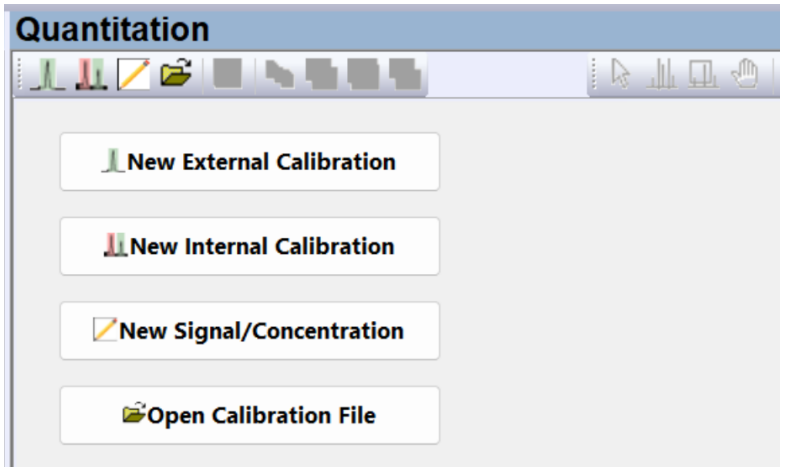
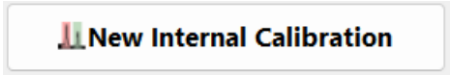
	Action	Result
10	Enter concentration and ratio values in the popup window: • **File**: Std Level 1 0.05, **Concentration**: 0.05 • **File**: Std Level 2 0.075, **Concentration**: 0.075 • **File**: Std Level 3 0.10, **Concentration**: 0.1 • **File**: Std Level 4 0.125, **Concentration**: 0.125 • **File**: Std Level 5 0.25, **Concentration**: 0.25 • **File**: Std Level 6 0.35, **Concentration**: 0.35 • **File**: Std Level 7 0.5, **Concentration**: 0.5 • **File**: Std Level 9 0.75, **Concentration**: 0.75 • **File**: Std Level 9 1.00, **Concentration**: 1 Ensure that the checkbox **for Internal standard concentration is constant** remains selected. Enter value for the internal standard concentration in the **Concentration** box “0.7625” ug/mL. Click the **Finish** button. 	The concentration settings for the samples and internal standard are shown:  Upon clicking Finish, the **Calibration Settings** popup window closes and the chromatograms display in **Quantitation** application.

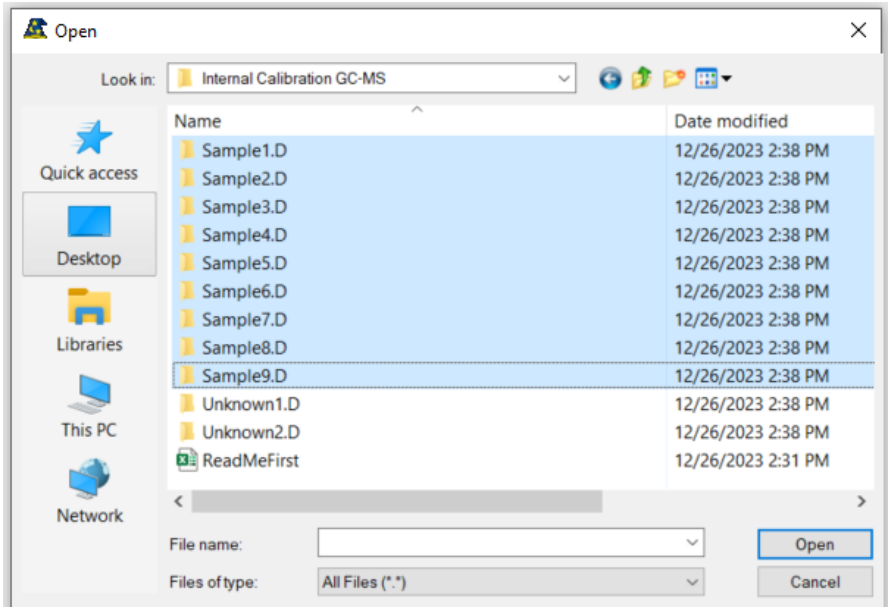
	Action	Result
11	Analyze the calibration results.	• Statistics are reported in the **Calibration Curve**. • The lower the value for **RMSE** (**Root Mean Squared Error)**, the better the curve fitting is. • The closer the **R^2** (**Coefficient of Determination**) is to 1**,** the better the curve fitting is. • The **Calibration Settings** button launches the **Calibration Settings** popup window, which allows for resetting the calibration parameters. • The calibration can be saved for future use or file sharing by clicking the **Save Calibration** button in the **Quantitative Analysis** panel. 

