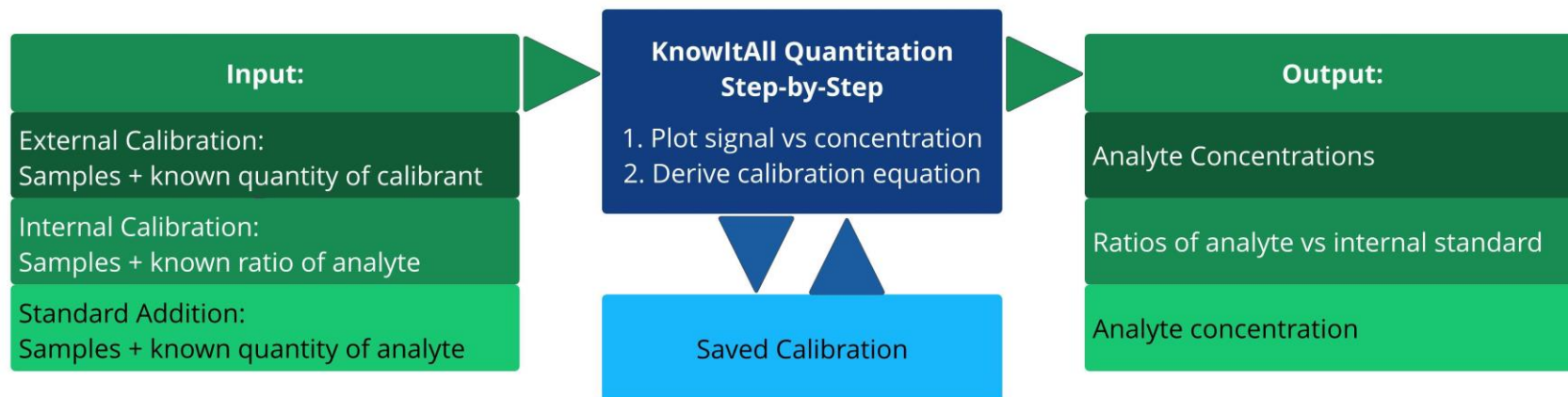


KnowItAll Informatics Training

Quantitation

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

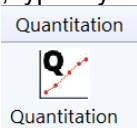
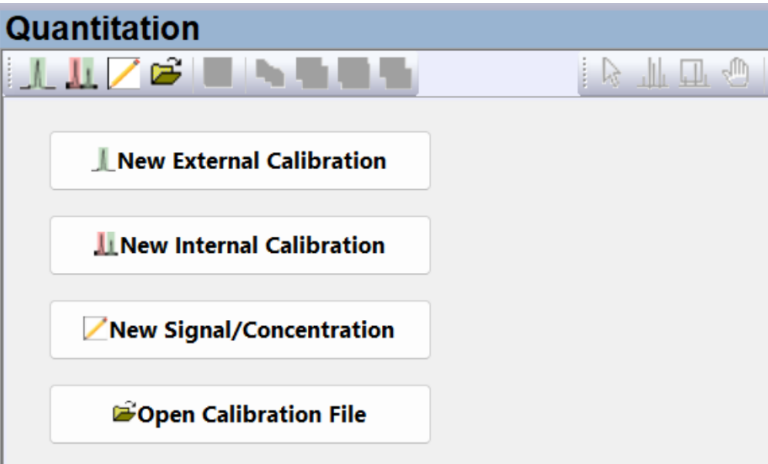
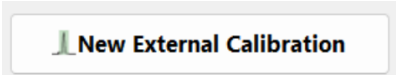
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

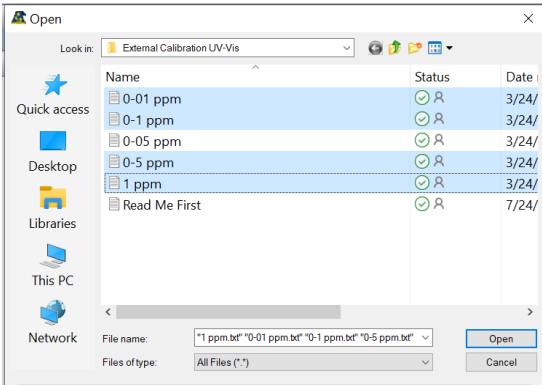
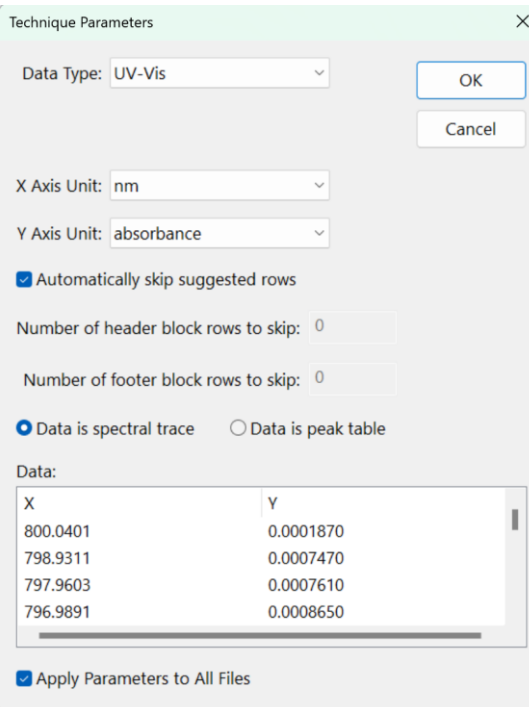
- External Calibration UV-Vis
- External Calibration IR

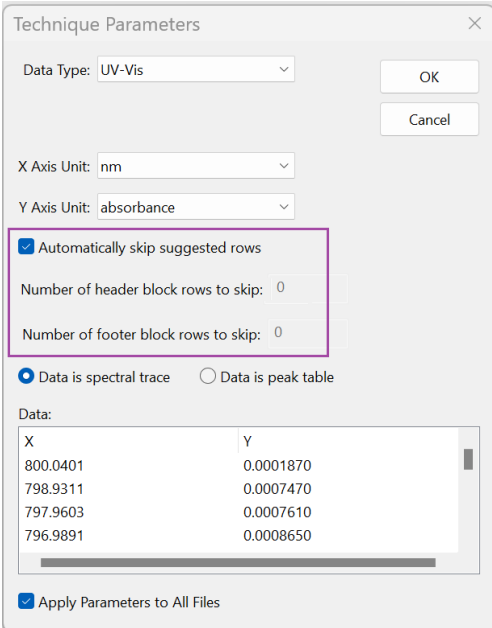
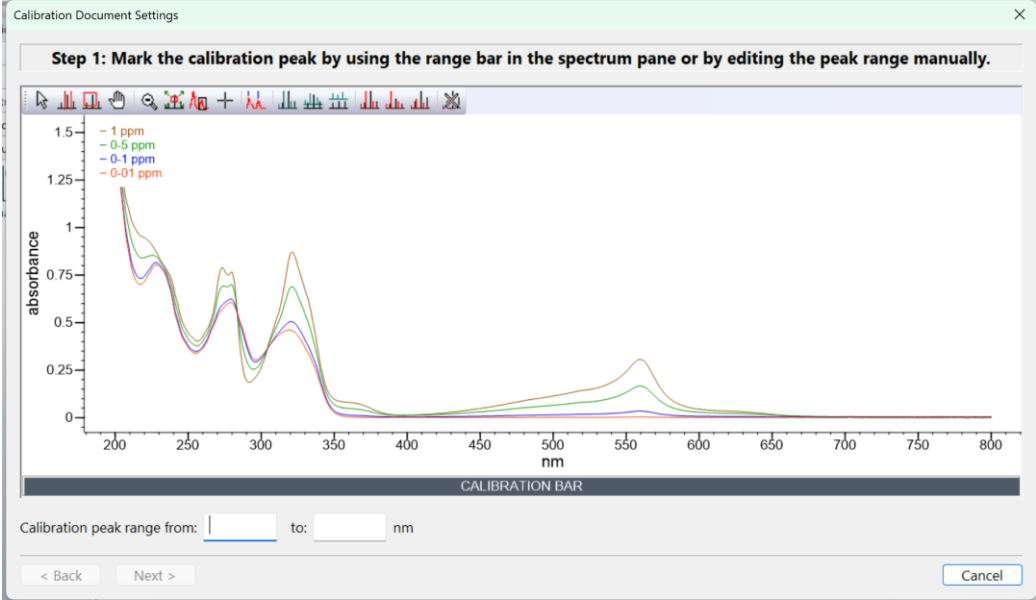
KnowItAll Applications Used

- Quantitation

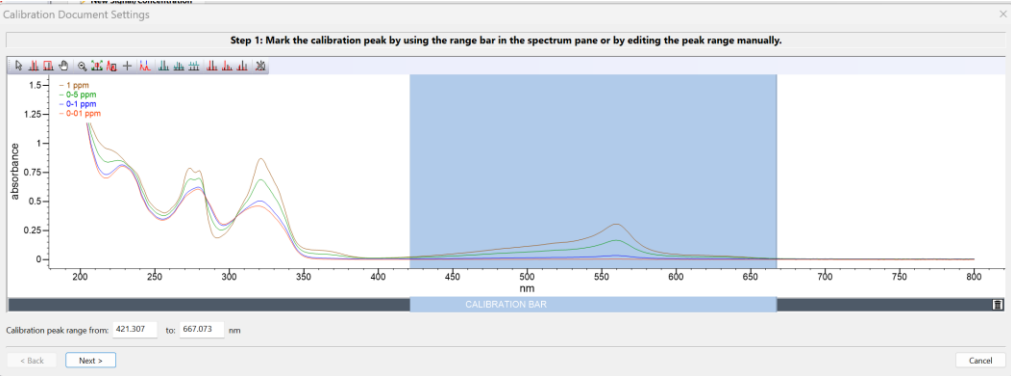
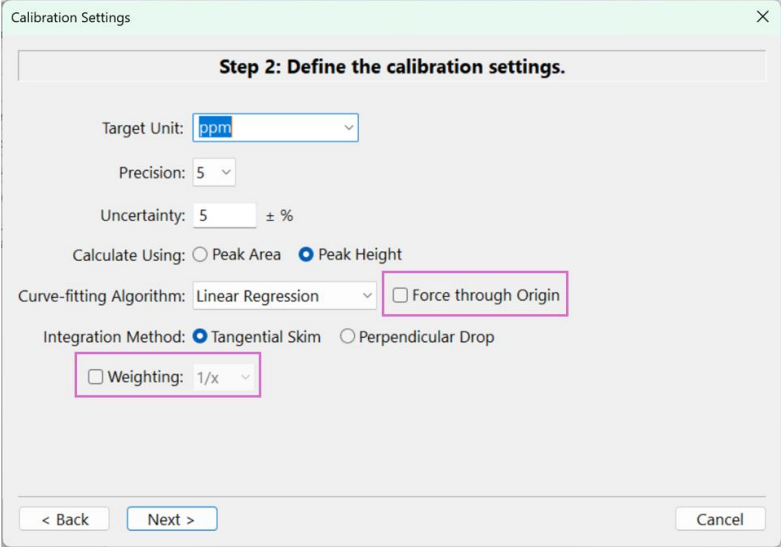
UV-Vis

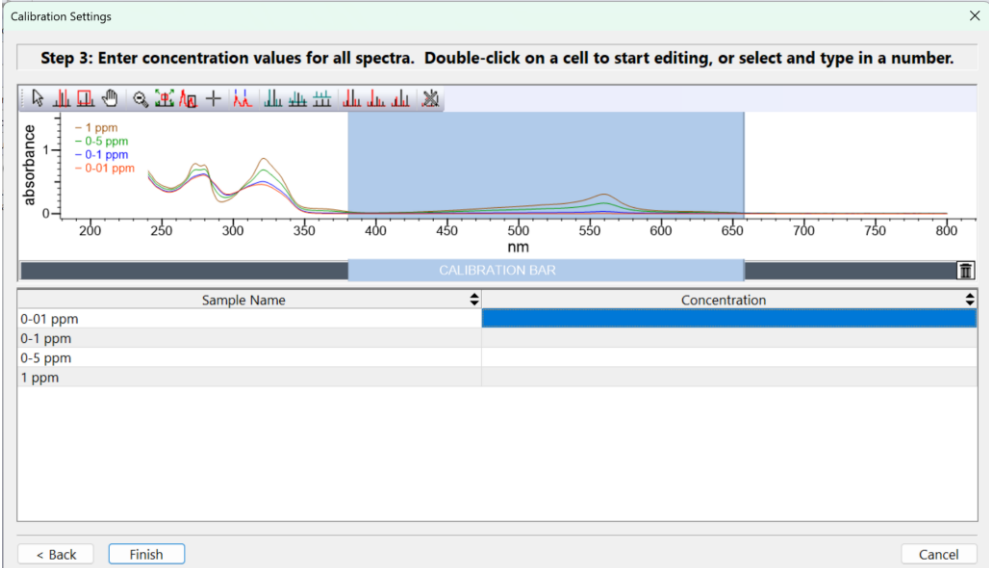
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p> 	<p>Quantitation application is displayed:</p> 
2	<p>Click New External Calibration button.</p> 	<p>KnowItAll prompts user to open calibrant files.</p>

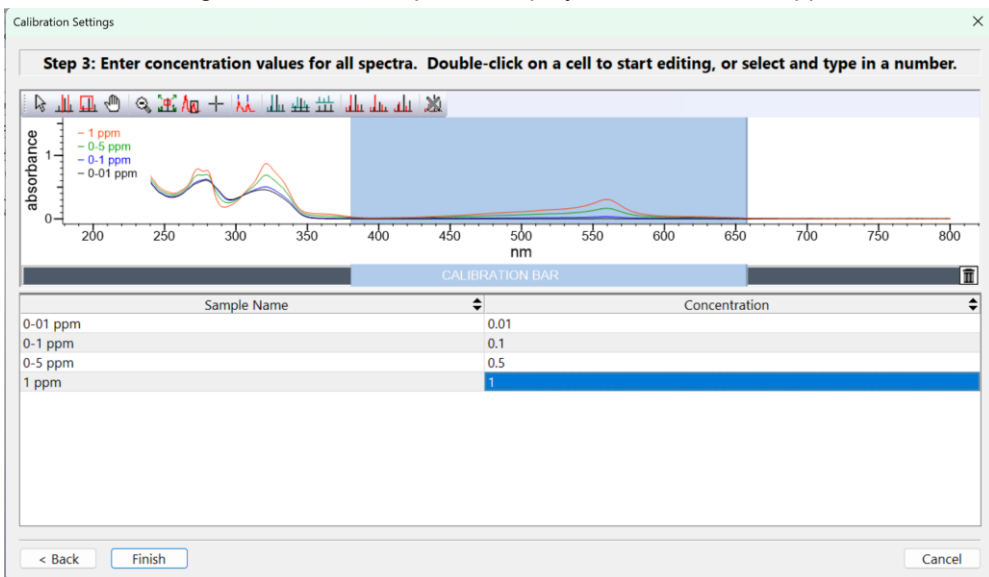
	Action	Result
3	<p>Navigate to the “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis” folder.</p> <p>Select 4 sample files and leave one out to be the unknown sample (e.g., as shown in image below).</p> <p>Click Open.</p> 	<p>The Technique Parameters dialog is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

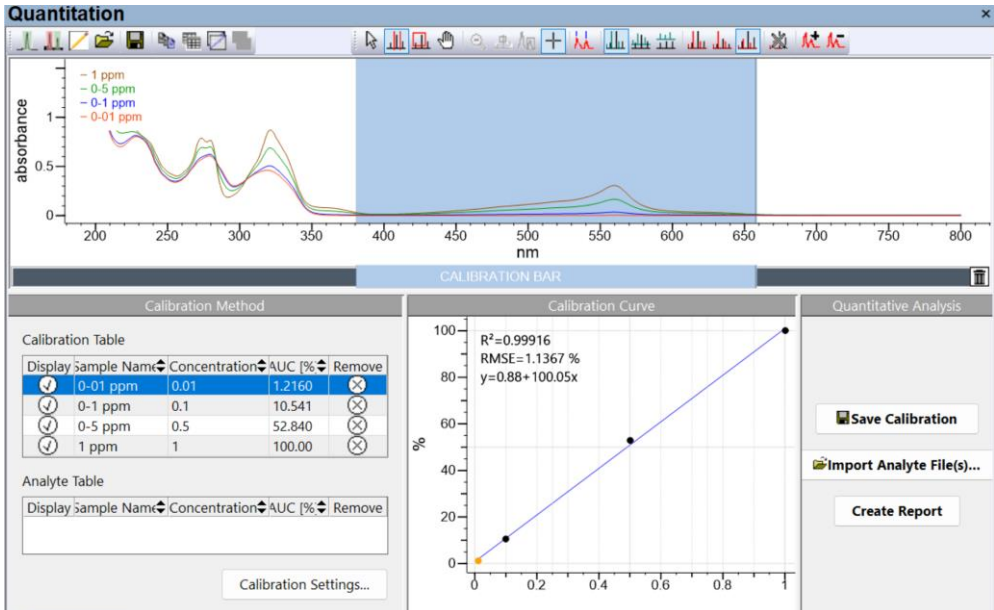
	Action	Result
4	<p>In Technique Parameters prompt window:</p> <ul style="list-style-type: none">Set Data Type to UV-Vis.Check Apply Parameters to All Files. <p>Click OK.</p> 	<p>The Calibration Document Settings popout window appears, displaying the selected UV-Vis files. The Step is identified as “Step 1”:</p> 

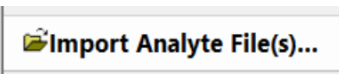
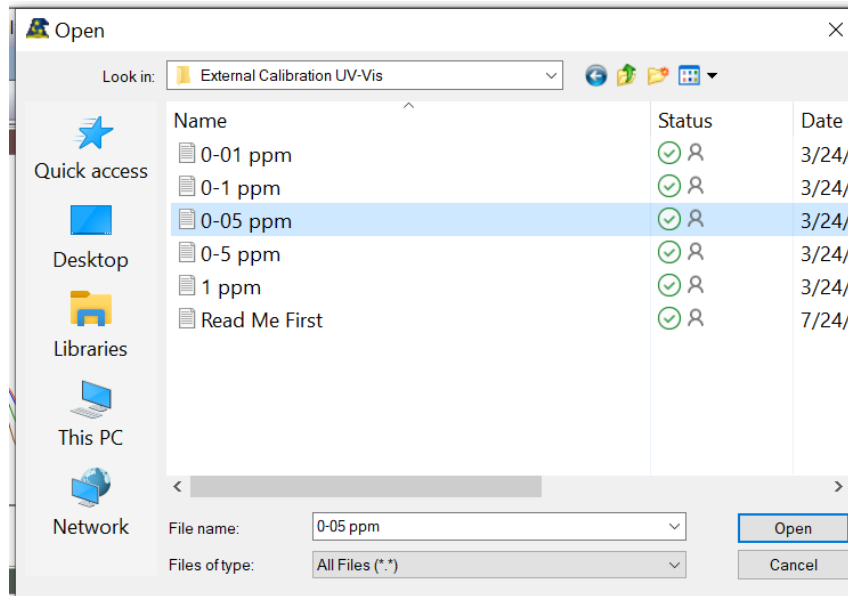
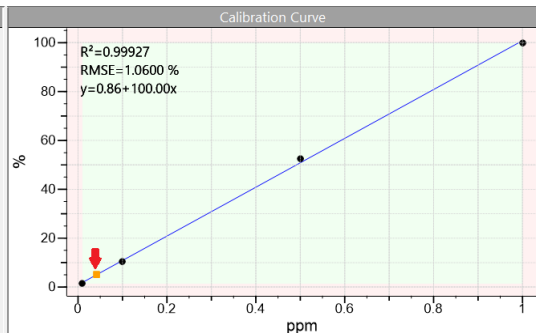
Note: The options highlighted above are added to skip lines which are not spectral x,y coordinates.

