MRM-DIFF tutorial

Edited in 2014/10/06

Introduction

MRM-DIFF is a data processing tool for multiple reaction monitoring (MRM)-based differential analysis. The main target of this application is '*lipidomics*'. The MRM transition, i.e. precursor-product *m/z* pair, can be theoretically determined from *in silico* MS/MS database such as LIPID MAPS and LipidBlast. This program hunts every peaks detected by each MRM transition by means of correlation optimized warping (COW) based non-linear alignment. In addition, pooled QC (quality control) data sets will be helpful for the automatic reference file picking and the peak detection method (see manuscript). The features of MRM-DIFF are:

1. Every peaks detected by each transition are utilized
2. The identification and quantification results can be manually curated by the graphical user interface (GUI).
3. It supports all data processing steps and pooled QC data sets would be helpful its processes.

Actually, the compound identifications should be performed by means of three or four transitions for the determination of lipid class and fatty acid compositions. Such targeted analysis can be performed by our reported program, MRMPROBS (see MRMPROBS section).

MRM-DIFF has been developed as the collaborative work among RIKEN, Osaka University, and Reifycs Incorporation.

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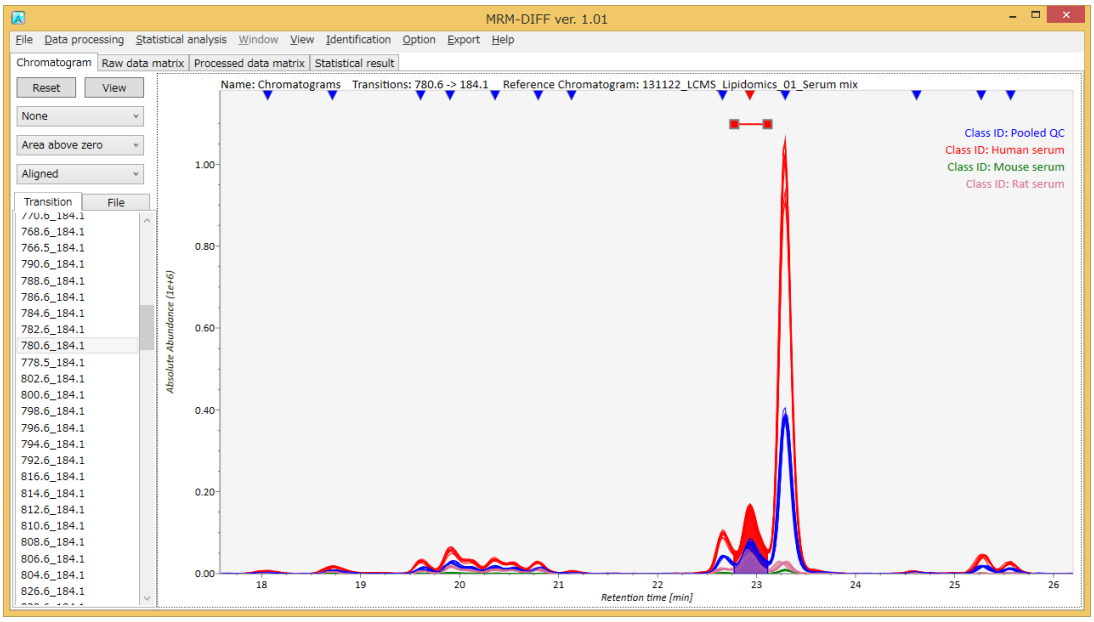


MRM-DIFF screenshot

Table of Contents

Software environments

- Microsoft Windows XP, -Vista, -7 or -8
- .NET Framework 4.0 or later

Required software programs and files

- Reifycs Analysis Base File Converter (ABF file converter)
  Download link: http://www.reifycs.com/english/AbfConverter/

- MRM-DIFF
  Download link: http://prime.psc.riken.jp/Metabolomics_Software/MRM-DIFF/index.html

- Reference library (tab-delimited text file)
  Example: http://prime.psc.riken.jp/Metabolomics_Software/MRM-DIFF/index.html

- Demonstration files
  Download link: http://prime.psc.riken.jp/Metabolomics_Software/MRM-DIFF/index.html

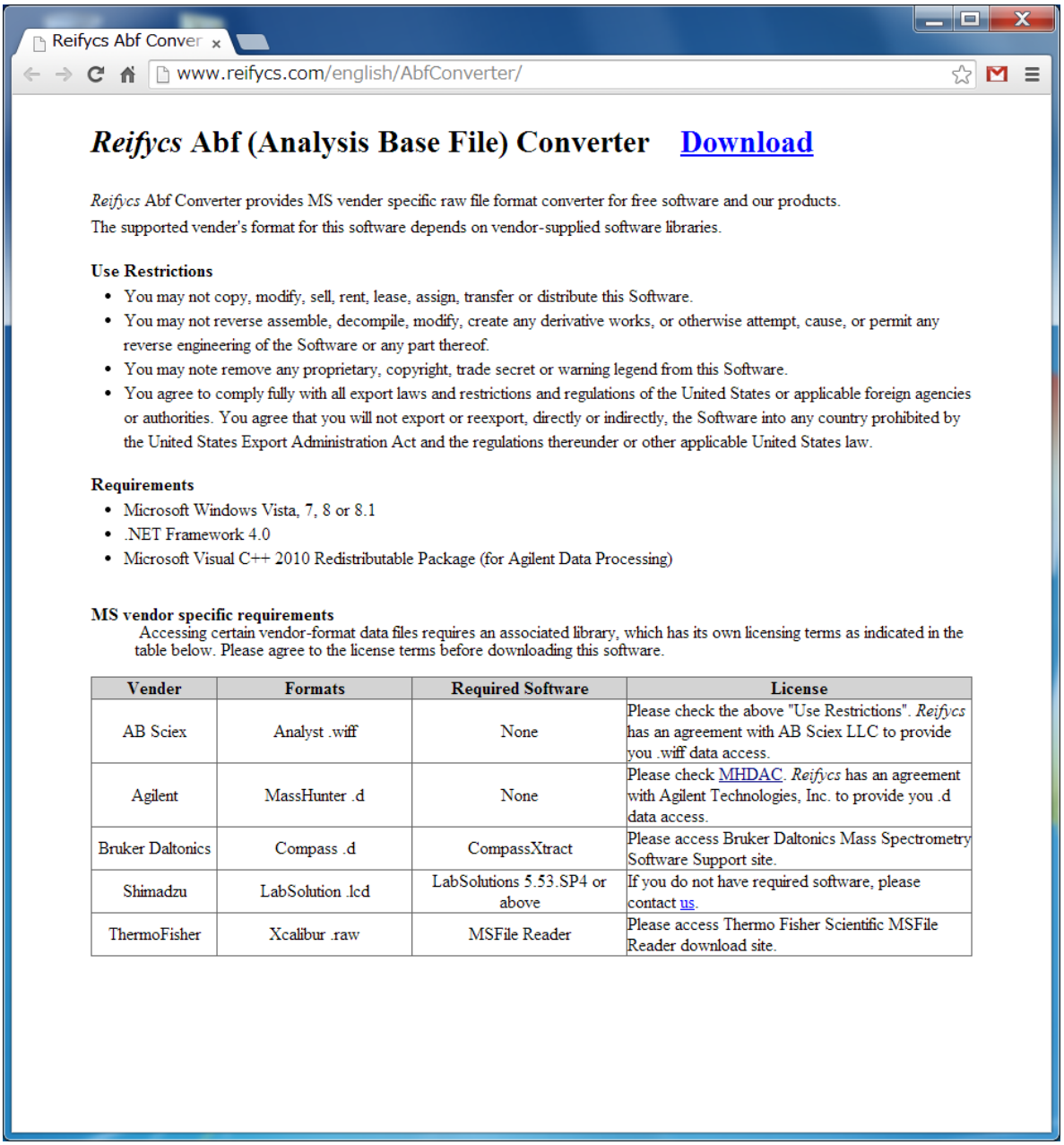
MRM-DIFF can import Analysis Base File (ABF) format data. This program extracts chromatogram data together with the reference library including the name of the target metabolite, its retention-time and amplitude information, and precursor *m/z* and product *m/z*. The supported formats for ABF conversion are Shimadzu Inc. (.LCD), Agilent Technologies (.D), AB Sciex (.WIFF), and Thermo Fisher Scientific (.RAW).

MRM-DIFF is also acceptable to mzML format file converted by an open source file translator ProteoWizard. Although our abf converter doesn't accept Waters (.RAW) file due to the license problem yet, MRM-DIFF can import Waters files via mzML.

MRM-DIFF program is implemented as a part of MRMPROBS software. Therefore, please select 'MRM-DIFF project' in the new project window.

Downloading the ABF converter from Reifycs Inc.

1. Go to http://www.reifycs.com/english/AbfConverter/.
2. Check the requirements and license terms, and download the converter.



Reifycs Abf (Analysis Base File) Converter Download

Reifycs Abf Converter provides MS vender specific raw file format converter for free software and our products.
The supported vender's format for this software depends on vendor-supplied software libraries.

Use Restrictions

- You may not copy, modify, sell, rent, lease, assign, transfer or distribute this Software.
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- You agree to comply fully with all export laws and restrictions and regulations of the United States or applicable foreign agencies or authorities. You agree that you will not export or reexport, directly or indirectly, the Software into any country prohibited by the United States Export Administration Act and the regulations thereunder or other applicable United States law.

Requirements

- Microsoft Windows Vista, 7, 8 or 8.1
- .NET Framework 4.0
- Microsoft Visual C++ 2010 Redistributable Package (for Agilent Data Processing)

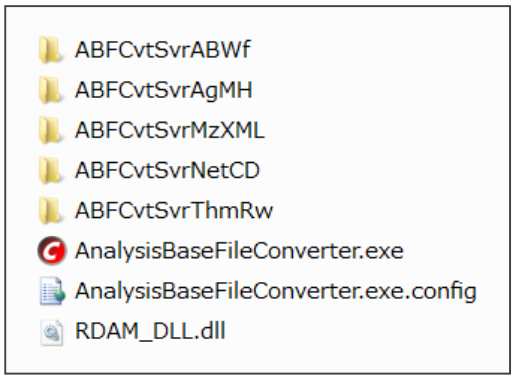
MS vendor specific requirements

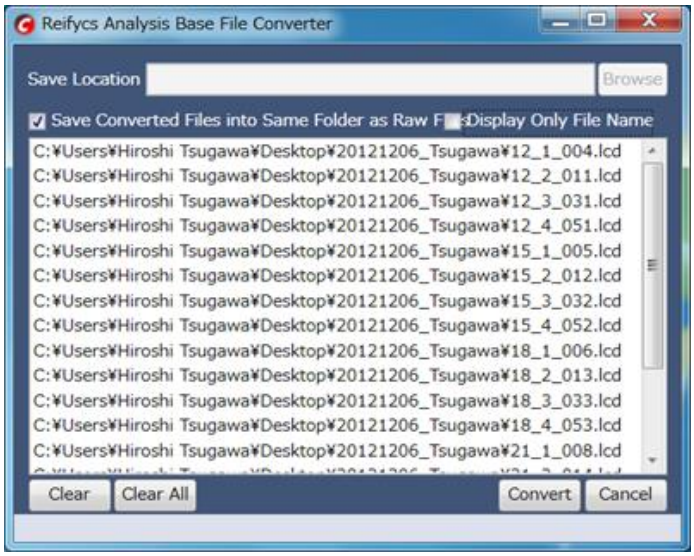
Accessing certain vendor-format data files requires an associated library, which has its own licensing terms as indicated in the table below. Please agree to the license terms before downloading this software.

Vender	Formats	Required Software	License
AB Sciex	Analyst .wiff	None	Please check the above "Use Restrictions". *Reifycs* has an agreement with AB Sciex LLC to provide you .wiff data access.
Agilent	MassHunter .d	None	Please check MHDAC. *Reifycs* has an agreement with Agilent Technologies, Inc. to provide you .d data access.
Bruker Daltonics	Compass .d	CompassXtract	Please access Bruker Daltonics Mass Spectrometry Software Support site.
Shimadzu	LabSolution .lcd	LabSolutions 5.53.SP4 or above	If you do not have required software, please contact us.
ThermoFisher	Xcalibur .raw	MSFile Reader	Please access Thermo Fisher Scientific MSFile Reader download site.

