225-45560A
May 2022

Smart Database

Smart Metabolites Database Ver.2

Instruction Manual

For GCMSsolution

1.8

Read the instruction manual thoroughly before you use the product.

Keep this instruction manual for future reference.

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Introduction

Read this Instruction Manual thoroughly before using the product.

Thank you for purchasing this product. This manual describes the installation, operation, usage cautions and accessories for this product. Read this manual thoroughly before using the product and operate the product in accordance with the instructions in this manual.

Also, keep this manual for future reference.

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Indications Used in This Instruction Manual

Throughout the text in this manual, warnings and other information essential when using this unit, such as cautionary or prohibited items, appear classified as per the following:

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.

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1. Warranty Period — Please contact your Shimadzu representative for information about the period of this warranty.
2. Description: — If a product/part failure occurs for reasons attributable to Shimadzu during the warranty period, Shimadzu will repair or replace the product/part free of charge. However, in the case of products which are usually available on the market only for a short time, such as personal computers and their peripherals/parts, Shimadzu may not be able to provide identical replacement products.
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   4) Computer viruses leading to device failures and damage to data and software, including the product's basic software
   5) Power failures, including power outages and sudden voltage drops, leading to device failures and damage to data and software, including the product's basic software
   6) Turning OFF the product without following the proper shutdown procedure leading to device failures and damage to data and software, including the product's basic software
   7) Reasons unrelated to the product itself
   8) Product use in harsh environments, such as those subject to high temperatures or humidity levels, corrosive gases, or strong vibrations
   9) Fires, earthquakes, or any other act of nature, contamination by radioactive or hazardous substances, or any other force majeure event, including wars, riots, and crimes
   10) Product movement or transportation after installation
   11) Consumables and equivalent items
       Recording media such as are considered consumable items.

- If there is a document such as a warranty provided with the product, or there is a separate contract agreed upon that includes warranty conditions, the provisions of those documents shall apply.
- Warranty periods for products with special specifications and systems are provided separately.

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1 About Smart Metabolites Database Ver.2

1.1 Overview

This product consists of method files, library files, the Smart Database, damlp files, an instruction manual, and a handbook. When analyzing metabolites such as amino acids, organic acids, fatty acids, and sugars, the work involved in evaluating the analysis conditions and creating compound tables containing data on the compounds targeted for analysis can be complicated and time-consuming. This product provides information on columns optimized to handle various analytes, and method files containing the appropriate analysis conditions and compound information. As a result, the work required for preparation is greatly reduced.

The method files in this product contain retention indices obtained from n-alkanes. Simply by making use of the Automatic Adjustment of Retention Time (AART) function of GCMSsolution and using n-alkane mixed standard samples, the retention time at which a metabolite will be detected can be easily predicted. Additionally, by using the library included with this product, similarity searches based on the retention index can be performed. The Smart Metabolites Database enables the creation of methods based on retention times that have been adjusted using the AART function, eliminating the need to analyze standard samples for measured compounds and calculate the retention times. An Excel file is used as the database file, allowing you to edit it and add compounds. Calibration curves for semiquantitation are registered in the methods for sugars, so semiquantitation values for detected components can be calculated.

NOTE

This Instruction Manual has been written assuming the user already has basic knowledge of handling GC-MS, GC-MS/MS, GCMSsolution, and LabSolutions Insight. There are many places where the special names and terminology used in the above products are used and explained. Therefore if there is anything that is unclear when first using the product, refer to the GC-MS, GC-MS/MS Instruction Manuals, the GCMS Operation Guide, LabSolutions Insight Instruction Manual, and GCMSsolution Help.

1.2 Operating Environment

The Smart Metabolites Database Ver.2 requires the following software environment.

OS	Microsoft Windows 10 Professional 64-bit version Microsoft Windows 7 Professional SP1 32/64-bit versions
Excel	Microsoft Excel 2016 (32-bit version) Excel 2019 (32-bit version or 64-bit version) Excel 2021 (32-bit version or 64-bit version)
Workstation software	GCMSsolution Ver4.53SP1 or later

1.3 Instruments Supported

The Smart Metabolites Database Ver.2 can be used with the following instruments.

GCMS-TQ series : GCMS-TQ8030, GCMS-TQ8040, GCMS-TQ8050,
GCMS-TQ8040NX, GCMS-TQ8050NX

GCMS-QP series : GCMS-QP2010 Ultra, GCMS-QP2010 SE,
GCMS-QP2020, GCMS-QP2020NX

1.4 Composition

1.4.1 Contents

This database includes a CD-ROM. If this is insufficient, kindly contact Shimadzu Corporation.

Part No.	Items	Quantity
225-45555-91 or 225-45555-92	CD-ROM	1

The CD-ROM includes the Smart Metabolites Database Ver.2 installer, the PDF version of the Instruction Manual and the Handbook.
(File location: C:\GCMSsolution\Manual\SmartDatabase\Metabolites)

NOTE

This product does not include columns. Be sure to obtain the specified ones.

1.4.2 Configuration of Method Files

The database contains the following method files for measuring components: 6 types for analysis of organic acids, fatty acids, amino acids, and sugars using trimethylsilylated(TMS) derivatives (scan: 3 types, MRM: 3 types); 1 type for analysis of sugars using acetyl derivatives; and 8 types for analysis of fatty acids using methyl ester(ME) derivatives (scan: 4 types, MRM: 4 types).

Different method files need to be used depending on the type of instrument being used. When the installer (Setup.exe) for this product is executed, the following method files are installed. During installation, the instrument type (QP or TQ) can be selected, and files will be installed in accordance with the type of instrument. (Here C: indicates the drive on which GCMSsolution is installed.)

(Method file location: C:\GCMSsolution\SmartDatabase\Metabolites\Method)

a) Method files for analysis of organic acids, fatty acids, amino acids, and sugars using trimethylsilylated(TMS) derivatives

File name (.qgm)	Mode	Column	Compounds	Contents
OA_TMS_DB5_37min_V4_Scan	Scan	DB-5	627	**Recommended (analysis time: 37 min)** Splitless analysis is supported.
OA_TMS_DB5_67min_V4_Scan				Improved separation (analysis time: 67 min) Splitless analysis is supported.
OA_TMS_BPX5_23min_V4_Scan		BPX5	547	Analysis time 23 min Only split analysis is supported.
OA_TMS_DB5_37min_V4_MRM	MRM	DB-5	526	Analysis time: 37 min Splitless analysis is supported.
OA_TMS_DB5_67min_V4_MRM				Improved separation (analysis time: 67 min) Splitless analysis is supported.
OA_TMS_BPX5_23min_V4_MRM		BPX5	540	**Recommended (analysis time: 23 min)** Only split analysis is supported.
OA_TMS_DB5_37min_V4_HC	Scan	DB-5	25	Retention time adjustment method for OA_TMS_DB5_37min_V4_ Scan(MRM).qgm
OA_TMS_DB5_67min_V4_HC				Retention time adjustment method for OA_TMS_DB5_67min_V4_ Scan(MRM).qgm
OA_TMS_BPX5_23min_V4_HC		BPX5		Retention time adjustment method for OA_TMS_BPX5_23min_V4_ Scan(MRM).qgm

b) Method files for analysis of sugars using acetyl derivatives

File name (.qgm)	Mode	Column	Compounds	Contents
Sugar_AC_BPX5_V4_Template	MRM /SIM	BPX5	24	Semiquantitation value calculation EI method
Sugar_AC_BPX5_V4_HC	Scan		19	Retention time correction method Sugar_AC_BPX5_V4_Template

c) Method files for analysis of fatty acids using methyl ester(ME) derivatives

File name (.qgm)	Mode	Column	Compounds	Contents
FA_ME_DB5MS_EI_V4_Scan	Scan	DB-5MS	50	Capable of analysis of very long chain fatty acids (C31:0) EI method
FA_ME_DB5MS_ PCI_V4_Scan				Capable of analysis of very long chain fatty acids (C31:0) PCI method
FA_ME_SP2560_EI_V4_Scan		SP2560	38	Supports high-resolution measurements EI method
FA_ME_SP2560_PCI_V4_Scan				Supports high-resolution measurements PCI method
FA_ME_DB5MS_EI_V4_MRM	MRM	DB-5MS	50	Capable of analysis of very long chain fatty acids (C31:0) EI method
FA_ME_DB5MS_PCI_V4_MRM				Capable of analysis of very long chain fatty acids (C31:0) PCI method
FA_ME_SP2560_EI_V4_MRM		SP2560	38	Supports high-resolution measurements EI method
FA_ME_SP2560_PCI_V4_MRM				Supports high-resolution measurements PCI method
FA_ME_DB5MS_EI_V4_HC	Scan	DB-5MS	27	Retention time adjustment method for FA_ME_DB5MS_EI(PCI)_Scan(MRM).qgm
FA_ME_SP2560_EI_V4_HC		SP2560	24	Retention time adjustment method for FA_ME_SP2560_EI(PCI)_Scan(MRM).qgm

d) Method file for analysis of blood plasma samples using surrogate internal standard

File name (.qgm)	Mode	Column	Compounds	Contents
SurrogateIS_OA_BPX	MRM	BPX5	133	Analysis time: 23 min Only split analysis is supported.

The columns in relation to each method file are detailed below.

DB-5 30m x 0.25mm I.D. df=1.00 um (Agilent Cat No. 122-5033)
BPX5 30m x 0.25mm I.D. df=0.25 um (SGE Cat No. 054101)
DB-5MS 30m x 0.25mm I.D. df=0.25 um (Agilent Cat No. 122-5532)
SP2560 100m x 0.25mm I.D. df=0.20 um (Supelco Cat No. 24056)

NOTE

When the injection mode is changed between the split and splitless modes, the retention index of components may be off by 1 to 5 if it is 1300 or less.

NOTE

In the case of analysis with “DB-5 column, analysis time:37 min” methods, for some of the components, the difference of the retention index between predicted values and measured values would be large compared to the case of analysis time: 67 min. However, it does not affect the data analysis significantly.

NOTE

In the case of analysis with SP2560 column, it is possible that the difference of the retention index between predicted values and measured values changes depending on the production lot of the column.

It is recommended to measure the standard reagent shown below, and adjust the retention times of the compound table for the analysis with SP2560 column. In this procedure, the retention times predicted by the retention index registered in this database can be used as the reference.

(Refer to “*Appendix2 Adjusting Retention Times of Compound Table by Measuring Standard*”)

Cat# CRM47885

Supelco® 37 Component FAME Mix certified reference material, TraceCERT®, in dichloromethane

SIGMA-ALDRICH

Hint

The trimethylsilyl derivatization method contains information on 38 stable isotopes. For a list of corresponding stable isotope reagents, see "*Appendix 6 List of Stable Isotope Reagents*."

Hint

The following glass inserts and CI reagent gas are recommended.

<Glass Insert>

Deactivated splitless insert with wool: Shimadzu Corporation P/N 221-48876-03
(GC-2030) P/N 227-35008-01

When performing analysis using a BPX-5 column or when increasing the split ratio above 1:10, use of the following is recommended.

Deactivated split insert with wool: Shimadzu Corporation P/N 225-20803-01
(GC-2030) P/N 227-35007-01

<CI Reagent Gas>

Isobutane gas (purity of 99.9 % or greater)

1.4.3 Configuration of Libraries

The database contains 7 types of libraries for scan measurements.
(Library file location: C:\GCMSsolution\library)

a) Libraries for analysis of organic acids, fatty acids, amino acids, and sugars using trimethylsilylated(TMS) derivatives.

File name(.lib)	Column	Compounds	Correspondence method files(.qgm)
OA_TMS_DB5_37min_V4	DB-5	627	OA_TMS_DB5_37min_V4_Scan
OA_TMS_DB5_67min_V4	DB-5	627	OA_TMS_DB5_67min_V4_Scan
OA_TMS_BPX5_23min_V4	BPX5	547	OA_TMS_BPX5_23min_V4_Scan

b) Libraries for analysis of fatty acids using methylester(ME) derivatives.

File name(.lib)	Column	Compounds	Correspondence method files(.qgm)
FA_ME_DB5MS_EI_V4	DB-5MS	50	FA_ME_DB5MS_EI_V4_Scan
FA_ME_DB5MS_PCI_V4			FA_ME_DB5MS_PCI_V4_Scan
FA_ME_SP2560_EI_V4	SP2560	38	FA_ME_SP2560_EI_V4_Scan
FA_ME_SP2560_PCI_V4			FA_ME_SP2560_PCI_V4_Scan

c) File composition of library

Each library consists of the following seven files. For example, “FA_ME_DB5MS_EI_V4” files are as shown below:

File name	Contents
FA_ME_DB5MS_EI_V4.LIB	Includes header information about the library and compound information, including molecular weights and CAS registration numbers.
FA_ME_DB5MS_EI_V4.FOM	Contains compound composition formulas.
FA_ME_DB5MS_EI_V4.SPC	Contains spectra information for compounds.
FA_ME_DB5MS_EI_V4.NAM	Contains compound names.
FA_ME_DB5MS_EI_V4.COM	Contains comments.
FA_ME_DB5MS_EI_V4.FLG	Contains information about search targets.
FA_ME_DB5MS_EI_V4.ADD	Contains link information with structure files.

- In the Shimadzu GC/MS software, any library is represented by the file extension .LIB. Files with this extension are the only library files the user need be concerned with. Note that the library can be used only when seven files are all in the same library folder.
- The file configuration of these libraries differs from that of the public libraries.
- This library will function as a library only when all seven of the files mentioned above, Meta1_Structure and Meta4_Structure folder are stored in the same folder. (C:\GCMSsolution\library)
- CAS numbers indicate the numbers of respective compounds before derivatization (native).

1.4.4 Configuration of Smart Database Files

The database contains 5 types of Smart Database files. For the procedure to create method files using Smart Database, refer to "*2.2 Operation Flow to Create Method Files Using Smart Database*". (C: \GCMSsolution\SmartDatabase)

a) Smart Database files for analysis of organic acids, fatty acids, amino acids, and sugars using trimethylsilylated(TMS) derivatives(Split).

File name (.xlsm)	Column	Compounds	Contents
OA_TMS_V4_Split	DB-5	627	Analysis time: 37 min, 67 min, Scan_627 compounds, MRM_526 compounds
	BPX5	547	Analysis time: 23 min, Scan_547 compounds, MRM_540 compounds

b) Smart Database files for analysis of organic acids, fatty acids, amino acids, and sugars using trimethylsilylated(TMS) derivatives(Splitless).

File name (.xlsm)	Column	Compounds	Contents
OA_TMS_V4_Splitless	DB-5	627	Analysis time: 37 min, 67 min Scan_627 compounds, MRM_526 compounds

c) Smart Database file for analysis of sugars using acetyl derivatization

File name (.xlsm)	Column	Compounds	Contents
Sugar_AC_V4	BPX5	24	SIM and MRM data EI method

d) Smart Database files for analysis of fatty acids using methyl ester(ME) derivatives

File name (.xlsm)	Column	Compounds	Contents
FA_ME_MRM_EI_V4	DB-5MS	50	Scan and MRM data EI method
	SP2560	38	
FA_ME_MRM_PCI_V4	DB-5MS	50	Scan and MRM data PCI method
	SP2560	38	

1.4.5 Batch Composition

File name (.qgb)	Contents
Sugar_AC_V4_Template	Batch file exclusively for sugar analysis. The dilution ratio is registered. By inputting the sample mass, semiquantitation values can be calculated.

(Batch file location: C:\GCMSsolution\ SmartDatabase\Metabolites\Batch)

1.4.6 flag ".damlp" File Composition

File name(.damlp)	Contents
Plant_Food	Foods derived from plants
Animal_Food	Foods derived from animals
Urine	Human urine
Blood	Human blood (plasma)
Cells	Human ES cells

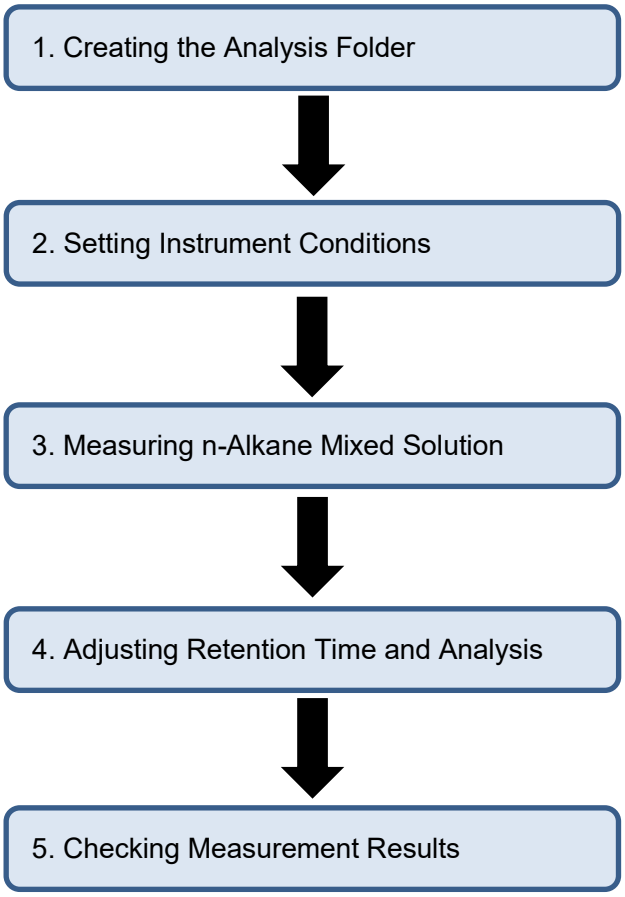
・damlp file location: C:\GCMSsolution\SmartDatabase\Metabolites\damlp

・contains compounds information to be displayed (hidden) as an analysis target when analyzing similar samples.

2 Usage Procedures

2.1 Operation flow to use method file

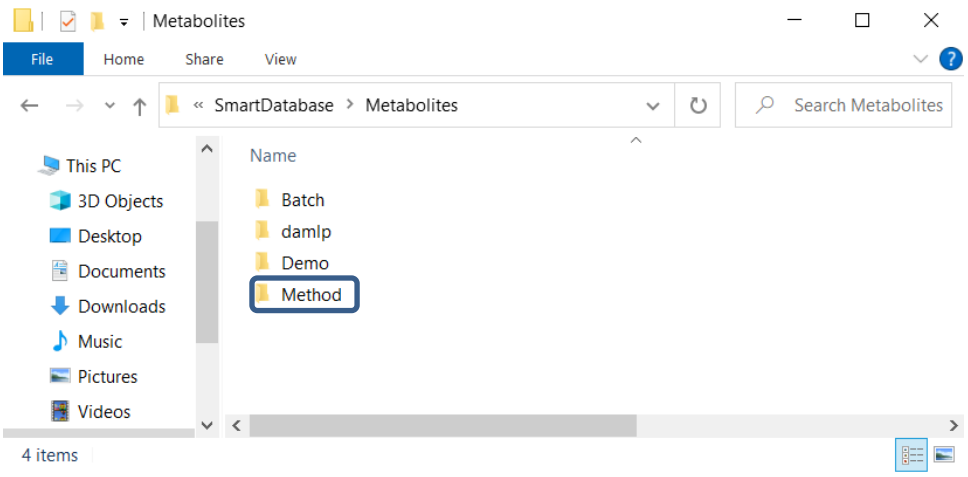
Follow the steps below to perform analysis using method files.



2.1.1 Creating the Analysis Folder

1) Create the folder for saving the analyzed data file.
(e.g.C: \GCMSsolution\Data\Project1)

2) Copy the necessary method files (.qgm extension) saved at C:\GCMSsolution\SmartDatabase\Metabolites\Method. Paste them to the folder created at step 1). To select the necessary files, refer to section "*1.4.2 Configuration of Method Files*".



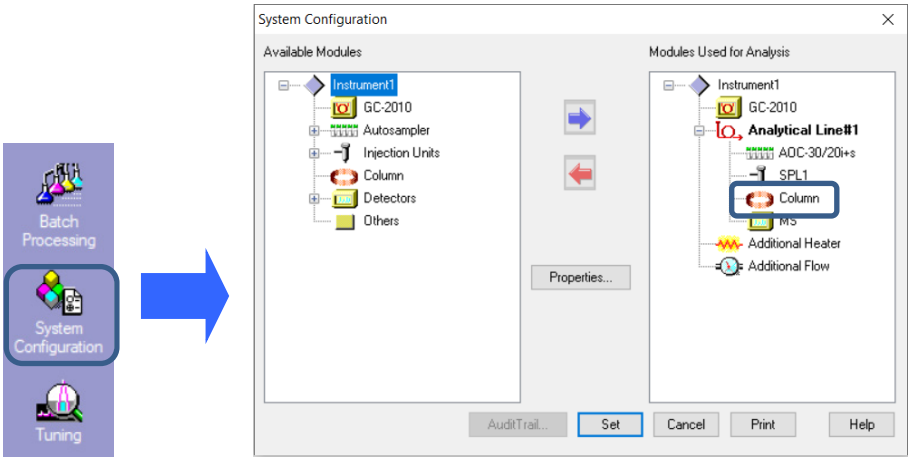
2.1.2 Setting Instrument Conditions

1) Use the column depending on the method file used in measurement.
Refer to "*1.4.2 Configuration of Method Files*".

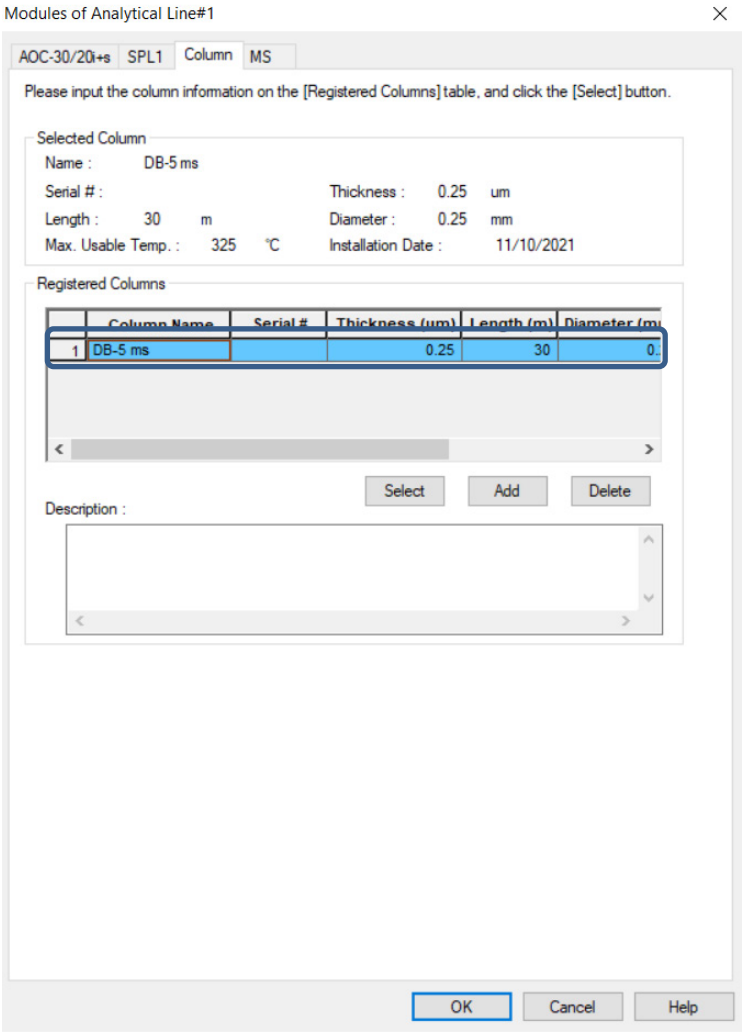
DB-5	30m x 0.25mm I.D. df=1.00 um (Agilent Cat No. 122-5033)
BPX5	30m x 0.25mm I.D. df=0.25 um (SGE Cat No. 054101)
DB-5MS	30m x 0.25mm I.D. df=0.25 um (Agilent Cat No. 122-5532)
SP2560	100m x 0.25mm I.D. df=0.20 um (Supelco Cat No. 24056)

The operational procedure will be explained for "FA_ME_DB5MS_EI_V4_Scan". The operation is the same for other methods.

2) Click the [System Configuration] icon on the [GCMS Real Time Analysis] assistant bar. The [System Configuration] window is displayed. Double-click the icon for the unit to be used for data acquisition (Column). The [Modules of Analytical Line] window is displayed.



Enter the column information correspond to the using method file at the column properties in the System Configuration window.



3) Perform auto-tuning.

1. Start up the vacuum system.

2. Wait 10 minutes after starting up the instrument. Perform leak check and confirm that there is no leakage.

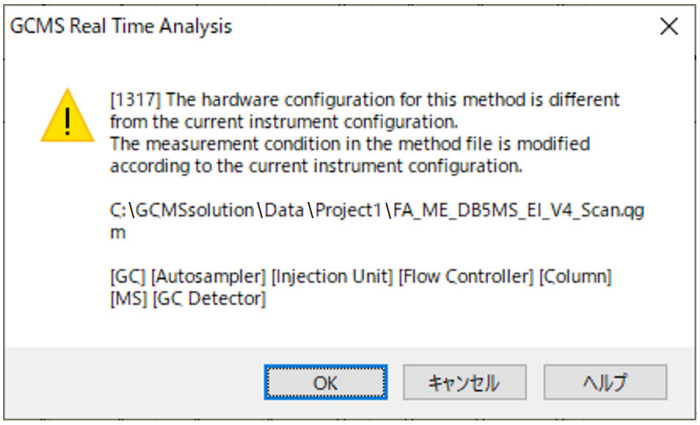
3. Wait 3 to 4 hours after starting up the instrument, perform auto-tuning.

NOTE

Execute tuning if the instrument has been shut down temporarily for reasons such as column replacement. The tuning should be performed about once per month, even if the instrument has not been shut down.

NOTE

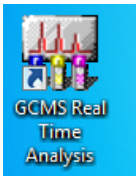
Load the method file for adjusting retention times and measurement parameters in the [GCMS Real Time Analysis] - [Acquisition] window. Here, click [OK] if the following confirmation message is displayed: "[1317] The hardware configuration for this method is different from the current configuration. The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



2.1.3 Measuring n-Alkane Mixed Solution

1) Measure n-alkanes using the method file for adjusting retention times.

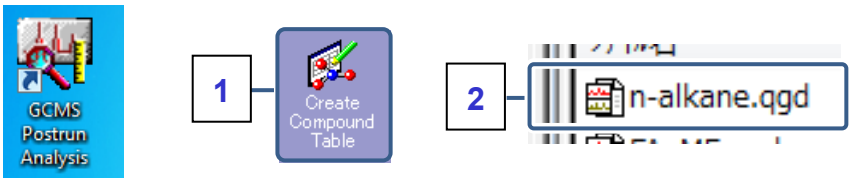
Reference concentration Injection mode Split: 100 µg/mL Splitless: 10 µg/mL

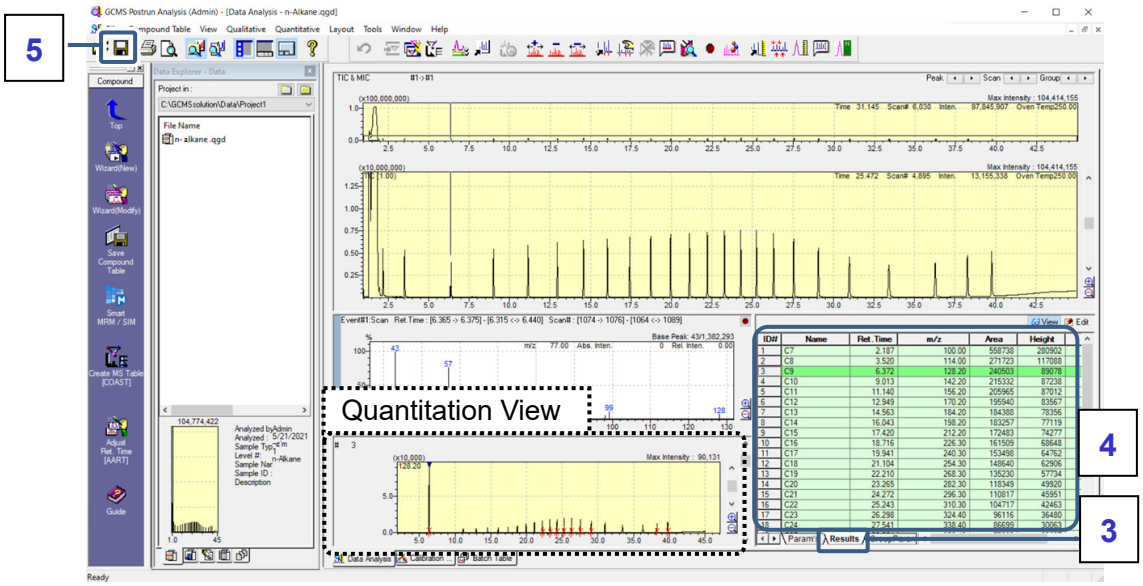


Folder: C:\GCMSsolution\Data\Project1

	Vial#	Sample Name	Sample Type	Analysis Type	Method File	Data File	Inj. Volume
1	1	n-alkane 10ug/mL	0:Unknown	IT QT	FA_ME_DB5MS_EI_V4_HC.qgm	n-alkane.qgd	1

2) Identify n-alkane.





