MRMkit

July 8, 2020

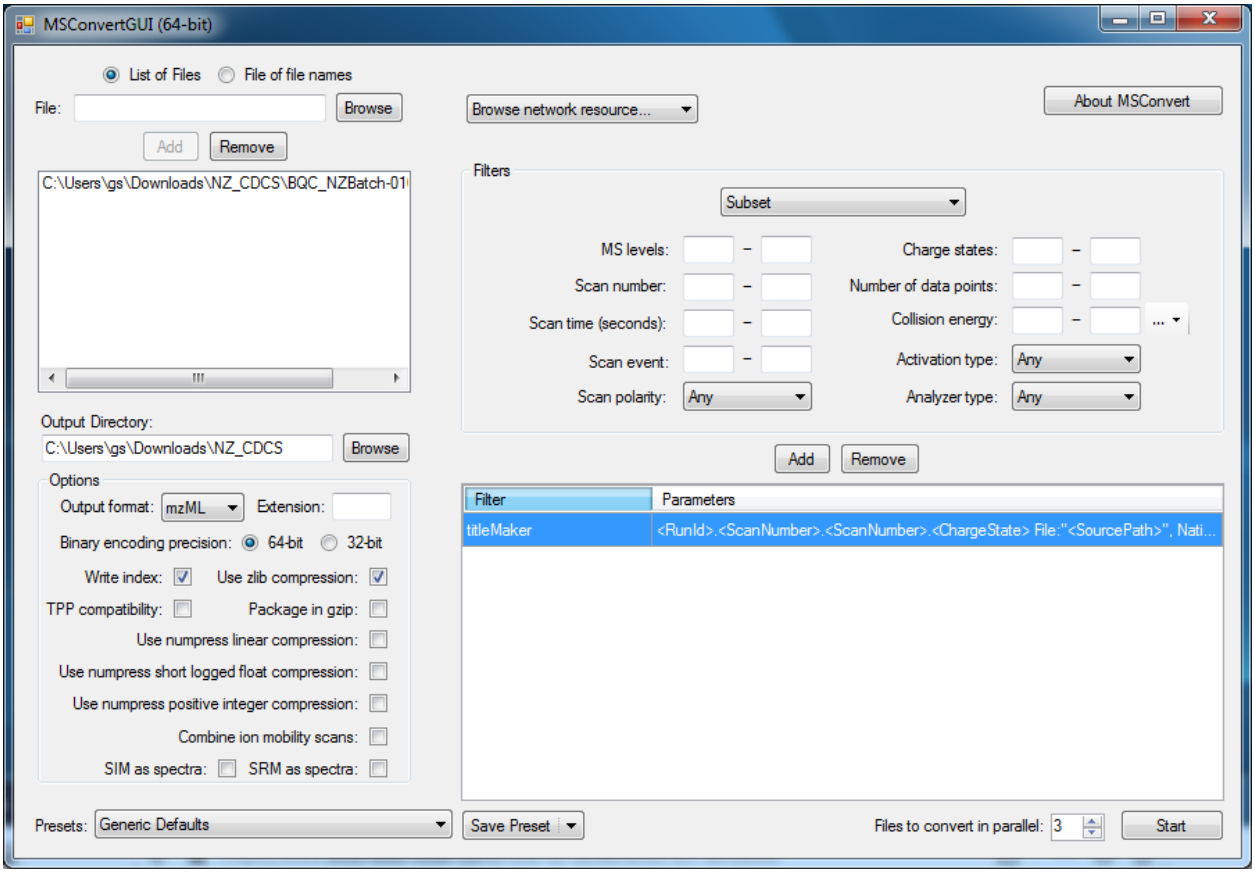
1 Raw Data Conversion into mzML

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
- **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).
- **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

