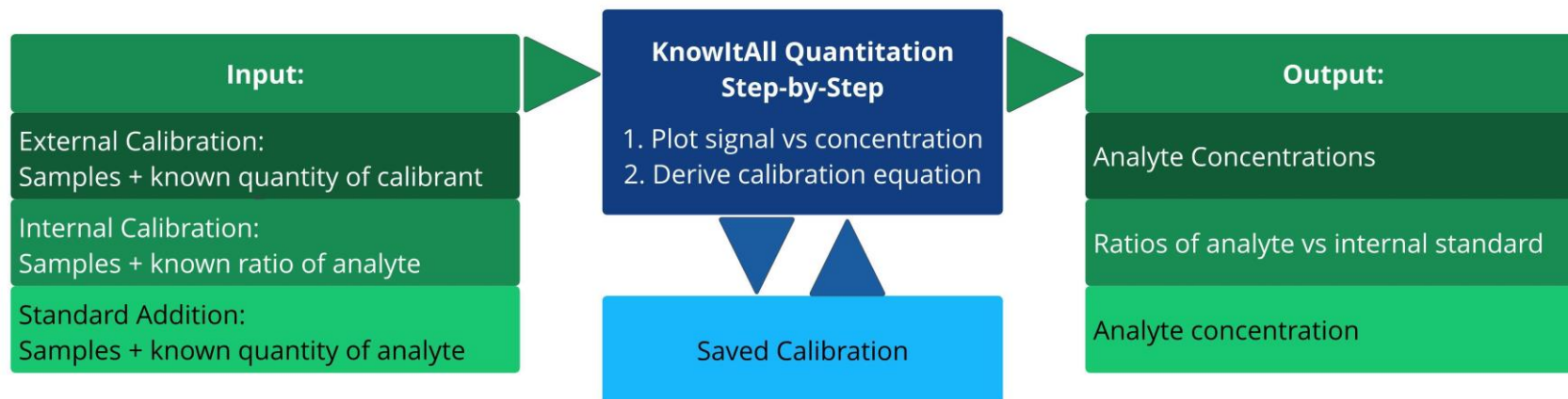


KnowItAll Informatics Training

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

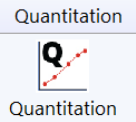
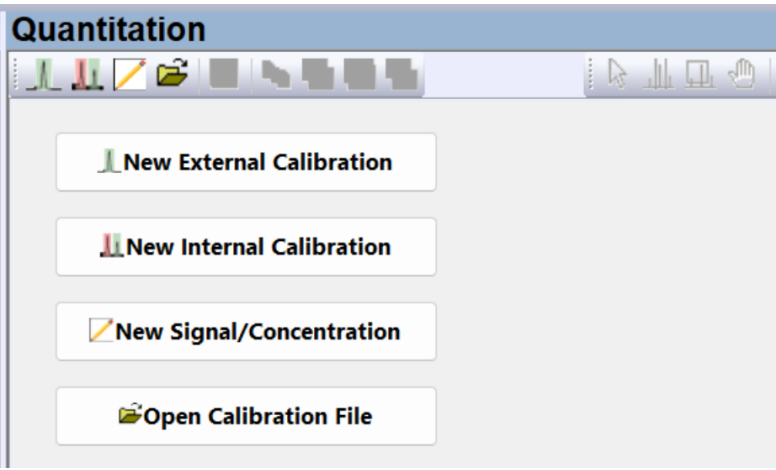
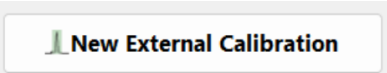
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

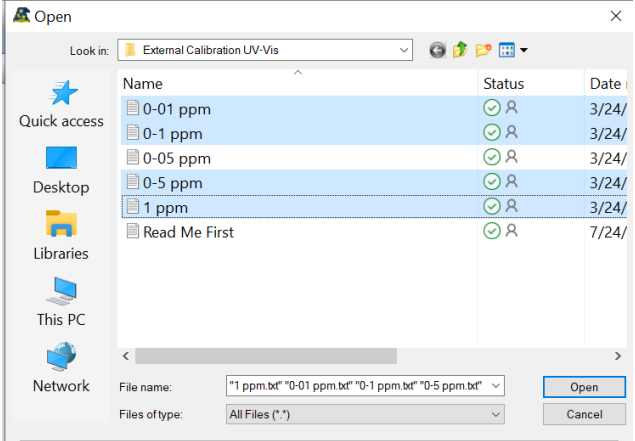
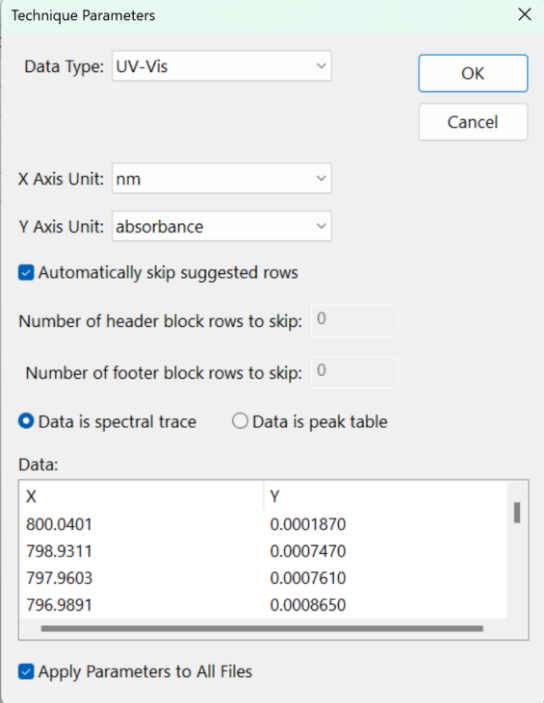
- External Calibration UV-Vis
- External Calibration IR

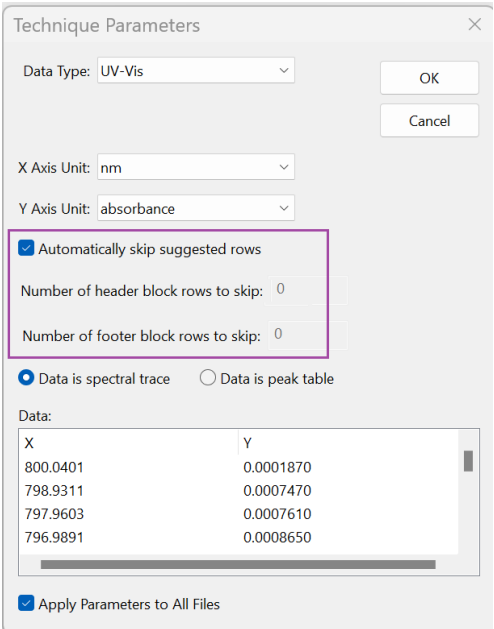
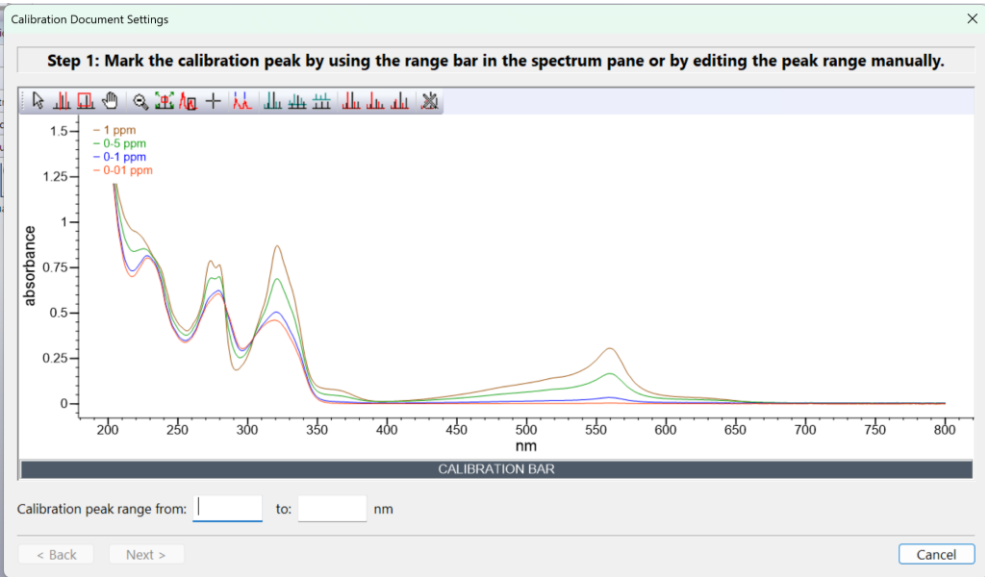
KnowItAll Applications Used

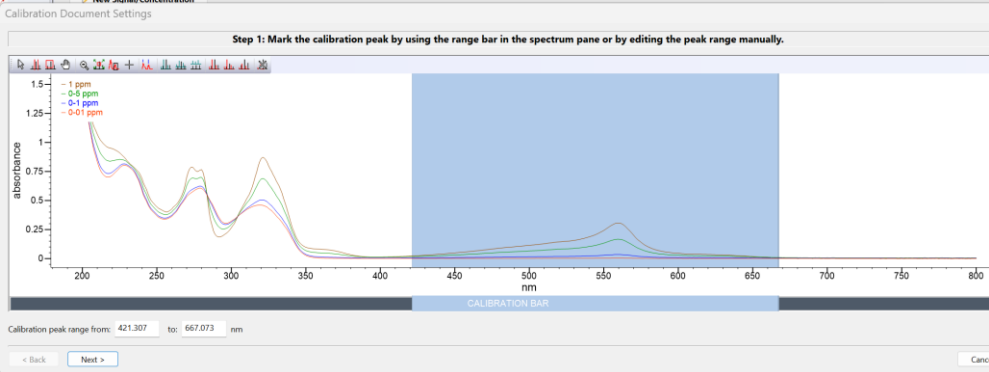
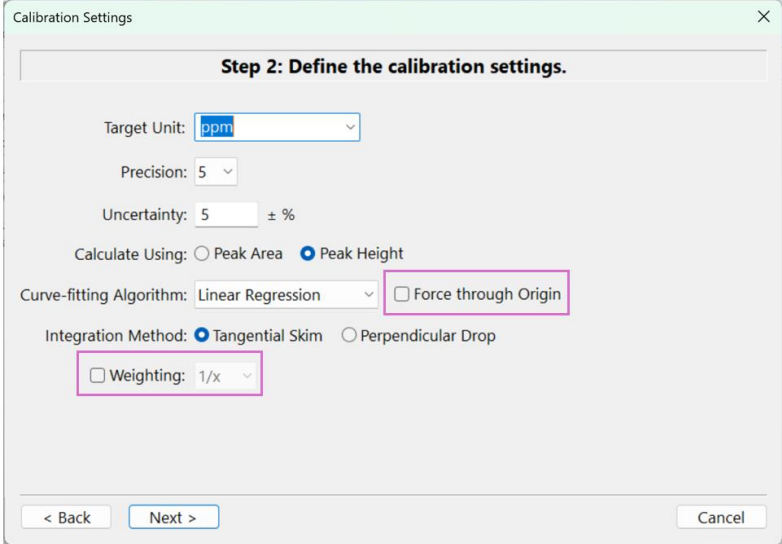
- Quantitation

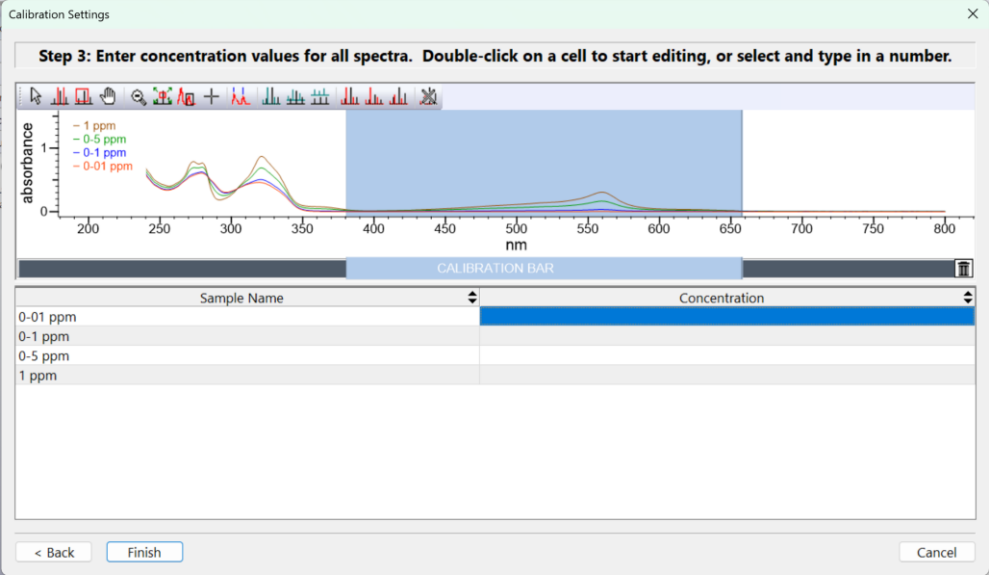
UV-Vis

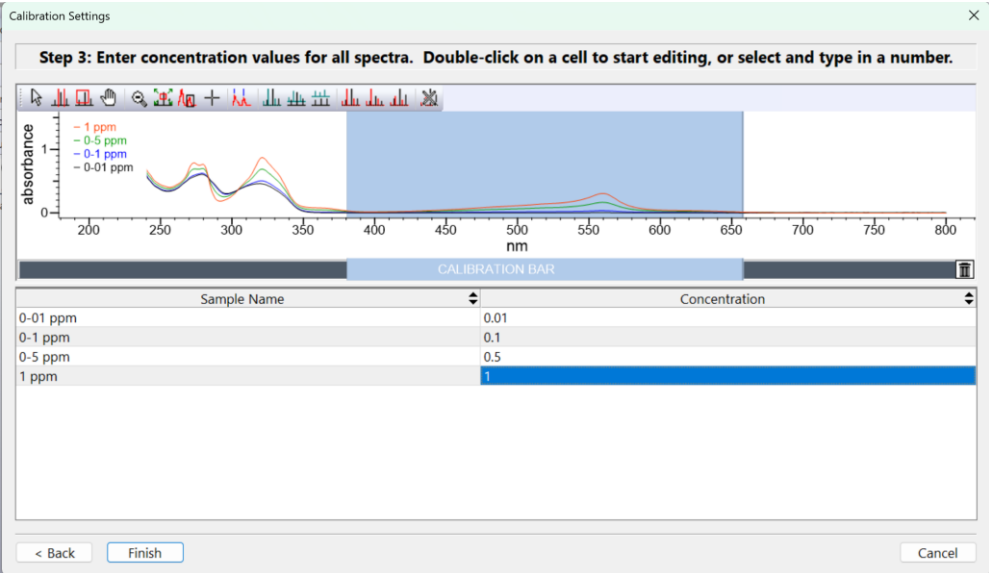
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p> 	<p>Quantitation application is displayed:</p> 
2	<p>Click New External Calibration button.</p> 	<p>KnowItAll prompts user to open calibrant files.</p>

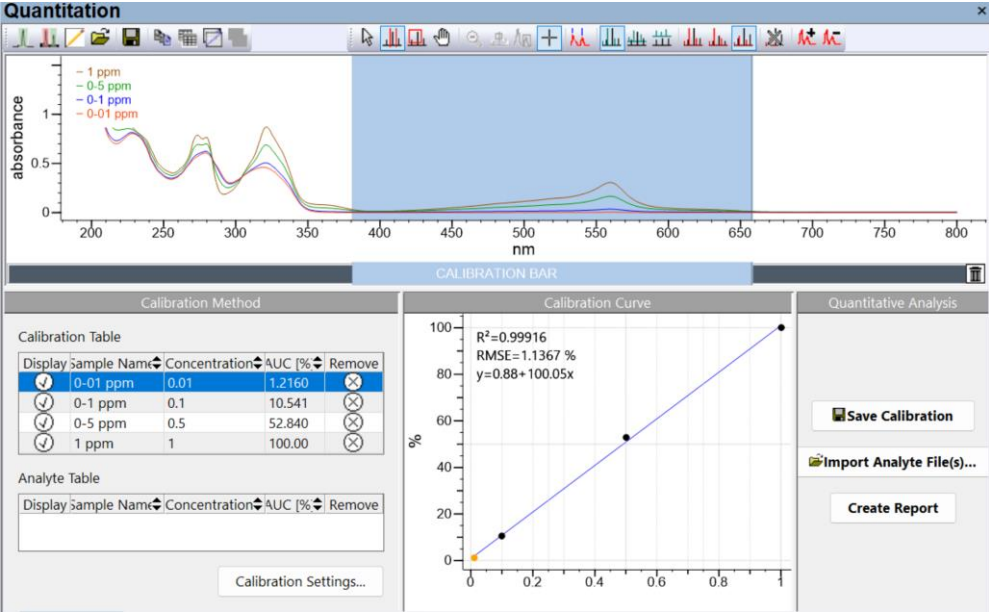
	Action	Result
3	<p>Navigate to the “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis” folder.</p> <p>Select 4 sample files and leave one out to be the unknown sample (e.g., as shown in image below).</p> <p>Click Open.</p> 	<p>The Technique Parameters dialog is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

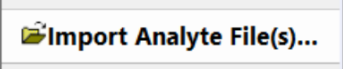
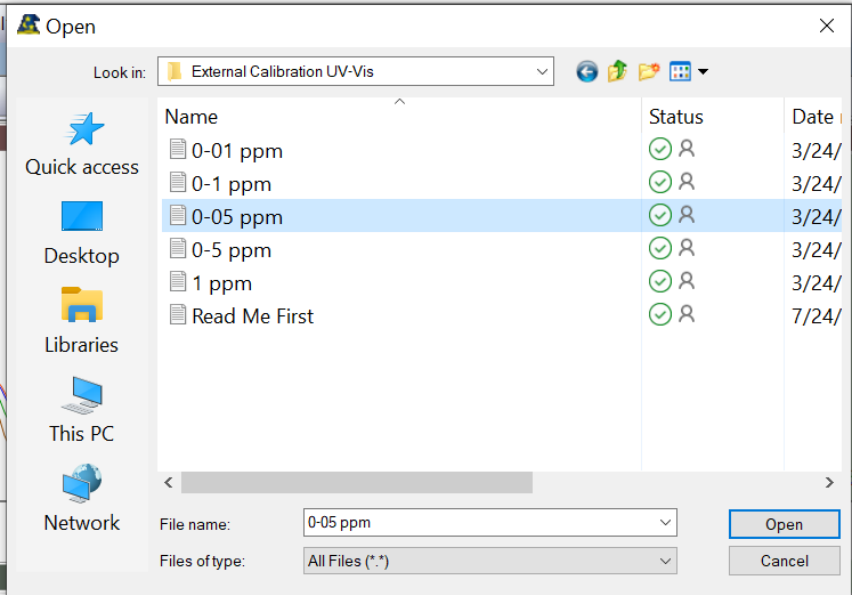
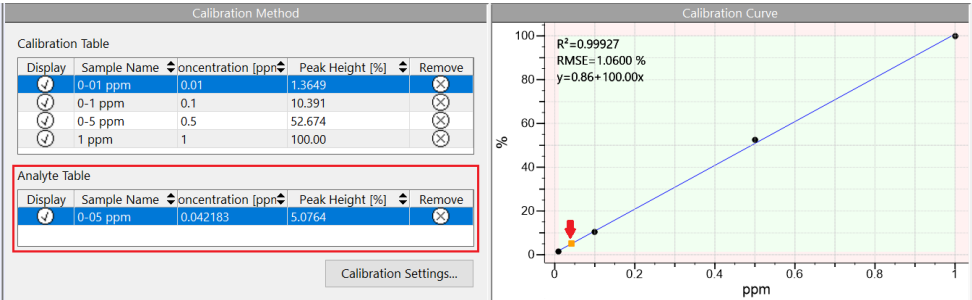
	Action	Result
4	<p>In Technique Parameters prompt window:</p> <ul style="list-style-type: none"> • Set Data Type to UV-Vis. • Check Apply Parameters to All Files. <p>Click OK.</p>  <p><i>Note:</i> The options highlighted above are added to skip lines which are not spectral x,y coordinates.</p>	<p>The Calibration Document Settings popout window appears, displaying the selected UV-Vis files. The Step is identified as “Step 1”:</p> 