1. Start the [GCMS Postrun Analysis] program and click the [Create Compound Table] icon on the [Postrun] assistant bar.
2. Double click the data file “n-alkane.qgd” on data explorer.
3. Click the [Results] tab in the [Compound Table View].
4. Click on a compound name in the compound table and check the chromatogram in the [Quantitation View]. If necessary, perform manual identification or peak integration.
5. Overwrite and save the data file.

Hint

The following of n-alkane is recommended.

C7-C33 Alkane Calibration Standard
Qualitative Retention Time Index Standard (Restek 31080)
(C15, C30 to C33 200 µg/mL, others 100 µg/mL) non-medical deleterious substances

⚠ CAUTION

Prepare the n-alkane standard sample in the exhaust epuipment such as a fume hood.

NOTE

Although mixed n-alkane standard samples are identified up to C33 (retention index 3300), the retention index of some compounds exceed 3300. In this case, the retention time is corrected by extrapolation, which may increase the difference between the corrected value and actual retention time.

NOTE

When measuring n-alkanes using the method file for adjusting retention times, the retention time can increase due to variances among column lots. As a result, all peaks up to C33 may not be eluted completely during analysis, some peaks could be partially clipped, or not be detected.

In such situations, modify the following two items of the method file for adjusting retention times so that all peaks up to C33 are completely eluted.

(1) Hold time of the GC temperature program prior to the end of analysis

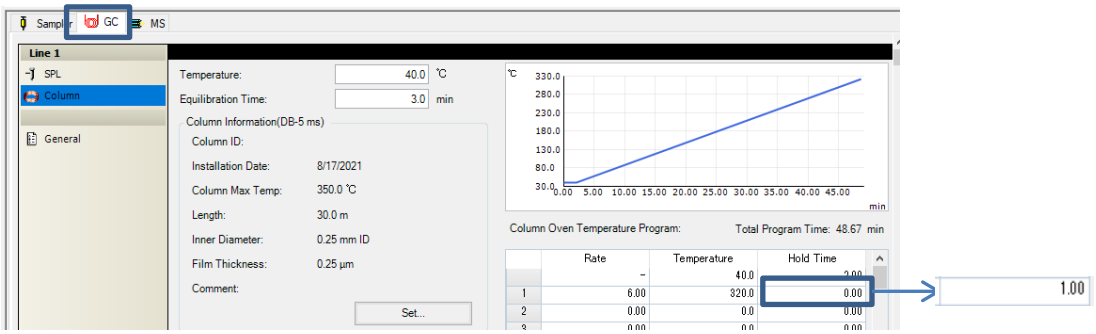
(2) MS analysis end time

Example) Extending by 1 minute the hold time of the GC temperature program prior to the end of analysis and the MS analysis end time in “FA_ME_DB5MS_EI_V4_HC.qgm”

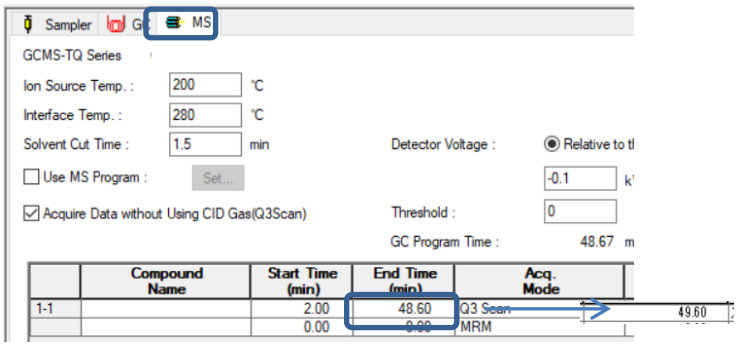
1) Start up the [GCMS Analysis Editor] program.

2) On the Data Explorer, double-click the method file that is to be modified.

3) Click the [GC] tab and extend the hold time of the column oven temperature prior to the end of analysis by 1 minute.



4) Click the [MS] tab, and extend the analysis end time by 1 minute.

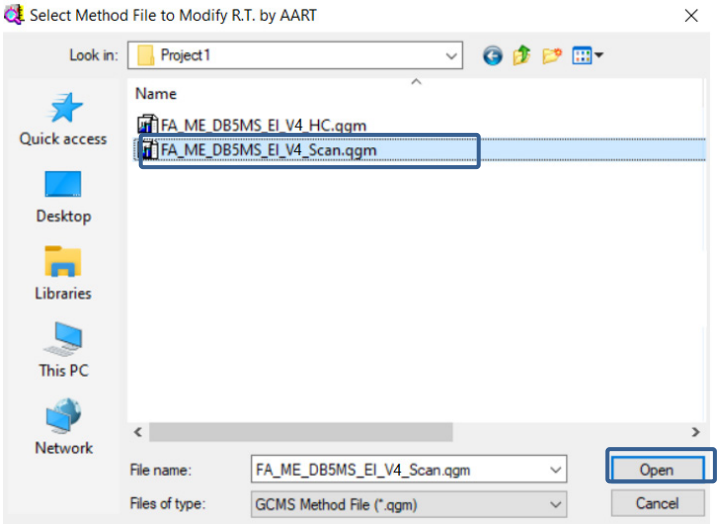


5) Overwrite and save the method file.

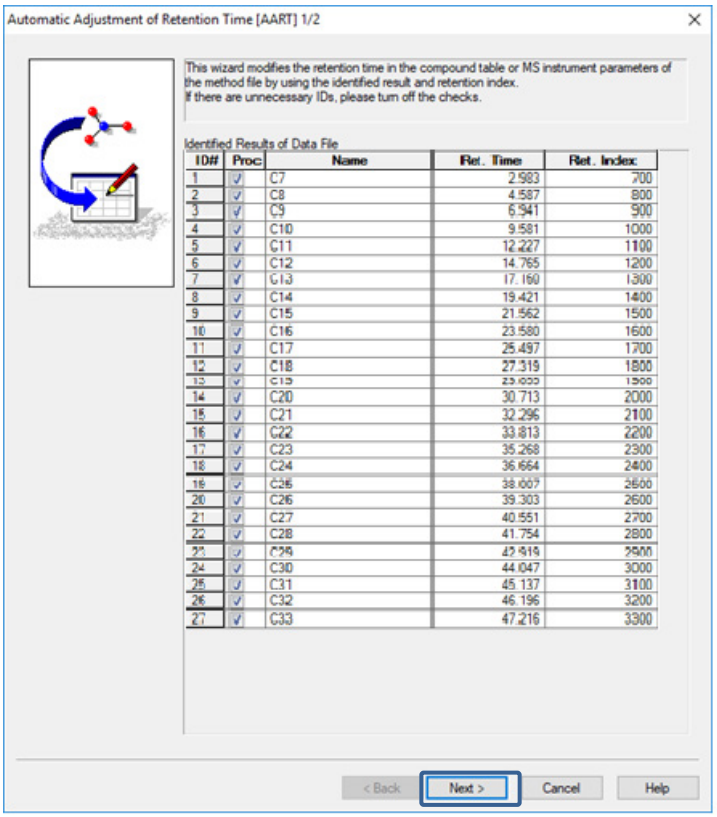
2.1.4 Adjusting Retention Time and Analysis

1) Click the [AART] icon on the [Compound] assistant bar.

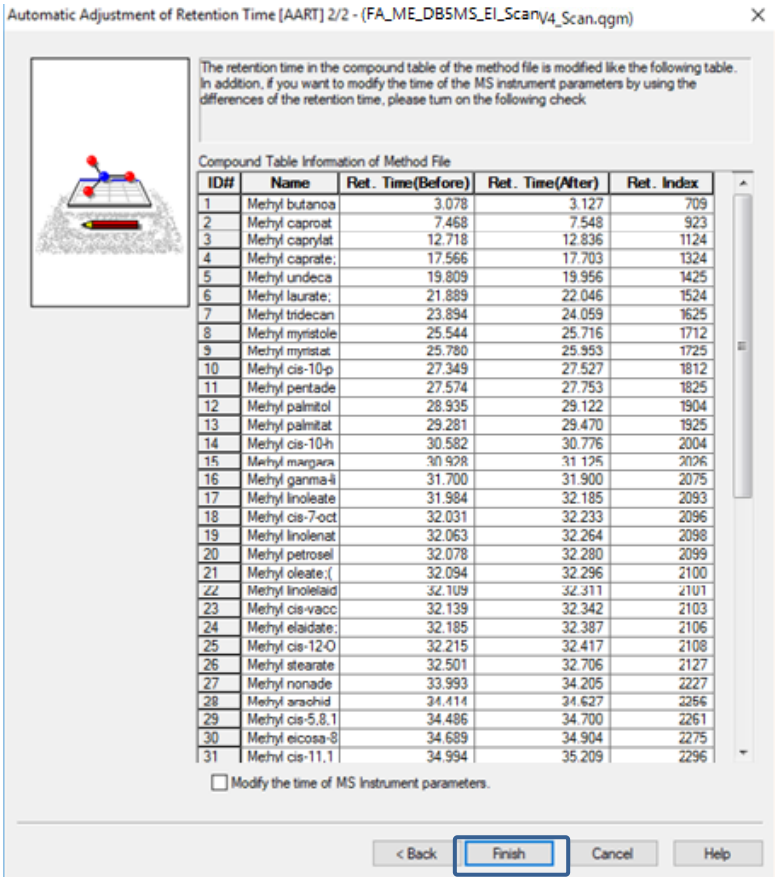
2) Select the method file that is to be modified and click [Open].



3) Check the results and click [Next].



4) Confirm that the retention times have been adjusted and click [Finish].



ID#	Name	Ret. Time(Before)	Ret. Time(After)	Ret. Index
1	Methyl butanoa	3.078	3.127	709
2	Methyl caproat	7.468	7.548	923
3	Methyl caprylat	12.718	12.836	1124
4	Methyl caprate;	17.566	17.703	1324
5	Methyl undeca	19.809	19.956	1425
6	Methyl laurate;	21.889	22.046	1524
7	Methyl tridecan	23.894	24.059	1625
8	Methyl myristole	25.544	25.716	1712
9	Methyl myristat	25.780	25.953	1725
10	Methyl cis-10-p	27.349	27.527	1812
11	Methyl pentade	27.574	27.753	1825
12	Methyl palmitol	28.935	29.122	1904
13	Methyl palmitat	29.281	29.470	1925
14	Methyl cis-10-h	30.582	30.776	2004
15	Methyl margara	30.928	31.125	2026
16	Methyl ganma-li	31.700	31.900	2075
17	Methyl linoleate	31.984	32.185	2093
18	Methyl cis-7-oct	32.031	32.233	2096
19	Methyl linolenat	32.063	32.264	2098
20	Methyl petrosel	32.078	32.280	2099
21	Methyl oleate;(	32.094	32.296	2100
22	Methyl linolelaid	32.109	32.311	2101
23	Methyl cis-vacc	32.139	32.342	2103
24	Methyl elaidate;	32.185	32.387	2106
25	Methyl cis-12-O	32.215	32.417	2108
26	Methyl stearate	32.501	32.706	2127
27	Methyl nonade	33.993	34.205	2227
28	Methyl arachid	34.414	34.627	2256
29	Methyl cis-5,8,1	34.486	34.700	2261
30	Methyl eicosa-8	34.689	34.904	2275
31	Methyl cis-11,1	34.994	35.209	2296

> **NOTE**
>
> When not performing a scan measurement, select the [Modify the time of MS Instrument parameters.] checkbox. When the checkbox is selected, the [MS] parameters of the analysis conditions are adjusted automatically. If the checkbox in a method file for MRM, SIM, or FAAST measurement is selected, automatic adjustment of the MRM or SIM table is also performed.

In this stage, the retention time of the method file has been adjusted using AART.

5) Measure the sample using the method with the corrected retention time.

2.1.5 Checking Measurement Results

The measurement results can be analyzed in the [GCMS Postrun Analysis] program or in the [LabSolutions Insight GCMS] program.

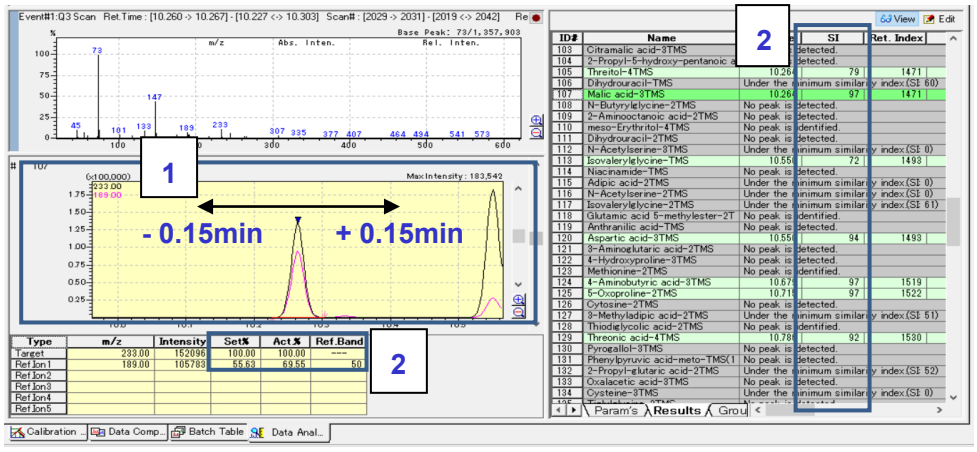
- GCMS Postrun Analysis: Enables library searches for compounds other than target compounds or additional compounds.
- LabSolutions Insight GCMS: Enables analysis of multiple samples, which is useful when there are statistical analyses such as multivariate analysis.

> **NOTE**
>
> During automatic peak detection performed by GCMSsolution, peaks may be incorrectly identified or not identified under the following conditions.
>
> - The concentration of the target component is extremely low.
> - Ion of the target component overlaps with the sample-derived matrix.
> - Instrument conditions are poor due to a contaminated glass insert, analytical column, or ion source.
> - Auto tuning has not been performed for a long time.

GCMS Postrun Analysis

1) Peak integration and peak identification are automatically performed on compounds registered in the compound table by the following criteria. The values shown below are for OA_TMS_BPX5_23min_V4_Scan.qgm as the example. Allowable deviation and Minimum similarity are different among the method files.

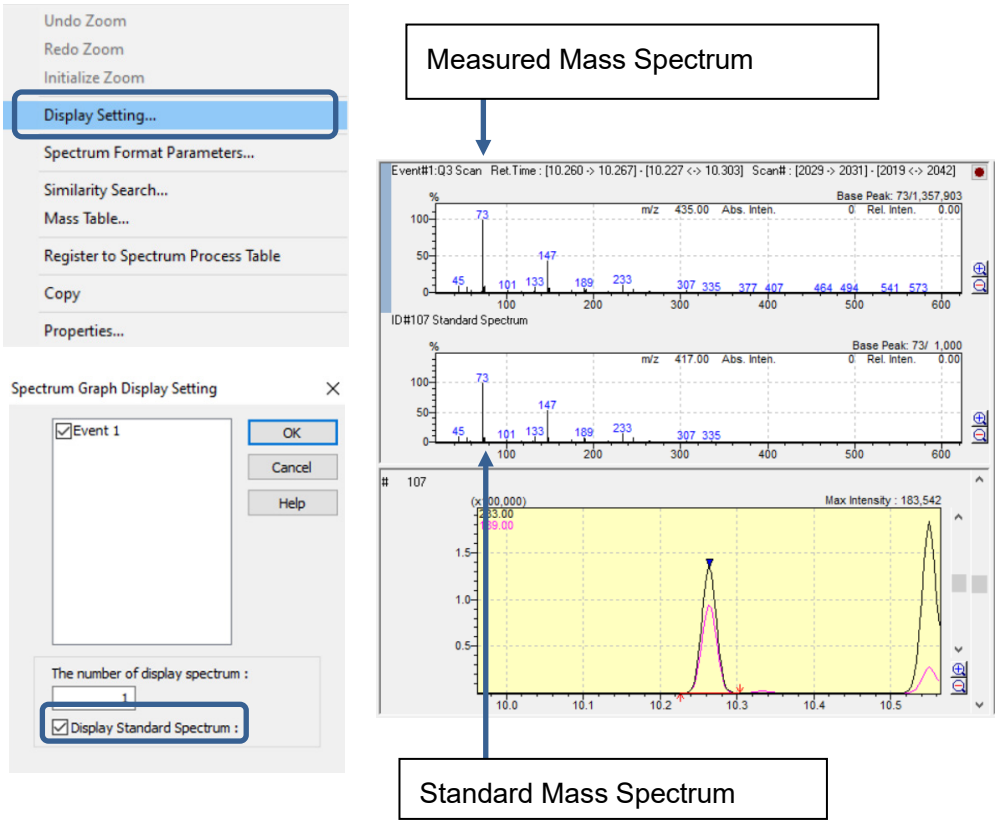
1. Peak integration and peak identification of mass chromatograms
   (Allowable deviation of registered retention time: BPX-5: ±0.15 min, DB-5: ±0.20 min)
2. MRM: Relative ion ratio of reference ion
   (Allowable deviation of the relative ion ratio to registered reference ion: ±50 %)
   Scan: Similarity to registered standard spectrum (SI) (Minimum similarity: 70)



Hint

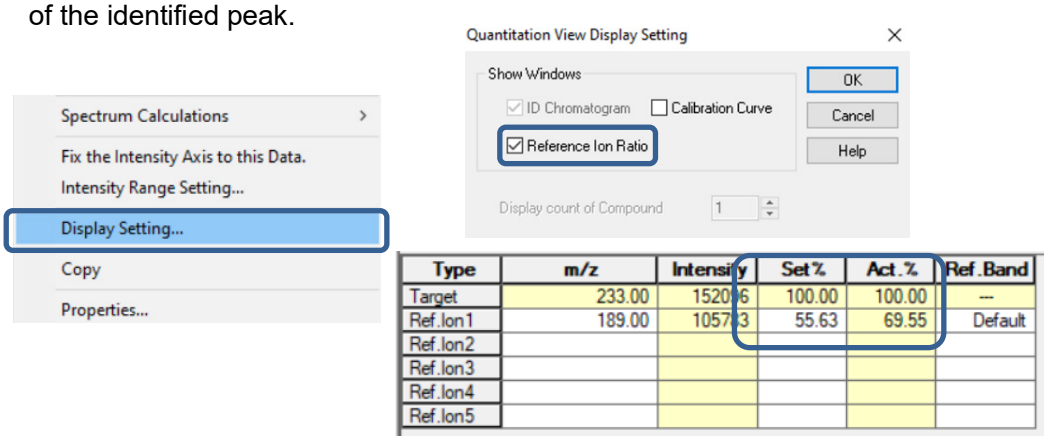
Displaying the standard spectrum

Select the compound that you want to refer to as the standard spectrum from the compound table, right-click the mouse on the [Spectrum Graph] and then select the [Display Setting] and check the [Display Standard Spectrum].



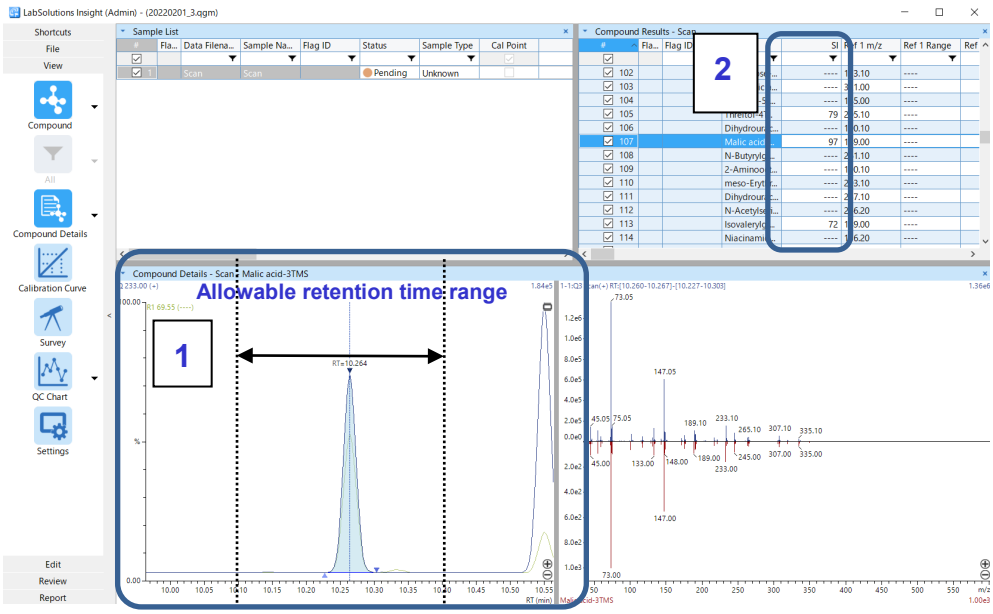
Displaying Reference Ion Ratios

In the compound table, select the compound for which you want to refer to the reference ion ratio, right-click the mouse on the [Quantitation view], and then select [Display Setting] and check the [Reference Ion Ratio]. [Set %] indicates the reference ion ratio registered in the method file and [Act. %] indicates the ion ratio of the identified peak.



LabSolutions Insight GCMS

1) Peak integration and peak identification are performed automatically in accordance with the following criteria for the compounds registered in the compound table.
   1. Peak integration and peak identification of mass chromatograms
      (Allowable deviation of registered retention time: BPX-5: ±0.15 min, DB-5: ±0.20 min)
   2. MRM: Relative ion ratio of reference ion
      (Allowable deviation of the relative ion ratio to registered reference ion: ±50 %)
      Scan: Similarity to registered standard spectrum (SI) (Minimum similarity: 70)



Hint

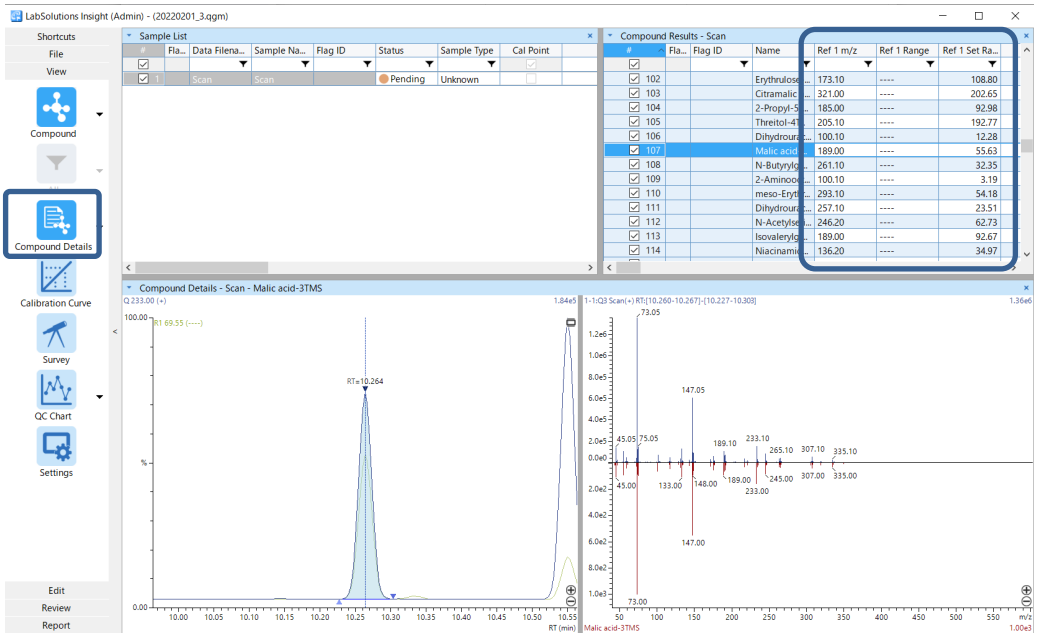
The compound information registered in the method file includes both mass spectral and reference ion ratio information. Results can be checked more easily by displaying and comparing results to that information.

How to Check Standard Spectra

Standard spectra can be checked by displaying the [Compound Details] tab page.

How to Display Reference Ion Ratios

Reference ion ratios can be checked in the [Compound Results] field.

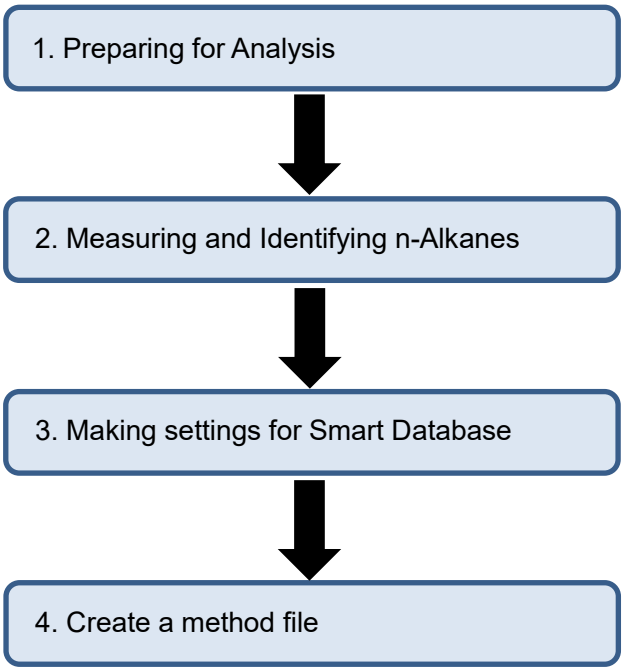


Hint

For the procedure to output the analysis result in list format so that it can be calculated by the statistical analysis software, refer to "*Appendix 4 Outputting the Area / Height Value from Multiple Data*".

2.2 Operation Flow to Create Method Files Using Smart Database

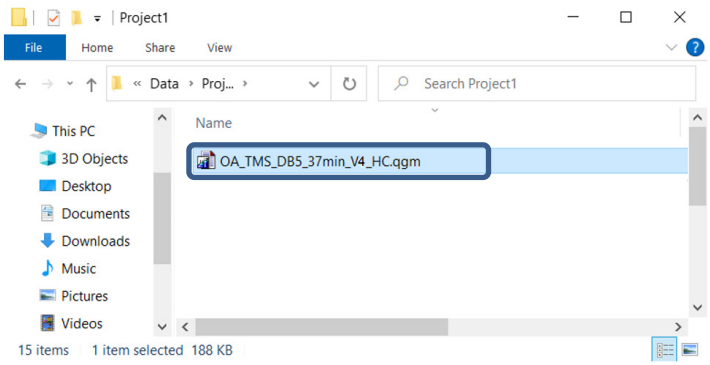
Follow the steps below to create a method file.



2.2.1 Preparing for Analysis

1) Create a folder to store the method file and data file.
(e.g. C:\GCMSsolution\Data\Project1)
The steps that follow show how to create a method using the Smart Database file "OA_TMS_V4_Splitless.xlsm" as one example.

2) Copy the necessary n-alkane method file that is stored in the C: \GCMSsolution\SmartDatabase\Metabolites\Method folder, and paste it in the folder that was created earlier.



3) Attach an analysis column appropriate for the Smart Database file to be used. Then configure the environmental settings and perform autotuning. For details on the relationship between columns and Smart Database, see "*1.4.4 Configuration of Smart Database Files.*"

> **NOTE**
>
> When a retention index registered in a database is used, be sure to use a column from the above-mentioned manufacturer matching the specified liquid phase type, length, internal diameter, and thickness.