File conversion

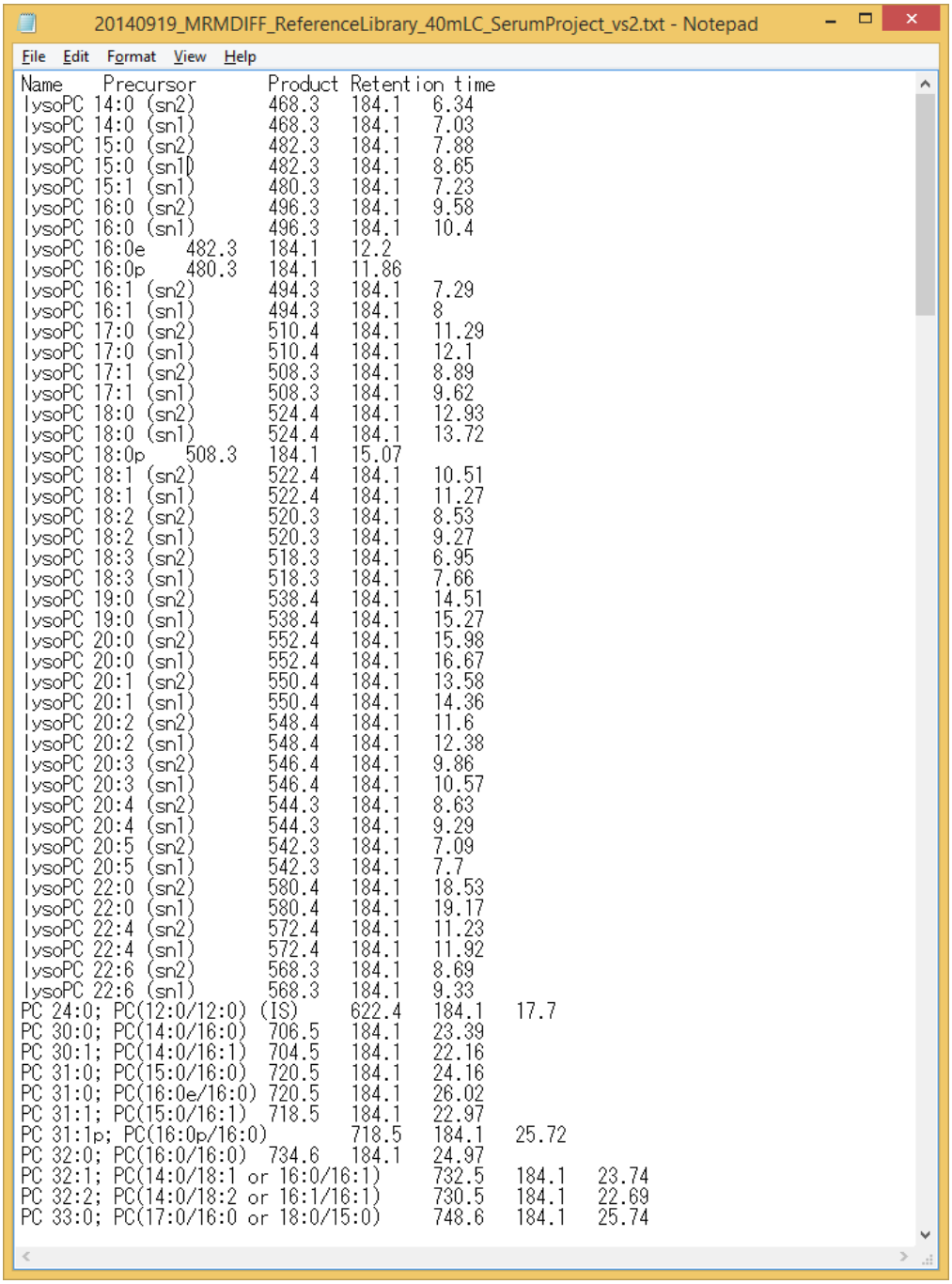
1. Start “AnalysisBaseFileConverter.exe”.
2. Drag & drop MS vendor files into this program.
3. Click “Convert”.
4. The ABF files are generated in the same directory as the raw data files.





Reference library for MRMPORBS program (tab-delimited text format)

Four items are required in the library file in tab-delimited format. The first header's name is flexible but the item order should be followed.



20140919_MRMDIFF_ReferenceLibrary_40mLC_SerumProject_vs2.txt - Notepad

File Edit Format View Help

Name	Precursor	Product	Retention time	
lysoPC 14:0 (sn2)		468.3	184.1	6.34
lysoPC 14:0 (sn1)		468.3	184.1	7.03
lysoPC 15:0 (sn2)		482.3	184.1	7.88
lysoPC 15:0 (sn1)		482.3	184.1	8.65
lysoPC 15:1 (sn1)		480.3	184.1	7.23
lysoPC 16:0 (sn2)		496.3	184.1	9.58
lysoPC 16:0 (sn1)		496.3	184.1	10.4
lysoPC 16:0e	482.3	184.1	12.2	
lysoPC 16:0p	480.3	184.1	11.86	
lysoPC 16:1 (sn2)		494.3	184.1	7.29
lysoPC 16:1 (sn1)		494.3	184.1	8
lysoPC 17:0 (sn2)		510.4	184.1	11.29
lysoPC 17:0 (sn1)		510.4	184.1	12.1
lysoPC 17:1 (sn2)		508.3	184.1	8.89
lysoPC 17:1 (sn1)		508.3	184.1	9.62
lysoPC 18:0 (sn2)		524.4	184.1	12.93
lysoPC 18:0 (sn1)		524.4	184.1	13.72
lysoPC 18:0p	508.3	184.1	15.07	
lysoPC 18:1 (sn2)		522.4	184.1	10.51
lysoPC 18:1 (sn1)		522.4	184.1	11.27
lysoPC 18:2 (sn2)		520.3	184.1	8.53
lysoPC 18:2 (sn1)		520.3	184.1	9.27
lysoPC 18:3 (sn2)		518.3	184.1	6.95
lysoPC 18:3 (sn1)		518.3	184.1	7.66
lysoPC 19:0 (sn2)		538.4	184.1	14.51
lysoPC 19:0 (sn1)		538.4	184.1	15.27
lysoPC 20:0 (sn2)		552.4	184.1	15.98
lysoPC 20:0 (sn1)		552.4	184.1	16.67
lysoPC 20:1 (sn2)		550.4	184.1	13.58
lysoPC 20:1 (sn1)		550.4	184.1	14.36
lysoPC 20:2 (sn2)		548.4	184.1	11.6
lysoPC 20:2 (sn1)		548.4	184.1	12.38
lysoPC 20:3 (sn2)		546.4	184.1	9.86
lysoPC 20:3 (sn1)		546.4	184.1	10.57
lysoPC 20:4 (sn2)		544.3	184.1	8.63
lysoPC 20:4 (sn1)		544.3	184.1	9.29
lysoPC 20:5 (sn2)		542.3	184.1	7.09
lysoPC 20:5 (sn1)		542.3	184.1	7.7
lysoPC 22:0 (sn2)		580.4	184.1	18.53
lysoPC 22:0 (sn1)		580.4	184.1	19.17
lysoPC 22:4 (sn2)		572.4	184.1	11.23
lysoPC 22:4 (sn1)		572.4	184.1	11.92
lysoPC 22:6 (sn2)		568.3	184.1	8.69
lysoPC 22:6 (sn1)		568.3	184.1	9.33
PC 24:0; PC(12:0/12:0) (IS)		622.4	184.1	17.7
PC 30:0; PC(14:0/16:0)	706.5	184.1	23.39	
PC 30:1; PC(14:0/16:1)	704.5	184.1	22.16	
PC 31:0; PC(15:0/16:0)	720.5	184.1	24.16	
PC 31:0; PC(16:0e/16:0)	720.5	184.1	26.02	
PC 31:1; PC(15:0/16:1)	718.5	184.1	22.97	
PC 31:1p; PC(16:0p/16:0)		718.5	184.1	25.72
PC 32:0; PC(16:0/16:0)	734.6	184.1	24.97	
PC 32:1; PC(14:0/18:1 or 16:0/16:1)		732.5	184.1	23.74
PC 32:2; PC(14:0/18:2 or 16:1/16:1)		730.5	184.1	22.69
PC 33:0; PC(17:0/16:0 or 18:0/15:0)		748.6	184.1	25.74

1 column. Compound name

2 column. Precursor *m/z* (accurate *m/z* information is rounded into nominal *m/z* information)

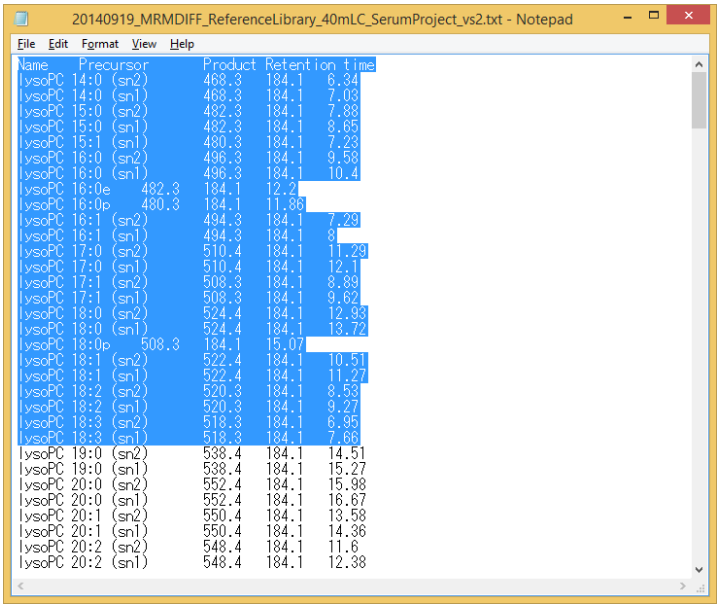
3 column. Product *m/z*

4 column. Retention time [min]

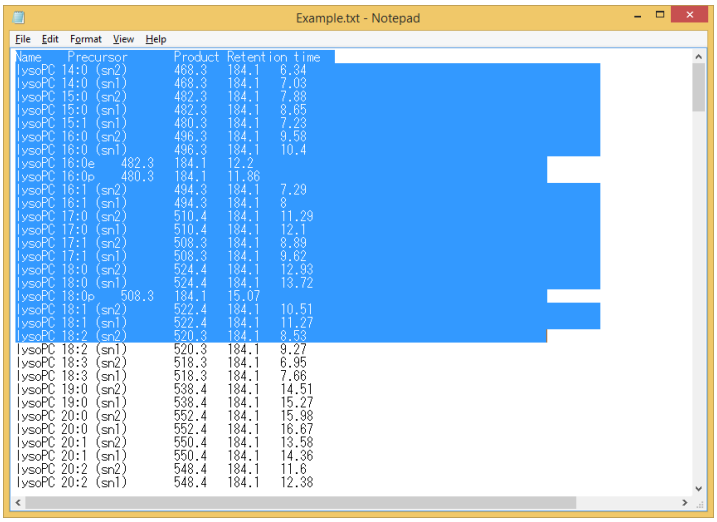
Notes 1: The compound name should be entered in English one-byte characters.

Note 2: Sometimes the tab-delimited file exported from Microsoft Excel includes unexpected hidden trailing columns. These unexpected columns after the ‘Retention time’ column cannot be handled by MRM-DIFF. You can inspect the exported file by selecting a few rows (see below). If there are selected characters after the last column (Retention time), edit the file in Excel to delete these columns and re-export it again.

Good example (no unexpected column)



Bad example (there are unexpected columns)



Starting MRM-DIFF

Note that again, MRM-DIFF is run as a part of MRMPROBS program. Therefore, the assembly name, i.e. EXE file name, is 'MRMPROBS.exe'.

1. Starting up your project
2. Importing Abf files
3. Setting parameters
4. Running the software (1-2 min / sample)

*The tutorial uses 37 demonstration files and the lipid reference library which are downloadable from the above link. The common measurement conditions of the demonstration files were as follows.

Liquid chromatography: total 45 min run per sample with InertSustain C18: 2.1×150 mm, 3 μm (GL sciences Co.).

