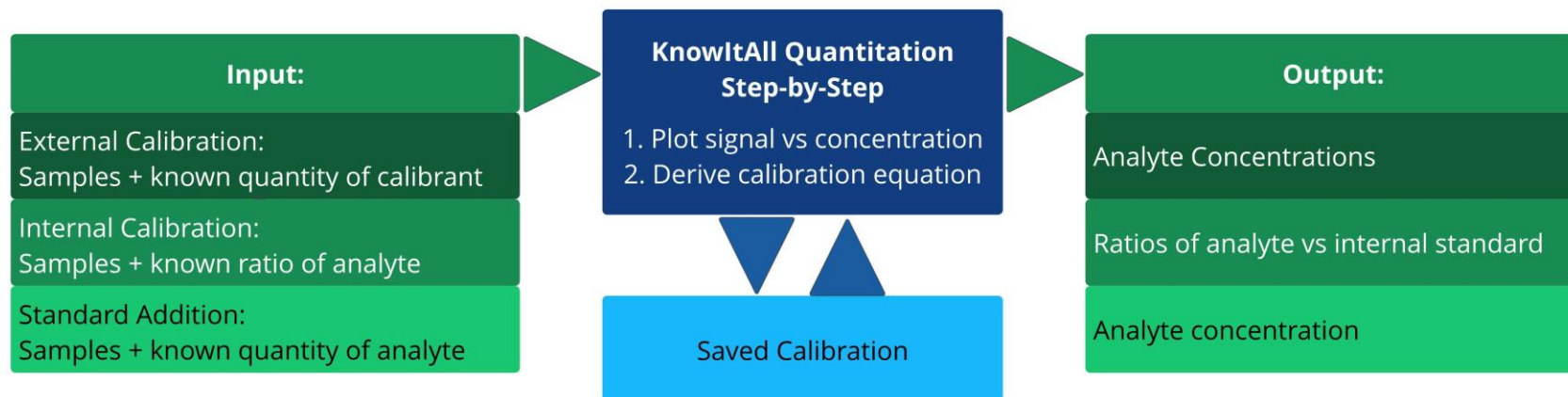


KnowItAll Informatics Training

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

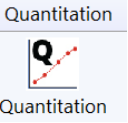
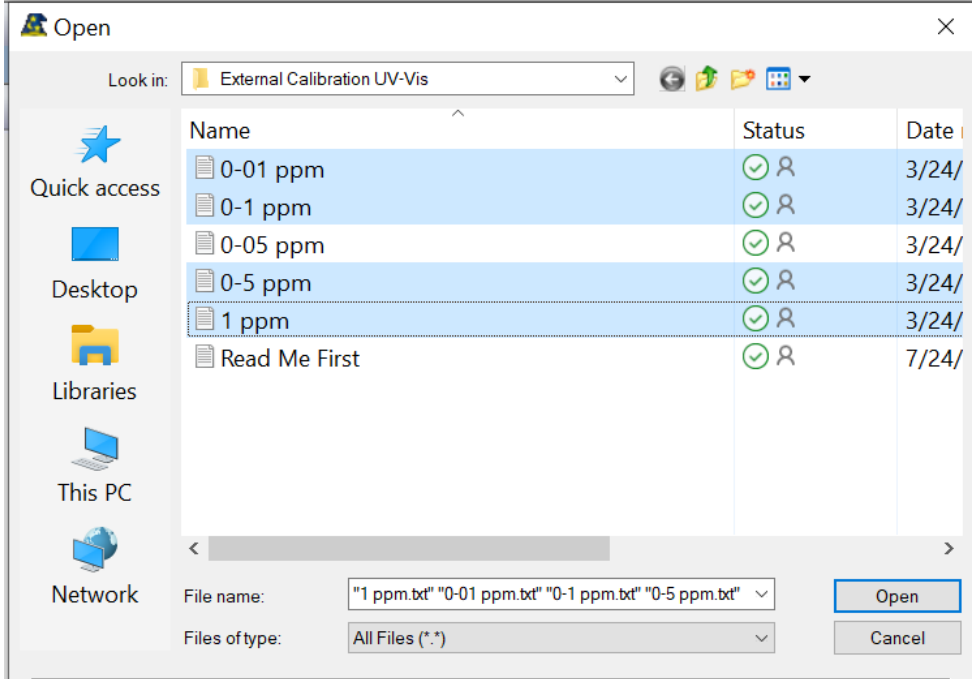
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation folder

- 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	<p>Navigate to the C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration UV-Vis folder.</p> <p>Select sample files and leave one out as the “unknown.”</p> <p>Click Open.</p>	

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

Technique Parameters

Data Type: IR

X Axis Unit: Vapor Phase IR

Y Axis Unit: Raman

Data is spectral Data is table

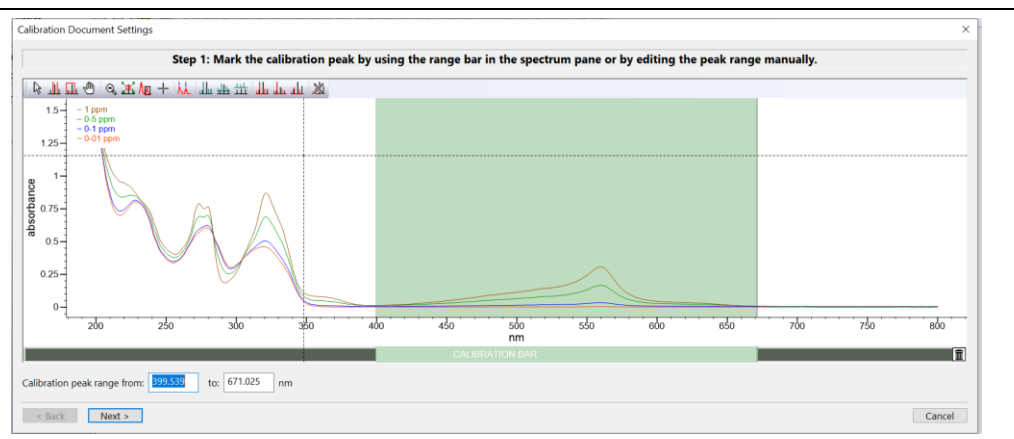
X	Y
800.0401	
798.9311	
797.9603	0.0007610
796.9891	0.0008650
796.0175	-2.15e-05
795.0456	0.000640
793.9344	0.0003440
792.9616	-0.0001240
791.9885	0.0001950
791.0150	0.001140

Apply Parameters to All Files

OK Cancel

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 >**.

Calibration Settings

Step 2: Define the calibration settings.

Target Unit:

Precision:

Uncertainty: ± %

Calculate Using: Peak Area Peak Height

Curve-fitting Algorithm:

Integration Method: Tangential Skim Perpendicular Drop

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.

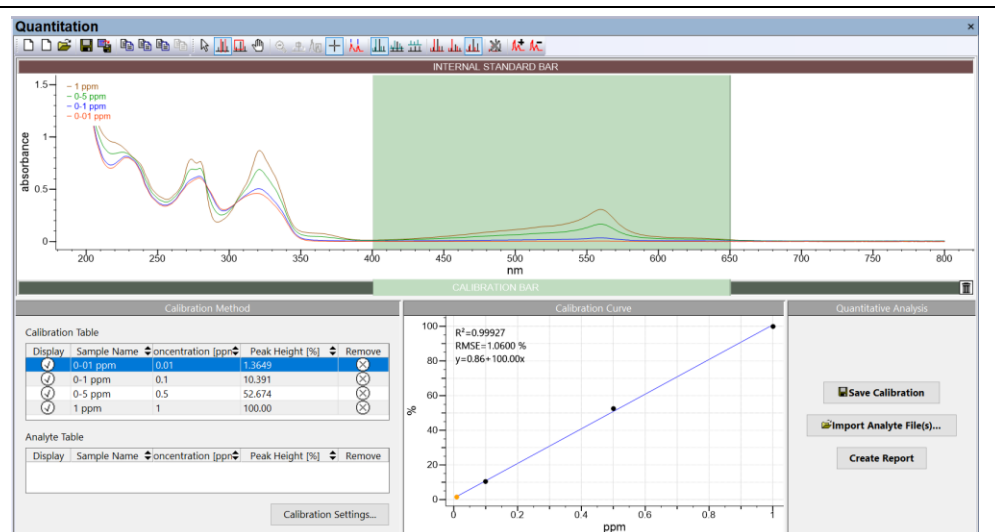
Calibration Settings

Step 3: Enter concentration values for all spectra. Double-click on a cell to start editing, or select and type in

Sample Name	Concentration [ppm]
0-01 ppm	0.01
0-1 ppm	0.1
0-5 ppm	0.5
1 ppm	1

< Back Finish

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 **R² (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.

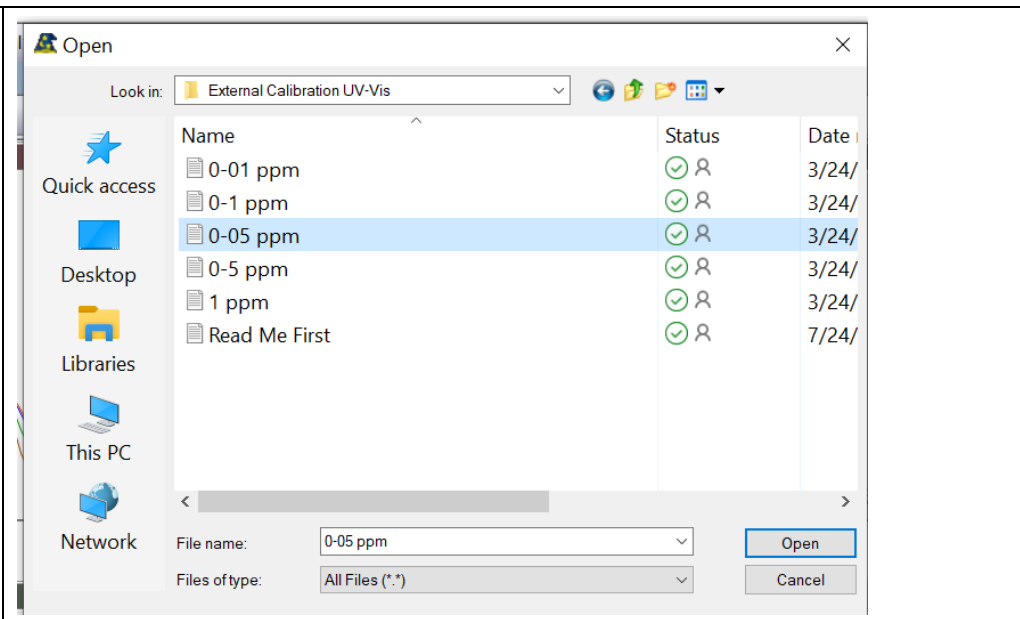
9 Click the **Import Analyte File(s)** button.

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.

Calibration Method

Display	Sample Name	Concentration [ppm]	Peak Height [%]	Remove
✓	0-01 ppm	0.01	1.3649	✕
✓	0-1 ppm	0.1	10.391	✕
✓	0-5 ppm	0.5	52.674	✕
✓	1 ppm	1	100.00	✕

Display	Sample Name	Concentration [ppm]	Peak Height [%]	Remove
✓	0-05 ppm	0.042183	5.0764	✕

Calibration Settings...