2.2.2 Measuring and Identifying n-Alkanes

Measure the mixed n-alkane standard sample in order to correct the retention times registered in Smart Database. Correct the retention times for the target compounds based on the n-alkane identification results and retention indice of target compounds.

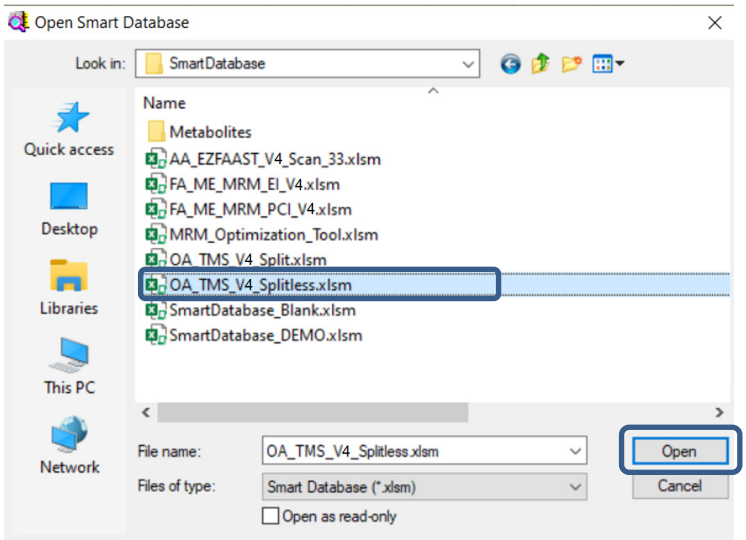
See "*2.1.3 Measuring n-Alkane Mixed Solution*" and measure and identify n-alkane mixed solution.

2.2.3 Making Settings for Smart Database

1) On the [Compound Table] assistant bar, click the 

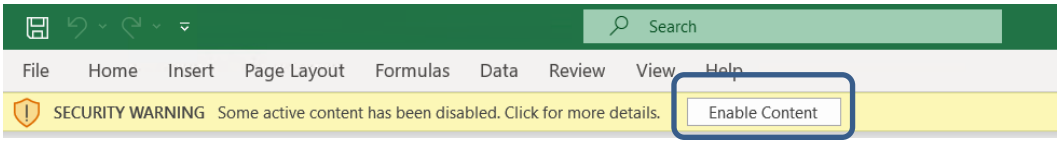
(Smart MRM/SIM) icon.

2) Select the Smart Database file (in Excel format), and click [Open].

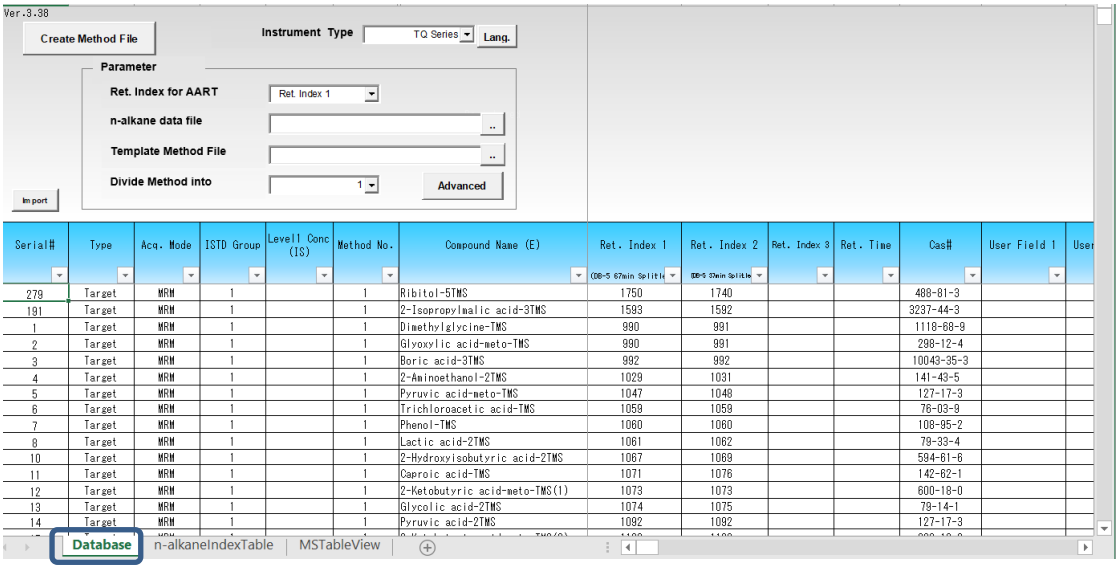


NOTE

If the following message is displayed on the message bar on Excel, Click [Enable Content].

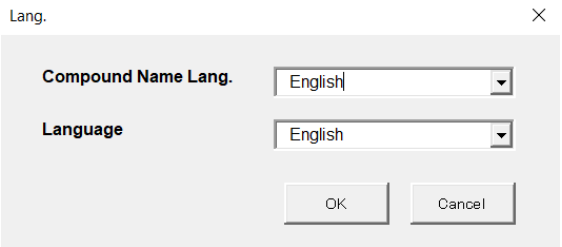


3) Open the [Database] sheet.



Serial#	Type	Acq. Mode	ISTD Group	Level1 Conc (IS)	Method No.	Compound Name (E)	Ret. Index 1	Ret. Index 2	Ret. Index 3	Ret. Time	Cas#	User Field 1
279	Target	MRM	1		1	Ribitol-5TMS	1750	1740			488-81-3	
191	Target	MRM	1		1	2-Isopropylmalic acid-3TMS	1593	1592			3237-44-3	
1	Target	MRM	1		1	Dimethylglycine-TMS	990	991			1118-68-9	
2	Target	MRM	1		1	Glyoxylic acid-meto-TMS	990	991			298-12-4	
3	Target	MRM	1		1	Boric acid-3TMS	992	992			10043-35-3	
4	Target	MRM	1		1	2-Aminoethanol-2TMS	1029	1031			141-43-5	
5	Target	MRM	1		1	Pyruvic acid-meto-TMS	1047	1048			127-17-3	
6	Target	MRM	1		1	Trichloroacetic acid-TMS	1059	1058			76-03-9	
7	Target	MRM	1		1	Phenol-TMS	1060	1060			108-95-2	
8	Target	MRM	1		1	Lactic acid-2TMS	1061	1062			79-33-4	
10	Target	MRM	1		1	2-Hydroxyisobutyric acid-2TMS	1067	1069			594-61-6	
11	Target	MRM	1		1	Caproic acid-TMS	1071	1076			142-62-1	
12	Target	MRM	1		1	2-Ketobutyric acid-meto-TMS(1)	1073	1073			600-18-0	
13	Target	MRM	1		1	Glycolic acid-2TMS	1074	1075			79-14-1	
14	Target	MRM	1		1	Pyruvic acid-2TMS	1092	1092			127-17-3	

4) Click the [Lang.] button, and set the language for compound name notation and the display.



Compound Name Lang: Select the language for compound name notation. Select from Japanese, English and Chinese.

Language: Select the display language for the Smart Database window.

This operation is required each file. Save the file after language setting.

> **Hint**
>
> The settings configured here will be stored by Smart Database.

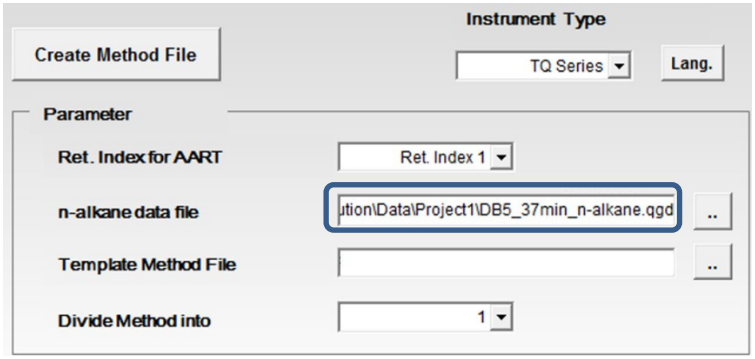
5) Set the instrument type at [Instrument Type].

> **NOTE**
>
> Note that an error message will be displayed if the instrument type set at [Instrument Type] is different from that set in the data files specified at [n-alkane data file] and [Template Method File], and that set in the [System Configuration] window for the method file.

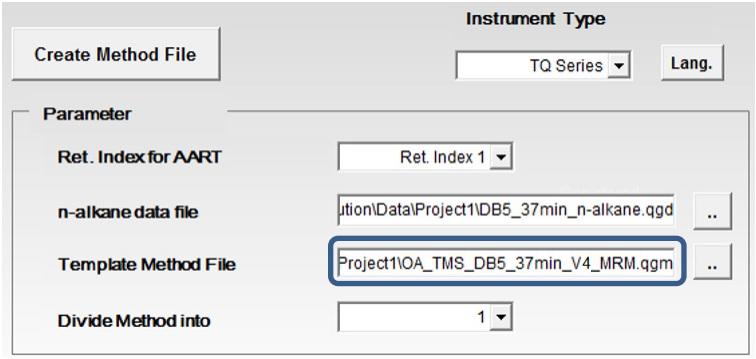
2.2.4 Create the Method File

2.2.4.1 Set Target Compounds.

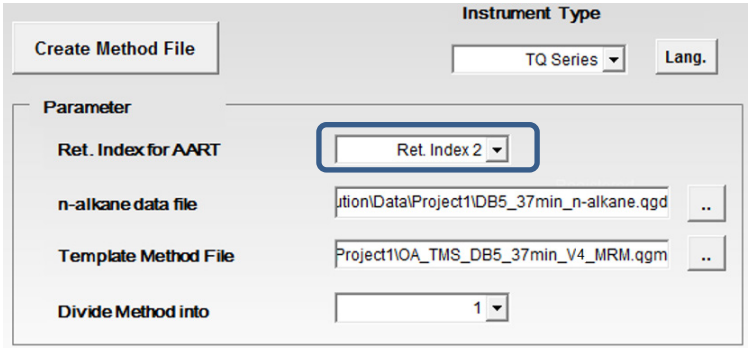
1) Check that the data file opened in section *"2.2.2 Measuring and Identifying n-Alkanes"* is loaded.



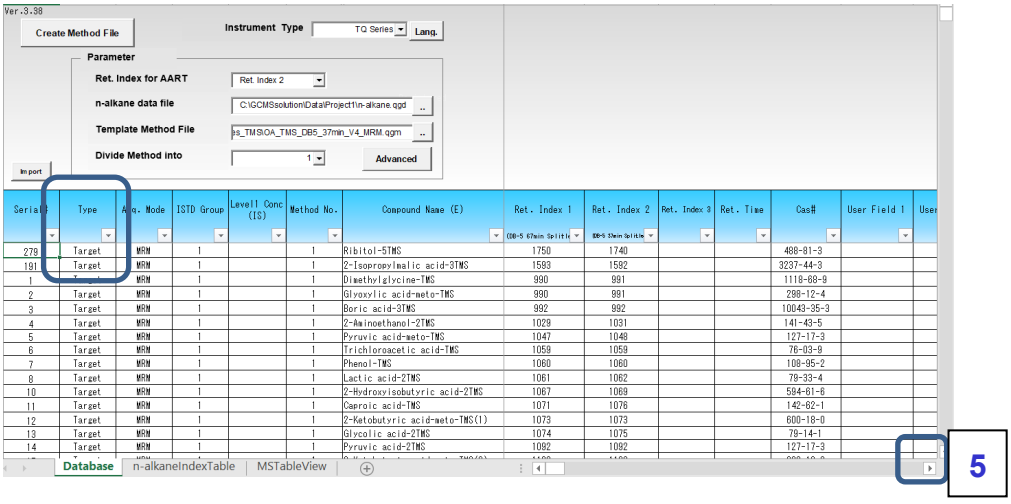
2) Of the method files stored in the C: \GCMSsolution\SmartDatabase\Metabolites\Method folder, copy the method file to be used as the template method file to the folder that was created earlier, and specify it at [Template Method File]. The data acquisition and analysis conditions of the method file that was specified here will be applied when a method is created.



3) Select the retention index appropriate for the method file to be created.



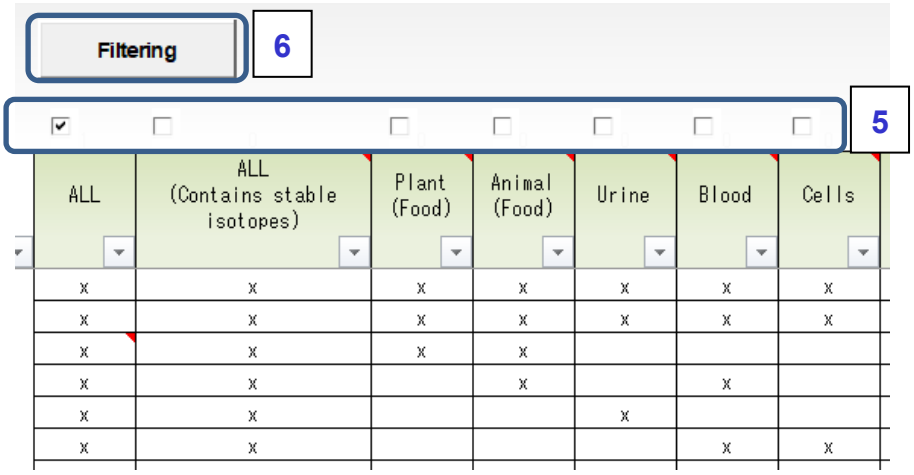
4) If the internal standard is used, change from “Target” to “ISTD” in the [Type] column. (Other compounds except for Ribitol and 2-Isopropylmailic acid also can be used as the internal standard.)



[Perform steps 5) and 6) when using the Smart Database files “OA_TMS_V4_Split” and “OA_TMS_V4_Splitless” for metabolite analysis.

5) Move to the FF column on the [Database] sheet, and click the appropriate boxes from among the “All”, “All (Contains stable isotope)”, “Plant (Food)”, “Animal (Food)”, “Urine”, “Blood”, “Cells” boxes.
Multiple checkboxes can be selected.

6) Click [Filtering].
Only compounds to which “x” is applied are automatically set to “Target,” and can be sorted.
Compounds to which “x” is not applied are not reflected in the compound table creation.



Note that when analyzing with the default setting “ALL”, skip steps 5) and 6).

NOTE

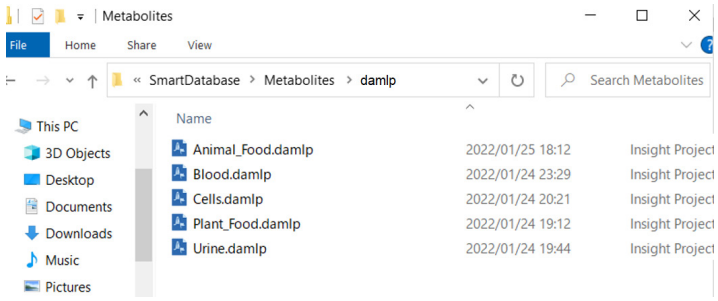
When using filters, check the compounds to be analyzed and modify as necessary. It is not guaranteed that the results will be equivalent to those obtained by analyzing all compounds.

Hint

Besides the method of using the above Smart Database filter function, another method of selecting the compounds to be subject to data analysis is to use the LabSolutions Insight flag filter function.

1) Click [Load Flags] of [File] on the menu bar.

2) Select files (.damlp), and read them in.
(File location: C:\GCMSsolution\SmartDatabase\Metabolites\damlp)



7) In the [Type] column, press the Delete key to erase "Target" from the fields for compounds that will not be measured, leaving these fields blank.
Rows in which the [Type] fields are left blank (for compounds that will not be measured) will not be included in the resulting compound table.

	Serial#	Type	Acq. Mode	Method No.	Compound Name (E)	Ret. Index 1	Ret. Index 2	Ret. Index 3	Ret. Time	Cas#
4	1	Target	MRM	1	Dimethylglycine-TMS	990	991			1118-68-9
5	2	Target	MRM	1	Glyoxylic acid-meto-TMS	990	991			298-12-4
6	3		MRM	1	Boric acid-3TMS	992	992			10043-35-3
7	4	Target	MRM	1	2-Aminoethanol-2TMS	1029	1031			141-43-5
8	5	Target	MRM	1	Pyruvic acid-meto-TMS	1047	1048			127-17-3
9	6		MRM	1	Trichloroacetic acid-TMS	1059	1059			76-03-9
10	7	Target	MRM	1	Phenol-TMS	1060	1060			108-95-2
11	8	Target	MRM	1	Lactic acid-2TMS	1061	1062			79-33-4
12	9	Target	MRM	1	Lactic acid-13C3-2TMS	1062	1062			
13	10		MRM	1	2-Hydroxyisobutyric acid-2TMS	1067	1069			594-61-6
14	11	Target	MRM	1	Caproic acid-TMS	1071	1076			142-62-1

> **Hint**
>
> It is also possible to create method files using already known retention times without using AART if the rention times of the target components are already obtained by the analysis of standard samples.
> For details, see "*Appendix 3 Directly Setting Retention Times with Smart Database Without Using AART*."

8) Set the analysis mode (“Scan”, “SIM”, “MRM”) in the [Acq. Mode] column for the target compounds.

Serial#	Type	Acq. Mode	Method No.	Compound Name (E)	Ret. Index 1	Ret. Index 2	Ret. Index 3	Ret. Time	Cas#
1	Target	MRM	1	Dimethylglycine-TMS	990	991			1118-68-9
2	Target	MRM	1	Glyoxylic acid-meto-TMS	990	991			298-12-4
3		MRM	1	Boric acid-3TMS	992	992			10043-35-3
4	Target	MRM	1	2-Aminoethanol-2TMS	1029	1031			141-43-5
5	Target	MRM	1	Pyruvic acid-meto-TMS	1047	1048			127-17-3
6		MRM	1	Trichloroacetic acid-TMS	1059	1059			76-03-9
7	Target	SIM	1	Phenol-TMS	1060	1060			108-95-2
8	Target	Scan	1	Lactic acid-2TMS	1061	1062			79-33-4
9	Target	MRM	1	Lactic acid-13C3-2TMS	1062	1062			
10		MRM	1	2-Hydroxyisobutyric acid-2TMS	1067	1069			594-61-6
11	Target	MRM	1	Caproic acid-TMS	1071	1076			142-62-1

2.2.4.2 Select the Ions.

Under default conditions, two MRM transitions and two *m/z* for SIM or Scan are selected for each component. To change these, make your selections while checking the masses and ion ratios.

If you set the fields in the [Type] column to "T," it will be used as a target ion. If you set the fields in the column to "Ref.," it will be used as a reference ion. You can configure one target ion and up to five reference ions.

MRM Transition

Ion1				Ion2				Ion3			
Type	m/z	CE	Ratio	Type	m/z	CE	Ratio	Type	m/z	CE	Ratio
Ref.1	175.1>58.0	6	100.00		175.1>73.0	27	0.82		175.1>56.0	30	15.65
T	160.1>59.0	12	100.00		160.1>116.1	9	35.17		160.1>89.0	9	27.23
	102.1>61.0	9	100.00	Ref.1	102.1>58.0	30	519.50	T	102.1>100.0	9	675.60
	158.1>130.1	9	100.00		158.1>89.0	12	66.10		158.1>61.0	21	159.21
	219.0>191.1	6	100.00	Ref.1	219.0>147.1	15	184.64		219.0>129.1	3	4.85
	222.1>193.1	6	100.00	Ref.1	222.1>147.1	15	216.08		222.1>132.1	3	4.27
Ref.1	233.2>147.1	18	100.00		233.2>163.1	6	5.17		233.2>205.2	9	63.40

m/z for SIM or Scan

Ion1			Ion2		
Type	m/z	Ratio	Type	m/z	Ratio
T	175.1	100.00	Ref.1	160.1	116.72
T	160.1	100.00	Ref.1	89.1	24.90
T	263	100.00	Ref.1	221	411.92
T	102.1	100.00	Ref.1	190.2	3.15
T	174	100.00	Ref.1	158.1	7.34
T	193	100.00	Ref.1	113	182.38
T	166	100.00	Ref.1	151	365.00
T	219	100.00	Ref.1	191	365.36
T	222.1	100.00	Ref.1	193.1	443.41
T	233	100.00	Ref.1	205	453.47

1) Check the registered transitions or ions.

2) To change or add ions, make your selection on the drop-down menu in the [Type] cell.

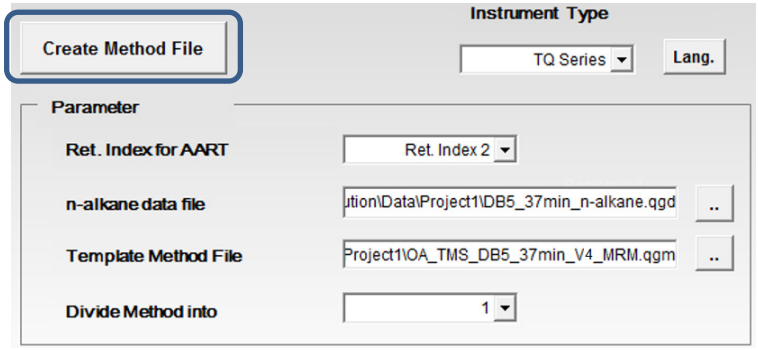
MRM Transition

Ion1				Ion2				Ion3			
Type	m/z	CE	Ratio	Type	m/z	CE	Ratio	Type	m/z	CE	Ratio
Ref.1	175.1>58.0	6	100.00		175.1>73.0	27	0.82		175.1>56.0	30	15.65
T	160.1>59.0	12	100.00		160.1>116.1	9	35.17		160.1>89.0	9	27.23
	102.1>61.0	9	100.00	Ref.1	102.1>58.0	30	519.50	T	102.1>100.0	9	675.60
	158.1>130.1	9	100.00		158.1>89.0	12	66.10		158.1>61.0	21	159.21
	219.0>191.1	6	100.00	Ref.1	219.0>147.1	15	184.64		219.0>129.1	3	4.85
	222.1>193.1	6	100.00	Ref.1	222.1>147.1	15	216.08		222.1>132.1	3	4.27
Ref.1	233.2>147.1	18	100.00		233.2>163.1	6	5.17		233.2>205.2	9	63.40
T	173.1>75.0	12	100.00		173.1>81.1	9	5.93		173.1>131.1	9	22.22
	188.1>89.0	15	100.00		188.1>59.0	24	93.83	Ref.1	188.1>74.0	21	104.72
T	177.1>147.1	6	100.00		177.1>149.1	6	34.76	Ref.1	177.1>73.0	27	53.36
	188.1>59.0	21	100.00	T	188.1>74.0	21	147.10	Ref.1	188.1>89.0	9	112.93
T	116.1>73.0	15	100.00		116.1>58.0	30	3.77		116.1>91.1	18	1.58
	186.1>170.1	9	100.00		186.1>96.0	6	267.29	T	186.1>73.0	18	861.05
	156.2>59.0	24	100.00		156.2>75.0	24	54.55	Ref.1	156.2>128.1	9	163.14
	204.1>176.1	9	100.00	Ref.1	204.1>147.1	18	105.89		204.1>103.1	15	5.68
	249.2>86.0	9	100.00		249.2>119.1	21	119.01		249.2>146.1	9	179.85

2.2.4.3 Create the MS Table.

Set the parameters required to create the MS table.

1) Click [Create Method File]. The [MS Table Parameter] window is displayed.



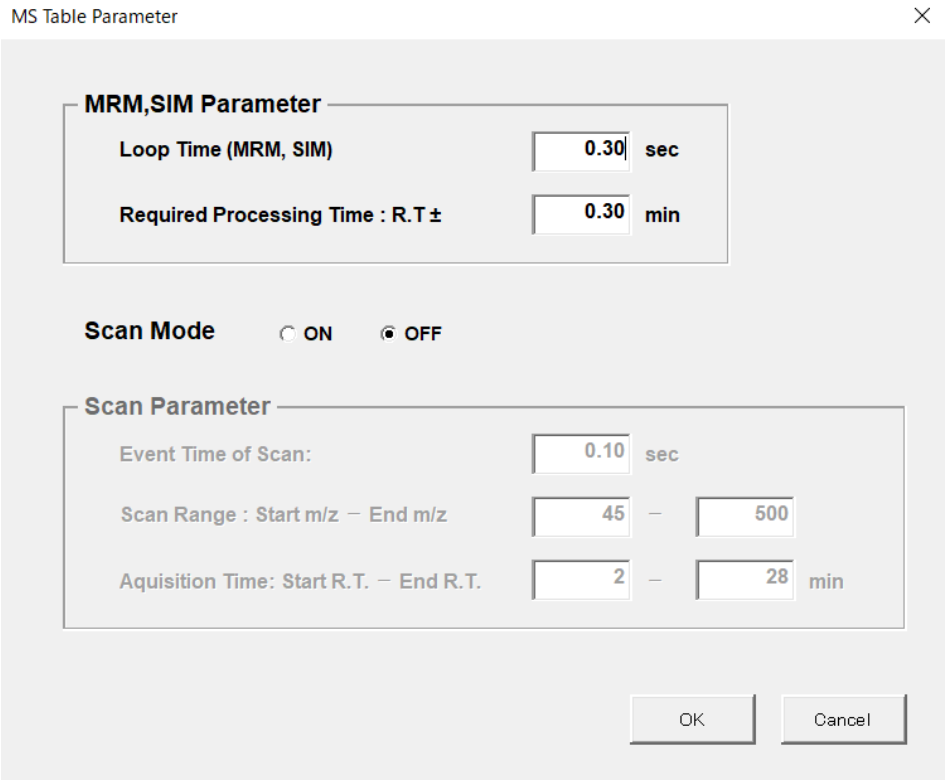
2) Set [MS Table Parameter].

Creating a Method File for MRM Mode or SIM Mode

Configure the settings in the [MRM, SIM Parameter] area. If performing simultaneous measurement with Scan/SIM or Scan/MRM, set [Scan Mode] to [ON], and configure the settings in the [Scan Parameter] area.

Creating a Method File for Scan Mode

Configure the settings in the [Scan Parameter] area.



Hint

- Loop Time (MRM, SIM)

This is the sum total of the event times for all compounds configured to one group. If "0.3 sec" is entered, for example, data for each compound will be acquired every 0.3 seconds. To ensure good reproducibility, at least 10 data points must be acquired per peak. However, if the loop time is shortened, the time required to collect a single transition or ion will be shortened, reducing the sensitivity.

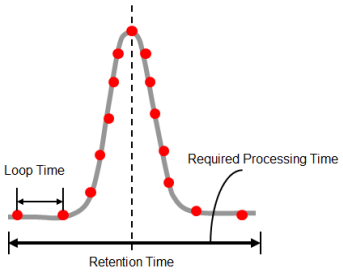
Recommended values:0.2 - 0.5 sec

- Required Processing Time

This indicates the time range for measurements, centered on the retention time for each compound. For example, if "0.3 min" is entered, data will be acquired for each compound within a range of the retention time 0.3 min (i.e. for 0.6 minutes).

Measure a sample spiked with the standard sample using the method file created, and check for retention time offset. Pay attention to retention time offset due to impurities and to the peak widths (affected by tailing or other deformation), and reconfigure the required processing time accordingly.

Recommended values: 0.3 - 0.5 min



Hint

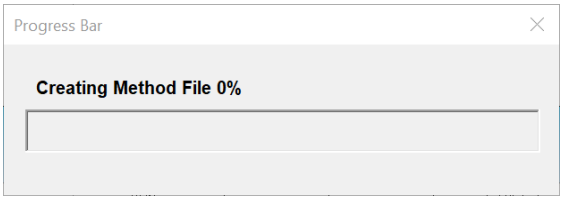
The loop times and required processing times of each MRM method in this product are described below. Use them as reference values.