Mass spectrometer: MRM method with positive and negative ion mode.
Target metabolite number: 284
Total transitions: 284

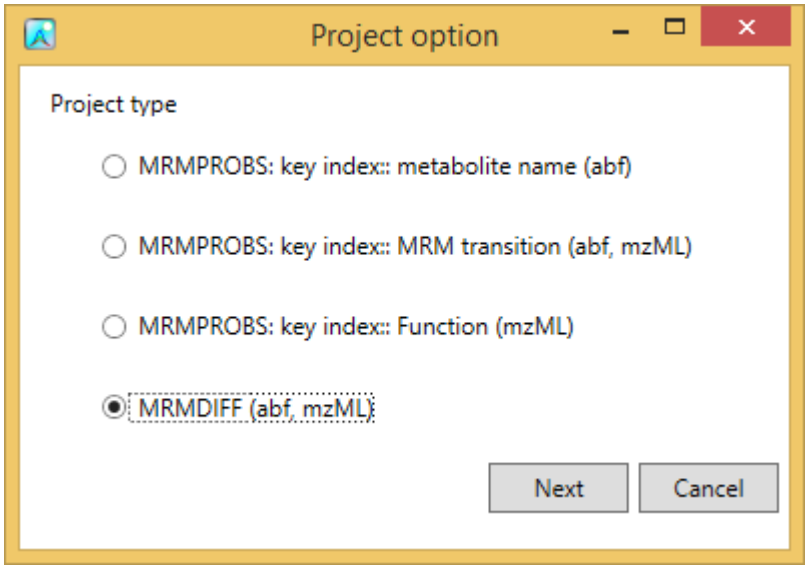
The detail of experimental conditions is downloadable at the MRM Database section (ODS-lipids).

http://prime.psc.riken.jp/Metabolomics_Software/MrmDatabase/index.html

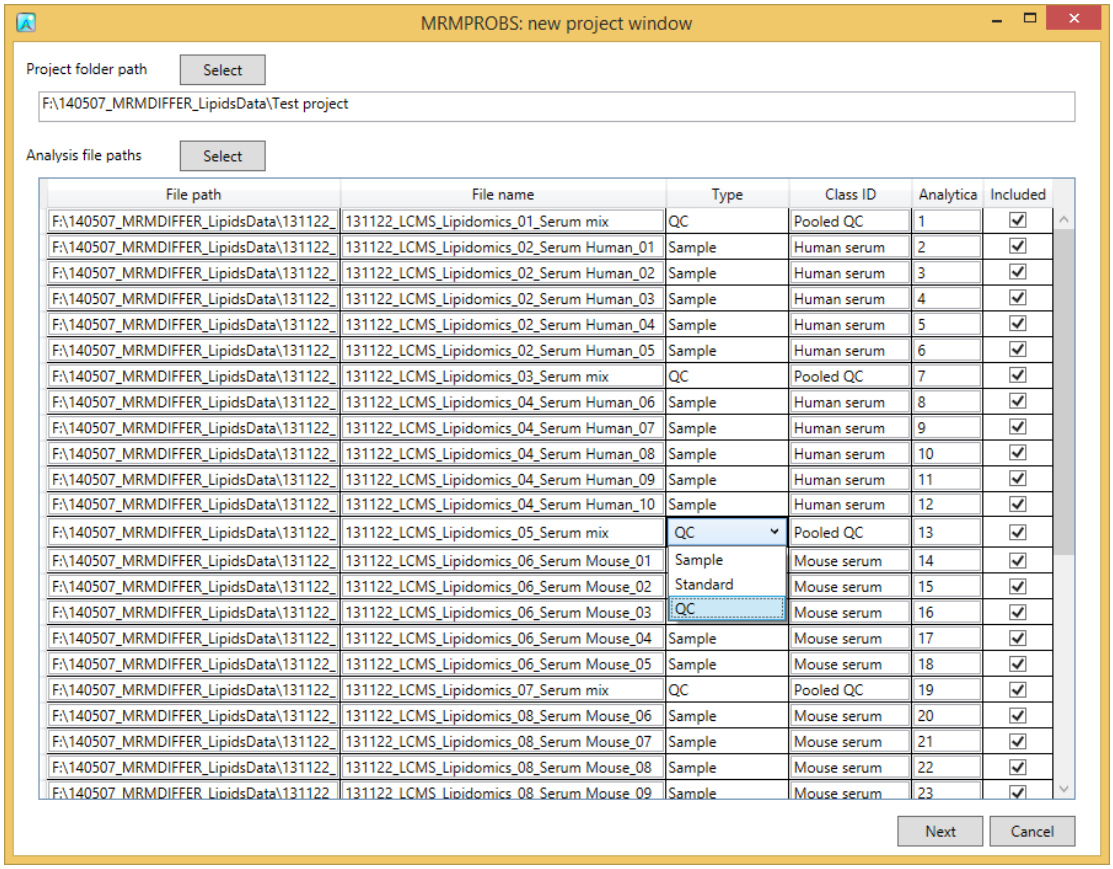
Starting up your project

1. File →New project.

2. Chose a project type (select the bottom one for this demonstration).



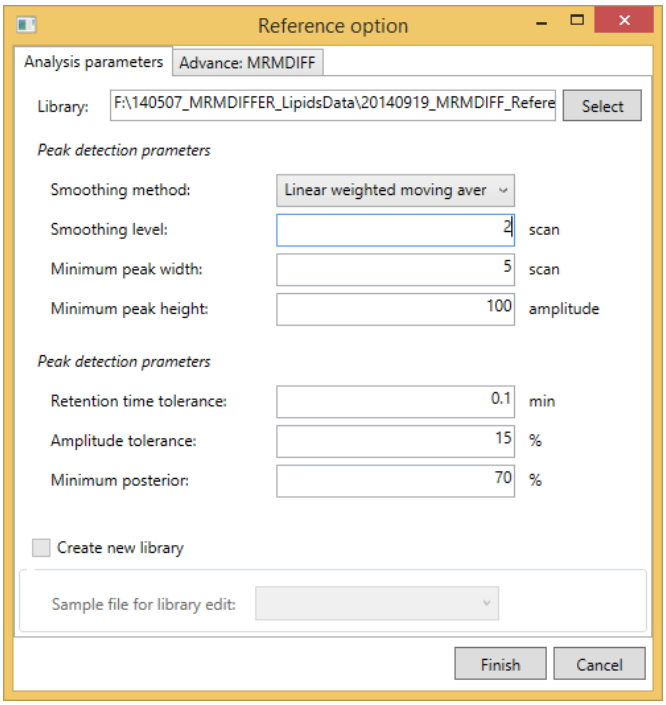
Importing Abf files

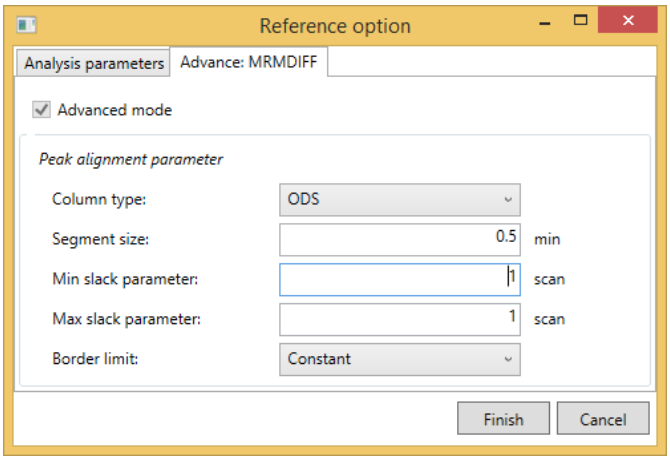


Note:

- We recommend that the project folder be made for each batch experiment. In the MRM-DIFF project, three folders (raw, processed, aligned) and one file (*.mth) are generated. They should be included in the same directory.
- The file name should be entered in half-width alphanumeric symbols.
- Select the file type of each file from “Sample”, “Standard”, and QC”. **QCs must be required for MRM-DILL program to perform chromatogram alignment- and peak detection methods as well as LOESS-based normalization method. If you don’t have pooled QC data sets, use the wild type (control) sample data sets as QCs. What matters is to select the (biological or technical) replicate data sets.** In such case, the LOESS-Cubic spline normalization will not work well, but the others such as chromatogram alignments and peak detections will be fine.
- Decide the class ID used for the color labels.
- The analytical order and class ID can be changed after data processing.

Setting parameters





Select '20140919_MRMDIFF_ReferenceLibrary_40mLC_SerumProject.txt' and set the above parameters for this demonstration.

[**Recommended**]

Peak detection

Smoothing method: linear weighted moving average.

Smoothing level: 1-2

Minimum peak width: 3-5

Minimum peak height: 50-100

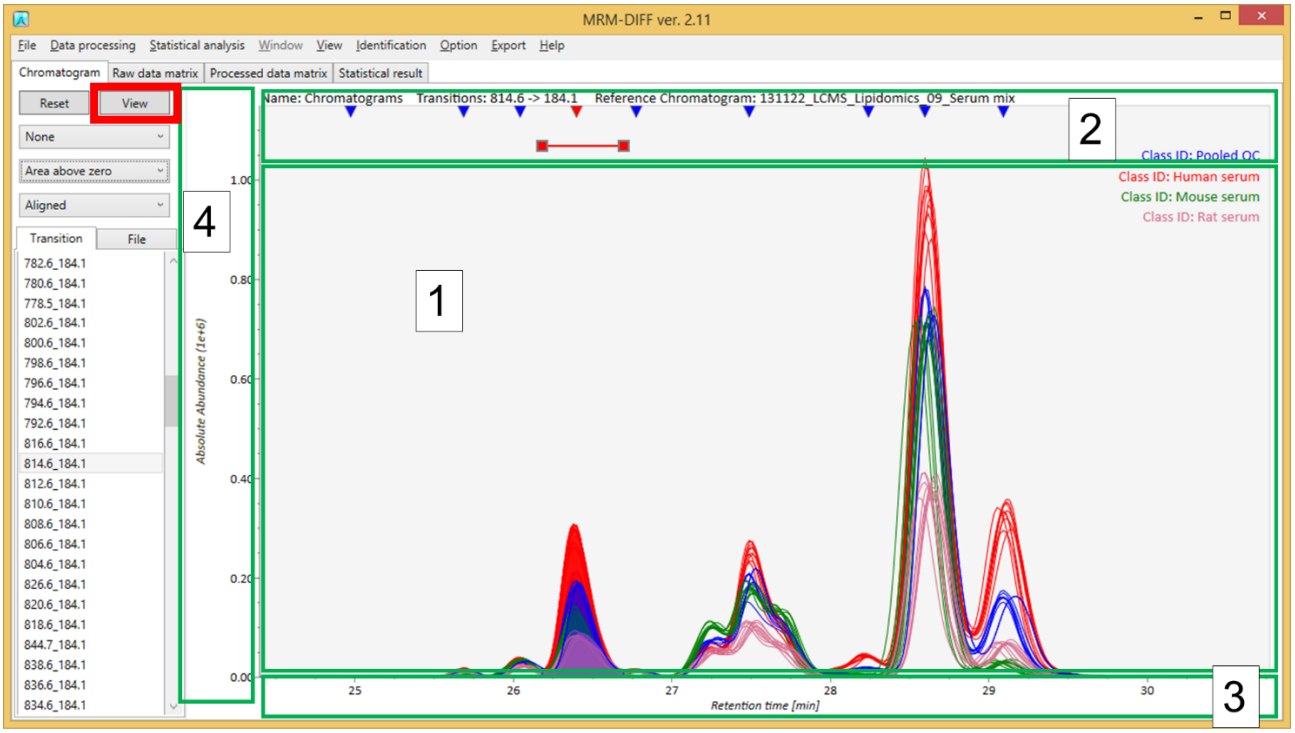
Peak identification

Retention time tolerance: As long as the reverse phase or hydrophilic interaction chromatography LC are used, 0.1-0.2 min is recommended.
Amplitude tolerance: non-meaningful
Minimum posterior: 50-70.

