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SHIMADZU

MassWorks Quick Guide

Read the instruction manual thoroughly before you use the product.

Keep this instruction manual for future reference.

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Introduction

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Mark	Description
⚠ WARNING	Indicates a potentially hazardous situation which, if not avoided, could result in serious injury or possibly death.
⚠ CAUTION	Indicates a potentially hazardous situation which, if not avoided, may result in minor to moderate injury or equipment damage.
NOTE	Emphasizes additional information that is provided to ensure the proper use of this product.
Hint	Provides useful information about operation of this system. Please read the description when required.
Reference	Indicates reference sections and pages.

⚠ WARNING

When the customer uses the CD-ROM
This is a CD-ROM disk. Do not play this on an audio CD player, as the high volume may damage your hearing or the audio speakers.

Contents

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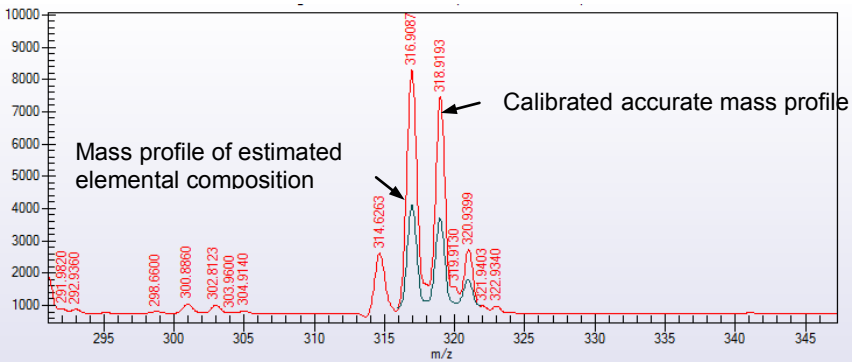
1 System Configuration

1.1 Overview

MassWorks enables highly-precise calculation of accurate mass and isotope spectra with a unique mass calibration technology based on cerno BIOSCIENCE's patented MSIntegrity technology to expand the capacity of unit mass resolution-base mass spectrometers. This feature enables the elemental composition estimation that can be usually performed only on expensive systems such as time-of-flight (TOF) mass spectrometers. This Quick Guide describes operation procedures for using the software with a GCMS-QP Series or GCMS-TQ Series system.



NOTE

This Quick Guide assumes that the reader already has a basic understanding with respect to the handling of GC-MS(/MS) and GCMSsolution. Names and terms specific to GCMSsolution are used in many places in this guide and so, if you are a first-time user or if there are any points that are not clear, refer to the GC-MS(/MS) instruction manual, the operation guide and the GCMSsolution help as necessary.

1.2 Operating Environment

OS	Microsoft Windows 7 Professional
Workstation software	GCMSsolution Ver. 4.42 or later

NOTE

If the GCMSsolution version is earlier than Ver. 4.42, upgrade the software.

1.3 Instruments Supported

GC/MS: GCMS-QP series, GCMS-TQ series

1.4 Files for Analysis Using MassWorks

Files for facilitating analysis using MassWorks are available in the folders shown below. To use them, copy these files and paste them in the folder for storing the analysis data.

Files for Analysis and data processing using MassWorks

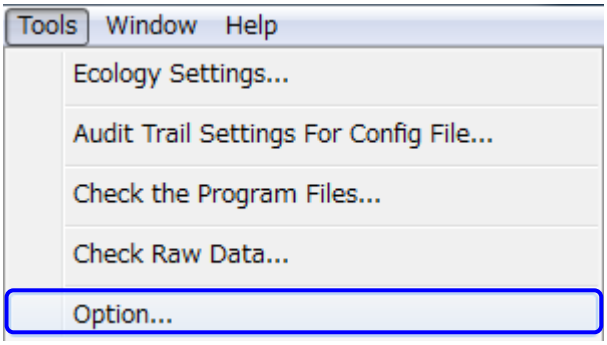
File Name	Description	Install folder
PFTBA_External.qgm	Method file for acquiring data for mass calibration	If the [GCMSsolution] program is installed in the C drive, C:GCMSsolution\Sample\MassWorks_Usage
Shimadzu_Default_CAL.mwc	File that stores PFTBA ion information for mass calibration	

2 Setting of GCMSsolution

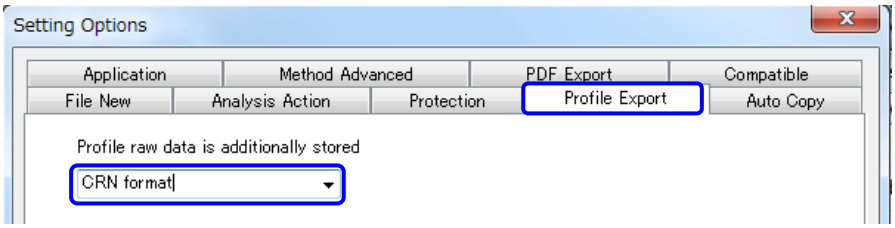
Configure settings in GCMSsolution to output mass profile data to be used on the MassWorks.

1) Execute the [GCMS Real Time Analysis] program.

2) Select "Option" from the [Tools] menubar.



3) Click the [Profile Export] tab, Set "CRN format" to the [profile raw data is additionally stored].



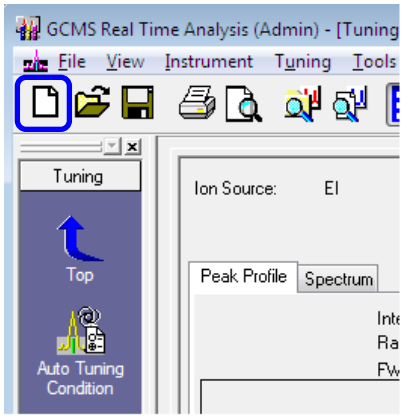
4) Click the [OK] button.

3 GC/MS Data Acquisition

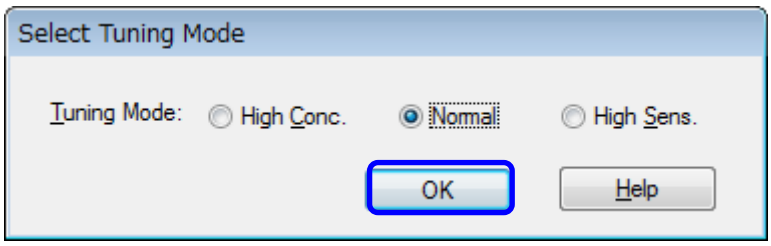
Acquire mass calibration data to be used on MassWorks. Two types of mass calibration data acquisition procedures are available; a procedure for acquiring data of the actual sample and mass calibration data separately (external calibration) and a procedure for acquiring data of the actual sample and then calibration data sequentially (internal calibration).

3.1 Auto-Tuning

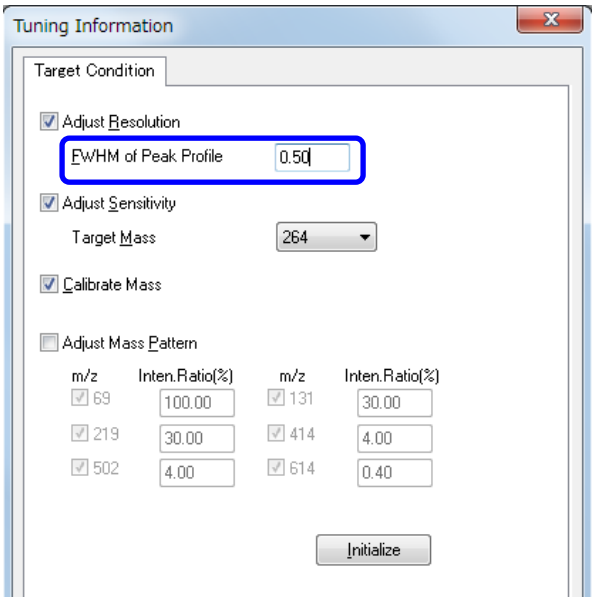
1) Perform auto-tuning. Open [Tuning] window from the [GCMS Real Time Analysis] program and click [New] icon.