	Action	Result
5	<p>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.</p> <p>Click Next > button.</p> <div data-bbox="247 592 413 641">Next ></div>	<p>The selected region is displayed in blue coloration:</p>  <p>Upon clicking Next button, "Step 2" loads in the popup window.</p>
6	<p>In the popup window, define calibration settings as shown in the image</p> <ul style="list-style-type: none">• Target Unit: ppm• Calculate Using: Peak Height <p>Remaining parameters can retain the default selection.</p> <p><i>Note:</i> New options (purple boxed) are added for additional control of data.</p>	

	Action	Result
7	Click Next > button.	<p>Upon clicking Next button, "Step 3" loads in the popup window:</p> 

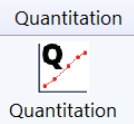

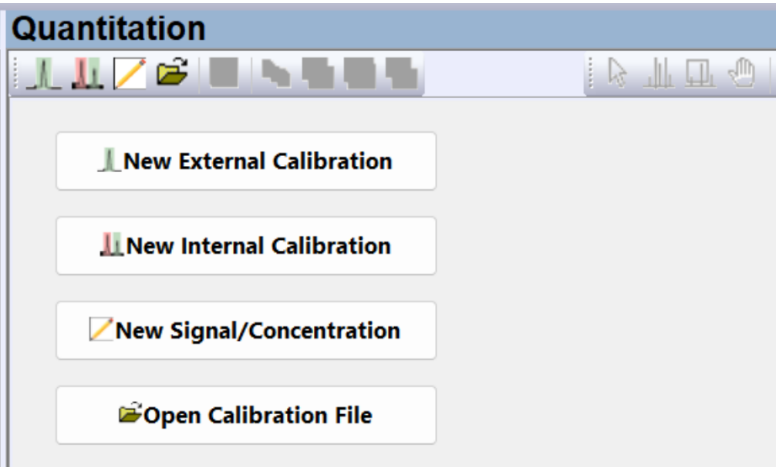
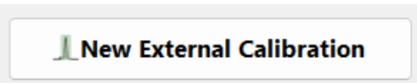
	Action	Result
8	<p>In the popup window, enter concentrations in the right column based on the file names:</p> <ul style="list-style-type: none">• File: 0-01 ppm, Concentration: 0.01 ppm• File: 0-1 ppm, Concentration: 0.1 ppm• File: 0-5 ppm, Concentration: 0.5 ppm• File: 1 ppm, Concentration: 1 ppm <p>Click Finish button.</p> <div>Finish</div>	<p>The manually entered Concentration values are shown in the table below. Upon clicking Finish, the dialog closes and the spectra display in Quantitation application.</p> 

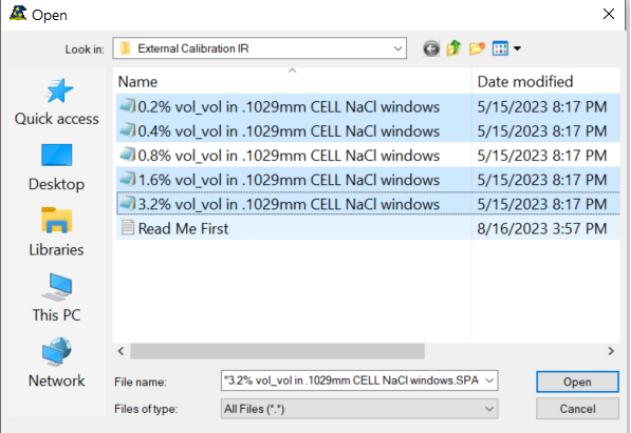
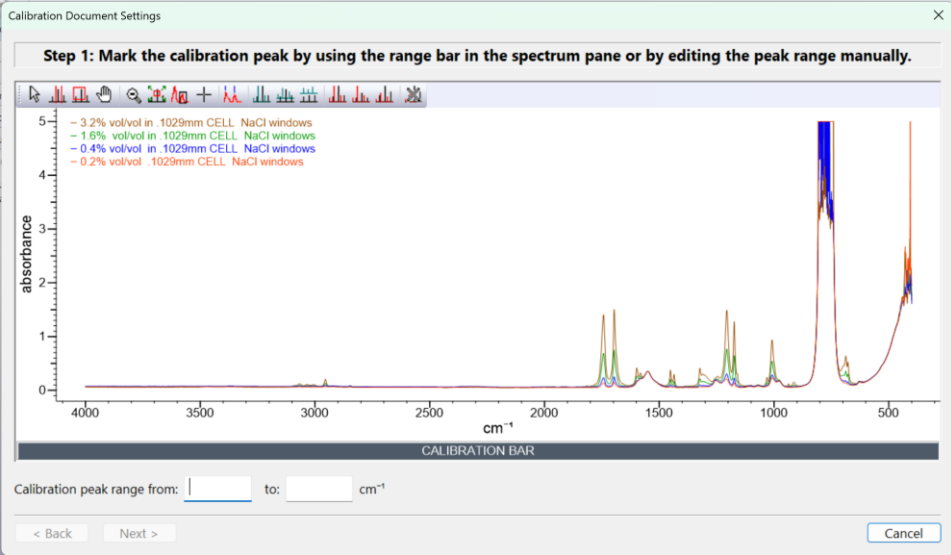
	Action	Result																																			
9	Analyze the results in Quantitation application.	<ul style="list-style-type: none">Statistics are reported in the Calibration Curve.The lower the value for RMSE (Root Mean Squared Error), the better the curve fitting is.The closer the R² (Coefficient 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.  <p>The screenshot displays the Quantitation application interface. At the top, a graph shows absorbance versus wavelength (nm) from 200 to 800 nm. Four curves are plotted for concentrations of 1 ppm (red), 0.5 ppm (green), 0.1 ppm (blue), and 0.01 ppm (orange). Below the graph is a 'CALIBRATION BAR'. The interface is divided into three main panels: 'Calibration Method', 'Calibration Curve', and 'Quantitative Analysis'.</p> <p>Calibration Table</p> <table><tr><th>Display</th><th>Sample Name</th><th>Concentration</th><th>AUC (%)</th><th>Remove</th></tr><tr><td><input checked="" type="checkbox"/></td><td>0-01 ppm</td><td>0.01</td><td>1.2160</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>0-1 ppm</td><td>0.1</td><td>10.541</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>0-5 ppm</td><td>0.5</td><td>52.840</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>1 ppm</td><td>1</td><td>100.00</td><td><input type="checkbox"/></td></tr></table> <p>Analyte Table</p> <table><tr><th>Display</th><th>Sample Name</th><th>Concentration</th><th>AUC (%)</th><th>Remove</th></tr><tr><td><input type="checkbox"/></td><td></td><td></td><td></td><td><input type="checkbox"/></td></tr></table> <p>Calibration Curve</p> <p>Graph showing absorbance (y-axis, 0 to 100) versus concentration (x-axis, 0 to 1). The curve is a straight line passing through the origin. Statistics displayed: $R^2 = 0.99916$, $RMSE = 1.1367\%$, and the equation $y = 0.88 + 100.05x$.</p> <p>Quantitative Analysis</p> <p>Buttons: Save Calibration, Import Analyte File(s)..., Create Report.</p>	Display	Sample Name	Concentration	AUC (%)	Remove	<input checked="" type="checkbox"/>	0-01 ppm	0.01	1.2160	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0-1 ppm	0.1	10.541	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0-5 ppm	0.5	52.840	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1 ppm	1	100.00	<input type="checkbox"/>	Display	Sample Name	Concentration	AUC (%)	Remove	<input type="checkbox"/>				<input type="checkbox"/>
Display	Sample Name	Concentration	AUC (%)	Remove																																	
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<input checked="" type="checkbox"/>	1 ppm	1	100.00	<input type="checkbox"/>																																	
Display	Sample Name	Concentration	AUC (%)	Remove																																	
<input type="checkbox"/>				<input type="checkbox"/>																																	

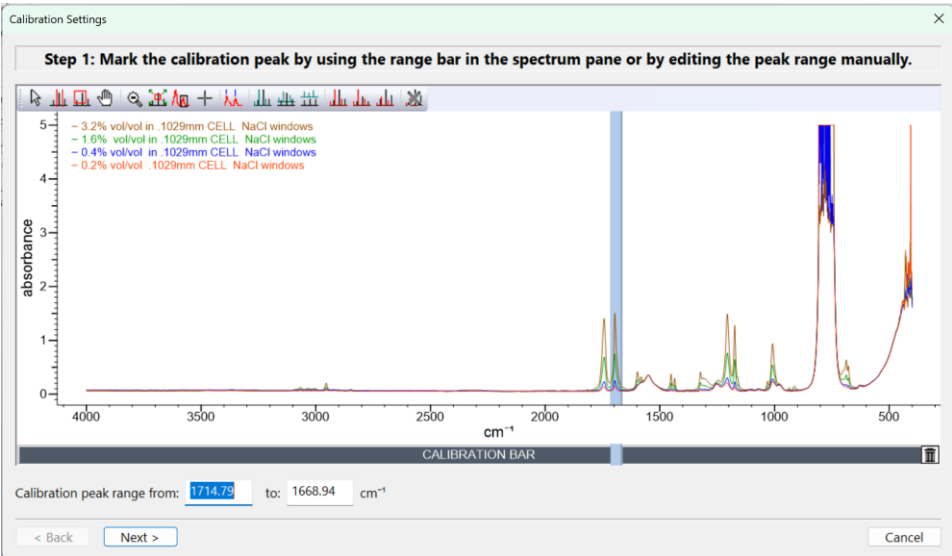
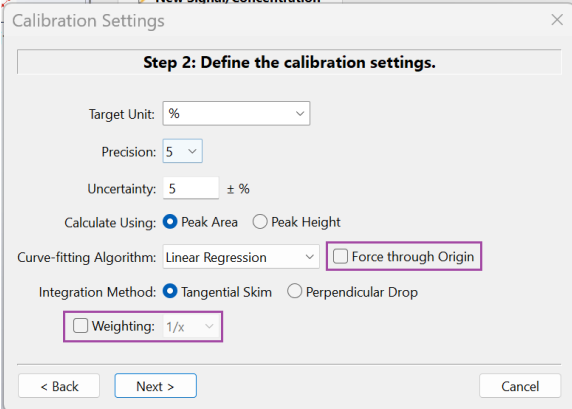
	Action	Result																																			
10	<p>Click the Import Analyte File(s) button.</p> <div></div> <p>Navigate to and select the file that was excluded in Step 3 “0-05 ppm”, located “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis”. Click Open.</p> <p>On the Technique Parameters dialog window, select Data Type to be UV-Vis. Click OK.</p>	<p>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.</p> <div></div>																																			
11	<p>Analyze the results of the unknown calculation by viewing the Analyte Table, selected in red in the image on the right.</p>	<p>The concentration of the unknown is calculated and marked.</p> <div><div><p>Calibration Method</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Concentration [ppm]</th><th>Peak Height [%]</th><th>Remove</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>0-01 ppm</td><td>0.01</td><td>1.3649</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>0-1 ppm</td><td>0.1</td><td>10.391</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>0-5 ppm</td><td>0.5</td><td>52.674</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>1 ppm</td><td>1</td><td>100.00</td><td><input type="checkbox"/></td></tr></tbody></table><p>Analyte Table</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Concentration [ppm]</th><th>Peak Height [%]</th><th>Remove</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>0-05 ppm</td><td>0.042183</td><td>5.0764</td><td><input type="checkbox"/></td></tr></tbody></table><p>Calibration Settings...</p></div><div><p>Calibration Curve</p><p>$R^2 = 0.99927$ $RMSE = 1.0600 \%$ $y = 0.86 + 100.00x$</p></div></div>	Display	Sample Name	Concentration [ppm]	Peak Height [%]	Remove	<input checked="" type="checkbox"/>	0-01 ppm	0.01	1.3649	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0-1 ppm	0.1	10.391	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0-5 ppm	0.5	52.674	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1 ppm	1	100.00	<input type="checkbox"/>	Display	Sample Name	Concentration [ppm]	Peak Height [%]	Remove	<input checked="" type="checkbox"/>	0-05 ppm	0.042183	5.0764	<input type="checkbox"/>
Display	Sample Name	Concentration [ppm]	Peak Height [%]	Remove																																	
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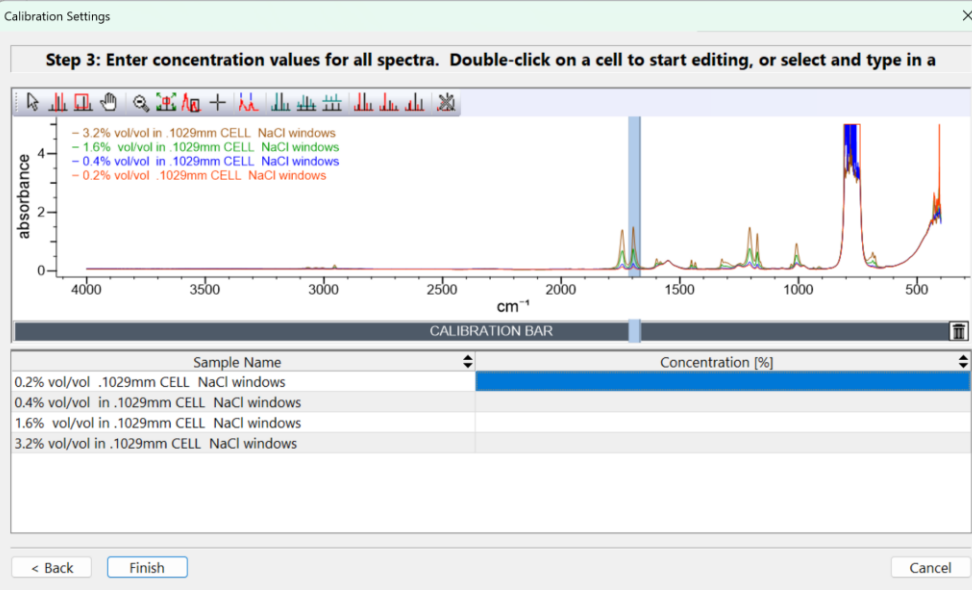
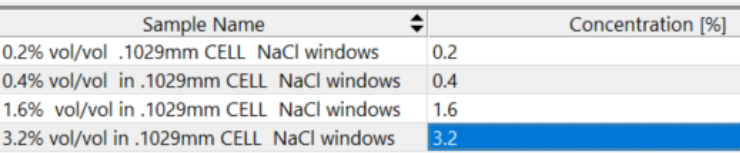
	Action	Result
12	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <div data-bbox="247 456 518 529" data-label="Image"> </div> <p>On the Select a Report Template dialog, retain the selection of “External Standard Landscape”. Click OK on the dialog window to create the report in the selected template.</p> <p><i>Note:</i> If a template is used for the first time, the user has to execute the following steps before transfer data to ReportIt application:</p> <ul style="list-style-type: none"> 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. 	<p>Upon clicking to generate the report, the Report Templates dialog window appears which prompts the user to choose the desired template for selection.</p> <div data-bbox="842 391 1824 940" data-label="Image"> <p>The dialog window titled "Select a Report Template" contains a list of templates with columns "Title" and "File Path". The "External Standard Landscape" template is selected. To the right is a preview of the report template, which includes a WILEY logo, a data table, a calibration curve graph with the equation $R^2=0.99916$, $RMSE=1.1367\%$, and $y=0.88+100.05x$, and a peak identification table.</p> </div> <p>Upon clicking OK on the Select a Report Template dialog window, the report is generated in ReportIt application.</p>