File Name (.qgm)	Loop Time (sec.)	Required Processing Time (min.)
OA_TMS_DB5_37min_V4_MRM	0.3	0.25
OA_TMS_DB5_67min_V4_MRM	0.3	0.3
OA_TMS_BPX5_23min_V4_MRM	0.25	0.15
FA_ME_DB5MS_EI_V4_MRM	0.3	0.5
FA_ME_DB5MS_PCI_V4_MRM	0.3	0.5
FA_ME_SP2560_EI_V4_MRM	0.3	0.5
FA_ME_SP2560_PCI_V4_MRM	0.3	0.5
Sugar_AC_BPX5_V4_Template	0.3	0.5

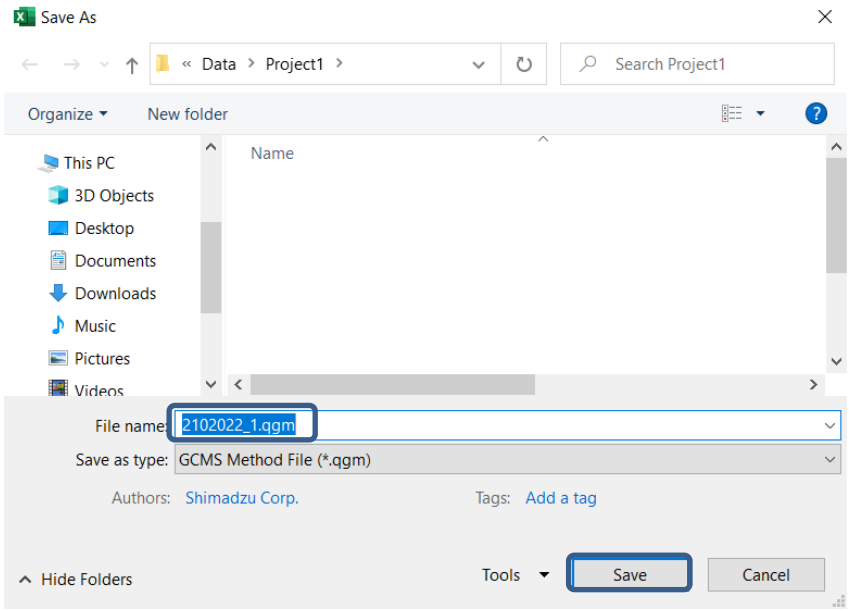
The scan parameters of each Scan method in this product are described below. Please use these values for reference.

File Name (.qgm)	Event Time (sec.)	Scan Range Starting *m/z* to Ending *m/z*	Analysis Time (min) Starting R.T to Ending R.T.
OA_TMS_DB5_37min_V4_Scan	0.3	45 - 600	4 - 37
OA_TMS_DB5_67min_V4_Scan	0.3	45 - 600	4 - 67
OA_TMS_BPX5_23min_V4_Scan	0.2	45 - 600	3.5 - 23
FA_ME_DB5MS_EI_V4_Scan	0.2	45 - 500	2.75 - 49.5
FA_ME_DB5MS_PCI_V4_Scan	0.2	90 - 550	2.75 - 49.5
FA_ME_SP2560_EI_V4_Scan	0.3	45 - 500	16 - 66
FA_ME_SP2560_PCI_V4_Scan	0.3	90 - 550	16 - 66
Sugar_AC_BPX5_V4_Template	0.3	40 - 700	6 - 41

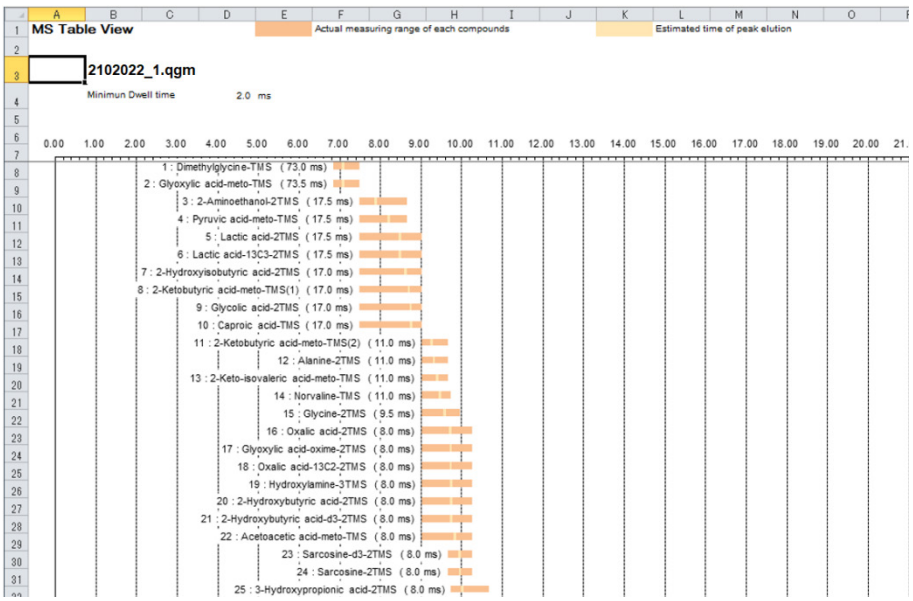
3) Click [OK]. The [Progress Bar] window is displayed. A method file is automatically created with the MS table and compound table set based on the parameters configured in step 2).



4) Enter a file name, and then click [Save].

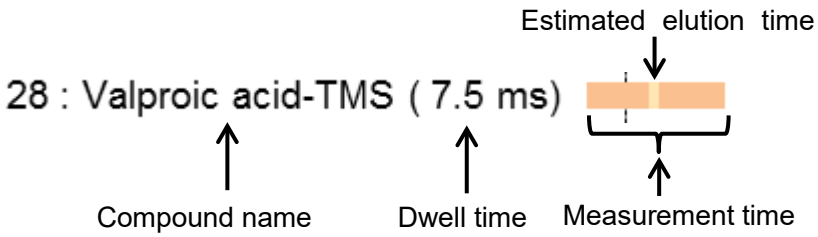


5) Check the time range for MRM or SIM measurements of target compounds.



Hint

You can use the [MSTableView] sheet to check the measurement time (hereinafter referred to as "dwell time") for each transition (ion) for each target compound.

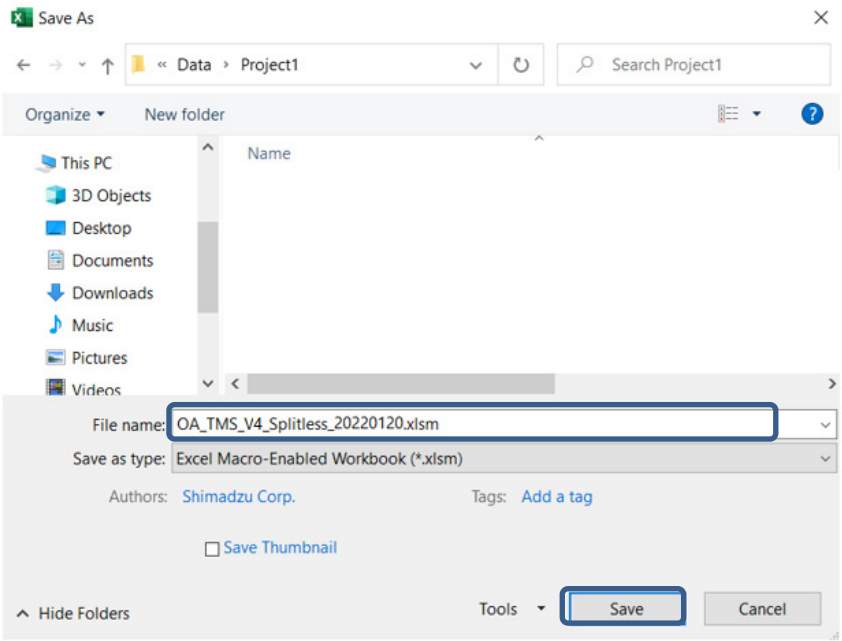


In addition, you can check the minimum value for the dwell time for all target compounds.

2102022_1.qgm

Minimun Dwell time 2.0 ms

6) Name the Smart Database file and save it in the folder that was created earlier.



7) Measure the samples using the created method file.

2.2.5 Editing the Database

Since the Smart Metabolites Database is created with an Excel file, it can be edited freely and compounds added.

If your column or GC analysis conditions differ from those specified in the database file, you can create a method appropriate for the conditions by adjusting the retention indices. Also, if you want to add information for new compounds or transitions, you can use the GCMSsolution MRM Optimization Tool to register the additional data.

Adjusting the Database Depending on Different GC Analysis Conditions

Follow the steps in Chapter 3 "*Registering Additional Compound Information in Smart Database*" in the GCMS Operation Guide - Method Development Guide, up to section 3.3.3 "*Register the retention indices in the compound table*," and then store the registered retention indices in the existing database. Save the database under a new name to finish the procedure.

Registering New Compounds and Transitions

To do this, follow the steps described in Chapter 3 "*Registering Additional Compound Information in Smart Database*" in the GCMS Operation Guide - Method Development Guide. After registration, save the database under a new name to finish the procedure

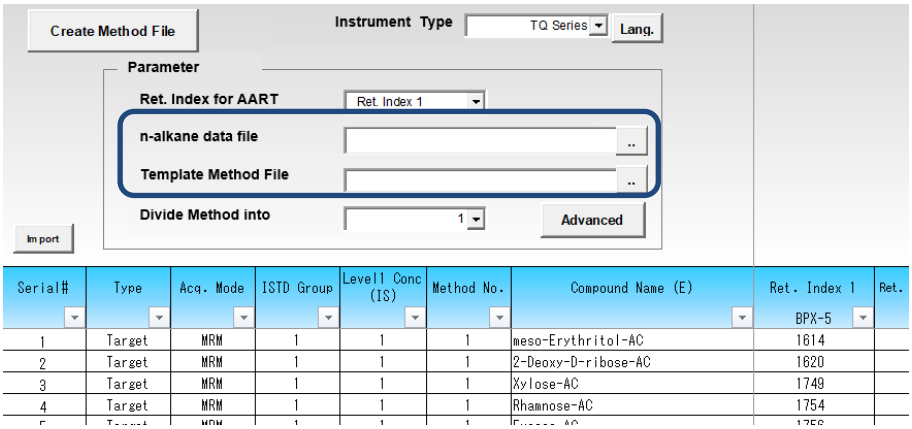
2.3 Sugars Semiquantitation Value Calculation

1) Prepare a folder for saving each file (method file and data file).
(Example of destination for creating the folder: C:\GCMSsolution\Data\Project1)

2) Copy the files that are stored in the C:\GCMSsolution\SmartDatabase\Metabolites\Method\Sugar_AC folder, and paste them in the folder that was created.

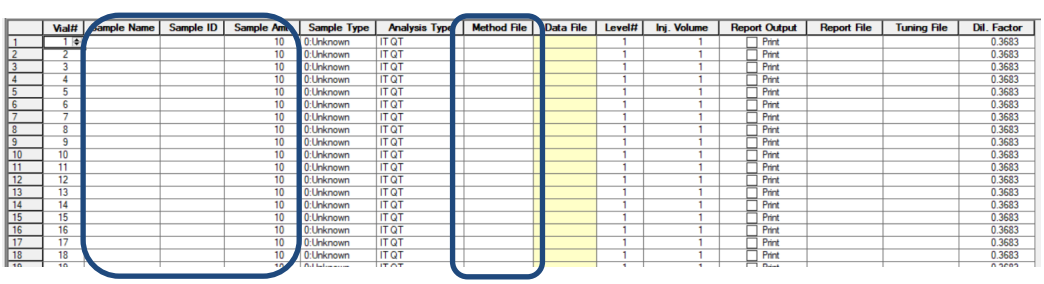
3) Perform measurement and identification for n-alkanes using Sugar_AC_BPX5_V4_HC.qgm.

4) Open Sugar_AC_V4.xlsm, and specify the data file for the n-alkanes identified in 3). Also, specify Sugar_AC_BPX5_V4_Template.qgm in the template method file, and create a method file. (Loop Time: 0.3 sec, Required Processing Time: 0.5 min)



Serial#	Type	Acq. Mode	ISTD Group	Level Conc (IS)	Method No.	Compound Name (E)	Ret. Index 1 BPX-5	Ret.
1	Target	MRM	1	1	1	meso-Erythritol-AC	1614	
2	Target	MRM	1	1	1	2-Deoxy-D-ribose-AC	1620	
3	Target	MRM	1	1	1	Xylose-AC	1749	
4	Target	MRM	1	1	1	Rhamnose-AC	1754	

5) Open the batch file (Sugar_AC_V4_Template.qgb), and enter the sample name and sample ID. The sample quantity is shown as 10 (mg). Correct this only when a change is required. Use the method file created in step 4).



NOTE

The dilution factor for calculation of the semiquantitation values are registered in the batch file, so be sure to copy and use Sugar_AC_V4_Template.qgb.
When the sample quantity and dilution factor are input, the concentrations in the sample are automatically calculated by the software.

6) The analysis starts.

7) When the analysis is completed, the quantities of sugar per 1 mg of sample are calculated.

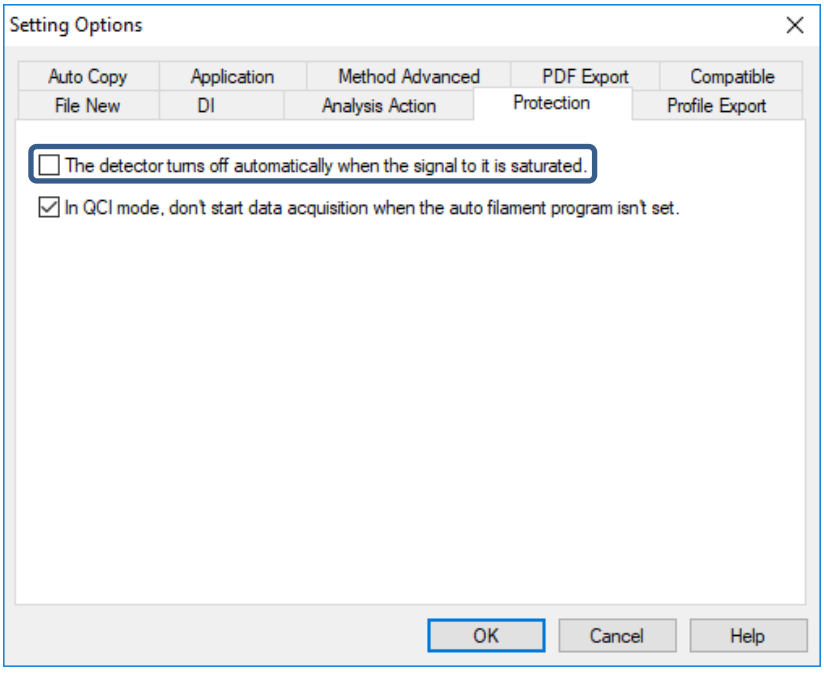
3 Caution

3.1 Detector Protective Function

The voltage of the detector is set beforehand. However, depending on the sample, the detector may become saturated. If the detector becomes saturated and the [The detector turms off automatically when the signal to it is saturated.] checkbox has been selected, the detector protection function is activated and the filament is switched OFF.

When this situation is encountered, lower the voltage of the detector first. If the detector still becomes saturated even after the voltage of the detector has been lowered, and analysis cannot be performed, clear the [The detector turms off automatically when the signal to it is saturated.] checkbox to turn OFF the detector protection function.

To display the window containing the detector protection function setting, click [Option] on the tool menu in the [GCMS Real Time Analysis] window.



NOTE

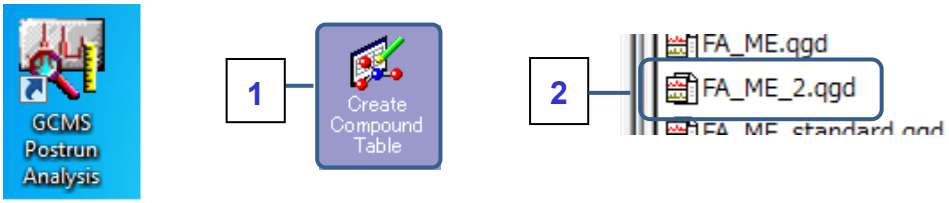
Note that there is a risk that turning OFF the detector protection function can cause a deterioration of the detector. Be sure to fully consider this point before using this function.

3.2 Fatty Acid Methyl Ester PCI Method File and Library

Due to the characteristics of ionization, the spectral pattern of the mass spectrum obtained by the PCI method can vary depending on the type of reagent gas that is used. This fatty acid methyl PCI method file library contains mass spectra obtained when using isobutane gas as the reagent gas. For this reason, it is recommended to use isobutane gases when using this method file library.

Appendix1 Adding and Deleting Quantitative Target Compounds

1. Opening the data file

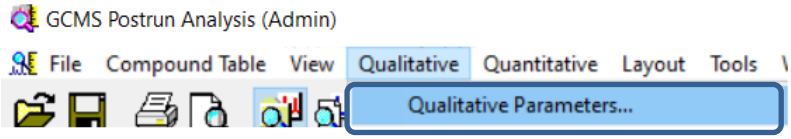


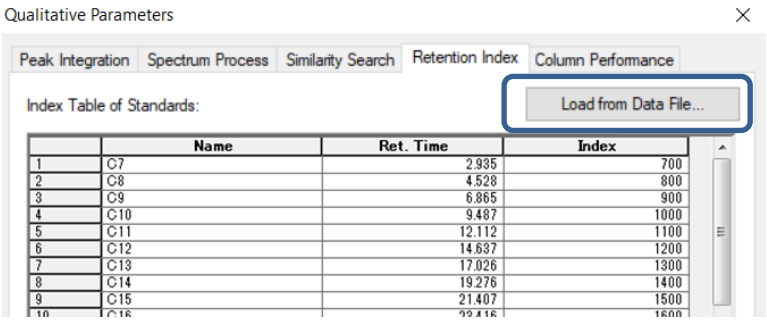
1) Start the [GCMS Postrun Analysis] program, and click [Create Compound Table] icon on the [Postrun] assistant bar.

2) Double-click the data file “FA_ME_2.qgd” from data explorer which you want to add the compounds.

2. Load the data of n-alkane to register the retention index.

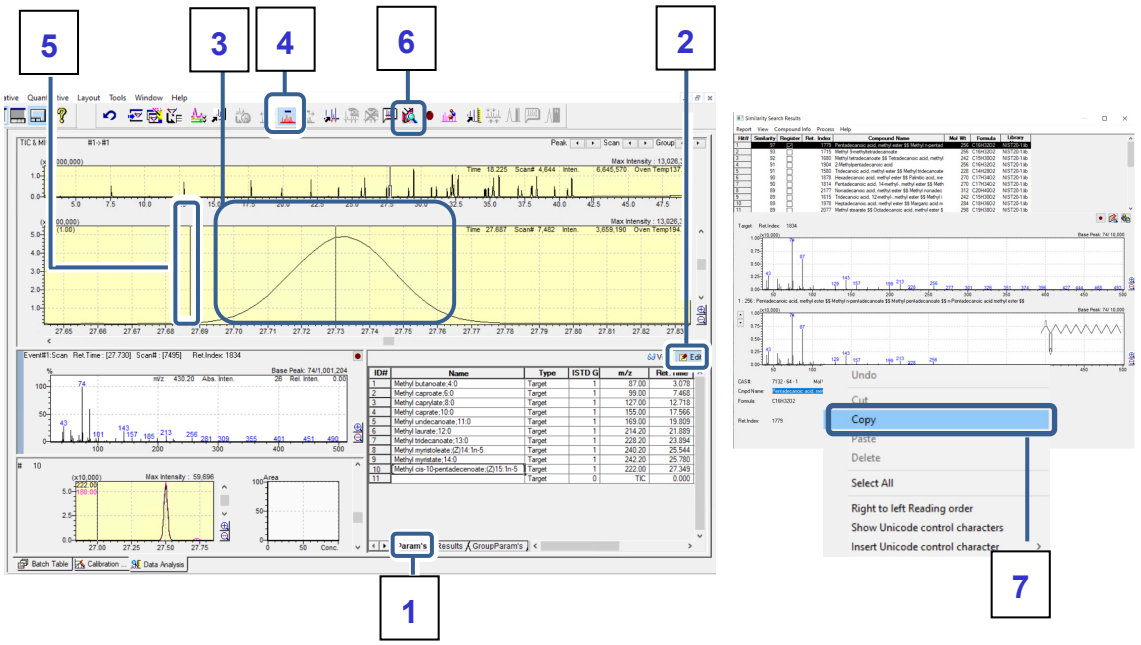
From the [Qualitative] menu, select [Qualitative Parameters] - [Retention Index] and load the data of n-alkane.





3. Adding quantitative target compounds

Display the mass spectrum of the target compound, and search it for similarity.



1) Click the [Param's] tab in the [Compound Table View].
2) Click the [Edit] button at the top-right corner of the table.
3) Move the mouse pointer to the peak and double-click.
4) Click the [Subtract Spectrum] on the toolbar.
5) Double-click at the background processing position.
6) Click [Similarity Search Result] on the toolbar.
7) Confirm quantitative target compounds. If the compound name registered in [Compound Table] refers to library, drug the name, right-click and copy.

NOTE

If the target compound is not confirmed by the similarity search, compare TIC of the analyzed data with that of blank test to confirm that the peak of the target compound is found only in the analyzed data.

It is possible that the target compound is not registered in the library. In this case, check the mass spectrum information with the other information source.

4. Set compound information to [Compound Table].

1) Click [Set Compound Information] on the [Compound Table] menu.

2) Check [Set name] and enter the compound name. If the name is copied at the operation of "*3. Adding quantitative target compounds*" 7), paste it.

3) Select the type and *m/z* value. To confirm the mass spectrum, click at the right corner of [*m/z*] cell.

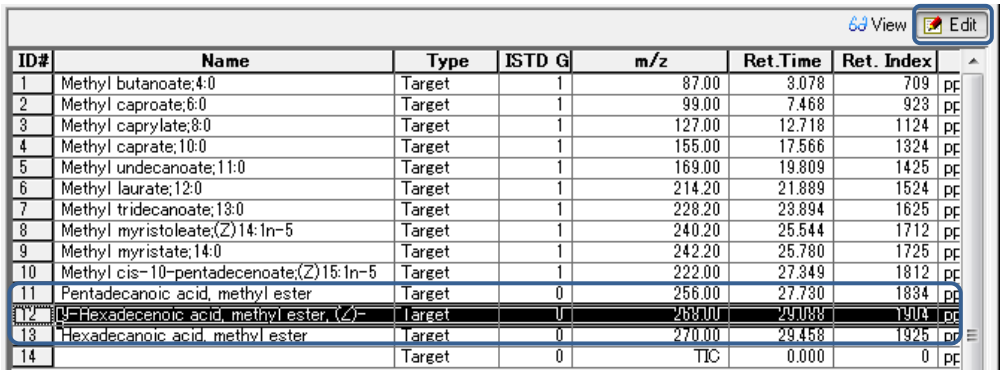
4) Click [Add To Tail].

5. Adding other quantitative target compounds by operating [3.] and [4.]

View Edit

ID#	Name	Type	ISTD G	m/z	Ret.Time	Ret. Index	
1	Methyl butanoate;4:0	Target	1	87.00	3.078	709	pp
2	Methyl caproate;6:0	Target	1	99.00	7.468	923	pp
3	Methyl caprylate;8:0	Target	1	127.00	12.718	1124	pp
4	Methyl caprate;10:0	Target	1	155.00	17.566	1324	pp
5	Methyl undecanoate;11:0	Target	1	169.00	19.809	1425	pp
6	Methyl laurate;12:0	Target	1	214.20	21.889	1524	pp
7	Methyl tridecanoate;13:0	Target	1	228.20	23.894	1625	pp
8	Methyl myristoleate;(Z)14:1n-5	Target	1	240.20	25.544	1712	pp
9	Methyl myristate;14:0	Target	1	242.20	25.780	1725	pp
10	Methyl cis-10-pentadecenoate;(Z)15:1n-5	Target	1	222.00	27.349	1812	pp
11	Pentadecanoic acid, methyl ester	Target	0	256.00	27.730	1834	pp
12	9-Hexadecenoic acid, methyl ester, (Z)-	Target	0	268.00	29.088	1904	pp
13	Hexadecanoic acid, methyl ester	Target	0	270.00	29.458	1925	pp
14		Target	0	TIC	0.000	0	pp

6. Deleting quantitative target compounds



ID#	Name	Type	ISTD G	m/z	Ret.Time	Ret. Index	
1	Methyl butanoate;4:0	Target	1	87.00	3.078	709	pp
2	Methyl caproate;6:0	Target	1	99.00	7.468	923	pp
3	Methyl caprylate;8:0	Target	1	127.00	12.718	1124	pp
4	Methyl caprate;10:0	Target	1	155.00	17.566	1324	pp
5	Methyl undecanoate;11:0	Target	1	169.00	19.809	1425	pp
6	Methyl laurate;12:0	Target	1	214.20	21.889	1524	pp
7	Methyl tridecanoate;13:0	Target	1	228.20	23.894	1625	pp
8	Methyl myristoleate;(Z)14:1n-5	Target	1	240.20	25.544	1712	pp
9	Methyl myristate;14:0	Target	1	242.20	25.780	1725	pp
10	Methyl cis-10-pentadecenoate;(Z)15:1n-5	Target	1	222.00	27.349	1812	pp
11	Pentadecanoic acid, methyl ester	Target	0	256.00	27.730	1834	pp
12	9-Hexadecenoic acid, methyl ester, (Z)-	Target	0	268.00	29.088	1904	pp
13	Hexadecanoic acid, methyl ester	Target	0	270.00	29.458	1925	pp
14		Target	0	TIC	0.000	0	pp

1) Click the [Edit] tab at the top-right corner of the compound table.

2) Click the row of target compound and press「Delete」key on the keyboard.

7. Save the method file.

1) Click the 

at the top-right corner of the table.

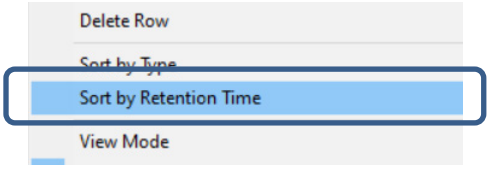
2) Click [Save Compound Table] on the [Compound] assistant bar.

3) Save the method file as other name or the same.

Hint

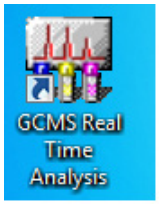
Click the 

at the top-right corner of the table. Right-click over the table and click [Sort by Retention Time].



Appendix2 Adjusting Retention Times of Compound Table by Measuring Standard

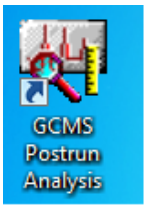
1. Measure standard solution using the modified method file.