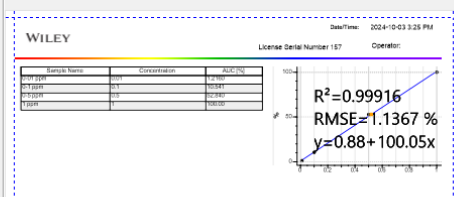
	Action	Result
5	<p>Select the region around the peak at 560 nm by clicking down the left mouse button on the CALIBRATION BAR and dragging the button over the peak region, e.g., from ~ 425 nm to ~ 650 nm. Release the mouse button at the end of the selection.</p> <p>Click Next > button.</p> <p><input type="button" value="Next >"/></p>	<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> 

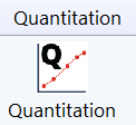
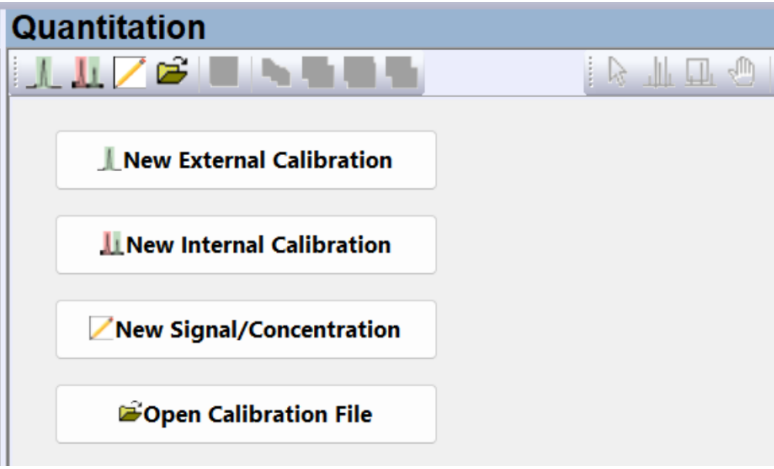
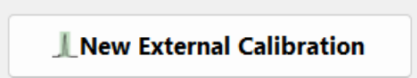
	Action	Result										
8	<p>In the popup window, enter concentrations in the right column based on the file names:</p> <ul style="list-style-type: none"> • File: 0-01 ppm, Concentration: 0.01 ppm • File: 0-1 ppm, Concentration: 0.1 ppm • File: 0-5 ppm, Concentration: 0.5 ppm • File: 1 ppm, Concentration: 1 ppm <p>Click Finish button.</p> <div style="border: 1px solid gray; padding: 5px; display: inline-block; margin-top: 10px;">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>  <table border="1" data-bbox="932 708 1887 938"> <thead> <tr> <th>Sample Name</th> <th>Concentration</th> </tr> </thead> <tbody> <tr> <td>0-01 ppm</td> <td>0.01</td> </tr> <tr> <td>0-1 ppm</td> <td>0.1</td> </tr> <tr> <td>0-5 ppm</td> <td>0.5</td> </tr> <tr> <td>1 ppm</td> <td>1</td> </tr> </tbody> </table>	Sample Name	Concentration	0-01 ppm	0.01	0-1 ppm	0.1	0-5 ppm	0.5	1 ppm	1
Sample Name	Concentration											
0-01 ppm	0.01											
0-1 ppm	0.1											
0-5 ppm	0.5											
1 ppm	1											

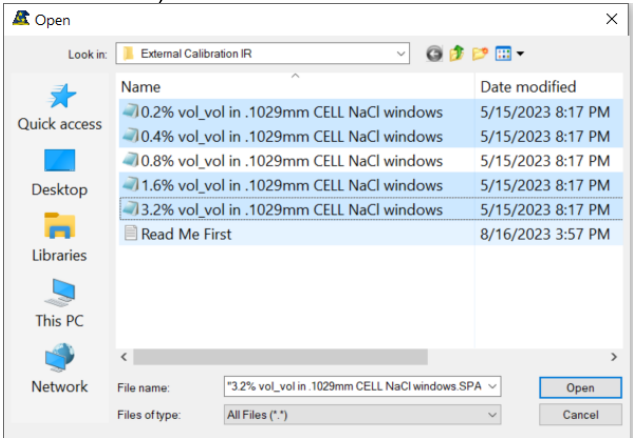
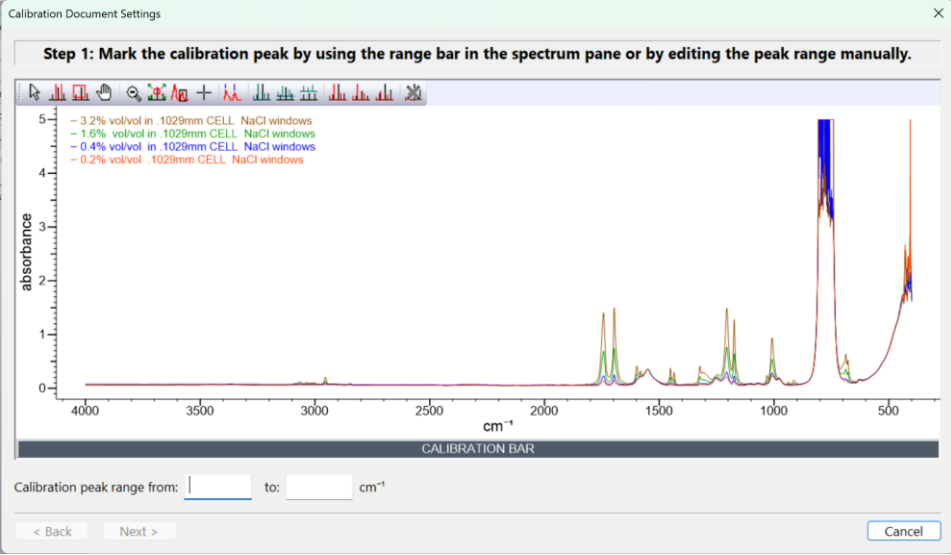
	Action	Result																									
9	Analyze the results in Quantitation application.	<ul style="list-style-type: none"> Statistics are reported in the Calibration Curve. The lower the value for RMSE (Root Mean Squared Error), the better the curve fitting is. The closer the R² (Coefficient of Determination) 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, there is a plot of absorbance versus wavelength (nm) from 200 to 800 nm. Four curves are shown for different concentrations: 1 ppm (red), 0.5 ppm (green), 0.1 ppm (blue), and 0.01 ppm (orange). A blue shaded region highlights the calibration range from approximately 400 nm to 650 nm. Below the plot is a 'CALIBRATION BAR'. The interface is divided into three main sections: 'Calibration Method', 'Calibration Curve', and 'Quantitative Analysis'. The 'Calibration Method' section contains a 'Calibration Table' with the following data:</p> <table border="1" data-bbox="932 971 1297 1101"> <thead> <tr> <th>Display</th> <th>Sample Name</th> <th>Concentration</th> <th>AUC [%]</th> <th>Remove</th> </tr> </thead> <tbody> <tr> <td><input checked="" type="checkbox"/></td> <td>0-01 ppm</td> <td>0.01</td> <td>1.2160</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> </tbody> </table> <p>The 'Calibration Curve' section shows a linear plot of % versus concentration with the following statistics: $R^2=0.99916$, $RMSE=1.1367\%$, and the equation $y=0.88+100.05x$. The 'Quantitative Analysis' panel includes buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'. A 'Calibration Settings...' button is also present at the bottom of the interface.</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 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"/>																							

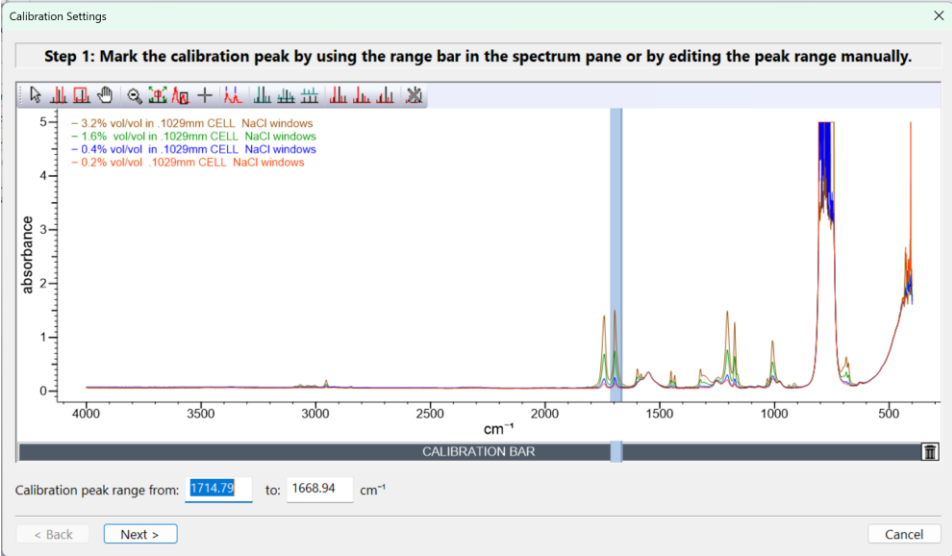
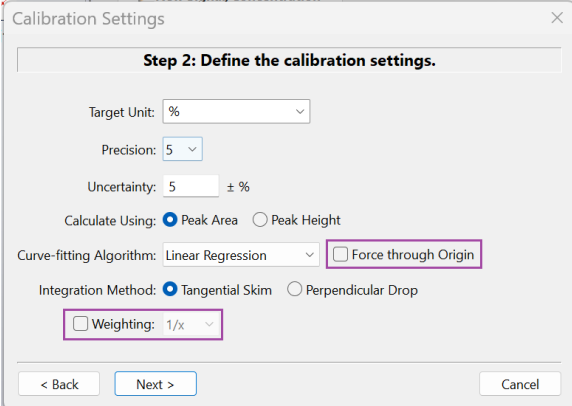
	Action	Result
10	<p>Click the Import Analyte File(s) button.</p>  <p>Navigate to and select the file that was excluded in Step 3 "0-05 ppm", located "C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis". Click Open.</p> <p>On the Technique Parameters dialog window, 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> 
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> 

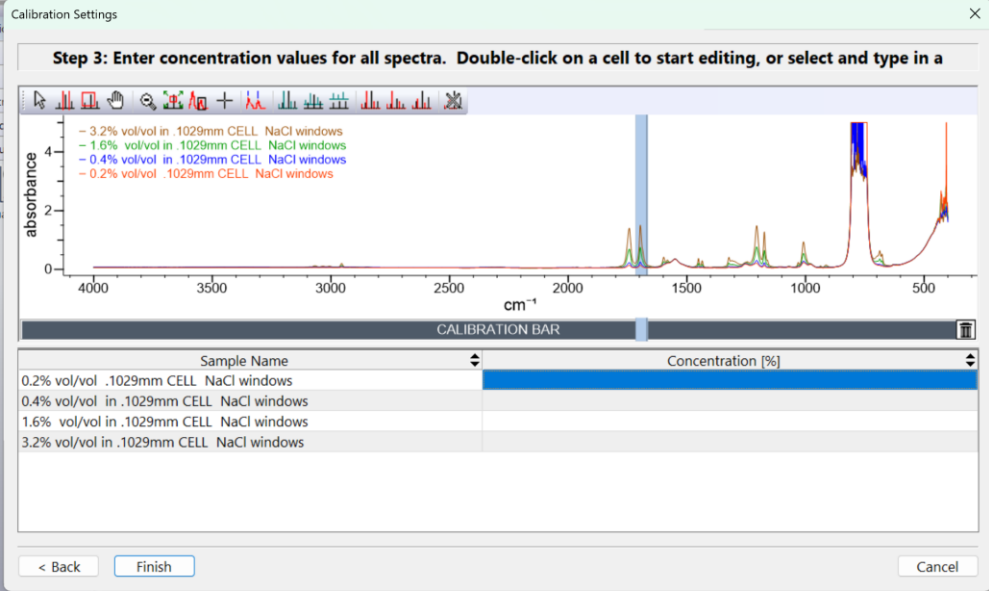
	Action	Result																							
12	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <div data-bbox="247 423 516 496" style="border: 1px solid gray; padding: 5px; text-align: center; margin: 10px 0;"> <p>Create Report</p> </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="919 391 1902 938" style="border: 1px solid gray; padding: 5px;"> <p>Select a Report Template</p> <p>Please select one of these templates:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Title</th> <th>File Path</th> </tr> </thead> <tbody> <tr style="background-color: #e0e0e0;"> <td>External Standard Landscape</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>External Standard Portrait</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Lan...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Por...</td> <td>C:\Users\Public\Documents\...</td> </tr> </tbody> </table> <div style="border: 1px dashed gray; padding: 5px; margin-top: 10px;"> <p>WILEY</p> <p>License Serial Number: 157 Operator:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Sample Name</th> <th>Concentration</th> <th>Area (%)</th> </tr> </thead> <tbody> <tr> <td>Standard</td> <td>100</td> <td>100.00</td> </tr> <tr> <td>Sample</td> <td>100</td> <td>100.00</td> </tr> </tbody> </table>  <p>X Axis region of Calibration peak: 380.617 - 667.294 nm</p> <p>Uncertainty (%): 5</p> <p style="text-align: right;">OK Cancel</p> </div> </div> <p>Upon clicking OK on the Select a Report Template dialog window, the report is generated in ReportIt application.</p>	Title	File Path	External Standard Landscape	C:\Users\Public\Documents\...	External Standard Portrait	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...	Internal Standard GC-MS Por...	C:\Users\Public\Documents\...	Sample Name	Concentration	Area (%)	Standard	100	100.00	Sample	100	100.00
Title	File Path																								
External Standard Landscape	C:\Users\Public\Documents\...																								
External Standard Portrait	C:\Users\Public\Documents\...																								
Internal Standard Chromatog...	C:\Users\Public\Documents\...																								
Internal Standard Chromatog...	C:\Users\Public\Documents\...																								
Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...																								
Internal Standard GC-MS Por...	C:\Users\Public\Documents\...																								
Sample Name	Concentration	Area (%)																							
Standard	100	100.00																							
Sample	100	100.00																							

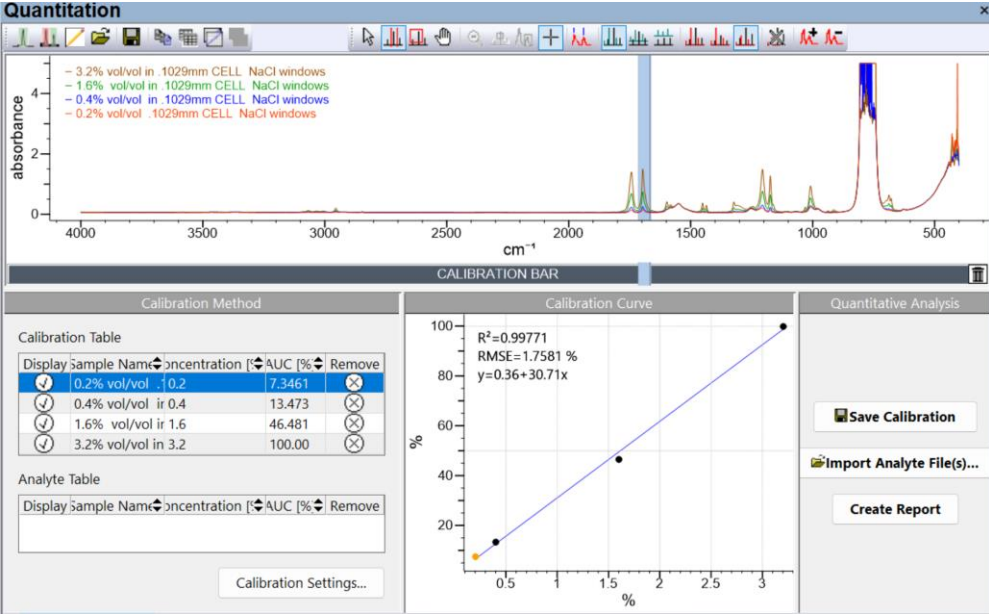
IR

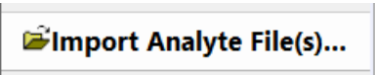
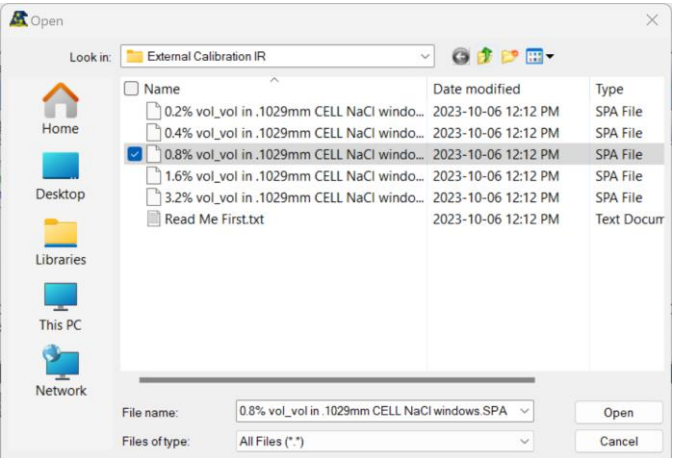
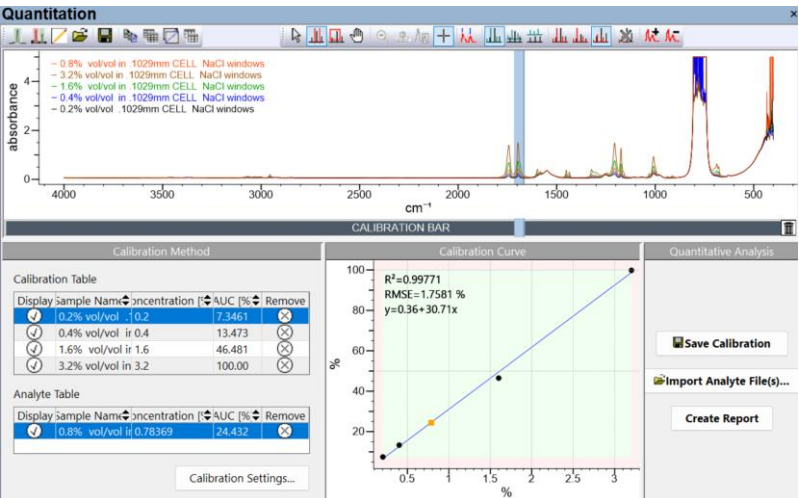
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note:</i> If a previous calibration study is displayed, it can be closed by clicking on the X icon (X) on the top right corner.</p>	<p>Quantitation application is displayed:</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 ~1650 cm^{-1}.</p> <p><i>Note:</i> In IR quantitation, one should avoid using the strongest peak.</p> <p>Click Next > button.</p> <div data-bbox="283 641 472 695" style="border: 1px solid blue; padding: 2px; display: inline-block;">Next ></div>	<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										
<p>6</p>	<p>Click Next > button.</p> <div data-bbox="285 367 447 410" style="border: 1px solid black; padding: 2px; display: inline-block;">Next ></div>	<p>Upon clicking Next button, “Step 3” loads in the popup window:</p> 										
<p>7</p>	<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> <div data-bbox="285 1154 468 1203" style="border: 1px solid black; padding: 2px; display: inline-block;">Finish</div>	<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> <table border="1" data-bbox="940 1078 1688 1224"> <thead> <tr> <th>Sample Name</th> <th>Concentration [%]</th> </tr> </thead> <tbody> <tr> <td>0.2% vol/vol .1029mm CELL NaCl windows</td> <td>0.2</td> </tr> <tr> <td>0.4% vol/vol in .1029mm CELL NaCl windows</td> <td>0.4</td> </tr> <tr> <td>1.6% vol/vol in .1029mm CELL NaCl windows</td> <td>1.6</td> </tr> <tr> <td>3.2% vol/vol in .1029mm CELL NaCl windows</td> <td>3.2</td> </tr> </tbody> </table>	Sample Name	Concentration [%]	0.2% vol/vol .1029mm CELL NaCl windows	0.2	0.4% vol/vol in .1029mm CELL NaCl windows	0.4	1.6% vol/vol in .1029mm CELL NaCl windows	1.6	3.2% vol/vol in .1029mm CELL NaCl windows	3.2
Sample Name	Concentration [%]											
0.2% vol/vol .1029mm CELL NaCl windows	0.2											
0.4% vol/vol in .1029mm CELL NaCl windows	0.4											
1.6% vol/vol in .1029mm CELL NaCl windows	1.6											
3.2% vol/vol in .1029mm CELL NaCl windows	3.2											