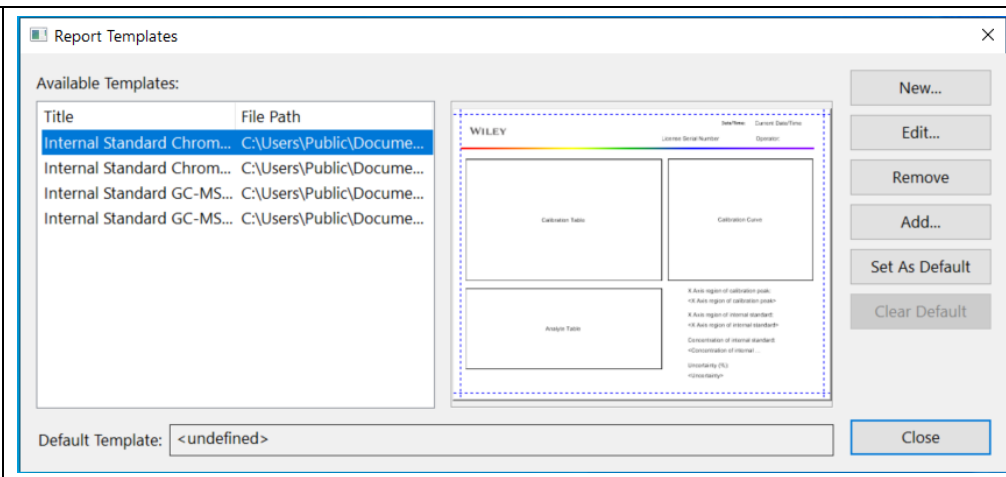
Calibration Curve

$R^2 = 0.99927$
 $RMSE = 1.0600\%$
 $y = 0.86 + 100.00x$

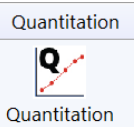
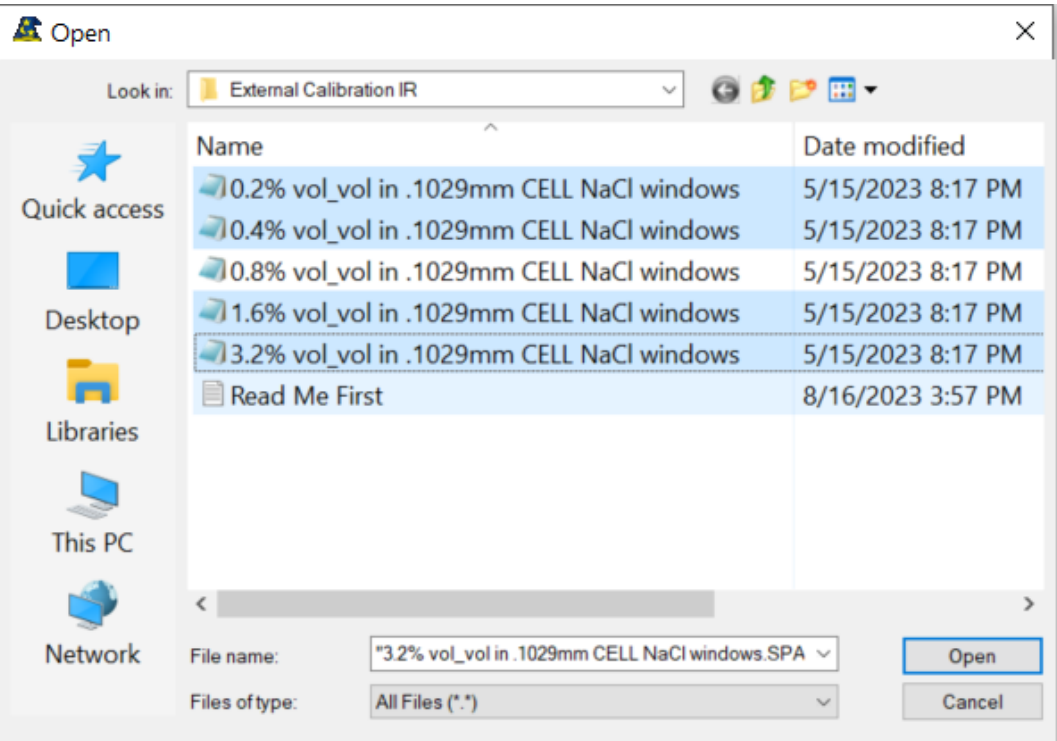
11 Click the **Create Report** button or use **Transfer to: ReportIt** to can generate a report in which objects can be copied/pasted into other desktop tools.

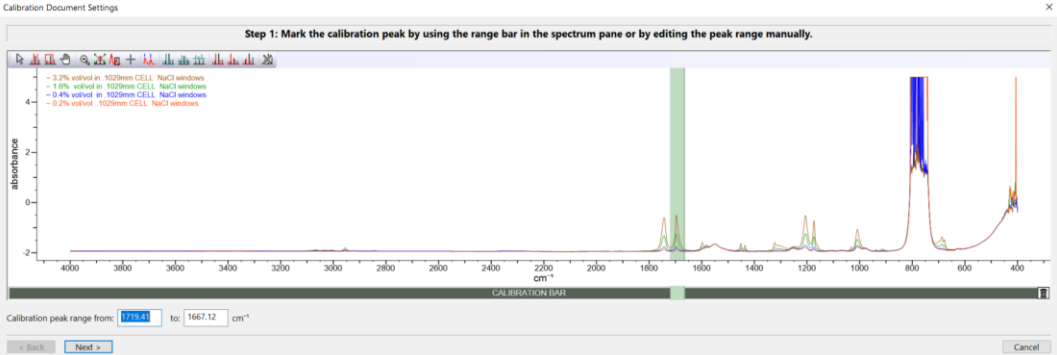
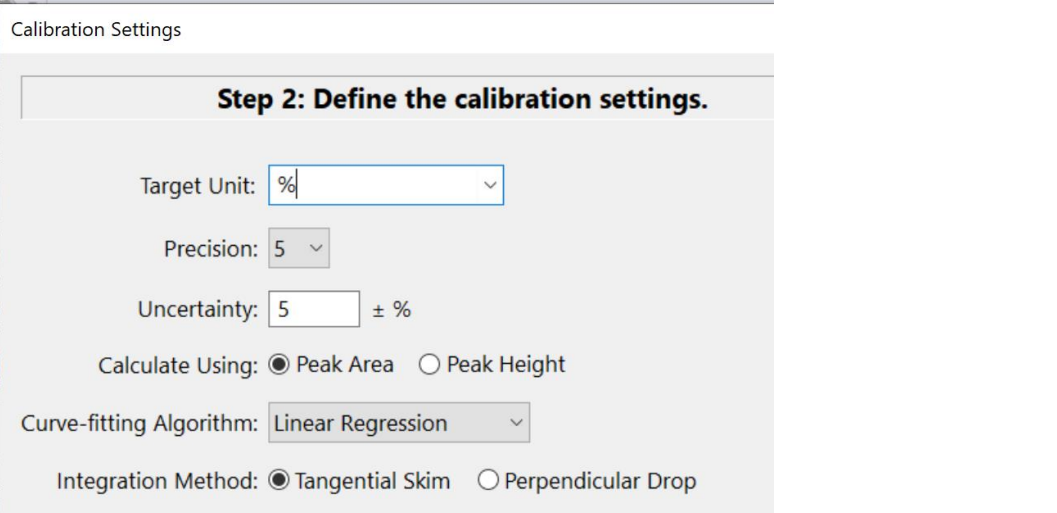
Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application:

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

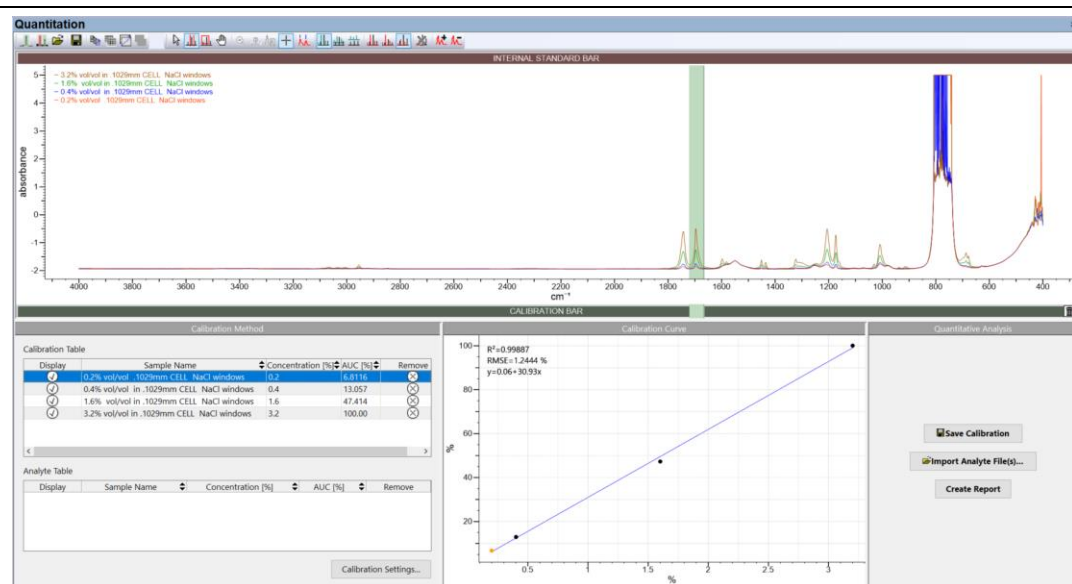


IR

	Action	Result														
1	Open the Quantitation application by clicking its icon, typically found in the Quantitation group.	 <p>Quantitation</p>														
2	Click New External Calibration button.	KnowItAll prompts user to open calibrant files.														
3	<p>Navigate to the C:\Users\Public\Documents\Wiley\KnowItAll\Samples\Quantitation\External Calibration IR folder.</p> <p>Select sample files and leave one (0.8%) out as the “unknown.”</p> <p>Click Open.</p>	 <p>The screenshot shows a Windows File Explorer window titled 'Open' with the address bar set to 'External Calibration IR'. The file list contains the following items:</p> <table border="1"> <thead> <tr> <th>Name</th> <th>Date modified</th> </tr> </thead> <tbody> <tr> <td>0.2% vol_vol in .1029mm CELL NaCl windows</td> <td>5/15/2023 8:17 PM</td> </tr> <tr> <td>0.4% vol_vol in .1029mm CELL NaCl windows</td> <td>5/15/2023 8:17 PM</td> </tr> <tr> <td>0.8% vol_vol in .1029mm CELL NaCl windows</td> <td>5/15/2023 8:17 PM</td> </tr> <tr> <td>1.6% vol_vol in .1029mm CELL NaCl windows</td> <td>5/15/2023 8:17 PM</td> </tr> <tr> <td>3.2% vol_vol in .1029mm CELL NaCl windows</td> <td>5/15/2023 8:17 PM</td> </tr> <tr> <td>Read Me First</td> <td>8/16/2023 3:57 PM</td> </tr> </tbody> </table> <p>The 'File name' field at the bottom contains '3.2% vol_vol in .1029mm CELL NaCl windows.SPA' and the 'Files of type' is set to 'All Files (*.*)'. The 'Open' button is highlighted.</p>	Name	Date modified	0.2% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM	0.4% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM	0.8% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM	1.6% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM	3.2% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM	Read Me First	8/16/2023 3:57 PM
Name	Date modified															
0.2% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM															
0.4% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM															
0.8% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM															
1.6% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM															
3.2% vol_vol in .1029mm CELL NaCl windows	5/15/2023 8:17 PM															
Read Me First	8/16/2023 3:57 PM															