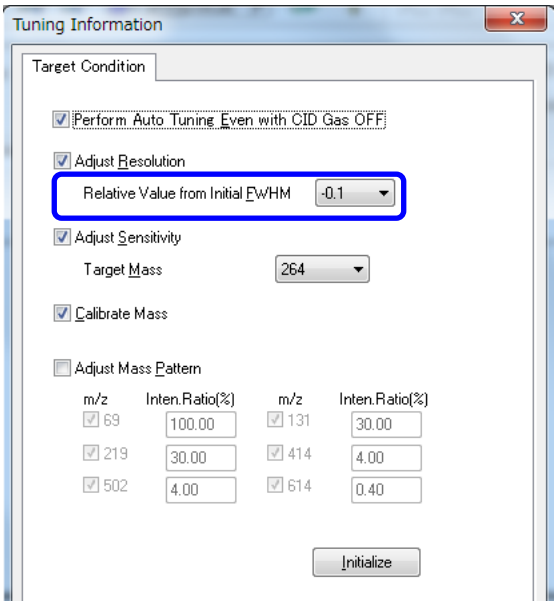
2) Set the tuning mode and click [OK] button.



3) In case of QP series, click [Auto Tuning Condition] icon and enter "**0.5**" in the "FWHM of Peak Profile".
In case of TQ series, click [Auto Tuning Condition] icon and set "**-0.1**" in the "Relative Value from initial FWHM".



In case of QP series



In case of TQ series

> **NOTE**
>
> Perform tuning before acquiring calibration data. If tuning is not performed for a long time, the accurate mass calculation result may not be accurate.

3.2 Acquiring Mass Calibration Data

3.2.1 Using External Calibration to Acquire Calibration Data

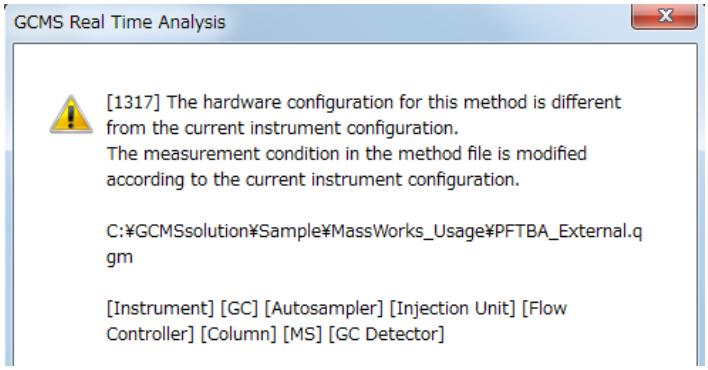
Data of the sample to be measured and mass calibration data are acquired separately. Mass calibration data ca be acquired by using PFTBA.

1) Copy the "PFTBA_External.qgm" method file described in "1.4 Files for Analysis Using MassWorks" and paste it on the folder that stores analysis data.

2) Load the "PFTBA_External.qgm" method file.

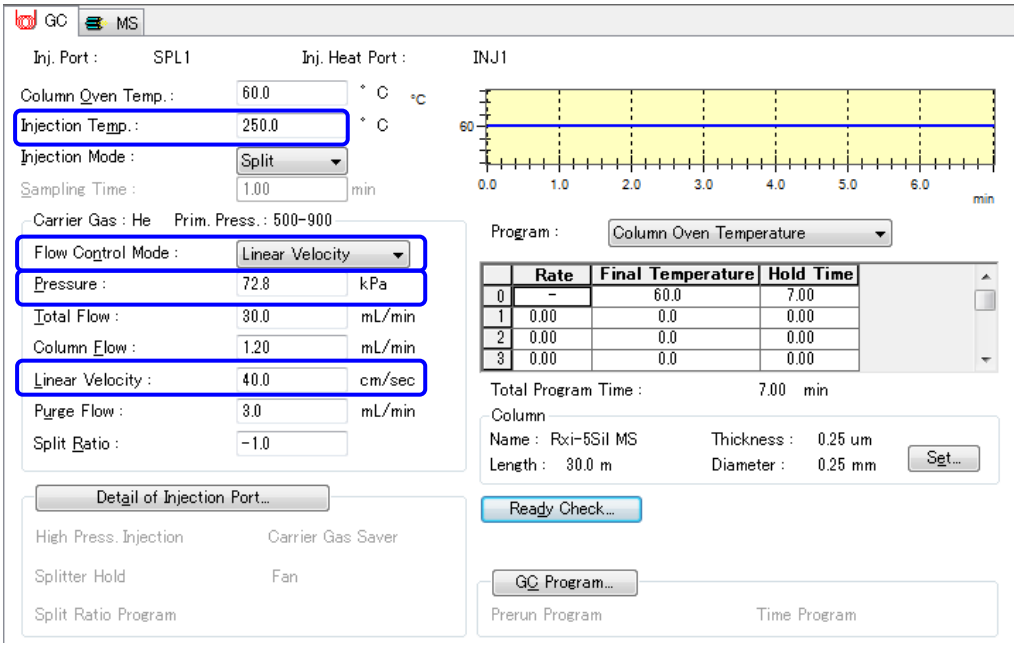
NOTE

Load the method file in the [GCMS Real Time Analysis] - [Acquisition] window. Here, click [OK] if the following confirmation message is displayed: "[1317] The hardware configuration for this method is different from the current configuration. The measurement condition in the method file is modified according to the current instrument configuration.

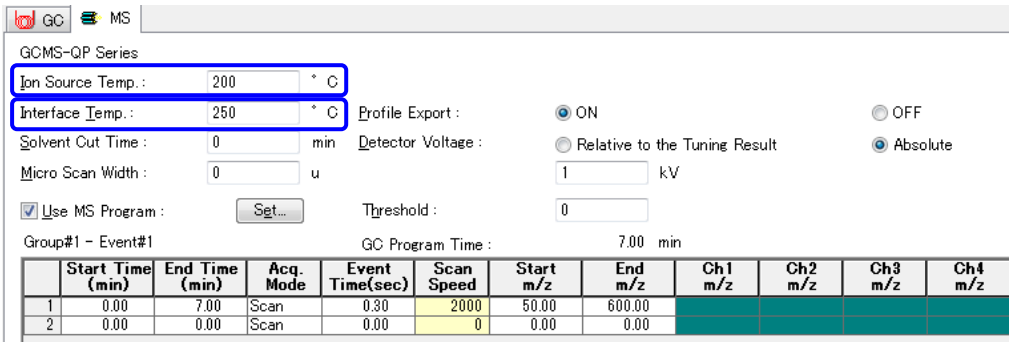


3) Specify the GC parameters below for actual analysis.

- Injection Temp.
- Flow Control Mode
- Pressure or Linear Velocity (Select either of these to match the control mode.)



4) Specify the MS parameters below for actual analysis.
   - Ion Source Temp.
   - Interface Temp.



NOTE

The detector voltage varies according to the instrument status. If the intensity of each ion of PFTBA used for calibration has been saturated, lower the detector voltage. If PFTBA ions are saturated, accurate mass calculation cannot be made correctly.

It's recommended to check the detector voltage that the intensity of m/z 69 for PFTBA is approximately 3,000,000 ~ 4,000,000 introducing PFTBA from the [Peak Monitor] of [Tuning] icon, before GC/MS measurement.

5) Click the (Save) icon on the toolbar to overwrite the method file.
6) Create a batch file to acquire mass calibration data and measure samples. It is recommended to acquire mass calibration data in each batch.
7) Configure batch settings for mass calibration and sample measurement.
8) Set **"0"** for the **vial #** on a row on which a mass calibration method has been set.

	Vial#	Sample Name	Sample ID	Sample Type	Analysis	Method File	Data File	Inj. Volum
1	0	PFTBA_EI		0:Unknown	IT QT	PFTBA_External.qgm	PFTBA_EI.qgd	1
2	1	Sample001		0:Unknown	IT QT	EI_Scan.qgm	Sample001.qgd	1
3	2	Sample002		0:Unknown	IT QT	EI_Scan.qgm	Sample002.qgd	1
4	3	Sample003		0:Unknown	IT QT	EI_Scan.qgm	Sample003.qgd	1
5	4	Sample004		0:Unknown	IT QT	EI_Scan.qgm	Sample004.qgd	1

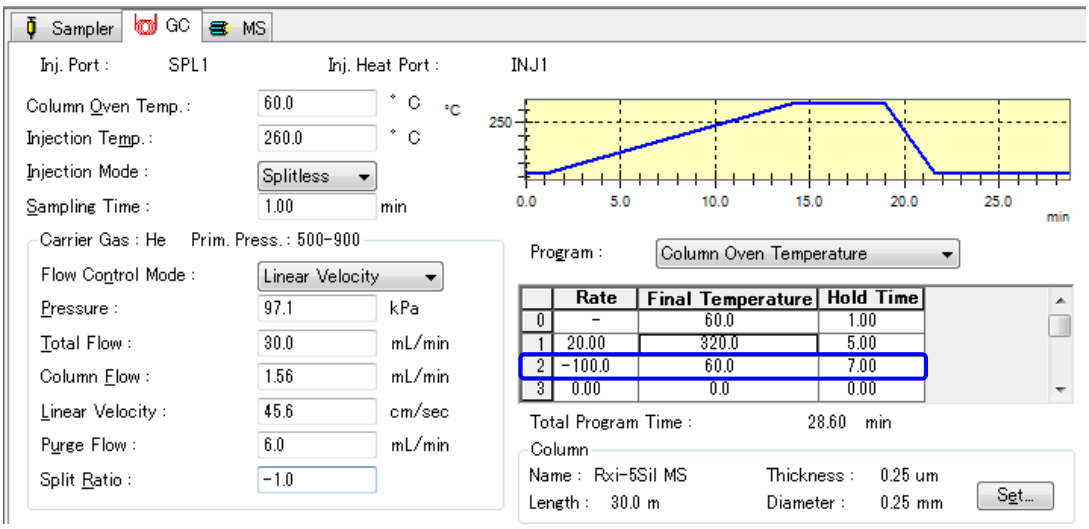
9) Save the batch file and measure samples.