Advance: MRMDIFF
Column type: select a type used in your experiment.
Segment size: add the average peak width in your data sets.
Others: Min slack, max slack, and border limit are automatically determined from the column type. These settings are derived from our pre-experiment. For the classical correlation optimized warping (COW), select ‘Constant’ for the border limit and in such case, max slack parameter is not meaningful.

MRM-DIFF viewer

Mouse operation in the chromatogram viewer



View mode

①. Chromatogram window: drag holding left click → chromatogram scroll, drag holding right click → chromatogram zoom.

②. Detected window: left double-click the reverse triangle → change the focused peak

③. Retention time window: drag holding right click → warping on retention time range.

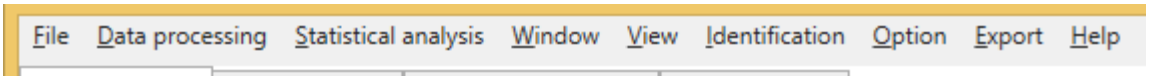
④. Intensity window: drag holding right click → warping on intensity range.

Edit mode

①. Right click and drag on un-detected peak area → detect new peak. Right click and drag on detected peak area → delete detected peaks.

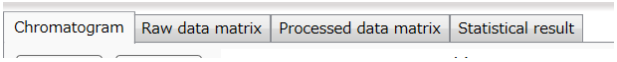
②. Left click and drag on the peak edge [red square] → change the location of the peak edge.

Tool button



- File: start new project, open existing project, save as a project, and save the project.
- Data processing: for data re-processing per file, per metabolite, or in all data sets.
- Statistical analysis: data normalization and statistical analysis.
- Window: non-meaningful in MRM-DIFF project.
- View: change focused chromatograms.
- Identification: Manual curation for identification results.
- Option: re-define class ID and analytical order, choose the internal standard, decide “include” or “exclude” data for statistical analysis.
- Export: The result is exported in tab-delimited text format.
- Help: show version information.

Tab

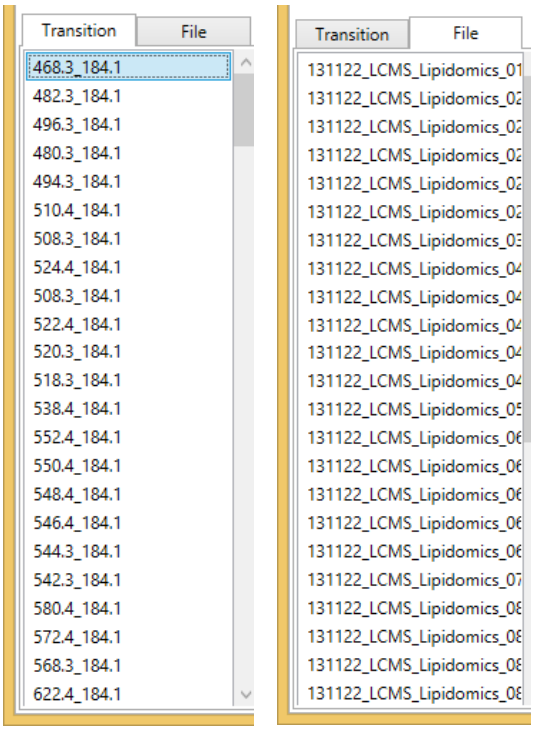


- Chromatogram: All data manipulation tasks are performed here.
- Raw data matrix: Not used in MRM-DIFF project.
- Processed data matrix: Not used in MRM-DIFF project.
- Statistical result: The result of statistical analysis is shown here.

Button

- Reset: Reset the display range of chromatograms.
- View: If you push this “View” button, the chromatogram viewer is changed to “Edit” mode. In the “Edit” mode you can modify the peak edge and detect new peaks manually
- None: The properties of detected peaks are shown in this ComboBox. You can confirm isotopic ions and identified lipids.
- Height: You can set the quantification mode. The default is set by peak height. Instead, you can change it to area mode. By using the “All” option, the quantification mode is reflected = implemented in all files and all metabolites.
- Aligned: You can see raw chromatograms as well as just smoothed chromatograms.

List Box



If you double-click a transition name, the chromatograms are generated in the chromatogram viewer. If you double-click a file name, the reference chromatogram used for alignments and peak detections is highlighted.

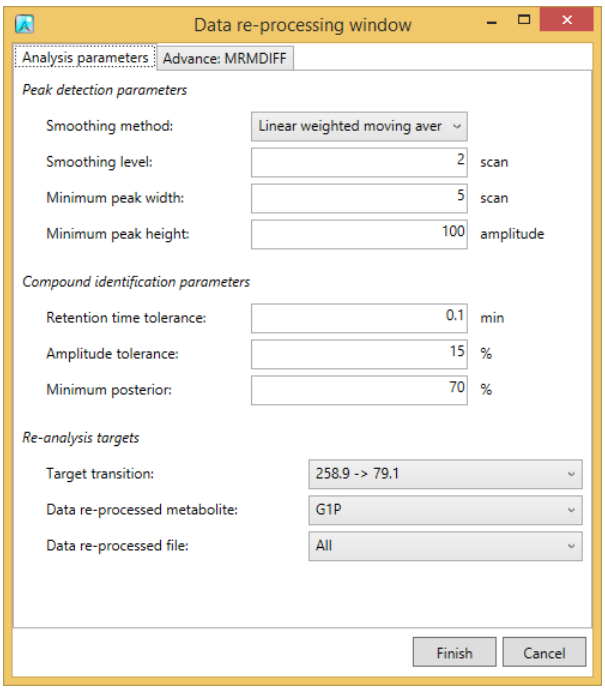
Details on the MRMPROBS function

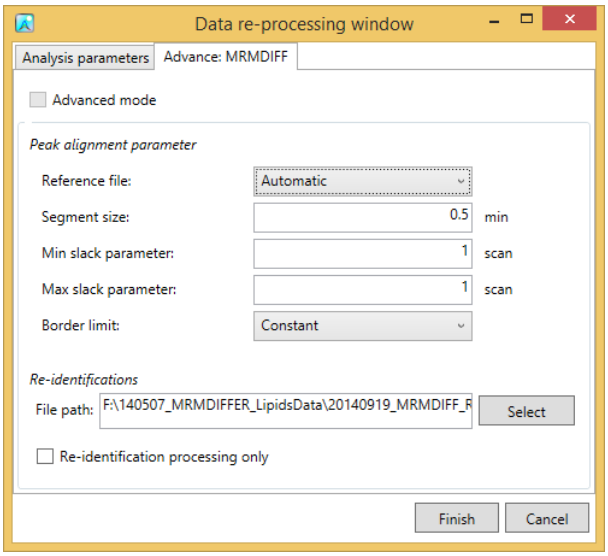
File menu

- New project: used for creating a new project.
- Open project: used for opening an existing project. Make sure that *.mth file, raw folder, and processed folder are included in the same directory.
- Save as: use to save as a new file.
- Save: use to overwrite an existing project.

Data processing menu

Data re-processing can be done by newly optimized parameters in this option. Re-processing is also performed per transition. Also, in MRM-DIFF program, you can re-set the compound library and the identifications can be only done by checking '*Re-identification processing only*'.

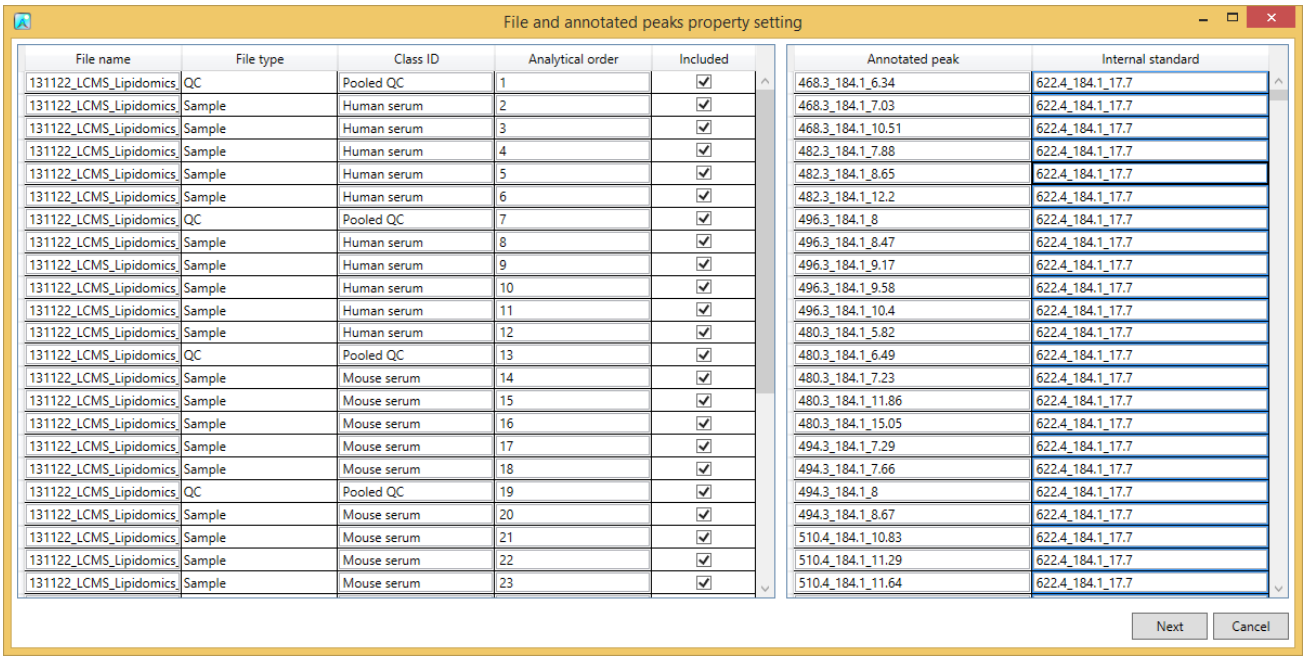




Statistical analysis menu

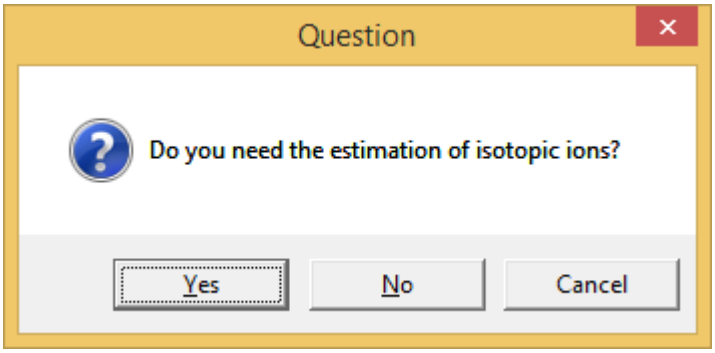
Normalization setting

At first, you can set properties of aligned peaks and files. In the file properties (left), you can reset file type, class ID, or analytical order. If you clear the check box of the “Included” column, the corresponding data are no longer used in the statistical analysis. In the alignment properties (right), you can set internal standard information for each aligned peak. Please make sure to assign “Annotated peak name” in the “internal standard” column.