<p>4</p>	<p>Select peak region around 1696 cm^{-1} by clicking down the CALIBRATION BAR (drag and drop).</p> <p>Note: In IR quantitation, one should avoid using the strongest peak.</p> <p>Click button Next >.</p>											
<p>6</p>	<p>In the following window, define calibration settings:</p> <p>(Target Unit: % Calculate Using: Peak Area)</p> <p>Click button Next >.</p>											
<p>7</p>	<p>Enter concentrations in the pop-up window based on the numbers in the sample names.</p> <p>Click Finish button.</p>	<table border="1" data-bbox="829 1193 1879 1404"> <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											

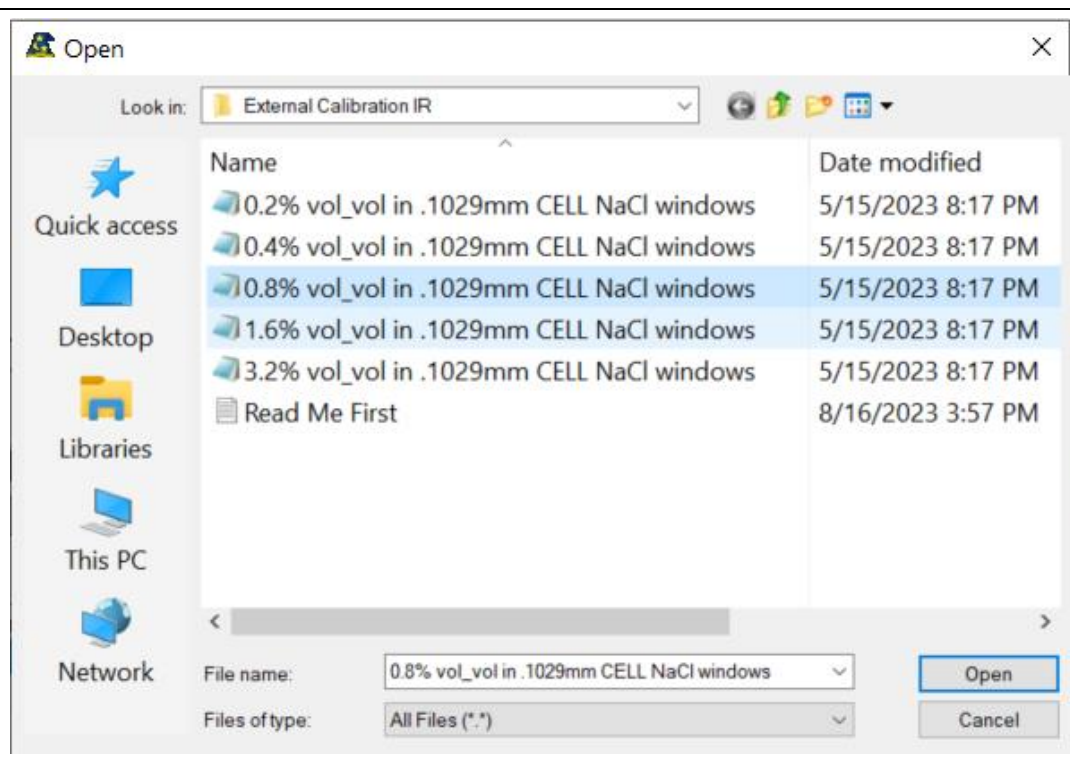
8



Statistics are reported in the calibration curve. The lower the **RMSE (root mean squared error)**, the better the curve is fitting. The closer the **R² (coefficient of determination)** is to 1, the better the curve is fitting.

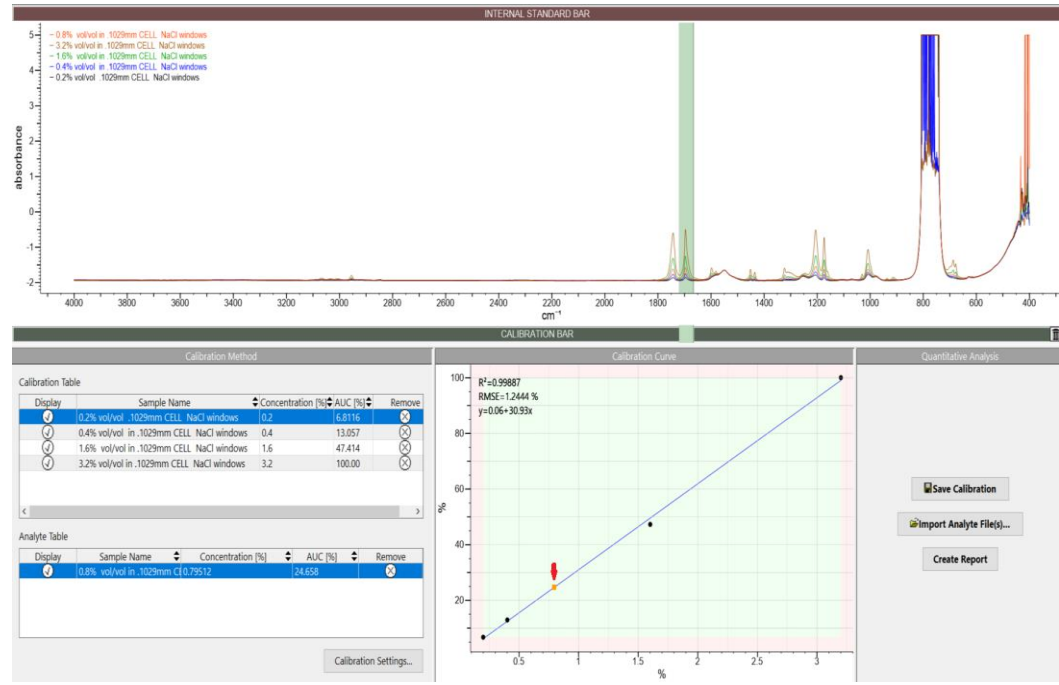
One can reset parameters using the **Calibration Settings** button. One can save this calibration for future use or sharing by clicking the **Save Calibration** button.

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



10

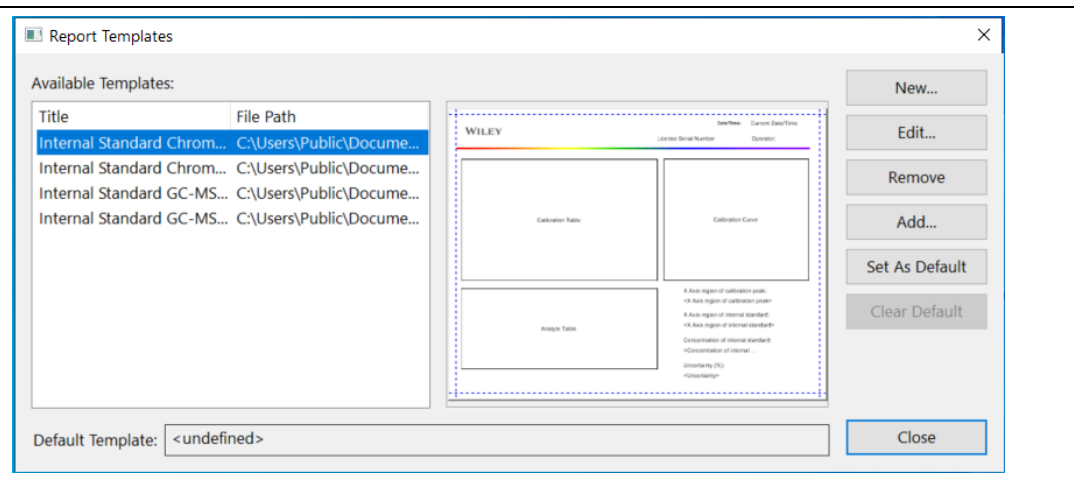
The concentration of the unknown is calculated and marked.



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:

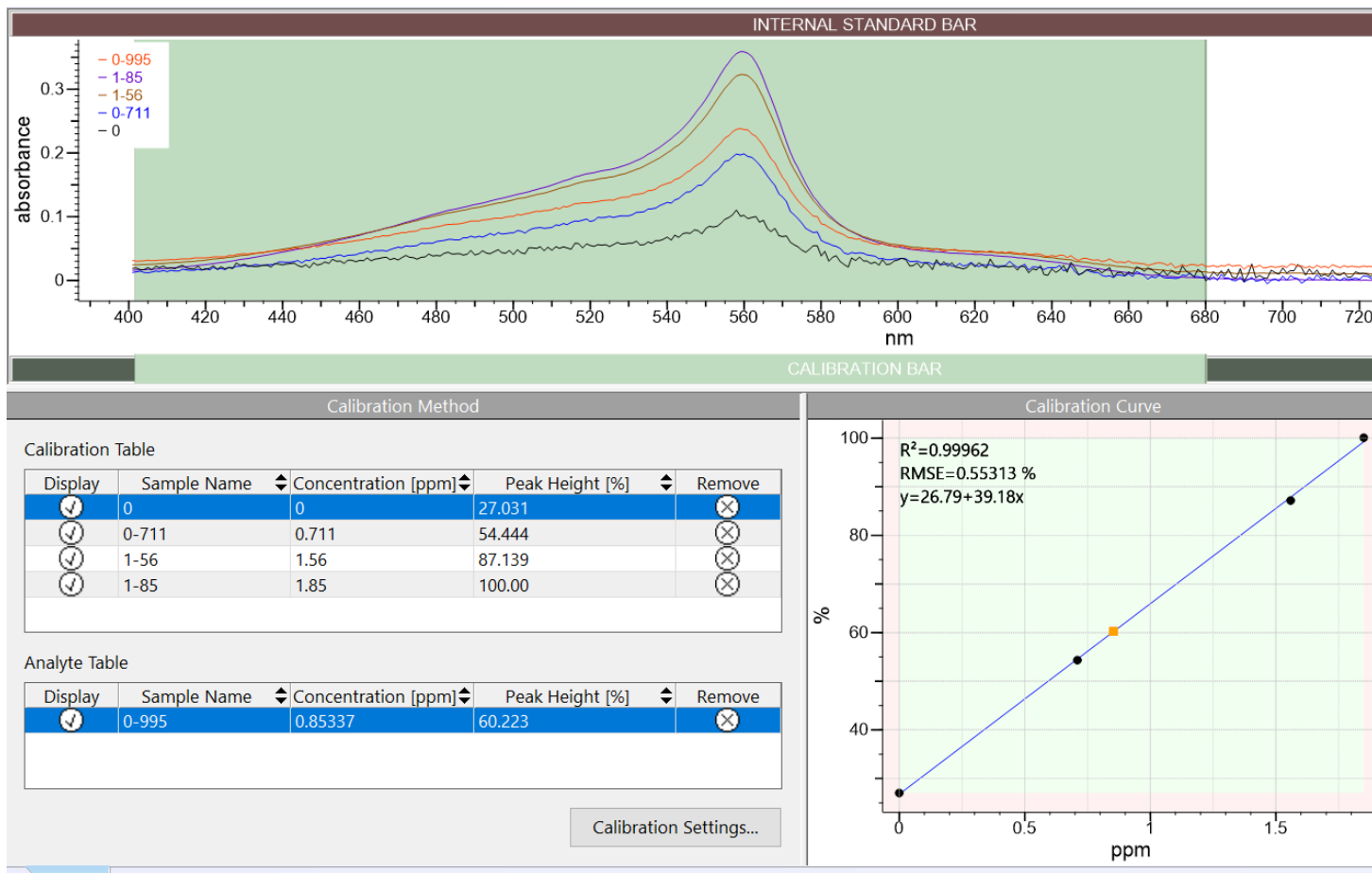
File > Edit Report Templates
Click Add button
Navigate to the template file
Open