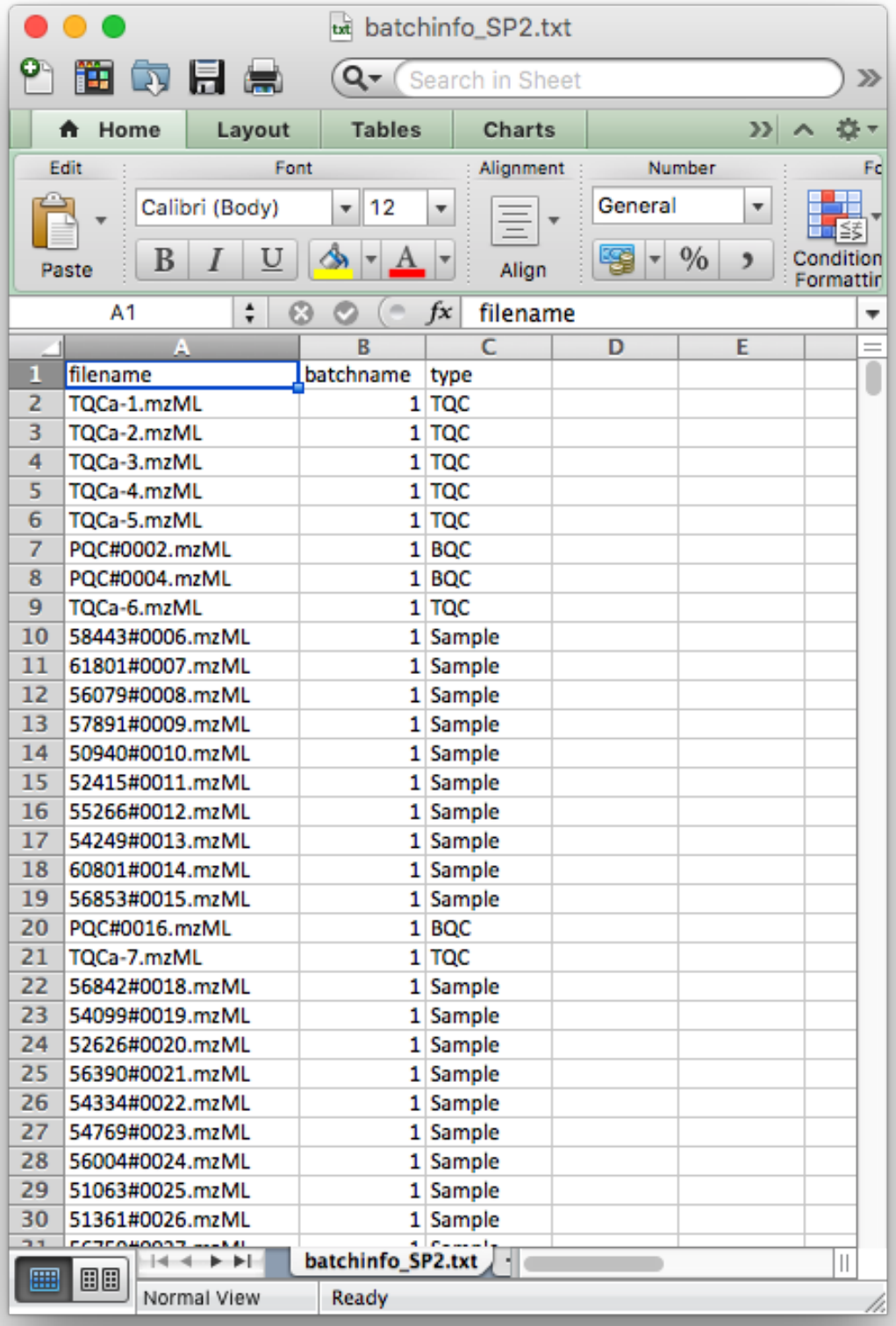
- **batch_adjusted.txt**: Quantitative data (compound peak area / ISTD) after correction of time trends and batch effects
- **quant_auc_rt.txt**: Integrated peak areas for target analytes and ISTDs
- **BQCtable.txt**: Batch specific and overall coefficient of variation using batch pooled QC samples (a 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)
- **IonC_{transition_name}.pdf**: Ion Chromatogram images with integrated peaks across all samples, per transition
- **users_RT.txt**: The RT selection table, refer to next section.
- **quant_table.txt**: The final quantification table after the user indicates the desired peak in "users_RT.txt".

