# MRMkit

August 1, 2020

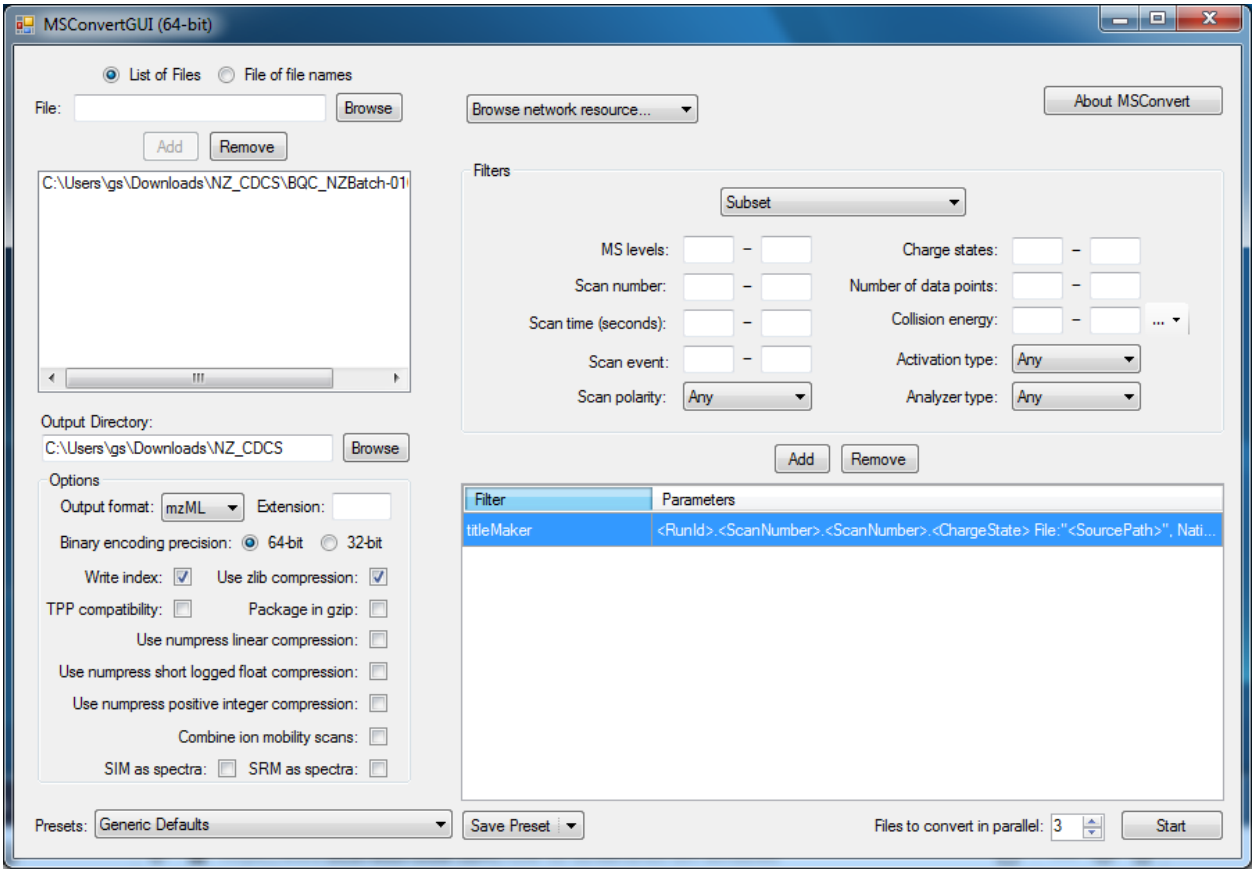
## 1 Raw Data Conversion into mzML

The input files need to be in mzML format for MRMkit. MSConvert, provided through the ProteoWizard software suite, enables conversion of proprietary raw data files (.wiff for SCIEX, .raw files for Thermo Fisher, .d folders for Agilent, etc) into the mzML format.

1. Download and install a recent version ProteoWizard on a Windows computer (the versions for other OS platforms do not work).

