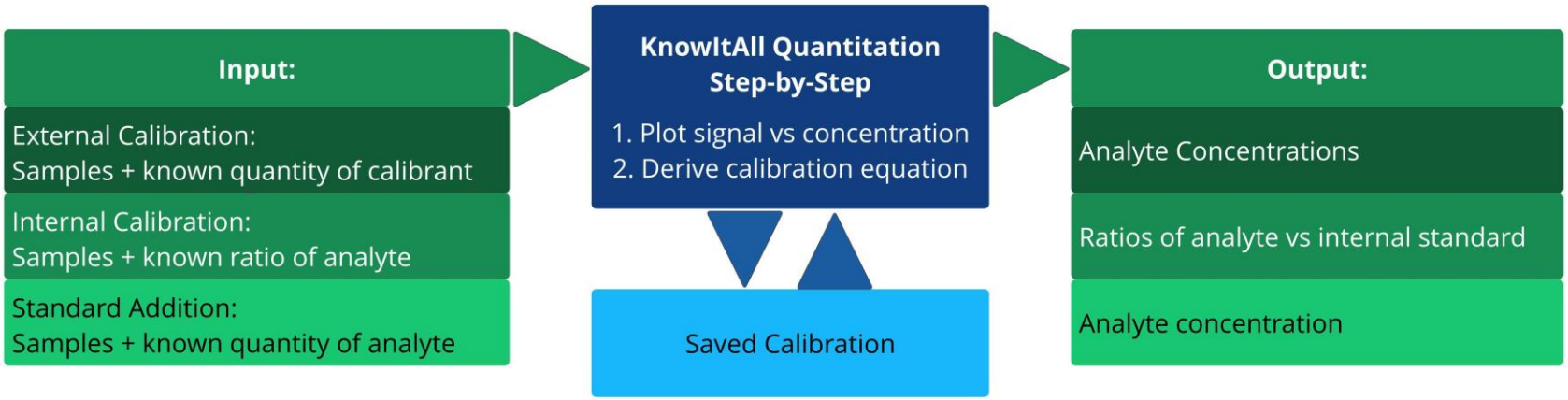
KnowItAll Informatics Training

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

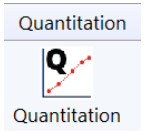
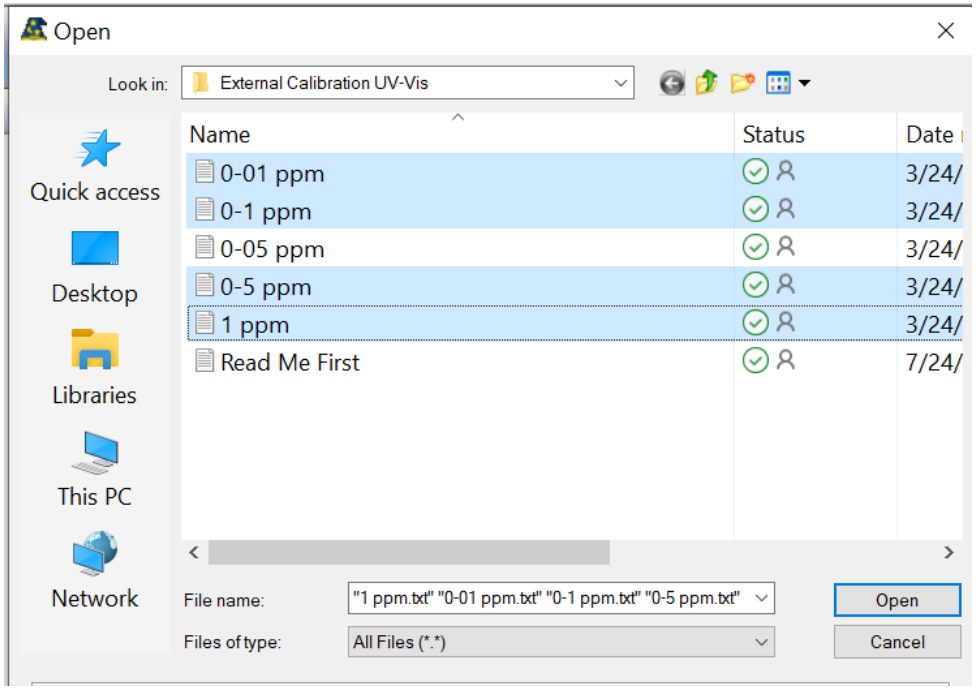
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

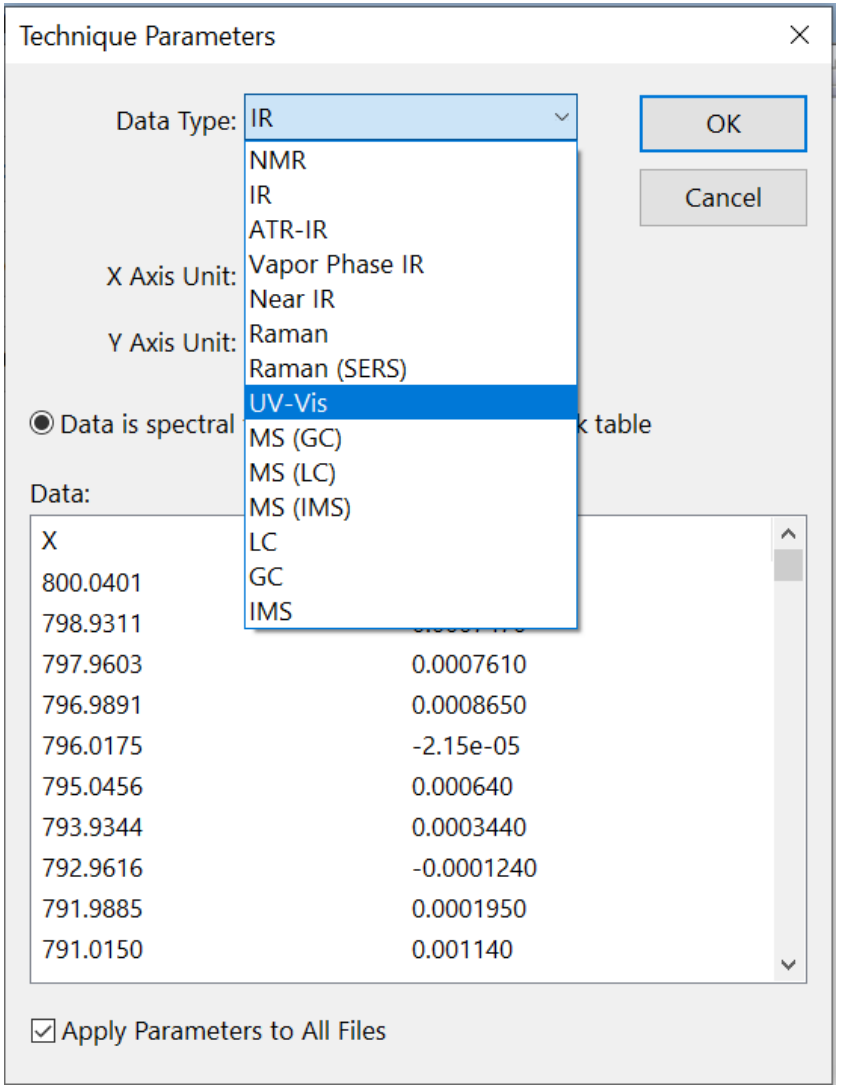
- External Calibration UV-Vis
- External Calibration IR

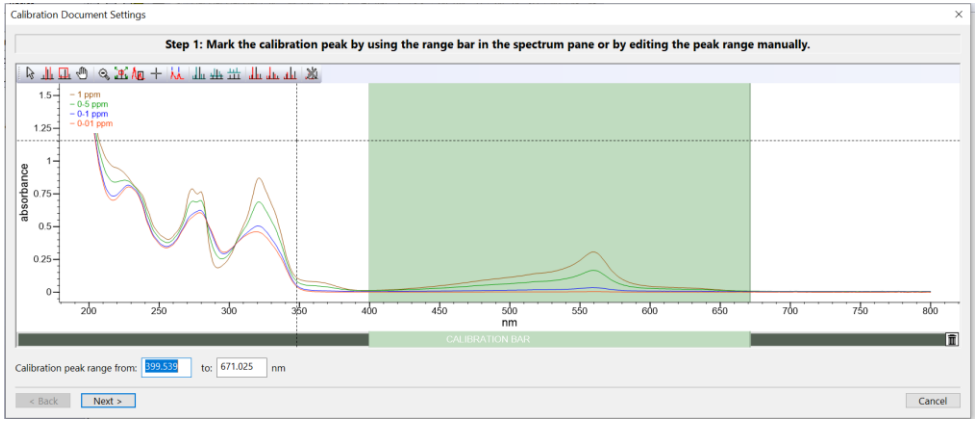
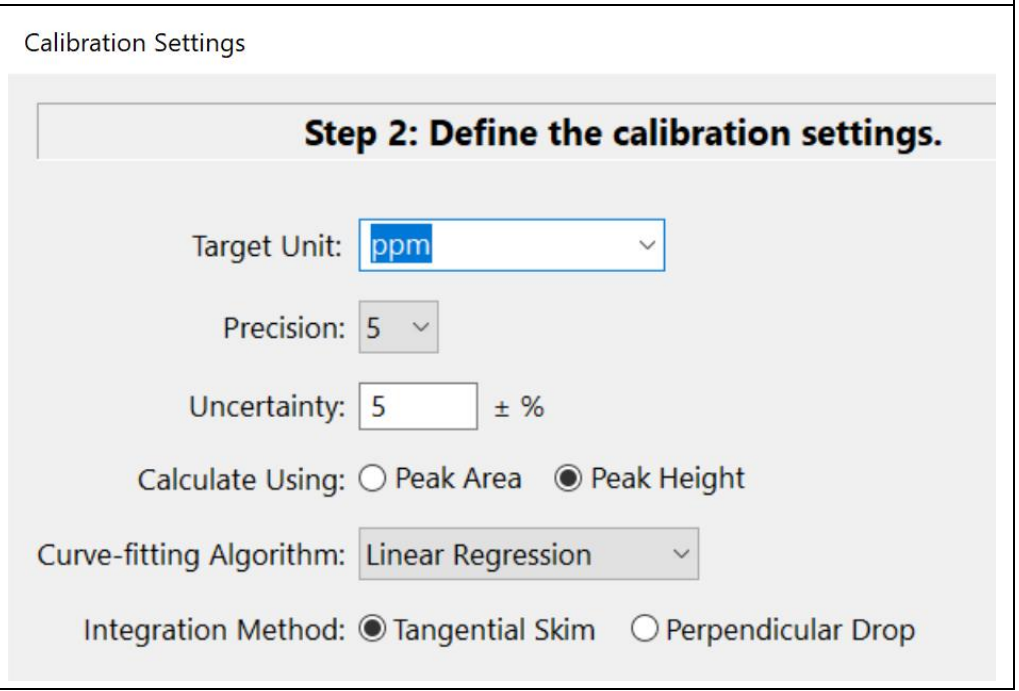
KnowItAll Applications Used