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:



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

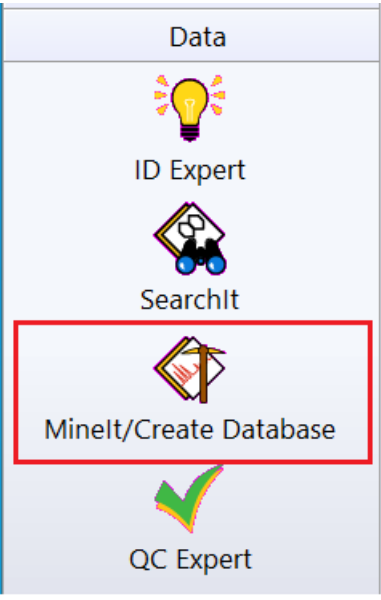
C:\Users\Public\Documents\Wiley\KnowItAll\Samples\
Quantitation folder

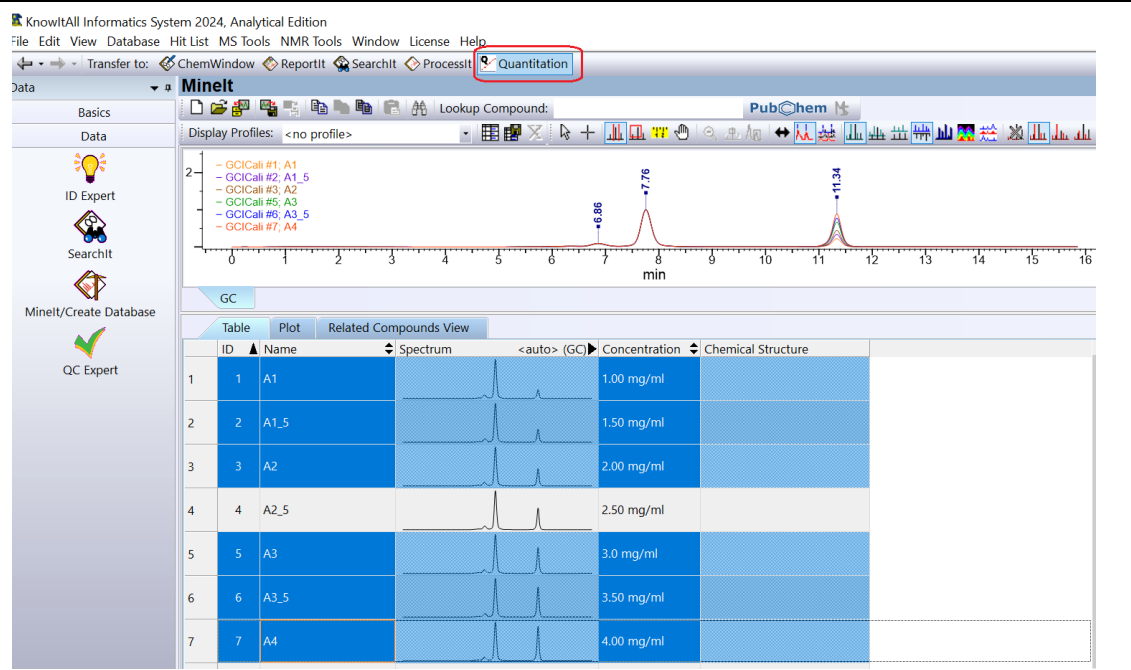
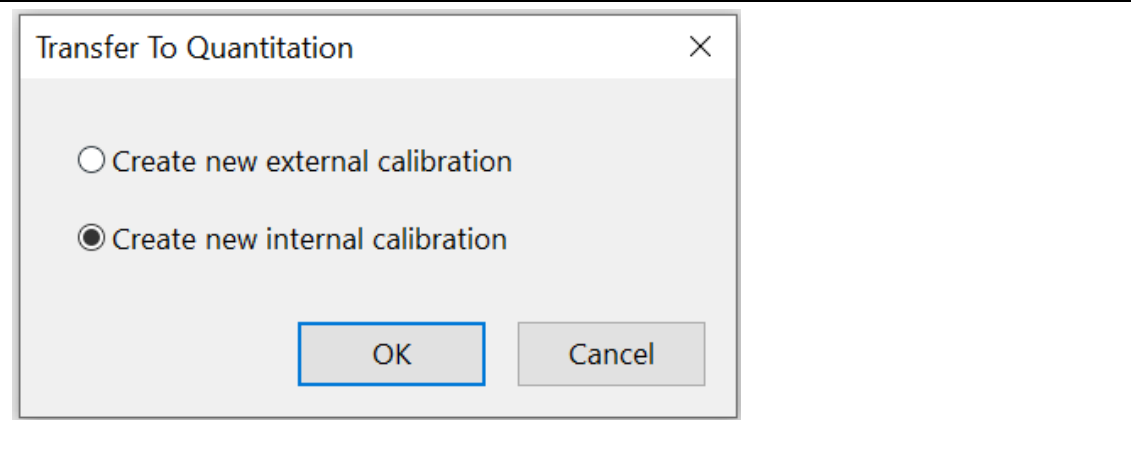
- Internal Calibration Chromatogram

KnowItAll Applications Used

- Quantitation

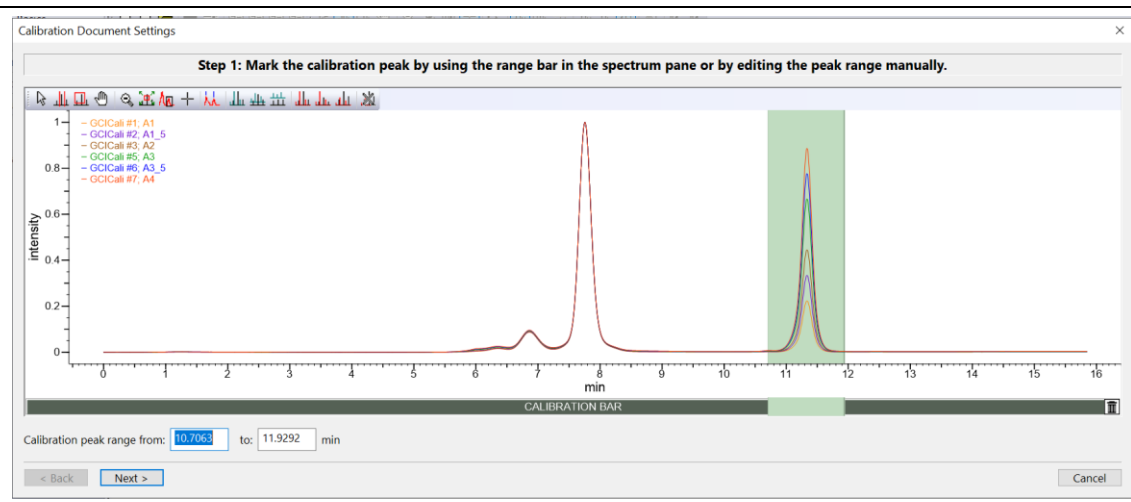
Chromatogram

	Action	Result
1	<p>Open the Minelt application by clicking its icon, typically found in the Data group.</p> <p>Choose Database > Open.</p> <p>Click the button Open By Browsing.</p>	 <p>The screenshot shows a vertical menu with the following items from top to bottom:</p> <ul style="list-style-type: none">DataID Expert (with a lightbulb icon)SearchIt (with a magnifying glass icon)Minelt/Create Database (with a document and pencil icon, highlighted by a red box)QC Expert (with a checkmark icon)

2	<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.</p> <p>Click Open.</p> <p>Select a set of records (leave one out to be the unknown), and then select Transfer to: Quantitation.</p>	 <p>The screenshot shows the Minelt software interface. The top menu bar includes File, Edit, View, Database, Hit List, MS Tools, NMR Tools, Window, License, and Help. The 'Transfer to:' dropdown menu is set to 'Quantitation'. The main window displays a chromatogram with peaks at 6.86, 7.76, and 11.34 minutes. Below the chromatogram is a table with the following data:</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></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><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																																							
3	<p>Choose Create new internal calibration at the prompt window.</p> <p>Click OK.</p>	 <p>The dialog box titled 'Transfer To Quantitation' has two radio button options: 'Create new external calibration' (unselected) and 'Create new internal calibration' (selected). There are 'OK' and 'Cancel' buttons at the bottom.</p>																																								

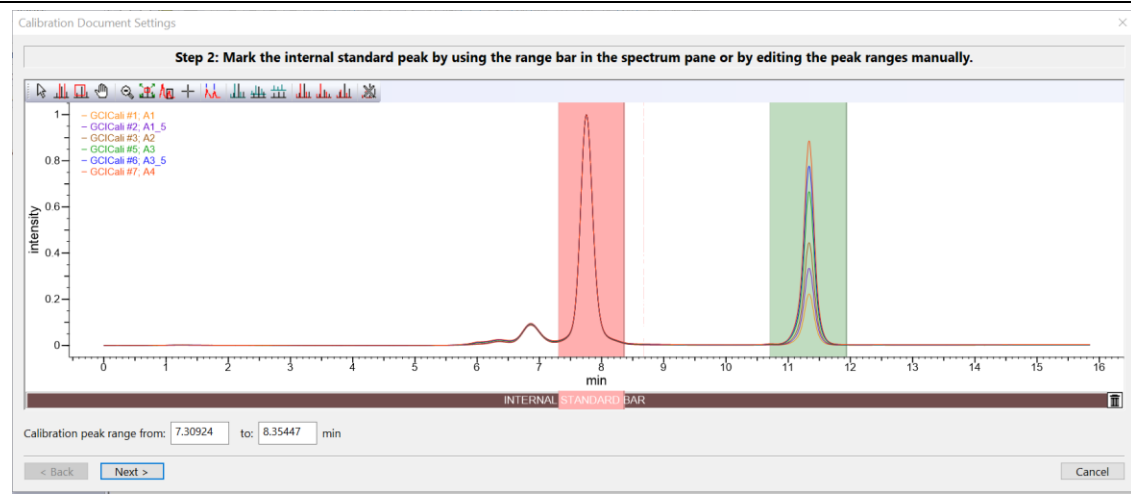
4 Select peak region around 11.3 as the calibrant peak by clicking down the CALIBRATION BAR (drag and drop).