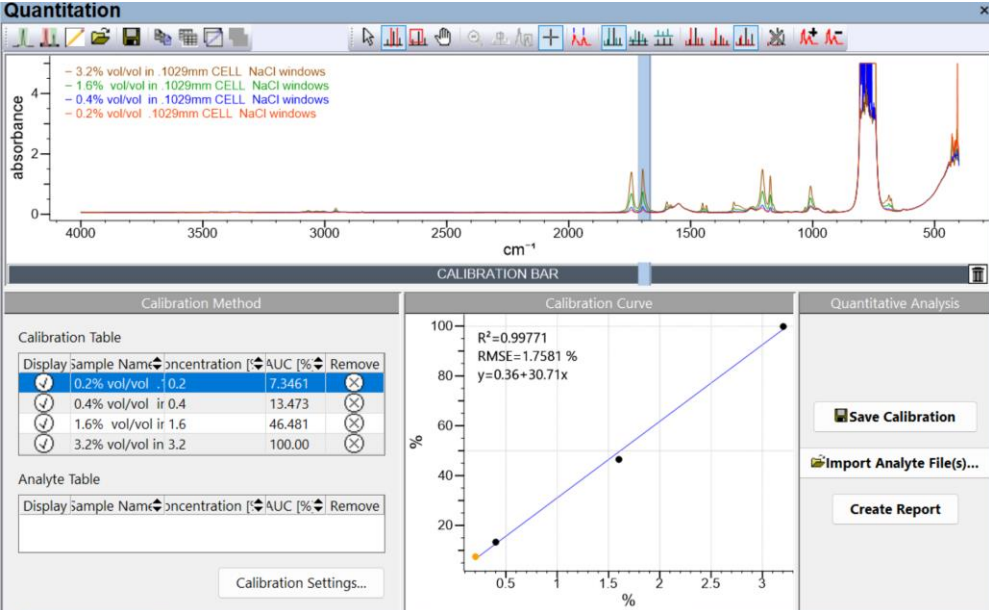
IR

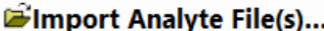
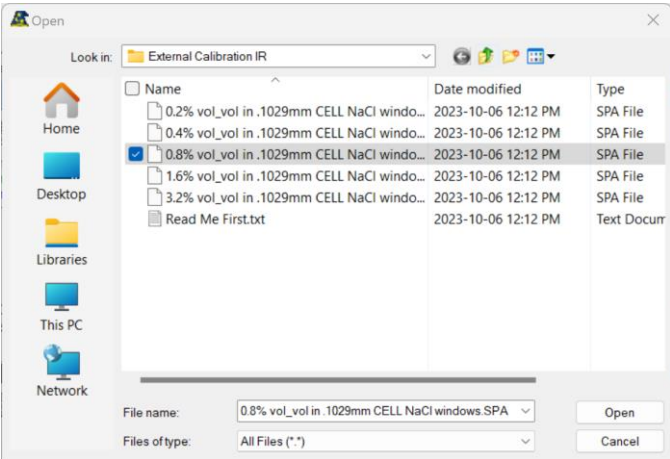
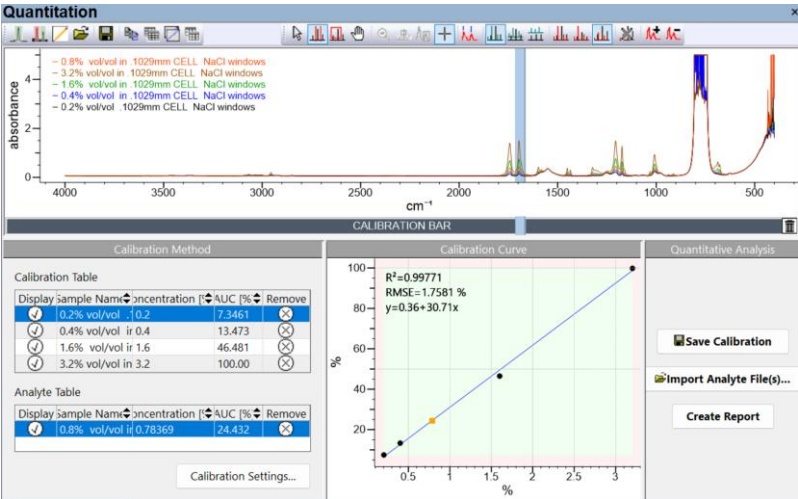
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note:</i> If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.</p>	<p>Quantitation application is displayed: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

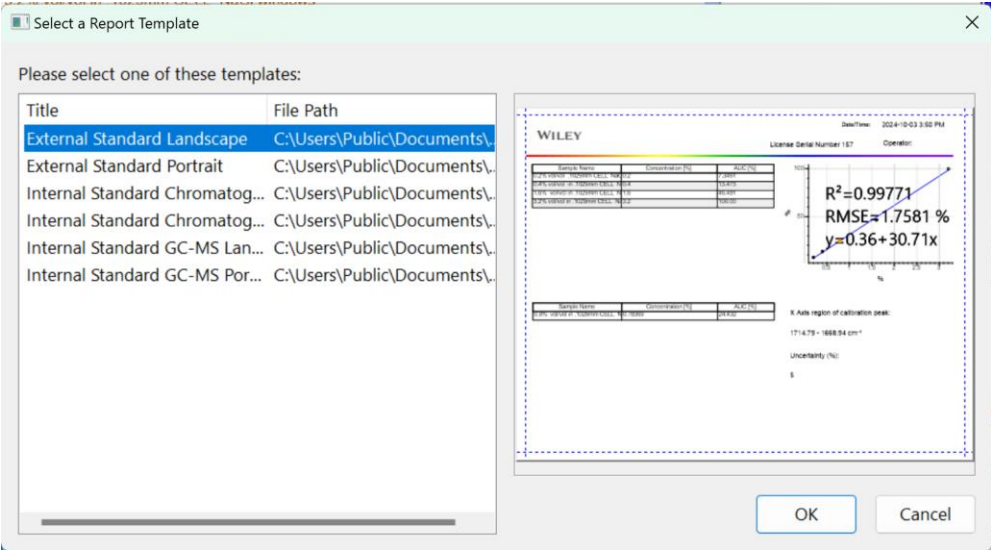
	Action	Result
3	<p>Navigate to the “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR” folder.</p> <p>Select sample files and leave the 0.8% file deselected to be the unknown sample file (e.g., as shown below).</p>  <p>Click Open.</p>	<p>The Calibration Document Settings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

	Action	Result
4	<p>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 $\sim 1650\text{ cm}^{-1}$.</p> <p><i>Note:</i> In IR quantitation, one should avoid using the strongest peak.</p> <p>Click Next > button.</p> <p>Next ></p>	<p>The selected region is displayed in blue coloration. Upon clicking Next button, “Step 2” loads in the popup window.</p> 
5	<p>In the following window, define calibration settings:</p> <ul style="list-style-type: none"> • Target Unit: % <p>Remaining parameters can retain the default selection.</p> <p><i>Note:</i> New options (purple boxed) are added for additional control of data.</p>	

	Action	Result
6	<p>Click Next > button.</p> <p>Next ></p>	<p>Upon clicking Next button, “Step 3” loads in the popup window:</p> 
7	<p>Enter concentrations in the popup window based on the numbers in the sample names (e.g., as shown in image on the righthand side).</p> <p>Click Finish button.</p> <p>Finish</p>	<p>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.</p> 

	Action	Result
8	Analyze the quantitation results in the application.	<ul style="list-style-type: none"> 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 R² (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.  <p>The screenshot displays the 'Quantitation' software window. At the top, an IR spectrum plot shows absorbance versus wavenumber (cm⁻¹) from 4000 to 500. Four curves are overlaid, representing different concentrations: 3.2% vol/vol (red), 1.6% vol/vol (green), 0.4% vol/vol (blue), and 0.2% vol/vol (orange). Below the spectrum is a 'CALIBRATION BAR'. The main interface is divided into three panels. The left panel, 'Calibration Method', contains a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Concentration [%]', 'AUC [%]', and 'Remove'. It lists four data points: 0.2% vol/vol (AUC 7.3461), 0.4% vol/vol (AUC 13.473), 1.6% vol/vol (AUC 46.481), and 3.2% vol/vol (AUC 100.00). Below this is an empty 'Analyte Table'. The middle panel, 'Calibration Curve', shows a scatter plot of AUC (%) versus concentration (%). The data points are (0.2, 7.3461), (0.4, 13.473), (1.6, 46.481), and (3.2, 100.00). A linear regression line is fitted to the data with the equation y = 0.36 + 30.71x. The statistics shown are R² = 0.99771 and RMSE = 1.7581 %. The right panel, 'Quantitative Analysis', contains three buttons: 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.</p>

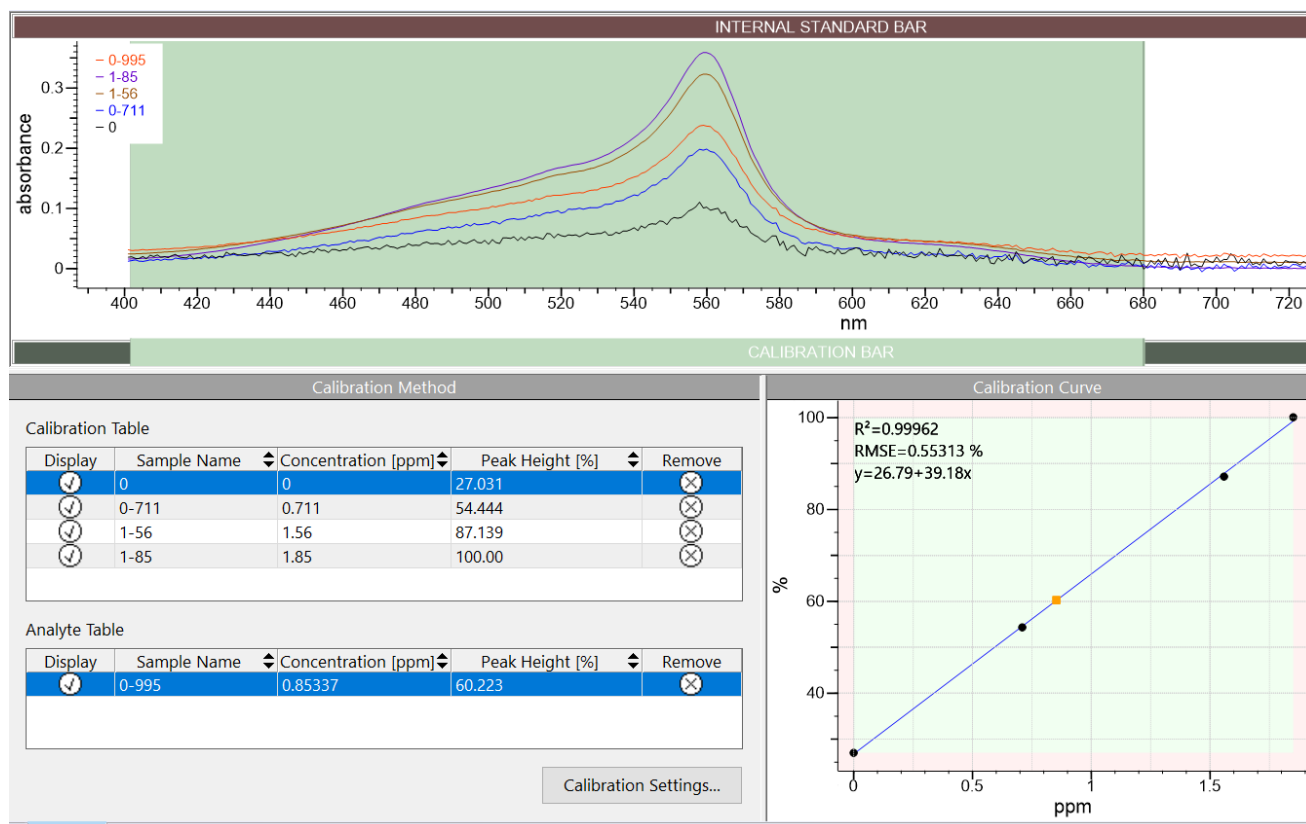
	Action	Result																												
9	<p>Click the Import Analyte File(s) button in the Quantitative Analysis panel.</p> <div></div> <p>Navigate to and select the file that was excluded in Step 3 “0.8%”, located “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR”.</p> <p>Click Open.</p>	<p>The Open file dialog window is shown. Upon clicking OK, the imported file opens in the previous calibration window.</p> <div></div>																												
10	<p>Analyze the results of the unknown calculation by viewing the Analyte Table located in the Calibration Method panel.</p>	<p>The concentration of the unknown is calculated and marked.</p> <div><p>Quantitation</p><p>absorbance</p><p>cm⁻¹</p><p>4000 3500 3000 2500 2000 1500 1000 500</p><p>CALIBRATION BAR</p><p>Calibration Method</p><p>Calibration Table</p><table><thead><tr><th>Display Sample Name</th><th>Concentration (%)</th><th>AUC (%)</th><th>Remove</th></tr></thead><tbody><tr><td>0.2% vol/vol</td><td>0.2</td><td>7.3461</td><td>X</td></tr><tr><td>0.4% vol/vol</td><td>0.4</td><td>13.473</td><td>X</td></tr><tr><td>1.6% vol/vol</td><td>1.6</td><td>46.481</td><td>X</td></tr><tr><td>3.2% vol/vol</td><td>3.2</td><td>100.00</td><td>X</td></tr></tbody></table><p>Analyte Table</p><table><thead><tr><th>Display Sample Name</th><th>Concentration (%)</th><th>AUC (%)</th><th>Remove</th></tr></thead><tbody><tr><td>0.8% vol/vol</td><td>0.78369</td><td>24.432</td><td>X</td></tr></tbody></table><p>Calibration Settings...</p><p>Calibration Curve</p><p>R²=0.99771 RMSE=1.7581 % y=0.36+30.71x</p><p>Quantitative Analysis</p><p>Save Calibration</p><p>Import Analyte File(s)...</p><p>Create Report</p></div>	Display Sample Name	Concentration (%)	AUC (%)	Remove	0.2% vol/vol	0.2	7.3461	X	0.4% vol/vol	0.4	13.473	X	1.6% vol/vol	1.6	46.481	X	3.2% vol/vol	3.2	100.00	X	Display Sample Name	Concentration (%)	AUC (%)	Remove	0.8% vol/vol	0.78369	24.432	X
Display Sample Name	Concentration (%)	AUC (%)	Remove																											
0.2% vol/vol	0.2	7.3461	X																											
0.4% vol/vol	0.4	13.473	X																											
1.6% vol/vol	1.6	46.481	X																											
3.2% vol/vol	3.2	100.00	X																											
Display Sample Name	Concentration (%)	AUC (%)	Remove																											
0.8% vol/vol	0.78369	24.432	X																											

	Action	Result
11	<p>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.</p> <p>Create Report</p> <p>On the Select a Report Template dialog window, retain selection “External Standard Landscape” and click OK to create the report in the selected template.</p> <p><i>Note:</i> If a template is used for the first time, the user has to execute the following steps before transfer data to ReportIt application:</p> <ul style="list-style-type: none"> Choose File > Edit Report Templates. Click Add button. Navigate to the template files located “C:\Users\Public\Documents\Wiley\KnowItAll\Report Templates\Quantitation”. <p>Highlight to select all of the templates in the folder, and click Open.</p>	<p>Upon clicking to generate the report, the Report Templates dialog window appears which prompts the user to choose the desired template for selection.</p>  <p>Upon clicking OK on the Select a Report Template dialog window, the report is generated in ReportIt application.</p>

Standard Addition Quantitation

Perform Standard Addition Quantitation

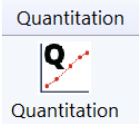
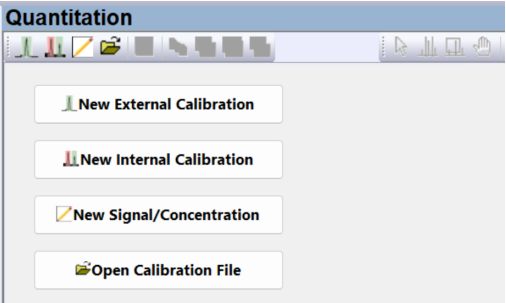

This screenshot shows a Standard Addition result, where when the added concentration is 0, the Y-axis value of 26.79 is the signal (due to iron in this case) in the original unknown sample:


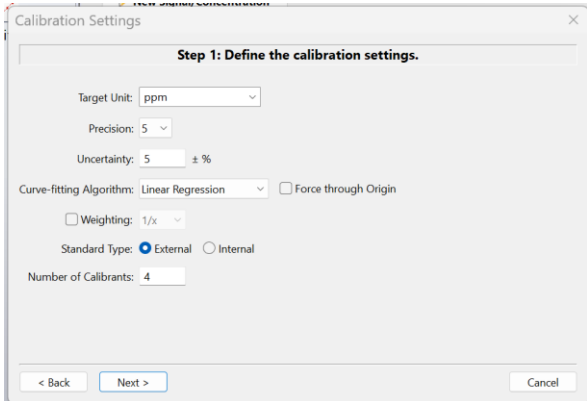
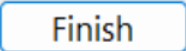
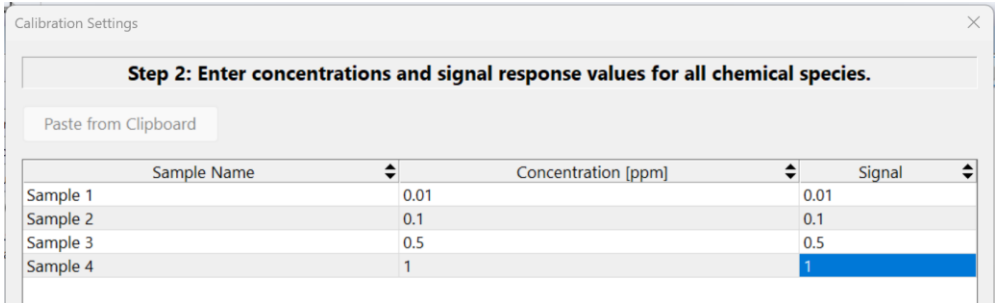


Directly Enter Signal Concentration Data for Quantitation

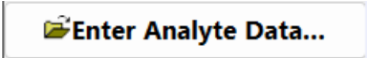
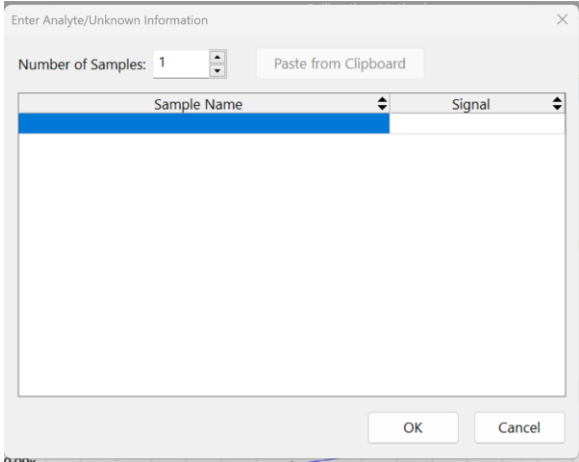
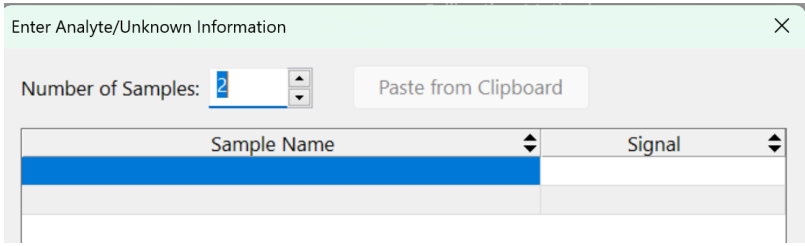
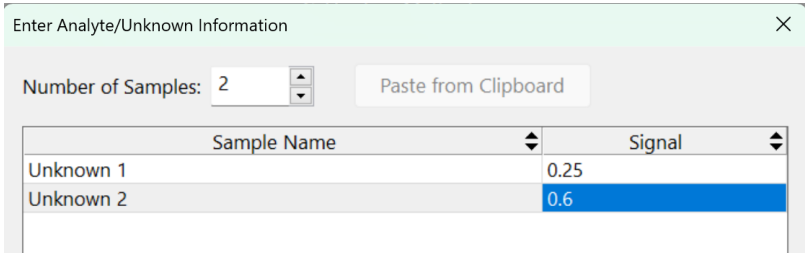
Directly Enter Signal Concentration Pairs to Create Calibration

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

	Action	Result
3	<p>Define the following parameters, as shown in the image on righthand side.</p> <ul style="list-style-type: none"> • Target Unit: ppm • Number of Calibrants: 4 <p>Remaining parameters can retain the default settings.</p> <p>Click Next > button to continue.</p> 	<p>The selected Calibration Settings are displayed. Upon clicking Next, Step 2 settings appear in the Calibration Settings dialog window.</p> 
4	<p>Enter calibration concentration-signal pairs:</p> <ul style="list-style-type: none"> • 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 <p>Tip: You can use the Tab button to move to the next field.</p> <p>Click Finish button.</p> 	<p>The Calibration Settings are displayed.</p> <ul style="list-style-type: none"> • 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.  <p>Upon clicking Finish, the dialog window closes.</p>

	Action	Result																																			
5	Analyze the manual calibration results.	<div><p>A calibration equation is created.</p><div><div>Quantitation</div><div><div><div>Calibration Method</div><div>Calibration Table</div><table><thead><tr><th>Sample Name</th><th>Concentration [ppm]</th><th>Signal</th><th>Relative Signal [%]</th><th>Remove</th></tr></thead><tbody><tr><td>Sample 1</td><td>0.010000</td><td>0.010000</td><td>1.0000</td><td></td></tr><tr><td>Sample 2</td><td>0.100000</td><td>0.100000</td><td>10.0000</td><td></td></tr><tr><td>Sample 3</td><td>0.500000</td><td>0.500000</td><td>50.0000</td><td></td></tr><tr><td>Sample 4</td><td>1.000000</td><td>1.000000</td><td>100.0000</td><td></td></tr></tbody></table><div>Analyte Table</div><table><thead><tr><th>Sample Name</th><th>Concentration [ppm]</th><th>Signal</th><th>Relative Signal [%]</th><th>Remove</th></tr></thead><tbody><tr><td></td><td></td><td></td><td></td><td></td></tr></tbody></table><div>Calibration Settings...</div></div><div><div>Calibration Curve</div><div><div><div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div></div><div></div></div></div><div><div><div></div><div></div></div><div><div><div><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Name	Concentration [ppm]	Signal	Relative Signal [%]	Remove	Sample 1	0.010000	0.010000	1.0000		Sample 2	0.100000	0.100000	10.0000		Sample 3	0.500000	0.500000	50.0000		Sample 4	1.000000	1.000000	100.0000		Sample Name	Concentration [ppm]	Signal	Relative Signal [%]	Remove					
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Sample Name	Concentration [ppm]	Signal	Relative Signal [%]	Remove																																	

	Action	Result
6	<p>To add an unknown sample for quantitation, click Enter Analyte Data button found in the Quantitative Analysis panel.</p> 	<p>The Enter Analyte/Unknown Information dialog window is launched:</p> 
7	<p>In the Number of Samples cell, enter “2” as the value.</p>	<p>Two rows appear in the Samples Table:</p> 
8	<p>Enter the following information regarding the unknown samples in the Samples Table:</p> <ul style="list-style-type: none"> • Sample Name: Unknown 1, Signal: 0.25 • Sample Name: Unknown 2, Signal: 0.6 	<p>The imported unknown information is displayed in the Samples Table:</p> 

	Action	Result																																								
9	<p>Click OK on the Enter Analyte/Unknown Information dialog window.</p> <p>Analyze the calibration results in application.</p>	<p>The unknown samples are quantified according to the calibration equation:</p> <div><div>Quantitation</div><div><div>Calibration Method</div><div>Calibration Table</div><table><thead><tr><th>Sample Name</th><th>Concentration [ppm]</th><th>Signal</th><th>Relative Signal [%]</th><th>Remove</th></tr></thead><tbody><tr><td>Sample 1</td><td>0.010000</td><td>0.010000</td><td>1.0000</td><td></td></tr><tr><td>Sample 2</td><td>0.100000</td><td>0.100000</td><td>10.0000</td><td></td></tr><tr><td>Sample 3</td><td>0.500000</td><td>0.500000</td><td>50.0000</td><td></td></tr><tr><td>Sample 4</td><td>1.000000</td><td>1.000000</td><td>100.0000</td><td></td></tr></tbody></table><div>Analyte Table</div><table><thead><tr><th>Sample Name</th><th>Concentration [ppm]</th><th>Signal</th><th>Relative Signal [%]</th><th>Remove</th></tr></thead><tbody><tr><td>Unknown 1</td><td>0.250000</td><td>0.250000</td><td>25.0000</td><td></td></tr><tr><td>Unknown 2</td><td>0.600000</td><td>0.600000</td><td>60.0000</td><td></td></tr></tbody></table><div>Calibration Settings...</div><div><div>Calibration Curve</div><div>Quantitative Analysis</div><div><div>Save Calibration</div><div>Enter Analyte Data...</div><div>Create Report</div></div></div></div></div>	Sample Name	Concentration [ppm]	Signal	Relative Signal [%]	Remove	Sample 1	0.010000	0.010000	1.0000		Sample 2	0.100000	0.100000	10.0000		Sample 3	0.500000	0.500000	50.0000		Sample 4	1.000000	1.000000	100.0000		Sample Name	Concentration [ppm]	Signal	Relative Signal [%]	Remove	Unknown 1	0.250000	0.250000	25.0000		Unknown 2	0.600000	0.600000	60.0000	
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Unknown 1	0.250000	0.250000	25.0000																																							
Unknown 2	0.600000	0.600000	60.0000																																							
10	<p>As described in previous sections, a report can be generated by clicking Create Report button.</p> <div>Create Report</div>																																									

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



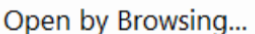
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

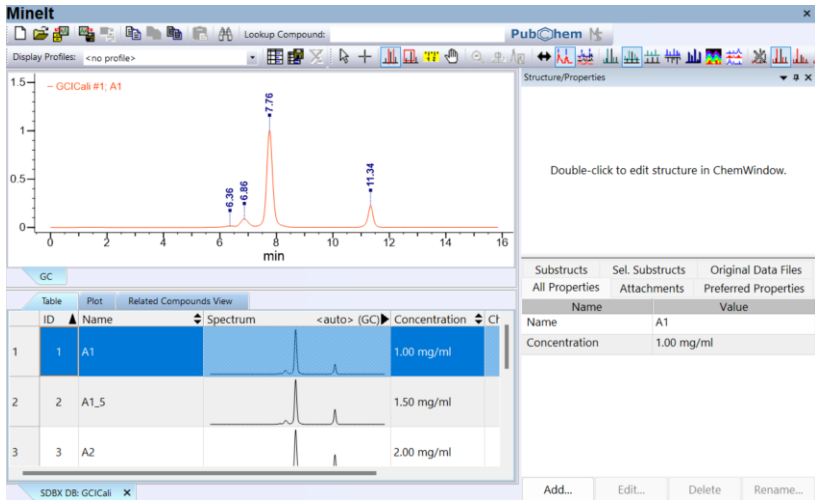
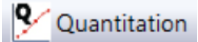
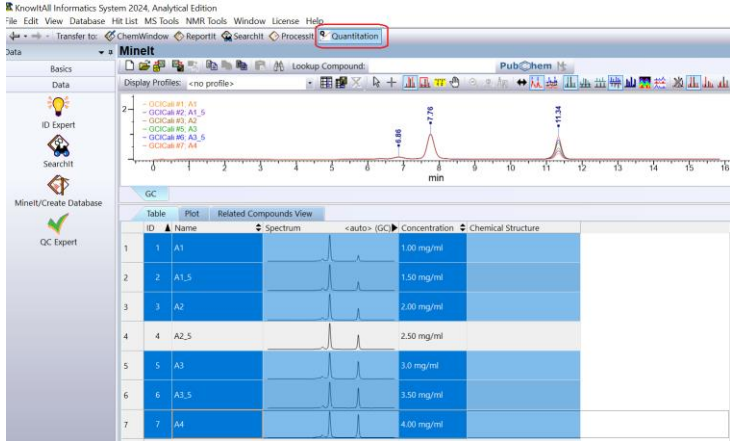
- Internal Calibration Chromatogram

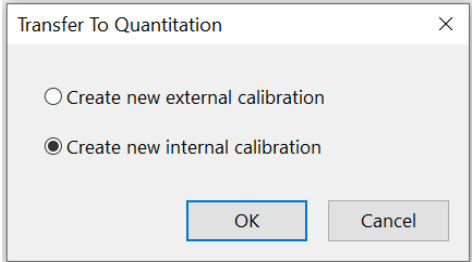
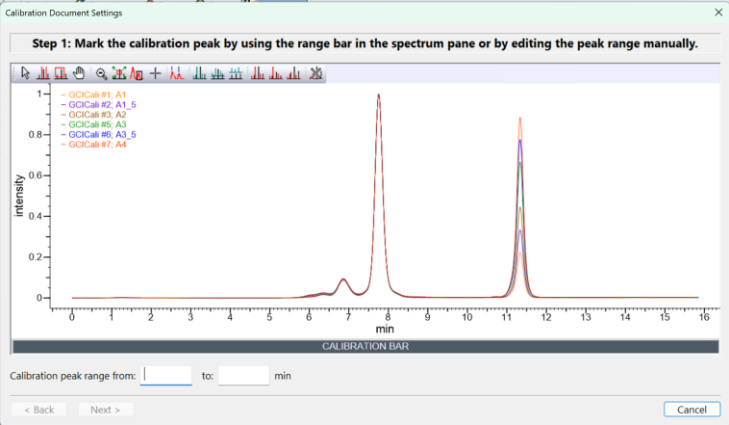
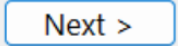
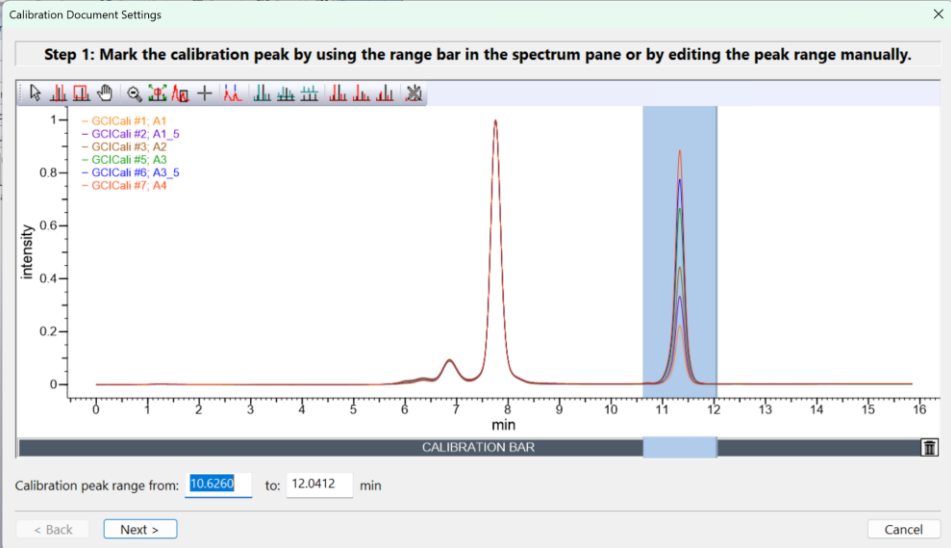
KnowItAll Applications Used