3.2.2 Using Internal Calibration to Acquire Calibration Data

Sample measurement and mass calibration data acquisition are performed at the same time. Increase the time to measure samples 10 minutes, introduce PFTBA, and acquire mass calibration data.

1) Load the analysis method for sample measurement.
2) Save the method file under a different name.
   (Ex.: xxxxxx_Internal_PFTBA.qgm)
3) Change the GC parameter below.
   Column oven program: Add the following program to the oven program for measuring samples.

Rate	Temperature	Hold Time
-100	Initial column temperature of analysis conditions for samples	7 (min)



NOTE

If the column with thicker film such as over 0.5 µm is used, set the cooling temperature rate lower.

4) Click the [MS] tab and select the [Profile Export] checkbox.

5) Change the MS parameters below.

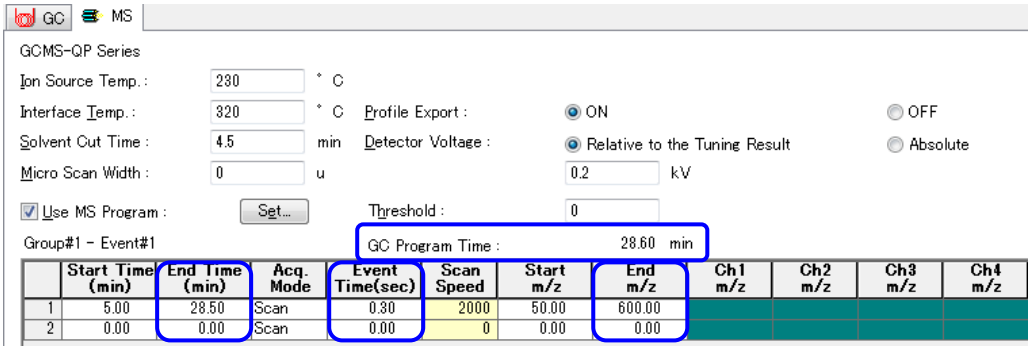
[End Time (min)]: Enter the GC program time.

[Start m/z]: 40 - 60

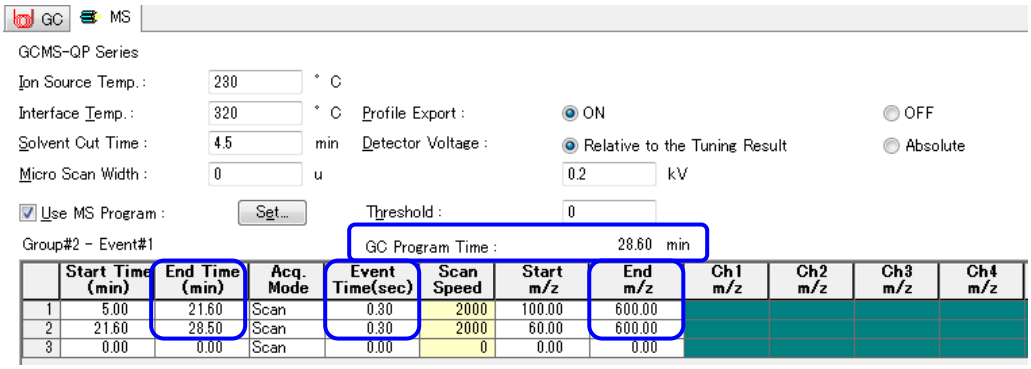
[End m/z]: 600 (to acquire the mass spectrum of PFTBA)

[Event Time (sec)]: Set this so that the scan speed becomes approximately 2000 u/sec.

[In case of usage with the EI mode]



[In case of usage with the CI mode]



NOTE

- When the [ON] checkbox of profile export is selected, the mass profile to be used on MassWorks is output. Due to the large size of mass profile files, it is recommended to clear this checkbox when you do not plan to use MassWorks.
- If the data is acquired in only SIM or MRM mode, the mass profile can not be loaded to MassWorks.

6) Click the [Set] button next to [Use MS Program].

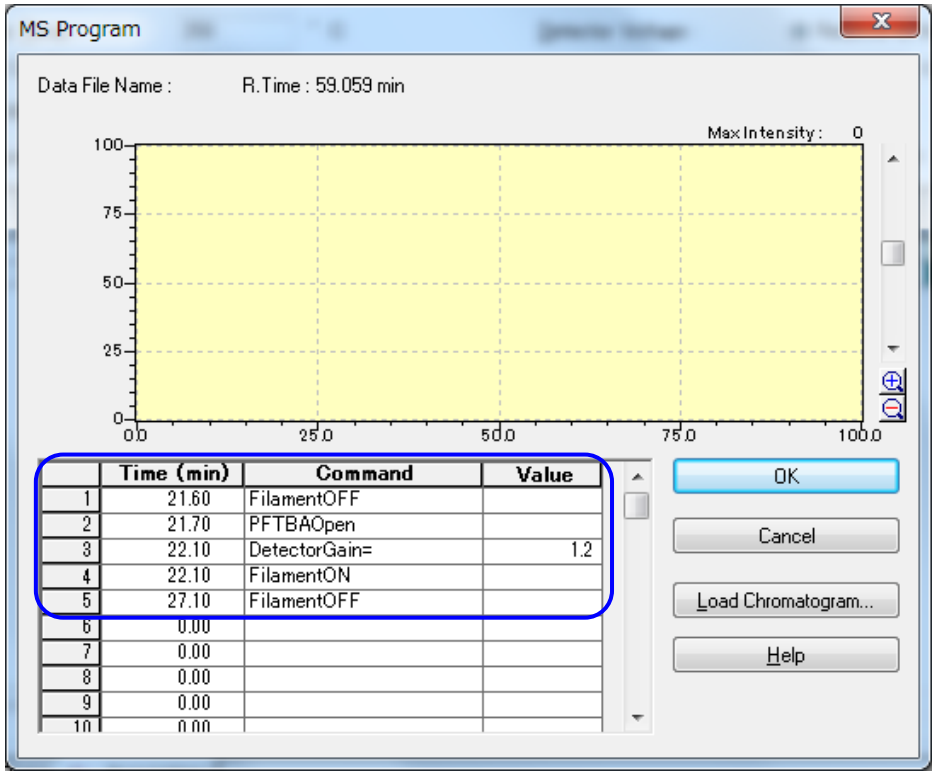
7) Set the program as shown in the table below.

[In case of usage with the EI mode]

No	Time (min)	Command	Value
1	GC program time – 6.5	FilamentOFF	
2	GC program time – 6.0	PFTBAOpen	
3	GC program time – 6.0	DetectorGain=	1.1 - 1.3
4	GC program time – 5.5	FilamentON	
5	GC program time – 0.5	FilamentOFF	

[In case of usage with the CI mode]

No	Time (min)	Command	Value
1	GC program time – 7.0	ReagentGasxClose (x means port number)	
2	GC program time – 6.5	FilamentOFF	
3	GC program time – 6.0	PFTBAOpen	
4	GC program time – 6.0	DetectorGain=	1.1 - 1.3
5	GC program time – 5.5	FilamentON	
6	GC program time – 0.5	FilamentOFF	



NOTE

The detector voltage varies according to the instrument status. If the intensity of each ion of PFTBA used for calibration has been saturated, lower the detector voltage. If PFTBA ions are saturated, accurate mass calculation cannot be made correctly.