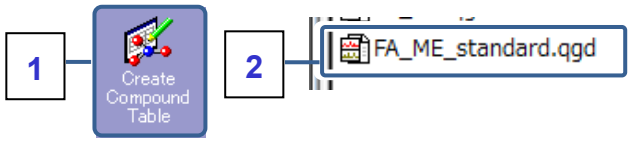


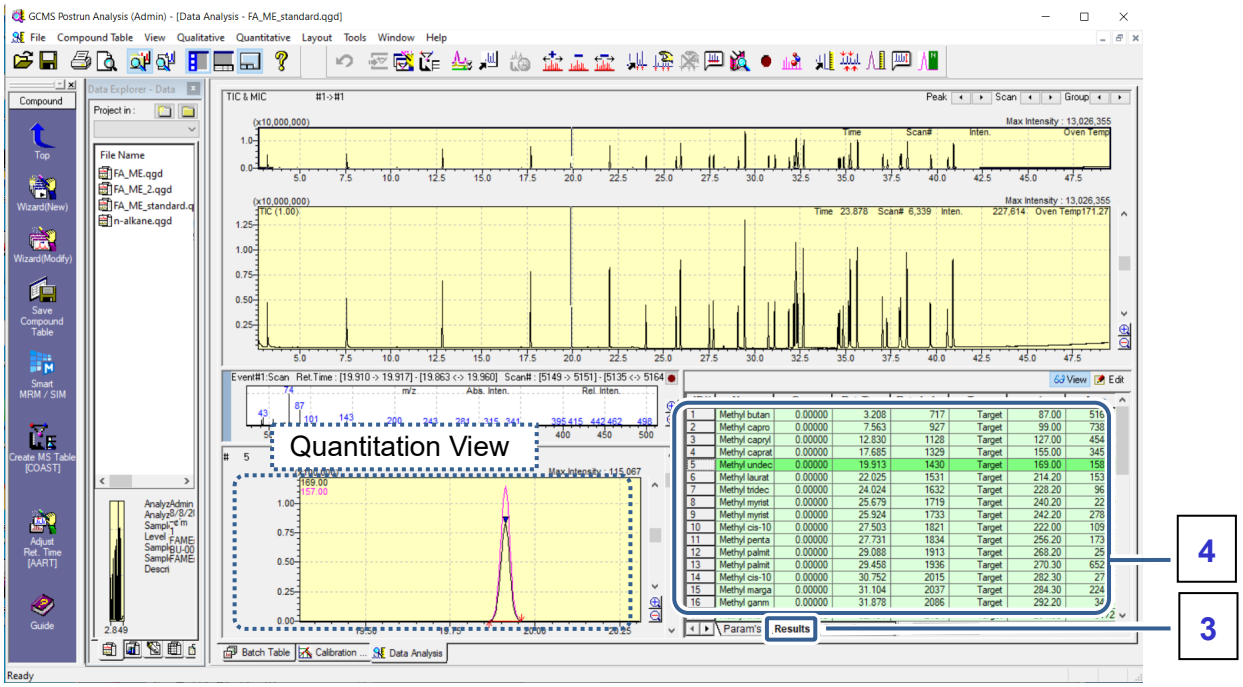
Folder: C:\GCMSsolution\Data\Project1

	Vial#	Sample Name	Sample Type	Analysis Ty	Method File	Data File	Inj. Volu
1	1	FA_ME standard 0.1mg/mL	0:Unknown	IT QT	FA_ME_DB5MS_EI_V4_Scan.qgm	FA_ME_standard.qgd	1

2. Identify target compounds.







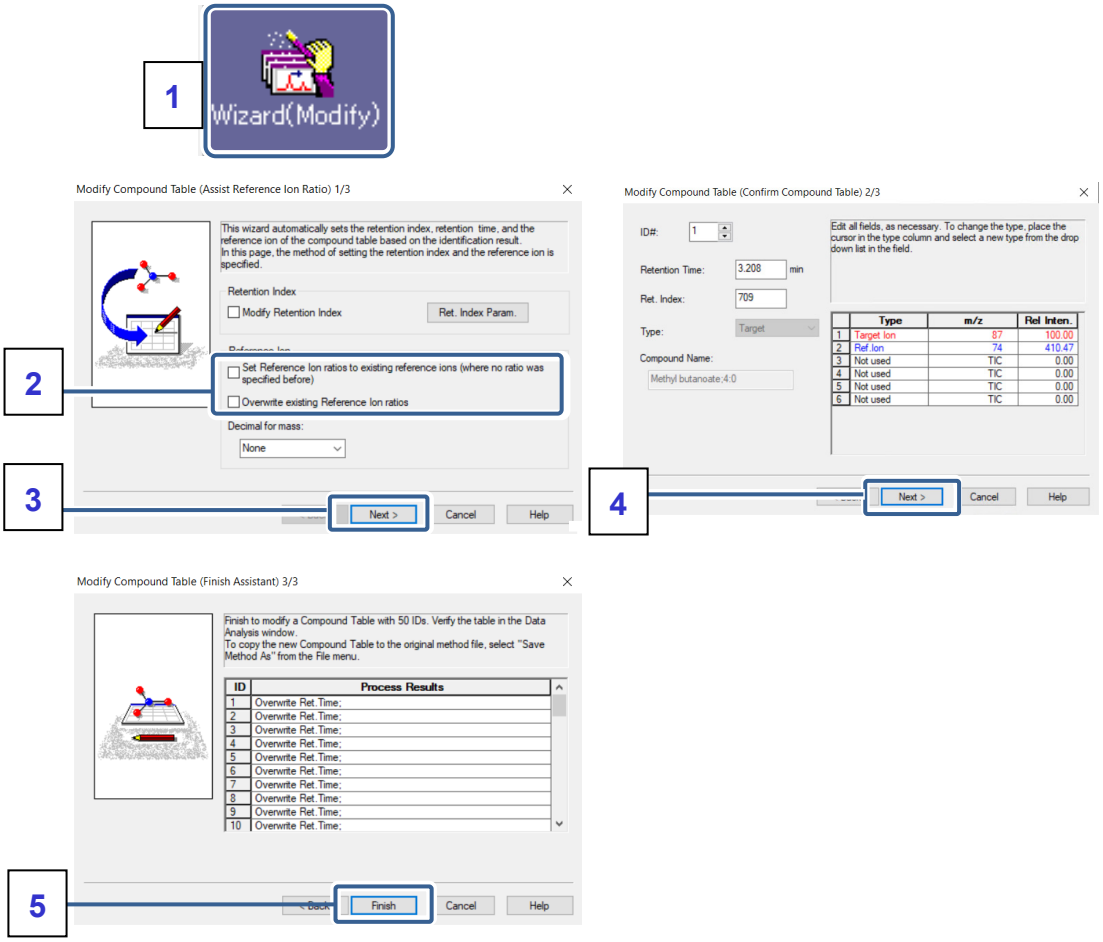
1) Start the [GCMS Postrun Analysis] program and click the [Create Compound Table] icon on the [Postrun] assistant bar.

2) Double click the data file “FA_ME_standard” on data explorer.

3) Click the [Results] tab in the [Compound Table View].

4) Click on a compound name in the compound table and check the chromatogram in the [Quantitation View]. If necessary, perform manual identification or peak integration.

3. Modify retention time based on identified results using [Wizard(Modify)].



1) Click [Wizard(Modify)] icon on the [Compound] assistant bar.

2) Uncheck all check boxes in this window and click [Next].

3) Click [Next].

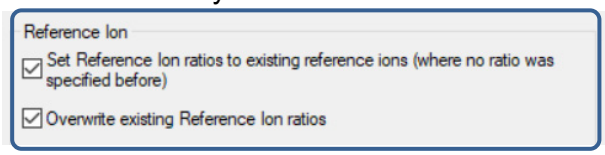
4) Click [Next].

5) Click [Finish].

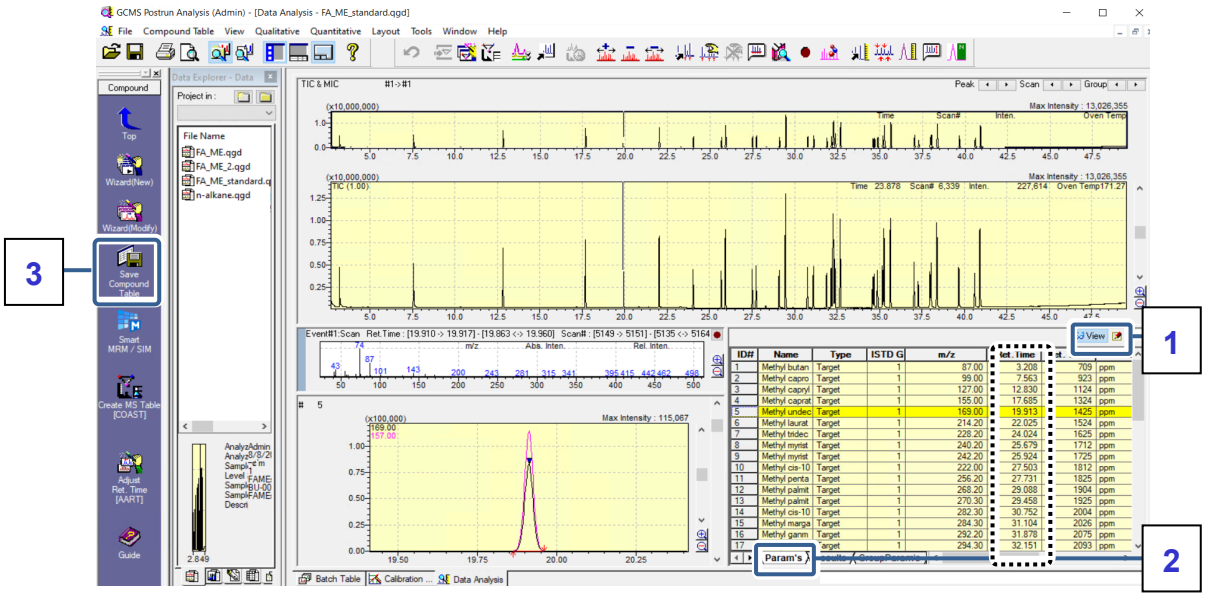
NOTE

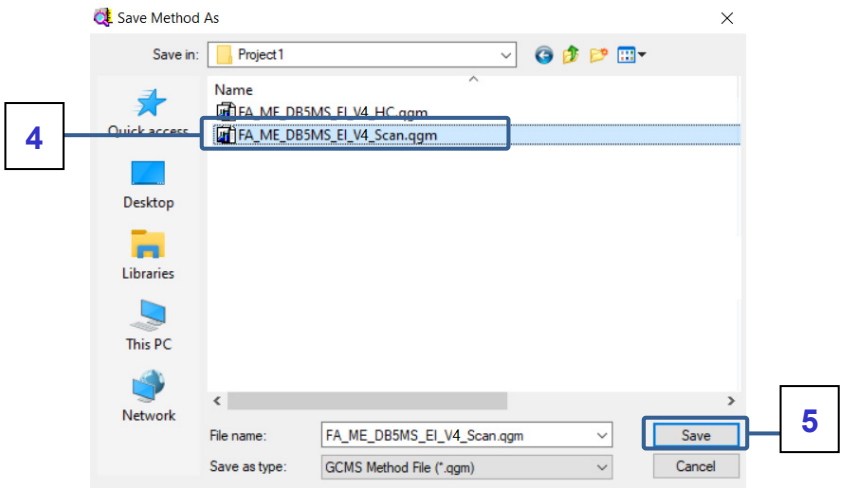
[Modify Compound Table(Assist Reference Ion Ratio) 1/3] Window

Normally these checkboxes are cleared in step 2. Select these checkboxes if it is necessary to revise the reference ion ratios set for the compound table so they reflect the data that are currently loaded.



4. Save [Compound Table].





1) Click the [View] button at the top-right corner of the table.

2) Click the [Param's] tab in the [Compound Table View]. The retention times in Param's are modified based on identified results.

3) Click the [Save Compound Table] icon on the [Compound] assistant bar.

4) Select the method file.

5) Click [Save].

Hint

It is possible to create method files with the Smart Database using the retention times that were corrected by the steps above.
For details, see "*Appendix 3 Directly Setting Retention Times with Smart Database Without Using AART.*"

Appendix3 Directly Setting Retention Times with Smart Database Without Using AART

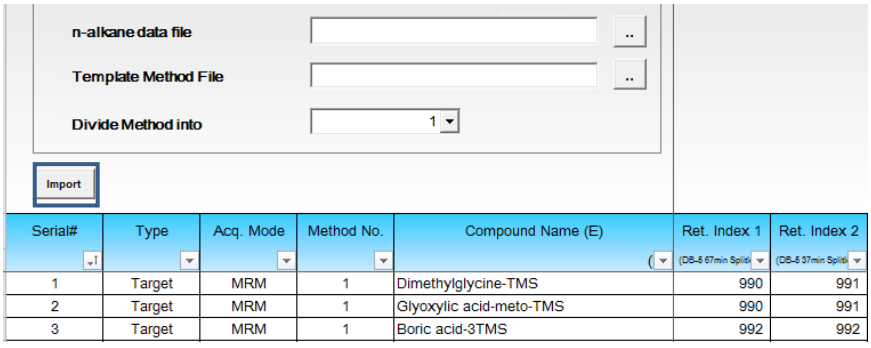
If the retention times of the target compounds are already known, it is possible to create measurement methods using already known retention times, without using retention indices.

1. Perform the steps in "*2.2.4.1 Set Target Compounds.*"
2. Enter the retention times of target components in the [Ret. Time] column.

Serial#	Type	Acq. Mode	Method No.	Compound Name (E)	Ret. Index 1 (DB-5 67min Split)	Ret. Index 2 (DB-5 37min Split)	Ret. Index 3	Ret. Time
1	Target	MRM	1	Dimethylglycine-TMS	990	991		6.979
2	Target	MRM	1	Glyoxylic acid-meto-TMS	990	991		6.979
3	Target	MRM	1	Boric acid-3TMS	992	992		7.000
4	Target	MRM	1	2-Aminoethanol-2TMS	1029	1031		7.875
5	Target	MRM	1	Pyruvic acid-meto-TMS	1047	1048		8.208
6	Target	MRM	1	Trichloroacetic acid-TMS	1059	1059		8.423
7	Target	MRM	1	Phenol-TMS	1060	1060		8.443
8	Target	MRM	1	Lactic acid-2TMS	1061	1062		8.482
9	Target	MRM	1	Lactic acid-13C3-2TMS	1062	1062		8.482
10	Target	MRM	1	2-Hydroxyisobutyric acid-2TMS	1067	1069		8.619
11	Target	MRM	1	Caproic acid-TMS	1071	1076		8.756

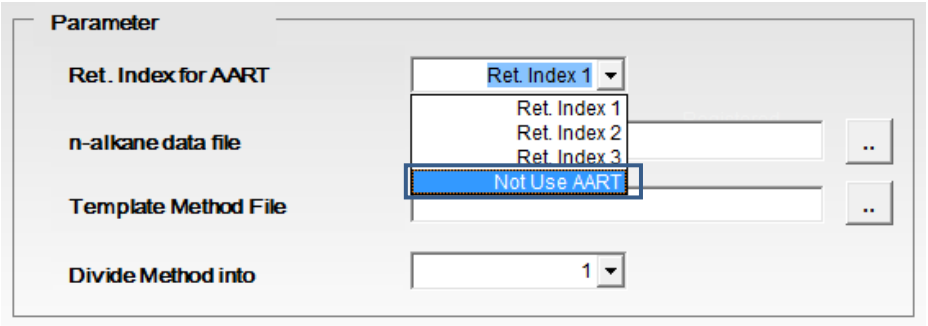
Hint

When a method file that has been created in accordance with "*Appendix 2 Adjusting Retention Times of Compound Table by Measuring Standard*" is imported into Smart Database, the corrected retention times will be entered in the [Ret. Time] column.



Serial#	Type	Acq. Mode	Method No.	Compound Name (E)	Ret. Index 1 (DB-5 67min Split)	Ret. Index 2 (DB-5 37min Split)
1	Target	MRM	1	Dimethylglycine-TMS	990	991
2	Target	MRM	1	Glyoxylic acid-meto-TMS	990	991
3	Target	MRM	1	Boric acid-3TMS	992	992

3. Set [Not Use AART] at [Ret. Index for AART].



4. Specify the method file including data acquisition and analysis conditions at [Template Method File].

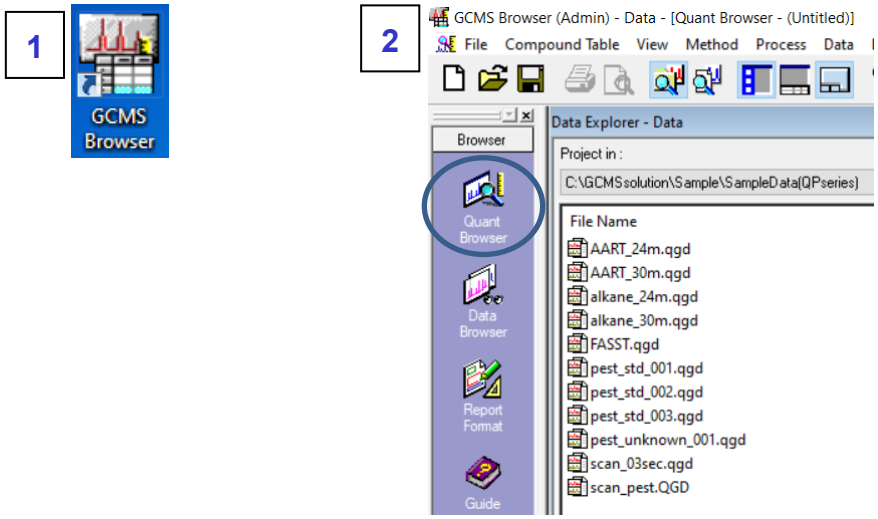
Hereafter, the standard procedures can be followed, so perform following of "*2.2.4.1 Set Target Compounds.*"

Appendix4 Outputting Area/Height Value from Multiple Data

(GCMSsolution)

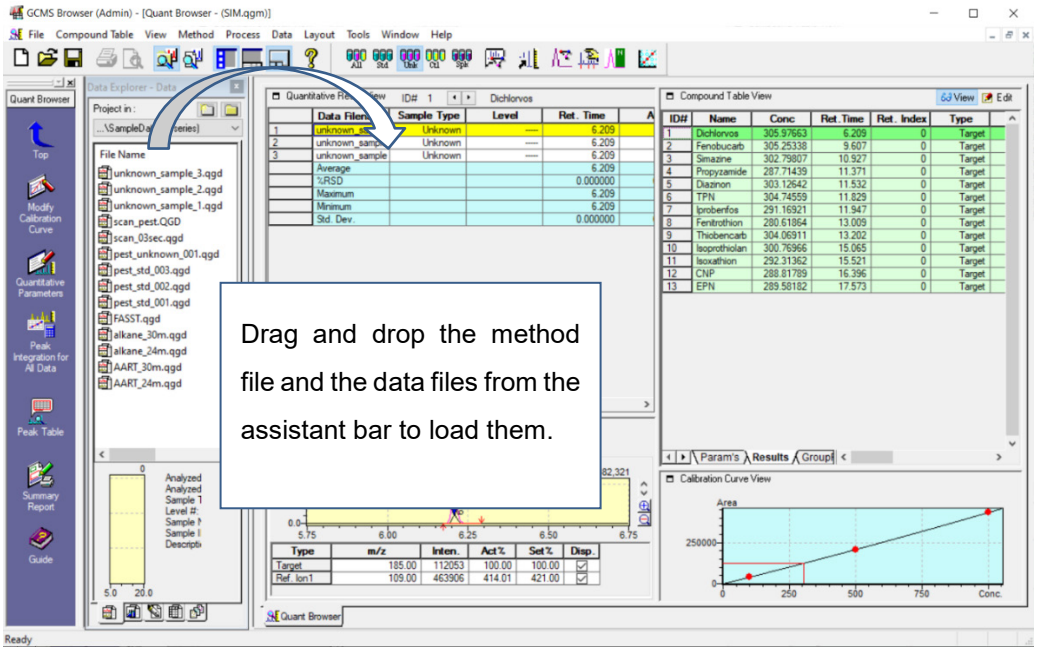
In order to perform statistical analyses such as multivariate analysis, using statistical analysis software, it is necessary to output the area values for each peak in multiple data in list format. Using GCMSsolution, it is possible to output in CSV file format by the following procedure.

1. Click [GCMS Browser] to display the [Quant Browser] window.

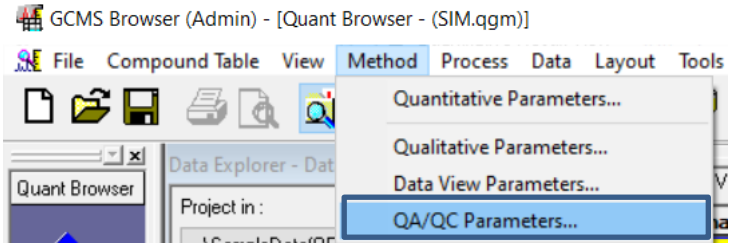


2. Load the method file for the analysis. Load the data files to output.

Note: It is possible to load these files by loading the batch file in which the data files were obtained from [Load from Batch file] on the [File] menu.



3. Click [QA/QC Parameters] from the [Method] menu.



4. Set the QA/QC Parameters.

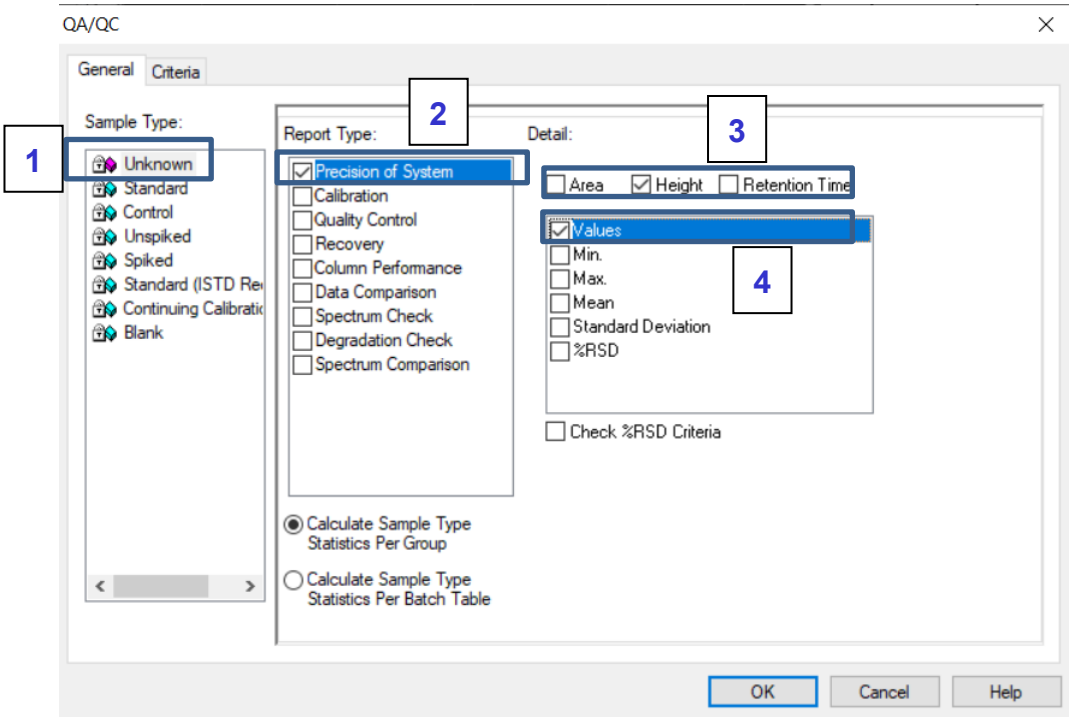
1) Click [Unknown] in the [Sample Type] field.

   Note: To output the sample information for the standard sample type, click [Standard].

2) Select the [Precision of System] checkbox.

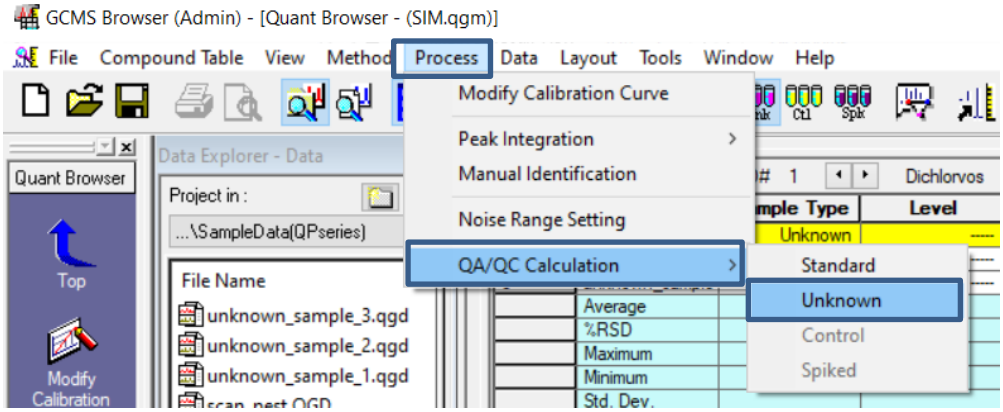
3) Select the [Area], [Height] and/or [Retention Time] checkboxes in accordance to the items to output.

4) Select the [Values] checkbox and then click [OK].



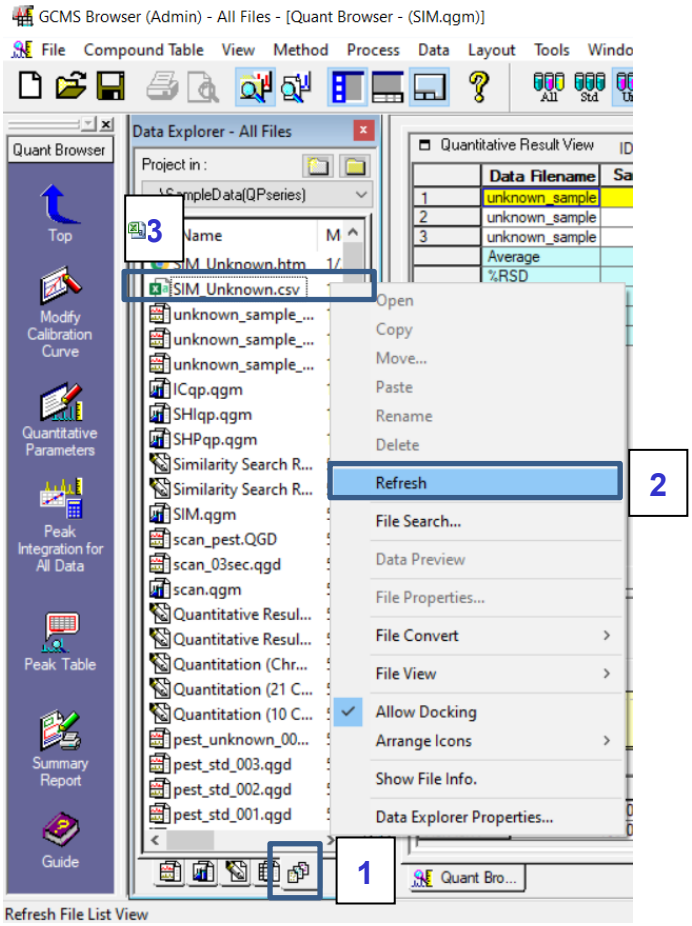
5. **Click the [Process] menu, point to [QA/QC Calculation], and click [Unknown].**

   Note: To output the sample information for the standard sample type, click [Standard].



6. **Once the QA/QC processing is completed, the file is output in .csv and .htm formats.**

   Click on the Data Explorer. Right-click on the assistant bar and click [Refresh]. A file with the extension ".csv" has been generated by the QA/QC processing. Double-click the file.



7. Height values (or area values) of all data are output as below.

	A	B	C	D	E	F	G
1	<Quality Assurance / Quality Control>						
2	<Create>	12/4/2014 11:45:39 AM					
3	<Report for Sample Type "Unknown">						
4	<Output Date>	12/4/2014 11:45:39 AM					
5	<Method File Name>	C:\GCMSsolution\Sample\SampleData(QP2010series)\SIM.qgm					
6	Precision of System						
7	Count	3					
8	Data	Data File Path	Sample Name	Sample ID	Analysis Date	Data File Status	
9	Data1:	C:\GCMSsolution\Sample\SampleData(	Pest13Compounds 1ug/mL		6/25/2001 7:00:12 PM	Normal	
10	Data2:	C:\GCMSsolution\Sample\SampleData(	Pesticides 13	Pest 002	10/3/2006 8:17:06 PM	Normal	
11	Data3:	C:\GCMSsolution\Sample\SampleData(	Pest13Compounds 1ug/mL	UNK-0004	7/1/2001 9:54:38 PM	Normal	
12	[Result](Height)						
13	ID	Compound Name	Data1 Height	Data2 Height	Data3 Height		
14	1	Dichlorvos	379708	13545	107713		
15	2	Fenobucarb	1021939	30807	215570		
16	3	Simazine	523481	21805	138757		
17	4	Propyzamide	595382	30697	161782		
18	5	Diazinon	319674	10750	62687		
19	6	TPN	832340	43543	196083		
20	7	Iprobenfos	830911	15252	180393		
21	8	Fenitrothion	189609	5499	49810		
22	9	Thiobencarb	2203314	89904	578678		
23	10	Isoprothiolane	216540	6966	52319		
24	11	Isoxathion	64076	1320	10519		
25	12	CNP	94847	2237	24267		
26	13	EPN	220996	5433	51801		

Only CSV file outputtede via LabSolutions Insight is available for multivariate analysis using the Multi-omics Analysis Package.

(LabSolutions Insight)

1) Click 

[Open] from the [File] menu bar, and open the method file to be used for analysis.