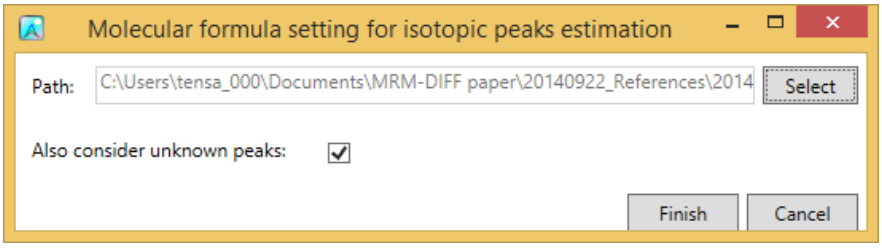


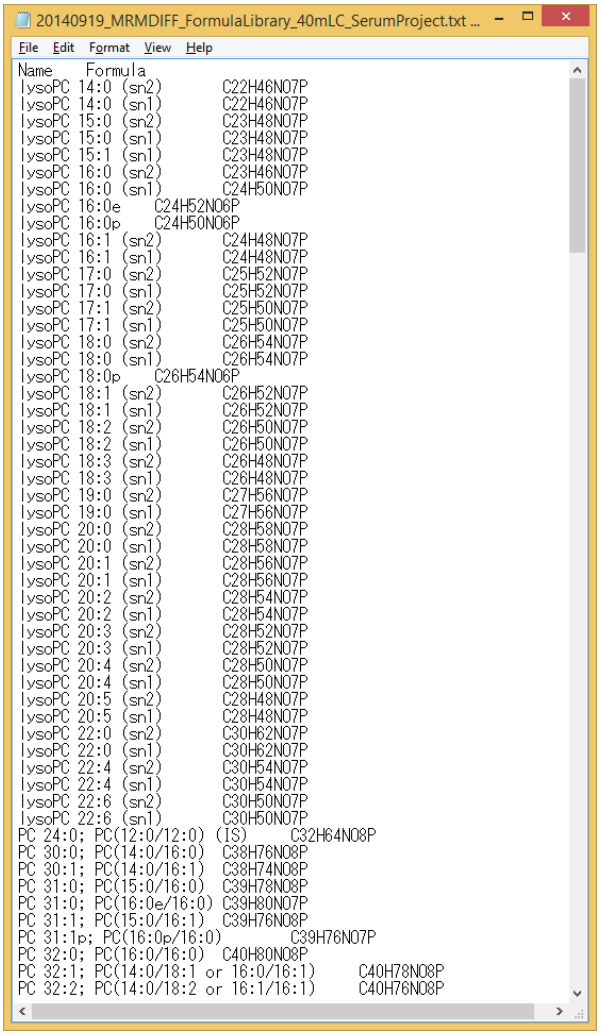
File name	File type	Class ID	Analytical order	Included	Annotated peak	Internal standard
131122_LCMS_Lipidomics	QC	Pooled QC	1	☑	468.3_184.1_6.34	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	2	☑	468.3_184.1_7.03	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	3	☑	468.3_184.1_10.51	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	4	☑	482.3_184.1_7.88	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	5	☑	482.3_184.1_8.65	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	6	☑	482.3_184.1_12.2	622.4_184.1_17.7
131122_LCMS_Lipidomics	QC	Pooled QC	7	☑	496.3_184.1_8	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	8	☑	496.3_184.1_8.47	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	9	☑	496.3_184.1_9.17	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	10	☑	496.3_184.1_9.58	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	11	☑	496.3_184.1_10.4	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Human serum	12	☑	480.3_184.1_5.82	622.4_184.1_17.7
131122_LCMS_Lipidomics	QC	Pooled QC	13	☑	480.3_184.1_6.49	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	14	☑	480.3_184.1_7.23	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	15	☑	480.3_184.1_11.86	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	16	☑	480.3_184.1_15.05	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	17	☑	494.3_184.1_7.29	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	18	☑	494.3_184.1_7.66	622.4_184.1_17.7
131122_LCMS_Lipidomics	QC	Pooled QC	19	☑	494.3_184.1_8	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	20	☑	494.3_184.1_8.67	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	21	☑	510.4_184.1_10.83	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	22	☑	510.4_184.1_11.29	622.4_184.1_17.7
131122_LCMS_Lipidomics	Sample	Mouse serum	23	☑	510.4_184.1_11.64	622.4_184.1_17.7

Then, in this demonstration, please choose ‘Yes’ from the below message.

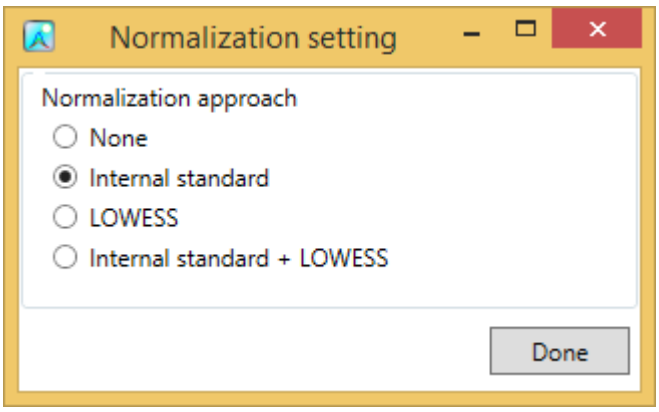


Select ‘20140919_MRMDIFF_FormulaLibrary_40mLC_SerumProject.txt’ as shown in below. The compound names, which should be the same as the identified name in the MRM-DIFF program, and formulas are utilized to estimate the peak abundance from isotopic ion. Moreover, the MRM-DIFF program can also estimate the isotopic abundances from unknown peaks as ‘alkane’. Checking ‘*Also consider unknown peaks*’ is to consider it.





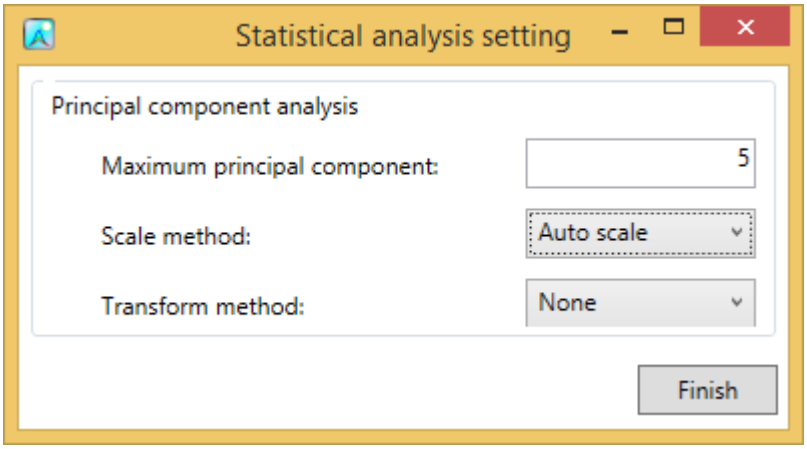
Finally, select a normalization approach.



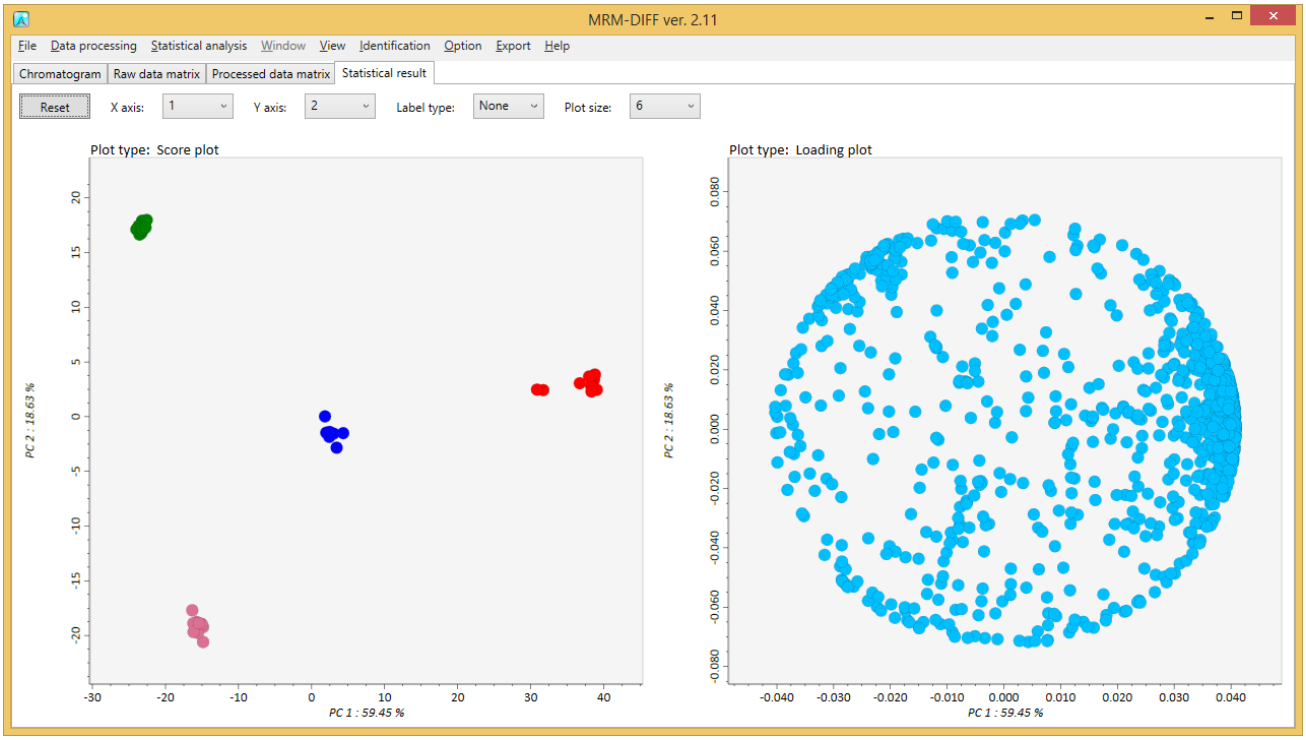
Note:

- None: after implementation of the missing value approach, the values of the raw data matrix are stored in the processed data matrix.
- Internal standard: after implementation of the missing value approach, the value divided by the internal standard value set in the “Option menu” is stored in the processed data matrix.
- LOESS: after implementation of the missing value approach, the signal intensities of each metabolite are normalized with the QC samples information by means of loess/cubic spline.
- Internal standard + LOESS: After internal standard normalization, loess/cubic spline based normalization is performed.

Statistical analysis setting

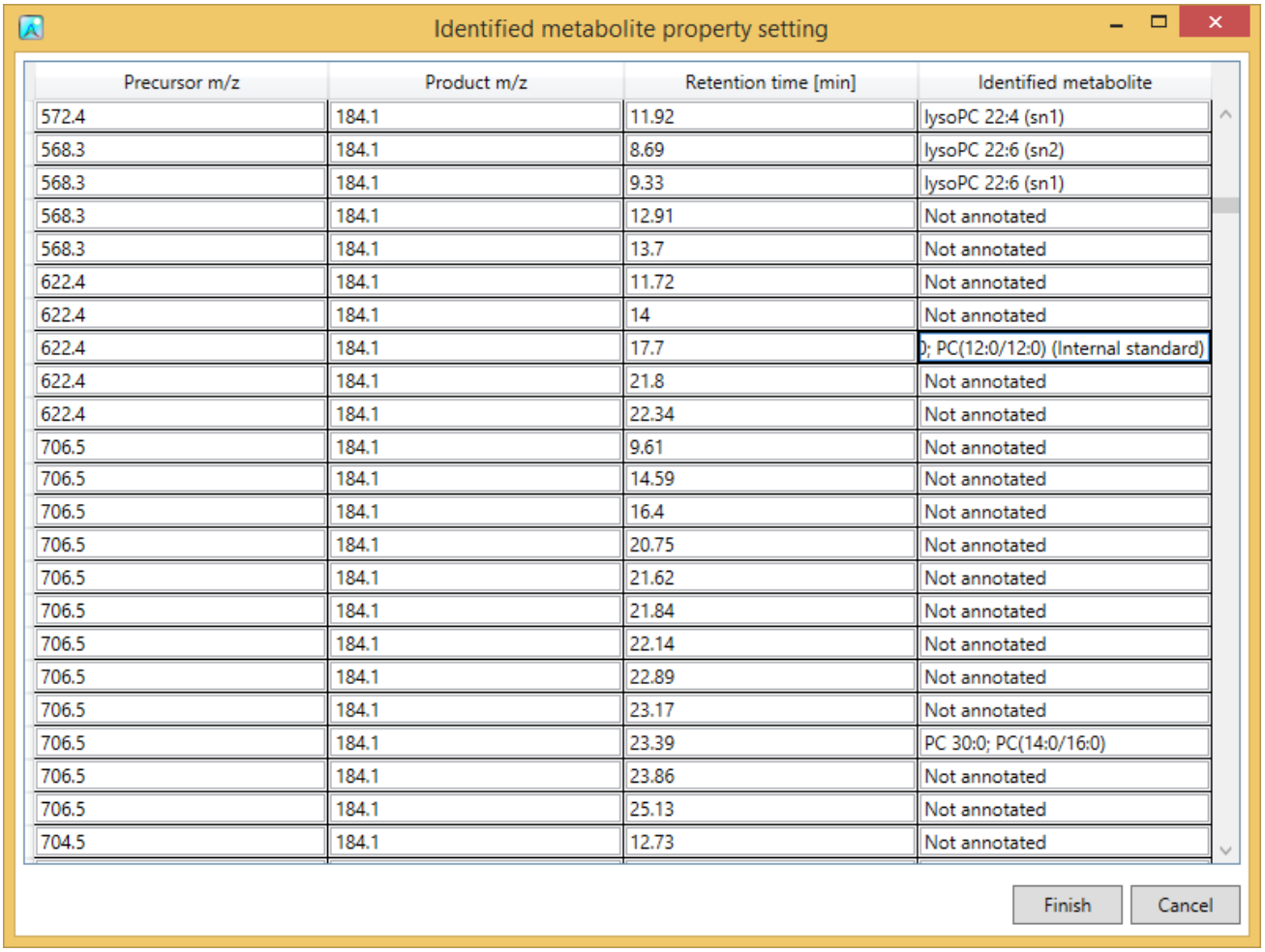


You can do principal component analysis. Add the calculated number of the principal components and choose the scale and transform method.