2. Start the software by opening the Start Menu, type "MSConvert" in the search field and click on "MSConvert".

3. In the MSConvert window, use the "Browse" button to select the raw files to be converted (the .wiff and .wiff.scan files need to be located in the same folder). Select "mzML" for "Output format", "64-bit" for "Binary encoding precision", check "Write index" and "Use zlib compression" checkbox (see figure below).




# 2 Input Parameters

Input parameters must be specified according to the user's preference in order to control various aspects of the data processing. Input parameters are to be specified in the "param.txt" file. MRMkit requires that the user provide the following options for data processing:

- **mzML_files**: Names of the mzML files. Use "*.mzML" to select all mzML files in the current working directory.
- **batch_info**: analytical sequence of samples with indication of metabolite extraction / sample preparation batches. An example shown in Figure 1.
- **length_of_ion_chromatogram**: range of ion chromatogram to be detected in each MRM window (in seconds)
- **transition_list**: assay information with compound name, Q1, Q3, retention time and the name of the designated internal standards (ISTD). An example shown in Figure 2.
- **ISTD_trace_all**: Set "1" to use the peak shape of the corresponding ISTD for peak integration for all transitions, "0" to directly take the area under peak for transitions

with unimodal chromatograms. Peak areas will still be extracted based on the peak shape of ISTD for multi-modal ion chromatograms

- **batch_correction**: Set "1" to enable time trend and batch effect correction, "0" to disable.

# 3 Analysis Output Table

- **batch_adjusted.txt**: Quantitative data (compound peak area / ISTD) after correction of time trends and batch effects
- **quant_auc_rt.txt**: Integrated peak areas for target analytes and ISTDs
- **BQCtable.txt**: Batch specific and overall coefficient of variation using batch pooled QC samples (a pooled sample with metabolite extraction performed along with the samples from a particular batch)
- **TQCtable.txt**: Batch specific and overall coefficient of variation using technical QC samples (a pooled sample formed at the beginning of the entire analytical sequence, intended to capture instrument variation only)
- **run_seq.pdf**: Visualization of concentration data before and after time trend and batch effect correction, per compound basis (produced from R script)
- **IonChromatogram**: A folder containing ion chromatogram images with integrated peaks across all samples, per transition
- **peak_picking.txt**: The peak picking table, refer to next section.
- **quant_table.txt**: The final quantification table after the user indicates the desired peak in "peak_picking.txt".