It's recommended to check the detector voltage that the intensity of m/z 69 for PFTBA is approximately 3,000,000 ~ 4,000,000 introducing PFTBA from the [Peak Monitor] of [Tuning] icon, before GC/MS measurement.

8) Click the [OK] button, overwrite the method file.
9) Create a batch file for measuring samples. Create it using the usual procedure to create a batch file.

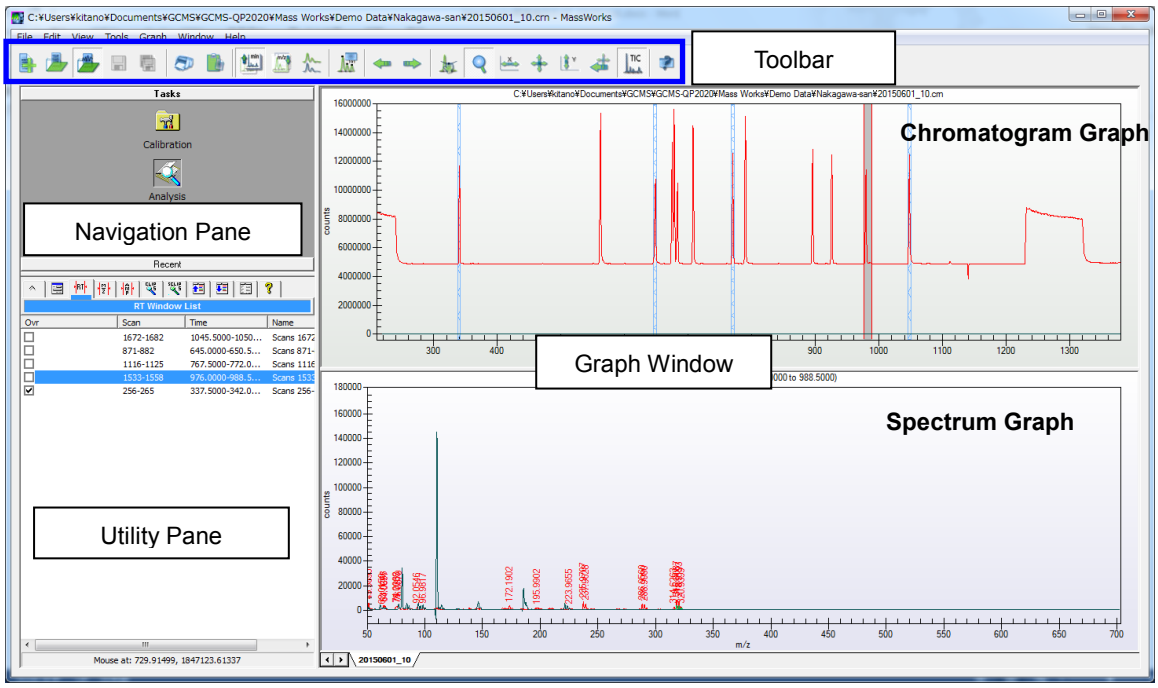
4 Data Processing Using MassWorks

> **NOTE**
>
> For details about functions of MassWorks, refer to MassWorks™ User's Guide.

4.1 User Interface Overview

4.1.1 MassWorks Application Screen

MassWorks provides an explorer style interface which consists of a left hand Navigation Pane to provide access to Calibration and Analysis tasks as well as various utility functions.



Name	Contents
Navigation Pane	The Navigation Pane allows selection between different functional panes which include "Tasks" "Recent" and "Explore". **Recent** provides a listing of the most recently opened data files or calibrations **Tasks** displays the key modes in MassWorks, **Calibration** Mode and **Analysis** Mode. Select the mode from the explorer panel by clicking on the icons.
Utility Pane	The Utility Pane shows additional information relating to information in the Graph Window including retention time or mass range windows, tables, shortcuts, and other information.
Graph Window	The graph window can include spectra, chromatograms, tables (such as standard ion tables), dialog boxes and buttons. It may be a tab style, with a different operation on each tab as in the Calibration mode, or it may contain multiple spectral windows (MDI).

The toolbar buttons on the MassWorks toolbar provide the following functionality:

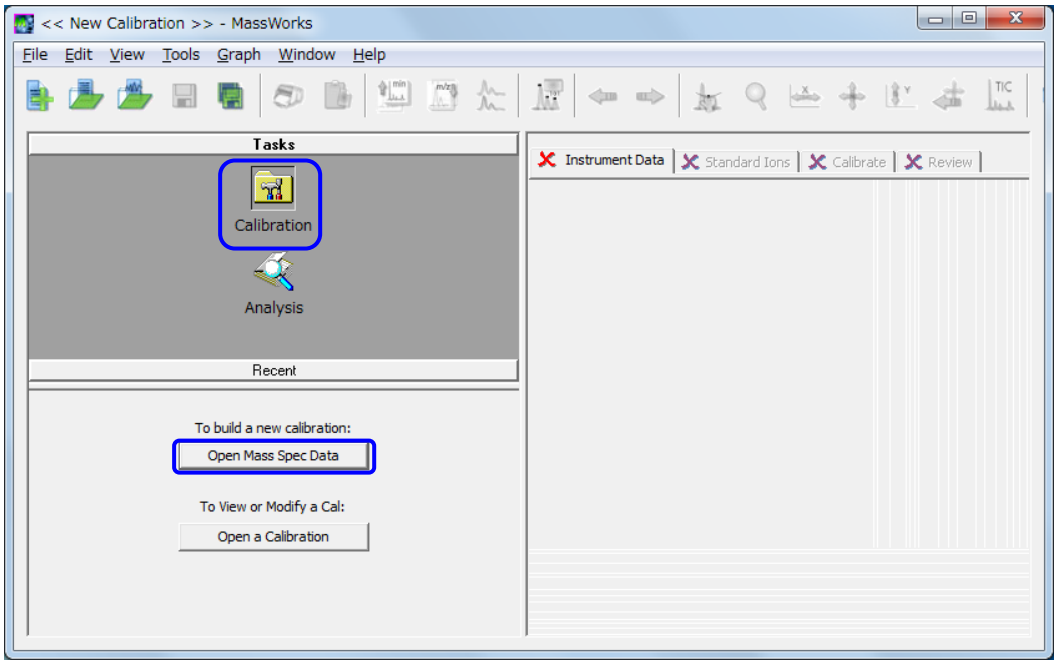
Icon	Functionality	Contents
	New Calibration	Close out the current calibration in the calibration task
	Open Calibration File	Open an existing calibration file and any associated calibration data
	Open MS Data File	Open an MS data file in either calibration mode or analysis mode
	Save Calibration File	Save the current calibration file
	Save Calibration File As	Save the calibration file to a new name
	Print Report	Print the analysis or calibration report to the printer
	Report to Clipboard	Send the analysis or calibration report to the clipboard
	Select Top Graph	When selected, all the toolbar buttons that operate on a graph will perform the operation on the top (chromatogram) graph
	Select Bottom Graph	When selected, all the toolbar buttons that operate on a graph will perform the operation on the bottom (spectrum) graph
	Stack Graphs	When depressed, if multiple spectra or chromatogram are present, the Y axes will be stacked above one another. When not depressed, the graphs will be overlaid. The select graph buttons determine which graph to apply.
	Edit Peak Picking Parameters	Brings up the chromatogram or spectrum peak parameters dialog. The select graph buttons determine which graph to apply.
	Previous Scan or Mass	Show the previous spectrum in the run or the previous XIC relative to the cursor. The select graph buttons determine which graph to apply.
	Next Scan or Mass	Show the next spectrum in the run or the previous XIC relative to the cursor. The select graph buttons determine which graph to apply.
	Pan Mode	When depressed, click and hold the left mouse button on the graph to pan the graph. The select graph buttons determine which graph to apply.
	Zoom Mode	When depressed, click and hold the left mouse button on draw zoom box and zoom into an area on the graph. The select graph buttons determine which graph to apply.
	Set Full X Scale	Scales the X axis to the full range. The select graph buttons determine which graph to apply
	Set Full Scale	Scales the X and Y axis to the full range. The select graph buttons determine which graph to apply.
	Set Full Y Scale	Scales the Y axis to the full range. The select graph buttons determine which graph to apply.
	Previous Scale	Will undo any previous scaling operation. You may click it multiple times to undo scaling multiple times. The select graph

Icon	Functionality	Contents
		buttons determine which graph to apply.
	Show TIC	Display the TIC. When an XIC is generated, the TIC is normally hidden on the display as the scale is much different. You can override this by clicking this button. This can also be done from the utility pane from the chromatogram legend tab.
	Help	Open the Help file.