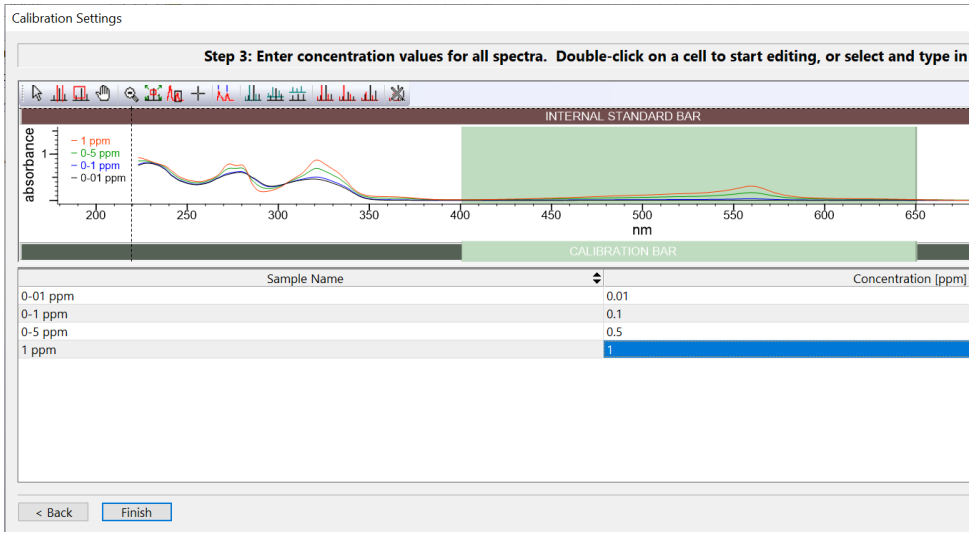
- Quantitation

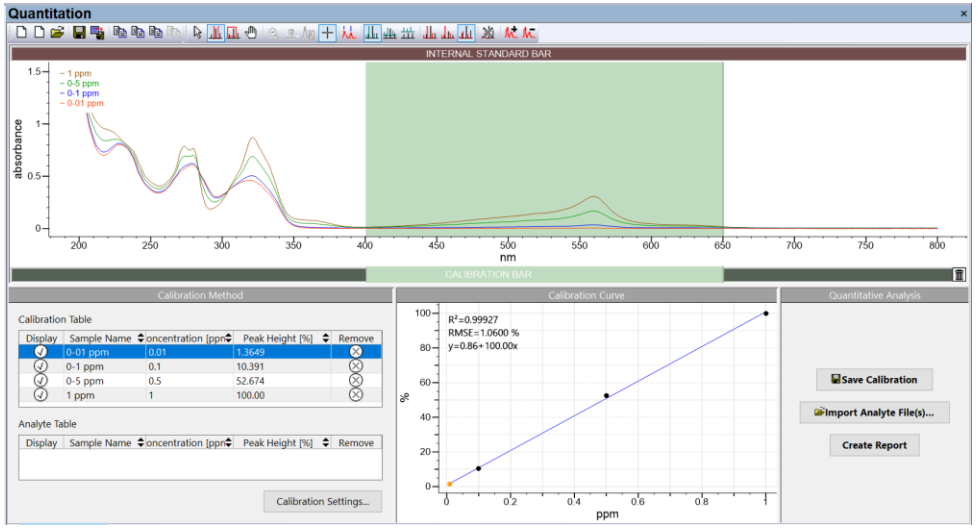
UV-Vis

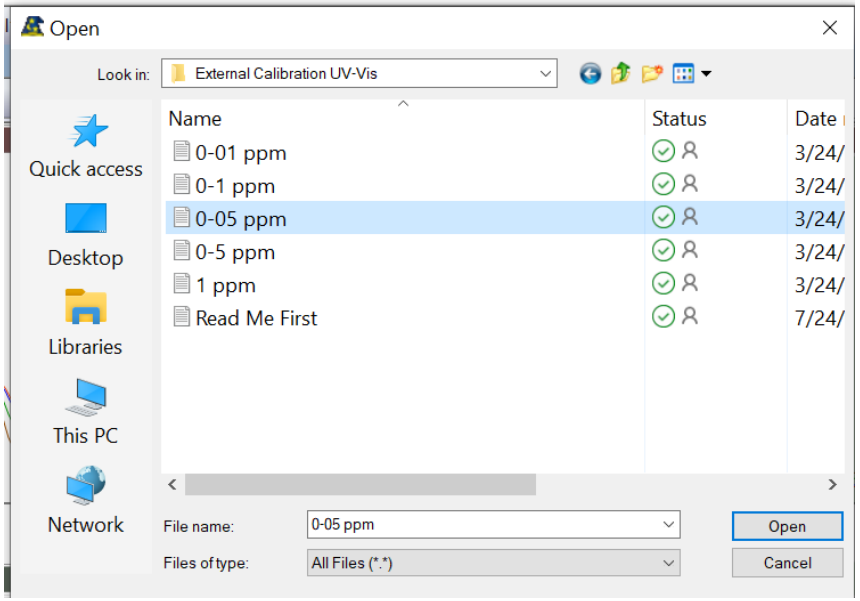
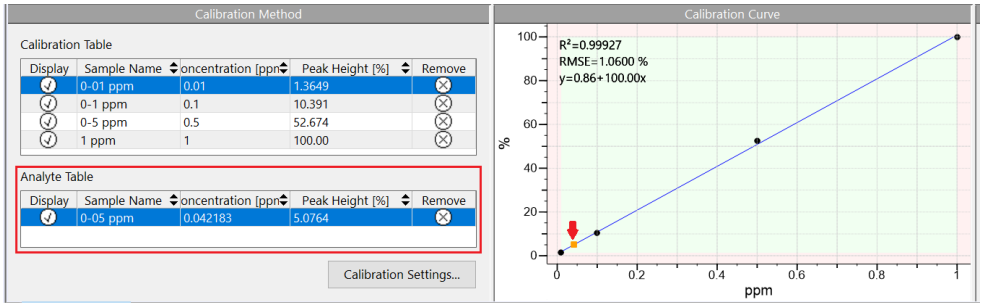
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.	
2	Click **New External Calibration** button	KnowItAll prompts user to open calibrant files.
3	Navigate to the **C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis** folder. Select sample files and leave one out as the "unknown." Click **Open.**	

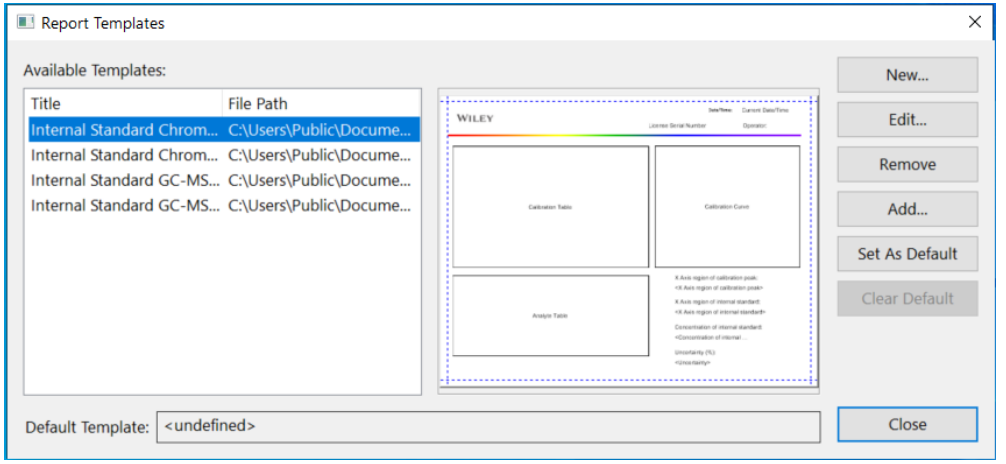
4	In **Technique Parameters** prompt window: • define file type **UV-Vis** • check **Apply Parameters to All Files** • click **OK**	

5	Select peak region around 560 nm by clicking down the CALIBRATION BAR (drag and drop). Click button **Next >**	
6	In the following window, define calibration settings as shown in the image to the right. Target Unit: **ppm** Calculate Using: **Peak Height** Click button **Next >.**	

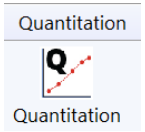
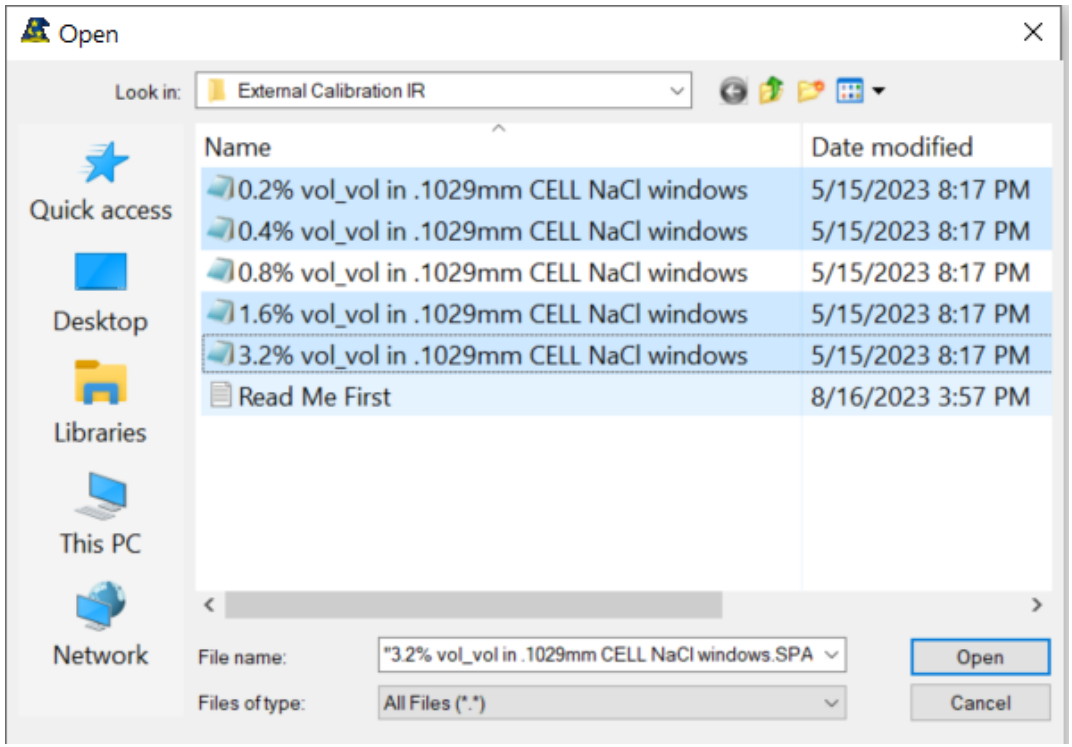
7	Enter concentrations in the right column based on the file names. (Note that in the sample files, dashes were used instead of decimals in the sample name. The file "0-01 ppm" has a concentration of 0.01 ppm.) Click **Finish** button.	

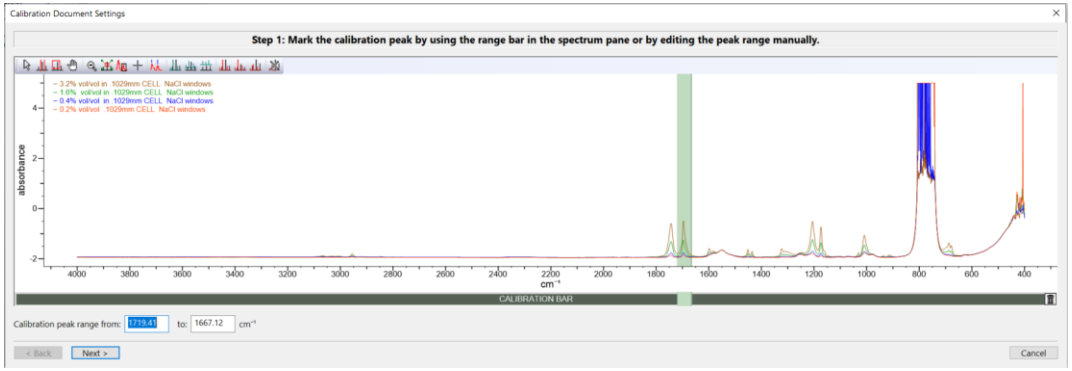
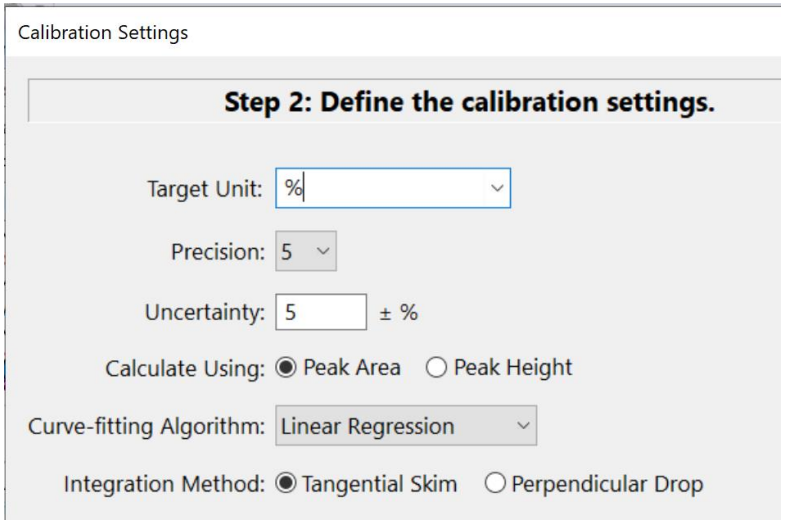
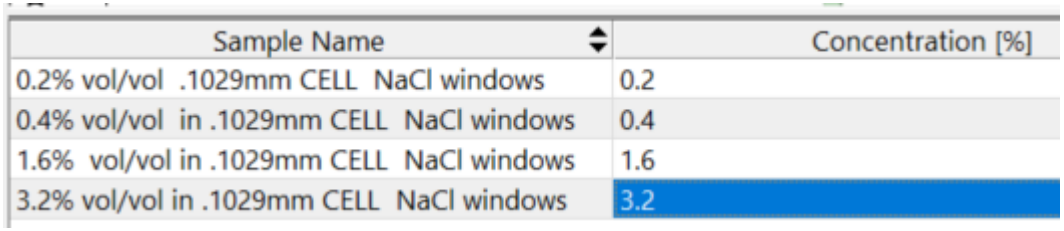
8		 Statistics are reported in the calibration curve. The lower the **RMSE** (**root mean squared error)**, the better the curve is fitting. The closer the **R^2** (**coefficient of determination**) is to 1**,** the better the curve is fitting. One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

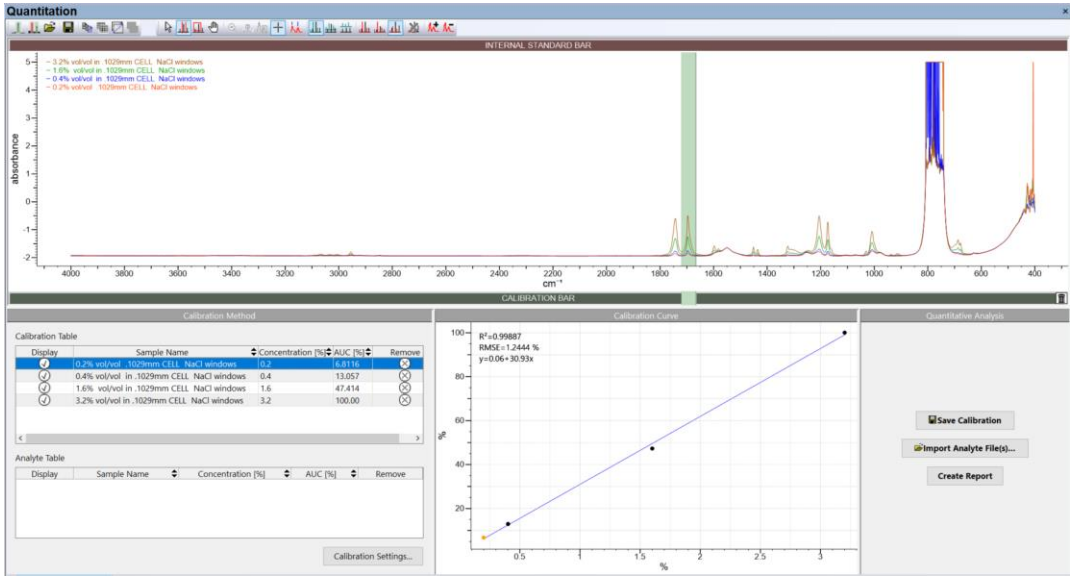
9	Click the **Import Analyte File(s)** button. Select the file that was left out (0-05 ppm). Click **Open.** Select file type to be **UV-Vis** at the prompt. Click **OK.**	
10		The concentration of the unknown is calculated and marked. 