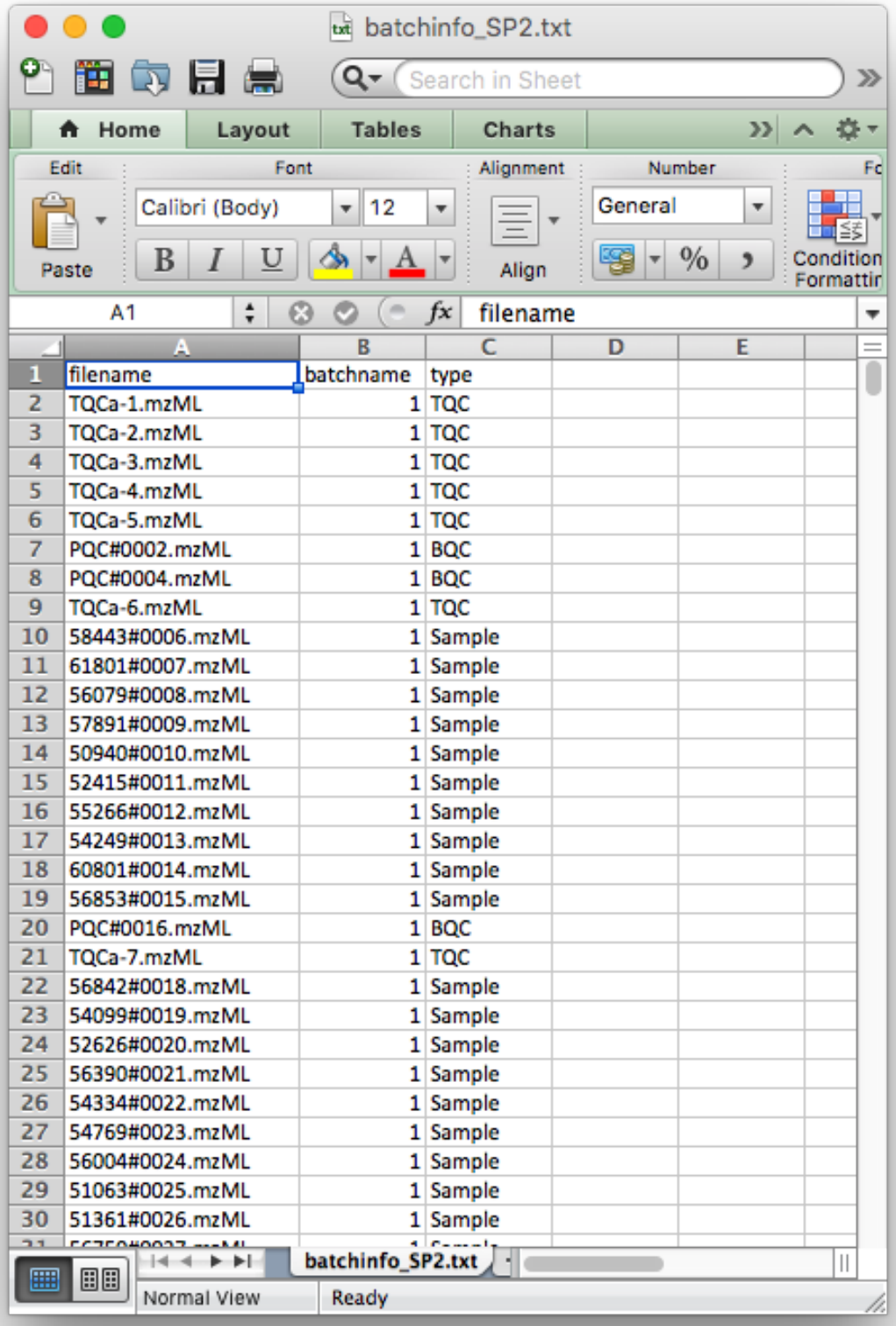
# 4 User's peak picking step

Upon running the analysis, MRMkit produces the aforementioned output files, but it also reports a peak picking table. This step is necessary: for transitions with a multi-modal ion chromatogram, the user must specify which peak he/she wishes to use to quantify the target compound. To facilitate the process, the template file shows the TQC/BQC-based coefficients of variation and their respective RTs. In addition, the user can refer to the ion chromatogram image files to see which peak corresponds to the "real" peak, based on the knowledge of the assay. See the template in Figure 3:

Once the user fills up this form, run the last command of the software **MRM_RT.py**.