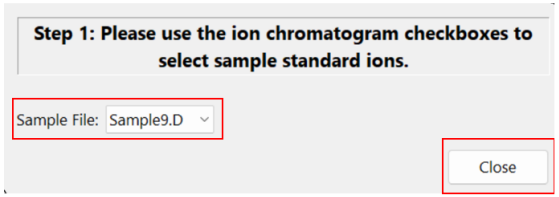
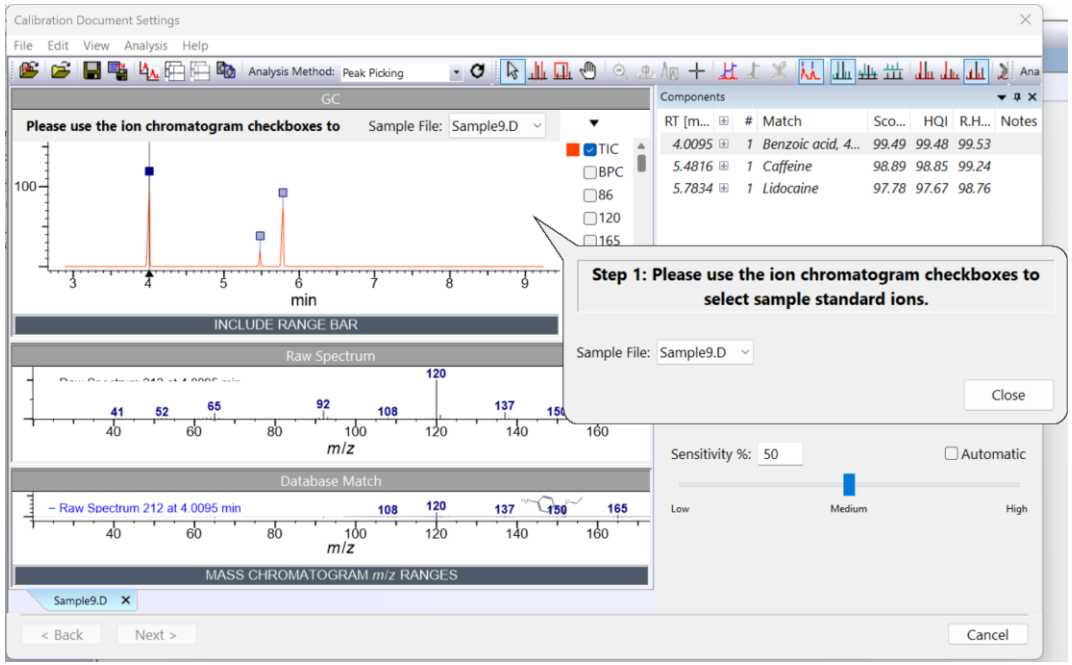
	Action	Result
12	Click the **Import Analyte File(s)** button found in the **Quantitative Analysis** panel.  On the **Open** popup window, hold CTRL button and click using left mouse button to select unknown file folder **Unknown1.D** and **Unknown2.D** (**"C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS"** folder). Click **Open.**	The selected sample files represent the unknown files for which the concentrations will be calculated from the calibration. Upon clicking **Open**, the dialog window closes and the unknown sample files open in the calibration window. 
13	Analyze the results of the unknown concentration calculation. Review the **Analyte Table** and **Calibration Curve**.	The concentration of the unknown file is calculated in the **Analyte Table**. The unknown concentration is displayed on the **Calibration Curve**. 