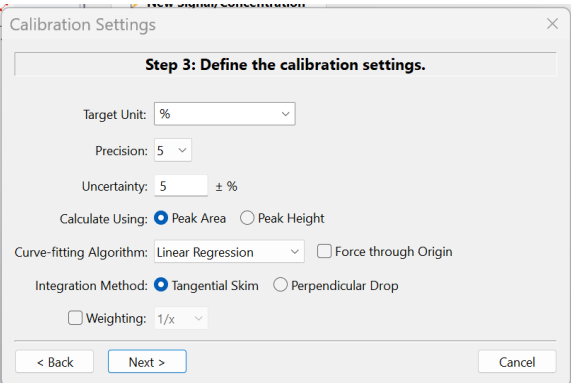
- Quantitation

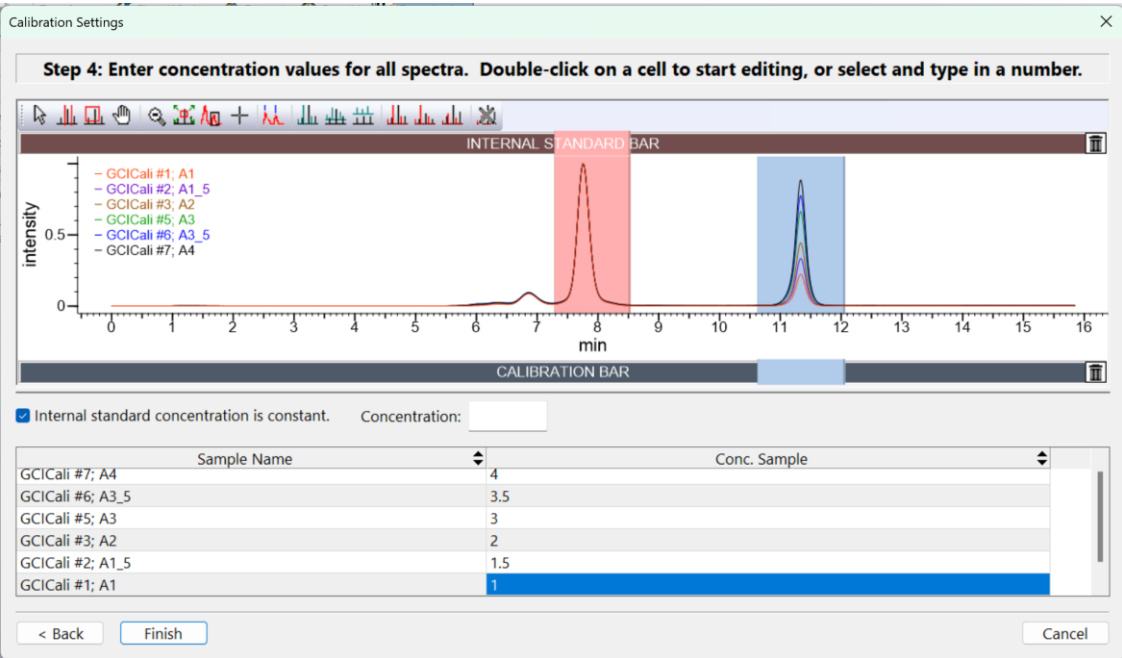
Chromatogram

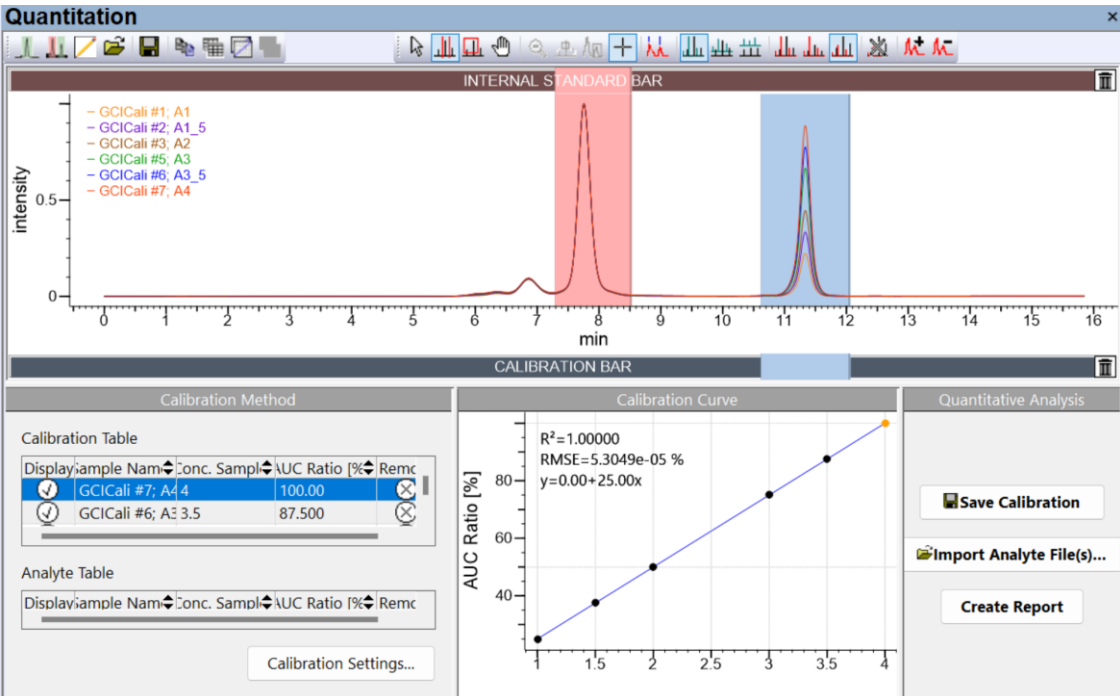
	Action	Result
1	Clear any calibration study presently open in Quantitation application by clicking on the X icon () on the top right corner.	
2	Open the Minelt application by clicking its icon, typically found in the Data group.  Minelt/Create Database Choose Database > Open .	The Select a Database popup 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.

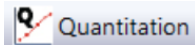
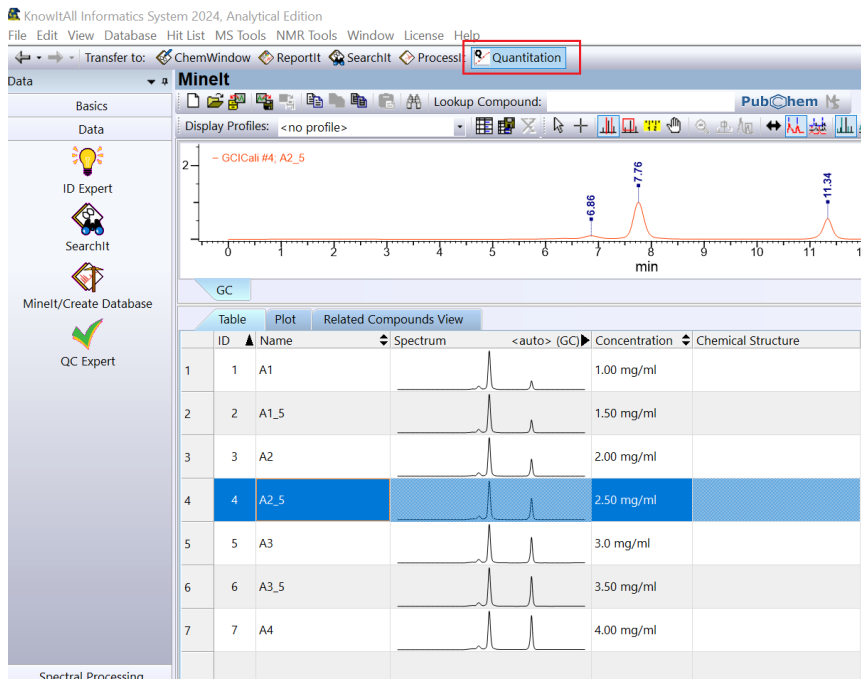
	Action	Result
4	<p>Navigate to the "C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration Chromatogram" folder.</p> <p>Select the Chromatograms For Internal Calibration Demo.sdbx file.</p> <p>Click Open.</p>	<p>The database file opens in Minelt:</p> 
5	<p>Hold the CTRL button 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.</p> 	<p>The series of selected records is shown in Minelt application:</p>  <p>Upon using the Transfer To button, KnowItAll loads Quantitation application with a Transfer to Quantitation popup window.</p>

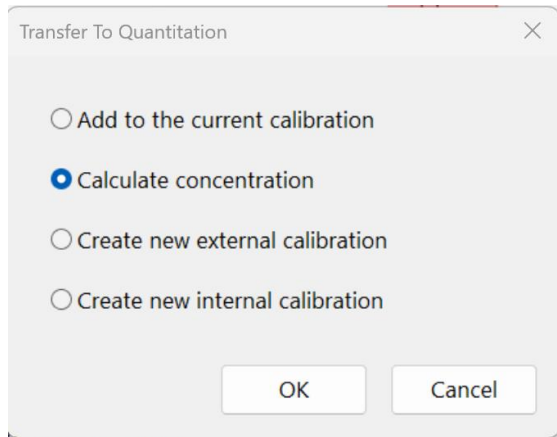
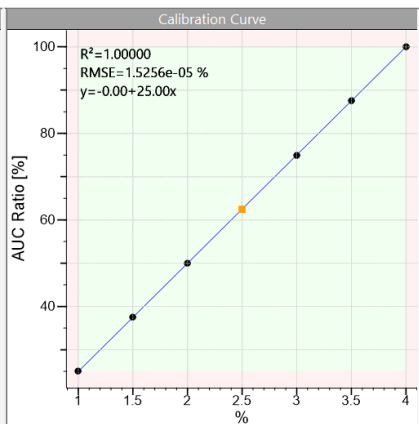
	Action	Result
6	<p>On the Transfer to Quantitation popup window, select Create new internal calibration at the prompt window.</p> <p>Click OK.</p> 	<p>The chromatograms load in the Calibration Document Settings popup window:</p> 
7	<p>Select peak region around 11.3 min as the calibrant peak by clicking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

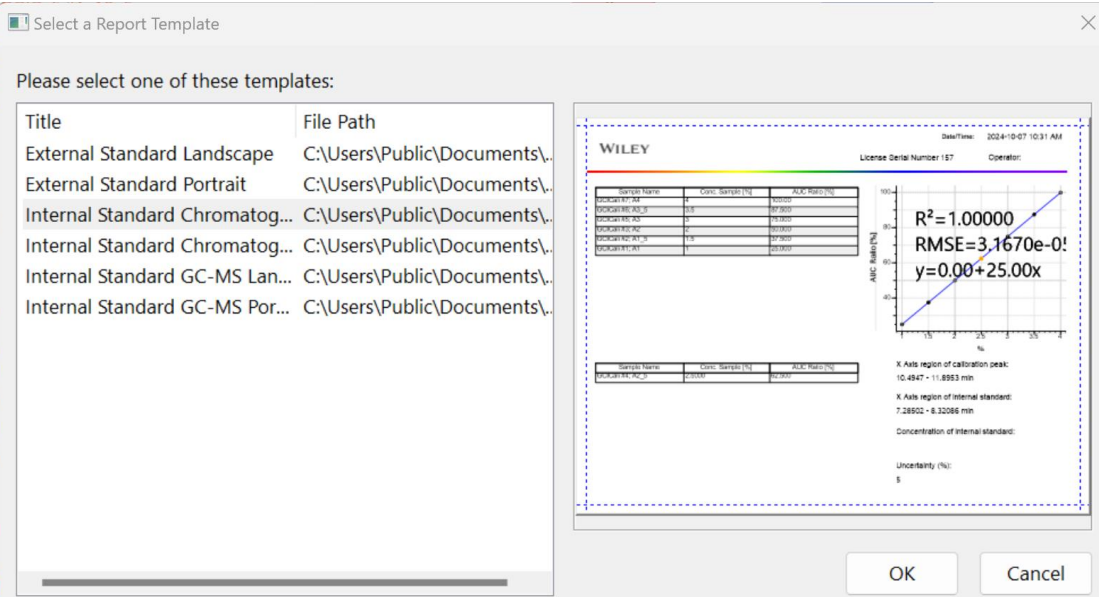
	Action	Result
8	<p>Select peak region around 7.8 min as the internal standard peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 7.3 – 8.5 min).</p> <p>Click Next > button.</p> <p>Next ></p>	<p>The selected region is displayed with red coloration:</p>  <p>Upon clicking Next button, “Step 3” loads in the popup window.</p>
9	<p>In the Calibration Settings window, define calibration the settings:</p> <ul style="list-style-type: none"> • Target Unit: % <p>Remaining options can retain the default selection.</p> <p>Click Next > button.</p> <p>Next ></p>	<p>The selected calibration settings are shown in the popup window. Upon selecting Next >, Step 4 settings load in the popup window.</p> 

	Action	Result
10	<p>Enter concentration ratios in the popup window:</p> <ul style="list-style-type: none"> • 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 <p>Ensure that the checkbox for Internal standard concentration is constant remains selected.</p> <p>Click the Finish button.</p>	<p>The calibration settings are shown in the popup window. Upon clicking Finish, the dialog closes and the chromatograms are displayed in Quantitation application.</p> 

	Action	Result
11	Analyze the calibration results in Quantitation application.	<ul style="list-style-type: none"> 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 R² (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.  <p>The screenshot displays the Quantitation application interface. At the top, a chromatogram shows intensity versus time (min) with several peaks. Below the chromatogram, there is a 'CALIBRATION BAR' and a 'Quantitative Analysis' panel. The 'Quantitative Analysis' panel includes a 'Calibration Table' with columns for Display, Sample Name, Conc., Sample, AUC Ratio [%], and Remc. It also features a 'Calibration Curve' plot showing AUC Ratio [%] versus concentration, with a linear fit equation $y = 0.00 + 25.00x$ and statistics $R^2 = 1.00000$ and $RMSE = 5.3049e-05 \%$. The 'Quantitative Analysis' panel also contains buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.</p>

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

	Action	Result																																													
13	<p>On the Transfer to Quantitation popup window, select Calculate concentration.</p> <p>Click OK.</p>	<p>The selection is shown on the popup window. Upon clicking OK, the unknown chromatogram loads in Quantitation application.</p> 																																													
14	<p>Analyze the results of the unknown concentration calculation. Review the Analyte Table and Calibration Curve.</p>	<p>The concentration of the unknown file is calculated in the Analyte Table. The unknown concentration is displayed on the Calibration Curve.</p> <div><div><p>Calibration Method</p><p>Calibration Table</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Conc. Sample [%]</th><th>AUC Ratio [%]</th><th>Remove</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #7; A4</td><td>4</td><td>100.00</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #6; A3_5</td><td>3.5</td><td>87.500</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #5; A3</td><td>3</td><td>75.000</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #3; A2</td><td>2</td><td>50.000</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #2; A1_5</td><td>1.5</td><td>37.500</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #1; A1</td><td>1</td><td>25.000</td><td><input type="checkbox"/></td></tr></tbody></table><p>Analyte Table</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Conc. Sample [%]</th><th>AUC Ratio [%]</th><th>Remove</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>GCI-Cali #4; A2_5</td><td>2.5000</td><td>62.715</td><td><input type="checkbox"/></td></tr></tbody></table><p>Calibration Settings...</p></div><div><p>Calibration Curve</p></div></div>	Display	Sample Name	Conc. Sample [%]	AUC Ratio [%]	Remove	<input checked="" type="checkbox"/>	GCI-Cali #7; A4	4	100.00	<input type="checkbox"/>	<input checked="" type="checkbox"/>	GCI-Cali #6; A3_5	3.5	87.500	<input type="checkbox"/>	<input checked="" type="checkbox"/>	GCI-Cali #5; A3	3	75.000	<input type="checkbox"/>	<input checked="" type="checkbox"/>	GCI-Cali #3; A2	2	50.000	<input type="checkbox"/>	<input checked="" type="checkbox"/>	GCI-Cali #2; A1_5	1.5	37.500	<input type="checkbox"/>	<input checked="" type="checkbox"/>	GCI-Cali #1; A1	1	25.000	<input type="checkbox"/>	Display	Sample Name	Conc. Sample [%]	AUC Ratio [%]	Remove	<input checked="" type="checkbox"/>	GCI-Cali #4; A2_5	2.5000	62.715	<input type="checkbox"/>
Display	Sample Name	Conc. Sample [%]	AUC Ratio [%]	Remove																																											
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<input checked="" type="checkbox"/>	GCI-Cali #2; A1_5	1.5	37.500	<input type="checkbox"/>																																											
<input checked="" type="checkbox"/>	GCI-Cali #1; A1	1	25.000	<input type="checkbox"/>																																											
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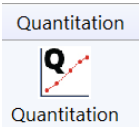
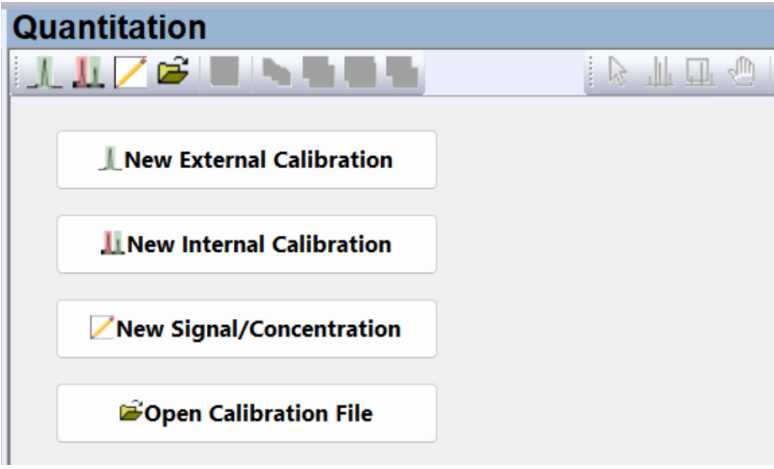
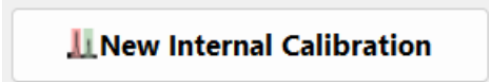
	Action	Result
15	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <p>On the Select a Report Template popup window, select “Internal Standard Chromatogram Landscape” report template. Click OK popup window to create the report in the selected template.</p>	<p>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.</p> 

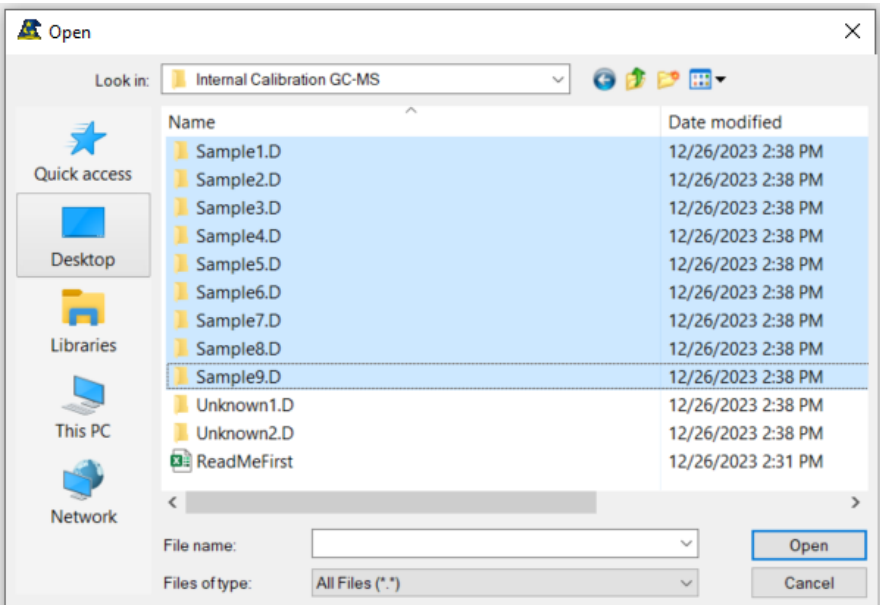
GC-MS

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.