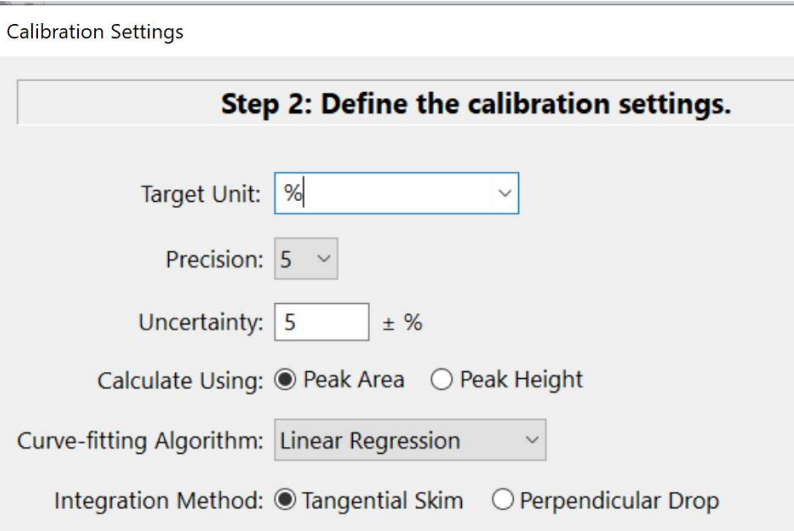
Click button **Next >**.



5 Select peak region around 7.8 as internal standard peak by clicking down the CALIBRATION BAR (drag and drop).

Click button **Next >**.



<p>6 In the following window, define calibration the settings as shown: (Target Unit: %)</p> <p>Click button Next >.</p>	 <p>Calibration Settings</p> <p>Step 2: Define the calibration settings.</p> <p>Target Unit: %</p> <p>Precision: 5</p> <p>Uncertainty: 5 ± %</p> <p>Calculate Using: <input checked="" type="radio"/> Peak Area <input type="radio"/> Peak Height</p> <p>Curve-fitting Algorithm: Linear Regression</p> <p>Integration Method: <input checked="" type="radio"/> Tangential Skim <input type="radio"/> Perpendicular Drop</p>
--	---

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.

Calibration Settings

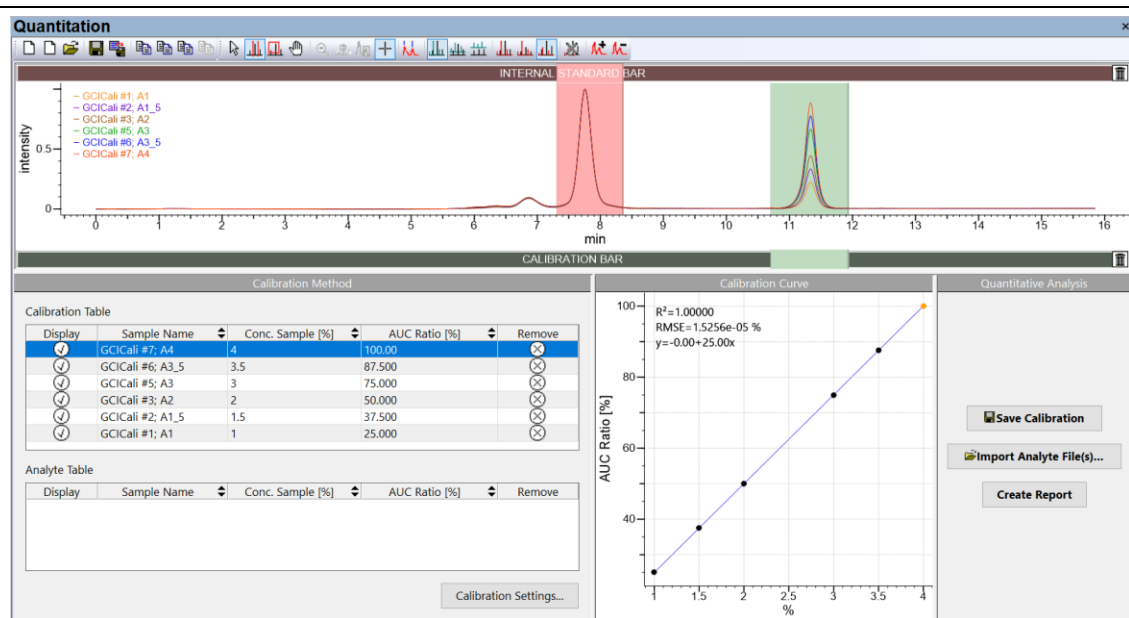
Step 4: Enter concentration values for all spectra. Double-click on a cell to start editing, or select and type in a cell.

Internal standard concentration is constant. Concentration: %

Sample Name	Conc. Sample [%]
GCICali #7; A4	4
GCICali #6; A3_5	3.5
GCICali #5; A3	3
GCICali #3; A2	2
GCICali #2; A1_5	1.5
GCICali #1; A1	1

< Back Finish

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 **R² (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 **Minelt** database.
- Select the file we have left out, **A2_5**.
- Select **Transfer to: Quantitation**.
- At the prompt, select **Calculate concentration**.
- Click **OK**.

KnowItAll Informatics System 2024, Analytical Edition

File Edit View Database Hit List MS Tools NMR Tools Window License Help

Transfer to: ChemWindow ReportIt SearchIt ProcessIt **Quantitation**

Data

Basics

Data

ID Expert

SearchIt

Minelt/Create Database

QC Expert

Spectral Processing

Display Profiles: <no profile>

PubChem

GC1Cali #4; A2_5

min

6.86 7.76 11.34

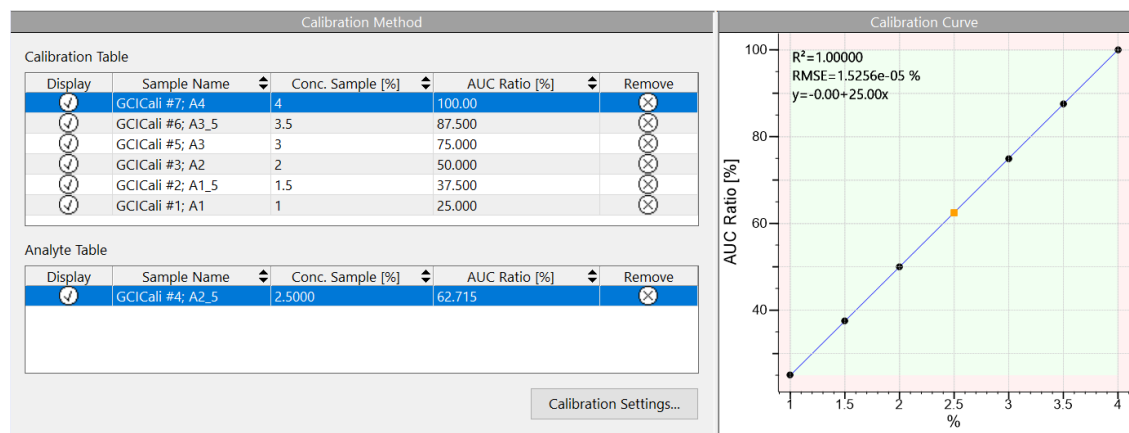
GC

Table Plot Related Compounds View

ID	Name	Spectrum	<auto> (GC)	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			3.0 mg/ml	
6	A3_5			3.50 mg/ml	
7	A4			4.00 mg/ml	

10

The concentration of the unknown is calculated and marked

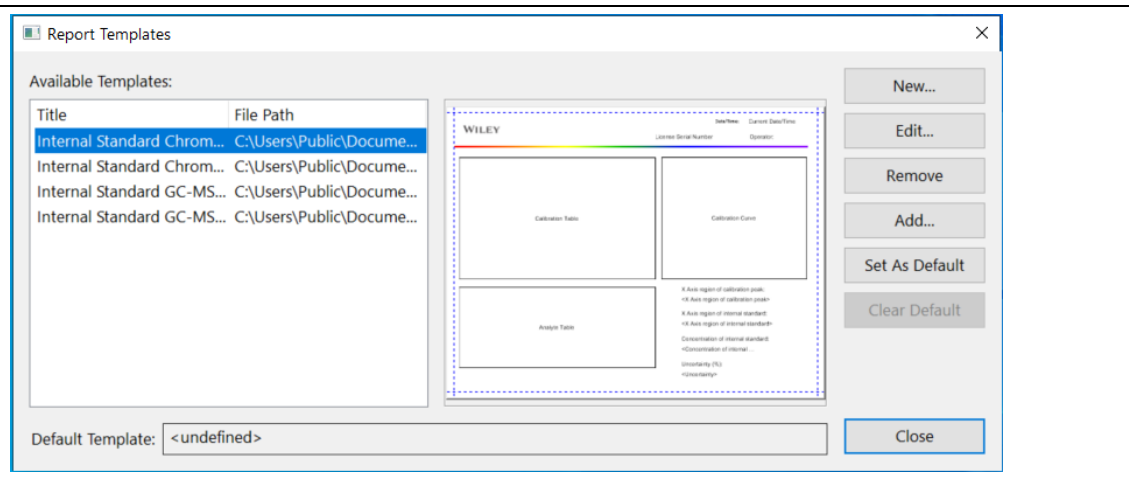


11

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

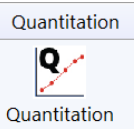


GC-MS

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.	 The image shows a button labeled 'Quantitation' with a square icon containing a red 'Q' and a red line graph with an upward arrow. Below the icon is the text 'Quantitation'.
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.

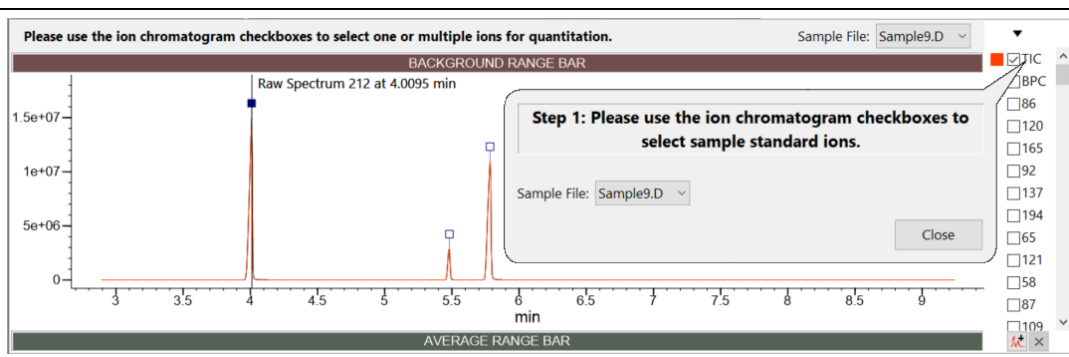
Click **Open**.