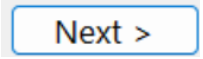
	Action	Result
14	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. **Create Report** In the **Select a Report Template** popup window, choose "**Internal Standard GC-MS Lanscape**" to use this report template. Click **OK** on the **Select a Report Template** dialog window to create the report in the selected template.	Upon clicking to generate the report, the **Report Templates** dialog window appears which prompts the user to choose the desired template for selection. Upon clicking **OK** on the **Select a Report Template** dialog window, the report is generated in **ReportIt** application. 

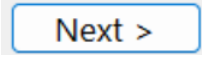
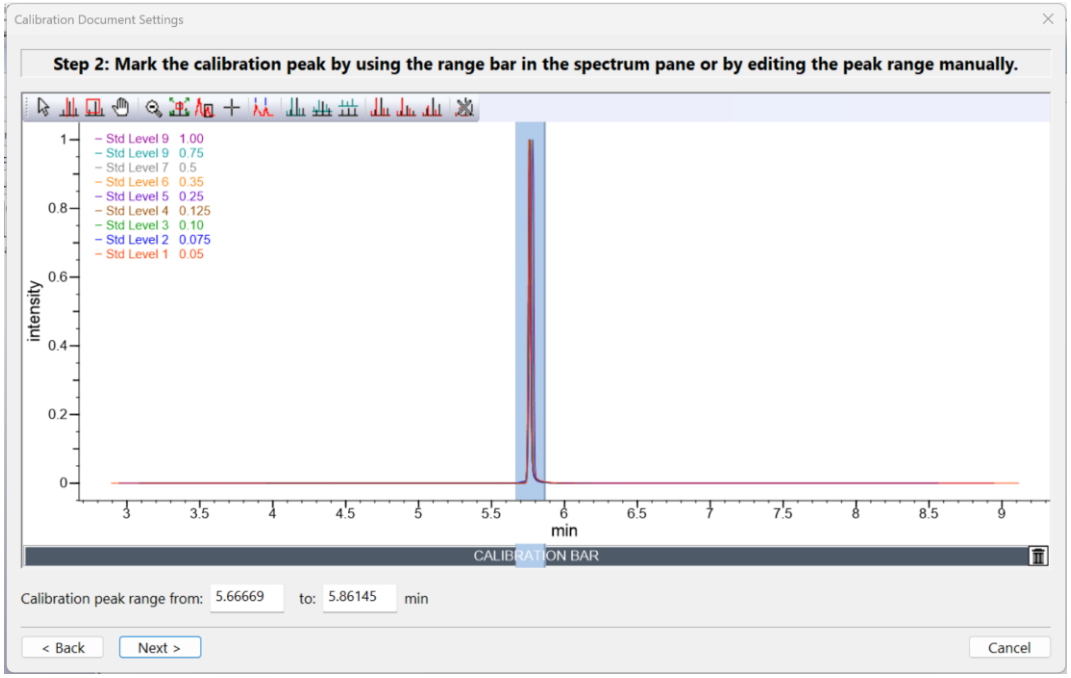
Lidocaine HCl

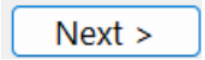
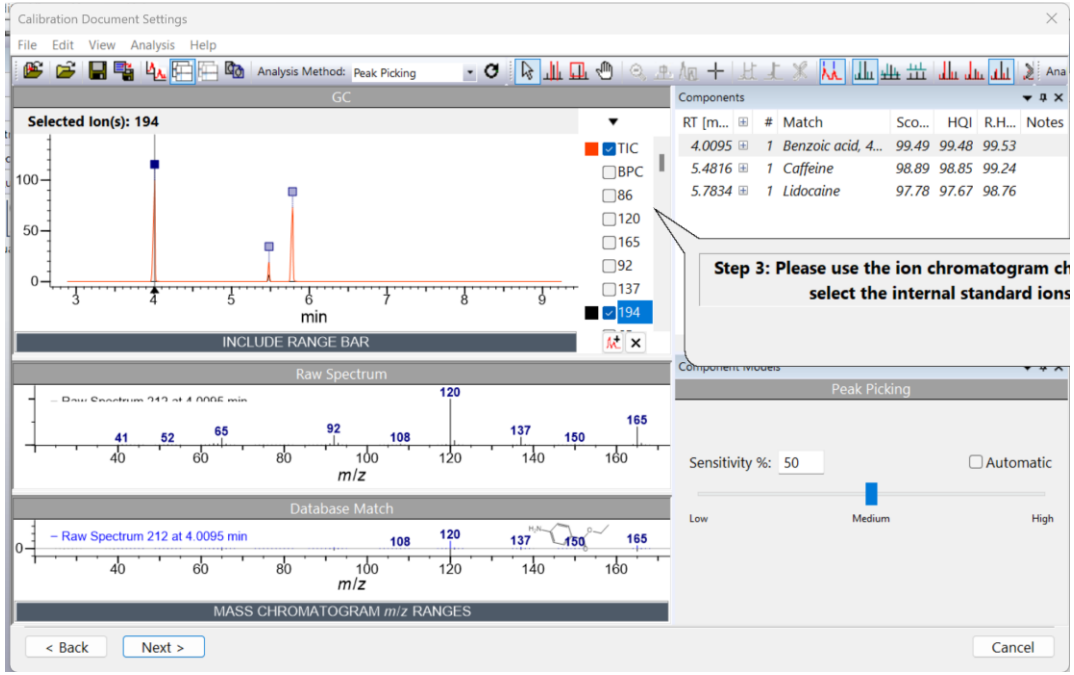
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.  *Note:* If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.	**Quantitation** application is displayed: 
2	Click **New Internal Calibration** button. 	**KnowItAll** prompts user to open calibrant files.