Identification menu

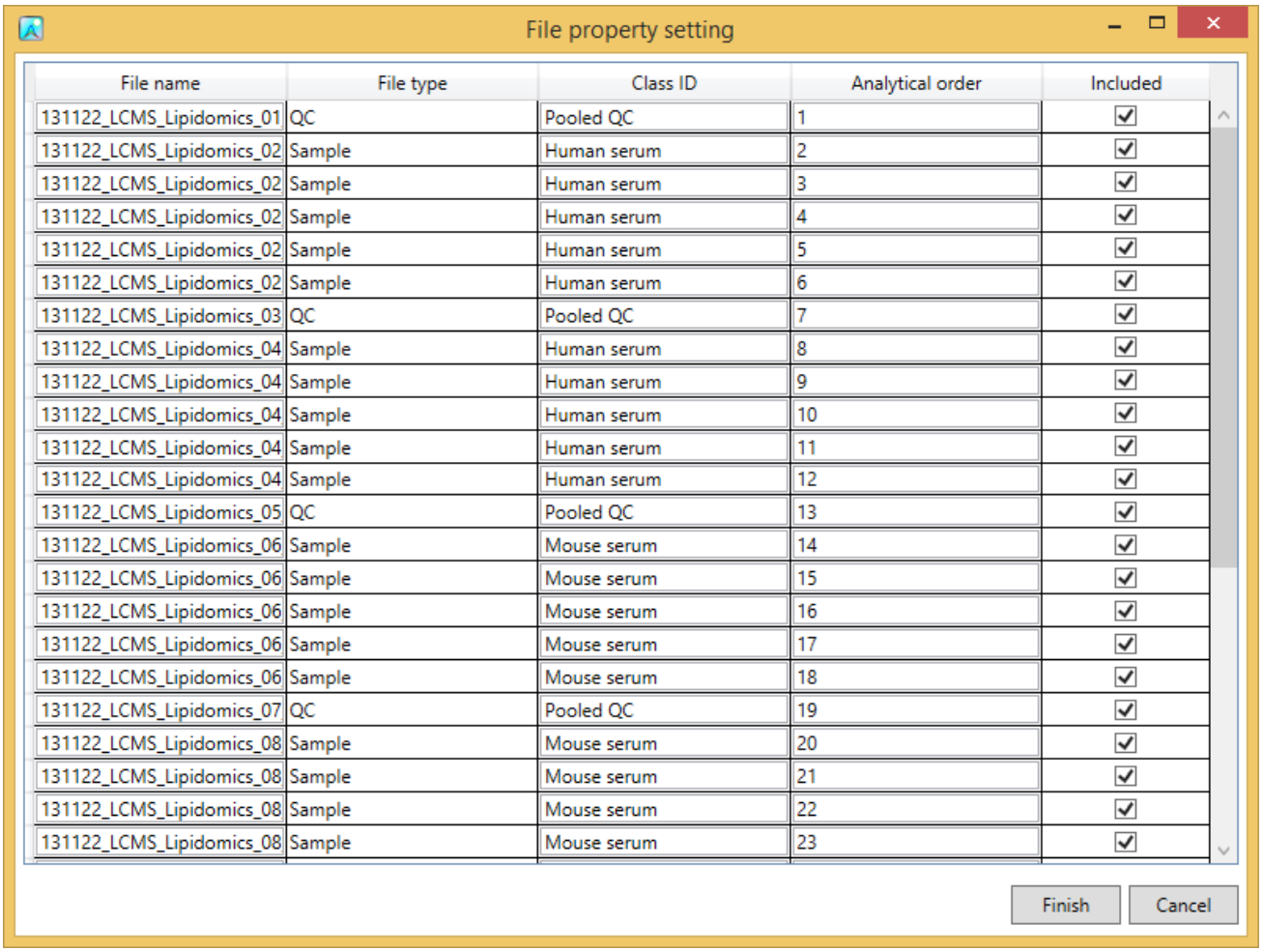
You can manually correct identification result. This option may be useful to check internal standards which are not included in the reference library.



Precursor m/z	Product m/z	Retention time [min]	Identified metabolite
572.4	184.1	11.92	lysoPC 22:4 (sn1)
568.3	184.1	8.69	lysoPC 22:6 (sn2)
568.3	184.1	9.33	lysoPC 22:6 (sn1)
568.3	184.1	12.91	Not annotated
568.3	184.1	13.7	Not annotated
622.4	184.1	11.72	Not annotated
622.4	184.1	14	Not annotated
622.4	184.1	17.7	); PC(12:0/12:0) (Internal standard)
622.4	184.1	21.8	Not annotated
622.4	184.1	22.34	Not annotated
706.5	184.1	9.61	Not annotated
706.5	184.1	14.59	Not annotated
706.5	184.1	16.4	Not annotated
706.5	184.1	20.75	Not annotated
706.5	184.1	21.62	Not annotated
706.5	184.1	21.84	Not annotated
706.5	184.1	22.14	Not annotated
706.5	184.1	22.89	Not annotated
706.5	184.1	23.17	Not annotated
706.5	184.1	23.39	PC 30:0; PC(14:0/16:0)
706.5	184.1	23.86	Not annotated
706.5	184.1	25.13	Not annotated
704.5	184.1	12.73	Not annotated

Option menu

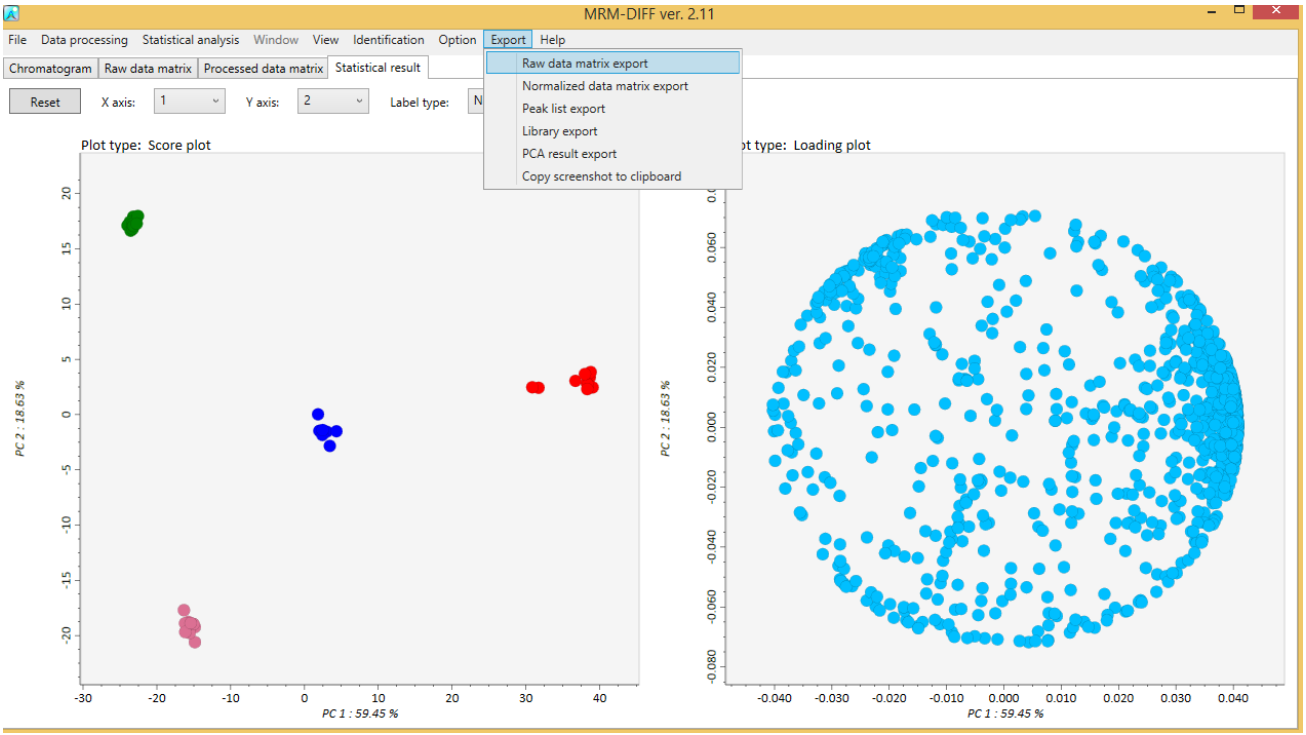
You can set properties of files. You can reset file type, class ID, or analytical order. If you clear the check box of the “Included” column, the corresponding data are no longer used in the statistical analysis.



File name	File type	Class ID	Analytical order	Included
131122_LCMS_Lipidomics_01	QC	Pooled QC	1	☑
131122_LCMS_Lipidomics_02	Sample	Human serum	2	☑
131122_LCMS_Lipidomics_02	Sample	Human serum	3	☑
131122_LCMS_Lipidomics_02	Sample	Human serum	4	☑
131122_LCMS_Lipidomics_02	Sample	Human serum	5	☑
131122_LCMS_Lipidomics_02	Sample	Human serum	6	☑
131122_LCMS_Lipidomics_03	QC	Pooled QC	7	☑
131122_LCMS_Lipidomics_04	Sample	Human serum	8	☑
131122_LCMS_Lipidomics_04	Sample	Human serum	9	☑
131122_LCMS_Lipidomics_04	Sample	Human serum	10	☑
131122_LCMS_Lipidomics_04	Sample	Human serum	11	☑
131122_LCMS_Lipidomics_04	Sample	Human serum	12	☑
131122_LCMS_Lipidomics_05	QC	Pooled QC	13	☑
131122_LCMS_Lipidomics_06	Sample	Mouse serum	14	☑
131122_LCMS_Lipidomics_06	Sample	Mouse serum	15	☑
131122_LCMS_Lipidomics_06	Sample	Mouse serum	16	☑
131122_LCMS_Lipidomics_06	Sample	Mouse serum	17	☑
131122_LCMS_Lipidomics_06	Sample	Mouse serum	18	☑
131122_LCMS_Lipidomics_07	QC	Pooled QC	19	☑
131122_LCMS_Lipidomics_08	Sample	Mouse serum	20	☑
131122_LCMS_Lipidomics_08	Sample	Mouse serum	21	☑
131122_LCMS_Lipidomics_08	Sample	Mouse serum	22	☑
131122_LCMS_Lipidomics_08	Sample	Mouse serum	23	☑