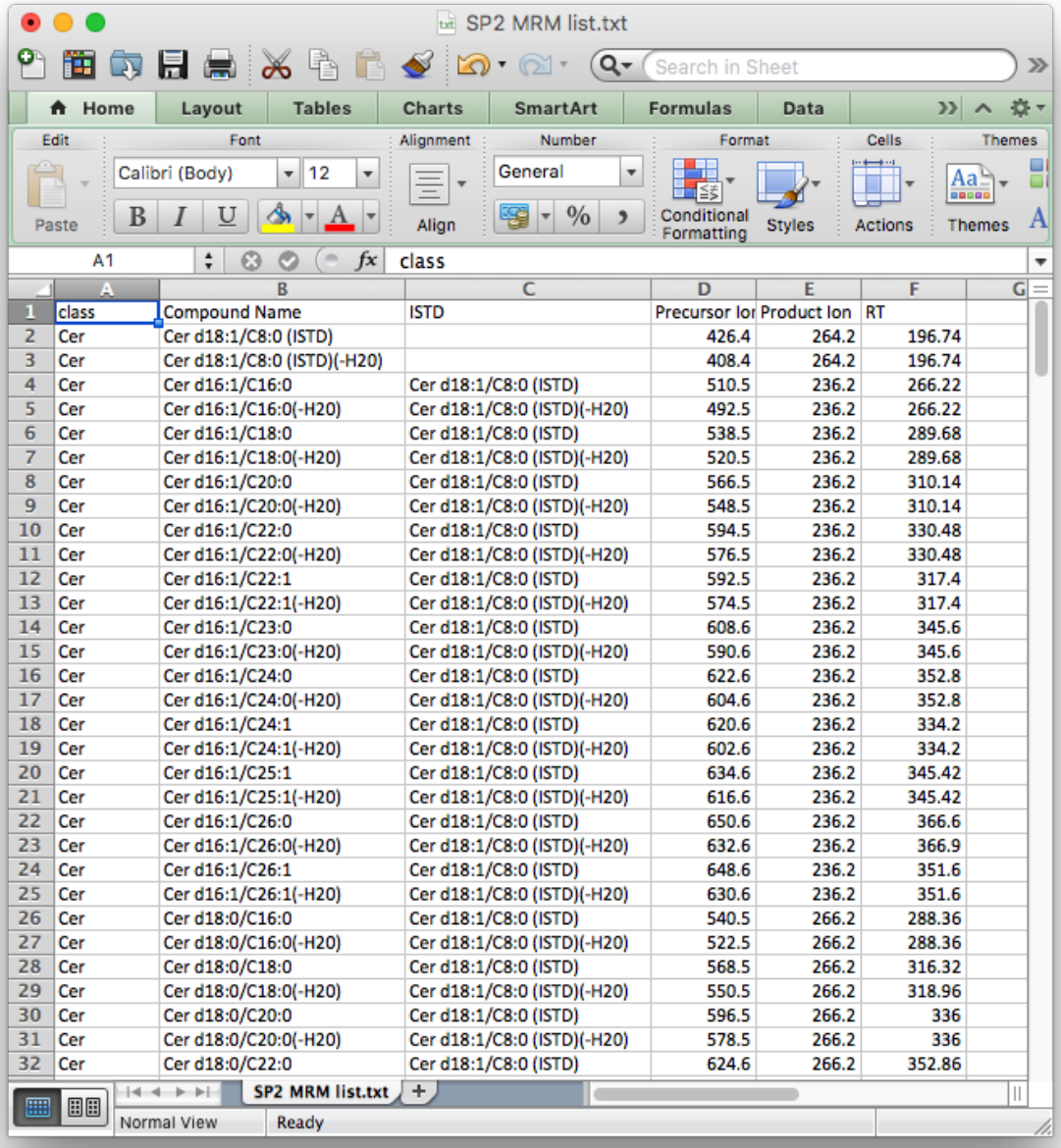
The user does not have to do this for every assay – as long as the MRM method stays the same and the LC-MS instrumentation stays the same, this template file can be applied to all future experiments.

Figure 1: **batch_info** file




| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | filename | batchname | type | | |
| 2 | TQCa-1.mzML | 1 | TQC | | |
| 3 | TQCa-2.mzML | 1 | TQC | | |
| 4 | TQCa-3.mzML | 1 | TQC | | |
| 5 | TQCa-4.mzML | 1 | TQC | | |
| 6 | TQCa-5.mzML | 1 | TQC | | |
| 7 | PQC#0002.mzML | 1 | BQC | | |
| 8 | PQC#0004.mzML | 1 | BQC | | |
| 9 | TQCa-6.mzML | 1 | TQC | | |
| 10 | 58443#0006.mzML | 1 | Sample | | |
| 11 | 61801#0007.mzML | 1 | Sample | | |
| 12 | 56079#0008.mzML | 1 | Sample | | |
| 13 | 57891#0009.mzML | 1 | Sample | | |
| 14 | 50940#0010.mzML | 1 | Sample | | |
| 15 | 52415#0011.mzML | 1 | Sample | | |
| 16 | 55266#0012.mzML | 1 | Sample | | |
| 17 | 54249#0013.mzML | 1 | Sample | | |
| 18 | 60801#0014.mzML | 1 | Sample | | |
| 19 | 56853#0015.mzML | 1 | Sample | | |
| 20 | PQC#0016.mzML | 1 | BQC | | |
| 21 | TQCa-7.mzML | 1 | TQC | | |
| 22 | 56842#0018.mzML | 1 | Sample | | |
| 23 | 54099#0019.mzML | 1 | Sample | | |
| 24 | 52626#0020.mzML | 1 | Sample | | |
| 25 | 56390#0021.mzML | 1 | Sample | | |
| 26 | 54334#0022.mzML | 1 | Sample | | |
| 27 | 54769#0023.mzML | 1 | Sample | | |
| 28 | 56004#0024.mzML | 1 | Sample | | |
| 29 | 51063#0025.mzML | 1 | Sample | | |
| 30 | 51361#0026.mzML | 1 | Sample | | |