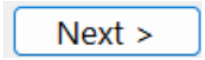
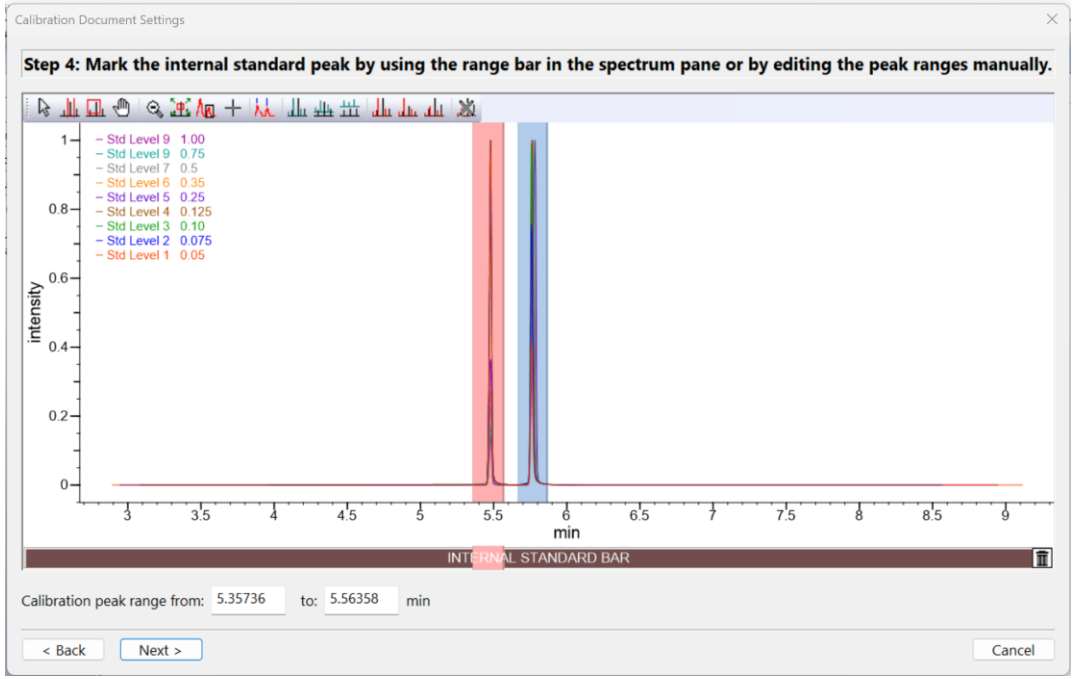
	Action	Result
3	Navigate to **"C:\Users\Public\Documents\Wiley\ KnowItAll\Samples\Quantitation\Internal Calibration GC-MS"** folder. Select all "**Sample_.D**" folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files. Click **Open.**	All sample files are selected in the **Open** popup window. Upon clicking **Open**, the **Calibration Document Settings** popup window appears. 