Export menu

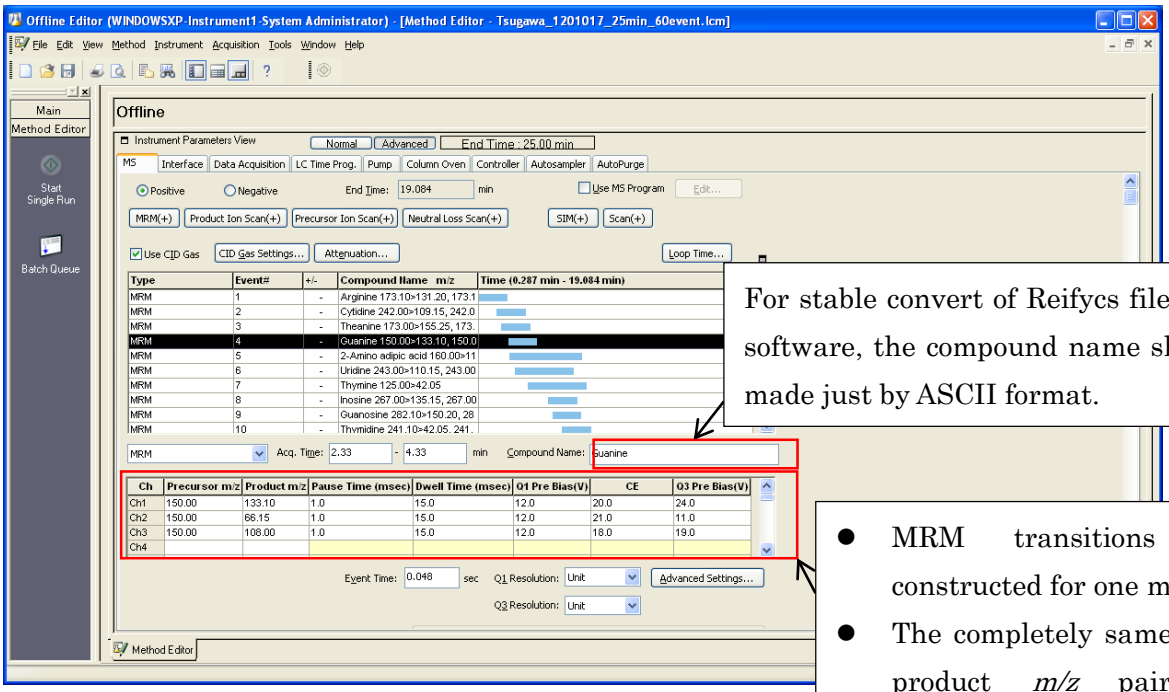
A tab-delimited text file can be exported for a raw data matrix, a processed data matrix, the updated library, detected peak information detail, and PCA results. Moreover, the PCA result can be exported by some image formats.



Appendix A: how to obtain appropriate file conversion of the Shimadzu .lcd file. Suitable method file (.lcm)

Although you can do a content change of the .lcd file after LC-QqQ/MS (MRM) analysis, it is very useful to construct a suitable method file (.lcm format file) for the successful file convert of the MRMPROBS software.

1. Event name and channel (MRM transitions) rule.

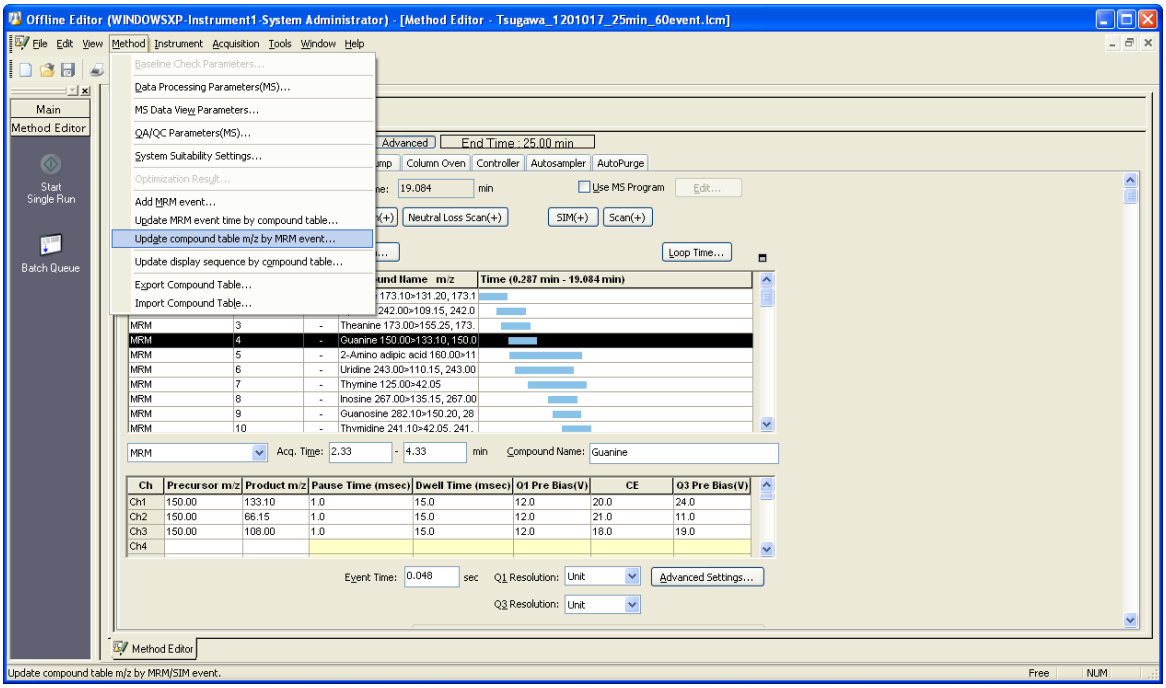


For stable convert of Reifycs file convert software, the compound name should be made just by ASCII format.

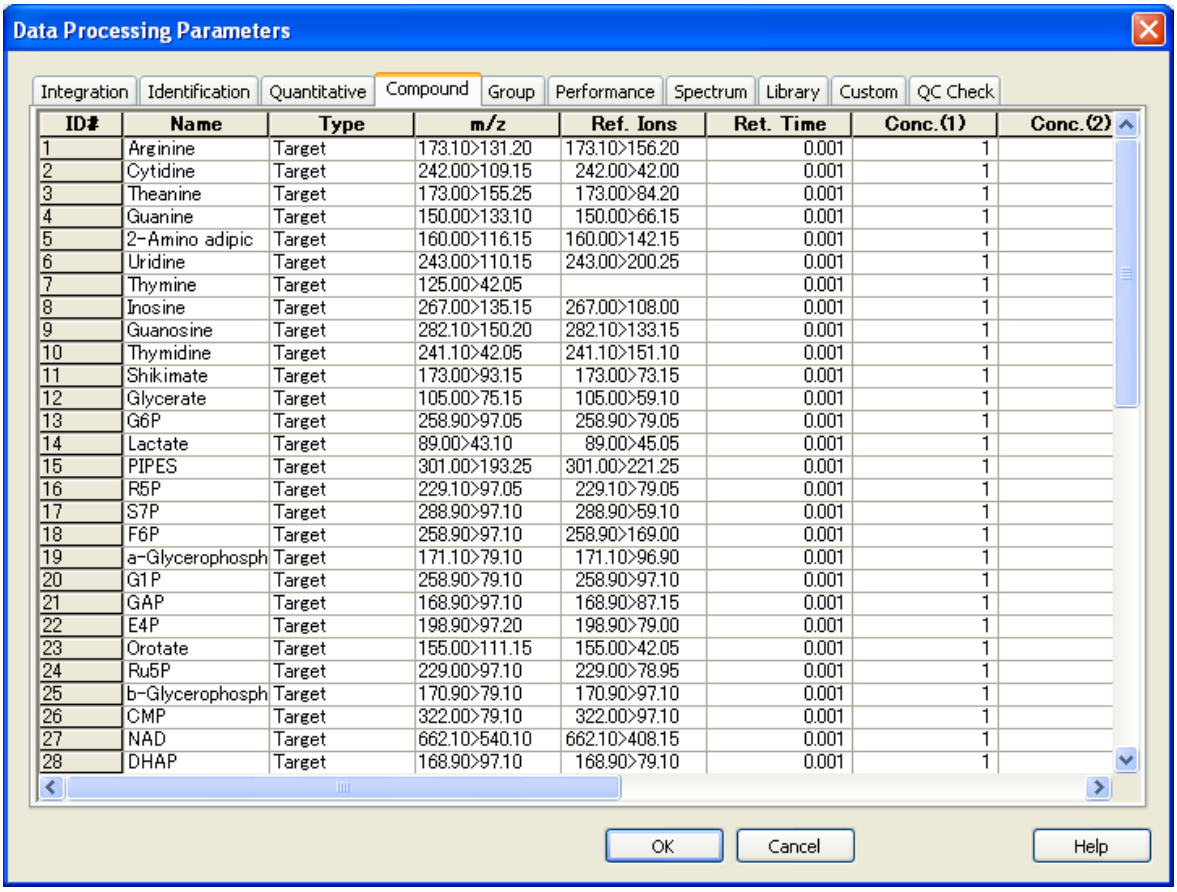
- MRM transitions should be constructed for one metabolite.
- The completely same precursor and product *m/z* pair cannot be acceptable in the file converter.

2. Update compound table

After the method construction of MRM transitions, you should update the compound table m/z by the MRM event. If you can analyze the samples by using the updated method file, you do not have to perform any other tasks for the stable file convert.

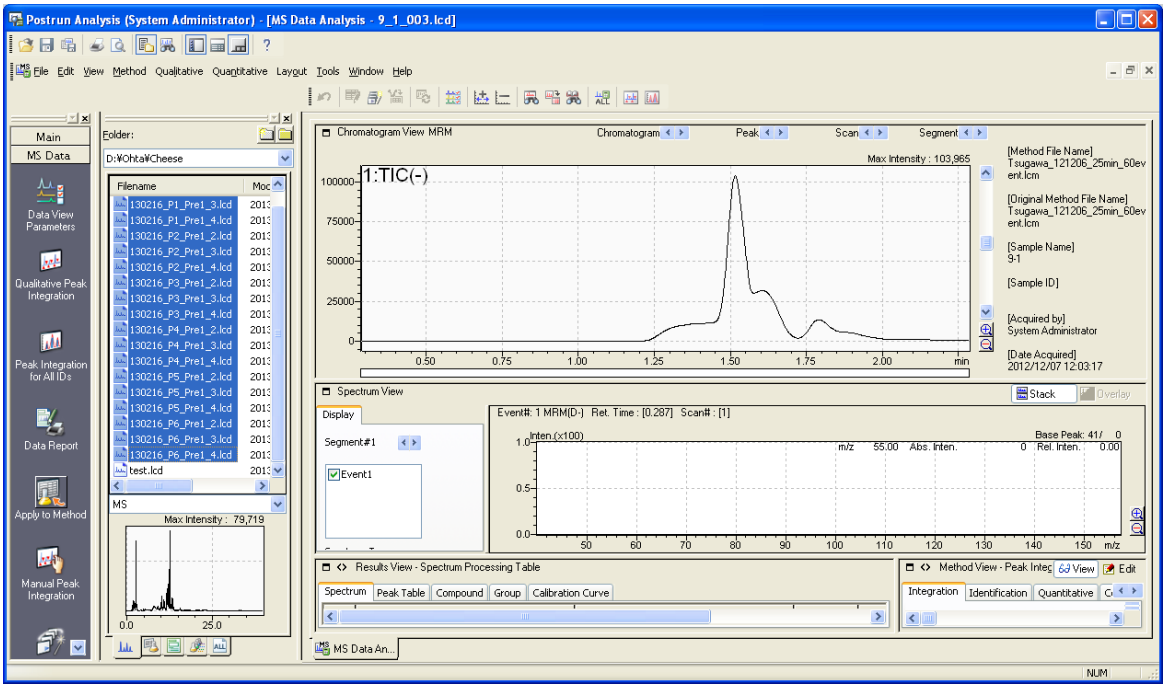


You can check the updated table by Method->Data Processing Parameters->Compound tab.