Figure 2: **transition_list** file


SP2 MRM list.txt

Search in Sheet

Home | Layout | Tables | Charts | SmartArt | Formulas | Data

Edit | Font | Alignment | Number | Format | Cells | Themes

Paste | Calibri (Body) | 12 | Align | General | Conditional Formatting | Styles | Actions | Themes

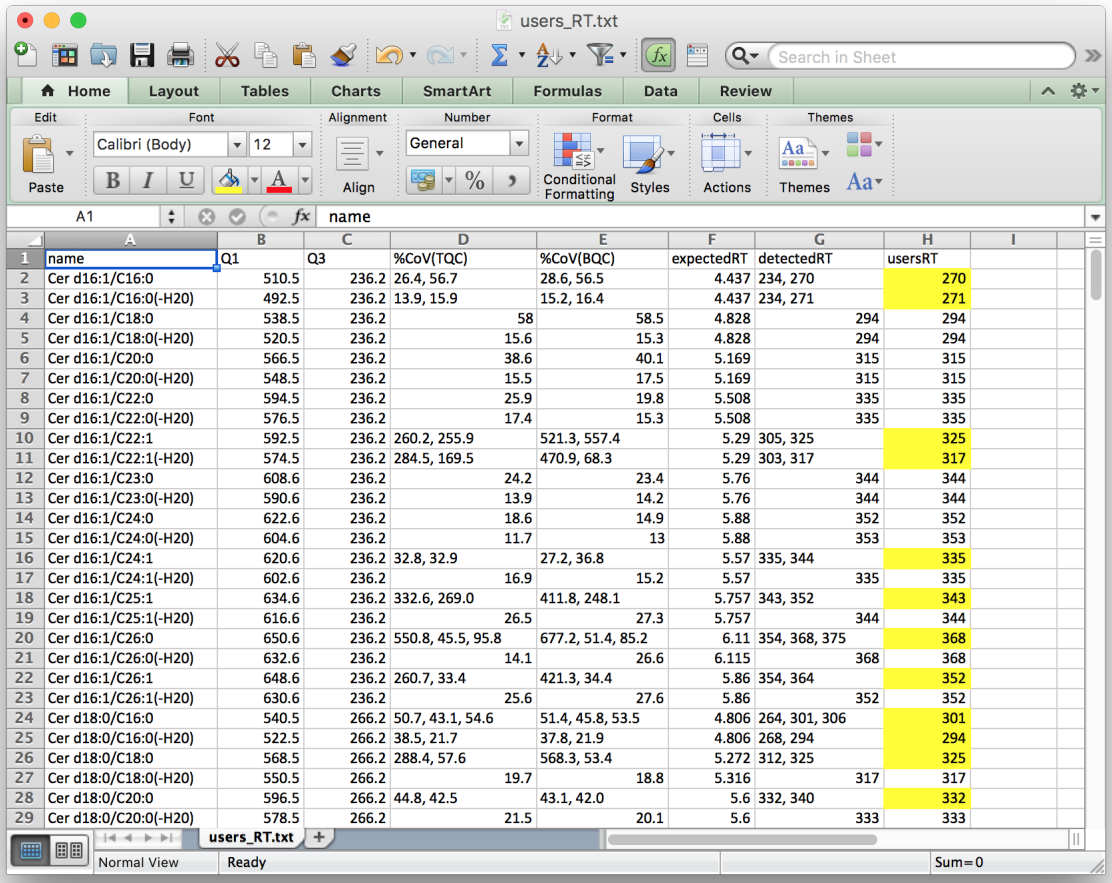
A1 | class

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | class | Compound Name | ISTD | Precursor Ion | Product Ion | RT |
| 2 | Cer | Cer d18:1/C8:0 (ISTD) | | 426.4 | 264.2 | 196.74 |
| 3 | Cer | Cer d18:1/C8:0 (ISTD)(-H20) | | 408.4 | 264.2 | 196.74 |
| 4 | Cer | Cer d16:1/C16:0 | Cer d18:1/C8:0 (ISTD) | 510.5 | 236.2 | 266.22 |
| 5 | Cer | Cer d16:1/C16:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 492.5 | 236.2 | 266.22 |
| 6 | Cer | Cer d16:1/C18:0 | Cer d18:1/C8:0 (ISTD) | 538.5 | 236.2 | 289.68 |
| 7 | Cer | Cer d16:1/C18:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 520.5 | 236.2 | 289.68 |
| 8 | Cer | Cer d16:1/C20:0 | Cer d18:1/C8:0 (ISTD) | 566.5 | 236.2 | 310.14 |
| 9 | Cer | Cer d16:1/C20:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 548.5 | 236.2 | 310.14 |
| 10 | Cer | Cer d16:1/C22:0 | Cer d18:1/C8:0 (ISTD) | 594.5 | 236.2 | 330.48 |
| 11 | Cer | Cer d16:1/C22:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 576.5 | 236.2 | 330.48 |
| 12 | Cer | Cer d16:1/C22:1 | Cer d18:1/C8:0 (ISTD) | 592.5 | 236.2 | 317.4 |
| 13 | Cer | Cer d16:1/C22:1(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 574.5 | 236.2 | 317.4 |
| 14 | Cer | Cer d16:1/C23:0 | Cer d18:1/C8:0 (ISTD) | 608.6 | 236.2 | 345.6 |
| 15 | Cer | Cer d16:1/C23:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 590.6 | 236.2 | 345.6 |