	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 interface. At the top, there's a toolbar with various icons. Below it is an IR spectrum plot with 'absorbance' on the y-axis and 'cm⁻¹' on the x-axis. The x-axis ranges from 4000 to 500 cm⁻¹. Several peaks are visible, with a prominent one around 1700 cm⁻¹. Below the spectrum is a 'CALIBRATION BAR'.</p> <p>Below the calibration bar are three panels:</p> <ul style="list-style-type: none"> Calibration Method: Contains a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Concentration', 'AUC', and 'Remove'. The table has four rows: <table border="1"> <thead> <tr> <th>Display</th> <th>Sample Name</th> <th>Concentration</th> <th>AUC</th> <th>Remove</th> </tr> </thead> <tbody> <tr> <td><input checked="" type="checkbox"/></td> <td>0.2% vol/vol in 1029mm CELL. NaCl windows</td> <td>0.2</td> <td>7.3461</td> <td><input type="checkbox"/></td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>0.4% vol/vol in 1029mm CELL. NaCl windows</td> <td>0.4</td> <td>13.473</td> <td><input type="checkbox"/></td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>1.6% vol/vol in 1029mm CELL. NaCl windows</td> <td>1.6</td> <td>46.481</td> <td><input type="checkbox"/></td> </tr> <tr> <td><input checked="" type="checkbox"/></td> <td>3.2% vol/vol in 1029mm CELL. NaCl windows</td> <td>3.2</td> <td>100.00</td> <td><input type="checkbox"/></td> </tr> </tbody> </table> Calibration Curve: Shows a scatter plot of 'AUC' vs 'Concentration (%)' with a linear regression line. The statistics shown are R²=0.99771 and RMSE=1.7581%. The equation of the line is y=0.36+30.71x. Quantitative Analysis: Contains buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'. There is also a 'Calibration Settings...' button at the bottom of this panel. 	Display	Sample Name	Concentration	AUC	Remove	<input checked="" type="checkbox"/>	0.2% vol/vol in 1029mm CELL. NaCl windows	0.2	7.3461	<input type="checkbox"/>	<input checked="" type="checkbox"/>	0.4% vol/vol in 1029mm CELL. NaCl windows	0.4	13.473	<input type="checkbox"/>	<input checked="" type="checkbox"/>	1.6% vol/vol in 1029mm CELL. NaCl windows	1.6	46.481	<input type="checkbox"/>	<input checked="" type="checkbox"/>	3.2% vol/vol in 1029mm CELL. NaCl windows	3.2	100.00	<input type="checkbox"/>
Display	Sample Name	Concentration	AUC	Remove																							
<input checked="" type="checkbox"/>	0.2% vol/vol in 1029mm CELL. NaCl windows	0.2	7.3461	<input type="checkbox"/>																							
<input checked="" type="checkbox"/>	0.4% vol/vol in 1029mm CELL. NaCl windows	0.4	13.473	<input type="checkbox"/>																							
<input checked="" type="checkbox"/>	1.6% vol/vol in 1029mm CELL. NaCl windows	1.6	46.481	<input type="checkbox"/>																							
<input checked="" type="checkbox"/>	3.2% vol/vol in 1029mm CELL. NaCl windows	3.2	100.00	<input type="checkbox"/>																							

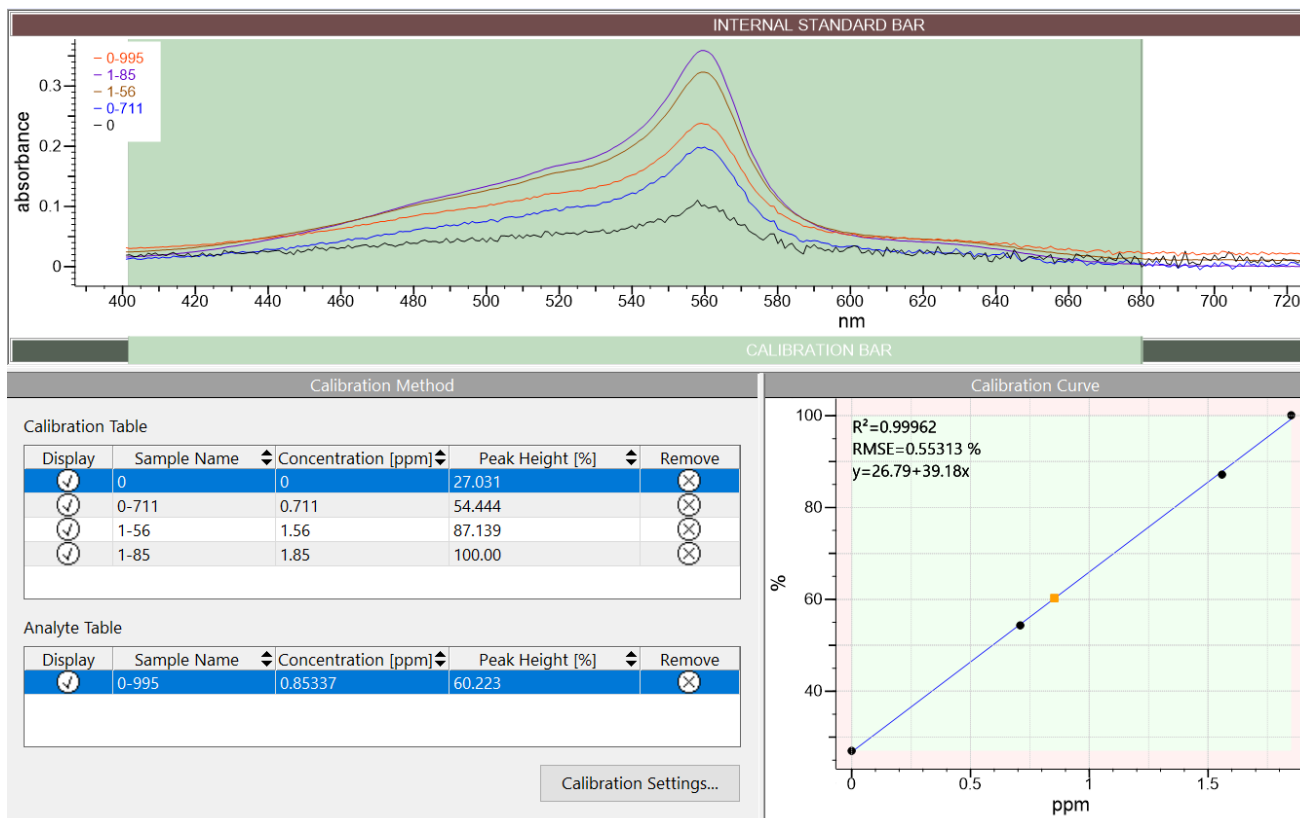
	Action	Result																		
<p>9</p>	<p>Click the Import Analyte File(s) button in the Quantitative Analysis panel.</p>  <p>Navigate to and select the file that was excluded in Step 3 "0.8%", located "C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR".</p> <p>Click Open.</p>	<p>The Open file dialog window is shown. Upon clicking OK, the imported file opens in the previous calibration window.</p> 																		
<p>10</p>	<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>  <p>The software interface displays the following data:</p> <table border="1"> <thead> <tr> <th>Sample Name</th> <th>Concentration (%)</th> <th>AUC (%)</th> </tr> </thead> <tbody> <tr> <td>0.2% vol/vol</td> <td>0.2</td> <td>7.3461</td> </tr> <tr> <td>0.4% vol/vol</td> <td>0.4</td> <td>13.473</td> </tr> <tr> <td>1.6% vol/vol</td> <td>1.6</td> <td>46.481</td> </tr> <tr> <td>3.2% vol/vol</td> <td>3.2</td> <td>100.00</td> </tr> <tr> <td>0.8% vol/vol (Unknown)</td> <td>0.78369</td> <td>24.432</td> </tr> </tbody> </table> <p>The Calibration Curve shows a linear relationship with the equation $y = 0.36 + 30.71x$ and $R^2 = 0.99771$. The RMSE is 1.7581%.</p>	Sample Name	Concentration (%)	AUC (%)	0.2% vol/vol	0.2	7.3461	0.4% vol/vol	0.4	13.473	1.6% vol/vol	1.6	46.481	3.2% vol/vol	3.2	100.00	0.8% vol/vol (Unknown)	0.78369	24.432
Sample Name	Concentration (%)	AUC (%)																		
0.2% vol/vol	0.2	7.3461																		
0.4% vol/vol	0.4	13.473																		
1.6% vol/vol	1.6	46.481																		
3.2% vol/vol	3.2	100.00																		
0.8% vol/vol (Unknown)	0.78369	24.432																		

	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> <div data-bbox="281 428 506 488" style="border: 1px solid gray; padding: 5px; width: fit-content; margin: 10px auto;"> <p>Create Report</p> </div> <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> <div data-bbox="940 391 1923 938" style="border: 1px solid gray; padding: 10px;"> <p>The screenshot shows a dialog box titled "Select a Report Template". It contains a table with the following data:</p> <table border="1"> <thead> <tr> <th>Title</th> <th>File Path</th> </tr> </thead> <tbody> <tr> <td>External Standard Landscape</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>External Standard Portrait</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Lan...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Por...</td> <td>C:\Users\Public\Documents\...</td> </tr> </tbody> </table> <p>Below the table is a preview of a report. The report includes a calibration curve with the following statistics:</p> <ul style="list-style-type: none"> $R^2 = 0.99771$ RMSE = 1.7581 % Equation: $y = 0.36 + 30.71x$ <p>The report also shows a table with columns for "Sample Name", "Concentration (ng)", and "Area (AU)".</p> </div> <p>Upon clicking OK on the Select a Report Template dialog window, the report is generated in ReportIt application.</p>	Title	File Path	External Standard Landscape	C:\Users\Public\Documents\...	External Standard Portrait	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...	Internal Standard GC-MS Por...	C:\Users\Public\Documents\...
Title	File Path															
External Standard Landscape	C:\Users\Public\Documents\...															
External Standard Portrait	C:\Users\Public\Documents\...															
Internal Standard Chromatog...	C:\Users\Public\Documents\...															
Internal Standard Chromatog...	C:\Users\Public\Documents\...															
Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...															
Internal Standard GC-MS Por...	C:\Users\Public\Documents\...															

Standard Addition Quantitation

Perform Standard Addition Quantitation

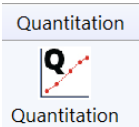

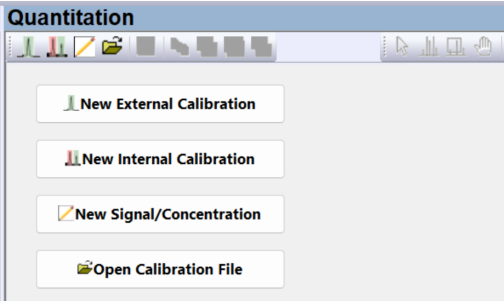

This screenshot shows a Standard Addition result, where when the added concentration is 0, the Y-axis value of 26.79 is the signal (due to iron in this case) in the original unknown sample:

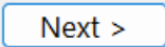
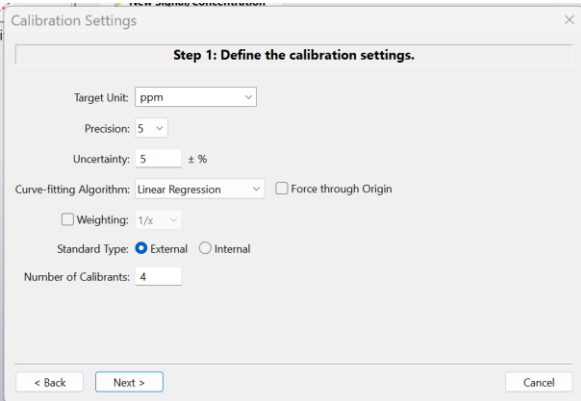
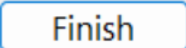
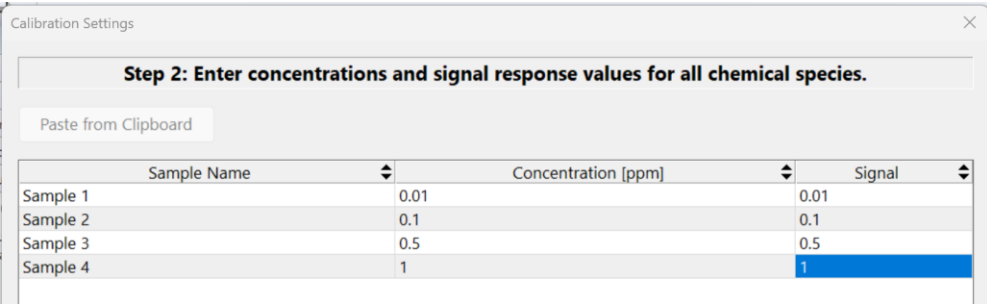


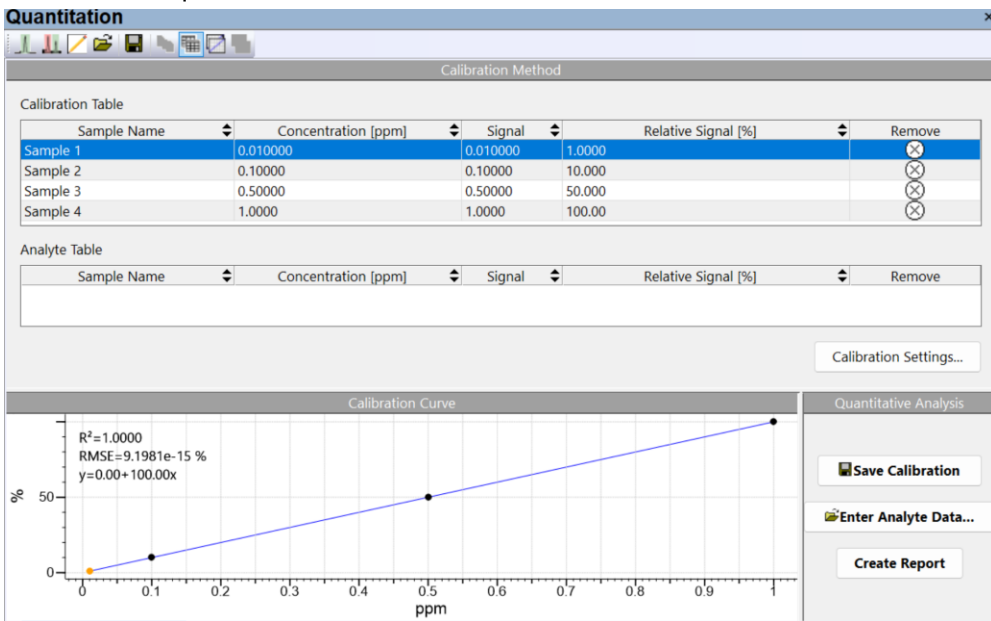
Directly Enter Signal Concentration Data for Quantitation

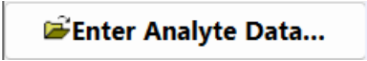
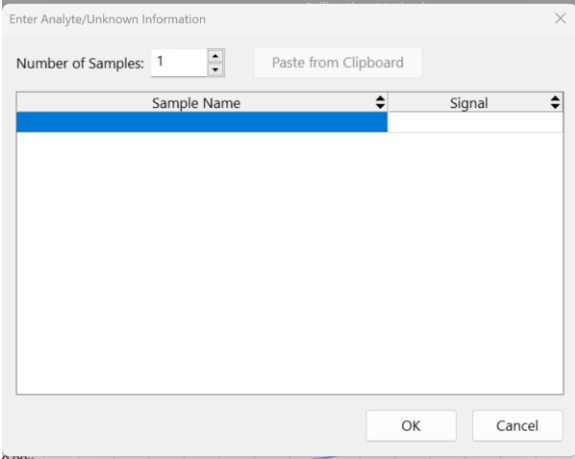
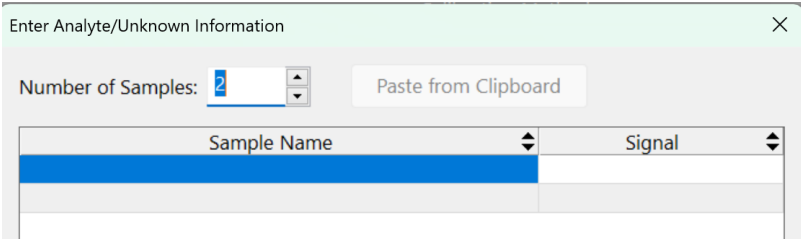
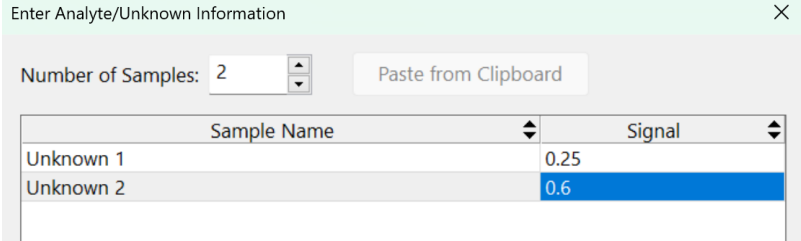
Directly Enter Signal Concentration Pairs to Create Calibration

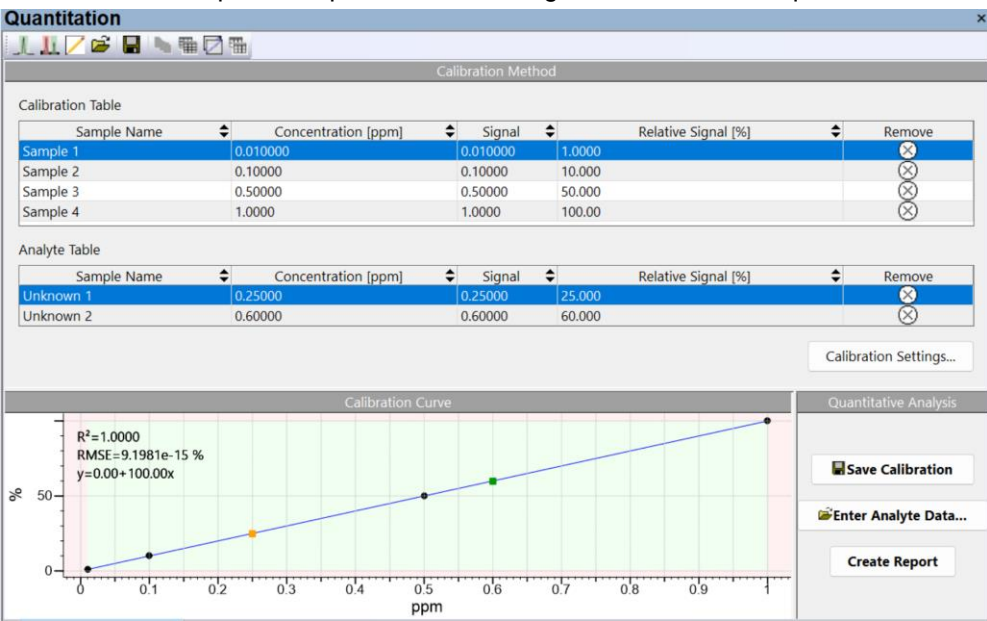
This is a new feature in **KnowItAll 2025**, which can be used to manually enter calibration values by importing calibrant and unknown information from reports.