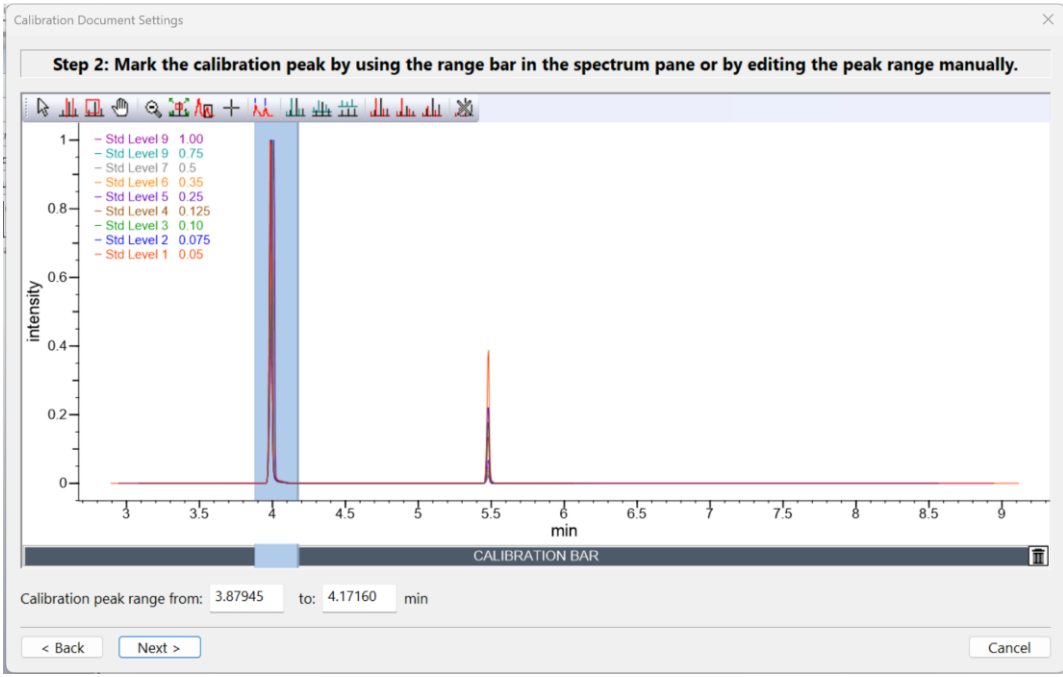
Benzocaine

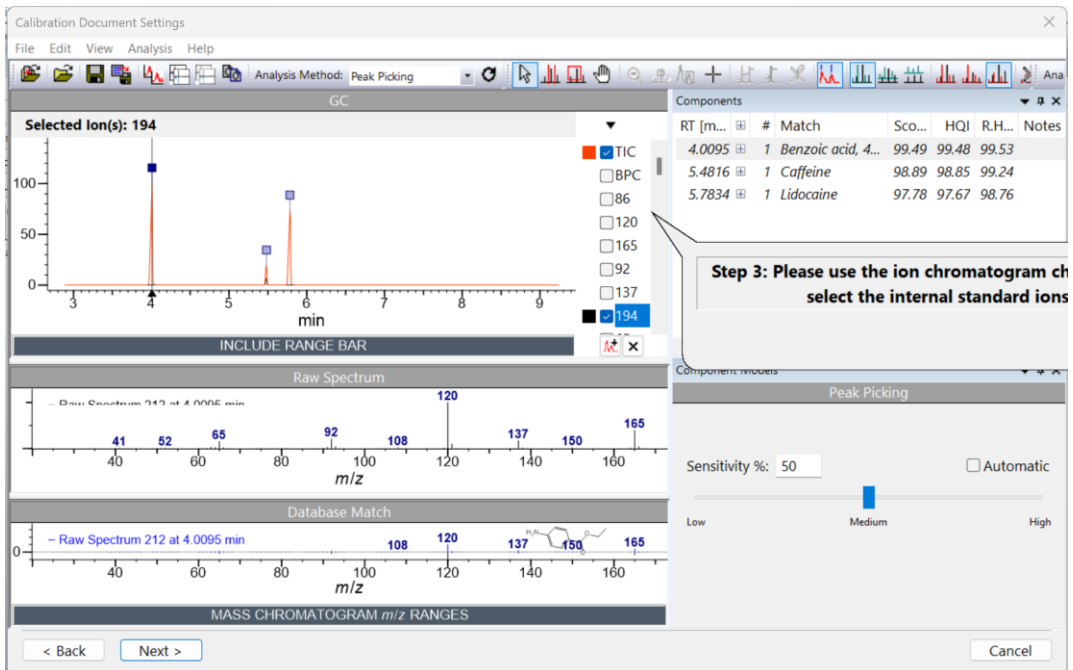
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note: If a previous calibration study is displayed, it can be closed by clicking on the X icon (X) on the top right corner.</i></p>	<p>Quantitation application is displayed: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

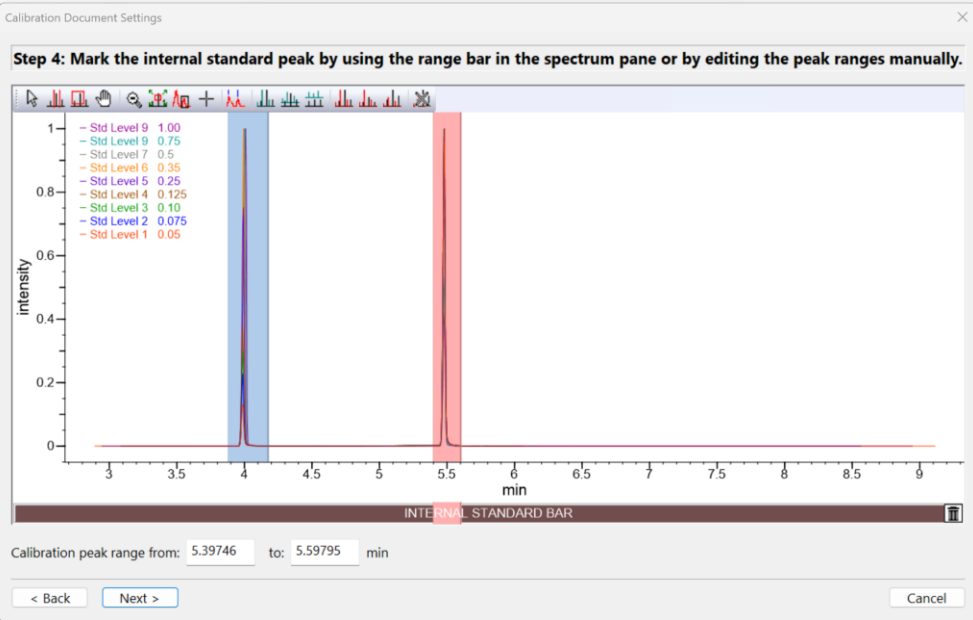
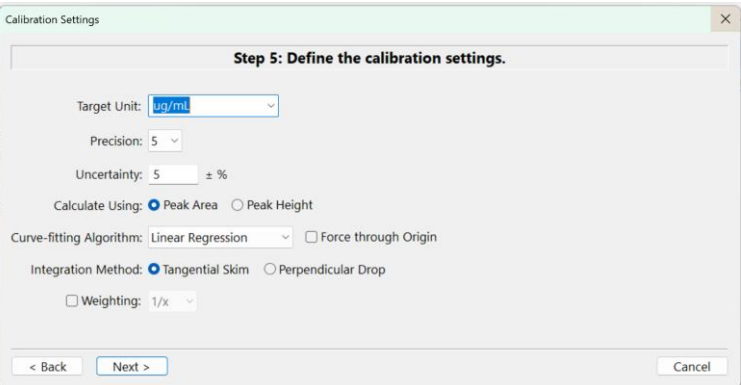
	Action	Result
3	<p>Navigate to “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS” folder.</p> <p>Select all “Sample_.D” folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files.</p> <p>Click Open.</p>	<p>All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

	Action	Result																												
4	<p>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.</p> <p>Click Close on the bubble window after making the file selection using the dropdown menu (e.g., see highlighted clicks below).</p> <div><p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p><div>Sample File: Sample9.D</div><div>Close</div></div>	<p>The chromatogram with the largest concentration, <i>i.e.</i>, Sample9.D., is used to select the component peak.</p> <div><p>Calibration Document Settings</p><p>File Edit View Analysis Help</p><p>Analysis Method: Peak Picking</p><p>GC</p><p>Please use the ion chromatogram checkboxes to select sample standard ions. Sample File: Sample9.D</p><p><input checked="" type="checkbox"/> TIC <input type="checkbox"/> BPC <input type="checkbox"/> 86 <input type="checkbox"/> 120 <input type="checkbox"/> 165</p><p>INCLUDE RANGE BAR</p><p>Raw Spectrum</p><p>Raw Spectrum 212 at 4.0095 min</p><p>Database Match</p><p>Raw Spectrum 212 at 4.0095 min</p><p>MASS CHROMATOGRAM m/z RANGES</p><p>Sample9.D</p><p>< Back Next ></p><p>Components</p><table><tr><th>RT [m...]</th><th>#</th><th>Match</th><th>Score</th><th>HQI</th><th>R.H...</th><th>Notes</th></tr><tr><td>4.0095</td><td>1</td><td>Benzoic acid, 4...</td><td>99.49</td><td>99.48</td><td>99.53</td><td></td></tr><tr><td>5.4816</td><td>1</td><td>Caffeine</td><td>98.89</td><td>98.85</td><td>99.24</td><td></td></tr><tr><td>5.7834</td><td>1</td><td>Lidocaine</td><td>97.78</td><td>97.67</td><td>98.76</td><td></td></tr></table><p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p><p>Sample File: Sample9.D</p><p>Close</p><p>Sensitivity %: 50 <input type="checkbox"/> Automatic</p><p>Low Medium High</p><p>Cancel</p></div>	RT [m...]	#	Match	Score	HQI	R.H...	Notes	4.0095	1	Benzoic acid, 4...	99.49	99.48	99.53		5.4816	1	Caffeine	98.89	98.85	99.24		5.7834	1	Lidocaine	97.78	97.67	98.76	
RT [m...]	#	Match	Score	HQI	R.H...	Notes																								
4.0095	1	Benzoic acid, 4...	99.49	99.48	99.53																									
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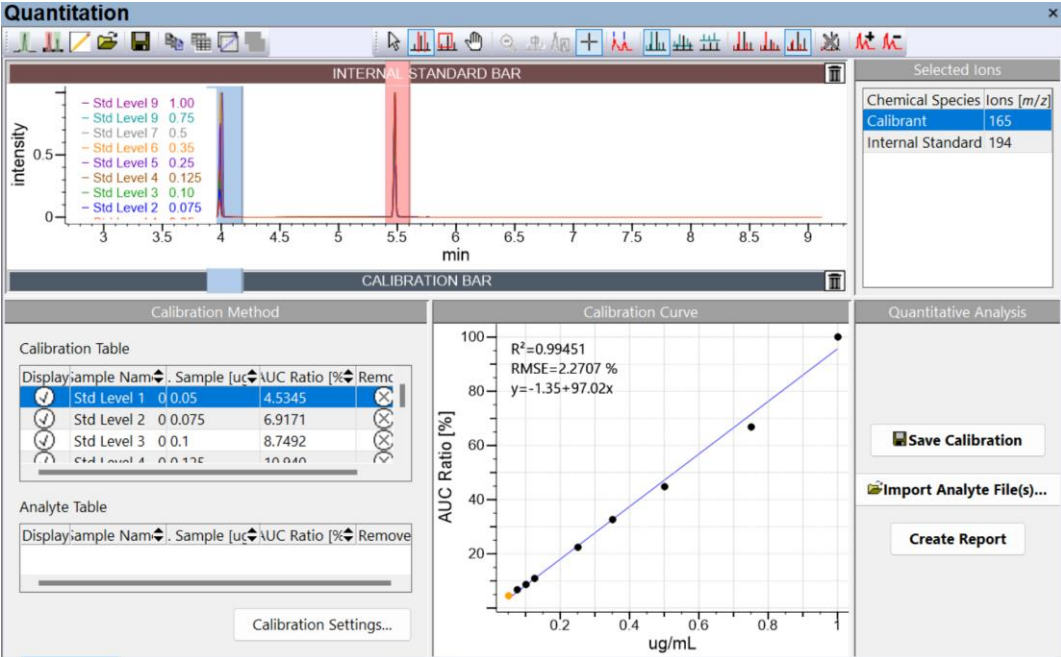
	Action	Result
5	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is "165". This represents the TIC component for the peak at 4.01 min.</p> <div><div><div><input checked="" type="checkbox"/> TIC</div><div><input type="checkbox"/> BPC</div><div><input type="checkbox"/> 86</div><div><input type="checkbox"/> 120</div><div><input checked="" type="checkbox"/> 165</div><div><input type="checkbox"/> 92</div><div><input type="checkbox"/> 137</div></div><div>Click Next > button.</div><div>Next ></div></div>	<p>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.</p> 

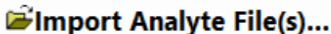
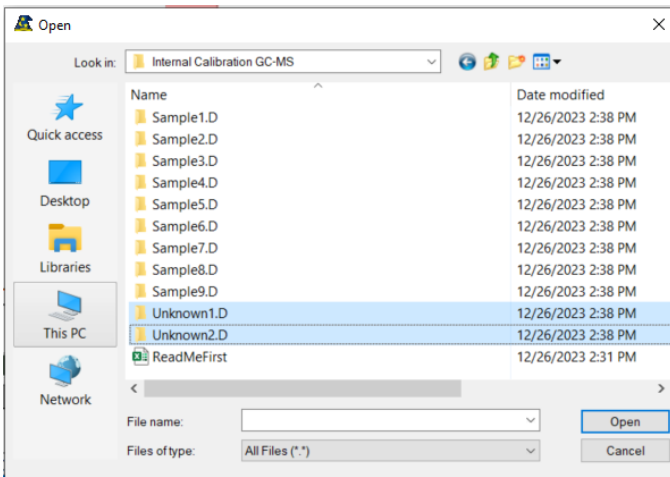
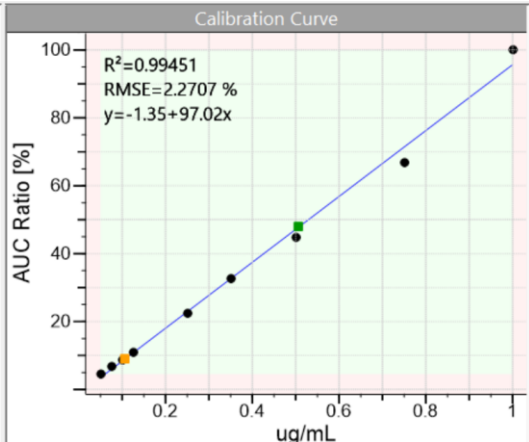
	Action	Result
6	<p>Select peak region around 4.0 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 3.9 – 4.2 min).</p> <p>Click Next > button.</p> <div><div>Next ></div></div>	<p>The selected region is shaded with blue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

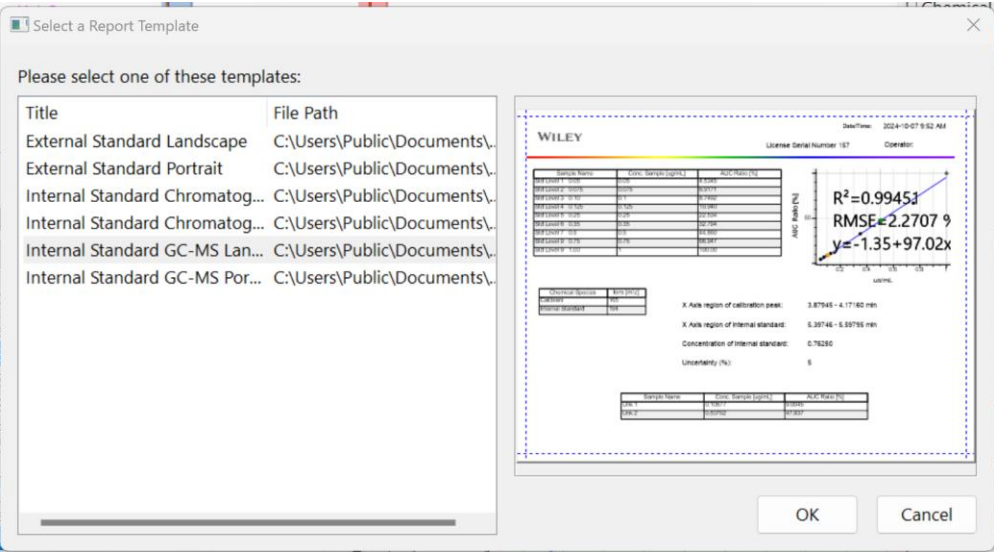
	Action	Result
7	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is "194". This represents the TIC internal standard for the peak at 5.48 min.</p> <p>Click Next > button.</p> <p>Next ></p>	<p>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.</p> 

	Action	Result
8	<p>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).</p> <p>Click Next > button.</p> <div data-bbox="254 565 443 613">Next ></div>	<p>The selected internal standard region is shaded with red coloration.</p> 
9	<p>In the Calibrations Settings popup window, define the calibration settings:</p> <ul style="list-style-type: none">• Target Unit: ug/mL (has to be manually typed in) <p>Remaining parameters can retain the default selection.</p> <p>Click Next > button.</p> <div data-bbox="254 1287 443 1336">Next ></div>	