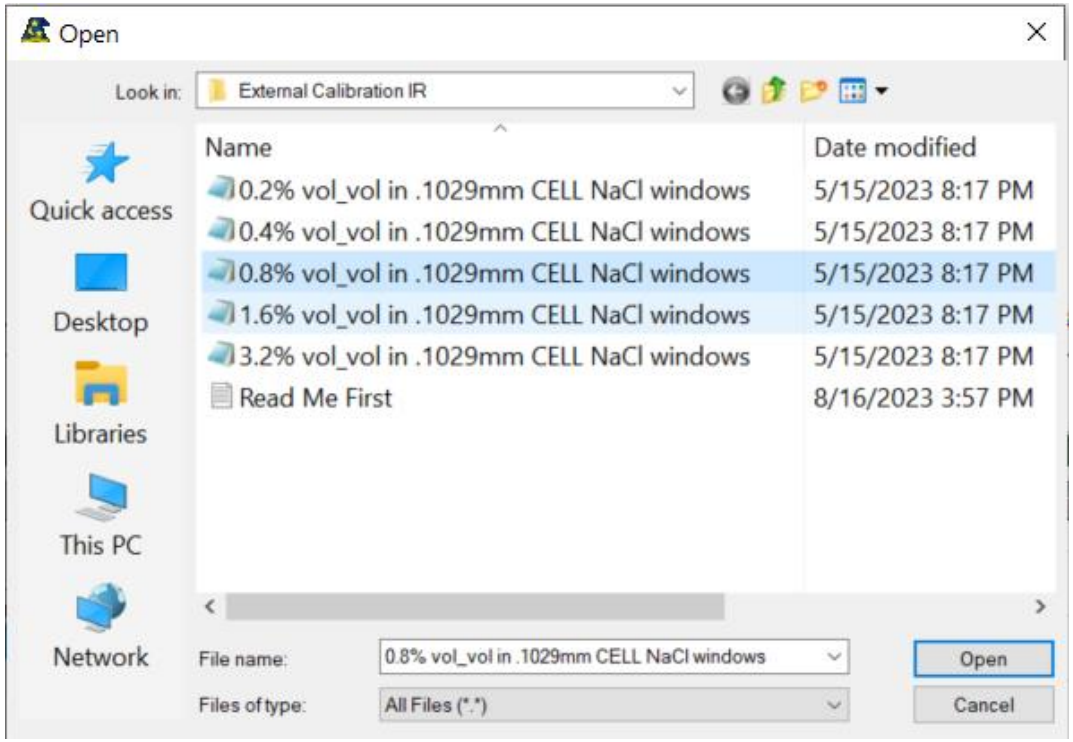
11	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application: **File > Edit Report Templates** **Click Add button** **Navigate to the template file** **Open**	

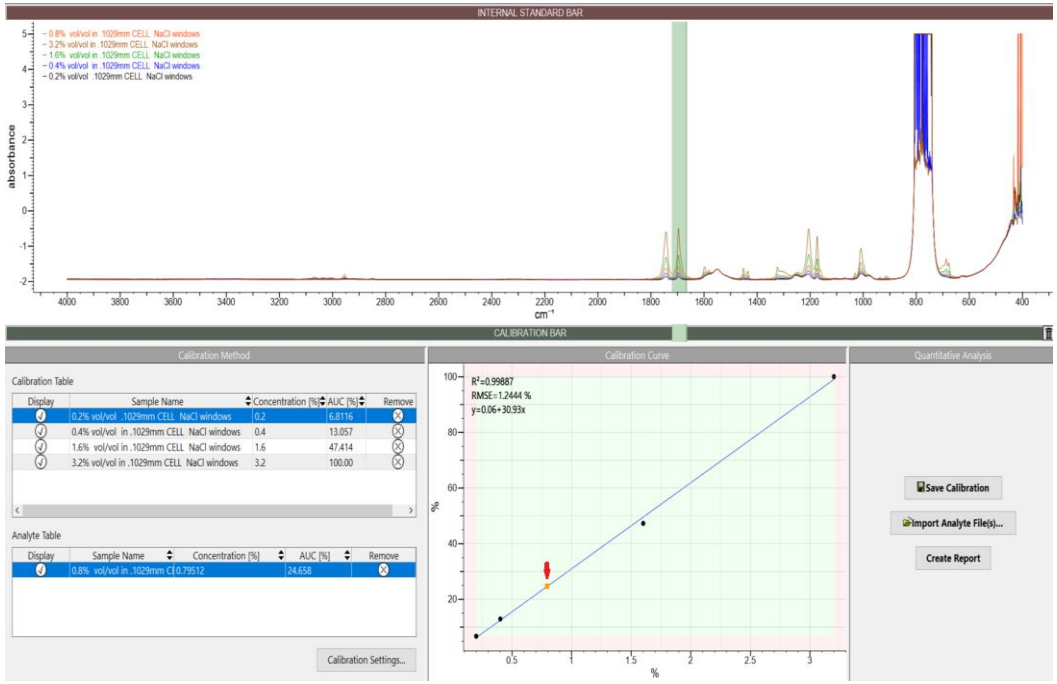
IR

	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.	
2	Click **New External Calibration** button.	KnowItAll prompts user to open calibrant files.
3	Navigate to the **C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR** folder. Select sample files and leave one (**0.8%**) out as the “unknown.” Click **Open.**	

4	Select peak region around 1696 cm^{-1} by clicking down the **CALIBRATION BAR** (drag and drop). ***Note**: In IR quantitation, one should avoid using the strongest peak.* Click button **Next >.**	
6	In the following window, define calibration settings: (Target Unit: **%** Calculate Using: **Peak Area**) Click button **Next >.**	
7	Enter concentrations in the pop-up window based on the numbers in the sample names. Click **Finish** button.	

8		 Statistics are reported in the calibration curve. The lower the **RMSE** (**root mean squared error)**, the better the curve is fitting. The closer the **R^2** (**coefficient of determination**) is to 1**,** the better the curve is fitting. One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

9	Click the **Import Analyte File(s)** button. Select the file that was left out (**0.8%**). Click **Open.**	

10		The concentration of the unknown is calculated and marked. 

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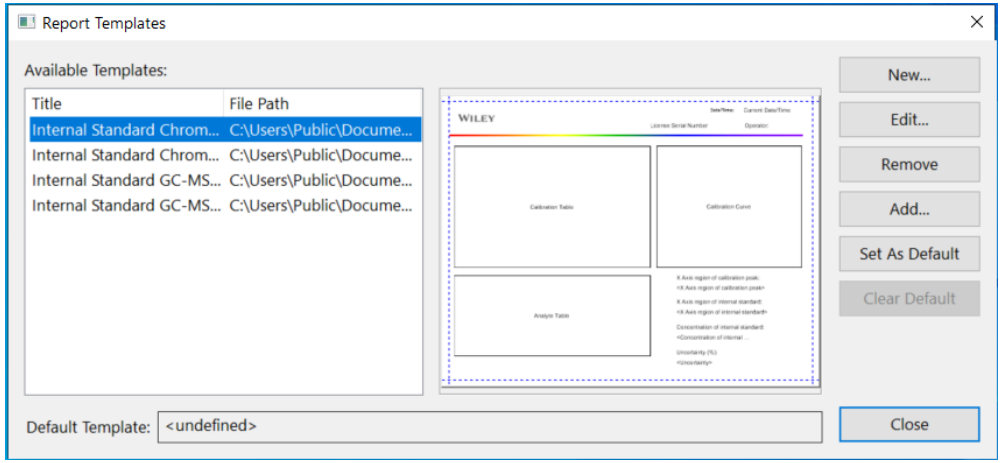
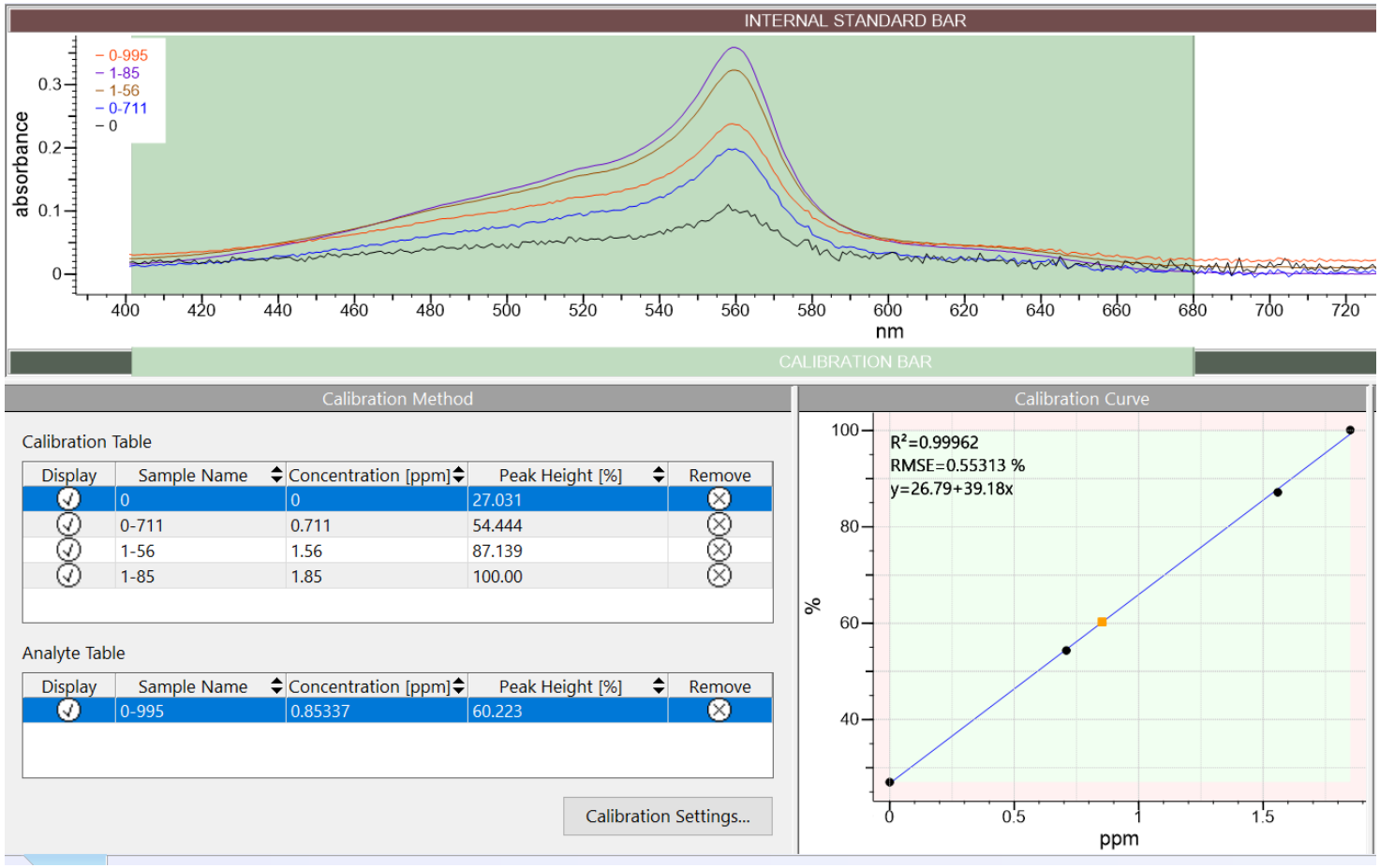
11	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application: **File > Edit Report Templates** **Click Add button** **Navigate to the template file** **Open**	

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:



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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

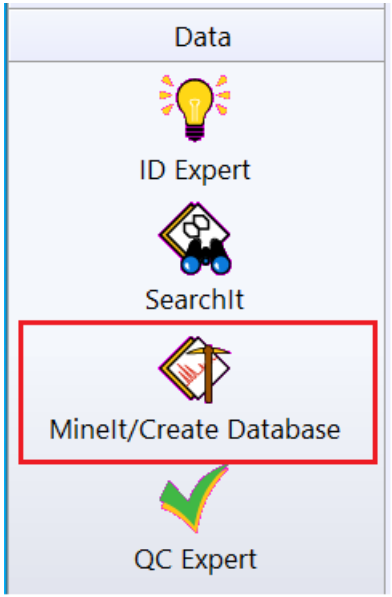
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

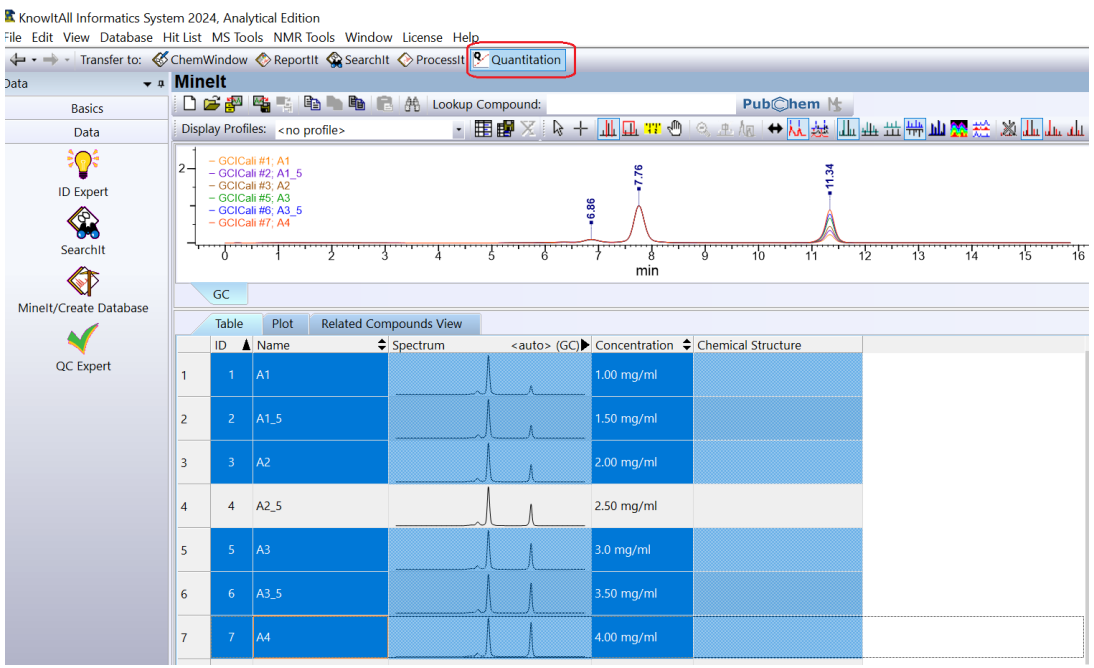
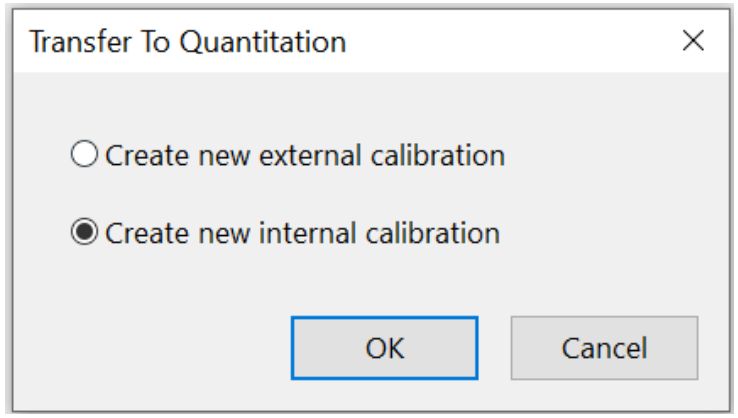
- Internal Calibration Chromatogram