| 16 | Cer | Cer d16:1/C24:0 | Cer d18:1/C8:0 (ISTD) | 622.6 | 236.2 | 352.8 |
| 17 | Cer | Cer d16:1/C24:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 604.6 | 236.2 | 352.8 |
| 18 | Cer | Cer d16:1/C24:1 | Cer d18:1/C8:0 (ISTD) | 620.6 | 236.2 | 334.2 |
| 19 | Cer | Cer d16:1/C24:1(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 602.6 | 236.2 | 334.2 |
| 20 | Cer | Cer d16:1/C25:1 | Cer d18:1/C8:0 (ISTD) | 634.6 | 236.2 | 345.42 |
| 21 | Cer | Cer d16:1/C25:1(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 616.6 | 236.2 | 345.42 |
| 22 | Cer | Cer d16:1/C26:0 | Cer d18:1/C8:0 (ISTD) | 650.6 | 236.2 | 366.6 |
| 23 | Cer | Cer d16:1/C26:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 632.6 | 236.2 | 366.9 |
| 24 | Cer | Cer d16:1/C26:1 | Cer d18:1/C8:0 (ISTD) | 648.6 | 236.2 | 351.6 |
| 25 | Cer | Cer d16:1/C26:1(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 630.6 | 236.2 | 351.6 |
| 26 | Cer | Cer d18:0/C16:0 | Cer d18:1/C8:0 (ISTD) | 540.5 | 266.2 | 288.36 |
| 27 | Cer | Cer d18:0/C16:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 522.5 | 266.2 | 288.36 |
| 28 | Cer | Cer d18:0/C18:0 | Cer d18:1/C8:0 (ISTD) | 568.5 | 266.2 | 316.32 |
| 29 | Cer | Cer d18:0/C18:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 550.5 | 266.2 | 318.96 |
| 30 | Cer | Cer d18:0/C20:0 | Cer d18:1/C8:0 (ISTD) | 596.5 | 266.2 | 336 |
| 31 | Cer | Cer d18:0/C20:0(-H20) | Cer d18:1/C8:0 (ISTD)(-H20) | 578.5 | 266.2 | 336 |
| 32 | Cer | Cer d18:0/C22:0 | Cer d18:1/C8:0 (ISTD) | 624.6 | 266.2 | 352.86 |

SP2 MRM list.txt

Normal View | Ready

Figure 3: **peak_picking.txt** file, the user will fill in RT of true peak where multiple peaks are detected (highlighted in yellow)