	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.	<p>A calibration equation is created.</p>  <p>The screenshot displays the 'Quantitation' window with the following components:</p> <ul style="list-style-type: none"> Calibration Table: <table border="1"> <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> Analyte Table: <table border="1"> <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> Calibration Curve: <p>Graph showing Relative Signal [%] vs Concentration [ppm].</p> <p>Equation: $y = 0.00 + 100.00x$</p> <p>Statistics: $R^2 = 1.0000$, $RMSE = 9.1981e-15 \%$</p> Quantitative Analysis Panel: <ul style="list-style-type: none"> Calibration Settings... Save Calibration Enter Analyte Data... Create Report 	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					
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																																	

	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>  <p>The screenshot displays the 'Quantitation' window with the following data:</p> <table border="1" data-bbox="909 467 1877 574"> <caption>Calibration Table</caption> <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> <table border="1" data-bbox="909 591 1877 678"> <caption>Analyte Table</caption> <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> <p>The Calibration Curve graph shows a linear relationship with the equation $y = 0.00 + 100.00x$, $R^2 = 1.0000$, and $RMSE = 9.1981e-15 \%$. The x-axis is labeled 'ppm' and the y-axis is labeled 'a.u.'.</p> <p>The Quantitative Analysis panel includes buttons for 'Save Calibration', 'Enter Analyte Data...', and 'Create Report'.</p>	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	⊗
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	⊗																																						
10	<p>As described in previous sections, a report can be generated by clicking Create Report button.</p> <p>Create Report</p>																																									

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



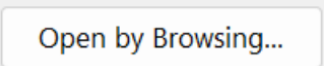
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\
Quantitation folder

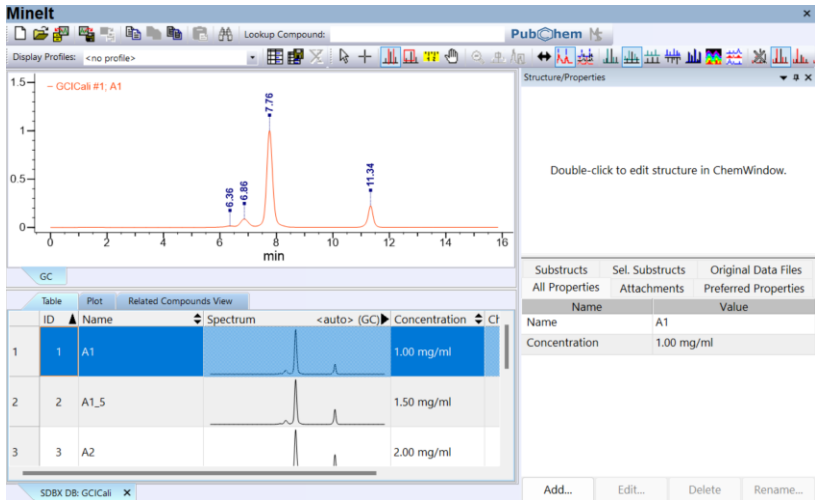
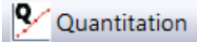
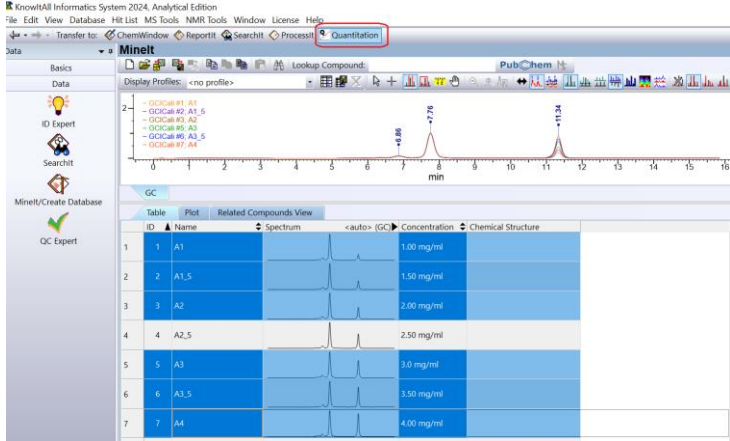
- Internal Calibration Chromatogram

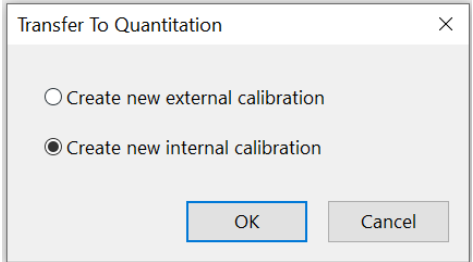
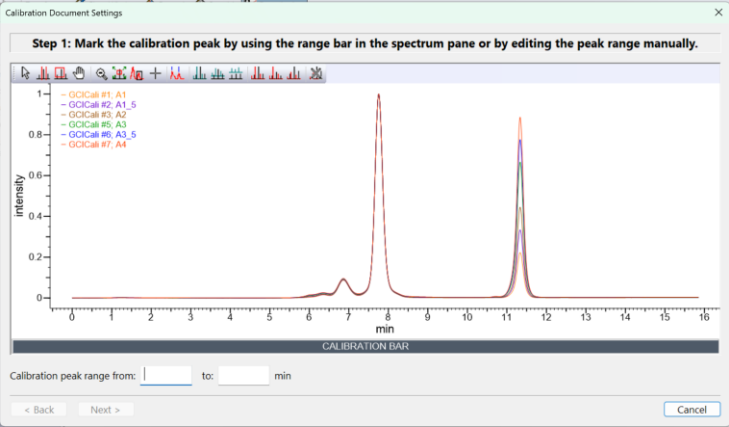
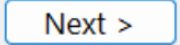
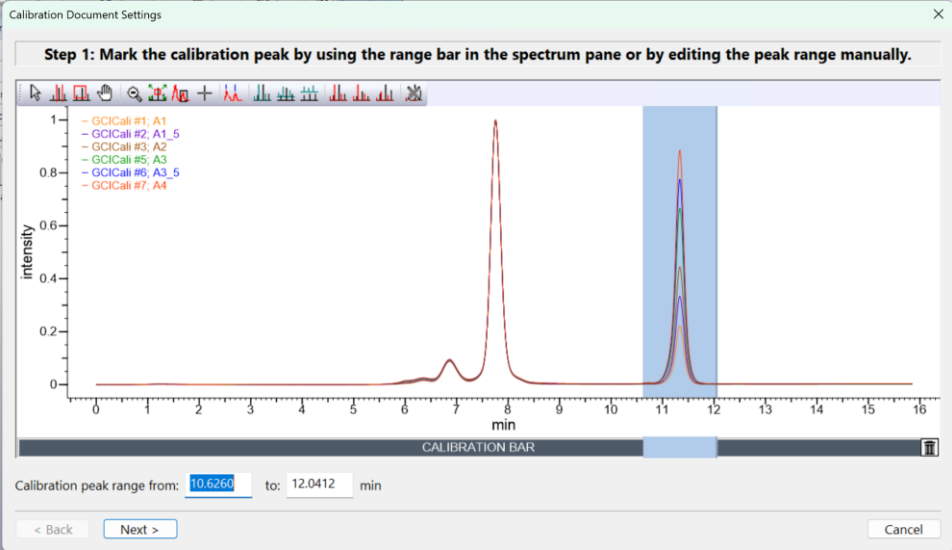
KnowItAll Applications Used

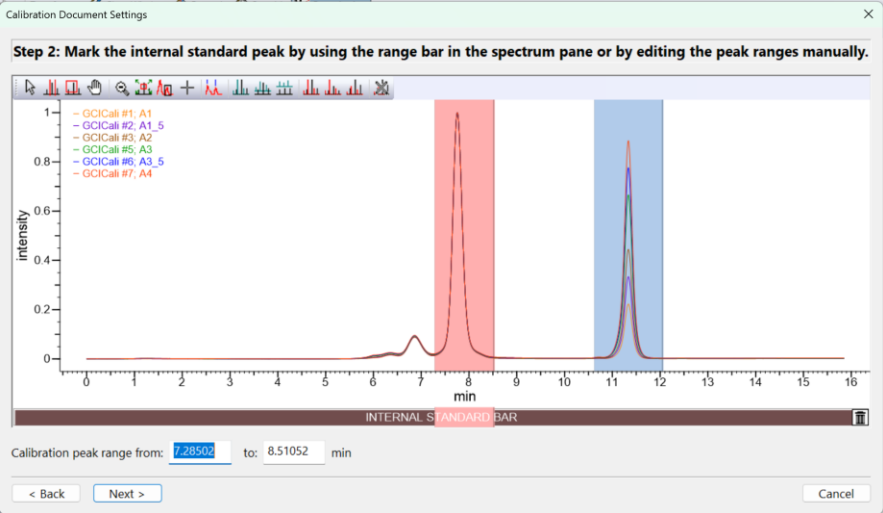
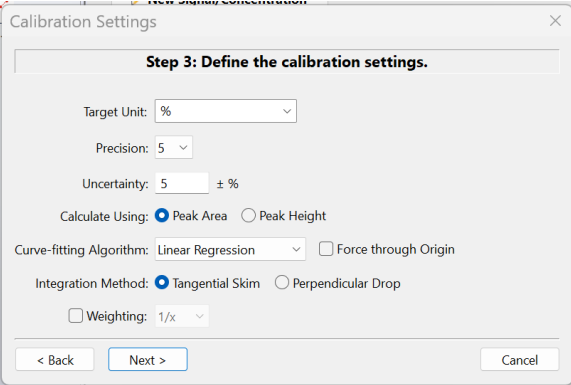
- Quantitation

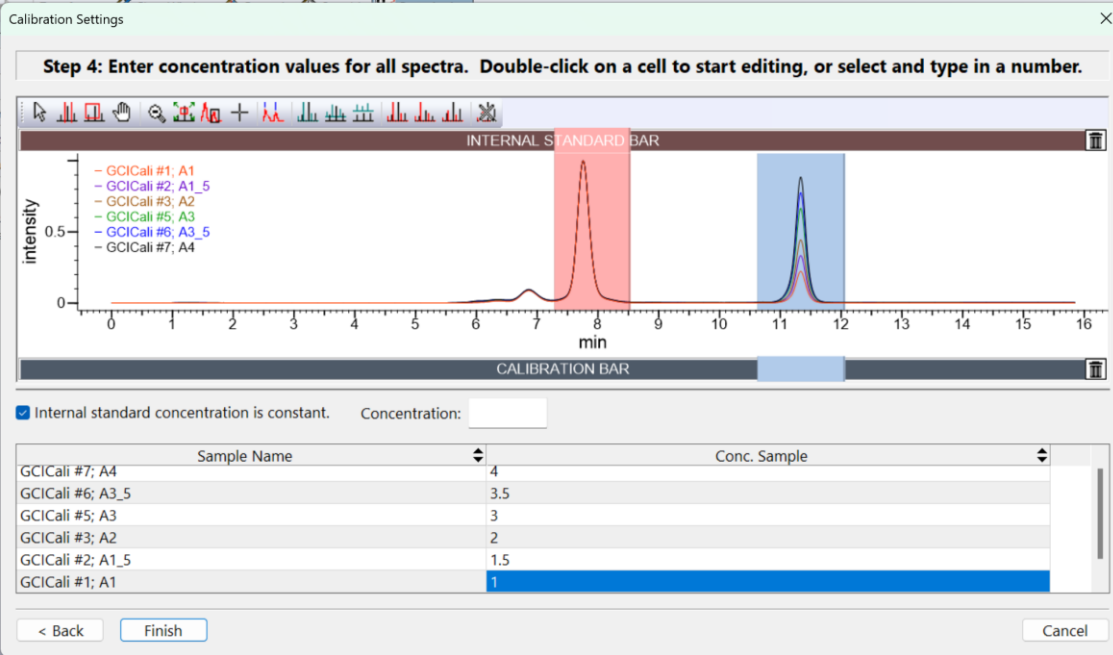
Chromatogram

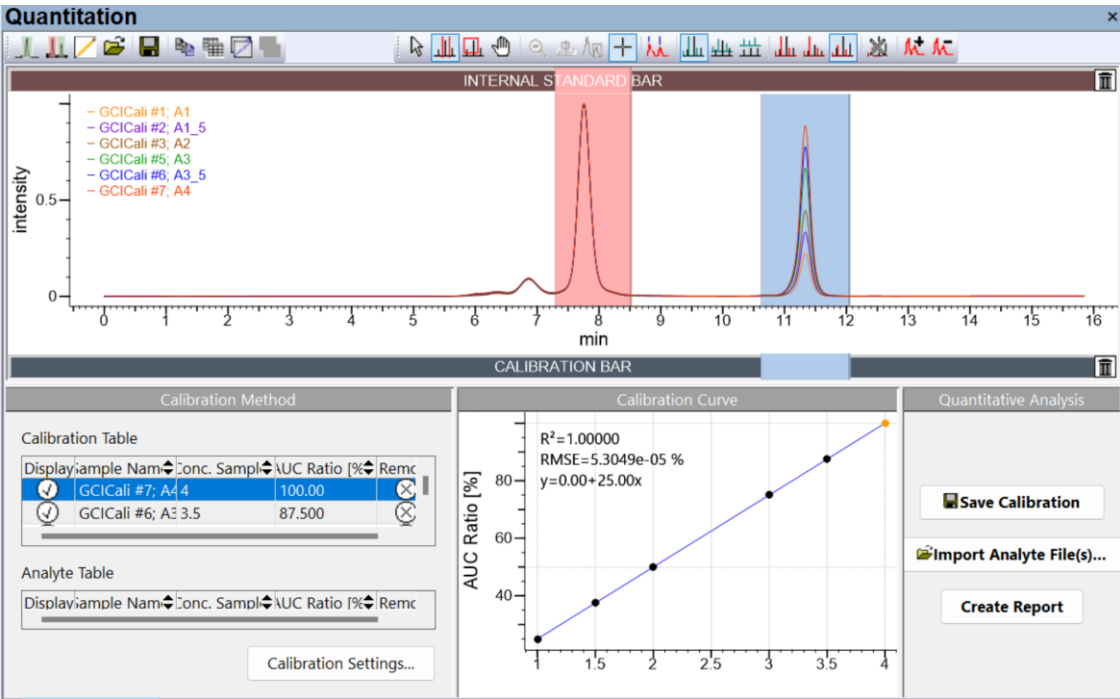
	Action	Result
1	Clear any calibration study presently open in Quantitation application by clicking on the X icon () on the top right corner.	
2	Open the Minelt application by clicking its icon, typically found in the Data group.  Minelt/Create Database Choose Database > Open .	The Select a Database popup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

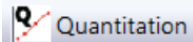
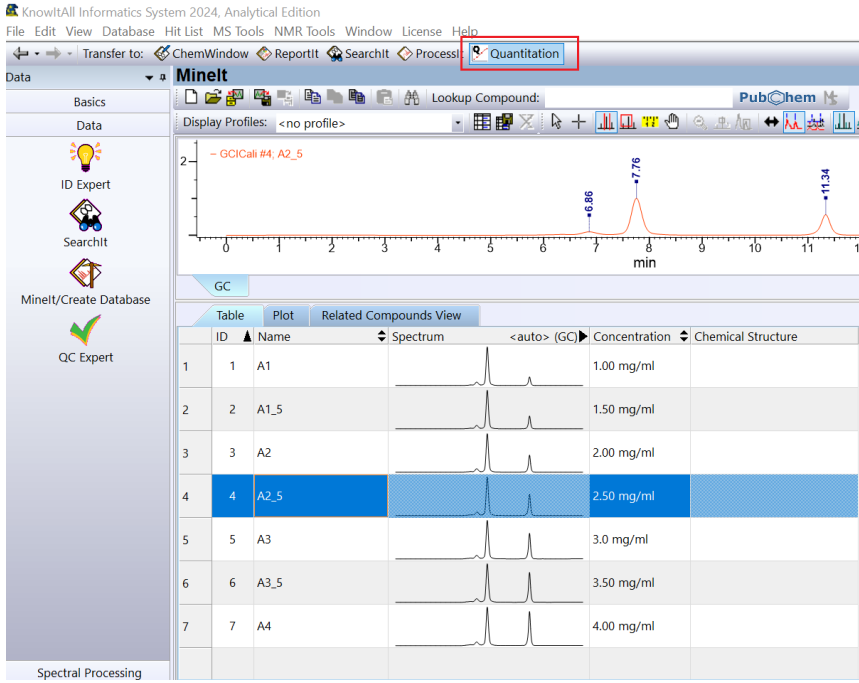
	Action	Result																																								
4	<p>Navigate to the “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration Chromatogram” folder.</p> <p>Select the Chromatograms For Internal Calibration Demo.sdbx file.</p> <p>Click Open.</p>	<p>The database file opens in Minelt:</p>  <p>The screenshot shows the Minelt interface with a chromatogram plot and a table of records. The table is as follows:</p> <table border="1"> <thead> <tr> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>Concentration</th> <th>CF</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>A1</td> <td>[Spectrum]</td> <td>1.00 mg/ml</td> <td></td> </tr> <tr> <td>2</td> <td>A1_5</td> <td>[Spectrum]</td> <td>1.50 mg/ml</td> <td></td> </tr> <tr> <td>3</td> <td>A2</td> <td>[Spectrum]</td> <td>2.00 mg/ml</td> <td></td> </tr> </tbody> </table>	ID	Name	Spectrum	Concentration	CF	1	A1	[Spectrum]	1.00 mg/ml		2	A1_5	[Spectrum]	1.50 mg/ml		3	A2	[Spectrum]	2.00 mg/ml																					
ID	Name	Spectrum	Concentration	CF																																						
1	A1	[Spectrum]	1.00 mg/ml																																							
2	A1_5	[Spectrum]	1.50 mg/ml																																							
3	A2	[Spectrum]	2.00 mg/ml																																							
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>The screenshot shows the Minelt interface with a series of selected records in a table. The table is as follows:</p> <table border="1"> <thead> <tr> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>Concentration</th> <th>Chemical Structure</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>A1</td> <td>[Spectrum]</td> <td>1.00 mg/ml</td> <td></td> </tr> <tr> <td>2</td> <td>A1_5</td> <td>[Spectrum]</td> <td>1.50 mg/ml</td> <td></td> </tr> <tr> <td>3</td> <td>A2</td> <td>[Spectrum]</td> <td>2.00 mg/ml</td> <td></td> </tr> <tr> <td>4</td> <td>A2_5</td> <td>[Spectrum]</td> <td>2.50 mg/ml</td> <td></td> </tr> <tr> <td>5</td> <td>A3</td> <td>[Spectrum]</td> <td>3.0 mg/ml</td> <td></td> </tr> <tr> <td>6</td> <td>A3_5</td> <td>[Spectrum]</td> <td>3.50 mg/ml</td> <td></td> </tr> <tr> <td>7</td> <td>A4</td> <td>[Spectrum]</td> <td>4.00 mg/ml</td> <td></td> </tr> </tbody> </table> <p>Upon using the Transfer To button, KnowItAll loads Quantitation application with a Transfer to Quantitation popup window.</p>	ID	Name	Spectrum	Concentration	Chemical Structure	1	A1	[Spectrum]	1.00 mg/ml		2	A1_5	[Spectrum]	1.50 mg/ml		3	A2	[Spectrum]	2.00 mg/ml		4	A2_5	[Spectrum]	2.50 mg/ml		5	A3	[Spectrum]	3.0 mg/ml		6	A3_5	[Spectrum]	3.50 mg/ml		7	A4	[Spectrum]	4.00 mg/ml	
ID	Name	Spectrum	Concentration	Chemical Structure																																						
1	A1	[Spectrum]	1.00 mg/ml																																							
2	A1_5	[Spectrum]	1.50 mg/ml																																							
3	A2	[Spectrum]	2.00 mg/ml																																							
4	A2_5	[Spectrum]	2.50 mg/ml																																							
5	A3	[Spectrum]	3.0 mg/ml																																							
6	A3_5	[Spectrum]	3.50 mg/ml																																							
7	A4	[Spectrum]	4.00 mg/ml																																							