The screenshot shows a Windows File Explorer window titled "Open" with the "Look in:" path set to "Internal Calibration GC-MS". The file list includes:

Name	Date modified
Sample1.D	12/26/2023 2:38 PM
Sample2.D	12/26/2023 2:38 PM
Sample3.D	12/26/2023 2:38 PM
Sample4.D	12/26/2023 2:38 PM
Sample5.D	12/26/2023 2:38 PM
Sample6.D	12/26/2023 2:38 PM
Sample7.D	12/26/2023 2:38 PM
Sample8.D	12/26/2023 2:38 PM
Sample9.D	12/26/2023 2:38 PM
Unknown1.D	12/26/2023 2:38 PM
Unknown2.D	12/26/2023 2:38 PM
ReadMeFirst	12/26/2023 2:31 PM

At the bottom of the window, a Total Ion Chromatogram (TIC) is displayed. The x-axis is labeled "min" and ranges from 3 to 9. The y-axis represents intensity. Two prominent peaks are visible at approximately 4.0 and 5.5 minutes. A red label above the first peak reads "- Std Level 9 0.75". The text "Data is 2D, only the TIC is shown." is overlaid on the plot area. The "File name:" field is empty, and "Files of type:" is set to "All Files (*.*)".

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.

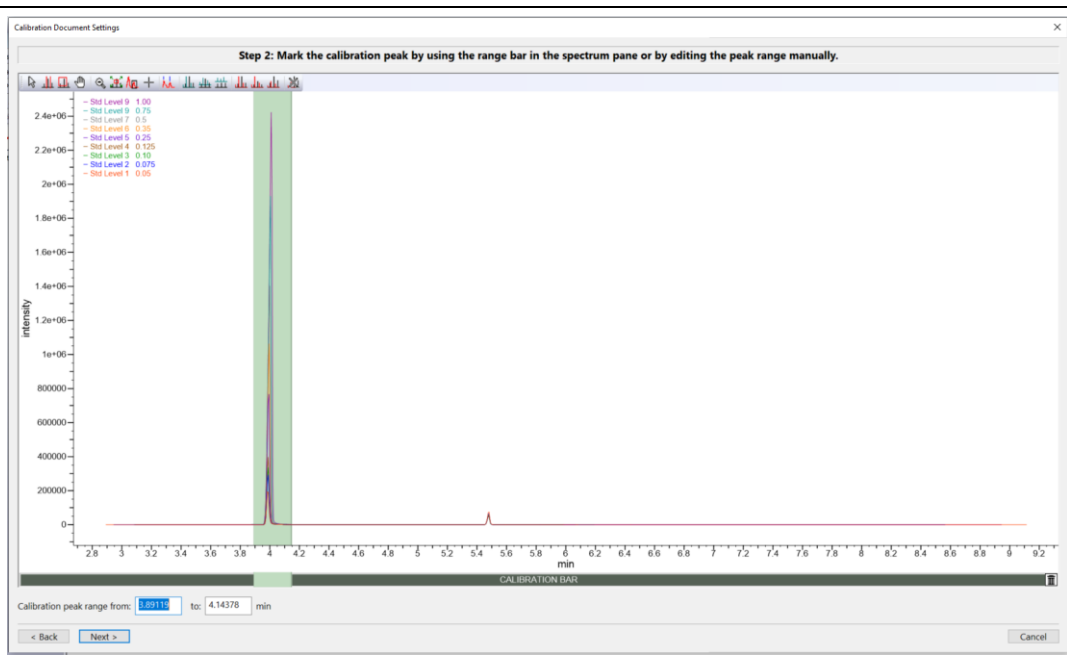
Select an ion, in this case, we select its **molecular m/z 165**.

Click **Next >** (bottom left corner)

- TIC
- BPC
- 86
- 120
- 165
- 92
- 137
- 194
- 65
- 121
- 58
- 87
- 109

6 Select peak region by clicking down the CALIBRATION BAR (drag and drop).

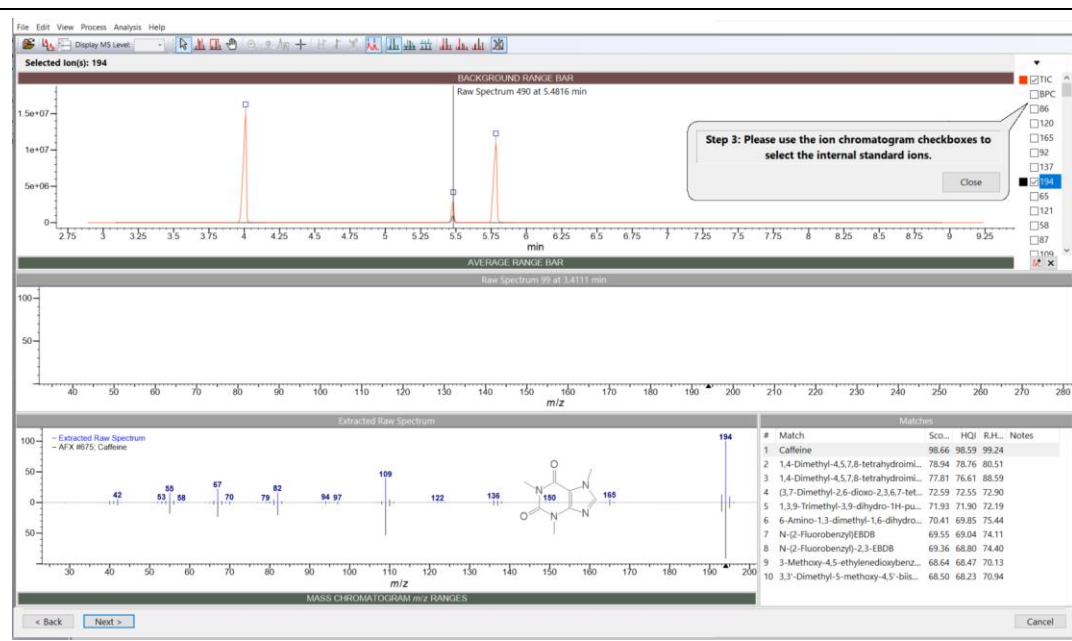
Click button **Next >**.



7 Select the internal standard peak from the top GC pane, in our example, **5.48 min.**

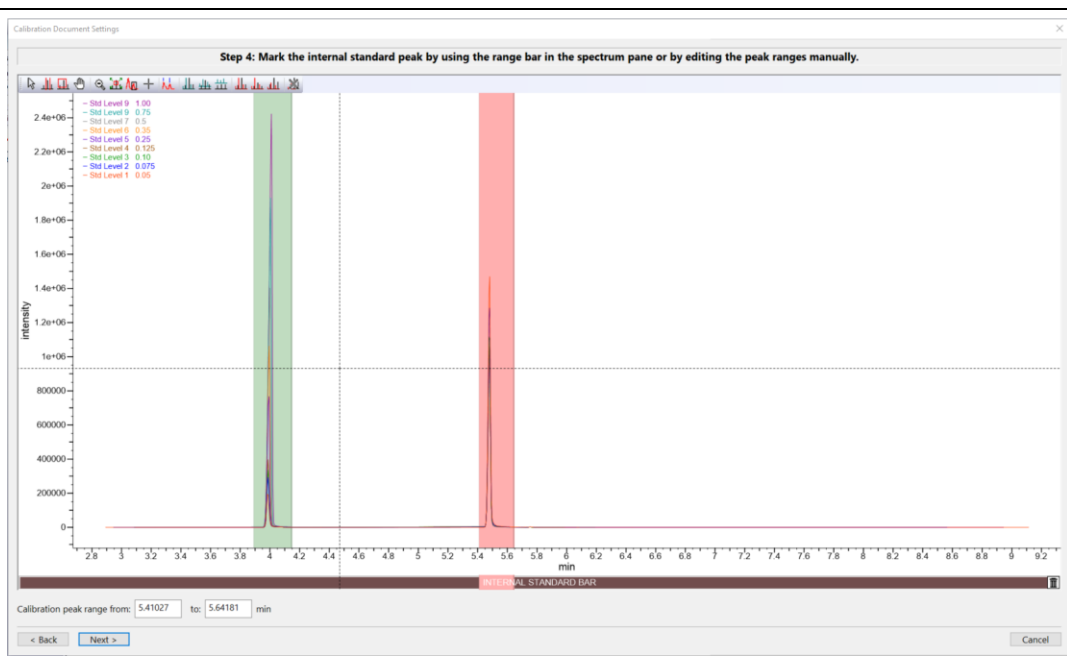
Select an ion, in this case, we select its **molecular m/z 194.**

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 >**.

Calibration Settings

Step 5: Define the calibration settings.

Target Unit: mg/ml

Precision: 5

Uncertainty: 5 ± %

Calculate Using: Peak Area Peak Height

Curve-fitting Algorithm: Linear Regression

Integration Method: Tangential Skim Perpendicular Drop

< Back Next > Cancel

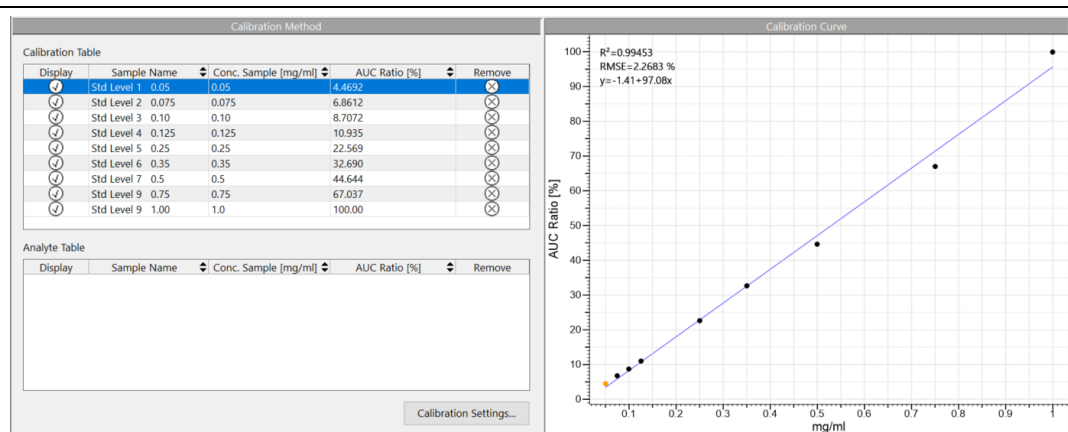
10 Enter concentration and ratio values in the pop-up window.

Internal standard concentration is constant. Concentration: mg/ml

Sample Name	Conc. Sample [mg/ml]
Std Level 1 0.05	0.0500
Std Level 2 0.075	0.0750
Std Level 3 0.10	0.100
Std Level 4 0.125	0.125
Std Level 5 0.25	0.250
Std Level 6 0.35	0.350
Std Level 7 0.5	0.500
Std Level 9 0.75	0.750
Std Level 9 1.00	1.00

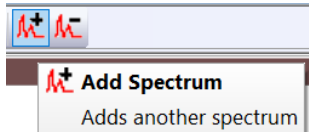
< Back Finish Cancel

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 **R² (coefficient of determination)** is to 1, the better the curve is fitting.

One can use \otimes 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.