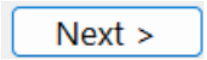
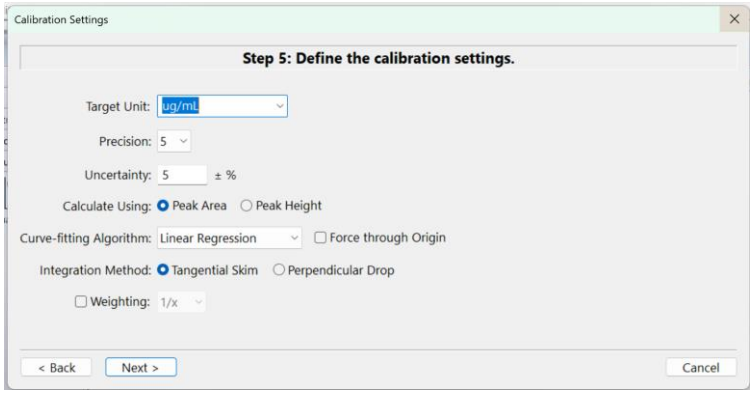
	Action	Result
4	On the **Calibration Document Settings** popup window, use the **Sample File** dropdown menu to choose "**Sample9.D**", which is the calibrant file in which the analyte concentration is the largest. Click **Close** on the bubble window after making the file selection using the dropdown menu (*e.g.,* see highlighted clicks below). 	The chromatogram with the largest concentration, *i.e.,* **Sample9.D.**, is used to select the component peak. 

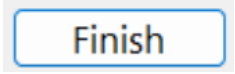
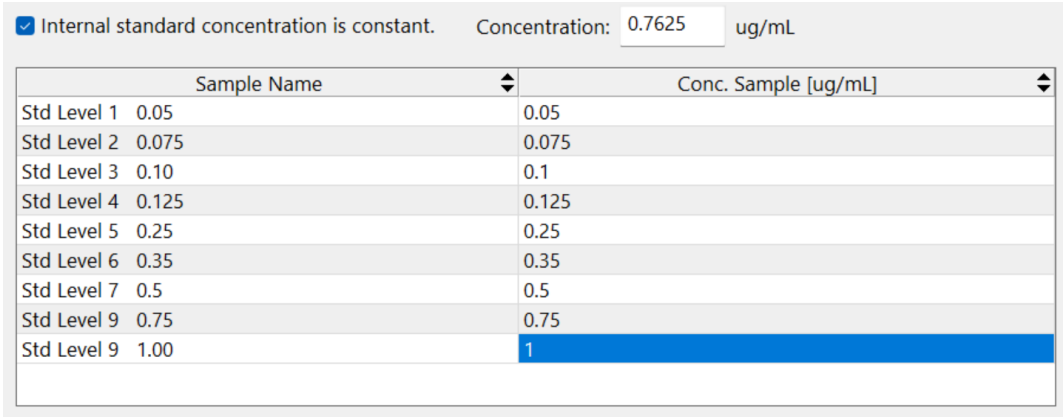
	Action	Result
5	Select a component from the **Raw Spectrum** pan by clicking on the numeric value in the **Mass Chromatogram List Pane**. In this example, the **Component Molecular m/z** to choose is “86”. This represents the TIC internal standard for the peak at 5.78 min. Click **Next >** button. 	The selected component is highlighted on chromatogram and **Mass Chromatogram List Pane**. Upon clicking **Next**, “Step 2” settings load in the **Calibration Document Settings** popup window. 