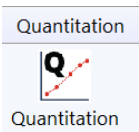
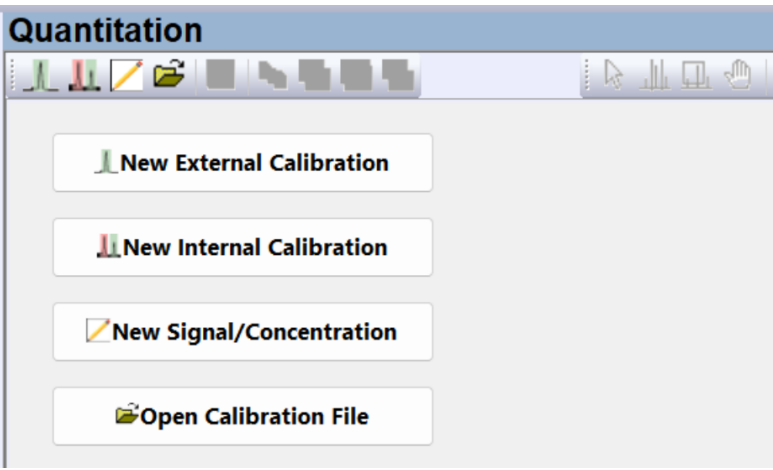
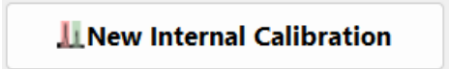
	Action	Result																				
10	<p>Enter concentration and ratio values in the popup window:</p> <ul style="list-style-type: none">• File: Std Level 1 0.05, Concentration: 0.05• File: Std Level 2 0.075, Concentration: 0.075• File: Std Level 3 0.10, Concentration: 0.1• File: Std Level 4 0.125, Concentration: 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 <p>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.</p> <p>Click the Finish button.</p> <div><div>Finish</div></div>	<p>The concentration settings for the samples and internal standard are shown:</p> <div><div><input checked="" type="checkbox"/> Internal standard concentration is constant. Concentration: <div>0.7625</div> ug/mL</div><table><thead><tr><th>Sample Name</th><th>Conc. Sample [ug/mL]</th></tr></thead><tbody><tr><td>Std Level 1 0.05</td><td>0.05</td></tr><tr><td>Std Level 2 0.075</td><td>0.075</td></tr><tr><td>Std Level 3 0.10</td><td>0.1</td></tr><tr><td>Std Level 4 0.125</td><td>0.125</td></tr><tr><td>Std Level 5 0.25</td><td>0.25</td></tr><tr><td>Std Level 6 0.35</td><td>0.35</td></tr><tr><td>Std Level 7 0.5</td><td>0.5</td></tr><tr><td>Std Level 9 0.75</td><td>0.75</td></tr><tr><td>Std Level 9 1.00</td><td>1</td></tr></tbody></table></div> <p>Upon clicking Finish, the Calibration Settings popup window closes and the chromatograms display in Quantitation application.</p>	Sample Name	Conc. Sample [ug/mL]	Std Level 1 0.05	0.05	Std Level 2 0.075	0.075	Std Level 3 0.10	0.1	Std Level 4 0.125	0.125	Std Level 5 0.25	0.25	Std Level 6 0.35	0.35	Std Level 7 0.5	0.5	Std Level 9 0.75	0.75	Std Level 9 1.00	1
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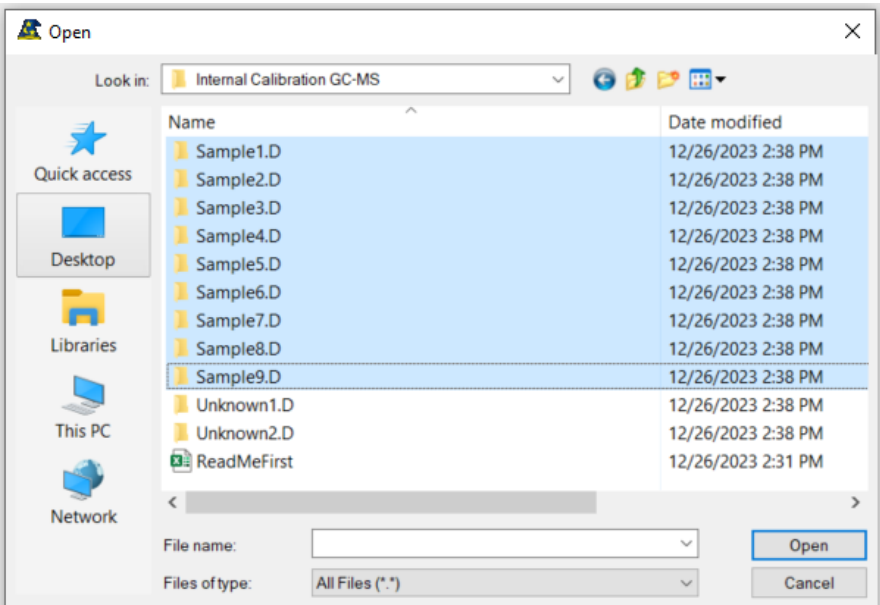
	Action	Result
11	Analyze the calibration results.	<ul style="list-style-type: none"> Statistics are reported in the Calibration Curve. The lower the value for RMSE (Root Mean Squared Error), the better the curve fitting is. The closer the R² (Coefficient of Determination) is to 1, the better the curve 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.  <p>The screenshot displays the Quantitation software interface. At the top, there's a toolbar with various icons. Below it, a chromatogram plot shows 'intensity' vs 'min' with a peak at approximately 5.5 minutes. A legend on the left lists standard levels from 1 to 9 with their respective concentrations. Below the chromatogram, a 'CALIBRATION BAR' is visible. The main panel is divided into three sections: 'Calibration Method' on the left, 'Calibration Curve' in the center, and 'Quantitative Analysis' on the right. The 'Calibration Method' section contains a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Sample [ug]', 'AUC Ratio [%]', and 'Remc'. It lists four standard levels with their corresponding AUC ratios. Below this is an 'Analyte Table' with similar columns. The 'Calibration Curve' section shows a scatter plot of 'AUC Ratio [%]' vs 'ug/mL' with a linear regression line. The statistics displayed are R²=0.99451 and RMSE=2.2707 %. The 'Quantitative Analysis' section contains buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.</p>

	Action	Result																																			
12	<p>Click the Import Analyte File(s) button found in the Quantitative Analysis panel.</p> <div></div> <p>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).</p> <p>Click Open.</p>	<p>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.</p> 																																			
13	<p>Analyze the results of the unknown concentration calculation. Review the Analyte Table and Calibration Curve.</p>	<p>The concentration of the unknown file is calculated in the Analyte Table. The unknown concentration is displayed on the Calibration Curve.</p> <div><div><p>Calibration Method</p><p>Calibration Table</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Sample [ug]</th><th>AUC Ratio [%]</th><th>Remc</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>Std Level 1</td><td>0.05</td><td>4.5345</td><td><input checked="" type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 2</td><td>0.075</td><td>6.9171</td><td><input checked="" type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 3</td><td>0.1</td><td>8.7492</td><td><input checked="" type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 4</td><td>0.125</td><td>10.040</td><td><input checked="" type="checkbox"/></td></tr></tbody></table><p>Analyte Table</p><table><thead><tr><th>Display</th><th>Sample Name</th><th>Sample [ug]</th><th>AUC Ratio [%]</th><th>Remc</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>Unk 1</td><td>0.10677</td><td>9.0045</td><td><input checked="" type="checkbox"/></td></tr></tbody></table><p>Calibration Settings...</p></div><div><p>Calibration Curve</p></div></div>	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	<input checked="" type="checkbox"/>	Std Level 1	0.05	4.5345	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 2	0.075	6.9171	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 3	0.1	8.7492	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 4	0.125	10.040	<input checked="" type="checkbox"/>	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	<input checked="" type="checkbox"/>	Unk 1	0.10677	9.0045	<input checked="" type="checkbox"/>
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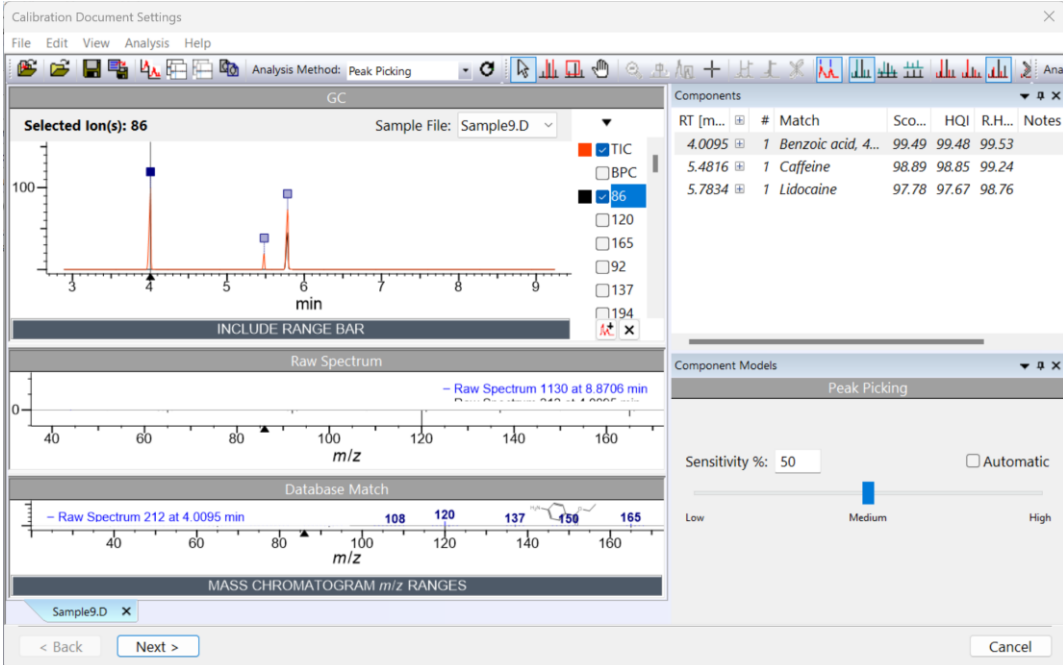
	Action	Result
14	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <div data-bbox="247 456 474 516" style="border: 1px solid black; padding: 5px; text-align: center; margin: 10px 0;"> Create Report </div> <p>In the Select a Report Template popup window, choose “Internal Standard GC-MS Landscape” to use this report template. Click OK on the Select a Report Template dialog window to create the report in the selected template.</p>	<p>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.</p>  <p>The screenshot shows the 'Select a Report Template' dialog box. On the left, a list of templates is displayed with columns 'Title' and 'File Path'. The 'Internal Standard GC-MS Landscape' template is selected. On the right, a preview of the report is shown, featuring a WILEY logo, a calibration curve graph with $R^2=0.9945$, and a table of data points.</p>

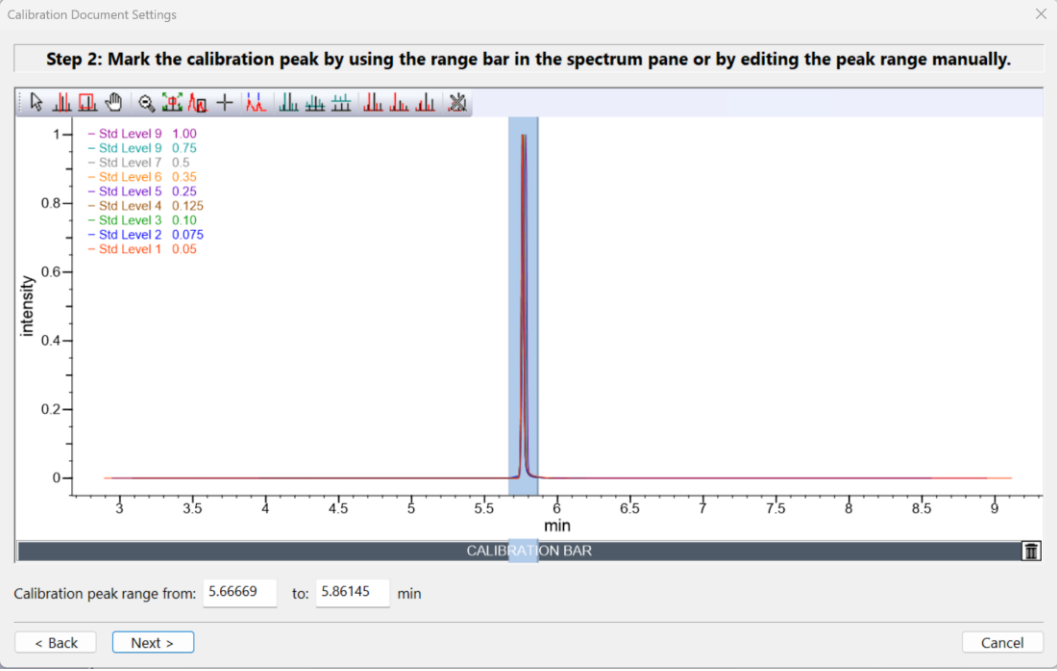
Lidocaine HCl

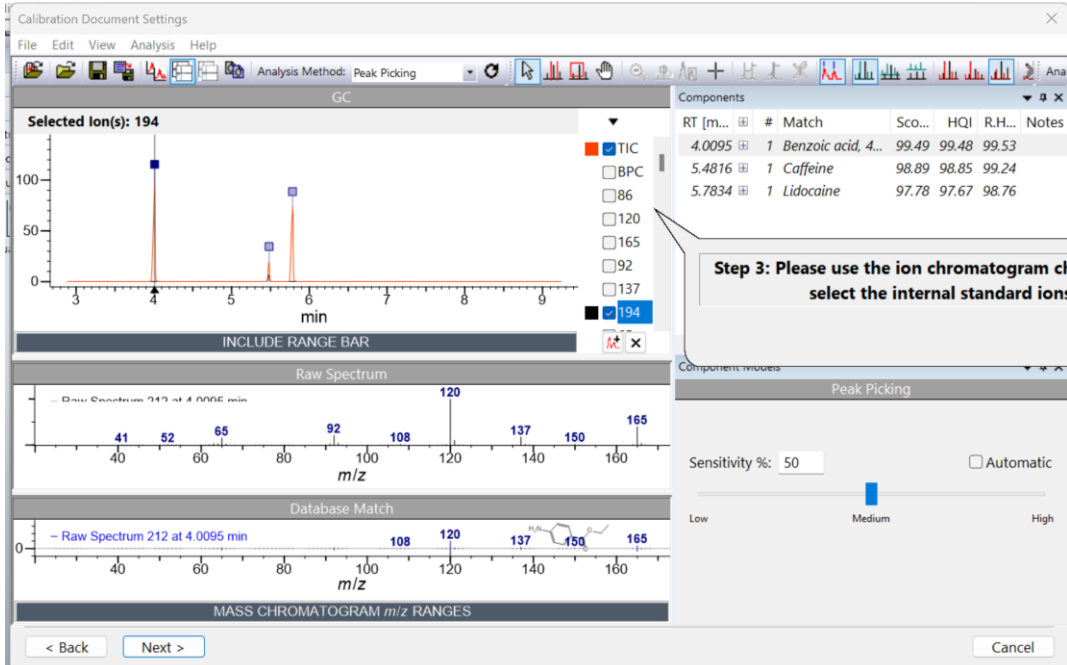
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note:</i> If a previous calibration study is displayed, it can be closed by clicking on the X icon (X) on the top right corner.</p>	<p>Quantitation application is displayed: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

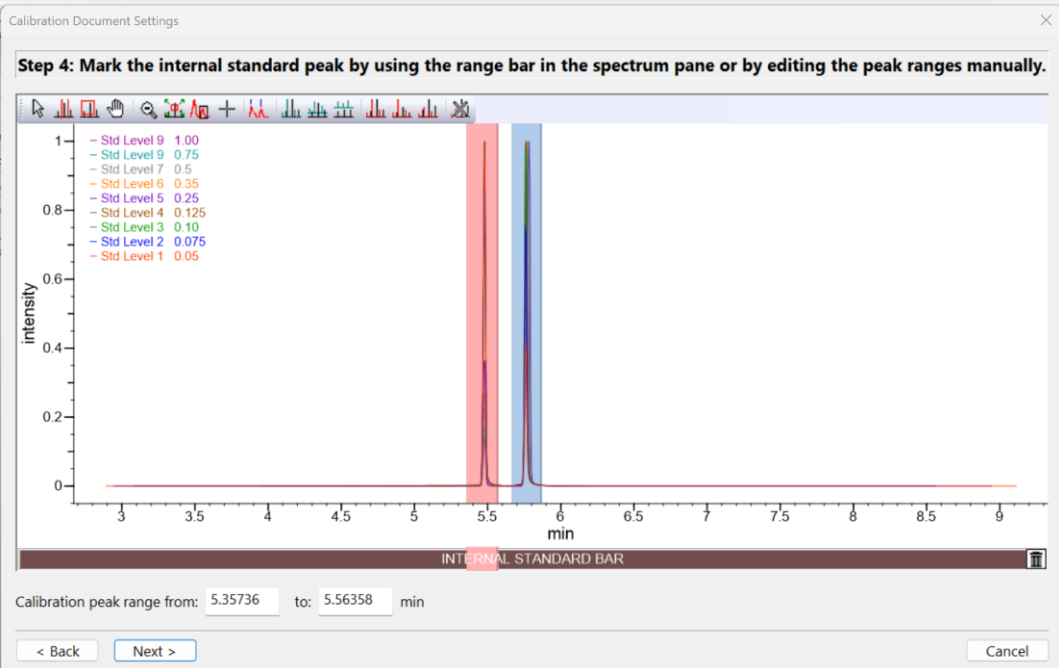
	Action	Result
3	<p>Navigate to “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS” folder.</p> <p>Select all “Sample_.D” folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files.</p> <p>Click Open.</p>	<p>All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