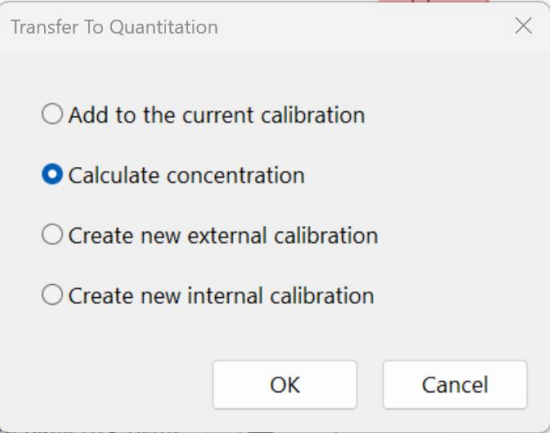
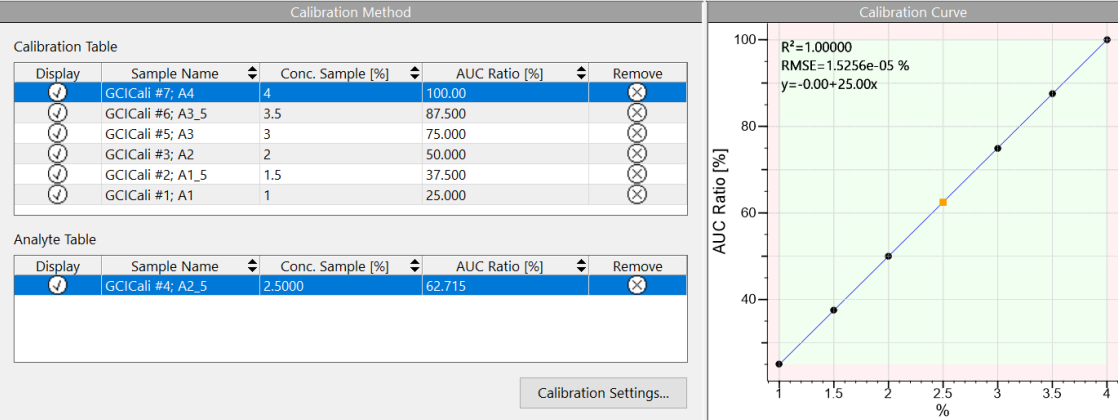
	Action	Result
6	<p>On the Transfer to Quantitation popup window, select Create new internal calibration at the prompt window.</p> <p>Click OK.</p> 	<p>The chromatograms load in the Calibration Document Settings popup window:</p> 
7	<p>Select peak region around 11.3 min as the calibrant peak by clicking 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> 

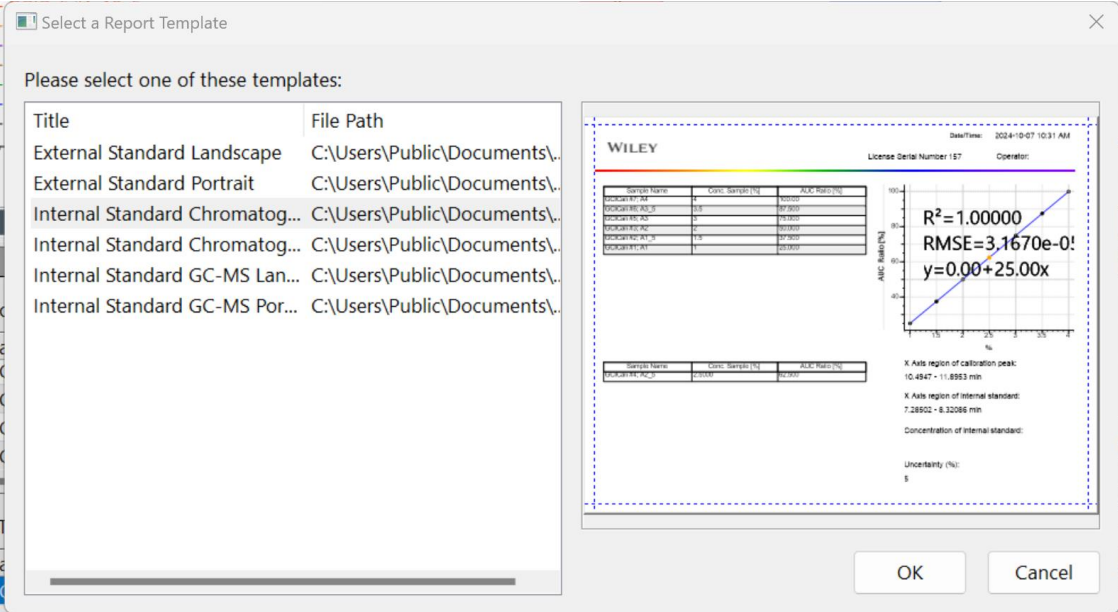
	Action	Result
8	<p>Select peak region around 7.8 min as the internal standard peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 7.3 – 8.5 min).</p> <p>Click Next > button.</p> <div data-bbox="254 565 428 613" style="border: 1px solid blue; padding: 2px; display: inline-block;">Next ></div>	<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> <div data-bbox="254 1190 428 1239" style="border: 1px solid blue; padding: 2px; display: inline-block;">Next ></div>	<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>  <p>Calibration Settings</p> <p>Step 4: Enter concentration values for all spectra. Double-click on a cell to start editing, or select and type in a number.</p> <p>INTERNAL STANDARD BAR</p> <p>intensity</p> <p>min</p> <p>CALIBRATION BAR</p> <p><input checked="" type="checkbox"/> Internal standard concentration is constant. Concentration: <input type="text"/></p> <table border="1"> <thead> <tr> <th>Sample Name</th> <th>Conc. Sample</th> </tr> </thead> <tbody> <tr> <td>GCCali #7; A4</td> <td>4</td> </tr> <tr> <td>GCCali #6; A3_5</td> <td>3.5</td> </tr> <tr> <td>GCCali #5; A3</td> <td>3</td> </tr> <tr> <td>GCCali #3; A2</td> <td>2</td> </tr> <tr> <td>GCCali #2; A1_5</td> <td>1.5</td> </tr> <tr> <td>GCCali #1; A1</td> <td>1</td> </tr> </tbody> </table> <p>< Back Finish Cancel</p>	Sample Name	Conc. Sample	GCCali #7; A4	4	GCCali #6; A3_5	3.5	GCCali #5; A3	3	GCCali #3; A2	2	GCCali #2; A1_5	1.5	GCCali #1; A1	1
Sample Name	Conc. Sample															
GCCali #7; A4	4															
GCCali #6; A3_5	3.5															
GCCali #5; A3	3															
GCCali #3; A2	2															
GCCali #2; A1_5	1.5															
GCCali #1; A1	1															

	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, there is a toolbar with various icons. Below it is a chromatogram titled 'INTERNAL STANDARD BAR' showing intensity versus time (min). The x-axis ranges from 0 to 16 minutes, and the y-axis represents intensity from 0 to 0.5. Several peaks are visible, with two prominent peaks highlighted by vertical bars: a red bar at approximately 8.5 minutes and a blue bar at approximately 11.5 minutes. Below the chromatogram is a 'CALIBRATION BAR'.</p> <p>The interface is divided into three main sections:</p> <ul style="list-style-type: none"> Calibration Method: Contains a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Conc. Sample', 'AUC Ratio [%]', and 'Remc'. Two entries are listed: 'GCIcali #7; A4 4' with an AUC Ratio of 100.00, and 'GCIcali #6; A3 3.5' with an AUC Ratio of 87.500. Below this is an 'Analyte Table' with similar columns. Calibration Curve: Shows a linear plot of 'AUC Ratio [%]' versus concentration. The regression equation is $y = 0.00 + 25.00x$ and the statistics are $R^2 = 1.00000$ and $RMSE = 5.3049e-05 \%$. Quantitative Analysis: Contains three buttons: 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.

	Action	Result																																								
12	<p>Return to Minelt application.</p> <p>Click to select the file that was left out, <i>i.e.</i>, A2_5.</p> <p>Select Transfer To: Quantitation.</p> 	<p>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.</p>  <table border="1" data-bbox="945 730 1625 1102"> <thead> <tr> <th>ID</th> <th>Name</th> <th>Spectrum</th> <th>Concentration</th> <th>Chemical Structure</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>A1</td> <td></td> <td>1.00 mg/ml</td> <td></td> </tr> <tr> <td>2</td> <td>A1_5</td> <td></td> <td>1.50 mg/ml</td> <td></td> </tr> <tr> <td>3</td> <td>A2</td> <td></td> <td>2.00 mg/ml</td> <td></td> </tr> <tr style="background-color: #4F81BD; color: white;"> <td>4</td> <td>A2_5</td> <td></td> <td>2.50 mg/ml</td> <td></td> </tr> <tr> <td>5</td> <td>A3</td> <td></td> <td>3.00 mg/ml</td> <td></td> </tr> <tr> <td>6</td> <td>A3_5</td> <td></td> <td>3.50 mg/ml</td> <td></td> </tr> <tr> <td>7</td> <td>A4</td> <td></td> <td>4.00 mg/ml</td> <td></td> </tr> </tbody> </table>	ID	Name	Spectrum	Concentration	Chemical Structure	1	A1		1.00 mg/ml		2	A1_5		1.50 mg/ml		3	A2		2.00 mg/ml		4	A2_5		2.50 mg/ml		5	A3		3.00 mg/ml		6	A3_5		3.50 mg/ml		7	A4		4.00 mg/ml	
ID	Name	Spectrum	Concentration	Chemical Structure																																						
1	A1		1.00 mg/ml																																							
2	A1_5		1.50 mg/ml																																							
3	A2		2.00 mg/ml																																							
4	A2_5		2.50 mg/ml																																							
5	A3		3.00 mg/ml																																							
6	A3_5		3.50 mg/ml																																							
7	A4		4.00 mg/ml																																							

	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>  <p>Calibration Table</p> <table border="1"> <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 border="1"> <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 Curve</p> <p>Graph showing AUC Ratio [%] vs. Concentration [%].</p> <p>Equation: $y = -0.00 + 25.00x$</p> <p>Statistics: $R^2 = 1.00000$, $RMSE = 1.5256e-05 \%$</p>	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																																											
<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"/>																																											

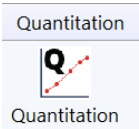

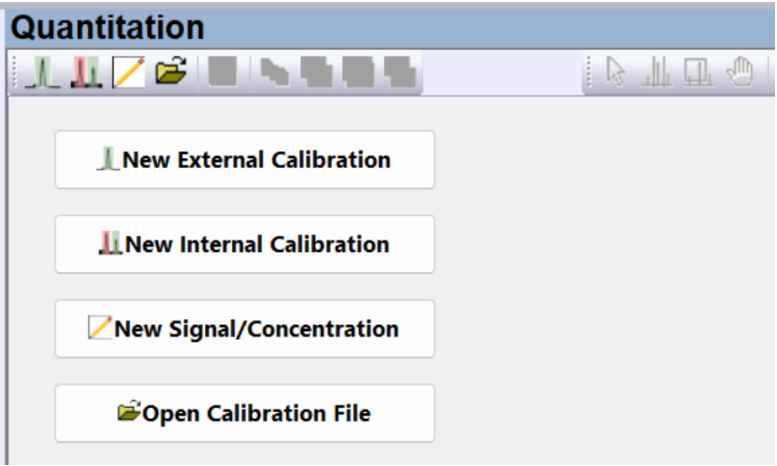
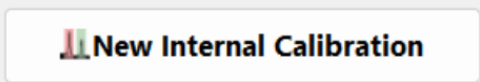
	Action	Result																																
15	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <div data-bbox="247 456 474 516" style="border: 1px solid gray; padding: 5px; width: fit-content; margin: 10px auto;"> <p style="text-align: center;">Create Report</p> </div> <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>  <p>The screenshot shows the 'Select a Report Template' dialog box with the following table of templates:</p> <table border="1" data-bbox="800 527 1333 738"> <thead> <tr> <th>Title</th> <th>File Path</th> </tr> </thead> <tbody> <tr> <td>External Standard Landscape</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>External Standard Portrait</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Lan...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Por...</td> <td>C:\Users\Public\Documents\...</td> </tr> </tbody> </table> <p>The report preview includes a table of sample data:</p> <table border="1" data-bbox="1375 609 1648 673"> <thead> <tr> <th>Sample Name</th> <th>Conc. Sample (ng)</th> <th>AUC (Response)</th> </tr> </thead> <tbody> <tr> <td>DOCAN101-101</td> <td>0.5</td> <td>12.5000</td> </tr> <tr> <td>DOCAN101-102</td> <td>1</td> <td>25.0000</td> </tr> <tr> <td>DOCAN101-103</td> <td>2</td> <td>50.0000</td> </tr> <tr> <td>DOCAN101-104</td> <td>4</td> <td>100.0000</td> </tr> <tr> <td>DOCAN101-105</td> <td>8</td> <td>200.0000</td> </tr> </tbody> </table> <p>The graph shows a linear calibration curve with the equation $R^2 = 1.00000$ and $y = 0.00 + 25.00x$. The X-axis is labeled 'Conc. (ng)' and the Y-axis is labeled 'AUC (Response)'.</p>	Title	File Path	External Standard Landscape	C:\Users\Public\Documents\...	External Standard Portrait	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...	Internal Standard GC-MS Por...	C:\Users\Public\Documents\...	Sample Name	Conc. Sample (ng)	AUC (Response)	DOCAN101-101	0.5	12.5000	DOCAN101-102	1	25.0000	DOCAN101-103	2	50.0000	DOCAN101-104	4	100.0000	DOCAN101-105	8	200.0000
Title	File Path																																	
External Standard Landscape	C:\Users\Public\Documents\...																																	
External Standard Portrait	C:\Users\Public\Documents\...																																	
Internal Standard Chromatog...	C:\Users\Public\Documents\...																																	
Internal Standard Chromatog...	C:\Users\Public\Documents\...																																	
Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...																																	
Internal Standard GC-MS Por...	C:\Users\Public\Documents\...																																	
Sample Name	Conc. Sample (ng)	AUC (Response)																																
DOCAN101-101	0.5	12.5000																																
DOCAN101-102	1	25.0000																																
DOCAN101-103	2	50.0000																																
DOCAN101-104	4	100.0000																																
DOCAN101-105	8	200.0000																																

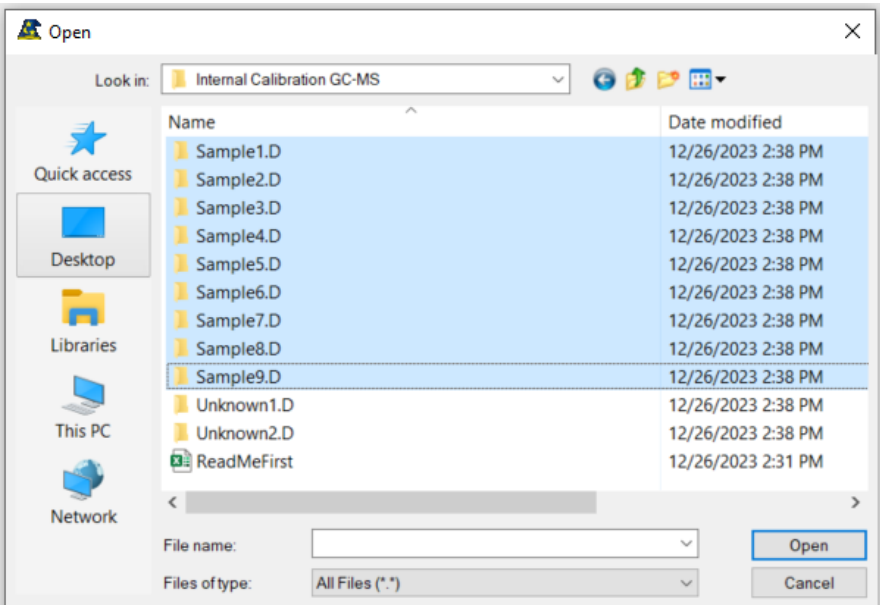
GC-MS

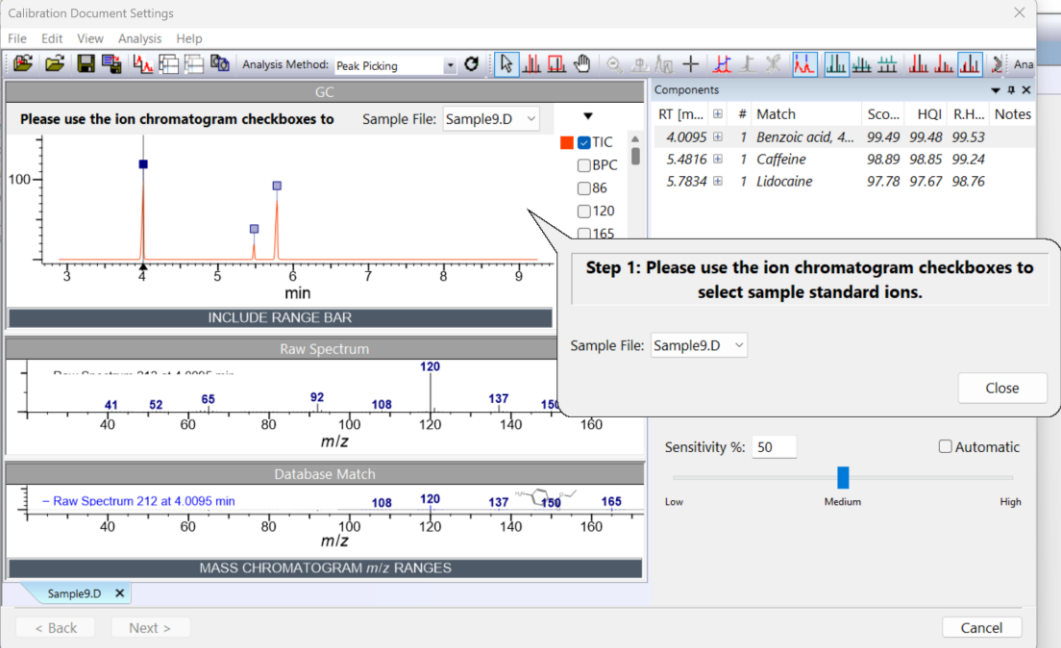
This dataset contains:


- 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.