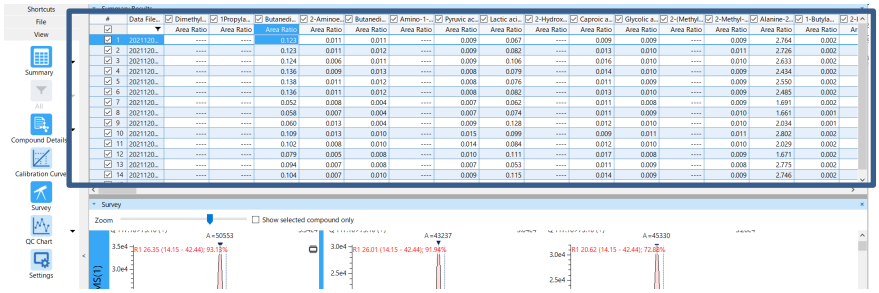
2) Click 

[Import] from the [File] menu bar, and load the method file to be output.

3) Check the analysis results.

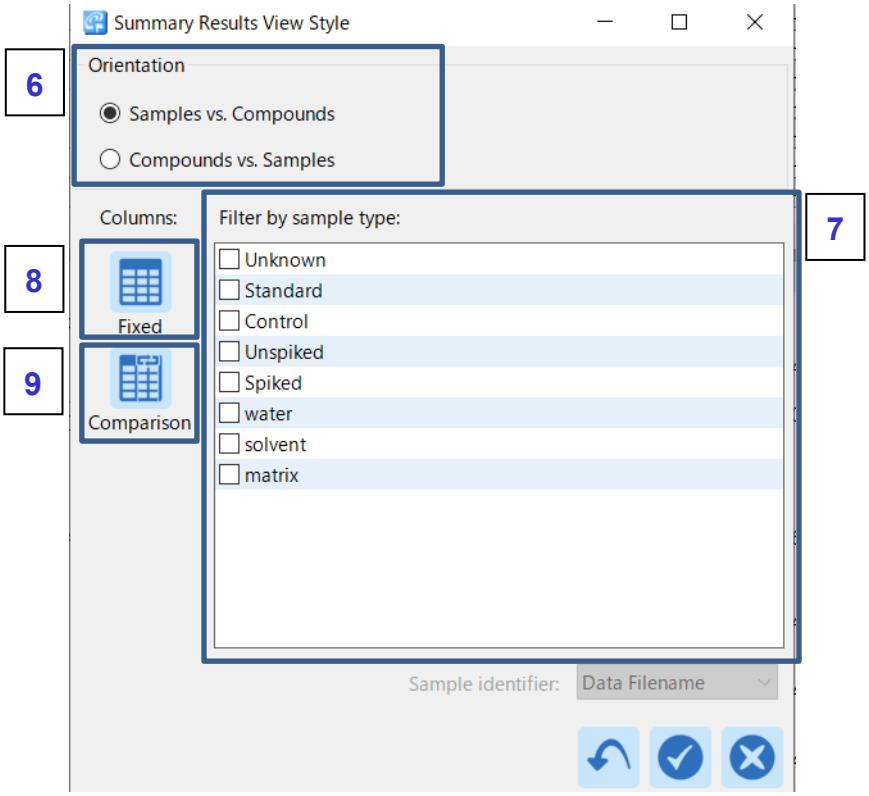
4) Display the summary.
(Select [View] → [Summary] from among the tabs)

5) Display [Summary Results View Style].
Right click (within the frame) in the Summary pane window → Properties)

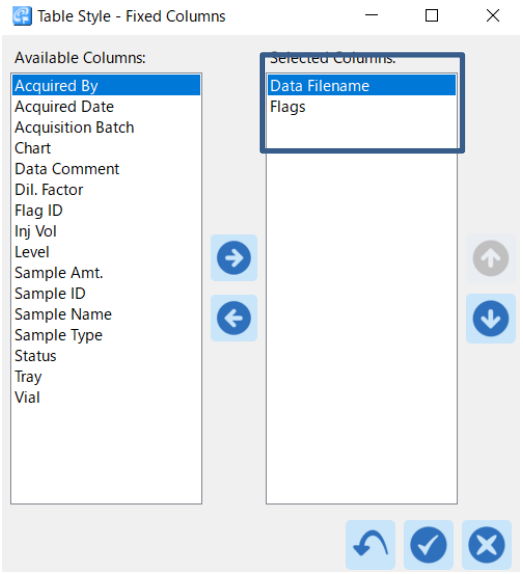


6) For [Orientation], select [Sample vs. Compounds].

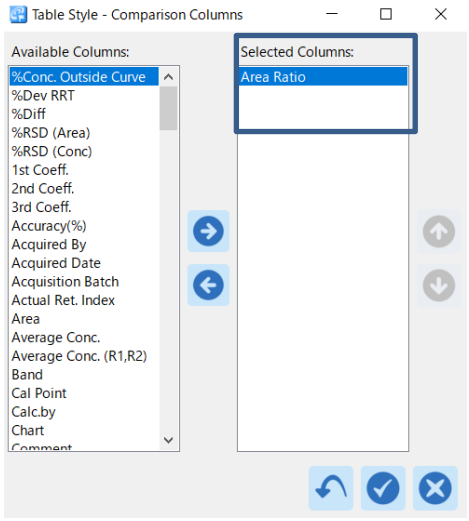
7) Do not select any checkbox for [Filter by sample type].



8) Click [Fixed], and select [Data Filename] and [Flags].



9) Click [Comparison], and select [Area] or [Area Ratio].
([Conc], [Height], and [Height Ratio] can also be selected.)

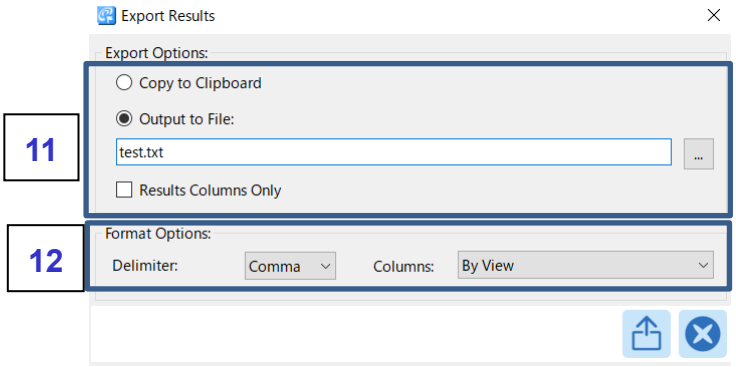


10) From among the tabs select [File] → [Export] 

→ [Results to File], and display [Export Results].

11) From the export options select [Output to File], and specify the save destination and filename.
Set [Comma-separated Values.csv] as the method of saving.
Also, do not select the [Results Columns Only] checkbox.

12) In the format options, select [Comma] as the demarcation character, and [By View] for the columns, and export the results.



Appendix5 FAQ

<Question 1>

How often should n-alkane be measured?

<Answer 1>

Measure n-alkane after cutting or replacing a column or approximately once a month to adjust the retention time.

<Question 2>

What type of cleaning solvent should be set on the autosampler?

<Answer 2>

It depends on the type of pretreatment and target sample to be measured. As the cleaning solvent to be used right before sample injection, use hexane. Although other types of organic solvents can be used, do not use a solvent that reacts with derivatization reagents (e.g. methanol) as the cleaning solvent right before sample injection.

<Question 3>

When the derivatization reagent was measured, many peaks were detected. Is that a problem?

<Answer 3>

No, it is not a problem. Components derived from the reagent, tools, and instrument are detected. However, blank measurement is preferable in order to distinguish from components of the sample.

<Question 4>

What is the quantitative analysis procedure components that have gone through qualitative measurement using the Smart Metabolites Database?

<Answer 4>

Enter information necessary for quantitative analysis on the method file used. (See the following figure.) Change the quantitative analysis procedure and concentration according to the type of analysis and the concentration of the unknown sample. After the creation of the method file, perform the procedure described in "*4.4 Sequential Analysis*" and after in the GCMS-QP Series / GCMS-TQ Series GCMS Operation Guide.

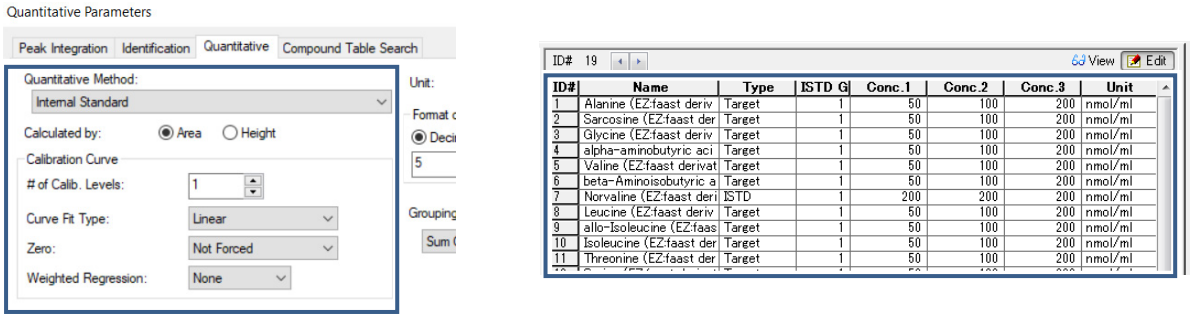


Fig. Example of Setting a Method File for Quantitative Analysis

Appendix6 List of Stable Isotope Reagents

The information on reagents presented here is current as of Febrary, 2022. In the future, these could change or be discontinued without prior notice. (＊: Dihydroxyacetone-13C3, Kynurenine-d6 and Cystamine-d8 are special order items)

Compound Name	P/N Reagent Name	Manufacture
Lactic acid-13C3	CLM-1579 SODIUM L-LACTATE (13C3, 98%) 20%W/W IN H2O	Cambridge Isotope Laboratories, Inc (CIL)
2-Hydroxybutyric acid-d3	D-7002 Sodium (±)-2-Hydroxybutyrate-2,3,3-d3	C/D/N Isotopes Inc
Oxalic acid-13C2	CLM-2002 OXALIC ACID (1,2-13C2, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Sarcosine-d3	DLM-6874 SARCOSINE: HCL(Methyl-D3, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Valine-d8	DLM-488 L-VALINE (D8, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Dihydroxyacetone-13C3＊	Custom Order 1,3-DIHYDROXYACETONE (1,2,3-13C3, 99%) 10% IN WATER	Cambridge Isotope Laboratories, Inc (CIL)
Isoleucine-d10	DLM-141 L-ISOLEUCINE (D10, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Fumaric acid-13C4	CLM-1529 FUMARIC ACID (13C4, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Malic acid-13C4	CLM-8065 L-MALIC ACID (13C4, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Aspartic acid-d3	DLM-546 L-ASPARTIC ACID (2,3,3-D3, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Glutamic acid-13C5	CLM-1800-H L-GLUTAMIC ACID (13C5, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
4-Hydroxybenzoic acid-13C6	CLM-4745 4-HYDROXYBENZOIC ACID (RING-13C6)	Cambridge Isotope Laboratories, Inc (CIL)
Lauric acid-d3	DLM-3062 LAURIC ACID (METHYL-D3, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Taurine-13C2	CLM-6622 TAURINE (1,2,-13C2, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Ribose-13C5	CLM-3652 D-RIBOSE (U-13C5, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Ornithine-d7	DLM-6669 L-ORNITHINE:HCL (D7, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Citric acid-d4	DLM-3487 CITRIC ACID (2,2,4,4-D4, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Tyrosine-13C6	CLM-1542 L-TYROSINE (RING-13C6, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Dopa-13C6	CLM-1007 L-DOPA (RING-13C6, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Kynurenine-d6＊	DLM-7842 (Custom-Order) L-KYNURENINE SULFATE:H2O (Ring-D4,3,3-D2 90-95%)	Cambridge Isotope Laboratories, Inc (CIL)
Cystamine-d8＊	Custom Order CYSTAMINE:2HCL (1,1,1',1',2,2,2',2'-D8 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Tryptophan-13C11	CLM-4290 L-TRYPTOPHAN (13C11, 99%)	Cambridge Isotope Laboratories, Inc (CIL)

Glucose - 13C6	CLM-1396-1 D-GLUCOSE (U-13C6, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Margaric acid - D33	DLM-6905-0.1 HEPTADECANOIC ACID (D33, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Glyoxylic acid - 13C2	CLM-6027-0.1 GLYOXYLIC ACID:H2O (13C2, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Palmitic acid - 13C16	CLM-409-0.1 PALMITIC ACID (U-13C16, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
3-Aminopropanoic acid - 13C3	CNLM-3946-0.1 BETA-ALANINE (13C3, 98%+; 15N, 96-99%)	Cambridge Isotope Laboratories, Inc (CIL)
3-Hydroxyisovaleric acid - D8	DLM-10603-0.1 3-HYDROXYISOVALERIC ACID (D8, 98%) 97% CHEMICAL PURITY	Cambridge Isotope Laboratories, Inc (CIL)
Succinic acid - 13C4	CLM-1571-0.1 SUCCINIC ACID (13C4, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Histidine-13C6	CLM-2264-PK L-HISTIDINE:HCL:H2O(<5% D)(13C6, 97-99%)	Cambridge Isotope Laboratories, Inc (CIL)
1,5-Anhydro-glucitol - 13C6	CLM-9657-0.1 1,5-ANHYDRO-D-GLUCITOL (U-13C6, 98%+)	Cambridge Isotope Laboratories, Inc (CIL)
Glutamine - 13C5	CLM-1822-H-0.1 L-GLUTAMINE (13C5, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Margaric acid - D3	DLM-1308-0.1 HEPTADECANOIC ACID (METHYL-D3, 98%)	Cambridge Isotope Laboratories, Inc (CIL)
Alanine -13C3	CLM-2184-H-0.25 L-ALANINE (13C3, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
2-Ketoglutaric acid - 13C4	CLM-4442-0.5 ALPHA-KETOGLUTARIC ACID, DISODIUM SALT (1,2,3,4-13C4, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Arabinose-13C5	CLM-8477-0.1 D-ARABINOSE(U-13C5, 99%)	Cambridge Isotope Laboratories, Inc (CIL)
Palmitoleic acid - 13C16	CLM-2241-0.005 PALMITOLEIC ACID (U-13C16, 98%) 97% CHEMICAL PURITY	Cambridge Isotope Laboratories, Inc (CIL)

Appendix7 Registered Compound Trimethylsilylated Metabolites GC-MS(Scan)

ID	Compound Name	ID	Compound Name
1	Dimethylglycine-TMS	51	2-Ketoisocaproic acid-meto-TMS(2)
2	Glyoxylic acid-meto-TMS	52	Methylmalonic acid-2TMS
3	Boric acid-3TMS	53	Valine-d8-2TMS
4	2-Aminoethanol-2TMS	54	2-Keto-isovaleric acid-oxime-2TMS(2)
5	Pyruvic acid-meto-TMS	55	Valine-2TMS
6	Trichloroacetic acid-TMS	56	Glyceraldehyde-meto-2TMS(2)
7	Phenol-TMS	57	Dihydroxyacetone-2TMS
8	Lactic acid-2TMS	58	Ethylhydracrylic acid-2TMS
9	Lactic acid-13C3-2TMS	59	Urea-2TMS
10	2-Hydroxyisobutyric acid-2TMS	60	4-Hydroxybutyric acid-2TMS
11	Caproic acid-TMS	61	3-Hydroxyvaleric acid-2TMS
12	2-Ketobutyric acid-meto-TMS(1)	62	2-Hydroxyisocaproic acid-2TMS
13	Glycolic acid-2TMS	63	Norvaline-2TMS
14	Pyruvic acid-2TMS	64	Acetoacetic acid-2TMS(2)
15	2-Ketobutyric acid-meto-TMS(2)	65	2-Hydroxy-3-methylvaleric acid-2TMS
16	Alanine-2TMS	66	Benzoic acid-TMS
17	2-Keto-isovaleric acid-meto-TMS	67	Dihydroxyacetone-meto-2TMS
18	Norvaline-TMS	68	Dihydroxyacetone-13C3-meto-2TMS
19	Glycine-2TMS	69	Acetoacetic acid-oxime-2TMS
20	Hydroxylamine-3TMS	70	Octanoic acid-TMS
21	Glyoxylic acid-oxime-2TMS	71	2-Keto-isovaleric acid-2TMS
22	Oxalic acid-2TMS	72	2-Aminoethanol-3TMS
23	2-Hydroxybutyric acid-2TMS	73	Cyclohexanediol-2TMS
24	Oxalic acid-13C2-2TMS	74	2-Methyl-3-hydroxyvaleric acid-2TMS
25	2-Hydroxybutyric acid-d3-2TMS	75	2-Propylhydroxyglutaric acid-oxime-2TMS
26	Acetoacetic acid-meto-TMS	76	Leucine-2TMS
27	2-Furoic acid-TMS	77	Glycerol-3TMS
28	Sarcosine-d3-2TMS	78	Acetylglycine-TMS
29	Sarcosine-2TMS	79	Phosphoric acid-3TMS
30	3-Hydroxypropionic acid-2TMS	80	Ethylmalonic acid-2TMS
31	2-Aminoisobutyric acid-2TMS	81	3-Hydroxypyruvic acid-meto-2TMS(1)
32	Valproic acid-TMS	82	2-Ketoisocaproic acid-oxime-2TMS
33	Pyruvic acid-oxime-2TMS	83	Isoleucine-d10-2TMS
34	4-Cresol-TMS	84	Nicotinic acid-TMS
35	3-Hydroxybutyric acid-2TMS	85	allo-Isoleucine-2TMS
36	3-Hydroxyisobutyric acid-2TMS	86	Isoleucine-2TMS
37	2-Hydroxyisovaleric acid-2TMS	87	Phenylacetic acid-TMS
38	2-Aminobutyric acid-2TMS	88	4-Aminobutyric acid-2TMS
39	2-Ketoisocaproic acid-meto-TMS(1)	89	Proline-2TMS
40	3-Methyl-2-oxovaleric acid-meto-TMS(1)	90	Maleic acid-2TMS
41	3-Aminopropanoic acid-2TMS	91	2-Octenoic acid-TMS
42	3-Methyl-2-oxovaleric acid-meto-TMS(2)	92	Succinic acid-2TMS
43	2-Methyl-3-hydroxybutyric acid-2TMS(1)	93	Glycine-3TMS
44	Malonic acid-2TMS	94	Catechol-2TMS
45	2-Methyl-3-hydroxybutyric acid-2TMS(2)	95	Methylsuccinic acid-2TMS
46	Glyceraldehyde-meto-2TMS(1)	96	3-Hydroxypyruvic acid-meto-2TMS(2)
47	Acetoacetic acid-2TMS(1)	97	Glyceric acid-3TMS
48	3-Aminoisobutyric acid-2TMS	98	Fumaric acid-2TMS
49	3-Hydroxyisovaleric acid-2TMS	99	Uracil-2TMS
50	2-Keto-isovaleric acid-oxime-2TMS(1)	100	Fumaric acid-13C4-2TMS

ID	Compound Name	ID	Compound Name
101	Citraconic acid-2TMS	151	p-Nitrophenol-TMS
102	Nonanoic acid-TMS	152	Isovalerylglycine-2TMS
103	Propionylglycine-TMS	153	Glutamic acid 5-methylester-2TMS
104	Serine-3TMS	154	3-Aminoglutaric acid-2TMS
105	Acetylglycine-2TMS	155	meso-Erythritol-4TMS
106	Mevalonic lactone-TMS	156	2-Hexenedioic acid-2TMS
107	2-Propyl-3-hydroxy-pentanoic acid-2TMS(1)	157	N-Acetylserine-3TMS
108	Isobutyrylglycine-TMS	158	Aspartic acid-d3-3TMS
109	2-Propyl-3-hydroxy-pentanoic acid-2TMS(2)	159	Aspartic acid-3TMS
110	2-Aminooctanoic acid-TMS	160	Methionine-2TMS
111	Threonine-3TMS	161	Cytosine-2TMS
112	Mesaconic acid-2TMS	162	5-Oxoproline-2TMS
113	5-Aminovaleric acid-2TMS	163	Thiodiglycolic acid-2TMS
114	O-Acetylserine-2TMS	164	4-Hydroxyproline-3TMS
115	Glutaric acid-2TMS	165	3-Methyladipic acid-2TMS
116	Hydroquinone-2TMS	166	Acetylsalicylic acid-TMS
117	Thymine-2TMS	167	Phenylpyruvic acid-meto-TMS(1)
118	3-Methylglutaconic acid-2TMS	168	4-Aminobutyric acid-3TMS
119	3-Methylglutaric acid-2TMS	169	7-Hydroxoctanoic acid-2TMS
120	Propionylglycine-2TMS	170	2-Propyl-glutaric acid-2TMS
121	Isobutyrylglycine-2TMS	171	Cinnamic acid-TMS
122	3-Aminopropanoic acid-3TMS	172	5-Hydroxymethyl-2-furoic acid-2TMS
123	2-Deoxytetronic acid-3TMS	173	Pyrogallol-3TMS
124	3-Methylglutaconic acid(E)-2TMS	174	Oxalacetic acid-3TMS
125	N-Butyrylglycine-TMS	175	3-Methylcrotonoylglycine-TMS
126	Glutaconic acid-2TMS	176	Tiglylglycine-2TMS
127	Succinylacetone-ox-origin fragment	177	Cysteine-3TMS
128	Dihydrouracil-TMS	178	Tiglylglycine-TMS
129	Decanoic acid-TMS	179	3-Hydroxybenzoic acid-2TMS
130	Homoserine-3TMS	180	Succinylacetone-meto-TMS(1)
131	Oxalacetic acid-meto-2TMS	181	Creatinine-3TMS
132	3-Aminoisobutyric acid-3TMS	182	Threonic acid-4TMS
133	Erythrulose-meto-3TMS(1)	183	3-Methylcrotonoylglycine-2TMS
134	3-Methylglutaconic acid(Z)-2TMS	184	2-Hydroxyphenylacetic acid-2TMS
135	2-Propyl-5-hydroxy-pentanoic acid-2TMS	185	2-Ketoglutaric acid-meto-2TMS
136	Citramalic acid-3TMS	186	2-Hydroxyglutaric acid-3TMS
137	Niacinamide-TMS	187	3-Hydroxyglutaric acid-3TMS
138	Mandelic acid-2TMS	188	Succinylacetone-meto-TMS(2)
139	Isovalerylglycine-TMS	189	O-Phosphoethanolamine-3TMS
140	Erythrulose-meto-3TMS(2)	190	Succinylacetone-meto-TMS(3)
141	N-Butyrylglycine-2TMS	191	2-Isopropylmalic acid-3TMS
142	Anthranilic acid-TMS	192	Phenylpyruvic acid-meto-TMS(2)
143	Malic acid-13C4-3TMS	193	Succinylacetone-meto-TMS(4)
144	Malic acid-3TMS	194	3-Phenyllactic acid-2TMS
145	2-Aminooctanoic acid-2TMS	195	Tropic acid-2TMS
146	Dihydrouracil-2TMS	196	Pimelic acid-2TMS
147	Adipic acid-2TMS	197	Phosphoenolpyruvic acid-3TMS
148	Threitol-4TMS	198	2-Ketoglutaric acid-3TMS
149	N-Acetylserine-2TMS	199	3-Hydroxy-3-methylglutaric acid-3TMS
150	2-Phenyllactic acid-2TMS	200	3-Hydroxyphenylacetic acid-2TMS

ID	Compound Name	ID	Compound Name
201	Hypotaurine-3TMS	251	3-Sulfinoalanine-3TMS
202	3-Aminoglutaric acid-3TMS	252	Fucose-4TMS(1)
203	Anthranilic acid-2TMS	253	Ribulose-meto-4TMS
204	Ornithine-3TMS	254	Ribose-meto-4TMS
205	Lyxose-4TMS(1)	255	Xylulose-meto-4TMS
206	Glutamic acid-13C5-3TMS	256	Ribose-13C5-meto-4TMS
207	Cadaverine-3TMS	257	Ribonolactone-3TMS
208	Glutamic acid-3TMS	258	Suberic acid-2TMS
209	Triethanolamine-3TMS	259	Ribulose-4TMS
210	Arabinose-4TMS(1)	260	Lysine-3TMS
211	4-Hydroxybenzoic acid-2TMS	261	Rhamnose-4TMS(2)
212	4-Hydroxybenzoic acid-13C6-2TMS	262	2-Ketoadipic acid-oxime-3TMS(1)
213	2-Ketoglutaric acid-oxime-3TMS(1)	263	Glyceraldehyde 3-phosphate-meto-3TMS(1)
214	Hexanoylglycine-TMS	264	1,6-Anhydroglucose-3TMS
215	2-Ketoadipic acid-meto-2TMS(1)	265	2-Aminoadipic acid-3TMS
216	5-Aminovaleric acid-3TMS	266	2-Ketoadipic acid-oxime-3TMS(2)
217	Phenylalanine-2TMS	267	Xylose-4TMS(1)
218	Asparagine-4TMS	268	Xylitol-5TMS
219	Rhamnose-4TMS(1)	269	Glyceraldehyde 3-phosphate-meto-3TMS(2)
220	4-Hydroxyphenylacetic acid-2TMS	270	Tricarballylic acid-3TMS
221	Lauric acid-d3-TMS	271	Fucose-4TMS(2)
222	Ureidopropionic acid-3TMS	272	Quinolinic acid-2TMS
223	Lauric acid-TMS	273	Glycerol 2-phosphate-4TMS
224	Tartaric acid-4TMS	274	Rhamnose-meto-4TMS(1)
225	Hexanoylglycine-2TMS	275	Arabitol-5TMS
226	2-Ketoglutaric acid-oxime-3TMS(2)	276	2-Deoxy-glucose-4TMS(1)
227	Ribose-4TMS(1)	277	Cysteic acid-3TMS
228	4-Aminobenzoic acid-TMS	278	Glutamine-4TMS
229	N-Acetylaspartic acid-2TMS	279	Ribitol-5TMS
230	Ribose-4TMS(2)	280	Rhamnose-meto-4TMS(2)
231	2-Ketoadipic acid-meto-2TMS(2)	281	Glutaconic acid-3TMS(2)
232	Arabinose-4TMS(2)	282	Aconitic acid-3TMS
233	Ureidopropionic acid-2TMS	283	Fucose-meto-4TMS(1)
234	Glutaconic acid-3TMS(1)	284	Orotic acid-3TMS
235	Lyxose-meto-4TMS(1)	285	Putrescine-4TMS
236	Ribose-4TMS(3)	286	Dihydroxyacetone phosphate-meto-3TMS(1)
237	Threo-b-hydroxyaspartic acid-4TMS	287	Fucose-meto-4TMS(2)
238	Lyxose-4TMS(2)	288	Mannose-5TMS(1)
239	Xylose-meto-4TMS	289	Dihydroxyacetone phosphate-meto-3TMS(2)
240	Homocysteine-3TMS	290	3-Hydroxyanthranilic acid-2TMS
241	N-Acetylaspartic acid-3TMS	291	3-Methoxy-4-hydroxybenzoic acid-2TMS
242	Lyxose-meto-4TMS(2)	292	5-Aminolevulinic acid-meto-3TMS(1)
243	Asparagine-3TMS	293	Glycerol 3-phosphate-4TMS
244	2-Hydroxyadipic acid-3TMS	294	Homovanillic acid-2TMS
245	Arabinose-meto-4TMS	295	Xylose-4TMS(2)
246	Octenedioic acid-2TMS	296	Glutamine-3TMS
247	Taurine-13C2-3TMS	297	Ribonic acid-5TMS
248	Taurine-3TMS	298	Dihydroorotic acid-3TMS
249	3-Hydroxyadipic acid-3TMS	299	3-Dehydroshikimic acid-meto-3TMS(1)
250	Ribose-4TMS(4)	300	Azelaic acid-2TMS