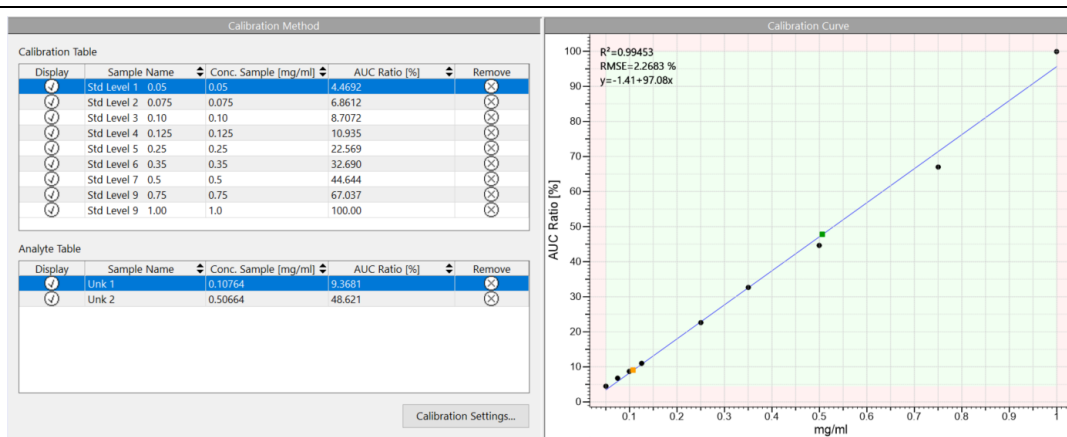
- 12 Click the **Import Analyte File(s)** button.
- Select unknown file folder **Unknown1.D** and **Unknown2.D** to calculate the concentrations.
- Click **Open**.

The screenshot shows a Windows File Explorer window titled 'Open' with the path 'Internal Calibration GC-MS'. The file list is as follows:

Name	Date modified
Sample1.D	12/26/2023 2:38 PM
Sample2.D	12/26/2023 2:38 PM
Sample3.D	12/26/2023 2:38 PM
Sample4.D	12/26/2023 2:38 PM
Sample5.D	12/26/2023 2:38 PM
Sample6.D	12/26/2023 2:38 PM
Sample7.D	12/26/2023 2:38 PM
Sample8.D	12/26/2023 2:38 PM
Sample9.D	12/26/2023 2:38 PM
Unknown1.D	12/26/2023 2:38 PM
Unknown2.D	12/26/2023 2:38 PM
ReadMeFirst	12/26/2023 2:31 PM

Below the file list, the 'File name' field is empty and the 'Files of type' is set to 'All Files (*.*)'. The 'Open' button is highlighted. At the bottom of the window, a chromatogram plot is displayed with the title '- Unk 1'. The x-axis is labeled 'min' and ranges from 3 to 9. The y-axis represents intensity. Two peaks are visible: one at approximately 4.2 minutes and another at approximately 5.8 minutes. The text 'Data is 2D, only the TIC is shown.' is overlaid on the plot. The 'Encoding' dropdown at the bottom is set to '<default>'. The 'Open' and 'Cancel' buttons are visible at the bottom right of the window.

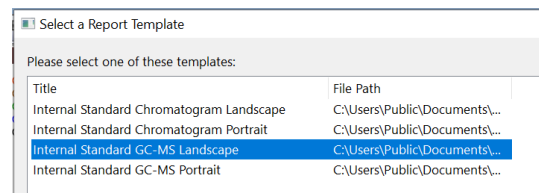
13



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

14 Click **Transfer to: Report**

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



Click **OK**

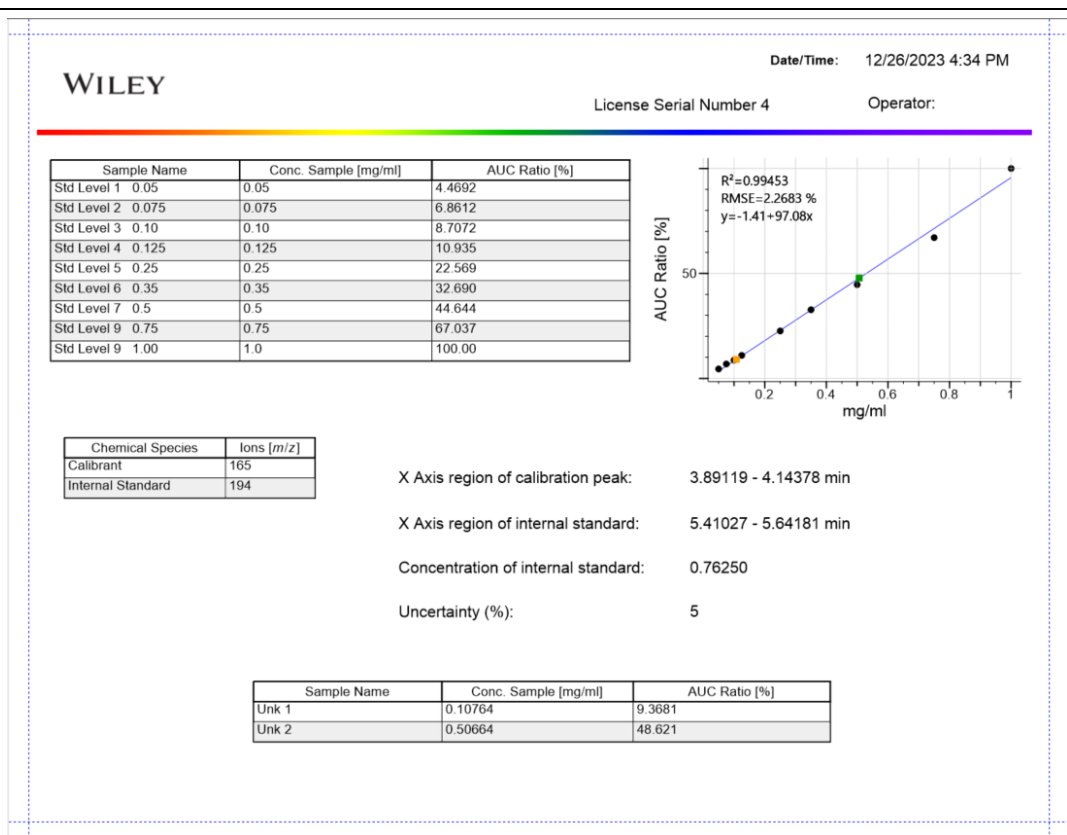
Note: if a template is used for the first time, user has to do the following before transfer data to ReportIt application:

File > Edit Report Templates

Click Add button

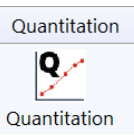
Navigate to the template file

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**.

Open

Look in: Internal Calibration GC-MS

Name	Date modified
Sample1.D	12/26/2023 2:38 PM
Sample2.D	12/26/2023 2:38 PM
Sample3.D	12/26/2023 2:38 PM
Sample4.D	12/26/2023 2:38 PM
Sample5.D	12/26/2023 2:38 PM
Sample6.D	12/26/2023 2:38 PM
Sample7.D	12/26/2023 2:38 PM
Sample8.D	12/26/2023 2:38 PM
Sample9.D	12/26/2023 2:38 PM
Unknown1.D	12/26/2023 2:38 PM
Unknown2.D	12/26/2023 2:38 PM
ReadMeFirst	12/26/2023 2:31 PM

File name:

Files of type: All Files (*.*)

Open Cancel

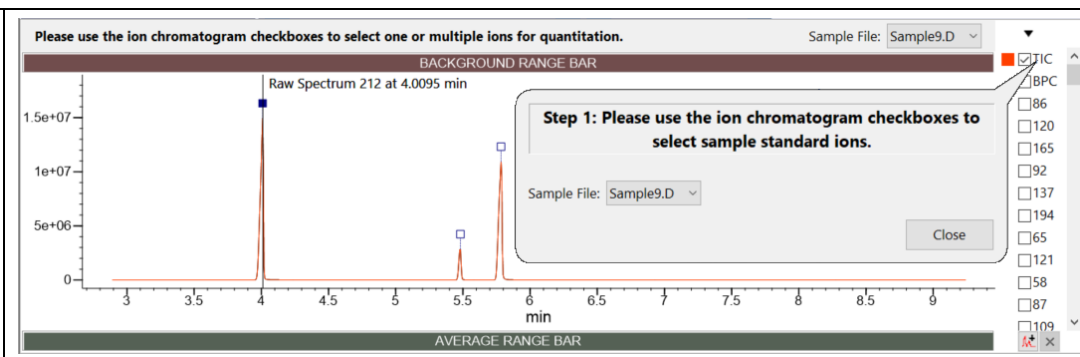
Std Level 9 0.75

Data is 2D, only the TIC is shown.

min

Encoding: <default>

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)

Selected Ion(s): 86 Sample File: Sample9.D

BACKGROUND RANGE BAR

Raw Spectrum 547 at 5.7834 min

AVERAGE RANGE BAR

Raw Spectrum 246 at 4.1896 min

Extracted Raw Spectrum

MSX #78557; Lidocaine

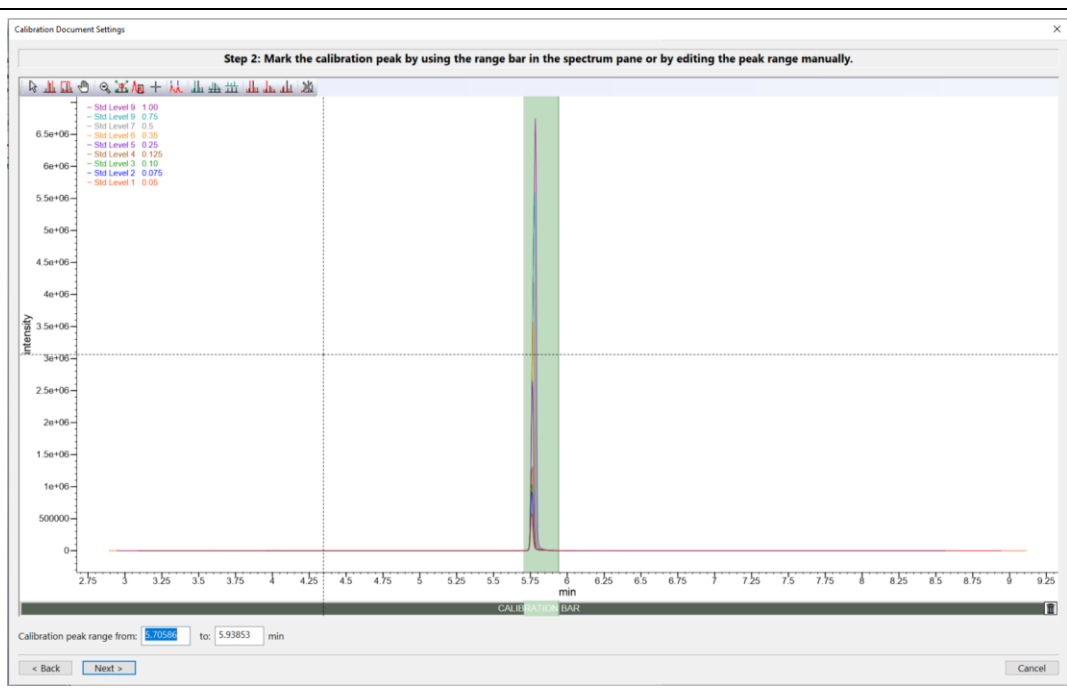
MASS CHROMATOGRAM *m/z* RANGES

#	Match	Sco...	HQI	R.H...	Notes
1	Lidocaine	97.75	97.64	98.76	
2	Tolycaine	97.60	97.52	98.31	
3	2-(Diethyl...	96.82	96.72	97.68	
4	Acetamide...	96.08	96.08	96.13	
5	4-Chloro...	89.46	89.45	89.49	
6	N,N-Dieth...	85.96	85.92	86.34	
7	N,N-Dieth...	85.96	85.91	86.40	
8	Trimecaine	84.64	83.96	90.79	
9	N,N-Dieth...	84.32	84.28	84.71	
10	2,4,5-TMM...	83.85	83.83	84.03	