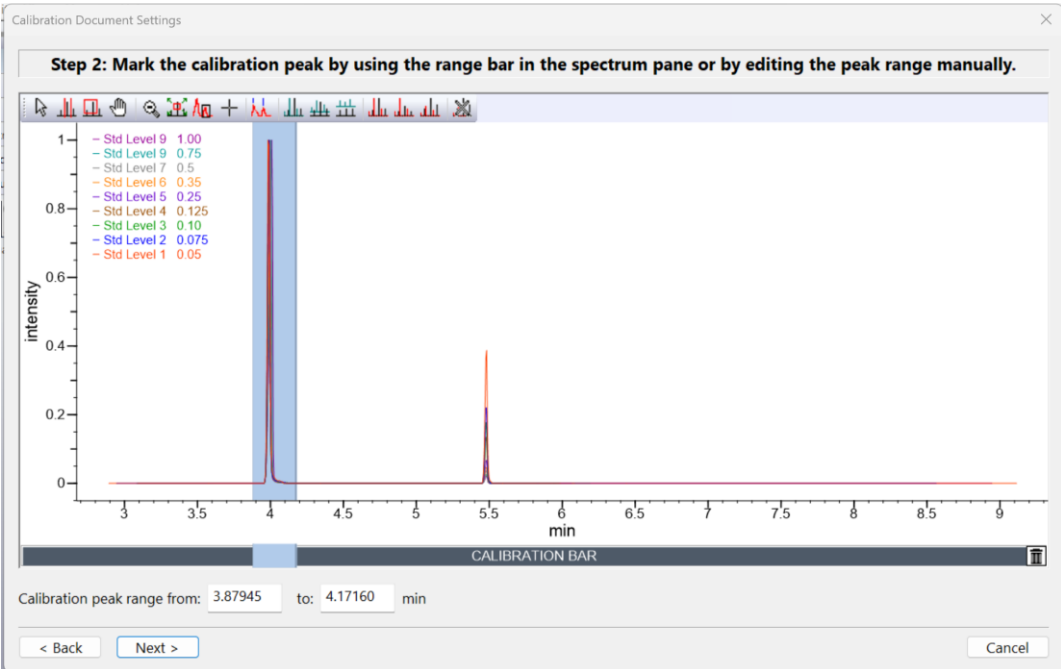
Benzocaine

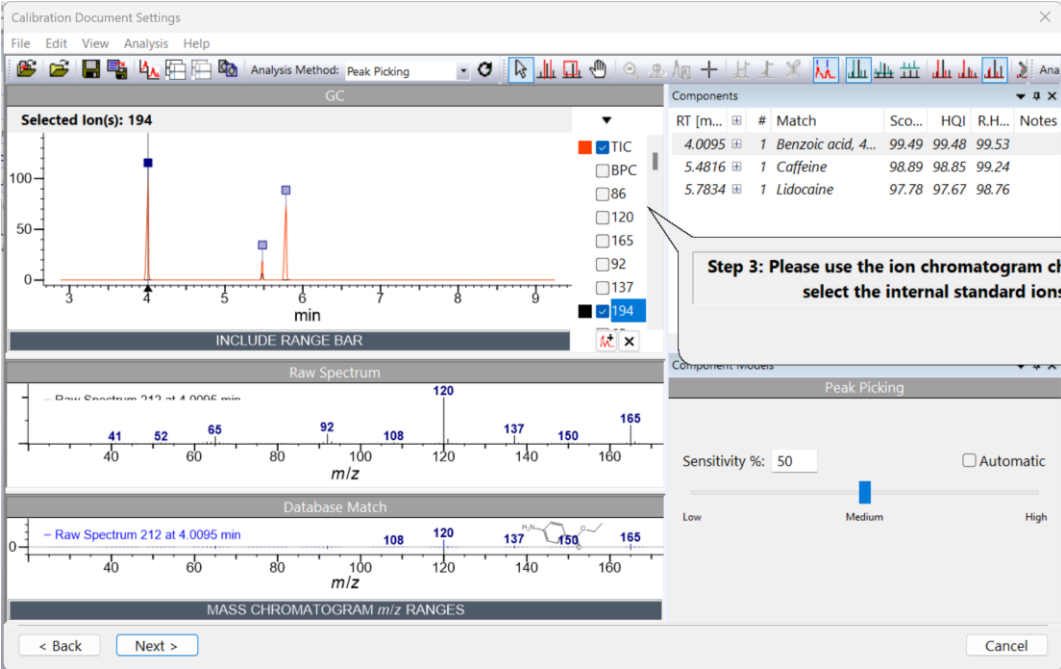
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note: If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.</i></p>	<p>Quantitation application is displayed: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

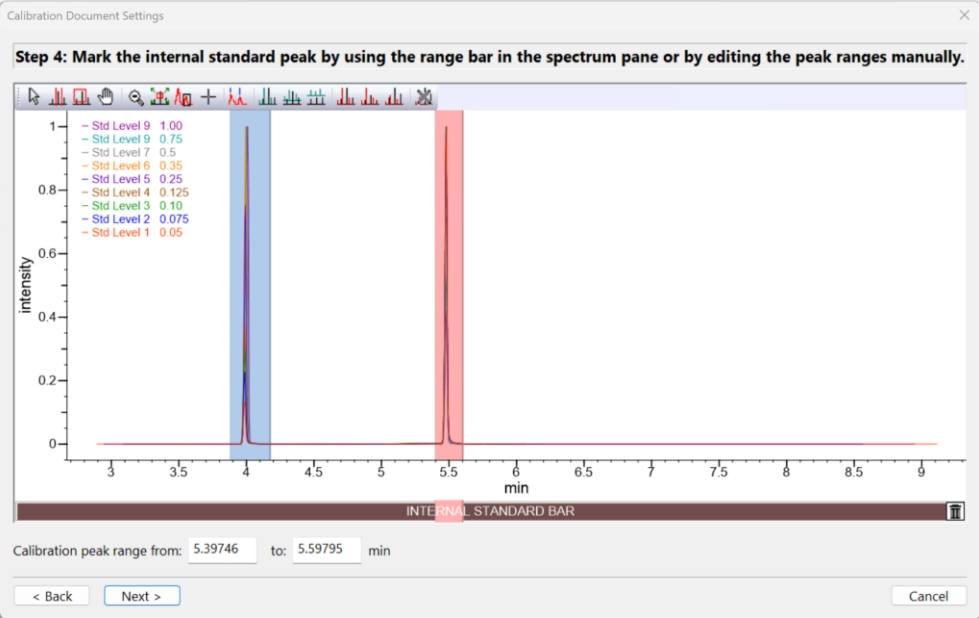
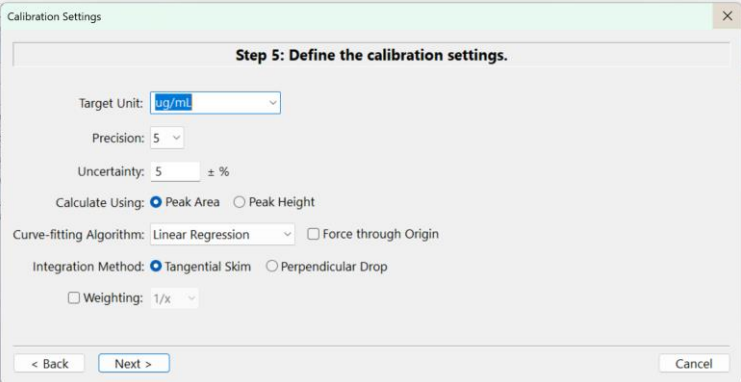
	Action	Result
3	<p>Navigate to “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS” folder.</p> <p>Select all “Sample_.D” folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files.</p> <p>Click Open.</p>	<p>All 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 data-bbox="247 623 793 813" style="border: 1px solid gray; padding: 5px; margin-top: 10px;"> <p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p> <p>Sample File: Sample9.D</p> <p style="text-align: right;">Close</p> </div>	<p>The chromatogram with the largest concentration, i.e., Sample9.D., is used to select the component peak.</p>  <p>The screenshot shows the 'Calibration Document Settings' window for 'Sample9.D'. It features a Total Ion Chromatogram (TIC) with peaks at approximately 4, 5.5, and 6 minutes. Below the chromatogram is a 'Raw Spectrum' plot showing intensity versus m/z, with major peaks at 120, 137, and 154. A 'Database Match' section shows a match for 'Benzoic acid, 4...' at 4.0095 minutes. A callout bubble points to the 'Step 1' instruction: 'Please use the ion chromatogram checkboxes to select sample standard ions.' The bubble also shows the 'Sample File' dropdown set to 'Sample9.D' and a 'Close' button.</p>

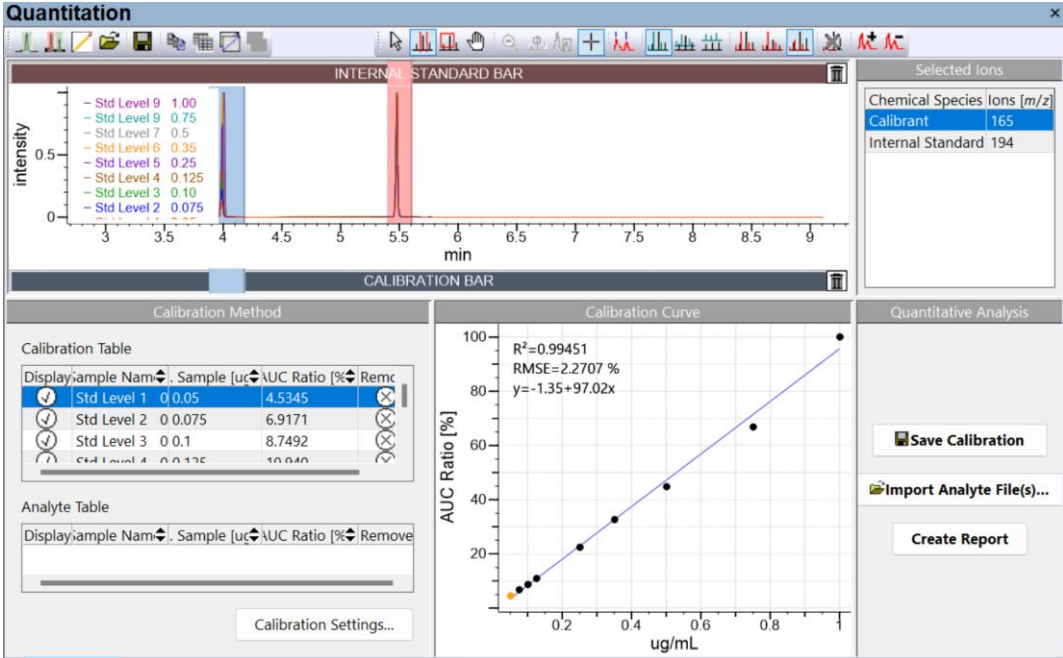
	Action	Result																												
5	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is "165". This represents the TIC component for the peak at 4.01 min.</p> <div style="border: 1px solid gray; padding: 5px; margin: 10px 0;"> <input checked="" type="checkbox"/> TIC <input type="checkbox"/> BPC <input type="checkbox"/> 86 <input type="checkbox"/> 120 <input checked="" type="checkbox"/> 165 <input type="checkbox"/> 92 <input type="checkbox"/> 137 </div> <p>Click Next > button.</p> <div style="border: 1px solid gray; padding: 5px; display: inline-block; margin: 10px 0;">Next ></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>  <p>The screenshot shows the 'Calibration Document Settings' window for 'Sample9.D'. The 'Selected Ion(s): 165' is highlighted. The 'Raw Spectrum' shows a peak at 4.0095 min. The 'Mass Chromatogram List Pane' shows a table of components:</p> <table border="1" data-bbox="1491 511 1881 625"> <thead> <tr> <th>RT [m...]</th> <th>#</th> <th>Match</th> <th>Score...</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>The 'Component Models' pane shows 'Peak Picking' with 'Sensitivity %: 50' and 'Automatic' unchecked. The 'Next >' button is visible at the bottom.</p>	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																									
5.4816	1	Caffeine	98.89	98.85	99.24																									
5.7834	1	Lidocaine	97.78	97.67	98.76																									


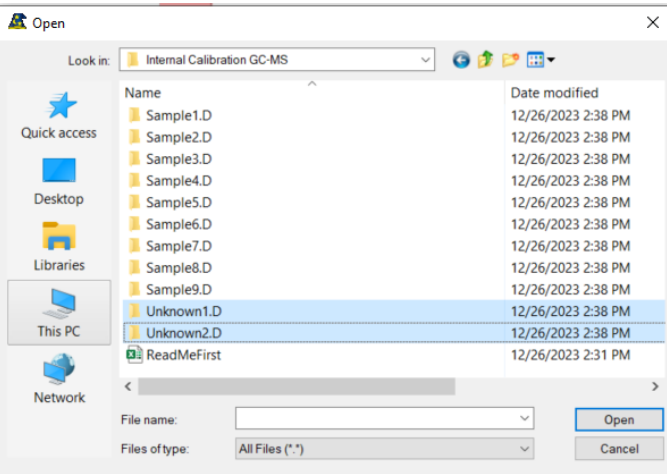
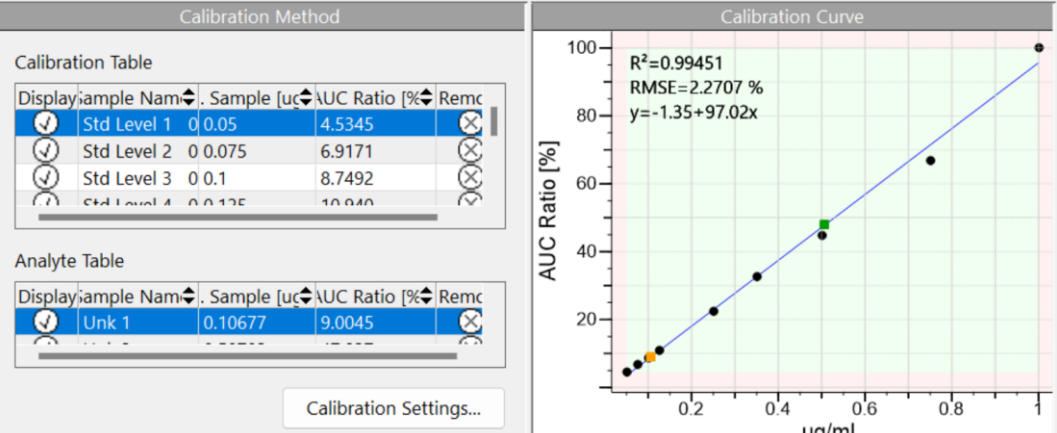
	Action	Result
6	<p>Select peak region around 4.0 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 3.9 – 4.2 min).</p> <p>Click Next > button.</p> <div data-bbox="254 565 457 621" style="border: 1px solid blue; padding: 2px; display: inline-block; margin-top: 10px;">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> 

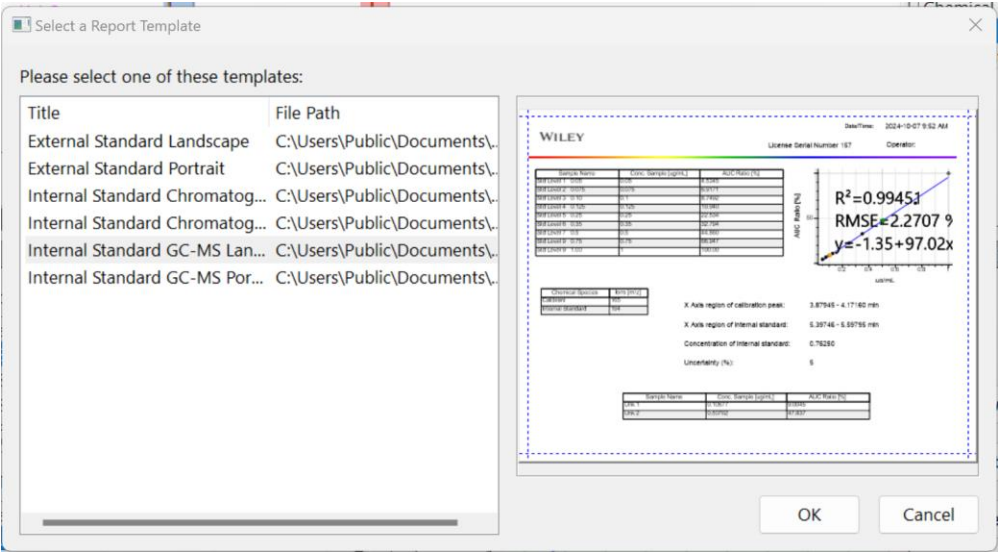
	Action	Result																												
7	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is "194". This represents the TIC internal standard for the peak at 5.48 min.</p> <p>Click Next > button.</p> <div data-bbox="254 565 464 621" style="border: 1px solid black; padding: 5px; display: inline-block;">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>The screenshot shows the 'Calibration Document Settings' window. It features a 'Selected Ion(s): 194' chromatogram at the top. Below it are three panels: 'Raw Spectrum' (m/z 40-160), 'Database Match' (m/z 40-160), and 'MASS CHROMATOGRAM m/z RANGES'. A 'Components' table is visible on the right side of the window.</p> <table border="1" data-bbox="1486 505 1879 646"> <thead> <tr> <th>RT [m...]</th> <th>#</th> <th>Match</th> <th>Score...</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>A callout box points to the 'Components' table with the text: Step 3: Please use the ion chromatogram ch select the internal standard ions. The 'Sensitivity %' is set to 50, and the 'Peak Picking' section has a slider set to 'Medium'.</p>	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																									
5.4816	1	Caffeine	98.89	98.85	99.24																									
5.7834	1	Lidocaine	97.78	97.67	98.76																									

	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="254 565 443 618" style="border: 1px solid blue; padding: 2px; display: inline-block; margin-top: 10px;">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 1341" style="border: 1px solid blue; padding: 2px; display: inline-block; margin-top: 10px;">Next ></div>	