ID	Compound Name	ID	Compound Name
301	2-Phosphoglyceric acid -4TMS	351	1,5-Anhydro-glucitol-4TMS
302	O-Phosphoethanolamine-4TMS	352	Tagatose-meto-5TMS(1)
303	5-Aminolevulinic acid-meto-3TMS(2)	353	N-Acetylglutamine-4TMS
304	3-Dehydroshikimic acid-meto-3TMS(2)	354	3-Hydroxyanthranilic acid-3TMS
305	2-Deoxy-glucose-meto-4TMS	355	Psicose-meto-5TMS(1)
306	2-Deoxy-glucose-4TMS(2)	356	Adenine-2TMS
307	Hippuric acid-2TMS	357	Histamine-3TMS
308	Shikimic acid-4TMS	358	N-Acetyl-Lysine-2TMS
309	2-Aminopimelic acid-3TMS	359	Vanilmandelic acid-3TMS
310	Hypoxanthine-2TMS	360	Sorbose-5TMS
311	Galactose-5TMS(1)	361	Urocanic acid-TMS
312	3-Phosphoglyceric acid-4TMS	362	Sebacic acid-2TMS
313	Ornithine-d7-4TMS	363	Pyridoxal-meto-2TMS(2)
314	Fructose-5TMS(1)	364	Tagatose-5TMS(3)
315	Protocatechuic acid-3TMS	365	2-Methylhippuric acid-TMS
316	Glycyl-Glycine-4TMS	366	Psicose-meto-5TMS(2)
317	Citric acid-d4-4TMS	367	Tagatose-meto-5TMS(2)
318	Arginine-3TMS	368	Vanillylamine-3TMS
319	Ornithine-4TMS	369	Sorbose-meto-5TMS(1)
320	Psicose-5TMS(1)	370	Fructose-meto-5TMS(1)
321	Citric acid-4TMS	371	Decadienedioic acid-2TMS
322	Isocitric acid-4TMS	372	Galactosamine-5TMS(1)
323	Fructose-5TMS(2)	373	Sorbose-meto-5TMS(2)
324	Dopamine-3TMS	374	5-Dehydroquinic acid-meto-4TMS
325	Glucuronic acid lactone-3TMS(1)	375	Glucono-1,5-lactone-4TMS
326	Psicose-5TMS(2)	376	Allose-meto-5TMS(1)
327	Tagatose-5TMS(1)	377	N6-Acetyllysine-3TMS
328	4-Aminobenzoic acid-2TMS	378	Allantoin-4TMS
329	Methionine sulfone-2TMS	379	Fructose-meto-5TMS(2)
330	Hippuric acid-TMS	380	Tagatose-5TMS(4)
331	Myristic acid-TMS	381	4-Hydroxyphenylpyruvic acid-meto-2TMS
332	Homogentisic acid-3TMS	382	4-Hydroxyphenyllactic acid-3TMS
333	2-Methylhippuric acid-2TMS	383	Mannose-meto-5TMS(1)
334	Fructose-5TMS(3)	384	Pyridoxine-3TMS
335	Tagatose-5TMS(2)	385	Tagatose-5TMS(5)
336	Glucuronic acid lactone-3TMS(2)	386	Glucose-5TMS(1)
337	O-Phospho-Serine-4TMS	387	Psicose-5TMS(4)
338	Pyridoxal-meto-2TMS(1)	388	Galactose-meto-5TMS(1)
339	Coniferyl aldehyde-TMS	389	Erythrose 4-phosphate-meto-4TMS(1)
340	Methylcitric acid-4TMS(1)	390	Glucose-meto-5TMS(1)
341	3-(3-Hydroxyphenyl)-3-hydroxypropionic acid-3TMS	391	N-Acetylglutamine-3TMS
342	Caffeine	392	Tyramine-3TMS
343	Epinephrine-3TMS	393	Indol-3-acetic acid-TMS
344	Hydroxylysine (2 isomers)-4TMS	394	Erythrose 4-phosphate-meto-4TMS(2)
345	Galactose-5TMS(2)	395	Theophylline-TMS
346	Histidinol-3TMS	396	Allose-meto-5TMS(2)
347	Methylcitric acid-4TMS(2)	397	Mannose-meto-5TMS(2)
348	Mannose-5TMS(2)	398	Lysine-4TMS
349	Allose-5TMS	399	Allantoin-5TMS
350	Psicose-5TMS(3)	400	Histidine-3TMS

ID	Compound Name	ID	Compound Name
401	Ureidosuccinic acid-3TMS	451	3-Hydroxysebacic acid -3TMS
402	3,4-Dihydroxymandelic acid-4TMS	452	2-Hydroxyhippuric acid-2TMS
403	5-Dehydroquinic acid-5TMS	453	Allantoin-3TMS
404	Glucosamine-5TMS(1)	454	Kynurenic acid-2TMS
405	N-Acetylglutamine-2TMS	455	Dodecanedioic acid-2TMS
406	Glucono-1,4-lactone-4TMS	456	Metoprolol-TMS
407	Galactose-meto-5TMS(2)	457	Naproxen-TMS
408	Coniferyl alcohol-2TMS	458	Dopamine-4TMS
409	N6-Acetyllysine-2TMS	459	Citrulline-3TMS
410	Glucose-meto-5TMS(2)	460	N-Acetylmannosamine-meto-4TMS(1)
411	Coniferyl aldehyde-meto-TMS(1)	461	N-Acetyltyrosine-3TMS
412	Tyrosine-13C6-3TMS	462	Dopa-13C6-4TMS
413	Tyrosine-3TMS	463	Dopa-4TMS
414	3,4-Dihydroxybenzylamine-4TMS	464	Uric acid-4TMS
415	1-Hexadecanol-TMS	465	Inositol-6TMS(2)
416	Coniferyl aldehyde-meto-TMS(2)	466	Methoprene acid-TMS
417	Lipoic acid-TMS	467	N-Acetylmannosamine-meto-4TMS(2)
418	Pyridoxamine-3TMS	468	Ribulose 5-phosphate-meto-5TMS(1)
419	Mannitol-6TMS	469	Pyridoxamine-4TMS
420	2-Hydroxyhippuric acid-3TMS	470	Ribose 5-phosphate-meto-5TMS(1)
421	Glucuronic acid-meto-5TMS(1)	471	Guanine-3TMS
422	Ascorbic acid-4TMS	472	Margaric acid-TMS
423	Glucosamine-5TMS(2)	473	Kynurenine-2TMS
424	Sorbitol-6TMS	474	N-Acetyltyrosine-2TMS
425	Galactitol-6TMS	475	Ribose 5-phosphate-meto-5TMS(2)
426	Galacturonic acid-meto-5TMS(1)	476	Ribulose 5-phosphate-meto-5TMS(2)
427	Tryptamine-2TMS	477	Octadecanol-TMS
428	ParaXanthine-TMS	478	3,6-Epoxydodecanedioic acid-2TMS
429	Indol-3-acetic acid-2TMS	479	Norepinephrine-5TMS
430	Galacturonic acid-5TMS(1)	480	7-Methylguanine-2TMS
431	Epinephrine-4TMS	481	Indolelactic acid-3TMS
432	Galactosamine-5TMS(2)	482	Kynurenine-d6-3TMS
433	Glucuronic acid-meto-5TMS(2)	483	Kynurenine-3TMS
434	Glucuronic acid-5TMS(1)	484	Linoleic acid-TMS
435	N-Acetyl-Ornithine-4TMS	485	Oleic acid-TMS
436	Galacturonic acid-meto-5TMS(2)	486	2,3-Bisphosphoglyceric acid-5TMS
437	Glucose-5TMS(2)	487	Elaidic acid-TMS
438	Urocanic acid-2TMS	488	Cystamine-d8-nTMS
439	Pantothenic acid-3TMS	489	Cystathionine-4TMS
440	S-Benzyl-Cysteine-4TMS	490	Cystamine-nTMS
441	Inositol-6TMS(1)	491	Stearic acid-TMS
442	Palmitoleic acid-TMS	492	Suberylglycine-3TMS
443	Xanthine-3TMS	493	Tryptophan-13C11-3TMS
444	Gluconic acid-6TMS	494	Tryptophan-3TMS
445	Glucuronic acid-5TMS(2)	495	Tryptamine-3TMS
446	Palmitic acid-TMS	496	3-Hydroxydodecanedioic acid-3TMS
447	Octopamine-4TMS	497	Suberylglycine-2TMS
448	2-Hydroxysebacic acid -3TMS	498	Spermidine-5TMS
449	Glucaric acid-6TMS	499	Metoprolol-2TMS
450	Galacturonic acid-5TMS(2)	500	Cystine-4TMS

ID	Compound Name	ID	Compound Name
501	Fructose 1-phosphate-meto-6TMS(1)	551	Guanosine-5TMS
502	Fructose 1-phosphate-meto-6TMS(2)	552	Trehalose-8TMS
503	Fructose 6-phosphate-meto-6TMS	553	Maltose-meto-8TMS(1)
504	Mannose 6-phosphate-meto-6TMS(1)	554	5'-Methylthioadenosine-3TMS
505	Glucose 6-phosphate-meto-6TMS(1)	555	Lactitol-9TMS
506	3-Hydroxy-kynurenine-3TMS	556	Maltose-meto-8TMS(2)
507	Mannose 6-phosphate-meto-6TMS(2)	557	Uridine monophosphate-5TMS
508	Arachidonic acid-TMS	558	Thymidine monophosphate-3TMS
509	Eicosapentaenoic acid-TMS	559	Maltitol-9TMS
510	Glucose 6-phosphate-meto-6TMS(2)	560	Isomaltose-meto-8TMS(1)
511	Juniperic acid-2TMS	561	Isomaltose-meto-8TMS(2)
512	Melatonin-2TMS	562	Inosine monophosphate-5TMS
513	Oleamide-TMS	563	Cholecalciferol-TMS
514	Thymidine-2TMS	564	Adenosine monophosphate-5TMS
515	Porphobilinogen-4TMS	565	Xanthosine monophosphate-6TMS
516	p-Aminohippuric acid-2TMS	566	Adenosine 3',5'-cyclic monophosphate-3TMS
517	5-Methoxytryptamine-2TMS	567	Cholesterol-TMS
518	5-Methoxytryptamine-3TMS	568	Trehalose 6-phosphate-9TMS
519	2'-Deoxyuridine-3TMS	569	Hydrocinnamic acid-TMS
520	5-Hydroxy-tryptophan-4TMS	570	Serotonin-4TMS
521	Uridine-4TMS	571	Serotonin-3TMS
522	Melatonin-TMS	572	Catechin-5TMS
523	Uridine-3TMS	573	Cholesta-3,5-diene
524	6-Phosphogluconic acid-7TMS	574	4-Coumaric acid-2TMS
525	Inositol phosphate-7TMS	575	Daidzein-2TMS
526	Saccharopine-4TMS	576	Epicatechin-5TMS
527	Biotin-3TMS	577	Epigallocatechin-6TMS
528	Chloramphenicol-2TMS	578	Ferulic acid-2TMS
529	Thymidine-3TMS	579	Gallic acid-4TMS
530	Fendiline	580	Genistein-3TMS
531	Homocystine-4TMS	581	Genistein-2TMS
532	Saccharopine-5TMS	582	Quinic acid-5TMS
533	Docosahexaenoic acid-TMS	583	Salicylic acid-2TMS
534	Docosapentaenoic acid-TMS	584	Sinapinic acid-2TMS
535	Inosine-4TMS	585	Theanine-2TMS
536	Sedoheptulose 7-phosphate-meto-7TMS	586	2-Deoxy-ribose-meto-4TMS
537	Fendiline-TMS	587	alpha-Tocopherol-TMS
538	Adenosine-4TMS	588	Caffeic acid-3TMS
539	Batyl alcohol-2TMS	589	Cellobiose-meto-8TMS(1)
540	N-Acetylneuraminic acid-6TMS	590	Cellobiose-meto-8TMS(2)
541	Sucrose-8TMS	591	Chlorogenic acid-6TMS
542	Xanthosine-5TMS	592	Cinnamyl alcohol-TMS
543	Cytidine-4TMS	593	Galactinol-9TMS
544	N-Acetylneuraminic acid-meto-7TMS	594	Gastrodin-5TMS
545	Lactose-meto-8TMS(1)	595	Isoascorbic acid-4TMS
546	Carnosine-4TMS	596	Itaconic acid-2TMS
547	Lactose-meto-8TMS(2)	597	N-Acetylglucosamine-meto-4TMS
548	5'-Methylthioadenosine-2TMS	598	Phytol-TMS
549	Monostearin-2TMS	599	Pinitol-5TMS
550	Spermine-6TMS	600	Pipecolic acid-TMS

ID	Compound Name
601	Pipecolic acid-2TMS
602	N-Acetyl-glutamic acid-2TMS
603	N-Acetyl-glutamic acid-3TMS
604	2,3-Butanediol-2TMS(1)
605	2,3-Butanediol-2TMS(2)
606	2-(Methylamino)ethanol-2TMS
607	2-Aminopropanol-2TMS
608	1-Butylamine-2TMS
609	Isobutylamine-2TMS
610	1-Propylamine-2TMS
611	Hydrocaffeic acid-3TMS
612	Glucose-13C6-meto-5TMS(1)
613	Glucose-13C6-meto-5TMS(2)
614	Margaric acid-d33-TMS
615	Glyoxylic acid-13C2-meto-TMS
616	Palmitic acid-13C16-TMS
617	3-Aminopropanoic acid-13C3-15N-3TMS
618	3-Hydroxyisovaleric acid-d8-2TMS
619	Succinic acid-13C4-2TMS
620	Histidine-13C6-3TMS
621	1,5-Anhydro-glucitol-13C6-4TMS
622	Glutamine-13C5-3TMS
623	Margaric acid-methyl-d3-TMS
624	Alanine-13C3-2TMS
625	2-Ketoglutaric acid-13C4-meto-2TMS
626	Arabinose-13C5-meto-4TMS
627	Palmitoleic acid-13C16-TMS

Appendix8 Registered Compound Trimethylsilylated Metabolites GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
1	Dimethylglycine-TMS	51	Glyceraldehyde-meto-2TMS(2)
2	Glyoxylic acid-meto-TMS	52	Dihydroxyacetone-2TMS
3	2-Aminoethanol-2TMS	53	2-Hydroxyisocaproic acid-2TMS
4	Pyruvic acid-meto-TMS	54	Norvaline-2TMS
5	Lactic acid-2TMS	55	Urea-2TMS
6	Lactic acid-13C3-2TMS	56	Acetoacetic acid-2TMS(2)
7	2-Hydroxyisobutyric acid-2TMS	57	Dihydroxyacetone-meto-2TMS
8	2-Ketobutyric acid-meto-TMS(1)	58	Dihydroxyacetone-13C3-meto-2TMS
9	Caproic acid-TMS	59	Acetoacetic acid-oxime-2TMS
10	Glycolic acid-2TMS	60	Serine-2TMS
11	Alanine-2TMS	61	Benzoic acid-TMS
12	2-Ketobutyric acid-meto-TMS(2)	62	2-Aminoethanol-3TMS
13	2-Keto-isovaleric acid-meto-TMS	63	Glycerol-3TMS
14	Hydroxylamine-3TMS	64	Octanoic acid-TMS
15	Norvaline-TMS	65	Leucine-2TMS
16	2-Hydroxybutyric acid-d3-2TMS	66	Phosphoric acid-3TMS
17	Glycine-2TMS	67	Ethylmalonic acid-2TMS
18	2-Hydroxybutyric acid-2TMS	68	2-Ketoisocaproic acid-oxime-2TMS
19	Glyoxylic acid-oxime-2TMS	69	3-Hydroxypyruvic acid-meto-2TMS(1)
20	Oxalic acid-2TMS	70	Isoleucine-d10-2TMS
21	Oxalic acid-13C2-2TMS	71	Isoleucine-2TMS
22	Sarcosine-d3-2TMS	72	Acetylglycine-TMS
23	Sarcosine-2TMS	73	Proline-2TMS
24	Acetoacetic acid-meto-TMS	74	4-Aminobutyric acid-2TMS
25	3-Hydroxypropionic acid-2TMS	75	Glycine-3TMS
26	2-Aminoisobutyric acid-2TMS	76	Maleic acid-2TMS
27	Pyruvic acid-oxime-2TMS	77	Nicotinic acid-TMS
28	Valproic acid-TMS	78	Phenylacetic acid-TMS
29	3-Hydroxyisobutyric acid-2TMS	79	Succinic acid-2TMS
30	2-Hydroxyisovaleric acid-2TMS	80	Glyceric acid-3TMS
31	3-Hydroxybutyric acid-2TMS	81	Catechol-2TMS
32	2-Aminobutyric acid-2TMS	82	Methylsuccinic acid-2TMS
33	2-Ketoisocaproic acid-meto-TMS(1)	83	3-Hydroxypyruvic acid-meto-2TMS(2)
34	3-Methyl-2-oxovaleric acid-meto-TMS(1)	84	Uracil-2TMS
35	Isoleucine-TMS	85	Fumaric acid-13C4-2TMS
36	2-Methyl-3-hydroxybutyric acid-2TMS(1)	86	Serine-3TMS
37	3-Aminopropanoic acid-2TMS	87	Fumaric acid-2TMS
38	2-Methyl-3-hydroxybutyric acid-2TMS(2)	88	Homoserine-2TMS
39	3-Methyl-2-oxovaleric acid-meto-TMS(2)	89	Nonanoic acid-TMS
40	Glyceraldehyde-meto-2TMS(1)	90	Acetylglycine-2TMS
41	Malonic acid-2TMS	91	2-Propyl-3-hydroxy-pentanoic acid-2TMS(1)
42	2-Keto-isovaleric acid-oxime-2TMS(1)	92	Threonine-3TMS
43	Acetoacetic acid-2TMS(1)	93	2-Propyl-3-hydroxy-pentanoic acid-2TMS(2)
44	Valine-d8-2TMS	94	O-Acetylserine-2TMS
45	3-Aminoisobutyric acid-2TMS	95	Mevalonic lactone-TMS
46	3-Hydroxyisovaleric acid-2TMS	96	2-Aminooctanoic acid-TMS
47	2-Keto-isovaleric acid-oxime-2TMS(2)	97	Mesaconic acid-2TMS
48	Valine-2TMS	98	5-Aminovaleric acid-2TMS
49	Methylmalonic acid-2TMS	99	Thymine-2TMS
50	2-Ketoisocaproic acid-meto-TMS(2)	100	Isobutyrylglycine-TMS

Appendix 8 Registered Compound Trimethylsilylated Metabolites GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
101	Glutaric acid-2TMS	151	3-Hydroxyglutaric acid-3TMS
102	Hydroquinone-2TMS	152	Succinylacetone-meto-TMS(1)
103	Isobutyrylglycine-2TMS	153	3-Methylcrotonoylglycine-2TMS
104	3-Methylglutaric acid-2TMS	154	2-Isopropylmalic acid-3TMS
105	3-Aminopropanoic acid-3TMS	155	O-Phosphoethanolamine-3TMS
106	Homoserine-3TMS	156	2-Ketoglutaric acid-meto-2TMS
107	Glutaconic acid-2TMS	157	3-Methylcrotonoylglycine-TMS
108	Erythrulose-meto-3TMS(1)	158	Succinylacetone-meto-TMS(2)
109	3-Aminoisobutyric acid-3TMS	159	Tiglylglycine-TMS
110	N-Butyrylglycine-TMS	160	3-Phenyllactic acid-2TMS
111	Decanoic acid-TMS	161	Succinylacetone-meto-TMS(3)
112	Oxalacetic acid-meto-2TMS	162	Tropic acid-2TMS
113	Erythrulose-meto-3TMS(2)	163	Phosphoenolpyruvic acid-3TMS
114	Citramalic acid-3TMS	164	3-Hydroxy-3-methylglutaric acid-3TMS
115	2-Propyl-5-hydroxy-pentanoic acid-2TMS	165	Succinylacetone-meto-TMS(4)
116	Malic acid-13C4-3TMS	166	Phenylpyruvic acid-meto-TMS(2)
117	Threitol-4TMS	167	2-Ketoglutaric acid-3TMS
118	Dihydrouracil-TMS	168	Pimelic acid-2TMS
119	Malic acid-3TMS	169	Hypotaurine-3TMS
120	N-Butyrylglycine-2TMS	170	3-Aminoglutaric acid-3TMS
121	2-Aminooctanoic acid-2TMS	171	Ornithine-3TMS
122	meso-Erythritol-4TMS	172	Glutamic acid-13C5-3TMS
123	Dihydrouracil-2TMS	173	Asparagine-4TMS
124	Isovalerylglycine-TMS	174	Glutamic acid-3TMS
125	Niacinamide-TMS	175	Triethanolamine-3TMS
126	Adipic acid-2TMS	176	Cadaverine-3TMS
127	N-Acetylserine-2TMS	177	3-Hydroxyphenylacetic acid-2TMS
128	Isovalerylglycine-2TMS	178	2-Ketoglutaric acid-oxime-3TMS(1)
129	Glutamic acid 5-methylester-2TMS	179	Tartaric acid-4TMS
130	Anthranilic acid-TMS	180	Anthranilic acid-2TMS
131	Aspartic acid-d3-3TMS	181	5-Aminovaleric acid-3TMS
132	Aspartic acid-3TMS	182	Lyxose-meto-4TMS(1)
133	3-Aminoglutaric acid-2TMS	183	Xylose-meto-4TMS(1)
134	4-Hydroxyproline-3TMS	184	2-Ketoadipic acid-meto-2TMS(1)
135	Methionine-2TMS	185	Phenylalanine-2TMS
136	4-Aminobutyric acid-3TMS	186	Ureidopropionic acid-3TMS
137	5-Oxoproline-2TMS	187	Xylose-meto-4TMS(2)
138	Cytosine-2TMS	188	4-Hydroxybenzoic acid-2TMS
139	3-Methyladipic acid-2TMS	189	4-Hydroxybenzoic acid-13C6-2TMS
140	Thiodiglycolic acid-2TMS	190	2-Ketoglutaric acid-oxime-3TMS(2)
141	Threonic acid-4TMS	191	Lyxose-meto-4TMS(2)
142	Pyrogallol-3TMS	192	Arabinose-meto-4TMS
143	Phenylpyruvic acid-meto-TMS(1)	193	Threo-b-hydroxyaspartic acid-4TMS
144	2-Propyl-glutaric acid-2TMS	194	Hexanoylglycine-2TMS
145	Oxalacetic acid-3TMS	195	4-Hydroxyphenylacetic acid-2TMS
146	Cysteine-3TMS	196	Xylulose-meto-4TMS
147	Tiglylglycine-2TMS	197	Hexanoylglycine-TMS
148	5-Hydroxymethyl-2-furoic acid-2TMS	198	Lauric acid-d3-TMS
149	Creatinine-3TMS	199	Ribose-13C5-meto-4TMS
150	2-Hydroxyglutaric acid-3TMS	200	Ribulose-meto-4TMS

Appendix 8 Registered Compound Trimethylsilylated Metabolites GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
201	Lauric acid-TMS	251	Homovanillic acid-2TMS
202	Ribose-meto-4TMS	252	5-Aminolevulinic acid-meto-3TMS(2)
203	N-Acetylaspartic acid-2TMS	253	3-Dehydroshikimic acid-meto-3TMS(2)
204	2-Ketoadipic acid-meto-2TMS(2)	254	3-Phosphoglyceric acid-4TMS
205	N-Acetylaspartic acid-3TMS	255	Shikimic acid-4TMS
206	Homocysteine-3TMS	256	Azelaic acid-2TMS
207	Asparagine-3TMS	257	Citric acid-d4-4TMS
208	Taurine-13C2-3TMS	258	Ornithine-d7-4TMS
209	Taurine-3TMS	259	Glycyl-Glycine-4TMS
210	3-Sulfinoalanine-3TMS	260	Isocitric acid-4TMS
211	Ribonolactone-3TMS	261	2-Aminopimelic acid-3TMS
212	4-Aminobenzoic acid-TMS	262	Citric acid-4TMS
213	Ureidopropionic acid-2TMS	263	Ornithine-4TMS
214	Xylitol-5TMS	264	Hippuric acid-2TMS
215	Arabitol-5TMS	265	Protocatechuic acid-3TMS
216	Rhamnose-meto-4TMS(1)	266	Arginine-3TMS
217	Glyceraldehyde 3-phosphate-meto-3TMS(1)	267	Histidinol-2TMS
218	Lysine-3TMS	268	Tagatose-meto-5TMS(1)
219	Ribitol-5TMS	269	O-Phospho-Serine-4TMS
220	Suberic acid-2TMS	270	Dopamine-3TMS
221	1,6-Anhydroglucose-3TMS	271	Hypoxanthine-2TMS
222	2-Ketoadipic acid-oxime-3TMS(1)	272	Glycyl-Glycine-3TMS
223	Glycerol 2-phosphate-4TMS	273	Psicose-meto-5TMS(1)
224	Rhamnose-meto-4TMS(2)	274	Homogentisic acid-3TMS
225	Fucose-meto-4TMS(1)	275	Cadaverine-4TMS
226	2-Aminoadipic acid-3TMS	276	Epinephrine-3TMS
227	2-Deoxy-glucose-4TMS(1)	277	Psicose-meto-5TMS(2)
228	Glyceraldehyde 3-phosphate-meto-3TMS(2)	278	1,5-Anhydro-glucitol-4TMS
229	Glutamine-4TMS	279	Tagatose-meto-5TMS(2)
230	2-Ketoadipic acid-oxime-3TMS(2)	280	Methionine sulfone-2TMS
231	Fucose-meto-4TMS(2)	281	Sorbose-meto-5TMS(1)
232	Cysteic acid-3TMS	282	2-Methylhippuric acid-2TMS
233	Putrescine-4TMS	283	Fructose-meto-5TMS(1)
234	Dihydroxyacetone phosphate-meto-3TMS(1)	284	Pyridoxal-meto-2TMS(1)
235	Ribonic acid-5TMS	285	Sorbose-meto-5TMS(2)
236	Quinolinic acid-2TMS	286	4-Aminobenzoic acid-2TMS
237	Aconitic acid-3TMS	287	Histidinol-3TMS
238	Orotic acid-3TMS	288	Galactosamine-5TMS(1)
239	Glycerol 3-phosphate-4TMS	289	Allose-meto-5TMS(1)
240	Dihydroxyacetone phosphate-meto-3TMS(2)	290	Myristic acid-TMS
241	5-Aminolevulinic acid-meto-3TMS(1)	291	Fructose-meto-5TMS(2)
242	2-Phosphoglyceric acid -4TMS	292	Mannose-meto-5TMS(1)
243	3-Hydroxyanthranilic acid-2TMS	293	Galactose-meto-5TMS(1)
244	Glutamine-3TMS	294	3-Hydroxyanthranilic acid-3TMS
245	2-Deoxy-glucose-meto-4TMS	295	5-Dehydroquinic acid-meto-4TMS
246	3-Methoxy-4-hydroxybenzoic acid-2TMS	296	Vanilmandelic acid-3TMS
247	O-Phosphoethanolamine-4TMS	297	Glucono-1,5-lactone-4TMS
248	Dihydroorotic acid-3TMS	298	Glucose-meto-5TMS(1)
249	3-Dehydroshikimic acid-meto-3TMS(1)	299	Hippuric acid-TMS
250	2-Deoxy-glucose-4TMS(2)	300	Mannose-meto-5TMS(2)