4.1.2 Creating the Calibration File

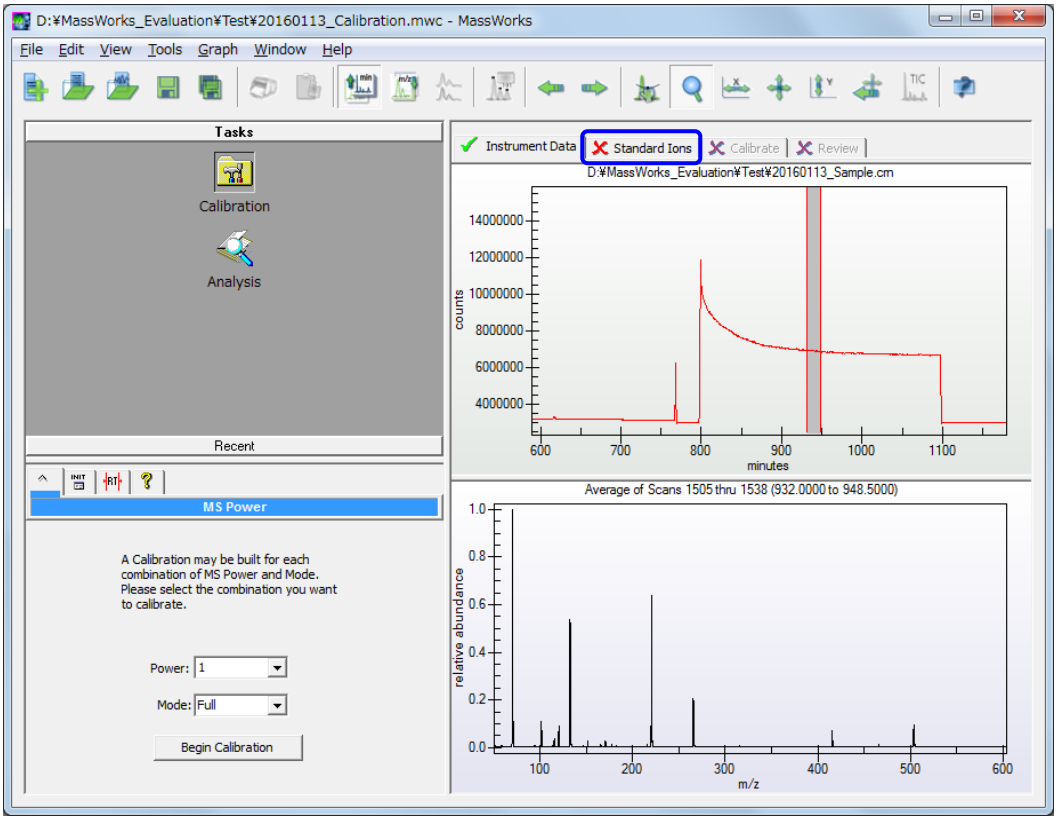
Create a calibration file to calibrate the mass and spectra using the mass calibration data acquired in "3. GC/MS Data Acquisition."

1) Start MassWorks
2) Select the [Calibration] icon from the navigation pane.
3) Click the [Open Mass Spec Data] button and open the PFTBA data (xxxx.crn) acquired by external or internal calibration.

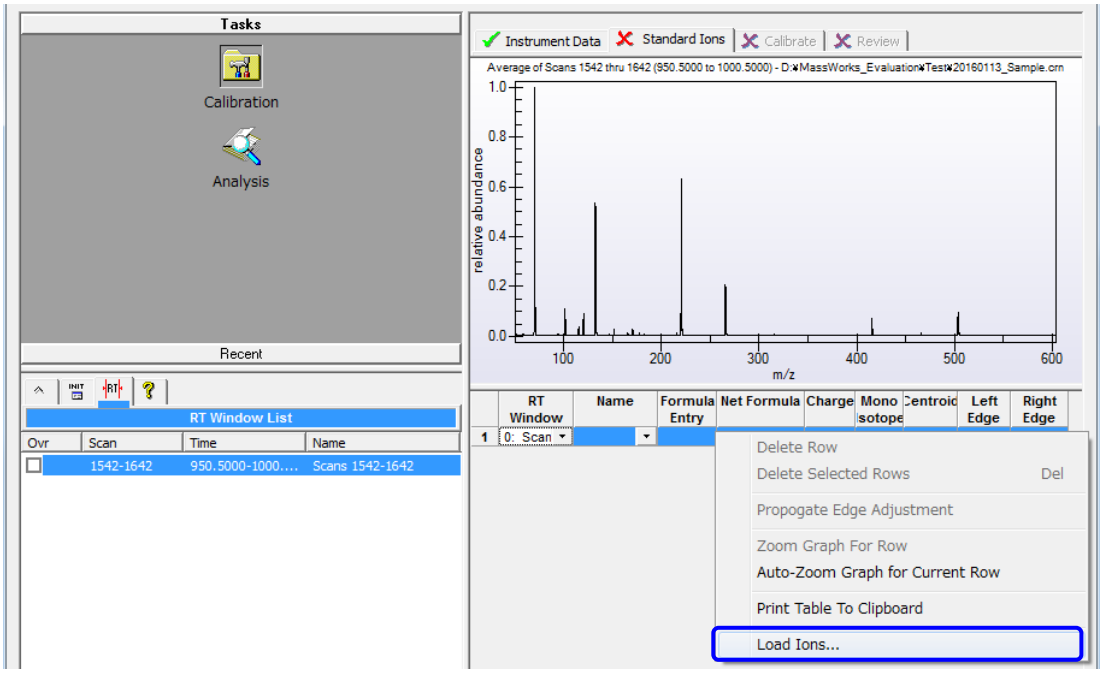


4) Right click the mouse on the TIC graph and from the drop down menu, select "Mark an RT window."

5) Specify a range for extracting the spectrum in an area where PFTBA is detected by clicking the mouse on the start and end points. Then, click the [Standard Ions] tab.

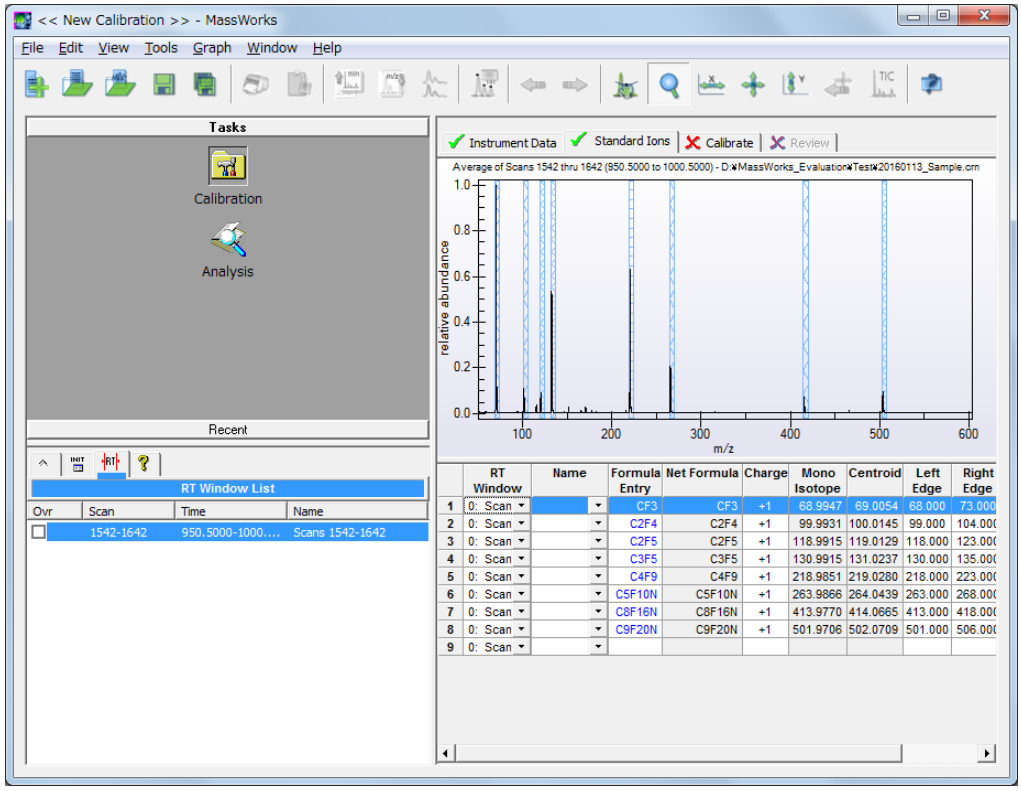


6) Right click the mouse on the table and choose "Load Ions…" from the pop up menu.