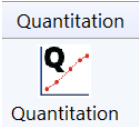

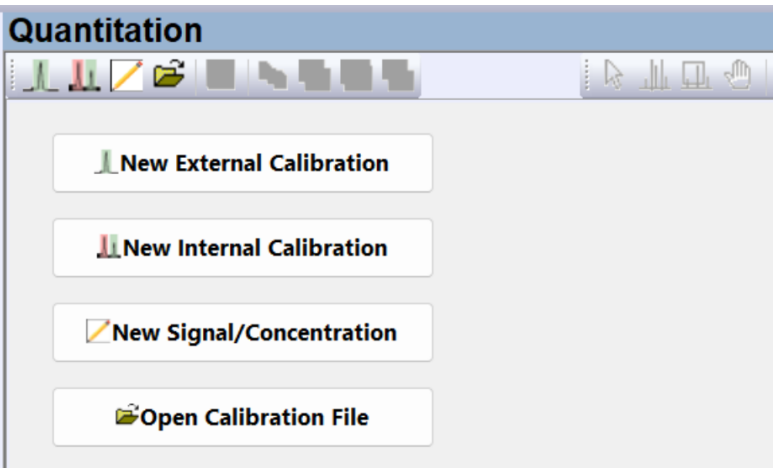
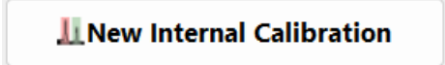
	Action	Result																				
10	<p>Enter concentration and ratio values in the popup window:</p> <ul style="list-style-type: none"> • File: Std Level 1 0.05, Concentration: 0.05 • File: Std Level 2 0.075, Concentration: 0.075 • File: Std Level 3 0.10, Concentration: 0.1 • File: Std Level 4 0.125, 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 data-bbox="254 1317 449 1377" style="border: 1px solid black; padding: 2px; display: inline-block;">Finish</div>	<p>The concentration settings for the samples and internal standard are shown:</p> <div data-bbox="831 358 1879 769" style="border: 1px solid gray; padding: 5px;"> <p><input checked="" type="checkbox"/> Internal standard concentration is constant. Concentration: <input type="text" value="0.7625"/> ug/mL</p> <table border="1" data-bbox="835 423 1875 761"> <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 style="background-color: #0070C0; color: white;"><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
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																					

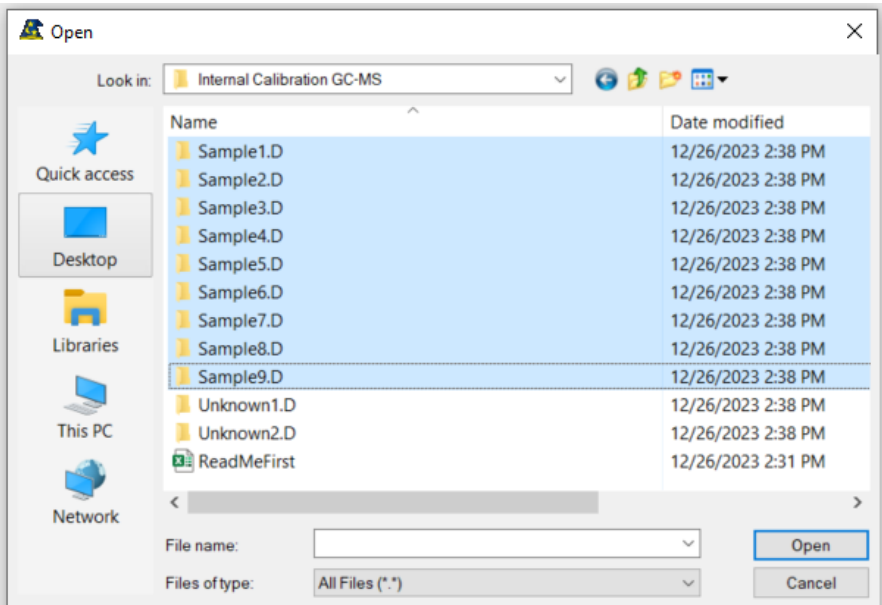
	Action	Result
11	Analyze the calibration results.	<ul style="list-style-type: none"> Statistics are reported in the Calibration Curve. The lower the value for RMSE (Root Mean Squared Error), the better the curve fitting is. The closer the R² (Coefficient of Determination) is to 1, the better the curve 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 is a toolbar with various icons. Below it is a chromatogram titled 'INTERNAL STANDARD BAR' showing intensity versus time (min). The x-axis ranges from 3 to 9 minutes, and the y-axis ranges from 0 to 0.5. A prominent peak is visible at approximately 5.5 minutes. Below the chromatogram is a 'CALIBRATION BAR'. The main interface is divided into several panels: 'Calibration Method' containing a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Sample [ug]', 'AUC Ratio [%]', and 'Remc'; 'Analyte Table' with columns for 'Display', 'Sample Name', 'Sample [ug]', 'AUC Ratio [%]', and 'Remove'; 'Calibration Curve' showing a scatter plot of 'AUC Ratio [%]' versus 'ug/mL' with a linear regression line and statistics: $R^2=0.99451$, $RMSE=2.2707\%$, and $y=-1.35+97.02x$; and 'Quantitative Analysis' containing buttons for 'Save Calibration', 'Import Analyte File(s)...', and 'Create Report'.</p>

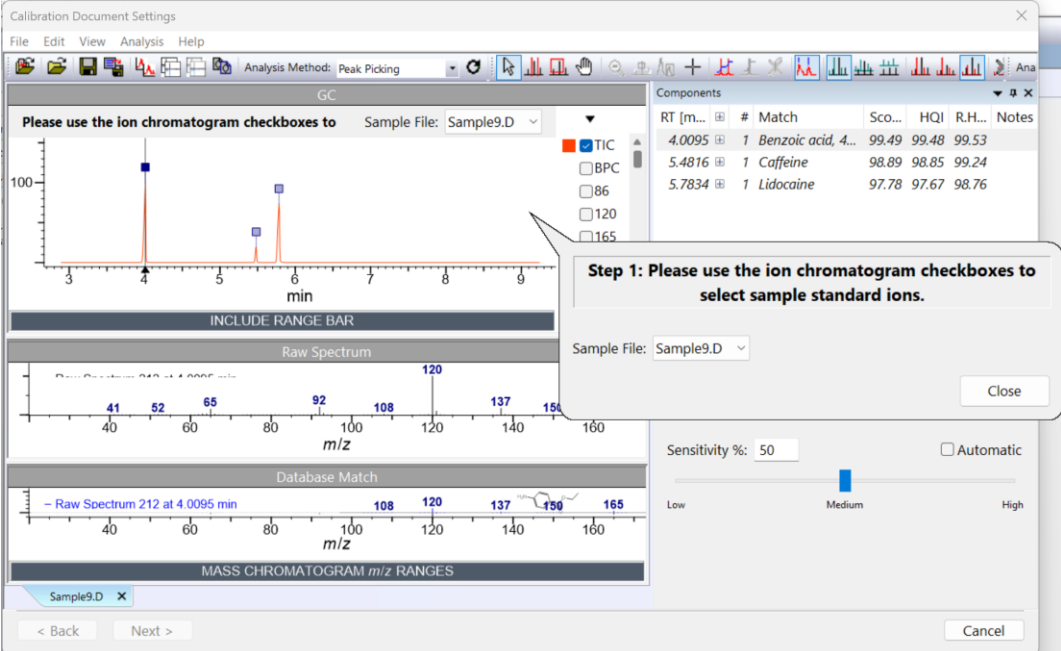
	Action	Result																																			
12	<p>Click the Import Analyte File(s) button found in the Quantitative Analysis panel.</p>  <p>On the Open popup window, hold CTRL button and click using left mouse button to select unknown file folder Unknown1.D and Unknown2.D (“C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal 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>  <table border="1" data-bbox="840 1023 1333 1193"> <caption>Calibration Table</caption> <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>✓</td> <td>Std Level 1</td> <td>0.005</td> <td>4.5345</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 2</td> <td>0.0075</td> <td>6.9171</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 3</td> <td>0.01</td> <td>8.7492</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 4</td> <td>0.0125</td> <td>10.040</td> <td>✕</td> </tr> </tbody> </table> <table border="1" data-bbox="840 1218 1333 1339"> <caption>Analyte Table</caption> <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>✓</td> <td>Unk 1</td> <td>0.10677</td> <td>9.0045</td> <td>✕</td> </tr> </tbody> </table>	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	✓	Std Level 1	0.005	4.5345	✕	✓	Std Level 2	0.0075	6.9171	✕	✓	Std Level 3	0.01	8.7492	✕	✓	Std Level 4	0.0125	10.040	✕	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	✓	Unk 1	0.10677	9.0045	✕
Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc																																	
✓	Std Level 1	0.005	4.5345	✕																																	
✓	Std Level 2	0.0075	6.9171	✕																																	
✓	Std Level 3	0.01	8.7492	✕																																	
✓	Std Level 4	0.0125	10.040	✕																																	
Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc																																	
✓	Unk 1	0.10677	9.0045	✕																																	

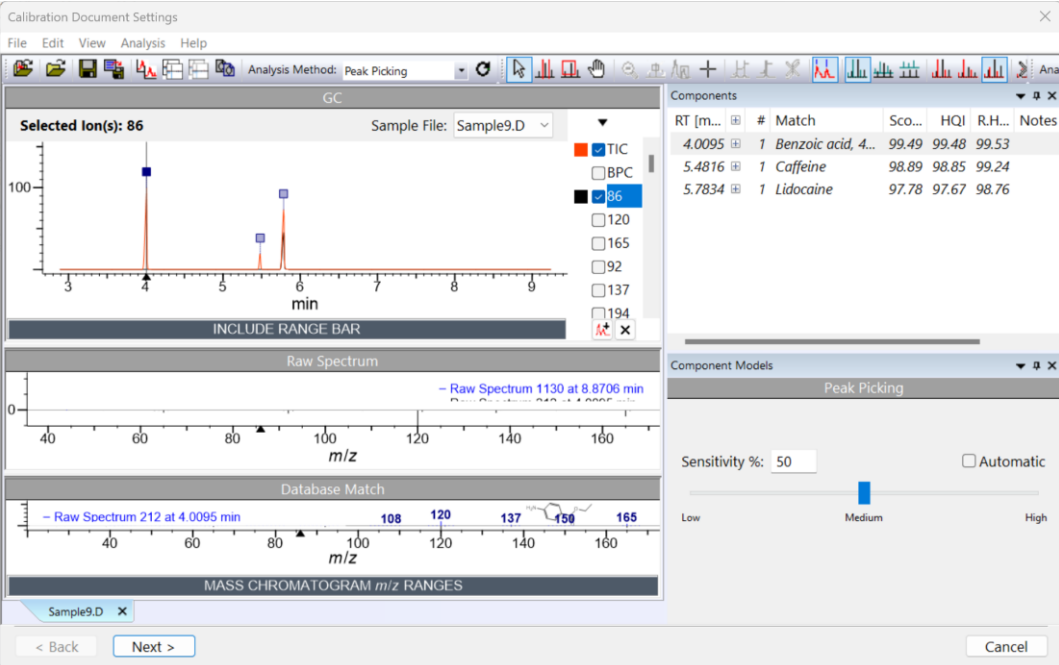
	Action	Result																																																																										
14	<p>Click the Create Report button or use Transfer to: ReportIt to can generate a report in which objects can be copied/pasted into other desktop tools.</p> <div data-bbox="247 456 474 516" style="border: 1px solid gray; padding: 5px; width: fit-content; margin: 10px auto;"> <p style="text-align: center;">Create Report</p> </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 with the following table of templates:</p> <table border="1" data-bbox="842 516 1318 711"> <thead> <tr> <th>Title</th> <th>File Path</th> </tr> </thead> <tbody> <tr> <td>External Standard Landscape</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>External Standard Portrait</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Lan...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Por...</td> <td>C:\Users\Public\Documents\...</td> </tr> </tbody> </table> <p>The report preview includes a calibration curve graph with the following data:</p> <table border="1" data-bbox="1344 516 1795 711"> <thead> <tr> <th>Sample Name</th> <th>Peak</th> <th>Retention Time (min)</th> <th>Area</th> </tr> </thead> <tbody> <tr><td>Internal Standard</td><td>1</td><td>3.87945</td><td>1000000</td></tr> <tr><td>Sample</td><td>1</td><td>3.87945</td><td>1000000</td></tr> <tr><td>Sample</td><td>2</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>3</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>4</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>5</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>6</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>7</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>8</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>9</td><td>6.58765</td><td>1000000</td></tr> <tr><td>Sample</td><td>10</td><td>6.58765</td><td>1000000</td></tr> </tbody> </table> <p>Calibration Curve Parameters:</p> <ul style="list-style-type: none"> X Axis region of calibration peak: 3.87945 - 4.17182 min X Axis region of internal standard: 6.38748 - 6.58765 min Concentration of internal standard: 0.76290 Uncertainty (%): 6 <p>Report Statistics:</p> <table border="1" data-bbox="1438 808 1690 841"> <thead> <tr> <th>Sample Name</th> <th>Peak</th> <th>Retention Time (min)</th> <th>Area</th> </tr> </thead> <tbody> <tr><td>Sample</td><td>1</td><td>3.87945</td><td>1000000</td></tr> <tr><td>Sample</td><td>2</td><td>6.58765</td><td>1000000</td></tr> </tbody> </table>	Title	File Path	External Standard Landscape	C:\Users\Public\Documents\...	External Standard Portrait	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...	Internal Standard GC-MS Por...	C:\Users\Public\Documents\...	Sample Name	Peak	Retention Time (min)	Area	Internal Standard	1	3.87945	1000000	Sample	1	3.87945	1000000	Sample	2	6.58765	1000000	Sample	3	6.58765	1000000	Sample	4	6.58765	1000000	Sample	5	6.58765	1000000	Sample	6	6.58765	1000000	Sample	7	6.58765	1000000	Sample	8	6.58765	1000000	Sample	9	6.58765	1000000	Sample	10	6.58765	1000000	Sample Name	Peak	Retention Time (min)	Area	Sample	1	3.87945	1000000	Sample	2	6.58765	1000000
Title	File Path																																																																											
External Standard Landscape	C:\Users\Public\Documents\...																																																																											
External Standard Portrait	C:\Users\Public\Documents\...																																																																											
Internal Standard Chromatog...	C:\Users\Public\Documents\...																																																																											
Internal Standard Chromatog...	C:\Users\Public\Documents\...																																																																											
Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...																																																																											
Internal Standard GC-MS Por...	C:\Users\Public\Documents\...																																																																											
Sample Name	Peak	Retention Time (min)	Area																																																																									
Internal Standard	1	3.87945	1000000																																																																									
Sample	1	3.87945	1000000																																																																									
Sample	2	6.58765	1000000																																																																									
Sample	3	6.58765	1000000																																																																									
Sample	4	6.58765	1000000																																																																									
Sample	5	6.58765	1000000																																																																									
Sample	6	6.58765	1000000																																																																									
Sample	7	6.58765	1000000																																																																									
Sample	8	6.58765	1000000																																																																									
Sample	9	6.58765	1000000																																																																									
Sample	10	6.58765	1000000																																																																									
Sample Name	Peak	Retention Time (min)	Area																																																																									
Sample	1	3.87945	1000000																																																																									
Sample	2	6.58765	1000000																																																																									

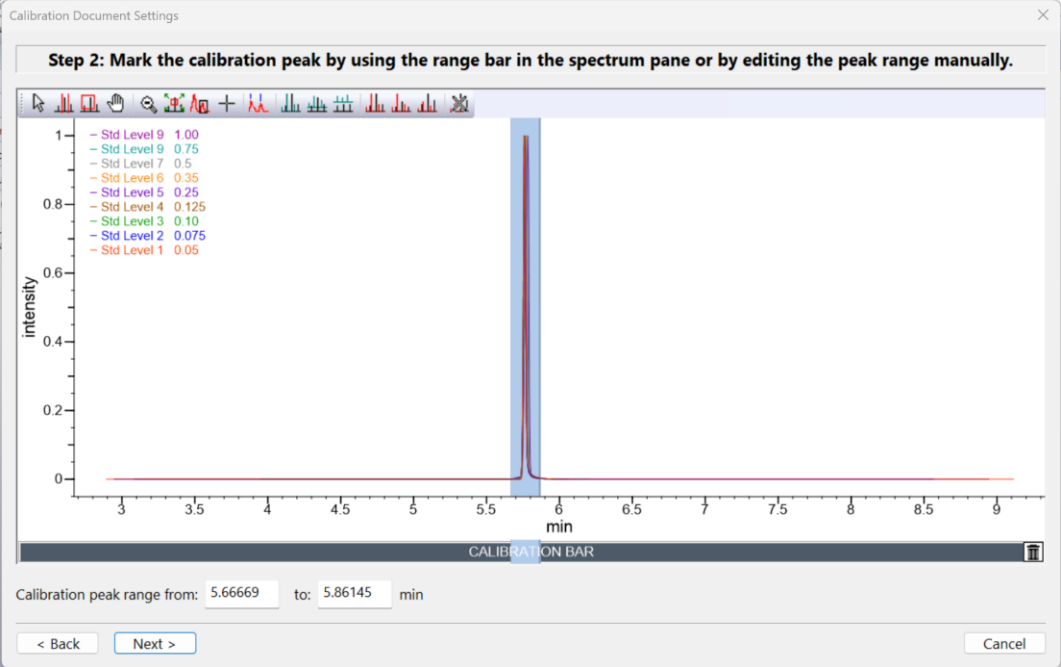
Lidocaine HCl

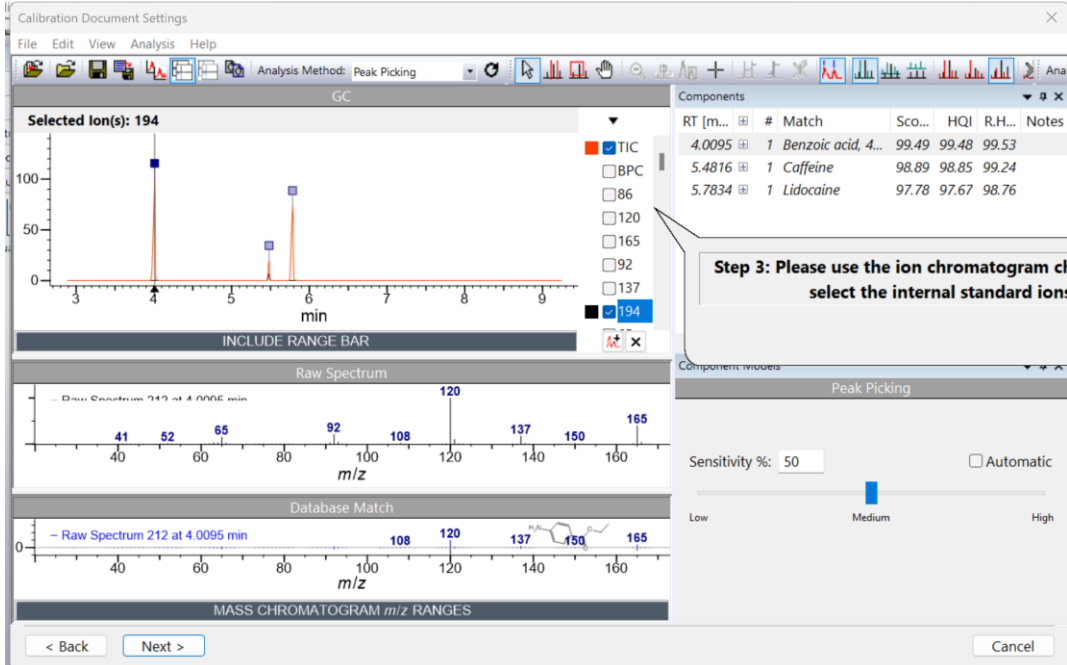
	Action	Result
1	<p>Open the Quantitation application by clicking its icon, typically found in the Quantitation group.</p>  <p><i>Note:</i> If a previous calibration study is displayed, it can be closed by clicking on the X icon () on the top right corner.</p>	<p>Quantitation application is displayed: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