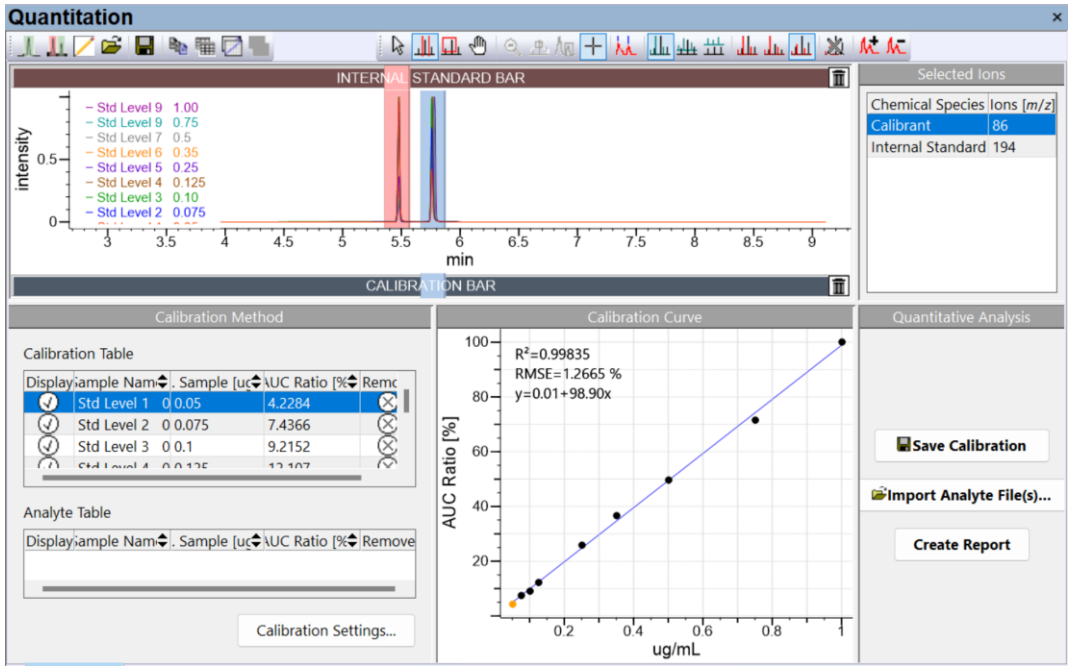
	Action	Result
6	Select peak region around 5.75 min as the calibrant peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.,* ~ 5.7 – 5.9 min). Click **Next >** button. 	The selected region is shaded with blue coloration. Upon clicking **Next >** button, “Step 3” settings load in the **Calibration Document Settings** popup window. 

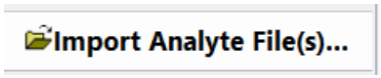
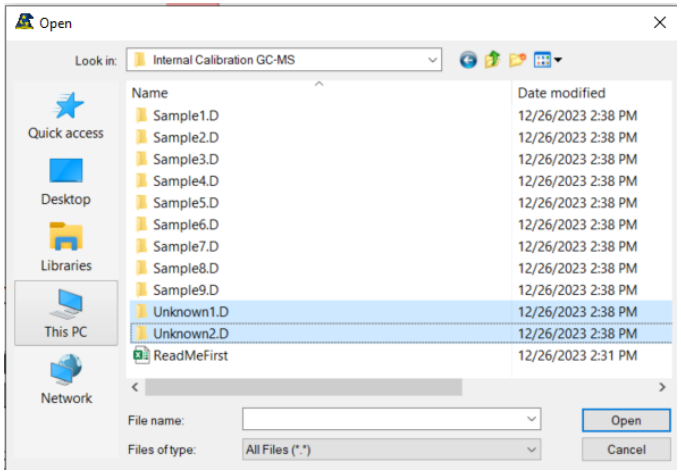
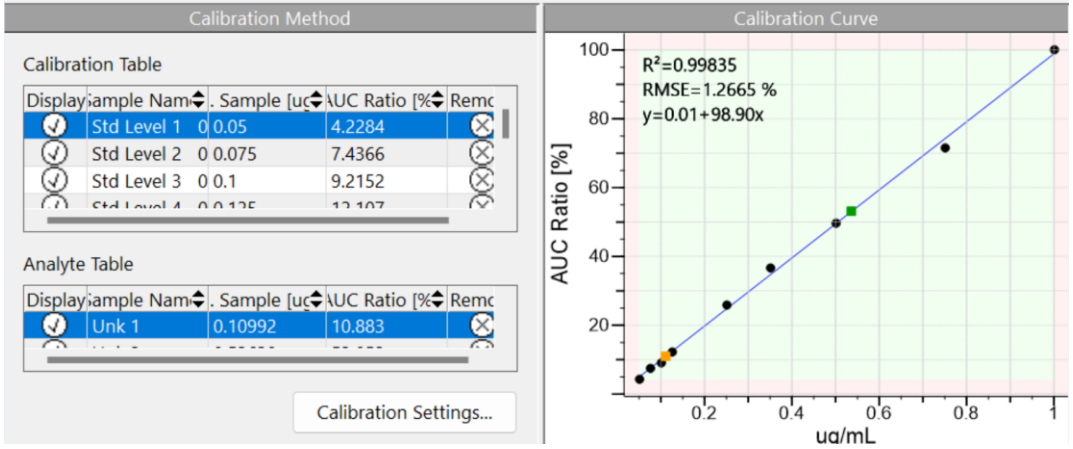
	Action	Result
7	Select a component from the **Raw Spectrum** pan by clicking on the numeric value in the **Mass Chromatogram List Pane**. In this example, the **Component Molecular m/z** to choose is “194”. This represents the TIC internal standard for the peak at 5.48 min. Click **Next >** button. 	The selected component is highlighted on chromatogram and **Mass Chromatogram List Pane**. Upon clicking **Next**, “Step 4” settings load in the **Calibration Document Settings** popup window. 