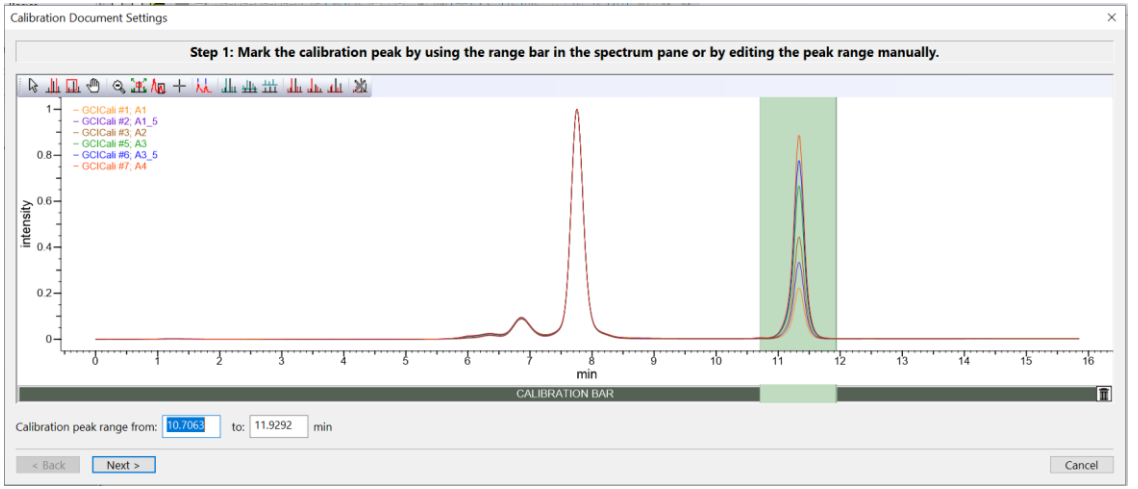
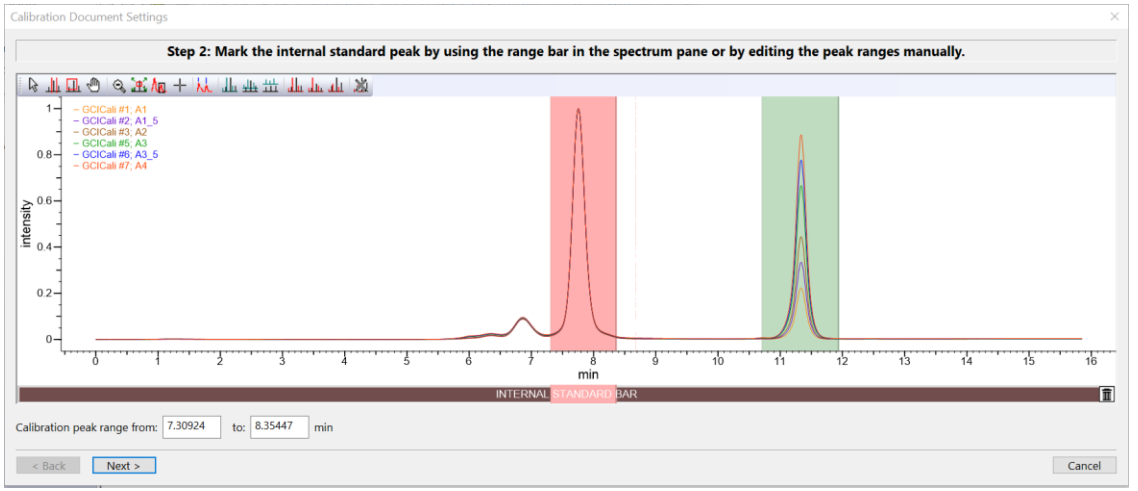
KnowItAll Applications Used

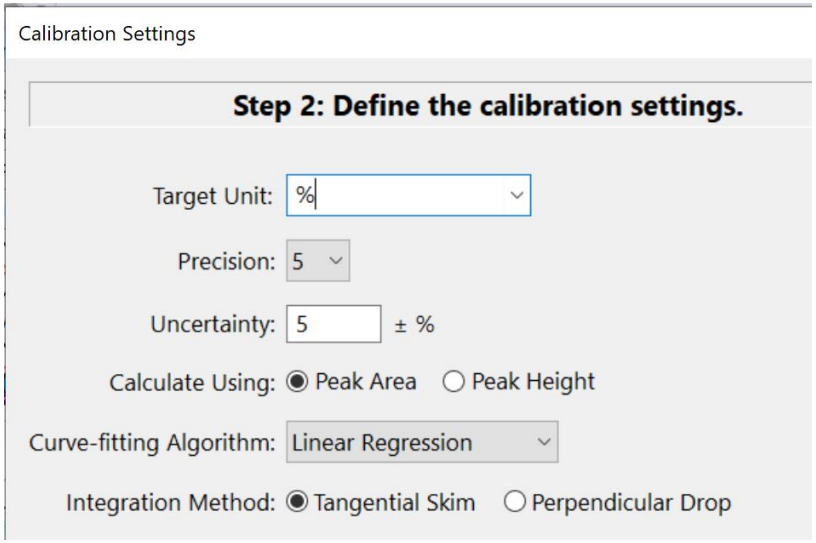
- Quantitation

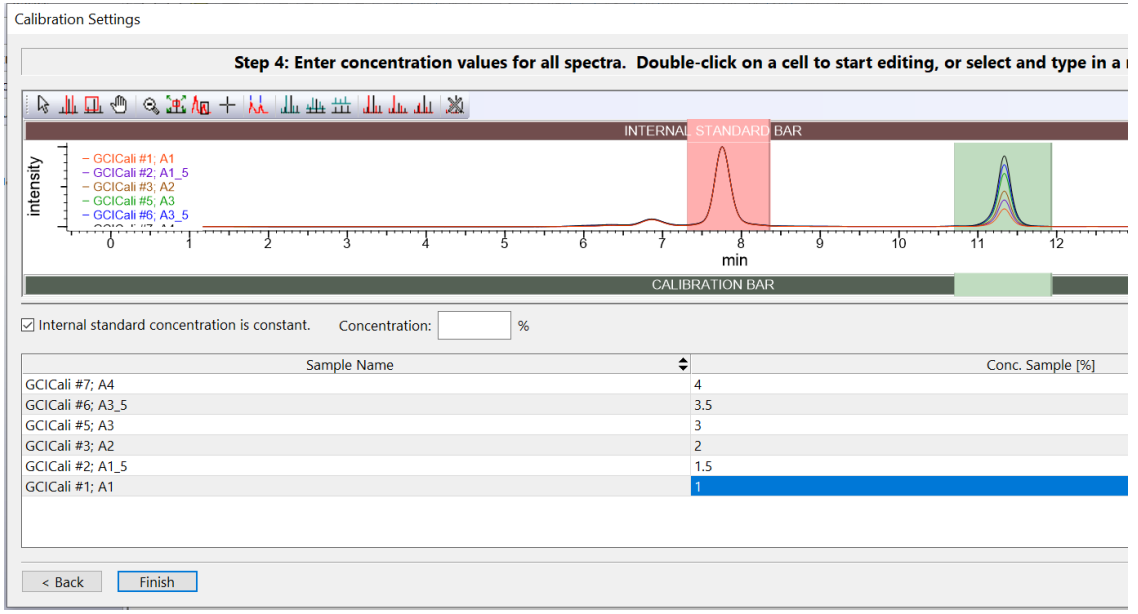
Chromatogram

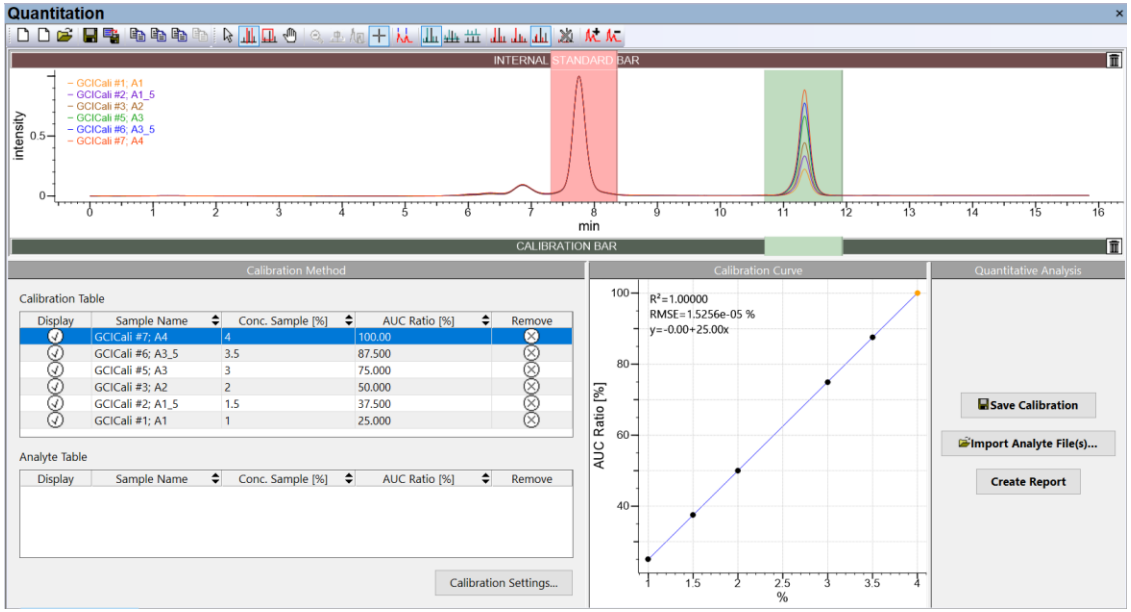
	Action	Result
1	Open the **MineIt** application by clicking its icon, typically found in the **Data** group. Choose **Database > Open.** Click the button **Open By Browsing.**	

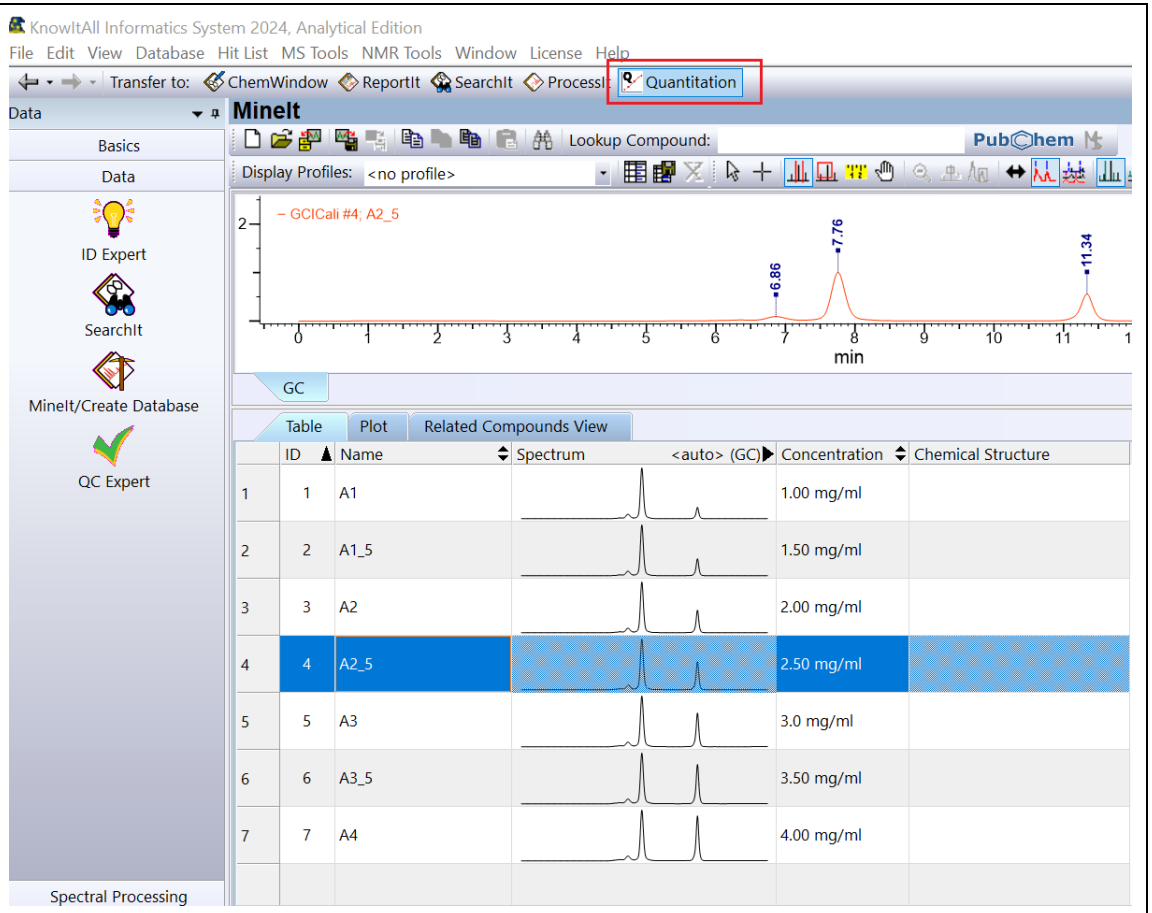
2	Navigate to the **C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration Chromatogram** folder. Select the **Chromatograms For Internal Calibration Demo.** Click **Open.** Select a set of records (leave one out to be the unknown), and then select **Transfer to: Quantitation.**	
3	Choose **Create new internal calibration** at the prompt window. Click **OK.**	

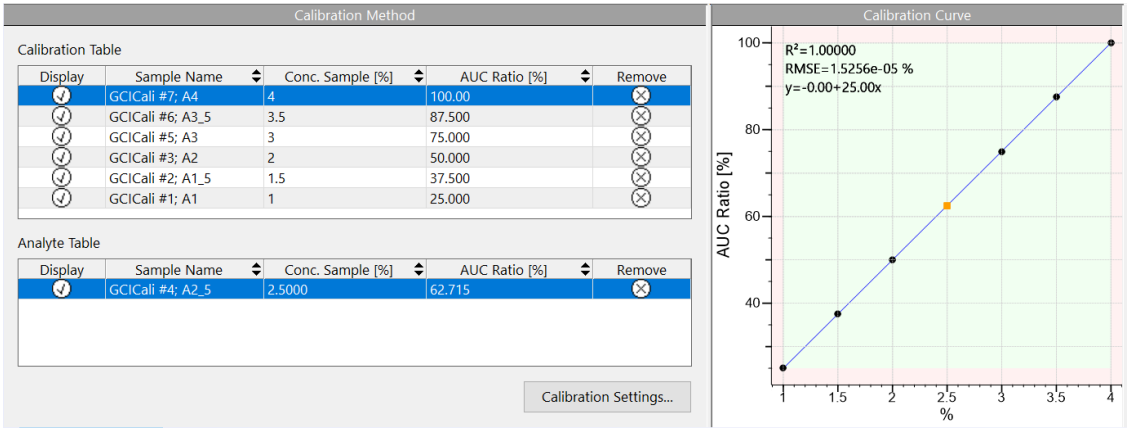
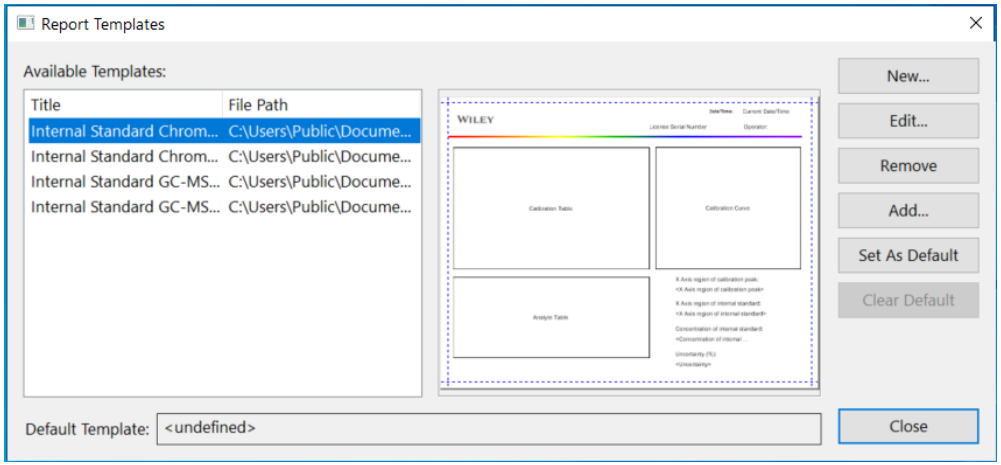
4	Select peak region around 11.3 as the calibrant peak by clicking down the CALIBRATION BAR (drag and drop). Click button **Next >.**	
5	Select peak region around 7.8 as internal standard peak by clicking down the CALIBRATION BAR (drag and drop). Click button **Next >.**	

6	In the following window, define calibration the settings as shown: (Target Unit: **%**) Click button **Next >.**	

7	Enter concentration ratios in the pop-up window. (Note that the sample names are based on concentrations but decimals have been replaced with underscores. The sample name GCICali #6; A3_5 has a sample concentration of 3.5%.) Click the **Finish** button.	