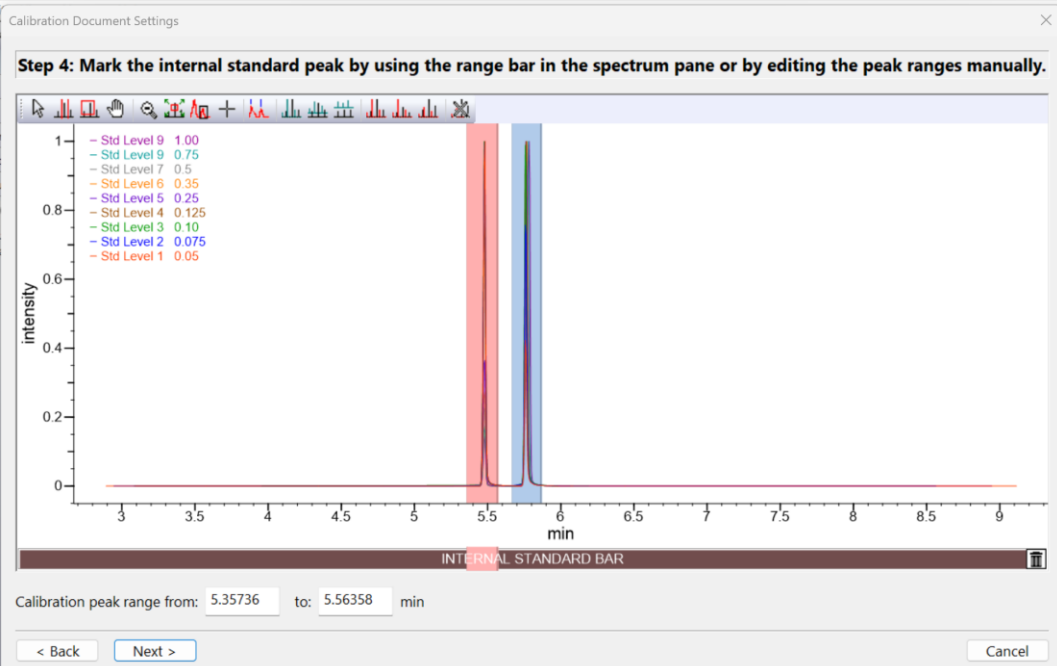
	Action	Result
3	<p>Navigate to “C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS” folder.</p> <p>Select all “Sample_.D” folders as shown in the right screenshot. Hold CTRL and click with left mouse button to select multiple files.</p> <p>Click Open.</p>	<p>All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

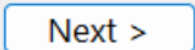
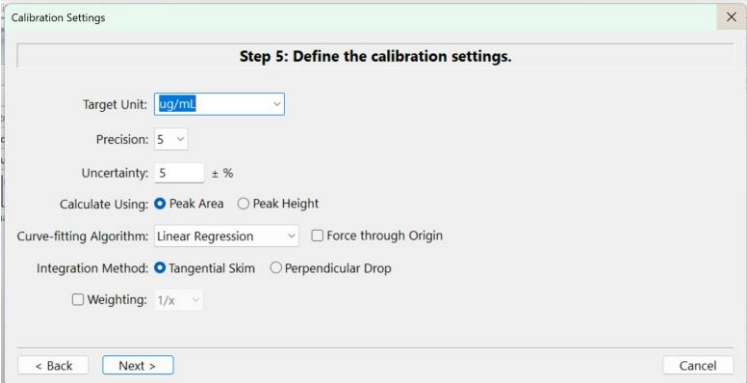
	Action	Result																												
4	<p>On the Calibration Document Settings popup window, use the Sample File dropdown menu to choose "Sample9.D", which is the calibrant file in which the analyte concentration is the largest.</p> <p>Click Close on the bubble window after making the file selection using the dropdown menu (e.g., see highlighted clicks below).</p> <div data-bbox="247 621 793 813" style="border: 1px solid gray; padding: 5px; margin-top: 10px;"> <p>Step 1: Please use the ion chromatogram checkboxes to select sample standard ions.</p> <p>Sample File: Sample9.D</p> <p style="text-align: right;">Close</p> </div>	<p>The chromatogram with the largest concentration, i.e., Sample9.D., is used to select the component peak.</p> <div data-bbox="825 394 1879 1040" style="border: 1px solid gray; padding: 5px; margin-top: 10px;">  <p>The screenshot shows the 'Calibration Document Settings' window for 'Sample9.D'. It features a Total Ion Chromatogram (TIC) with peaks at approximately 4, 5.5, and 6 minutes. Below the chromatogram is a 'Raw Spectrum' plot showing intensity versus m/z, with major peaks at 120, 137, and 154. A 'Database Match' section shows a match for 'Benzoic acid, 4...' at 4.0095 minutes. A 'Components' table is visible in the top right corner of the window.</p> <table border="1" data-bbox="1470 470 1858 592"> <thead> <tr> <th>RT [m...]</th> <th>#</th> <th>Match</th> <th>Score</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>A callout bubble from the 'Action' column points to the 'Sample File' dropdown menu (set to 'Sample9.D') and the 'Close' button in the 'Step 1' instruction box.</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																									
5.4816	1	Caffeine	98.89	98.85	99.24																									
5.7834	1	Lidocaine	97.78	97.67	98.76																									

	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> <div data-bbox="254 565 443 613" style="border: 1px solid blue; padding: 2px; display: inline-block;">Next ></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>  <p>The screenshot shows the 'Calibration Document Settings' window for 'Sample9.D'. It features a 'Selected Ion(s): 86' panel with a chromatogram showing peaks at approximately 4, 5.78, and 6 minutes. Below this are three sub-panels: 'Raw Spectrum' (m/z 40-160), 'Database Match' (m/z 40-160), and 'MASS CHROMATOGRAM m/z RANGES'. The 'Database Match' panel shows peaks at m/z 108, 120, 137, 150, and 165. On the right, the 'Components' table lists: <table border="1" data-bbox="1486 508 1881 621"> <thead> <tr> <th>RT [m...]</th> <th>#</th> <th>Match</th> <th>Score</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> The 'Component Models' section shows 'Peak Picking' with 'Sensitivity %' set to 50 and 'Automatic' unchecked. The 'Next >' button is highlighted at the bottom. </p>	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																									
5.4816	1	Caffeine	98.89	98.85	99.24																									
5.7834	1	Lidocaine	97.78	97.67	98.76																									

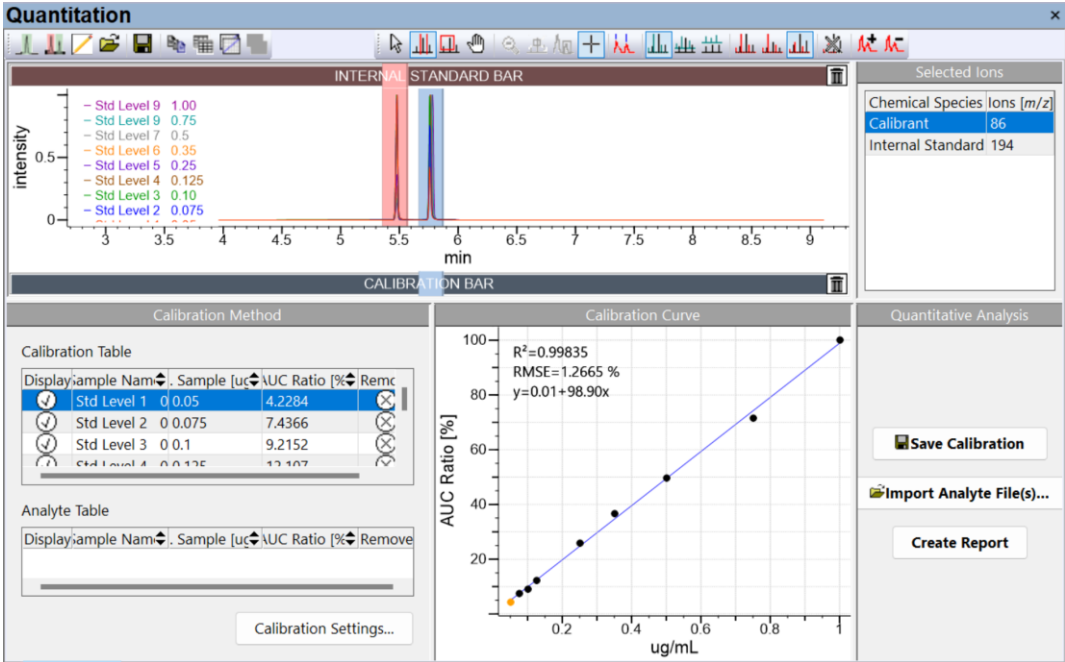
	Action	Result
6	<p>Select peak region around 5.75 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 5.7 – 5.9 min).</p> <p>Click Next > button.</p> <div data-bbox="254 558 447 610" style="border: 1px solid blue; padding: 2px; display: inline-block; margin: 10px 0;">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> 

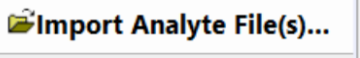
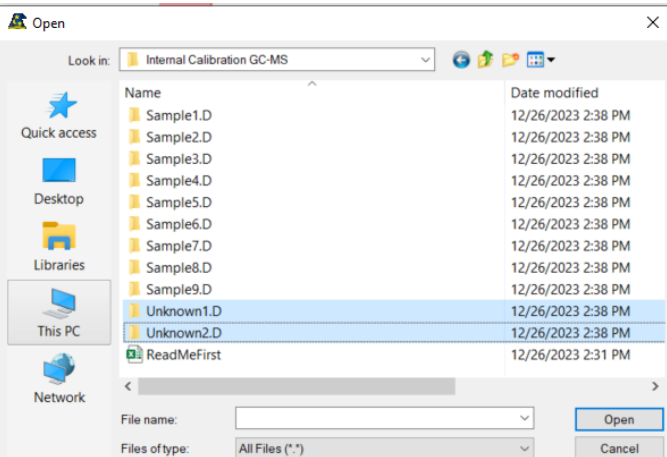
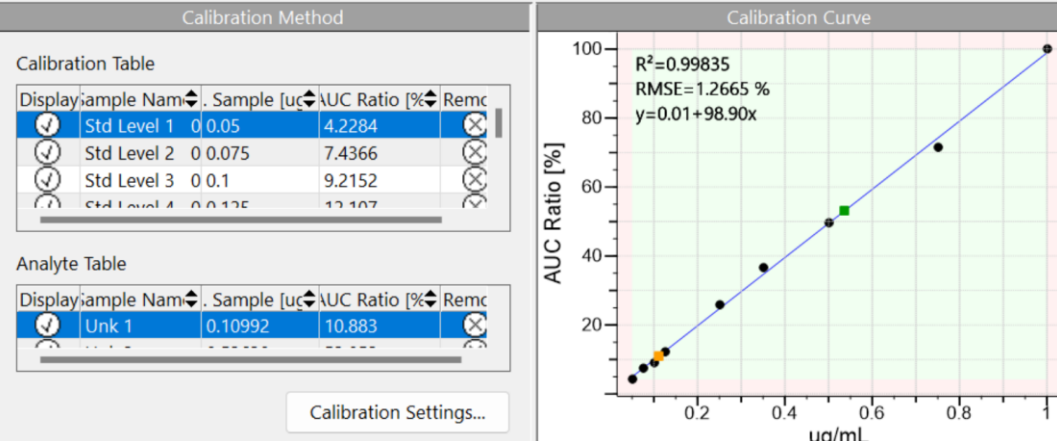
	Action	Result
7	<p>Select a component from the Raw Spectrum pan by clicking on the numeric value in the Mass Chromatogram List Pane. In this example, the Component Molecular m/z to choose is "194". This represents the TIC internal standard for the peak at 5.48 min.</p> <p>Click Next > button.</p> <div data-bbox="254 565 447 613" style="border: 1px solid blue; padding: 2px; display: inline-block;">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>The screenshot shows the 'Calibration Document Settings' window. The top panel displays a chromatogram with a peak at 5.48 minutes. The 'Selected Ion(s): 194' is indicated. Below the chromatogram are three mass spectrum panels: 'Raw Spectrum' (m/z 40-160), 'Database Match' (m/z 40-160), and 'MASS CHROMATOGRAM m/z RANGES'. A 'Components' table is visible on the right, listing peaks at 4.0095, 5.4816, and 5.7834 minutes. A callout box points to the 'Peak Picking' section, stating: 'Step 3: Please use the ion chromatogram chart to select the internal standard ions'. The 'Sensitivity %' is set to 50, and the 'Automatic' checkbox is unchecked. The 'Next >' button is highlighted at the bottom.</p>

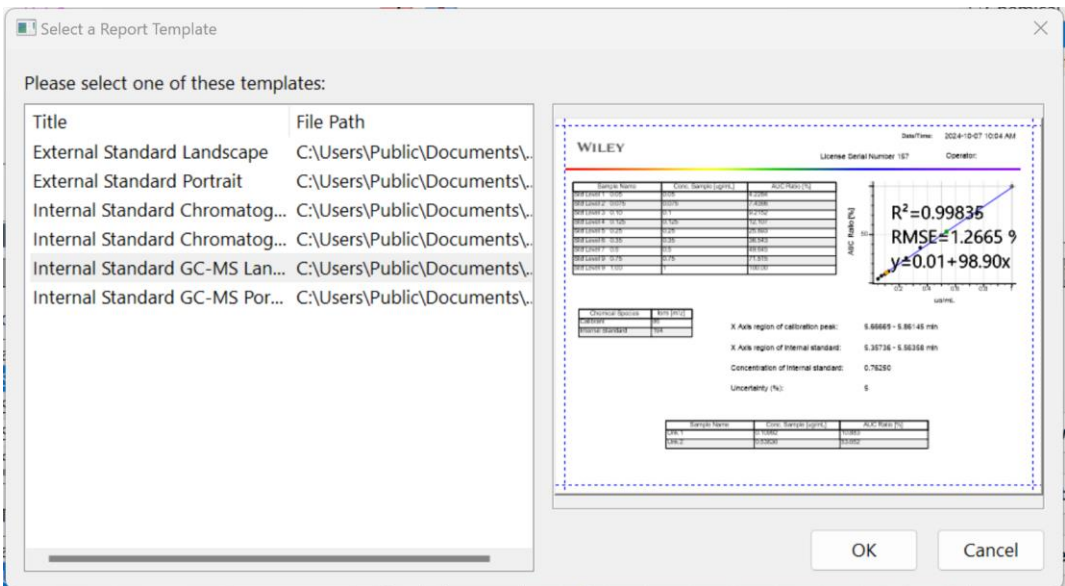
	Action	Result
8	<p>Select peak region around 5.5 min as the calibrant peak by clicking down on the CALIBRATION BAR with left mouse button and release after selecting a region (e.g., ~ 5.4 – 5.6 min).</p> <p>Click Next > button.</p> <p><input type="button" value="Next >"/></p>	<p>The selected internal standard region is shaded with red coloration.</p>  <p>The screenshot shows a 'Calibration Document Settings' dialog box. 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 'Std Level' values from 1 to 9. A red vertical bar highlights a peak at approximately 5.5 minutes. Below the plot is an 'INTERNAL STANDARD BAR' with a range input field showing '5.35736' to '5.56358' min. At the bottom are '< Back', 'Next >', and 'Cancel' buttons.</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>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 data-bbox="254 1317 468 1377" style="border: 1px solid black; padding: 5px; display: inline-block;">Finish</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 data-bbox="831 423 1879 829" style="border: 1px solid gray; padding: 5px;"> <p><input checked="" type="checkbox"/> Internal standard concentration is constant. Concentration: <input type="text" value="0.7625"/> ug/mL</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 60%;">Sample Name</th> <th style="width: 40%;">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 style="background-color: #0070C0; color: white;"><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
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																					

	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 is a toolbar and a title bar. Below this is a chromatogram showing intensity versus time (min) with several peaks labeled as 'Std Level 1' through 'Std Level 9'. The x-axis ranges from 3 to 9 minutes, and the y-axis ranges from 0 to 0.5 intensity. Below the chromatogram is a 'CALIBRATION BAR'. The main interface is divided into three panels: 'Calibration Method' on the left, 'Calibration Curve' in the center, and 'Quantitative Analysis' on the right. The 'Calibration Method' panel contains a 'Calibration Table' with columns for 'Display', 'Sample Name', 'Sample [ug]', 'UC Ratio [%]', and 'Remc'. The table lists four standard levels. The 'Calibration Curve' panel shows a scatter plot of 'AUC Ratio [%]' versus 'ug/mL' with a linear regression line. The statistics shown are R²=0.99835, RMSE=1.2665 %, and the equation y=0.01+98.90x. The 'Quantitative Analysis' panel 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>  <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>  <table border="1" data-bbox="840 1015 1333 1193"> <caption>Calibration Table</caption> <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>✓</td> <td>Std Level 1</td> <td>0.005</td> <td>4.2284</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 2</td> <td>0.0075</td> <td>7.4366</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 3</td> <td>0.01</td> <td>9.2152</td> <td>✕</td> </tr> <tr> <td>✓</td> <td>Std Level 4</td> <td>0.0125</td> <td>12.107</td> <td>✕</td> </tr> </tbody> </table> <table border="1" data-bbox="840 1209 1333 1323"> <caption>Analyte Table</caption> <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>✓</td> <td>Unk 1</td> <td>0.10992</td> <td>10.883</td> <td>✕</td> </tr> </tbody> </table>	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	✓	Std Level 1	0.005	4.2284	✕	✓	Std Level 2	0.0075	7.4366	✕	✓	Std Level 3	0.01	9.2152	✕	✓	Std Level 4	0.0125	12.107	✕	Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc	✓	Unk 1	0.10992	10.883	✕
Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc																																	
✓	Std Level 1	0.005	4.2284	✕																																	
✓	Std Level 2	0.0075	7.4366	✕																																	
✓	Std Level 3	0.01	9.2152	✕																																	
✓	Std Level 4	0.0125	12.107	✕																																	
Display	Sample Name	Sample [ug]	AUC Ratio [%]	Remc																																	
✓	Unk 1	0.10992	10.883	✕																																	

	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; width: fit-content; margin: 10px auto;"> <p style="text-align: center;">Create Report</p> </div> <p>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.</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 two windows. On the left is the 'Select a Report Template' dialog box with a list of templates:</p> <table border="1" data-bbox="846 521 1350 727"> <thead> <tr> <th>Title</th> <th>File Path</th> </tr> </thead> <tbody> <tr> <td>External Standard Landscape</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>External Standard Portrait</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard Chromatog...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Lan...</td> <td>C:\Users\Public\Documents\...</td> </tr> <tr> <td>Internal Standard GC-MS Por...</td> <td>C:\Users\Public\Documents\...</td> </tr> </tbody> </table> <p>On the right is the 'WILEY' report window showing a calibration curve graph with the equation $R^2=0.99836$ and $RMSE=1.26659$. The graph also shows the linear regression equation $y=0.01+98.90x$. Below the graph, there are fields for 'X Axis region of calibration peak' (5.86689 - 5.86148 min), 'X Axis region of internal standard' (5.35738 - 5.36358 min), 'Concentration of internal standard' (0.76280), and 'Uncertainty (%)' (5). There are also small tables for 'Chemical Species' and 'Sample Name'.</p>	Title	File Path	External Standard Landscape	C:\Users\Public\Documents\...	External Standard Portrait	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard Chromatog...	C:\Users\Public\Documents\...	Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...	Internal Standard GC-MS Por...	C:\Users\Public\Documents\...
Title	File Path															
External Standard Landscape	C:\Users\Public\Documents\...															
External Standard Portrait	C:\Users\Public\Documents\...															
Internal Standard Chromatog...	C:\Users\Public\Documents\...															
Internal Standard Chromatog...	C:\Users\Public\Documents\...															
Internal Standard GC-MS Lan...	C:\Users\Public\Documents\...															
Internal Standard GC-MS Por...	C:\Users\Public\Documents\...															