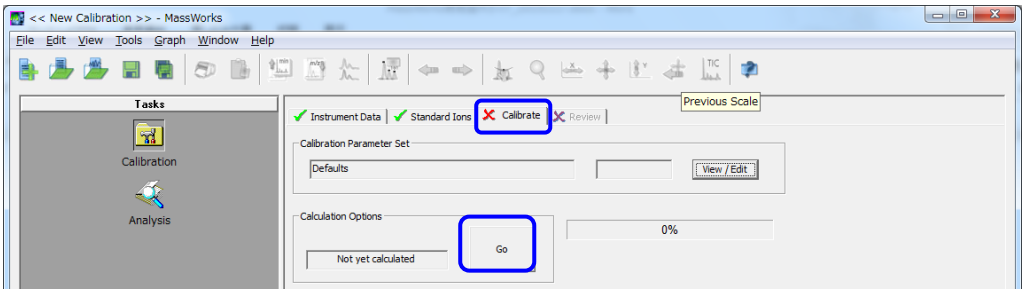


7) Select the "Shimadzu_Default_CAL.mwc" file that contains the PFTBA ion information.

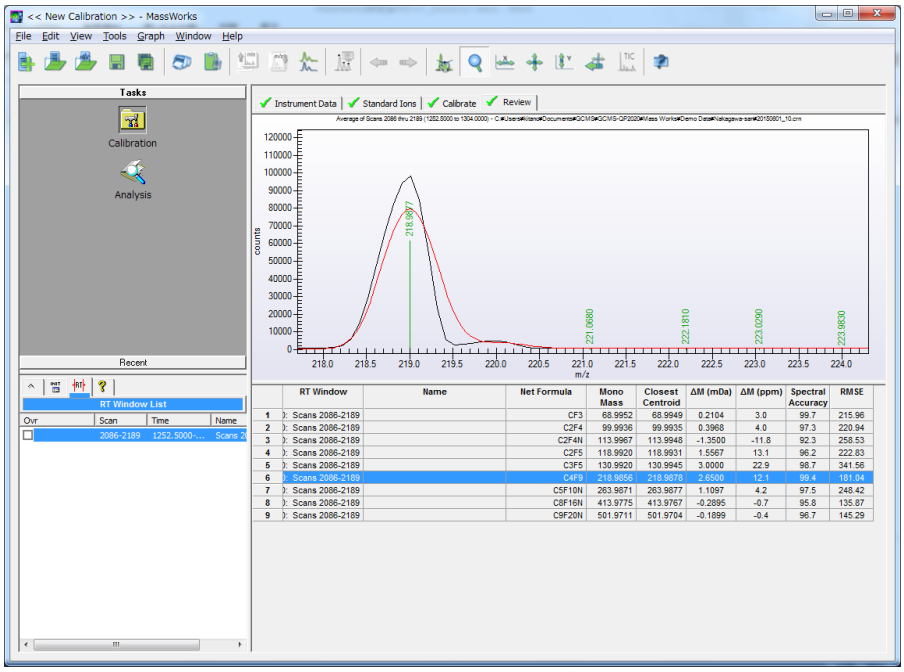
8) The selected information is input to the lower table and corresponding peaks are shaded. Confirm that each peak is within the shaded area.



9) Click on the [Calibration"] tab and then click [Go] button.

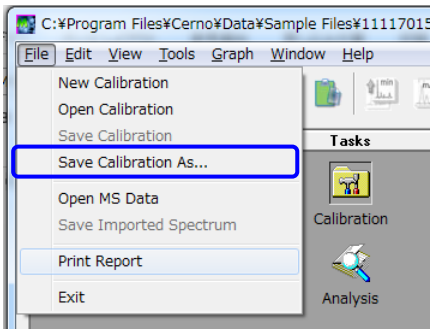


10) When data processing is completed, move to the [Review] tab and check the results. Confirm that each m/z peak is recognized. If the peaks are not recognized, go back to the [Standard Ions] tab, set the ion and calibration range again, and perform calibrate again. If the setting is changed and the peaks isn't recognized, acquire the data again after increasing the detector voltage.



RMSE = Root mean squared error

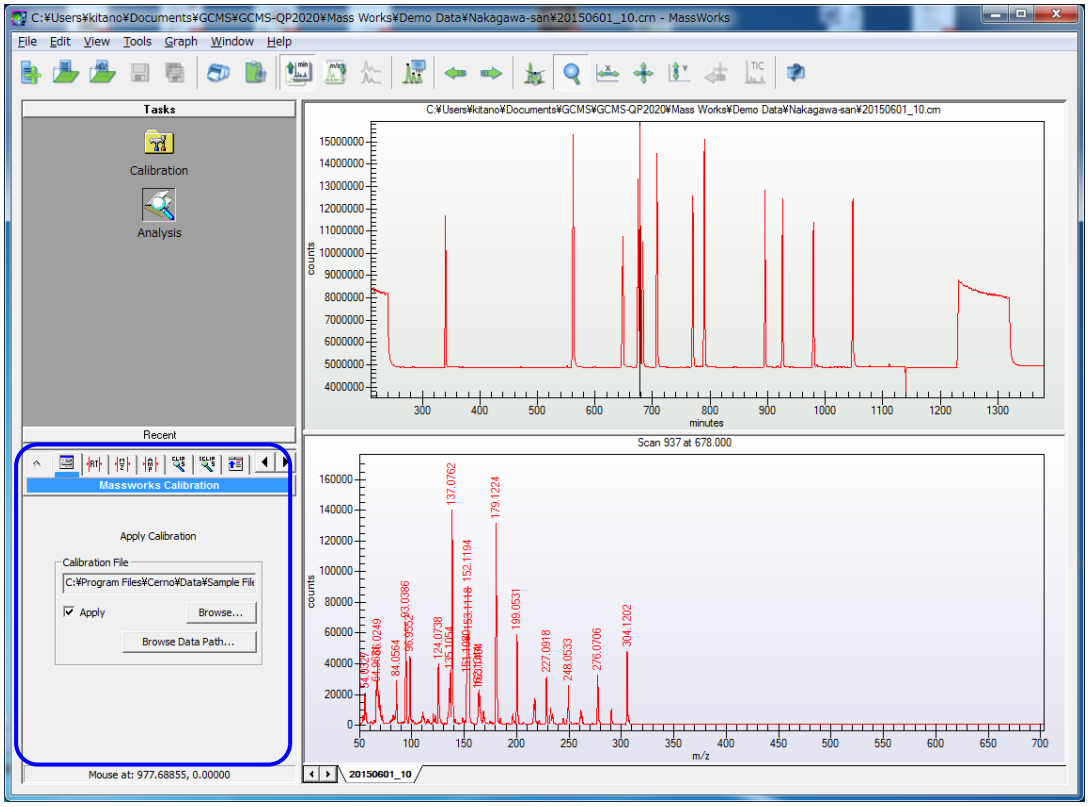
11) Click [File] - [Save Calibration File As] to save the created calibration file under a new name. Click [File] - [Print Report] if you want to output the results as a report.



4.1.3 Processing the Acquired Data

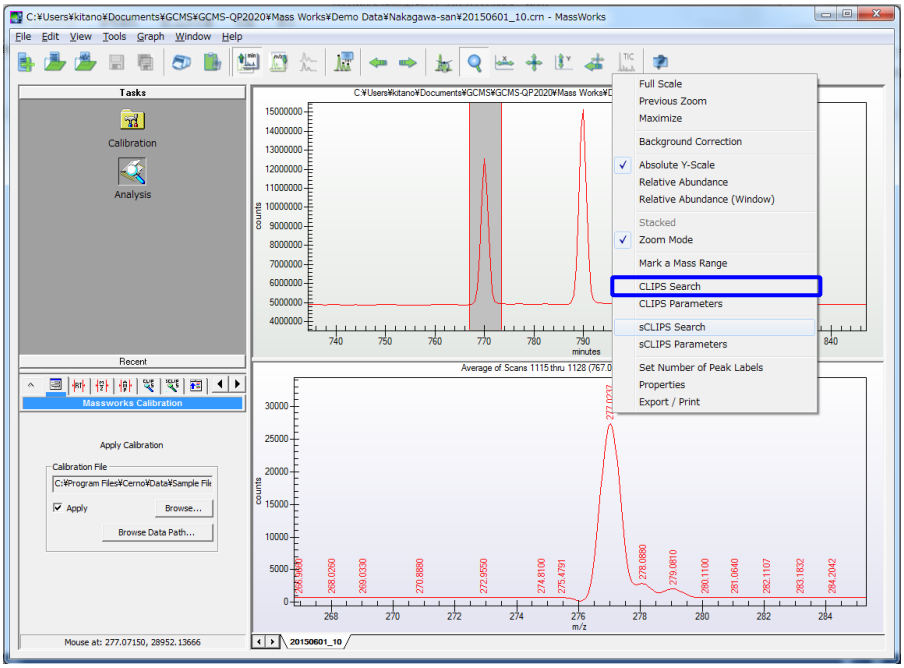
Calculate accurate mass of the peaks within the acquired data and estimate the elemental composition using the mass calibration file created by the procedure described in "4.1.2 Creating the Calibration File."