8		 Statistics are reported in the calibration curve. The lower the **RMSE** (**root mean squared error)**, the better the curve is fitting. The closer the **R^2** (**coefficient of determination**) is to 1**,** the better the curve is fitting. One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

9	Go back to the **MineIt** database. Select the file we have left out, **A2_5.** Select **Transfer to: Quantitation.** At the prompt, select **Calculate concentration.** Click **OK.**	

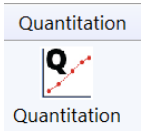
10		The concentration of the unknown is calculated and marked 
11	Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools. Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application: **File > Edit Report Templates** **Click Add button** **Navigate to the template file** **Open**	

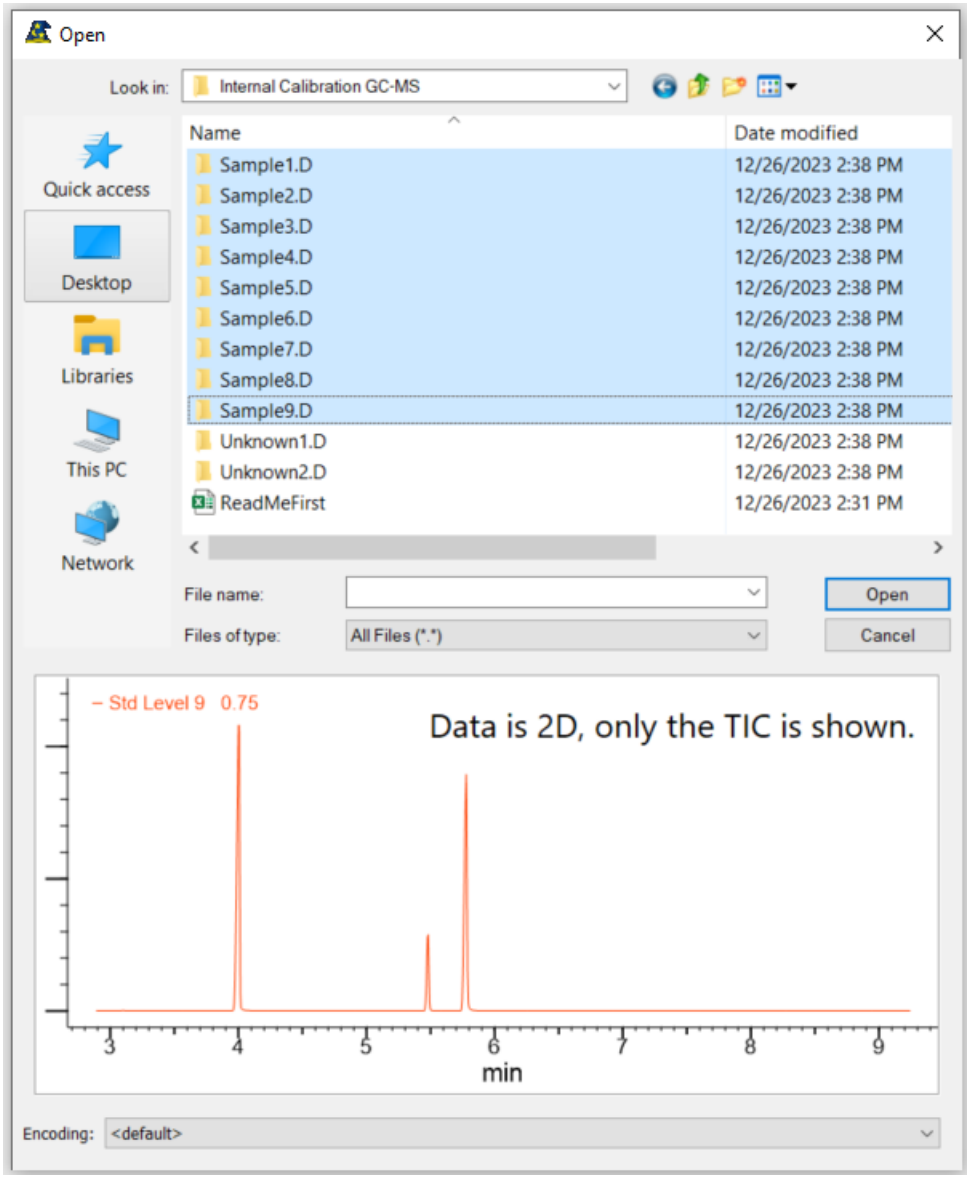
GC-MS

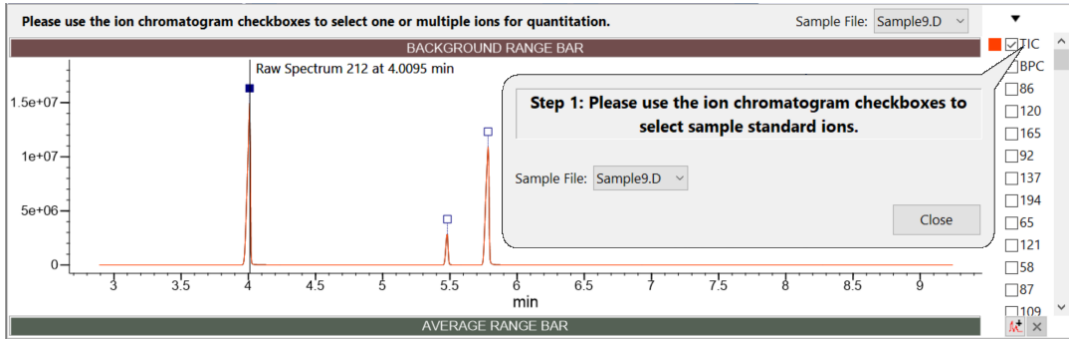
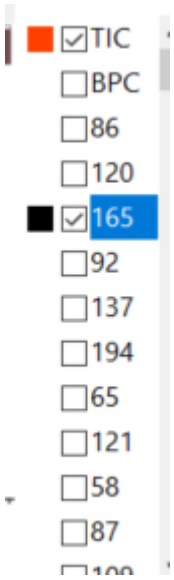
This dataset contains

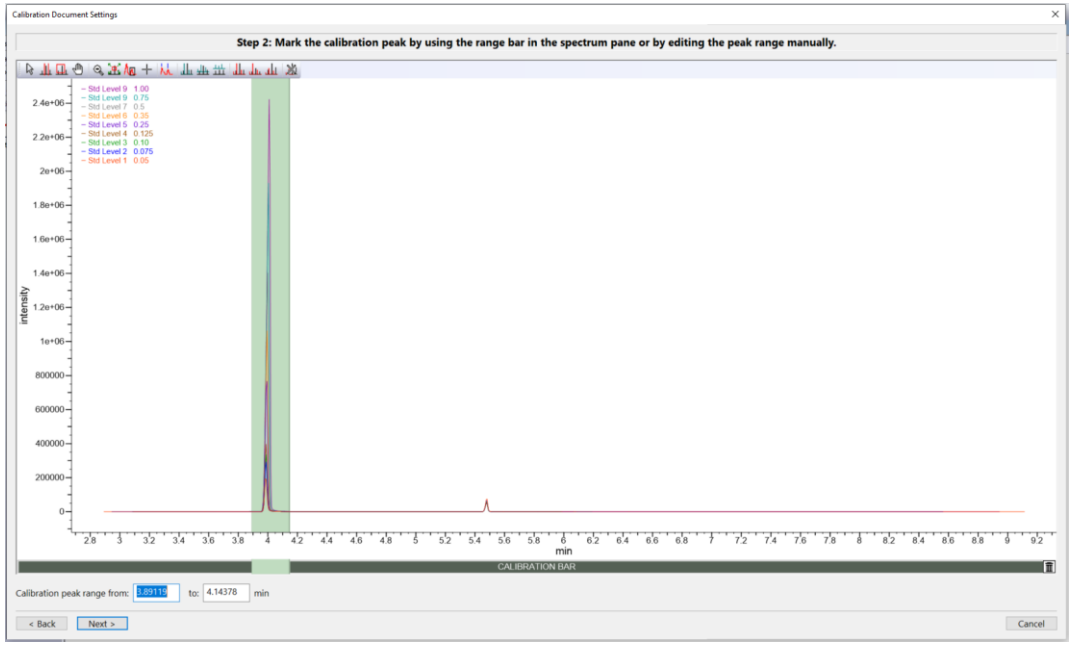
- two calibrants
  - Benzocaine - GC retention time 4.01 min, MS ion to use: 165
  - Lidocaine - GC retention time 5.78 min, MS ion to use: 86 (it breaks down in GC, therefore does not have molecular ion)
- one internal standard – Caffeine at 0.7625 mg/mL, GC retention time 5.48 min, MS ion to use: 194
- the dataset to use pick MS ion(s) is the Sample 9.

Benzocaine

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

3	Navigate to "C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS" folder. Select folders as shown in the right screenshot. Click **Open.**	

4	In the drop-down list, select the calibrant file where analyte concentration is the largest, in this example, it is the **Sample9.D**	
5	Select a component from the **Raw Spectrum** pane, in our example, the interested component has a TIC peak at **4.01** min. Select an ion, in this case, we select its **molecular m/z 165.** Click **Next >** (bottom left corner)	