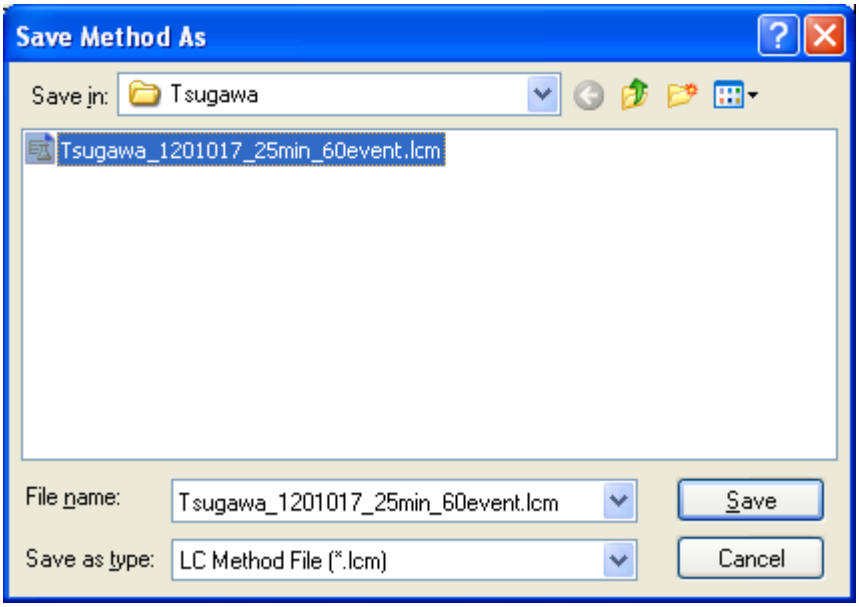


ID#	Name	Type	m/z	Ref. Ions	Ret. Time	Conc.(1)	Conc.(2)
1	Arginine	Target	173.10>131.20	173.10>156.20	0.001	1	
2	Cytidine	Target	242.00>109.15	242.00>42.00	0.001	1	
3	Theanine	Target	173.00>155.25	173.00>84.20	0.001	1	
4	Guanine	Target	150.00>133.10	150.00>66.15	0.001	1	
5	2-Amino adipic	Target	160.00>116.15	160.00>142.15	0.001	1	
6	Uridine	Target	243.00>110.15	243.00>200.25	0.001	1	
7	Thymine	Target	125.00>42.05		0.001	1	
8	Inosine	Target	267.00>135.15	267.00>108.00	0.001	1	
9	Guanosine	Target	282.10>150.20	282.10>133.15	0.001	1	
10	Thymidine	Target	241.10>42.05	241.10>151.10	0.001	1	
11	Shikimate	Target	173.00>93.15	173.00>73.15	0.001	1	
12	Glycerate	Target	105.00>75.15	105.00>59.10	0.001	1	
13	G6P	Target	258.90>97.05	258.90>79.05	0.001	1	
14	Lactate	Target	89.00>43.10	89.00>45.05	0.001	1	
15	PIPES	Target	301.00>193.25	301.00>221.25	0.001	1	
16	R5P	Target	229.10>97.05	229.10>79.05	0.001	1	
17	S7P	Target	288.90>97.10	288.90>59.10	0.001	1	
18	F6P	Target	258.90>97.10	258.90>169.00	0.001	1	
19	a-Glycerophosph	Target	171.10>79.10	171.10>96.90	0.001	1	
20	G1P	Target	258.90>79.10	258.90>97.10	0.001	1	
21	GAP	Target	168.90>97.10	168.90>87.15	0.001	1	
22	E4P	Target	198.90>97.20	198.90>79.00	0.001	1	
23	Orotate	Target	155.00>111.15	155.00>42.05	0.001	1	
24	Ru5P	Target	229.00>97.10	229.00>78.95	0.001	1	
25	b-Glycerophosph	Target	170.90>79.10	170.90>97.10	0.001	1	
26	CMP	Target	322.00>79.10	322.00>97.10	0.001	1	
27	NAD	Target	662.10>540.10	662.10>408.15	0.001	1	
28	DHAP	Target	168.90>97.10	168.90>79.10	0.001	1	

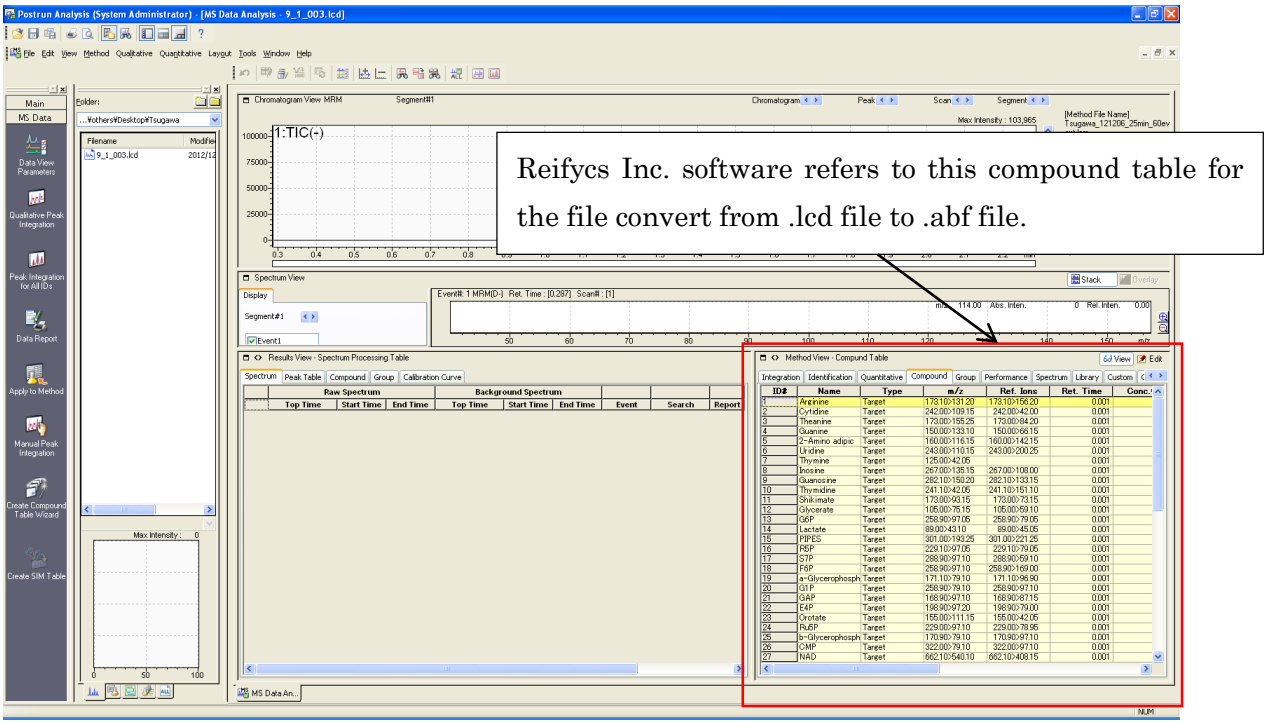
3. If your data (.lcd) were not collected by a suitable method described above, you can improve the .lcd file by using the method file modified in the above way. After the construction of the modified method file, please open “Postrun Analysis” of LabSolutions.



After selecting the analysis files (.lcd) push the “Apply to Method” button.



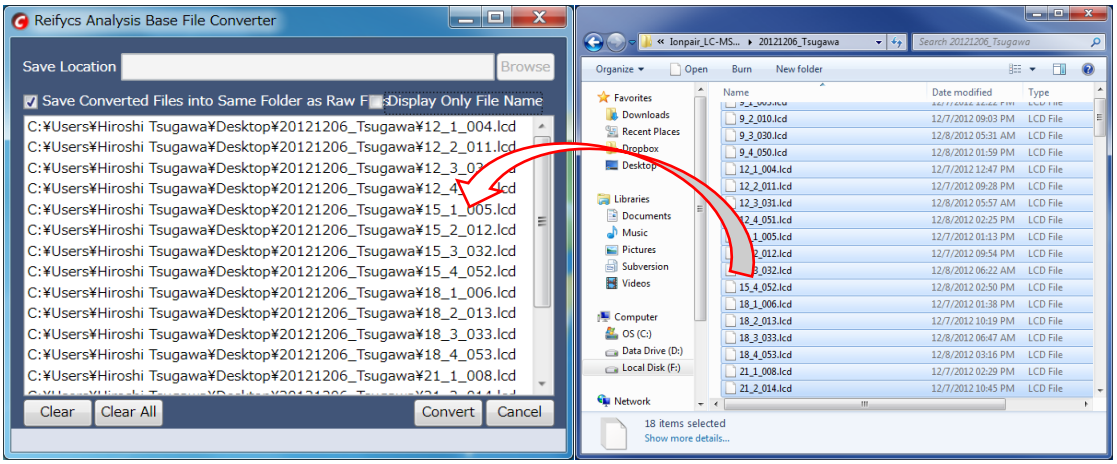
Select the modified method file and improve your .lcd file including the compound table m/z. If you can do this, the file (.lcd) is successfully converted by Reifycs Inc. software.



4. File convert

Conditions: You can convert from .lcd files to .abf files on your computer by installing LabSolutions software. “TTFLDataExportVer5.dll” of LabSolutions ver. 5.53 SP4 or later is required for the file convert. Check the “TTFLDataExportVer5.dll” (Program Files (or *86)>LabSolutions) file property. If the file size is less than 577,536 bytes, contact Shimadzu Inc. for a file change.

After “AnalysisBaseFileConverter.exe” is opened, drag and drop the .lcd files to this converter.



Push the “Convert” button. The ABF format files will be generated in the same folder as the .lcd files.

Appendix B: mzML file conversion via ProteoWizard.

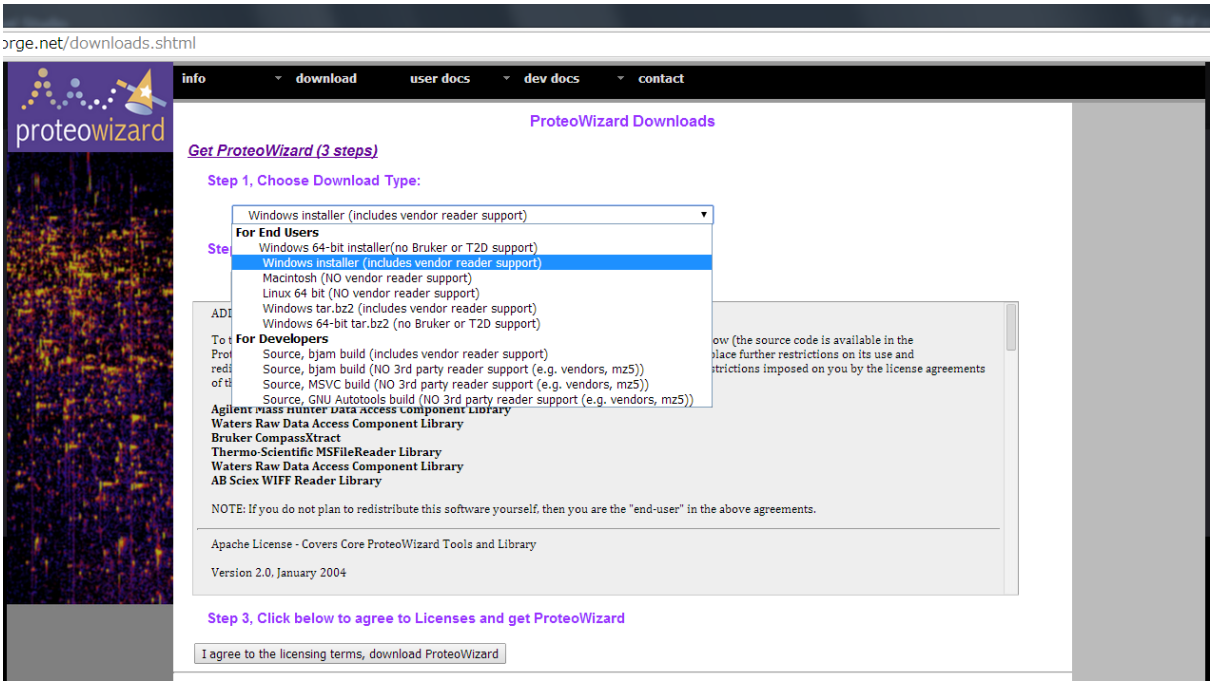
Required software and file

- MSConvert

  Download link: http://proteowizard.sourceforge.net/

Download ProteoWizard

1. Select download type: Windows installer (includes vendor reader support) is recommended.
2. Read license agreements and download the proteowizard.



(http://proteowizard.sourceforge.net/downloads.shtml)

Setup ProteoWizard

1. Follow the wizard windows. (Maybe you don't miss it.)
2. "SeeMS" should be also imported.

Convert the vendor's MS file to mzML via ProteoWizard

1. Open the MSConvertGUI.exe.
2. Select "List of Files".
3. Select the vendor's file via "Browse" button.
4. In the "Options", never check any additional compression including "Use numpress linear compression", "Use numpress short logged float compression", and "Use numpress short positive

integer compression". Each of binary encoding precision is available.

5. Click "Start" button.