1) Click the [Analysis] icon in the navigation pane. Then click [File] - [Open MS Data] to open a crm format file.
2) Click the [MassWorks Calibration] tab in the utility pane and select the created calibration file.

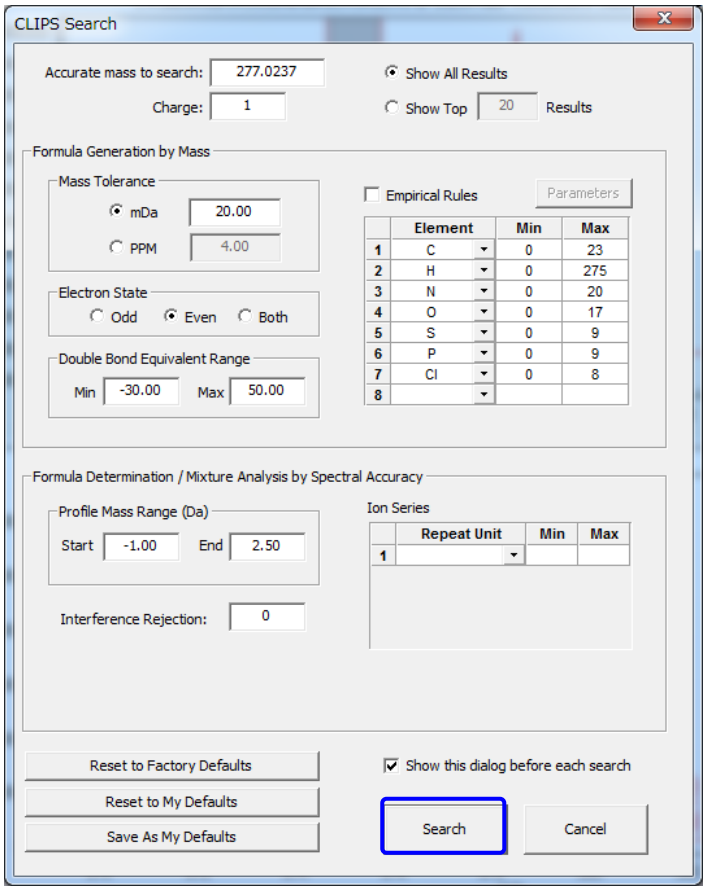


3) Right-click the mouse in the chromatogram subwindow. In the popup menu, click [Mark an RT window] and specify the peak analysis range by clicking the mouse on the start and end points.

4) Enlarge the molecular ion peak in the spectrum subwindow and right-click the mouse on the m/z value displayed at the peak top, and select [CLIPS Search].



5) Set the parameters in the [CLIPS Search] window and click [Search].



Functionality	Contents
Accurate mass to search	The accurate mass derived from clicking the monoisotope peak of the ion the search.
Charge	The charge state of the ion.
Show All Results	Displays all elemental compositions searched.
Show Top xxx Results	Displays a specified number of components from the top in the search result.
Mass Tolerance	The mass window in milli-Daltons or PPM about the monoisotope peak to search for formulas.
Electron State	The Electron State specifies the preferred types of molecule to be included or excluded from the results. Odd: Includes odd electron types only Even: Includes even electron types only. Both: Includes both odd and even electron types.
Double Bond Equivalent Range	The Double Bond Equivalent (DBE) specifies the maximum and minimum number of double bonds per molecule considered to be acceptable in producing a valid formula.
Element table	The Element table defines what elements and what number of each, minimum to maximum, to allow for the search. It is best to restrict this as best you can to limit the search candidates.
Empirical Rules	Check box to indicate whether to use empirical elemental rules to eliminate formulas that are unfeasible or unlikely, according to typically recognized chemical rules and/or existing known compound libraries. By using these empirical rules, you may be able to work with only a limited number of possible formula candidates and quickly arrive at the correct identification. On the other hand, some new and unique chemical compounds may fall outside of the statistical boundaries and be eliminated from consideration, leading to incorrect identifications. Another case where these rules may not apply is in MS/MS or EI experiments where ion fragments instead of molecular ions are the unknowns to be determined. It is recommended to always confirm your formula identification results by also considering high spectral accuracy hits with these empirical rules unchecked.
Profile Mass Range	Sets the mass range for calculating the spectral accuracy taking isotope ions into account.
Interference Rejection	Sets a threshold to minimize interferences between the isotope peaks. Set from 0 - 1 where 1 is the maximum peak height.
Ion Series	Ion Series (formerly Mixture Analysis) Allows the inclusion of up to two additional ion series which are interfering with the ion of interest. The input can be a formula, or a modification of the search formula.
Reset to Factory Defaults	Resets the parameters to factory defaults and clears the element table.
Reset to My Defaults	Resets the parameters and element table to user saved values.
Save As My Defaults	Saves the current parameters and element table to for future application to new searches or for recalling from the "Reset to My Default" button.
Show this dialog before each search	Shows the CLIPS parameters dialog every time a search is executed. If you do not want this to show, uncheck this box. This will force CLIPS to immediately search without presenting the dialog using the existing parameters.

Hint

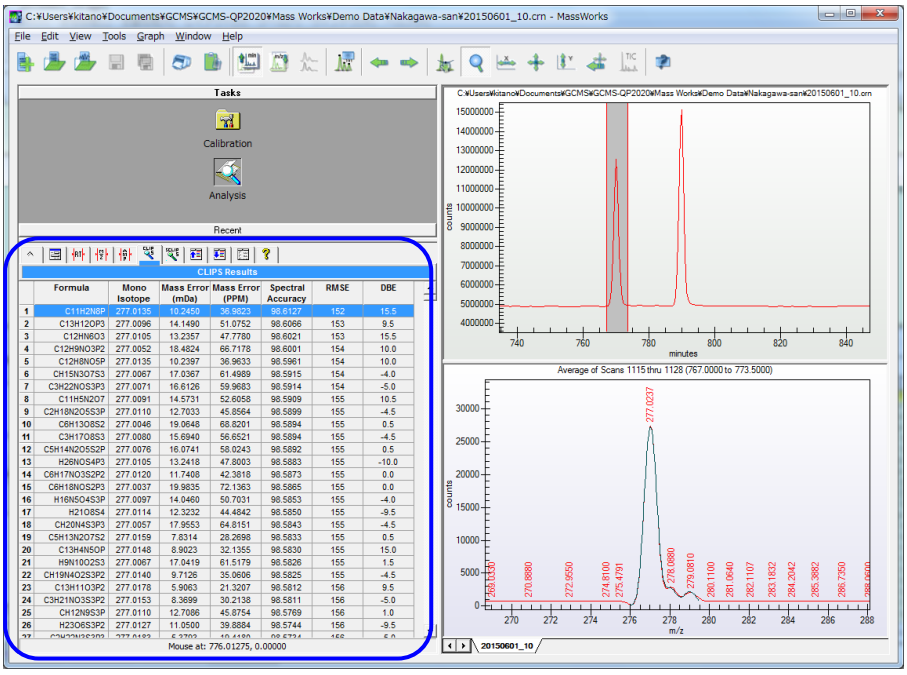
Elements set for CLIPS search

The more the number of elements registered for composition estimation using calculated accurate mass, the more the candidate composition formulas. The table below shows the natural isotopic ratios of elements detected by GC/MS. Some elements such as silicon (Si), sulfur (S), chlorine (Cl), and bromine (Br) have a higher natural isotopic ratio than other elements. Check the molecular ions (M^+) of the acquired peak and investigate the relative intensity ratio of M+2. Then set elements as search parameters if that ratio is relatively high (for example 10 % or more). Doing so will reduce the number of search candidates and make the search process easier.