6	Select peak region by clicking down the CALIBRATION BAR (drag and drop). Click button **Next >.**	

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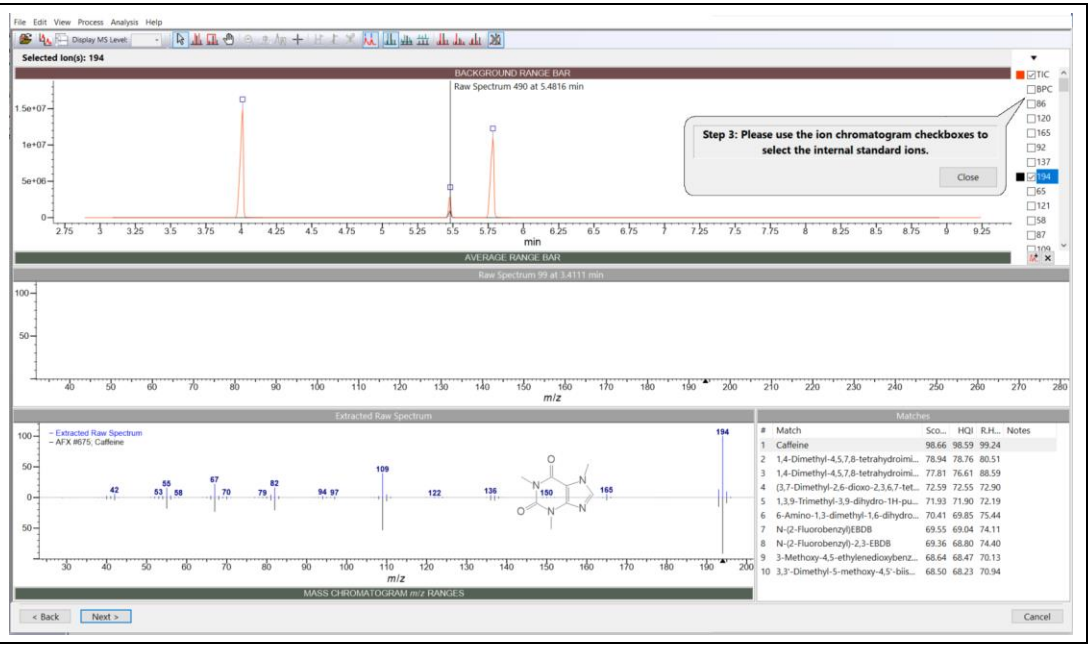
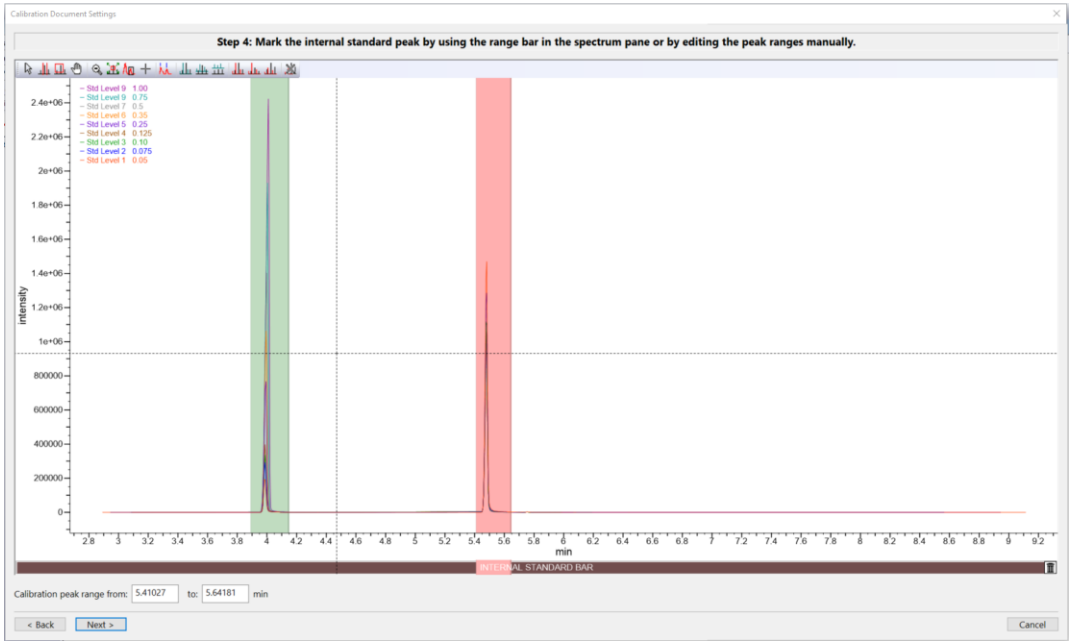
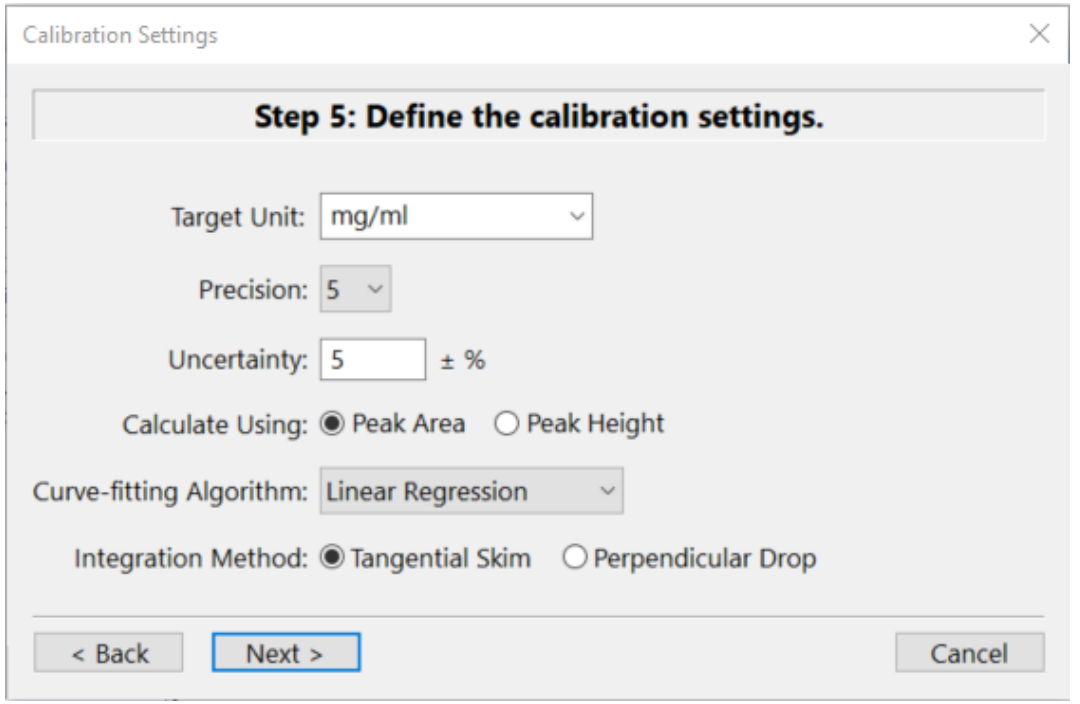
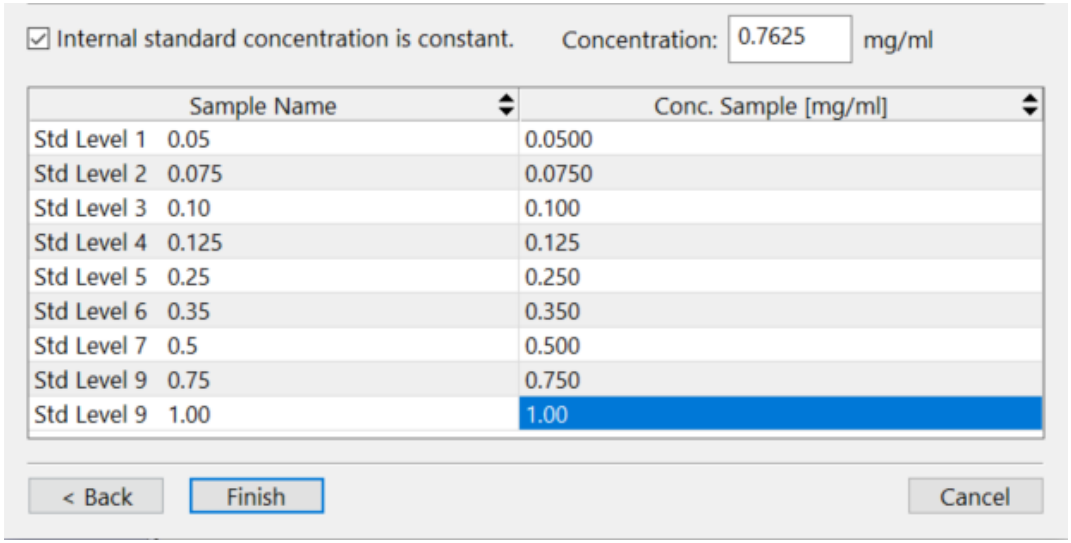
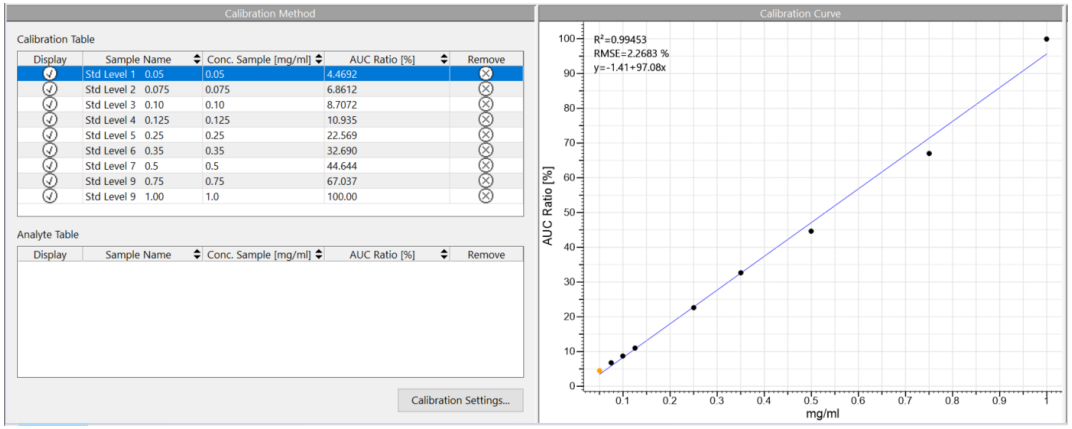
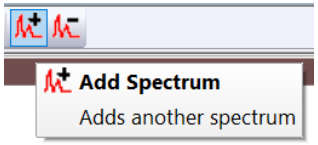
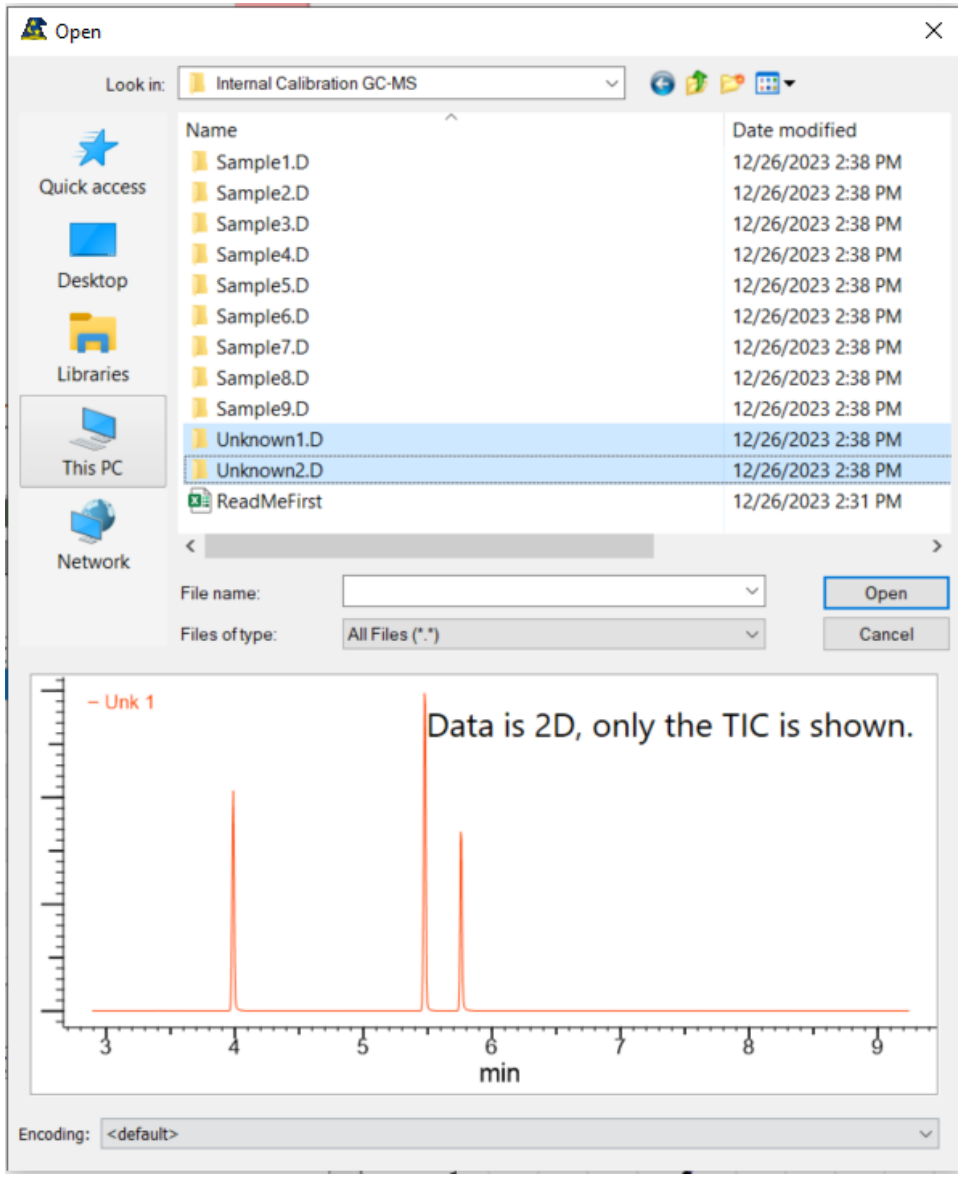
7	Select the internal standard peak from the top GC pane, in our example, **5.48** min. Select an ion, in this case, we select its **molecular m/z 194.** Click **Next >** (bottom left corner)	

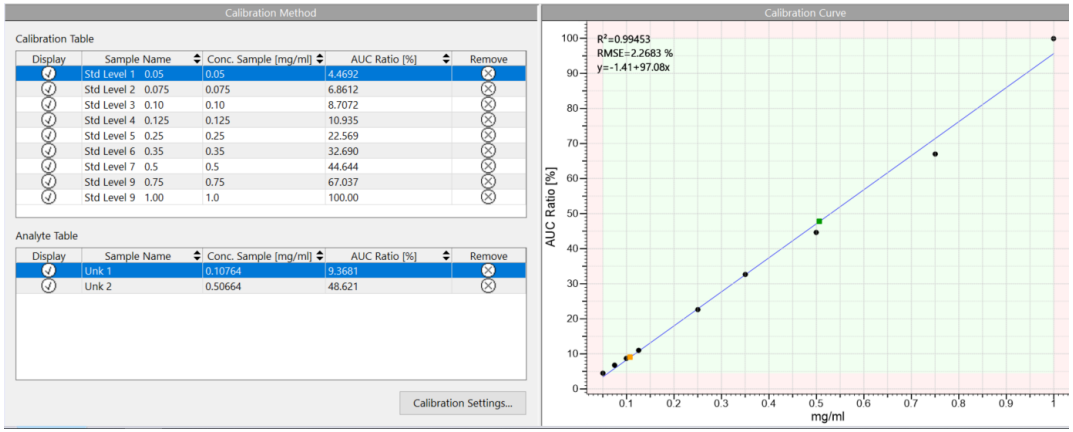
10	Select peak region by clicking down the INTERNAL STANDARD BAR (drag and drop). Click button **Next >.**	

9	In the following window, define calibration settings. Target Unit: **ug/ml** (you have to type in) Calculate Using: **Peak Area** Click button **Next >.**	

10	Enter concentration and ratio values in the pop-up window.	

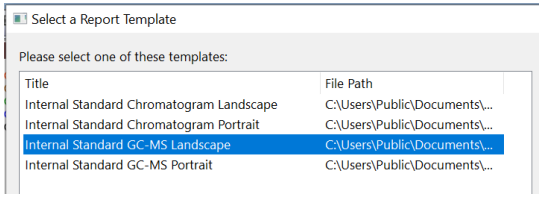
11	Click **Finish** button.	 Statistics are reported in the calibration curve. The lower the **RMSE** (**root mean squared error)**, the better the curve is fitting. The closer the **R^2** (**coefficient of determination**) is to 1**,** the better the curve is fitting. One can use ⊗ in the **Remove** cell to remove samples from calibration; and use **Add Spectrum**  to add new calibrants, One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

12	Click the **Import Analyte File(s)** button. Select unknown file folder **Unknown1.D** and **Unknown2.D** to calculate the concentrations. Click **Open.**	

13		 The concentration ratio of analyte to internal standard is shown in the **Analyte Table** and as a sqaure spot in the **Calibration Curve**.