Appendix 8 Registered Compound Trimethylsilylated Metabolites GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
301	Allantoin-4TMS	351	Coniferyl aldehyde-meto-TMS(2)
302	Allose-meto-5TMS(2)	352	Tryptamine-2TMS
303	Vanillylamine-3TMS	353	Indol-3-acetic acid-2TMS
304	Erythrose 4-phosphate-meto-4TMS(1)	354	Pantothenic acid-3TMS
305	Pyridoxal-meto-2TMS(2)	355	Lipoic acid-TMS
306	Histamine-3TMS	356	Glucaric acid-6TMS
307	N6-Acetyllysine-3TMS	357	ParaXanthine-TMS
308	Erythrose 4-phosphate-meto-4TMS(2)	358	S-Benzyl-Cysteine-4TMS
309	Galactose-meto-5TMS(2)	359	Octopamine-4TMS
310	Adenine-2TMS	360	Xanthine-3TMS
311	N-Acetyl-Lysine-2TMS	361	Urocanic acid-2TMS
312	Glucose-meto-5TMS(2)	362	Palmitoleic acid-TMS
313	4-Hydroxyphenyllactic acid-3TMS	363	Inositol-6TMS(1)
314	Pyridoxine-3TMS	364	Palmitic acid-TMS
315	5-Dehydroquinic acid-5TMS	365	N-Acetylmannosamine-meto-4TMS(1)
316	Glucosamine-5TMS(1)	366	Inositol-6TMS(2)
317	Sebacic acid-2TMS	367	Dopamine-4TMS
318	Mannitol-6TMS	368	Ribulose 5-phosphate-meto-5TMS(1)
319	N-Acetylglutamine-3TMS	369	Ribose 5-phosphate-meto-5TMS(1)
320	Sorbitol-6TMS	370	N-Acetylmannosamine-meto-4TMS(2)
321	Galactitol-6TMS	371	Ribose 5-phosphate-meto-5TMS(2)
322	Lysine-4TMS	372	Allantoin-3TMS
323	4-Hydroxyphenylpyruvic acid-meto-2TMS	373	Dopa-13C6-4TMS
324	Glucuronic acid-meto-5TMS(1)	374	Dopa-4TMS
325	Glucono-1,4-lactone-4TMS	375	Ribulose 5-phosphate-meto-5TMS(2)
326	2-Methylhippuric acid-TMS	376	2-Hydroxyhippuric acid-2TMS
327	Tyramine-3TMS	377	Dodecanedioic acid-2TMS
328	Galacturonic acid-meto-5TMS(1)	378	N-Acetyltyrosine-3TMS
329	Histidine-3TMS	379	Kynurenic acid-2TMS
330	Glucosamine-5TMS(2)	380	Uric acid-4TMS
331	3,4-Dihydroxybenzylamine-4TMS	381	Metoprolol-TMS
332	Urocanic acid-TMS	382	Pyridoxamine-4TMS
333	Glucuronic acid-meto-5TMS(2)	383	Citrulline-3TMS
334	Ureidosuccinic acid-3TMS	384	Methoprene acid-TMS
335	Galactosamine-5TMS(2)	385	Guanine-3TMS
336	Tyrosine-13C6-3TMS	386	Norepinephrine-5TMS
337	Ascorbic acid-4TMS	387	N-Acetyltyrosine-2TMS
338	Tyrosine-3TMS	388	Margaric acid-TMS
339	Pyridoxamine-3TMS	389	Kynurenine-2TMS
340	Galacturonic acid-meto-5TMS(2)	390	Octadecanol-TMS
341	N-Acetylglutamine-2TMS	391	2,3-Bisphosphoglyceric acid-5TMS
342	N6-Acetyllysine-2TMS	392	Kynurenine-d6-3TMS
343	Epinephrine-4TMS	393	Kynurenine-3TMS
344	Coniferyl alcohol-2TMS	394	Cystathionine-4TMS
345	2-Hydroxyhippuric acid-3TMS	395	Cystamine-d8-nTMS
346	Indol-3-acetic acid-TMS	396	Cystamine-nTMS
347	1-Hexadecanol-TMS	397	7-Methylguanine-2TMS
348	N-Acetyl-Ornithine-4TMS	398	Linoleic acid-TMS
349	Coniferyl aldehyde-meto-TMS(1)	399	Oleic acid-TMS
350	Gluconic acid-6TMS	400	Tryptophan-3TMS

Appendix 8 Registered Compound Trimethylsilylated Metabolites GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
401	Suberylglycine-3TMS	451	Xanthosine-5TMS
402	Tryptophan-13C11-3TMS	452	Batyl alcohol-2TMS
403	Elaidic acid-TMS	453	Maltose-meto-8TMS(1)
404	Spermidine-5TMS	454	Trehalose-8TMS
405	Tryptamine-3TMS	455	Cytidine-4TMS
406	Stearic acid-TMS	456	Lactitol-9TMS
407	Fructose 1-phosphate-meto-6TMS(1)	457	Maltose-meto-8TMS(2)
408	Fructose 1-phosphate-meto-6TMS(2)	458	Spermine-6TMS
409	Fructose 6-phosphate-meto-6TMS	459	Carnosine-4TMS
410	Mannose 6-phosphate-meto-6TMS(1)	460	Guanosine-5TMS
411	Suberylglycine-2TMS	461	Monostearin-2TMS
412	Glucose 6-phosphate-meto-6TMS(1)	462	Maltitol-9TMS
413	Cystine-4TMS	463	5'-Methylthioadenosine-2TMS
414	Mannose 6-phosphate-meto-6TMS(2)	464	5'-Methylthioadenosine-3TMS
415	Metoprolol-2TMS	465	Isomaltose-meto-8TMS(1)
416	Glucose 6-phosphate-meto-6TMS(2)	466	Uridine monophosphate-5TMS
417	3-Hydroxy-kynurenine-3TMS	467	Isomaltose-meto-8TMS(2)
418	2'-Deoxyuridine-2TMS	468	Thymidine monophosphate-3TMS
419	Arachidonic acid-TMS	469	Inosine monophosphate-5TMS
420	6-Phosphogluconic acid-7TMS	470	Xanthosine monophosphate-6TMS
421	Juniperic acid-2TMS	471	Cholecalciferol-TMS
422	Eicosapentaenoic acid-TMS	472	Adenosine monophosphate-5TMS
423	Porphobilinogen-4TMS	473	Trehalose 6-phosphate-9TMS
424	Inositol phosphate-7TMS	474	Adenosine 3',5'-cyclic monophosphate-3TMS
425	Thymidine-2TMS	475	Cholesterol-TMS
426	5-Hydroxy-tryptophan-4TMS	476	Hydrocinnamic acid-TMS
427	Oleamide-TMS	477	Serotonin-4TMS
428	Uridine-4TMS	478	Serotonin-3TMS
429	5-Methoxytryptamine-3TMS	479	beta-Sitosterol-TMS
430	2'-Deoxyuridine-3TMS	480	Campesterol-TMS
431	Saccharopine-4TMS	481	Catechin-5TMS
432	Uridine-3TMS	482	Cholesta-3,5-diene
433	p-Aminohippuric acid-2TMS	483	4-Coumaric acid-2TMS
434	5-Methoxytryptamine-2TMS	484	Cycloartenol-TMS
435	Thymidine-3TMS	485	Daidzein-2TMS
436	Biotin-3TMS	486	Epicatechin-5TMS
437	Melatonin-TMS	487	Epigallocatechin-6TMS
438	Sedoheptulose 7-phosphate-meto-7TMS	488	Ferulic acid-2TMS
439	Saccharopine-5TMS	489	Gallic acid-4TMS
440	Homocystine-4TMS	490	Genistein-3TMS
441	Inosine-4TMS	491	Genistein-2TMS
442	Fendiline	492	Quinic acid-5TMS
443	Docosahexaenoic acid-TMS	493	Raffinose-nTMS
444	Sucrose-8TMS	494	Salicylic acid-2TMS
445	N-Acetylneuraminic acid-6TMS	495	Sinapinic acid-2TMS
446	Docosapentaenoic acid-TMS	496	Stigmasterol-TMS
447	Adenosine-4TMS	497	Theanine-2TMS
448	Lactose-meto-8TMS(1)	498	2-Deoxy-ribose-meto-4TMS
449	Fendiline-TMS	499	alpha-Tocopherol-TMS
450	Lactose-meto-8TMS(2)	500	Caffeic acid-3TMS

ID	Compound Name
501	Caffeine
502	Cellobiose-meto-8TMS(1)
503	Cellobiose-meto-8TMS(2)
504	Chlorogenic acid-6TMS
505	Cinnamyl alcohol-TMS
506	Galactinol-9TMS
507	Gastrodin-5TMS
508	Isoascorbic acid-4TMS
509	Itaconic acid-2TMS
510	N-Acetylglucosamine-meto-4TMS
511	N-Acetylglucosamine-4TMS
512	Phytol-TMS
513	Pinitol-5TMS
514	Pipecolic acid-TMS
515	Pipecolic acid-2TMS
516	N-Acetyl-glutamic acid-2TMS
517	N-Acetyl-glutamic acid-3TMS
518	2,3-Butanediol-2TMS(1)
519	2,3-Butanediol-2TMS(2)
520	2-(Methylamino)ethanol-2TMS
521	2-Aminopropanol-2TMS
522	1-Butylamine-2TMS
523	Isobutylamine-2TMS
524	1-Propylamine-2TMS
525	Hydrocaffeic acid-3TMS
526	Glucose-13C6-meto-5TMS(1)
527	Glucose-13C6-meto-5TMS(2)
528	Margaric acid-d33-TMS
529	Glyoxylic acid-13C2-meto-TMS
530	Palmitic acid-13C16-TMS
531	3-Aminopropanoic acid-13C3-15N-3TMS
532	3-Hydroxyisovaleric acid-d8-2TMS
533	Succinic acid-13C4-2TMS
534	Histidine-13C6-3TMS
535	1,5-Anhydro-glucitol-13C6-4TMS
536	Glutamine-13C5-3TMS
537	Margaric acid-methyl-d3-TMS
538	Alanine-13C3-2TMS
539	2-Ketoglutaric acid-13C4-meto-2TMS
540	Arabinose-13C5-meto-4TMS

Appendix9 Registered Compound Fatty Acid Methyl Esters GC-MS(Scan), GC-MS/MS(MRM)

ID	Compound Name	ID	Compound Name
1	Methyl butanoate;4:0	40	Methyl behenate;22:0
2	Methyl caproate;6:0	41	Methyl tricosanoate;23:0
3	Methyl caprylate;8:0	42	Methyl nervonate;(Z)24:1n-9
4	Methyl caprate;10:0	43	Methyl lignocerate;24:0
5	Methyl undecanoate;11:0	44	Methyl pentacosanoate;25:0
6	Methyl laurate;12:0	45	Methyl cerotate;26:0
7	Methyl tridecanoate;13:0	46	Methyl heptacosanoate;27:0
8	Methyl myristoleate;(Z)14:1n-5	47	Methyl octacosanoate;28:0
9	Methyl myristate;14:0	48	Methyl nonacosanonate;29:0
10	Methyl cis-10-pentadecenoate;(Z)15:1n-5	49	Methyl melissate;30:0
11	Methyl pentadecanoate;15:0	50	Methyl hentriacontanoate;31:0
12	Methyl palmitoleate;(Z)16:1n-7		
13	Methyl palmitate;16:0		
14	Methyl cis-10-heptadecenoate;(Z)17:1n-7		
15	Methyl margarate;17:0		
16	Methyl ganma-linolenate;(Z)18:3n-6		
17	Methyl linoleate;(Z)18:2n-6		
18	Methyl cis-7-octadecenoate;(Z)18:1n-11		
19	Methyl linolenate;(Z)18:3n-3		
20	Methyl petroselate;(Z)18:1n-12		
21	Methyl oleate;(Z)18:1n-9		
22	Methyl linolelaidate;(E)18:2n-6		
23	Methyl cis-vaccenate;(Z)18:1n-7		
24	Methyl elaidate;(E)18:1n-9		
25	Methyl cis-12-Octadecenoate;(Z)18:1n-6		
26	Methyl stearate;18:0		
27	Methyl nonadecanoate;19:0		
28	Methyl arachidonate;(Z)20:4n-6		
29	Methyl cis-5,8,11,14,17-Eicosapentaenoate;(Z)20:5n-3		
30	Methyl eicosa-8,11,14-trienoate;20:3n-6		
31	Methyl cis-11,14-Icosadienoate;(Z)20:2n-6		
32	Methyl cis-11-icosenoate;(Z)20:1n-9		
33	Methyl cis-11,14,17-Icosatrienoate;(Z)20:3n-3		
34	Methyl arachisate;20:0		
35	Methyl heneicosanoate;21:0		
36	Methyl cis-4,7,10,13,16,19-Docosahexaenoate;(Z)22:6n-3		
37	Methyl cis-7,10,13,16,19-docosapentaenoate;(Z)22:5n-3		
38	Methyl cis-13,16-Docosadienate;(Z)22:2n-6		
39	Methyl erucate;22:1n-9		

Appendix10 Registered Compound Acetylated sugars GC-MS(SIM) GC-MS/MS(MRM)

ID	Compound Name
1	meso-Erythritol-AC
2	2-Deoxy-D-ribose-AC
3	Xylose-AC
4	Rhamnose-AC
5	Fucose-AC
6	Arabinose-AC
7	Ribose-AC
8	Ribitol-AC
9	Arabitol-AC
10	Xylitol-AC
11	Fructose-AC
12	Galactose-AC
13	Sorbose-AC
14	Mannose-AC
15	Glucose-AC
16	myo-Inositol-AC
17	Mannitol-AC
18	Sorbitol-AC
19	Galactitol-AC
20	Sucrose-AC
21	Trehalose-AC
22	Maltose-AC (1)
23	Maltose-AC (2)
24	Lactose-AC

Appendix11 Database Installation

Follow the steps below to install the database. The same steps are followed to perform the installation when using the upgrade version of the installer.

The explanations given here assume use of Windows 10.

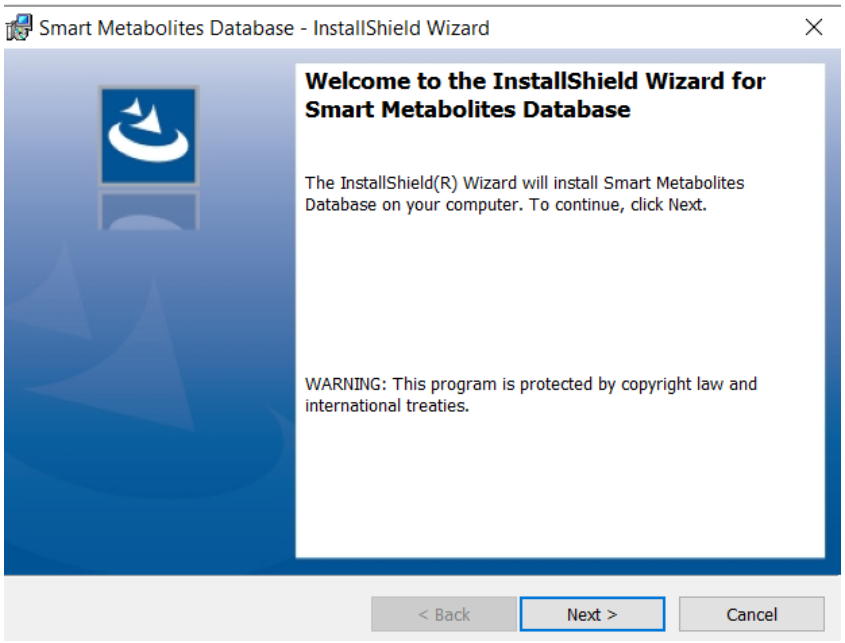
NOTE

To install the database, GCMSsolution needs to be installed in advance.

NOTE

When using the upgrade version of the installer, make sure that the GC/MS Metabolite Database or Smart Metabolites Database is installed beforehand.

1) Insert the database installation disk into the CD-ROM drive. The installer starts up automatically.



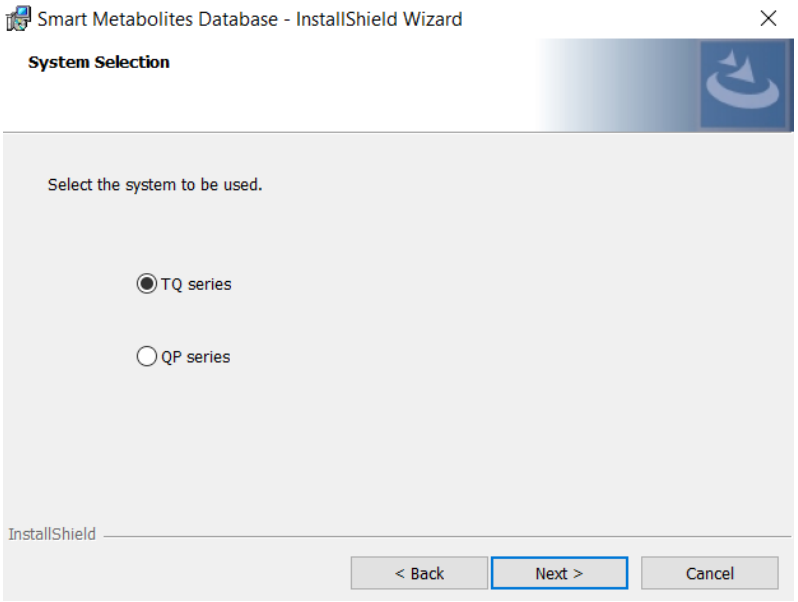
NOTE

If it does not, click the Windows Start menu. Enter "E:\setup.exe," and press the [Enter] key. (where E: is the CD-ROM drive)

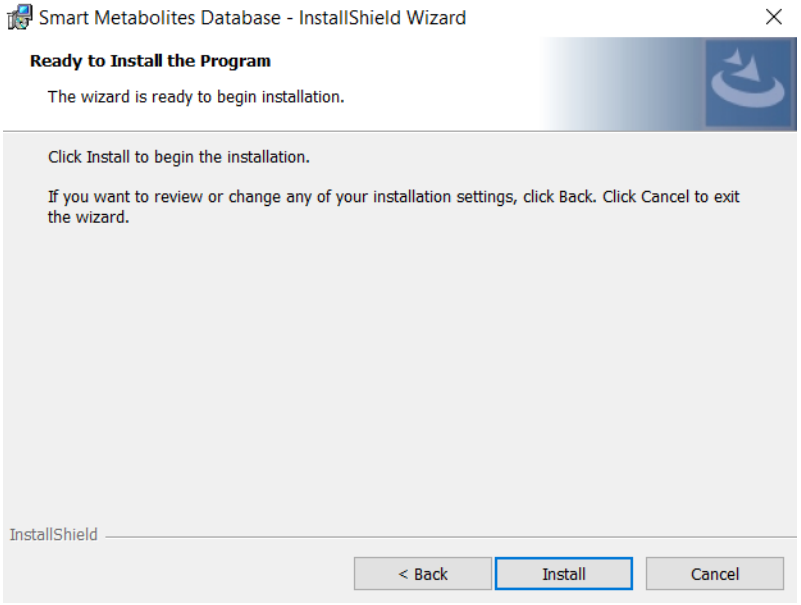
NOTE

The dialog box "Do you want to allow the following program to make changes to this computer?" may be displayed. In this case, click the [Yes] button to proceed.

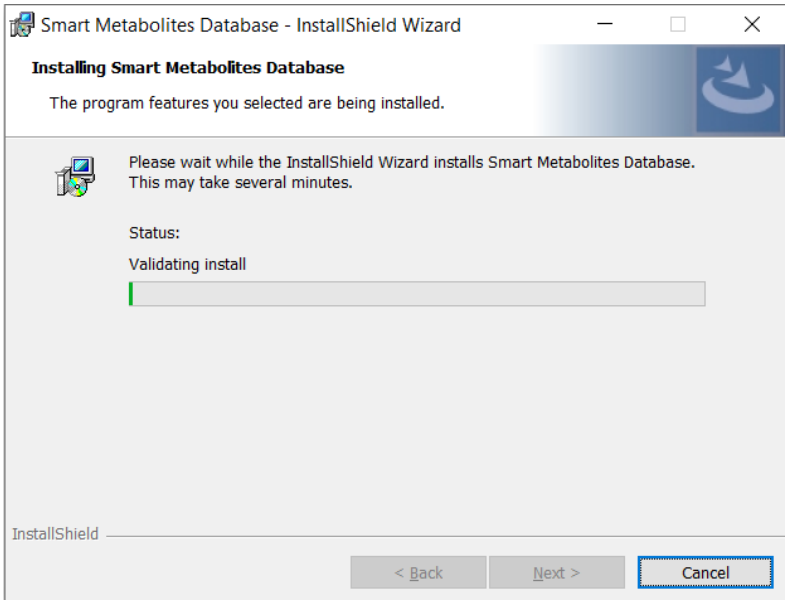
2) Select the system to be used in the [System Selection] window and click the [Next >] button.



3) Click [Next >] in the [Wellcome] window. The window will be replaced by the [Ready to Install the Program] window.

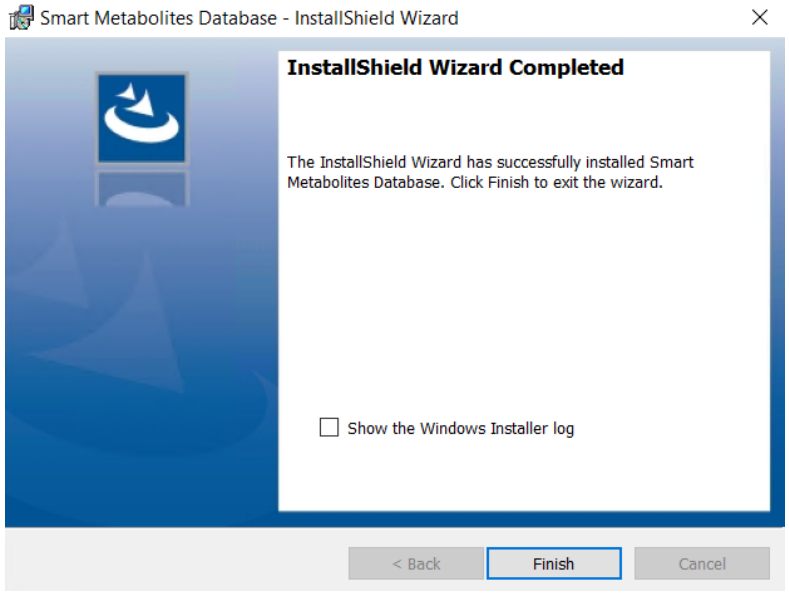


4) Click [Install] in the [Ready to Install the Program] window. The database files are installed in the designated locations.



Normally, the method file and database file are installed in the "C:\GCMSsolution\SmartDatabase" folder.

5) When installation completes, the [InstallShield Wizard Completed] window opens. Click [Finish] to complete the installation.



Appendix12 Changes in Analysis Conditions from Smart Metabolites Database

Concerning the below-listed analysis methods, certain portions of the default settings for the analysis conditions in the Smart Metabolites Database have been changed. Please take note of these differences when transitioning from Smart Metabolites Database.

File Name (.qgm)	Corresponding file name in Smart Metabolites Database	Changes
OA_TMS_DB5_37min_V4_MRM	OA_TMS_DB5_37min_V3_MRM	Detector voltage Changed the relative value from the tuning result to 0.3 kV
OA_TMS_DB5_67min_V4_MRM	OA_TMS_DB5_67min_ V3_MRM	
OA_TMS_BPX5_23min_V4_MRM	OA_TMS_BPX5_23min_ V3_MRM	

Appendix13 Calculation of Area Ratios Using a Surrogate Internal Standard

The results measured under conditions in which the analysis environment such as GCMS instrument and columns are different will naturally produce different area values. With the Surrogate IS_OA_BPX method, when human blood plasma is analyzed under different analysis environment conditions, surrogate internal standards are registered as an IS group that show the same variation tendency as each metabolite. By adding a surrogate internal standard registered for blood plasma for pretreatment and analyzing using this method, it is possible to perform analysis with high reproducibility even when the analysis environment is different.

NOTE

This method has only been validated with human blood plasma samples.
The extraction efficiency is not taken into consideration in the pretreatment, so there is a possibility that the results will differ due to the pretreatment conditions used.
The area ratios calculated are only approximate, and compared with the correction method using compounds labeled with stable isotopes as the target compounds, the reproducibility is inferior. Therefore, to obtain a more accurate area ratios, consider separately using stable isotope reagents of the target compounds.

Also, in the compounds detected when analyzing human blood plasma, compounds with low detection sensitivity and low reproducibility are excluded from the measurement.

Hint

The following is used as the surrogate internal standard used for sensitivity correction. Note that the reagents listed here are those as of Febrary 2022, and in the future, these could change or be discontinued without prior notice.

Compound Name	Reagent Name	P/N	Manufacturer
Glyoxylic acid-13C2-meto-TMS	GLYOXYLIC ACID:H2O (13C2, 99%)	CLM-6027-PK	Cambridge Isotope Laboratories, Inc (CIL)
Lactic acid-13C3-2TMS	SODIUM L-LACTATE (13C3, 98%) 20% W/W IN H2O	CLM-1579-PK	Cambridge Isotope Laboratories, Inc (CIL)
2-Hydroxybutyric acid-d3-2TMS	Sodium (±)-2-Hydroxybutyrate-2,3,3-d3	D-7002	C/D/N Isotopes Inc
Oxalic acid-13C2-2TMS	OXALIC ACID (1,2-13C2, 99%)	CLM-2002-PK	Cambridge Isotope Laboratories, Inc (CIL)
Isoleucine-d10-2TMS	L-ISOLEUCINE (D10, 98%)	DLM-141-PK	Cambridge Isotope Laboratories, Inc (CIL)
Fumaric acid-13C4-2TMS	FUMARIC ACID (13C4, 99%)	CLM-1529-PK	Cambridge Isotope Laboratories, Inc (CIL)
Aspartic acid-d3-3TMS	L-ASPARTIC ACID (2,3,3-D3, 98%)	DLM-546-PK	Cambridge Isotope Laboratories, Inc (CIL)
2-Isopropylmalic acid-3TMS	2-Isopropylmalic acid	333115	Sigma-Aldrich
2-Ketoglutaric acid-13C4-2TMS	ALPHA-KETOGLUTARIC ACID, DISODIUM SALT (1,2,3,4-13C4, 99%)	CLM-4442-PK	Cambridge Isotope Laboratories, Inc (CIL)
Ribose-13C5-meto-4TMS	D-RIBOSE (U-13C5, 98%)	CLM-3652-PK	Cambridge Isotope Laboratories, Inc (CIL)
Glutamine-13C5-3TMS	L-GLUTAMINE (13C5, 99%)	CLM-1822-H-PK	Cambridge Isotope Laboratories, Inc (CIL)
Citric acid-d4-4TMS	CITRIC ACID (2,2,4,4-D4, 98%)	DLM-3487-PK	Cambridge Isotope Laboratories, Inc (CIL)
Ornithine-d7-4TMS	L-ORNITHINE:HCL (D7, 98%)	DLM-6669-PK	Cambridge Isotope Laboratories, Inc (CIL)
1,5-Anhydro-D-glucitol-13C6-4TMS	1,5-ANHYDRO-D-GLUCITOL (U-13C6, 98%+)	CLM-9657-PK	Cambridge Isotope Laboratories, Inc (CIL)
Glucose-13C6-meto-5TMS(1)	D-GLUCOSE (U-13C6, 99%)	CLM-1396-PK	Cambridge Isotope Laboratories, Inc (CIL)
Glucose-13C6-meto-5TMS(2)			
Histidine-13C6-3TMS	L-HISTIDINE:HCL:H2O(<5% D) (13C6, 97-99%)	CLM-2264-PK	Cambridge Isotope Laboratories, Inc (CIL)
Tyrosine-13C6-3TMS	L-TYROSINE (RING-13C6, 99%)	CLM-1542-PK	Cambridge Isotope Laboratories, Inc (CIL)
Palmitoleic acid-13C16-TMS	PALMITOLEIC ACID (U-13C16, 98%) 97% CHEMICAL PURITY	CLM-2241-PK	Cambridge Isotope Laboratories, Inc (CIL)
Margaric acid-methyl-d3-TMS	HEPTADECANOIC ACID (METHYL-D3, 98%)	DLM-1308-PK	Cambridge Isotope Laboratories, Inc (CIL)
Tryptophan-13C11-3TMS	L-TRYPTOPHAN (13C11, 99%)	CLM-4290-H-PK	Cambridge Isotope Laboratories, Inc (CIL)

Appendix 13.1 Correction of Retention Time in the Method Files

To correct the retention time, measure the n-alkanes and correct the retention time in the method.

1) Analyze and identify the n-alkanes in accordance with "*2.1.3 Measuring n-Alkane Mixed Solution*".

2) Correct the retention time of the method file (SurrogateIS_OA_BPX) for analyzing human blood plasma in accordance with "*2.1.4 Adjusting Retention time and Analysis*".

3) Apply any name to the corrected method file, and click the [Save] button.

Appendix 13.2 Analysis of Sample and Calculation of Area Ratios

Analyze the sample using the prepared method file, and calculate the area ratio of the surrogate internal standard from the measurement results.

1) Set the sample to be measured in the sampler, and perform the analysis using the prepared method file.

2) Open the [GCMS Browser], and open the quantitation browser window.

3) Read the method file used for the analysis, and read in the measured data.

4) Right click on the table, and click [Table style] on the pop-up menu. The [Table style] window opens.

5) Select [Internal standard area] and [Area ratio] in the [Items to hide], click [Add>>], and these are moved to the [Items to display].

6) The area ratio for the surrogate internal standard for each of the compounds in the table is calculated.

NOTE

If the area ratios are not calculated even though the method and the measured data have been read in, check that the quantitation parameter [Quantittative method] in the method is [Internal standard method].