Element	Isotope	Isotopic Composition (%)
H	^{1}H	99.9885
	^{2}H	0.0115
C	^{12}C	98.93
	^{13}C	1.07
N	^{14}N	99.636
	^{15}N	0.364
O	^{16}O	99.757
	^{17}O	0.038
	^{18}O	0.205
F	^{19}F	100.000
Si	**^{28}Si**	**92.223**
	^{29}Si	**4.685**
	^{30}Si	**3.092**
P	^{31}P	100.000
S	**^{32}S**	**95.99**
	^{33}S	**0.75**
	^{34}S	**4.25**
	^{36}S	**0.01**
Cl	**^{35}Cl**	**75.76**
	^{37}Cl	**24.24**
Br	**^{79}Br**	**50.69**
	^{81}Br	**49.31**
I	^{127}I	100.000

Reference: NIST Atomic Weights and Isotopic Compositions for All Elements

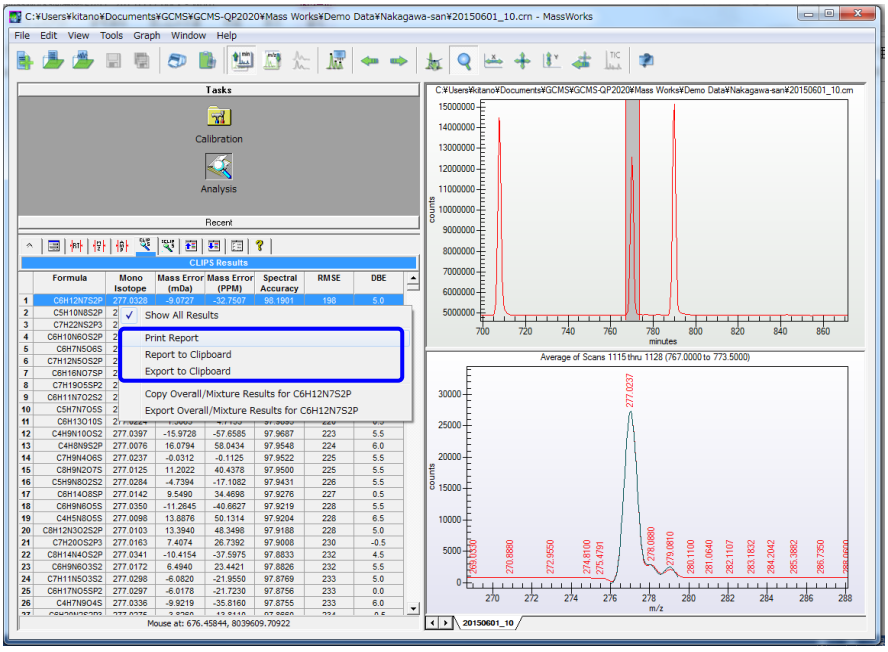
6) Move to the [CLIPS Results] tab in the utility pane and check the search results. Click the list to display the calculated mass profile for the selected composition on the spectrum in an overlay manner.



RMSE = Root mean squared error

DBE = Double bond equivalent

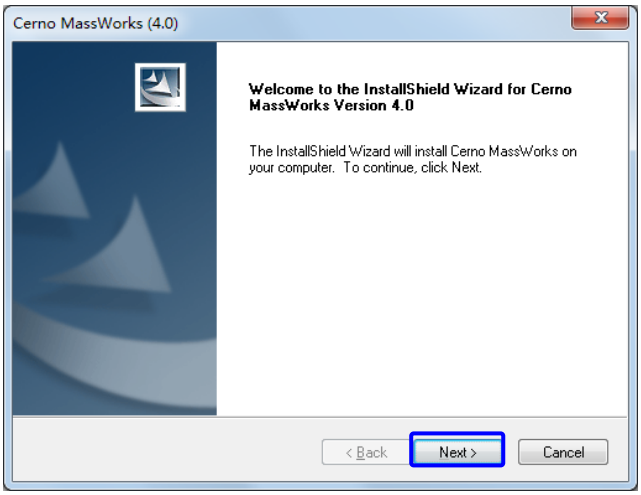
7) To print the graph subwindow (chromatogram and spectrum), click [Print Report] in the [File] menu. To print out search results, right-click the mouse on the table in the utility pane and click [Print Report]. Or click [Report to Clipboard] to copy the search results in print image. If clicking [Export to Clipboard], the search results will be copied as ASCII value.



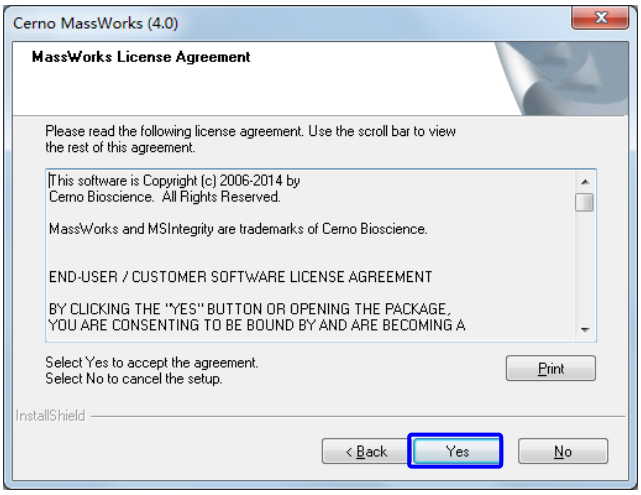
Appendix 1 Installing MassWorks Software

Install MassWorks Software in accordance with the following procedures.
Explanation in this section is given for a Windows 7-based computer.

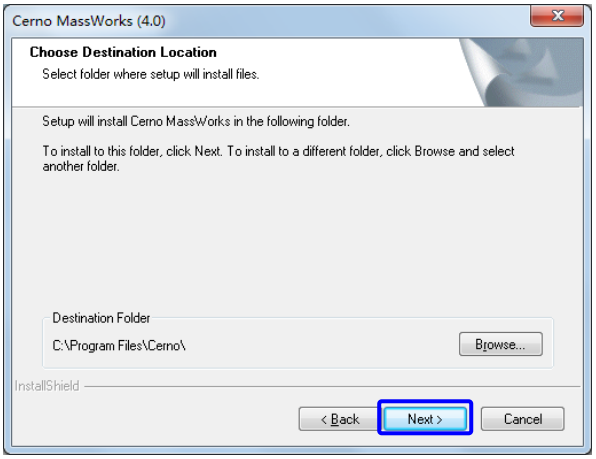
1) Insert MassWorks CD and right click "Setup.exe" to run as Administrator.
2) Click [Next] button.



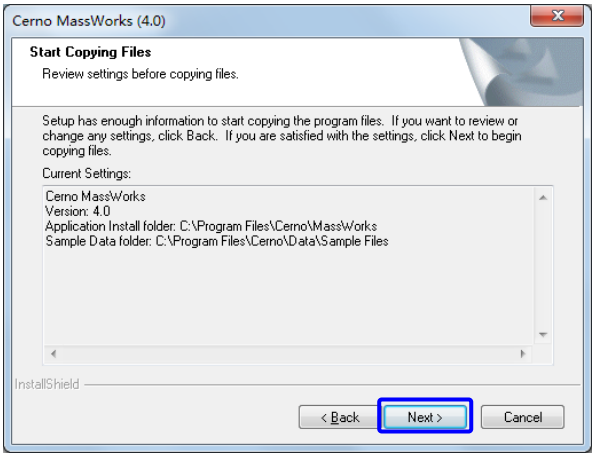
3) [MassWorks License Agreement] is displayed. Click [Yes] button.



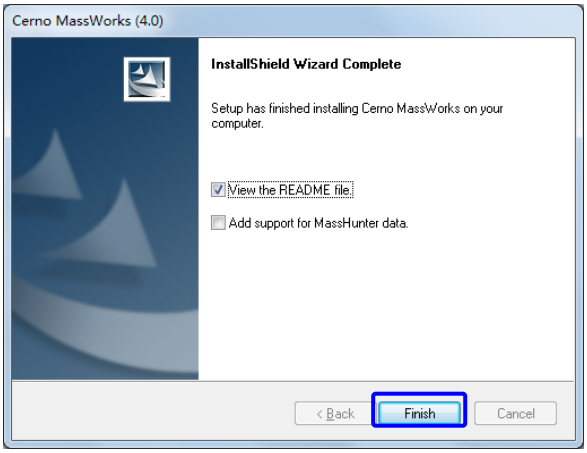
4) [Choose Destination Location] is displayed. Click [Next] button.



5) [Start Copying Files] is displayed. Click [Next] button.



6) [InstallShield Wizard Complete] is displayed. Click [Finish] button.



7) Insert the USB security key into a USB port on your computer.