14 Click **Transfer to**: Report

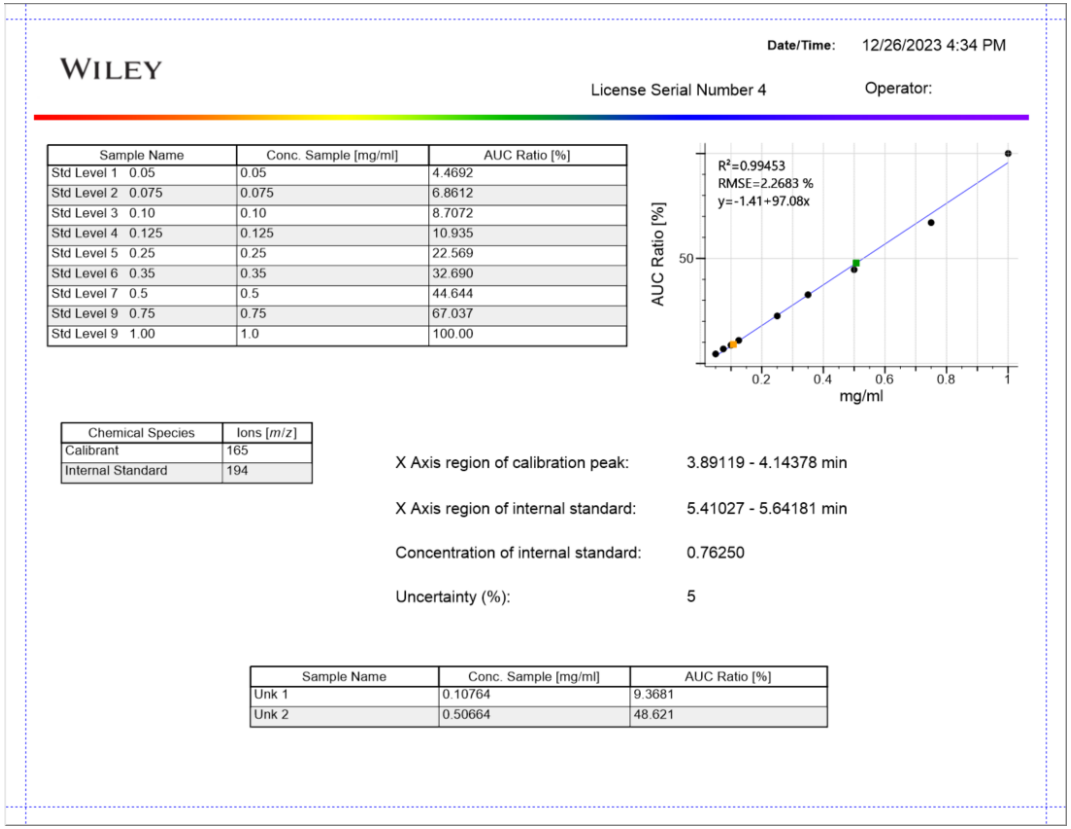
Select the **Internal Standard GC-MS Landscape** template



Click **OK**

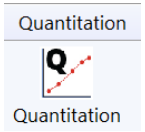
Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application:

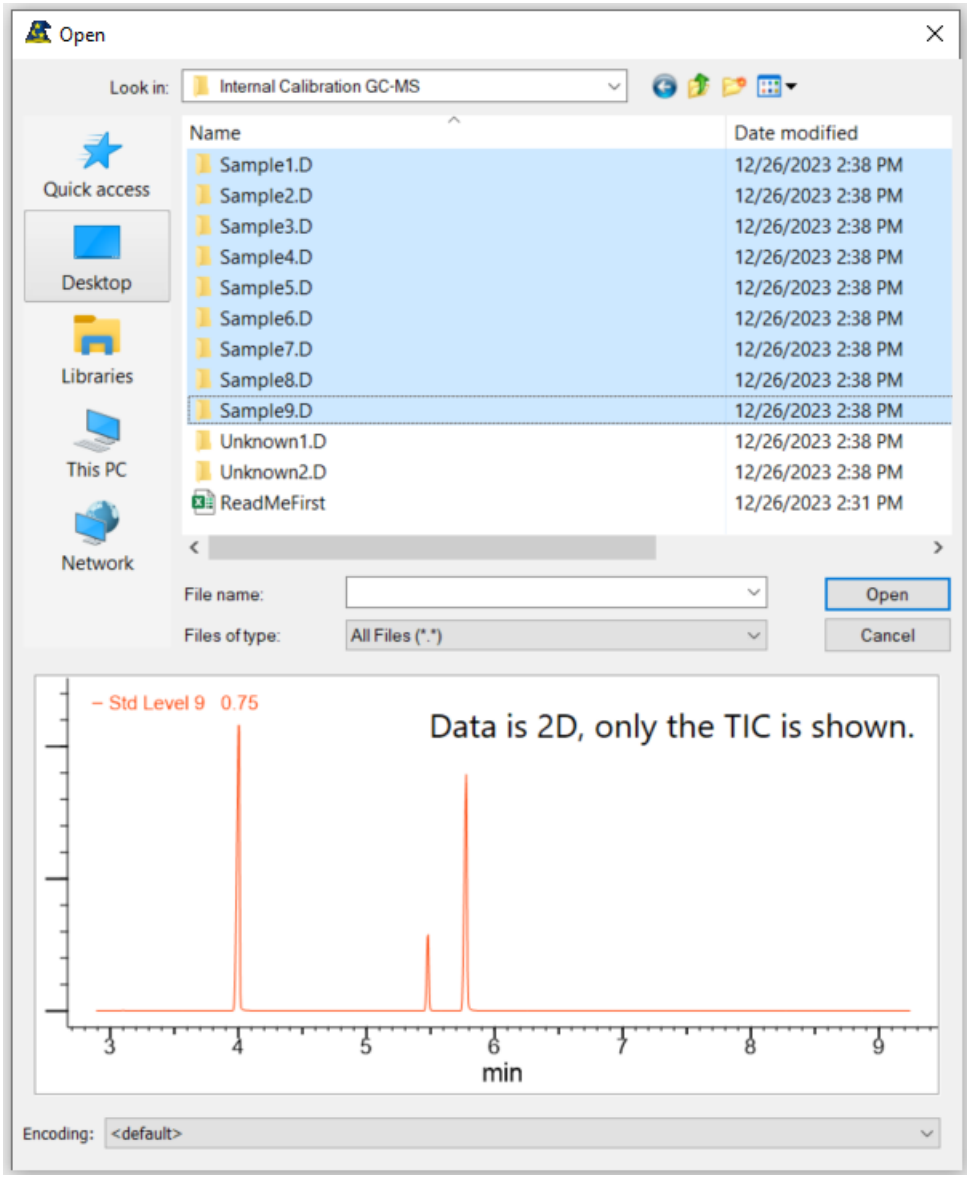
File > Edit Report Templates
Click Add button
Navigate to the template file
Open

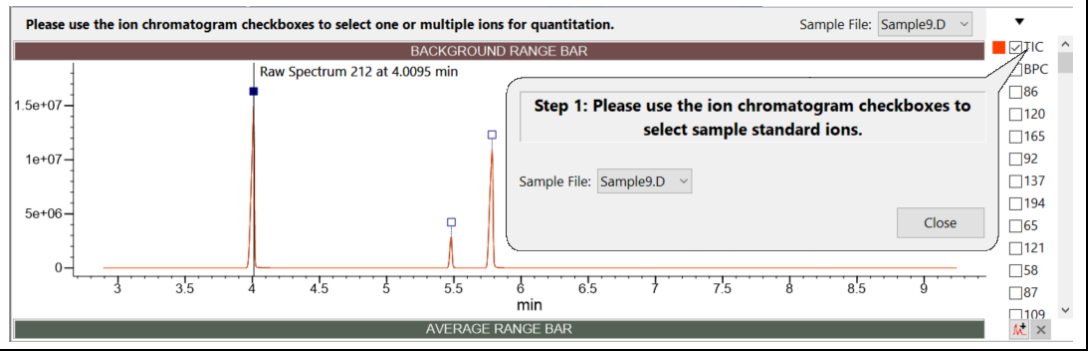


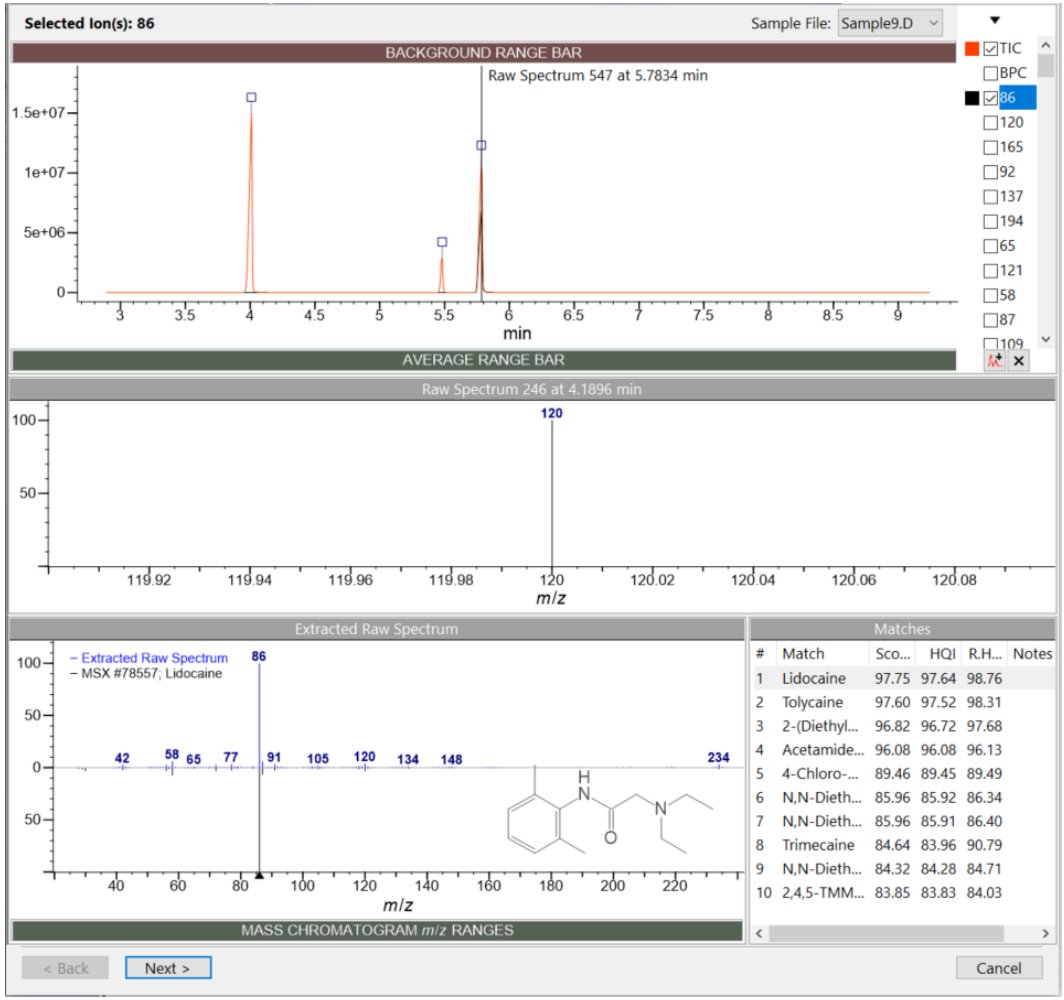
This is a basic report, one can select these objects and copy/paste to other applications.

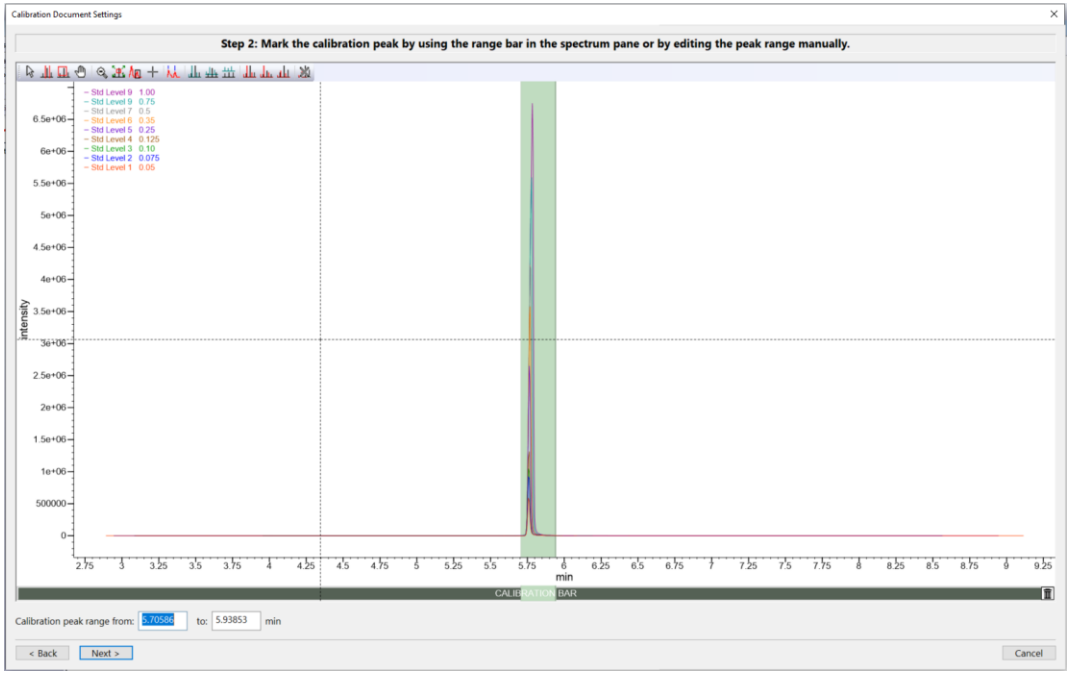
Lidocaine HCl

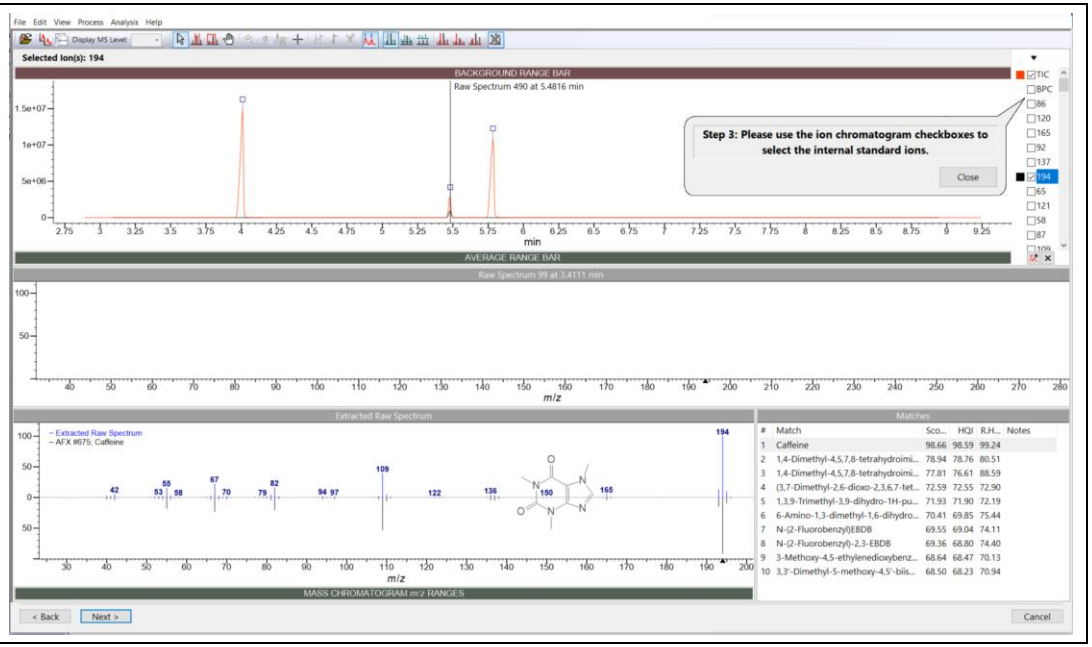
	Action	Result
1	Open the **Quantitation** application by clicking its icon, typically found in the **Quantitation** group.	
2	Click **New Internal Calibration** button.	KnowItAll prompts user to open calibrant files.