	Action	Result
8	Select peak region around 5.5 min as the calibrant peak by clicking down on the **CALIBRATION BAR** with left mouse button and release after selecting a region (*e.g.*, ~ 5.4 – 5.6 min). Click **Next >** button. 	The selected internal standard region is shaded with red coloration. 

	Action	Result
9	In the **Calibrations Settings** popup window, define the calibration settings: • **Target Unit:** ug/mL (has to be manually typed in) Remaining parameters can retain the default selection. Click **Next >** button. 	The applied calibration settings are shown: 

	Action	Result
10	Enter concentration and ratio values in the popup window: • **File**: Std Level 1 0.05, **Concentration**: 0.05 • **File**: Std Level 2 0.075, **Concentration**: 0.075 • **File**: Std Level 3 0.10, **Concentration**: 0.100 • **File**: Std Level 4 0.125, **Concentration**: 0.125 • **File**: Std Level 5 0.25, **Concentration**: 0.25 • **File**: Std Level 6 0.35, **Concentration**: 0.35 • **File**: Std Level 7 0.5, **Concentration**: 0.5 • **File**: Std Level 9 0.75, **Concentration**: 0.75 • **File**: Std Level 9 1.00, **Concentration**: 1 Ensure that the checkbox **for Internal standard concentration is constant** remains selected. Enter value for the internal standard concentration in the **Concentration** box “0.7625” ug/mL. Click the **Finish** button. 	The concentration settings for the samples and internal standard are shown. Upon clicking Finish, the **Calibration Settings** popup window closes and the chromatograms display in **Quantitation** application. 

	Action	Result
11	Analyze the calibration results.	• Statistics are reported in the **Calibration Curve**. • The lower the value for **RMSE** (**Root Mean Squared Error)**, the better the curve fitting is. • The closer the **R^2** (**Coefficient of Determination**) is to 1**,** the better the curve fitting is. • The **Calibration Settings** button launches the **Calibration Settings** popup window, which allows for resetting the calibration parameters. • The calibration can be saved for future use or file sharing by clicking the **Save Calibration** button in the **Quantitative Analysis** panel. 

	Action	Result
12	Click the **Import Analyte File(s)** button found in the **Quantitative Analysis** panel.  On the **Open** popup window, hold CTRL button and click using left mouse button to select unknown file folder **Unknown1.D** and **Unknown2.D** (**"C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS"** folder). Click **Open.**	The selected sample files represent the unknown files for which the concentrations will be calculated from the calibration. Upon clicking **Open**, the dialog window closes and the unknown sample files open in the calibration window. 
13	Analyze the results of the unknown concentration calculation. Review the **Analyte Table** and **Calibration Curve**.	The concentration of the unknown file is calculated in the **Analyte Table**. The unknown concentration is displayed on the **Calibration Curve**. 

WILEY

	Action	Result
14	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. **Create Report** In the **Select a Report Template** popup window, choose “Internal Standard GC-MS Lanscape” to use this report template. Click **OK** on the **Select a Report Template** dialog window to create the report in the selected template.	Upon clicking to generate the report, the **Report Templates** dialog window appears which prompts the user to choose the desired template for selection. Upon clicking **OK** on the **Select a Report Template** dialog window, the report is generated in **ReportIt** application.