users_RT.txt

| | name | Q1 | Q3 | %CoV(TQC) | %CoV(BQC) | expectedRT | detectedRT | usersRT |
|---|---|---|---|---|---|---|---|---|
| 2 | Cer d16:1/C16:0 | 510.5 | 236.2 | 26.4, 56.7 | 28.6, 56.5 | 4.437 | 234, 270 | 270 |
| 3 | Cer d16:1/C16:0(-H20) | 492.5 | 236.2 | 13.9, 15.9 | 15.2, 16.4 | 4.437 | 234, 271 | 271 |
| 4 | Cer d16:1/C18:0 | 538.5 | 236.2 | 58 | 58.5 | 4.828 | 294 | 294 |
| 5 | Cer d16:1/C18:0(-H20) | 520.5 | 236.2 | 15.6 | 15.3 | 4.828 | 294 | 294 |
| 6 | Cer d16:1/C20:0 | 566.5 | 236.2 | 38.6 | 40.1 | 5.169 | 315 | 315 |
| 7 | Cer d16:1/C20:0(-H20) | 548.5 | 236.2 | 15.5 | 17.5 | 5.169 | 315 | 315 |
| 8 | Cer d16:1/C22:0 | 594.5 | 236.2 | 25.9 | 19.8 | 5.508 | 335 | 335 |
| 9 | Cer d16:1/C22:0(-H20) | 576.5 | 236.2 | 17.4 | 15.3 | 5.508 | 335 | 335 |
| 10 | Cer d16:1/C22:1 | 592.5 | 236.2 | 260.2, 255.9 | 521.3, 557.4 | 5.29 | 305, 325 | 325 |
| 11 | Cer d16:1/C22:1(-H20) | 574.5 | 236.2 | 284.5, 169.5 | 470.9, 68.3 | 5.29 | 303, 317 | 317 |
| 12 | Cer d16:1/C23:0 | 608.6 | 236.2 | 24.2 | 23.4 | 5.76 | 344 | 344 |
| 13 | Cer d16:1/C23:0(-H20) | 590.6 | 236.2 | 13.9 | 14.2 | 5.76 | 344 | 344 |
| 14 | Cer d16:1/C24:0 | 622.6 | 236.2 | 18.6 | 14.9 | 5.88 | 352 | 352 |
| 15 | Cer d16:1/C24:0(-H20) | 604.6 | 236.2 | 11.7 | 13 | 5.88 | 353 | 353 |
| 16 | Cer d16:1/C24:1 | 620.6 | 236.2 | 32.8, 32.9 | 27.2, 36.8 | 5.57 | 335, 344 | 335 |
| 17 | Cer d16:1/C24:1(-H20) | 602.6 | 236.2 | 16.9 | 15.2 | 5.57 | 335 | 335 |
| 18 | Cer d16:1/C25:1 | 634.6 | 236.2 | 332.6, 269.0 | 411.8, 248.1 | 5.757 | 343, 352 | 343 |
| 19 | Cer d16:1/C25:1(-H20) | 616.6 | 236.2 | 26.5 | 27.3 | 5.757 | 344 | 344 |
| 20 | Cer d16:1/C26:0 | 650.6 | 236.2 | 550.8, 45.5, 95.8 | 677.2, 51.4, 85.2 | 6.11 | 354, 368, 375 | 368 |
| 21 | Cer d16:1/C26:0(-H20) | 632.6 | 236.2 | 14.1 | 26.6 | 6.115 | 368 | 368 |
| 22 | Cer d16:1/C26:1 | 648.6 | 236.2 | 260.7, 33.4 | 421.3, 34.4 | 5.86 | 354, 364 | 352 |
| 23 | Cer d16:1/C26:1(-H20) | 630.6 | 236.2 | 25.6 | 27.6 | 5.86 | 352 | 352 |
| 24 | Cer d18:0/C16:0 | 540.5 | 266.2 | 50.7, 43.1, 54.6 | 51.4, 45.8, 53.5 | 4.806 | 264, 301, 306 | 301 |
| 25 | Cer d18:0/C16:0(-H20) | 522.5 | 266.2 | 38.5, 21.7 | 37.8, 21.9 | 4.806 | 268, 294 | 294 |
| 26 | Cer d18:0/C18:0 | 568.5 | 266.2 | 288.4, 57.6 | 568.3, 53.4 | 5.272 | 312, 325 | 325 |
| 27 | Cer d18:0/C18:0(-H20) | 550.5 | 266.2 | 19.7 | 18.8 | 5.316 | 317 | 317 |
| 28 | Cer d18:0/C20:0 | 596.5 | 266.2 | 44.8, 42.5 | 43.1, 42.0 | 5.6 | 332, 340 | 332 |
| 29 | Cer d18:0/C20:0(-H20) | 578.5 | 266.2 | 21.5 | 20.1 | 5.6 | 333 | 333 |