< Back Next > Cancel

6 Select peak region by clicking down the CALIBRATION BAR (drag and drop).

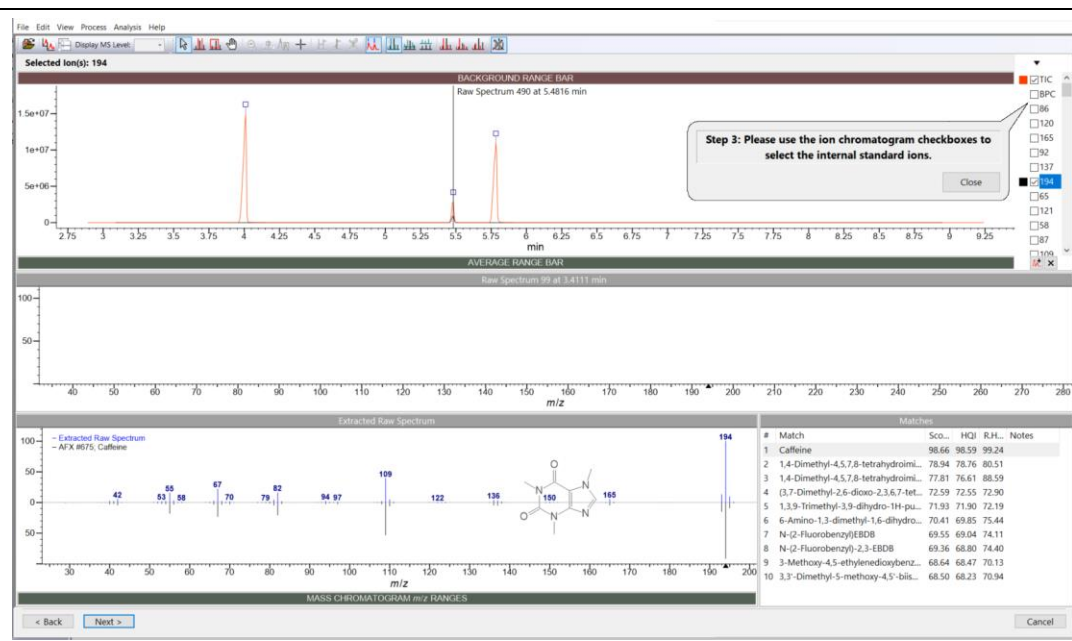
Click button **Next >**.



7 Select the internal standard peak from the top GC pane, in our example, **5.48 min.**

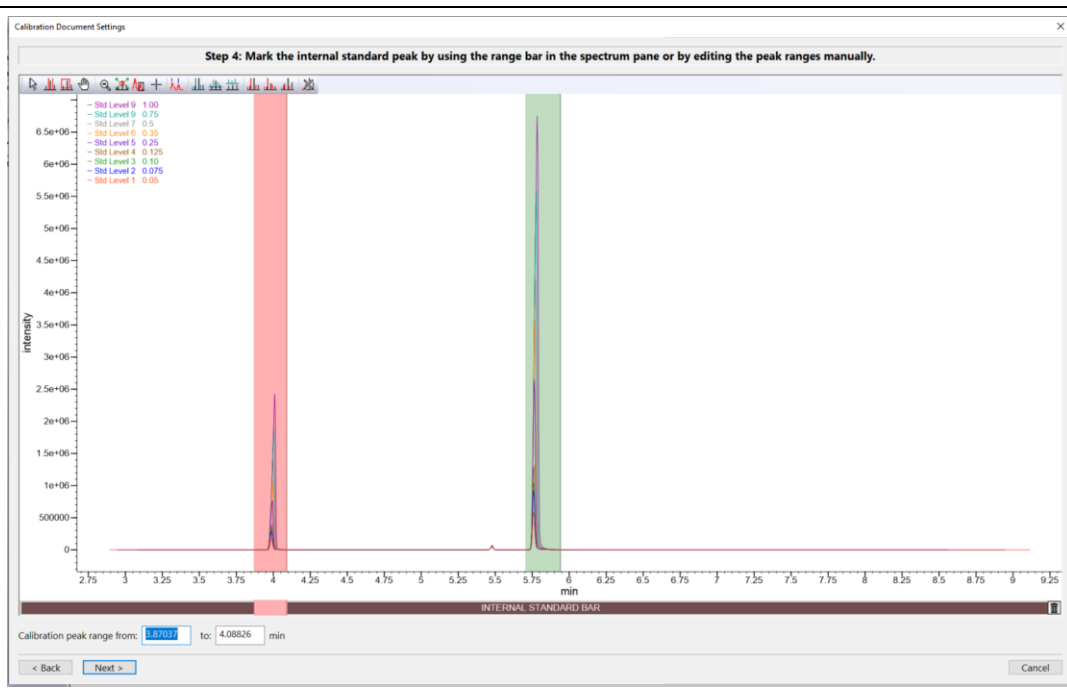
Select an ion, in this case, we select its **molecular m/z 194.**

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 >**.

Calibration Settings

Step 5: Define the calibration settings.

Target Unit: mg/ml

Precision: 5

Uncertainty: 5 ± %

Calculate Using: Peak Area Peak Height

Curve-fitting Algorithm: Linear Regression

Integration Method: Tangential Skim Perpendicular Drop

< Back Next > Cancel

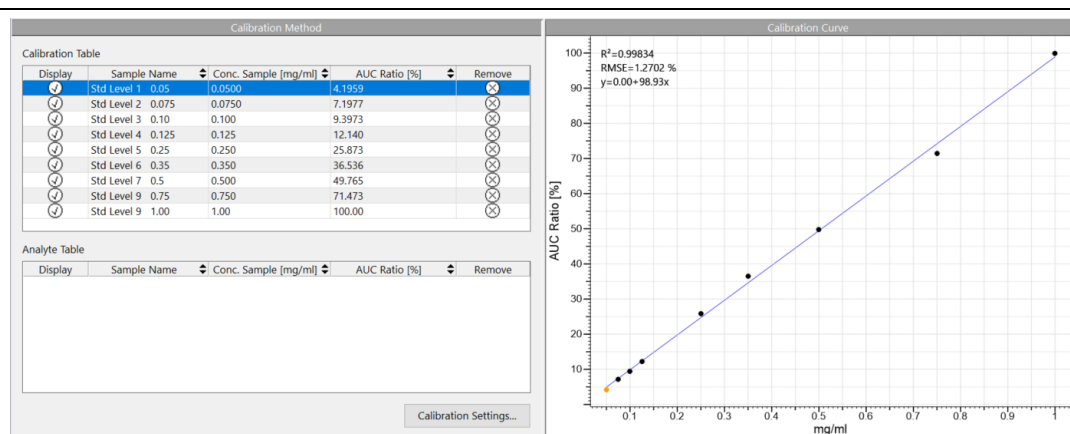
10 Enter concentration and ratio values in the pop-up window.

Internal standard concentration is constant. Concentration: mg/ml

Sample Name	Conc. Sample [mg/ml]
Std Level 1 0.05	0.0500
Std Level 2 0.075	0.0750
Std Level 3 0.10	0.100
Std Level 4 0.125	0.125
Std Level 5 0.25	0.250
Std Level 6 0.35	0.350
Std Level 7 0.5	0.500
Std Level 9 0.75	0.750
Std Level 9 1.00	1.00

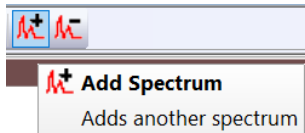
< Back Finish Cancel

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 **R² (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.

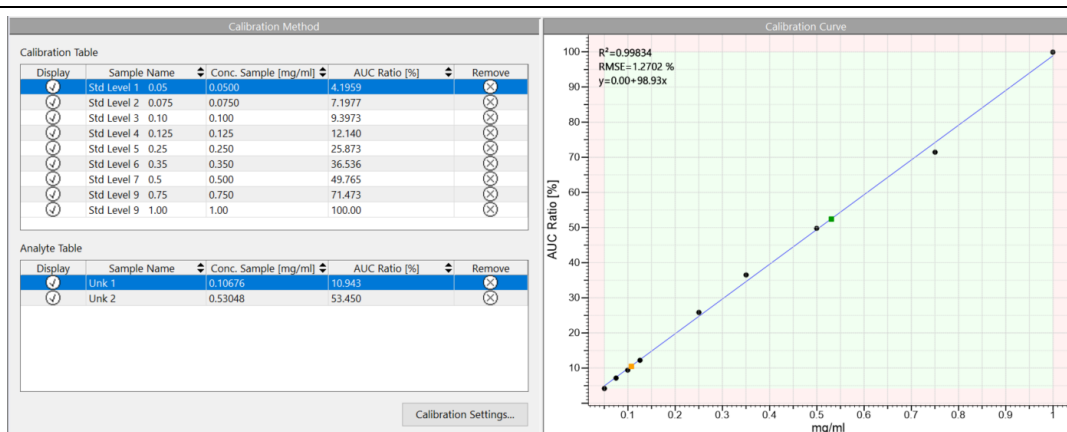
- 12 Click the **Import Analyte File(s)** button.
- Select unknown file folder **Unknown1.D** and **Unknown2.D** to calculate the concentrations.
- Click **Open**.

The screenshot shows a Windows File Explorer window titled 'Open' with the address bar set to 'Internal Calibration GC-MS'. The file list is as follows:

Name	Date modified
Sample1.D	12/26/2023 2:38 PM
Sample2.D	12/26/2023 2:38 PM
Sample3.D	12/26/2023 2:38 PM
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Sample7.D	12/26/2023 2:38 PM
Sample8.D	12/26/2023 2:38 PM
Sample9.D	12/26/2023 2:38 PM
Unknown1.D	12/26/2023 2:38 PM
Unknown2.D	12/26/2023 2:38 PM
ReadMeFirst	12/26/2023 2:31 PM

Below the file list, the 'File name' field is empty, and the 'Files of type' is set to 'All Files (*.*)'. The 'Open' button is highlighted. At the bottom of the window, a chromatogram plot is displayed with the x-axis labeled 'min' ranging from 3 to 9. The plot shows three distinct peaks at approximately 4.2, 5.5, and 5.8 minutes. The plot is titled '- Unk 1' and contains the text 'Data is 2D, only the TIC is shown.'

13



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