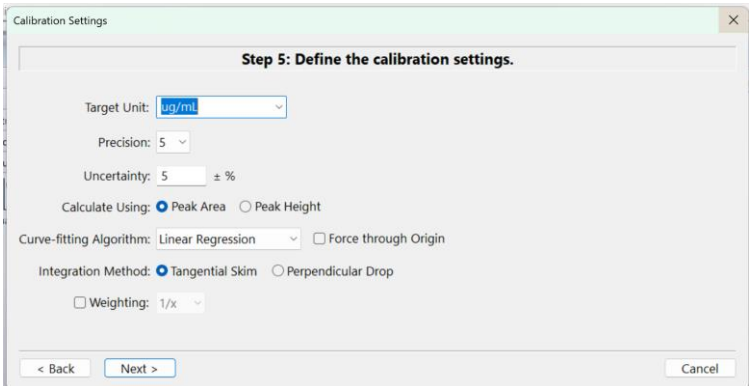
	Action	Result																												
4	<p>On the Calibration Document Settings popup window, use the Sample File dropdown menu to choose “Sample9.D”, which is the calibrant file in which the analyte concentration is the largest.</p> <p>Click Close on the bubble window after making the file selection using the dropdown menu (e.g., see highlighted clicks below).</p> <div><p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p><p>Sample File: Sample9.D</p><p>Close</p></div>	<p>The chromatogram with the largest concentration, <i>i.e.</i>, Sample9.D., is used to select the component peak.</p> <div><p>Calibration Document Settings</p><p>File Edit View Analysis Help</p><p>Analysis Method: Peak Picking</p><p>GC</p><p>Please use the ion chromatogram checkboxes to select sample standard ions. Sample File: Sample9.D</p><p><input checked="" type="checkbox"/> TIC <input type="checkbox"/> BPC <input type="checkbox"/> 86 <input type="checkbox"/> 120 <input type="checkbox"/> 165</p><p>100</p><p>min</p><p>INCLUDE RANGE BAR</p><p>Raw Spectrum</p><p>Database Match</p><p>MASS CHROMATOGRAM m/z RANGES</p><p>Sample9.D</p><p>< Back Next ></p><p>Components</p><table><tr><th>RT [m...]</th><th>#</th><th>Match</th><th>Score</th><th>HQI</th><th>R.H...</th><th>Notes</th></tr><tr><td>4.0095</td><td>1</td><td>Benzoic acid, 4...</td><td>99.49</td><td>99.48</td><td>99.53</td><td></td></tr><tr><td>5.4816</td><td>1</td><td>Caffeine</td><td>98.89</td><td>98.85</td><td>99.24</td><td></td></tr><tr><td>5.7834</td><td>1</td><td>Lidocaine</td><td>97.78</td><td>97.67</td><td>98.76</td><td></td></tr></table><p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p><p>Sample File: Sample9.D</p><p>Close</p><p>Sensitivity %: 50 <input type="checkbox"/> Automatic</p><p>Low Medium High</p><p>Cancel</p></div>	RT [m...]	#	Match	Score	HQI	R.H...	Notes	4.0095	1	Benzoic acid, 4...	99.49	99.48	99.53		5.4816	1	Caffeine	98.89	98.85	99.24		5.7834	1	Lidocaine	97.78	97.67	98.76	
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	Action	Result
5	<p>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.</p> <p>Click Next > button.</p> <p>Next ></p>	<p>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.</p> 

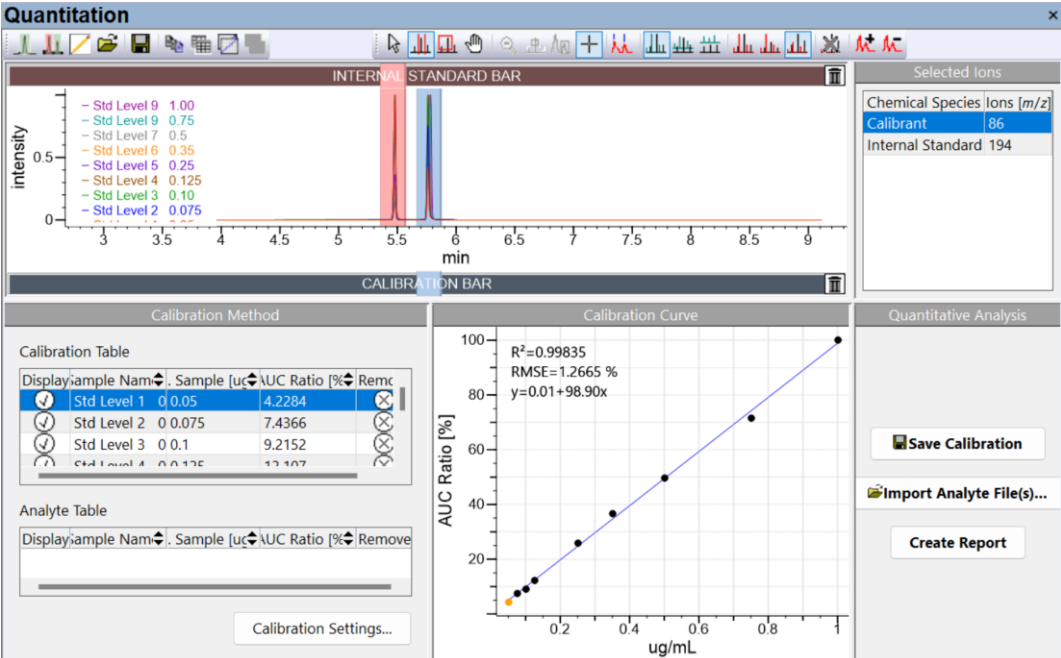
	Action	Result
6	<p>Select peak region around 5.75 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 5.7 – 5.9 min).</p> <p>Click Next > button.</p> <div>Next ></div>	<p>The selected region is shaded with blue coloration.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


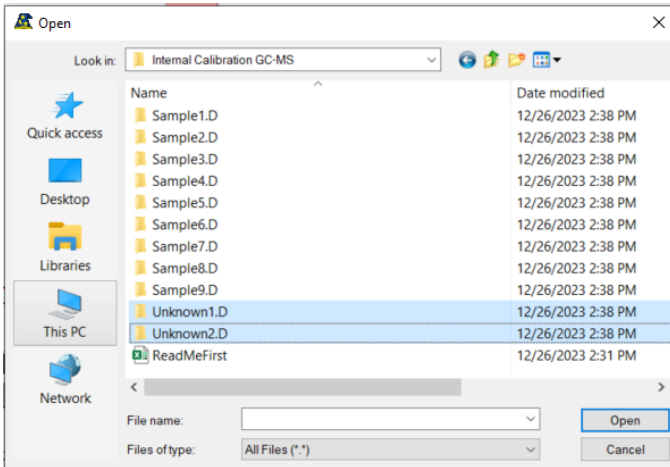
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7	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is “194”. This represents the TIC internal standard for the peak at 5.48 min.</p> <p>Click Next > button.</p> <div>Next ></div>	<p>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.</p>  <p>Calibration Document Settings</p> <p>File Edit View Analysis Help</p> <p>Analysis Method: Peak Picking</p> <p>Selected Ion(s): 194</p> <p>min</p> <p>INCLUDE RANGE BAR</p> <p>Raw Spectrum</p> <p>Raw Spectrum 212 at 4.0095 min</p> <p>m/z</p> <p>Database Match</p> <p>Raw Spectrum 212 at 4.0095 min</p> <p>m/z</p> <p>MASS CHROMATOGRAM m/z RANGES</p> <p>Components</p> <table><thead><tr><th>RT [m...]</th><th>#</th><th>Match</th><th>Sco...</th><th>HQI</th><th>R.H...</th><th>Notes</th></tr></thead><tbody><tr><td>4.0095</td><td>1</td><td>Benzoic acid, 4...</td><td>99.49</td><td>99.48</td><td>99.53</td><td></td></tr><tr><td>5.4816</td><td>1</td><td>Caffeine</td><td>98.89</td><td>98.85</td><td>99.24</td><td></td></tr><tr><td>5.7834</td><td>1</td><td>Lidocaine</td><td>97.78</td><td>97.67</td><td>98.76</td><td></td></tr></tbody></table> <p>Step 3: Please use the ion chromatogram to select the internal standard ions</p> <p>Component: 194</p> <p>Peak Picking</p> <p>Sensitivity %: 50</p> <p>Low Medium High</p> <p>< Back Next > Cancel</p>	RT [m...]	#	Match	Sco...	HQI	R.H...	Notes	4.0095	1	Benzoic acid, 4...	99.49	99.48	99.53		5.4816	1	Caffeine	98.89	98.85	99.24		5.7834	1	Lidocaine	97.78	97.67	98.76	
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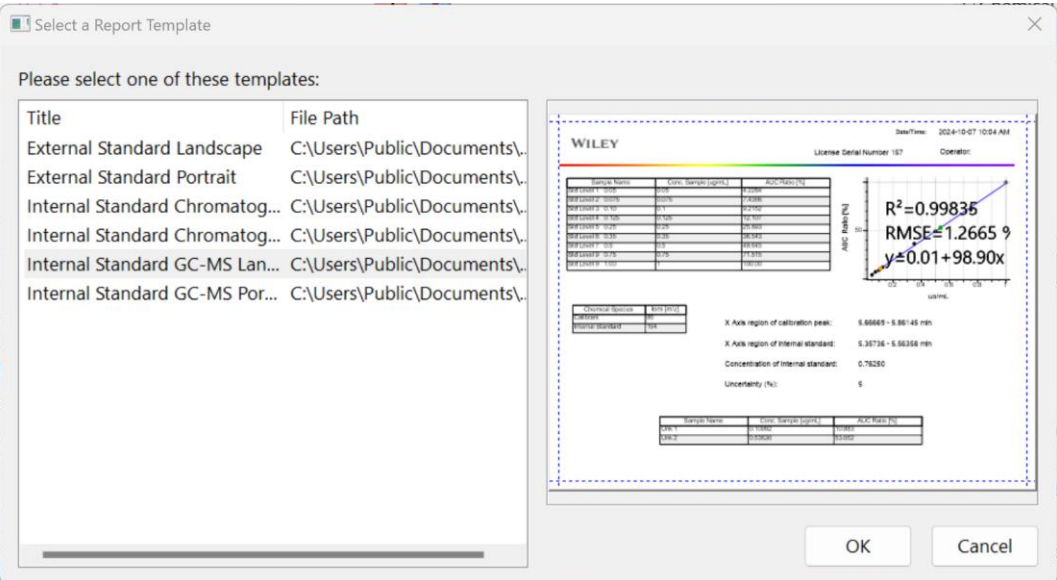
	Action	Result
8	<p>Select peak region around 5.5 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 5.4 – 5.6 min).</p> <p>Click Next > button.</p> <div data-bbox="254 565 443 613">Next ></div>	<p>The selected internal standard region is shaded with red coloration.</p>  <p>The screenshot shows a 'Calibration Document Settings' window. At the top, it says 'Step 4: Mark the internal standard peak by using the range bar in the spectrum pane or by editing the peak ranges manually.' Below this is a chromatogram plot with 'intensity' on the y-axis (0 to 1) and 'min' on the x-axis (3 to 9). A legend on the left lists standard levels: Std Level 9 (1.00), Std Level 8 (0.75), Std Level 7 (0.5), Std Level 6 (0.35), Std Level 5 (0.25), Std Level 4 (0.125), Std Level 3 (0.10), Std Level 2 (0.075), and Std Level 1 (0.05). A red shaded region is visible around 5.5 min. Below the plot is an 'INTERNAL STANDARD BAR' with a range from 5.35736 to 5.56358 min. At the bottom are buttons for '< Back', 'Next >', and 'Cancel'.</p>

	Action	Result
9	<p>In the Calibrations Settings popup window, define the calibration settings:</p> <ul style="list-style-type: none">• Target Unit: ug/mL (has to be manually typed in) <p>Remaining parameters can retain the default selection.</p> <p>Click Next > button.</p> <p>Next ></p>	<p>The applied calibration settings are shown:</p> 

	Action	Result																				
10	<p>Enter concentration and ratio values in the popup window:</p> <ul style="list-style-type: none">• 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 <p>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.</p> <p>Click the Finish button.</p> <div><div>Finish</div></div>	<p>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.</p> <div><div><input checked="" type="checkbox"/> Internal standard concentration is constant. Concentration: <div>0.7625</div> ug/mL</div><table><thead><tr><th>Sample Name</th><th>Conc. Sample [ug/mL]</th></tr></thead><tbody><tr><td>Std Level 1 0.05</td><td>0.05</td></tr><tr><td>Std Level 2 0.075</td><td>0.075</td></tr><tr><td>Std Level 3 0.10</td><td>0.1</td></tr><tr><td>Std Level 4 0.125</td><td>0.125</td></tr><tr><td>Std Level 5 0.25</td><td>0.25</td></tr><tr><td>Std Level 6 0.35</td><td>0.35</td></tr><tr><td>Std Level 7 0.5</td><td>0.5</td></tr><tr><td>Std Level 9 0.75</td><td>0.75</td></tr><tr><td>Std Level 9 1.00</td><td>1</td></tr></tbody></table></div>	Sample Name	Conc. Sample [ug/mL]	Std Level 1 0.05	0.05	Std Level 2 0.075	0.075	Std Level 3 0.10	0.1	Std Level 4 0.125	0.125	Std Level 5 0.25	0.25	Std Level 6 0.35	0.35	Std Level 7 0.5	0.5	Std Level 9 0.75	0.75	Std Level 9 1.00	1
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11	Analyze the calibration results.	<ul style="list-style-type: none"> 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 R² (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.  <p>The screenshot displays the Quantitation software interface. At the top, there's a toolbar with various icons. Below it, a chromatogram plot shows 'Intensity' vs 'min' with several peaks labeled 'Std Level 1' through 'Std Level 9'. A 'CALIBRATION BAR' is visible below the plot. To the right, a 'Selected Ions' table lists 'Chemical Species' as 'Calibrant' with 'Ions [m/z]' as 86 and 'Internal Standard' as 194. Below the chromatogram, there's a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Sample [ug]', 'UC Ratio [%]', and 'Remc'. It lists four standard levels. To the right of the table is a 'Calibration Curve' plot showing 'AUC Ratio [%]' vs 'ug/mL' with a linear fit line and statistics: $R^2=0.99835$, $RMSE=1.2665\%$, and $y=0.01+98.90x$. On the far right, the 'Quantitative Analysis' panel contains buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.</p>

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13	<p>Analyze the results of the unknown concentration calculation. Review the Analyte Table and Calibration Curve.</p>	<p>The concentration of the unknown file is calculated in the Analyte Table. The unknown concentration is displayed on the Calibration Curve.</p> <div><div><div>Calibration Method</div><div><div>Calibration Table</div><table><thead><tr><th>Display</th><th>Sample Name</th><th>Sample [ug]</th><th>AUC Ratio [%]</th><th>Remc</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>Std Level 1</td><td>0.05</td><td>4.2284</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 2</td><td>0.075</td><td>7.4366</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 3</td><td>0.1</td><td>9.2152</td><td><input type="checkbox"/></td></tr><tr><td><input checked="" type="checkbox"/></td><td>Std Level 4</td><td>0.125</td><td>12.107</td><td><input type="checkbox"/></td></tr></tbody></table><div>Analyte Table</div><table><thead><tr><th>Display</th><th>Sample Name</th><th>Sample [ug]</th><th>AUC Ratio [%]</th><th>Remc</th></tr></thead><tbody><tr><td><input checked="" type="checkbox"/></td><td>Unk 1</td><td>0.10992</td><td>10.883</td><td><input type="checkbox"/></td></tr></tbody></table><div>Calibration Settings...</div></div></div><div><div>Calibration Curve</div><div><div><div><div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></div><div></d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Name	Sample [ug]	AUC Ratio [%]	Remc	<input checked="" type="checkbox"/>	Std Level 1	0.05	4.2284	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 2	0.075	7.4366	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 3	0.1	9.2152	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Std Level 4	0.125	12.107	<input type="checkbox"/>	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	<input checked="" type="checkbox"/>	Unk 1	0.10992	10.883	<input type="checkbox"/>
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	Action	Result
14	<p>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.</p> <p>Create Report</p> <p>In the Select a Report Template popup window, choose “Internal Standard GC-MS Landscape” to use this report template. Click OK on the Select a Report Template dialog window to create the report in the selected template.</p>	<p>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.</p>  <p>The screenshot shows the 'Select a Report Template' dialog box. On the left, a list of templates is displayed with columns 'Title' and 'File Path'. The 'Internal Standard GC-MS Landscape' template is selected. On the right, a preview of the report is shown, featuring a WILEY logo, a calibration curve graph with $R^2=0.99835$ and $RMSE=1.2665$, and a table of results.</p>