# 5 Example "param.txt"

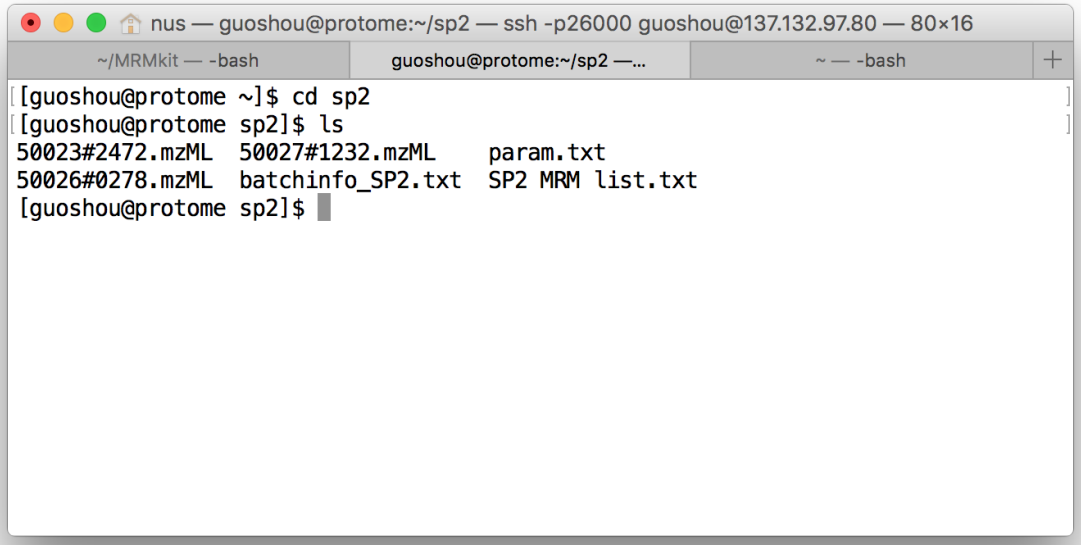
```
mzML_files
*.mzML

batch_info
batchinfo_SP2.txt

length_of_ion_chromatogram
4 40

transition_list
SP2 MRM list.txt

ISTD_trace_all
1

batch_correction
1
```

# 6 Command line for unix/macOS users

Installation of python3 (>= 3.7), numpy, matplotlib and R (>= 4.0) is required.

```
cd {path_to_folder_with_mzML_files,_param.txt,_assay_info_and_batch_info}

# internal standards (ISTDs) peak extraction
python3 {path_to_MRMkit}/MRMistd.py

# peak detection
python3 {path_to_MRMkit}/MRMgetpeak.py

# draw ion chromatograms
Rscript {path_to_MRMkit}/MRMionc.r

# batch correction
python3 {path_to_MRMkit}/MRMcorrect.py

# peak picking (optional)
# users to input RT of true peaks in user_RT.txt before running the command below
python3 {path_to_MRMkit}/MRM_RT.py
```

1. Change directory to the folder (e.g. sp2) containing mzML files (a truncated list of mzML files shown here), param.txt, assay info and batch info files.




2. internal standards (ISTDs) peak extraction, MRMkit's path in this example was "~/02srm".

3. peak detection


4. draw ion chromatograms


A series of IonC2_{transition_name}.pdf will appear upon completion.

5. batch correction


"batch_adjusted.txt", "peak_picking.txt" will appear upon completion.

# 7 Windows users

Installation of R (>= 4.0) is required.
Users can copy and paste the provided executables (.exe) and batch files (.bat) to the folder containing the mzML files, param.txt, assay info and batch info files.
Then, double-click on the batch files in the sequence of "MRMistd.bat", "MRMgetpeak.bat", "MRMcorrect.bat" and "MRM_RT.bat".

1. internal standards (ISTDs) peak extraction
   MRMistd.

2. peak detection
   MRMgetpeak.

3. draw ion chromatograms
   MRMionc.r. Run this R script using R
   A series of IonC2_{transition_name}.pdf will appear upon completion.

4. batch correction
   MRMcorrect.

"batch_adjusted.txt", "peak_picking.txt" will appear upon completion.

# 8 Copyrights