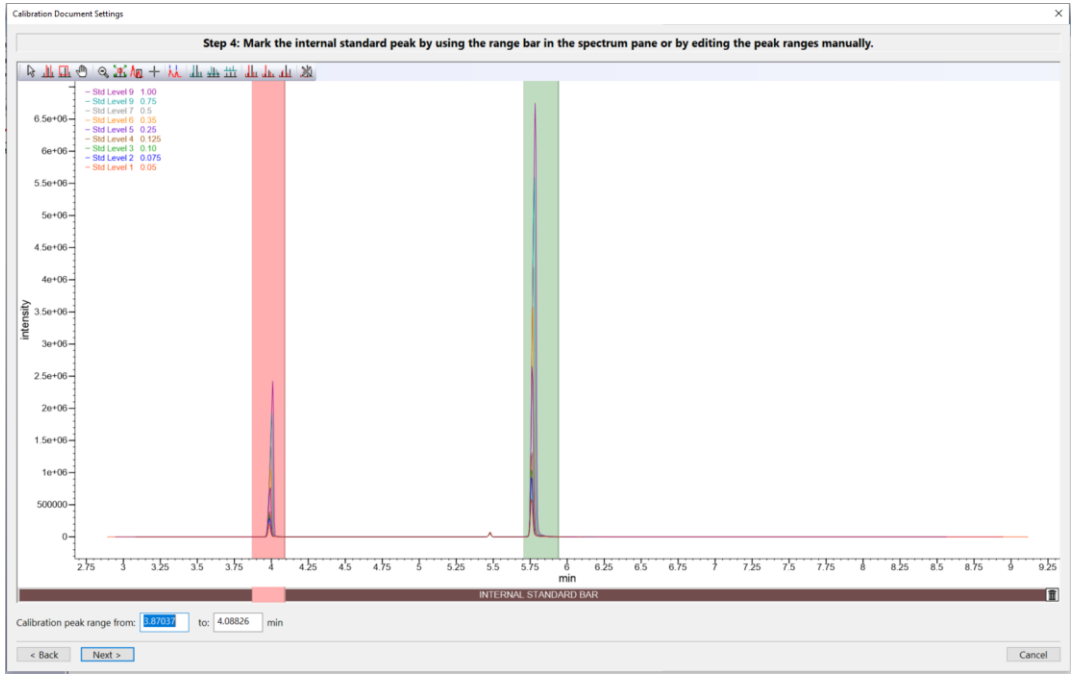
3	Navigate to "C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\Internal Calibration GC-MS" folder. Select folders as shown in the right screenshot. Click **Open.**	

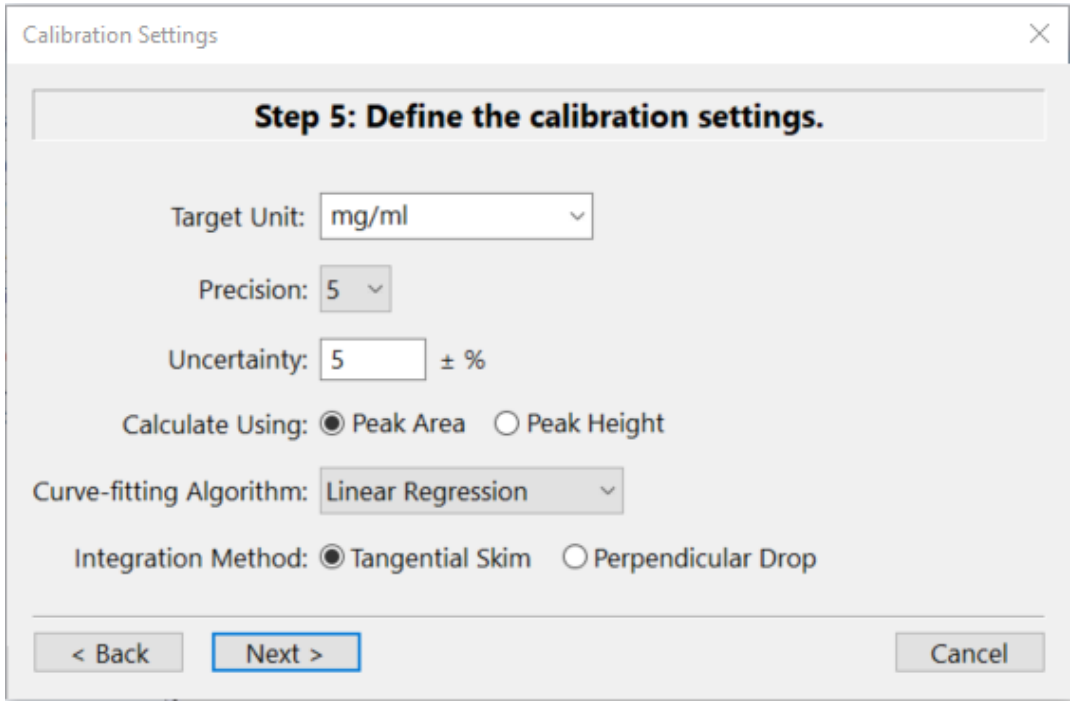
4	In the drop-down list, select the calibrant file where analyte concentration is the largest, in this example, it is the **Sample9.D**	

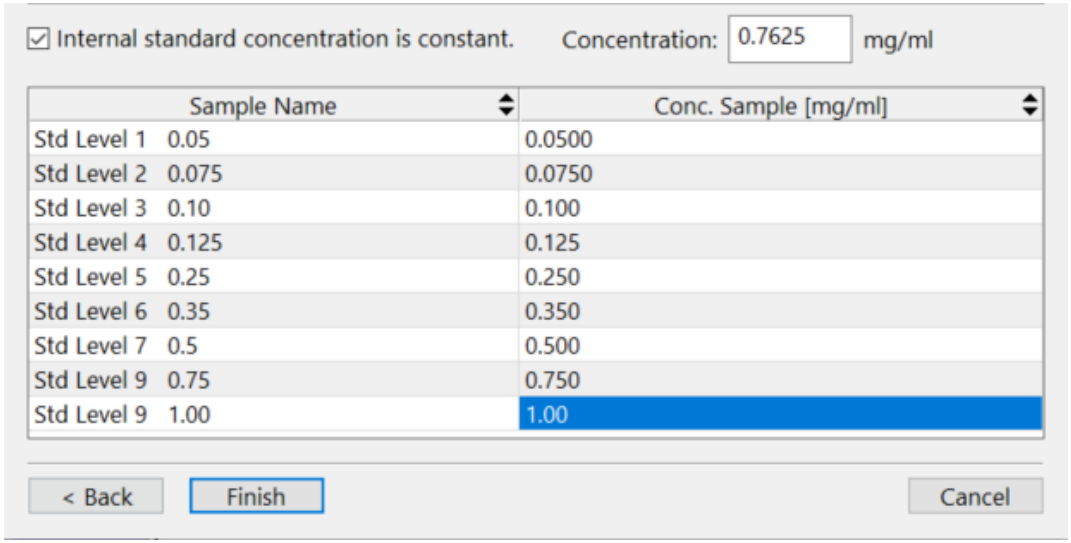
5	Select a component from the **Raw Spectrum** pane, in our example, the interested component has a TIC peak at **5.78** min. This sample breaks down in GC, so there is no molecular ion in extracted MS. We select its strongest ion **86.** Click **Next >** (bottom left corner)	

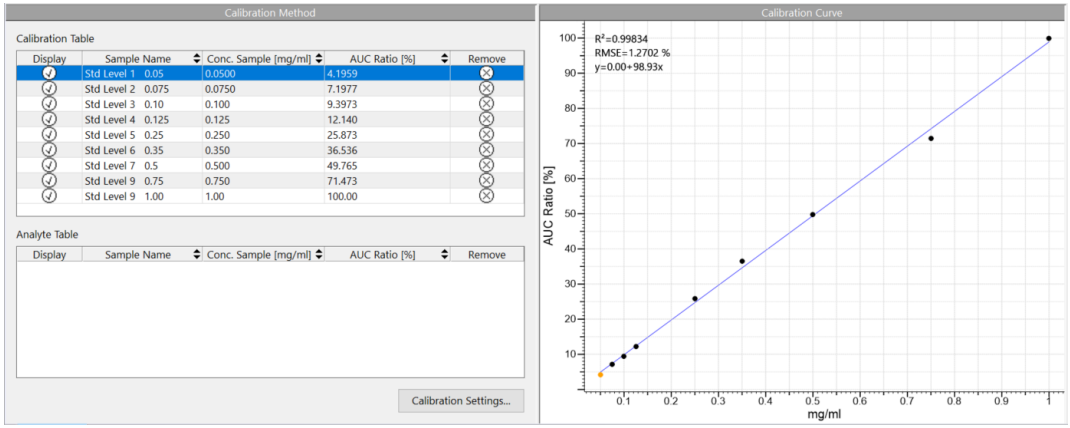
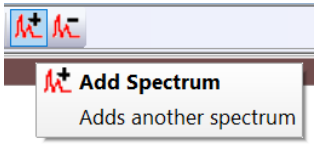
6	Select peak region by clicking down the CALIBRATION BAR (drag and drop). Click button **Next >.**	

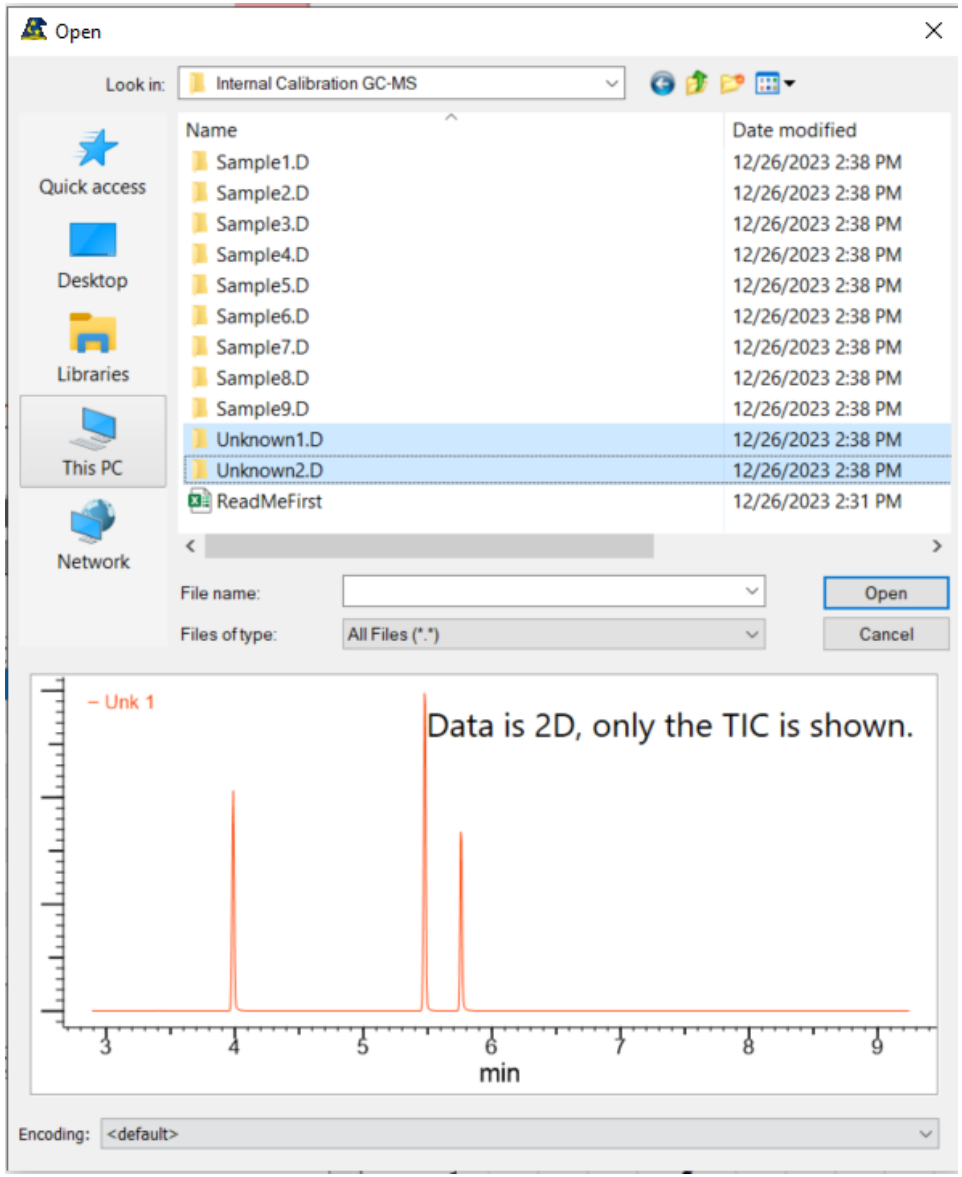
7	Select the internal standard peak from the top GC pane, in our example, **5.48** min. Select an ion, in this case, we select its **molecular m/z 194.** Click **Next >** (bottom left corner)	

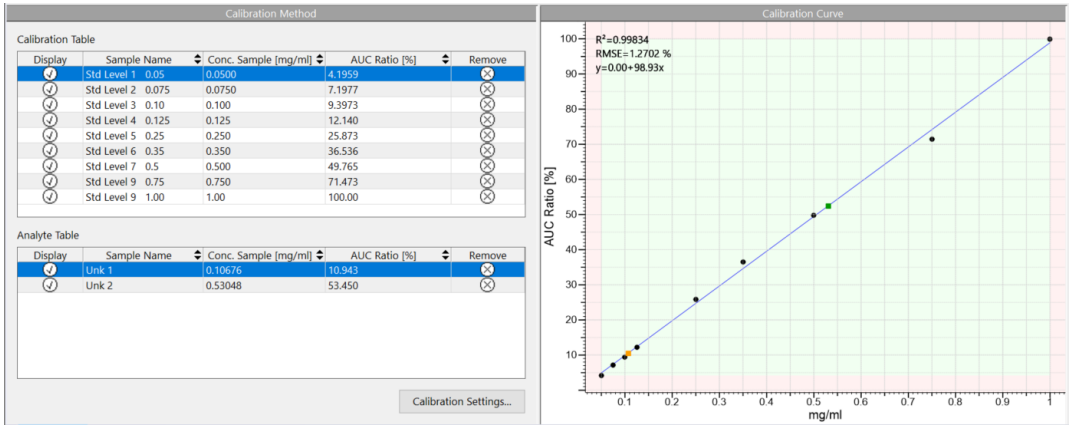
10	Select peak region by clicking down the INTERNAL STANDARD BAR (drag and drop). Click button **Next >.**	

9	In the following window, define calibration settings. Target Unit: **ug/ml** (you have to type in) Calculate Using: **Peak Area** Click button **Next >.**	

10	Enter concentration and ratio values in the pop-up window.	

11	Click **Finish** button.	 Statistics are reported in the calibration curve. The lower the **RMSE** (**root mean squared error)**, the better the curve is fitting. The closer the **R^2** (**coefficient of determination**) is to 1**,** the better the curve is fitting. One can use ⊗ in the **Remove** cell to remove samples from calibration; and use **Add Spectrum**  to add new calibrants, One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

12	Click the **Import Analyte File(s)** button. Select unknown file folder **Unknown1.D** and **Unknown2.D** to calculate the concentrations. Click **Open.**	

13		 The concentration ratio of analyte to internal standard is shown in the **Analyte Table** and as a sqaure spot in the **Calibration Curve**.