4 User's RT selection step

Upon running the analysis, MRMkit produces the aforementioned output files, but it also reports a RT selection 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 below:

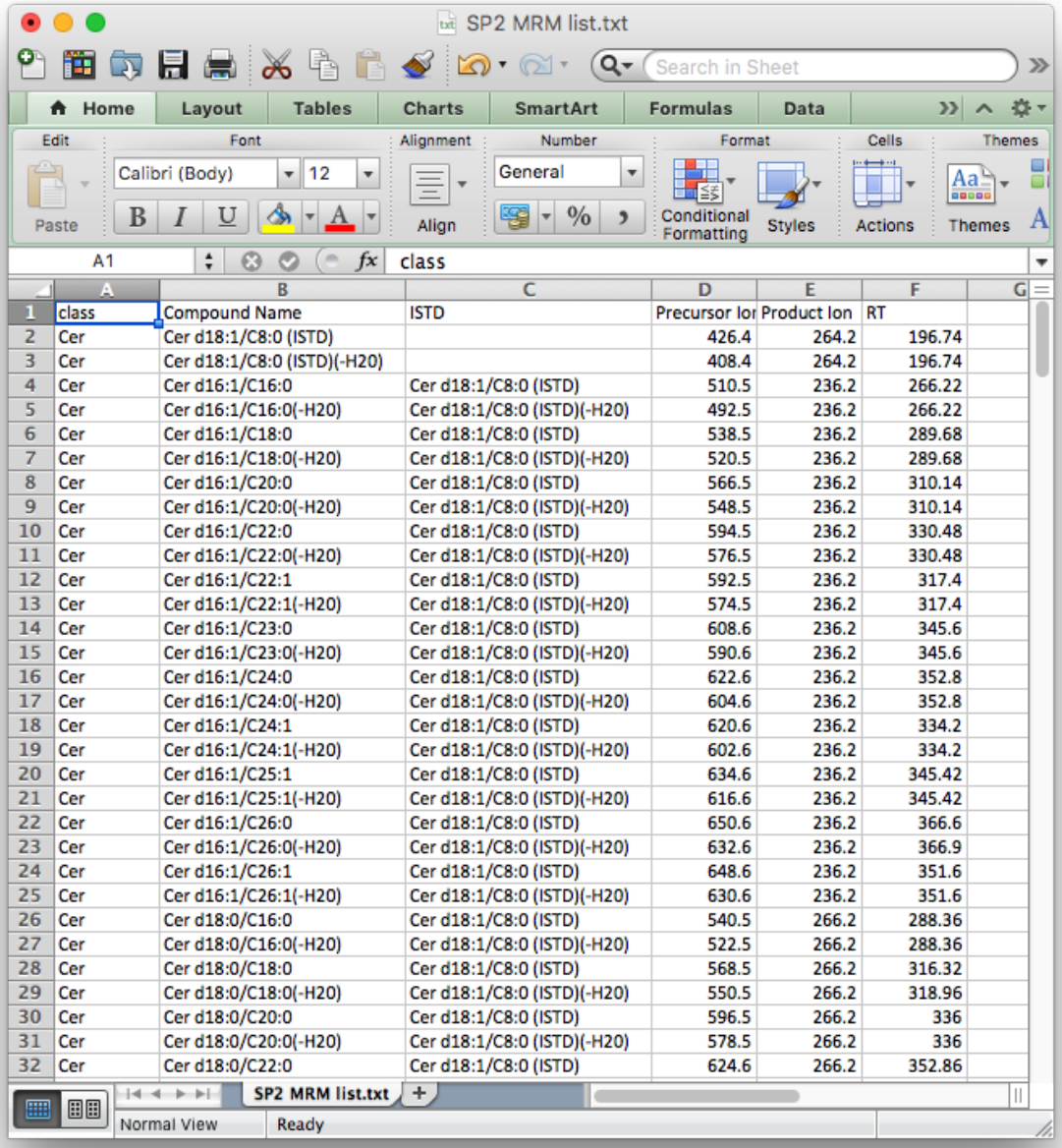
Once the user fills up this form, run the following to produce the final quantification table.

Figure 1: **batch_info** file



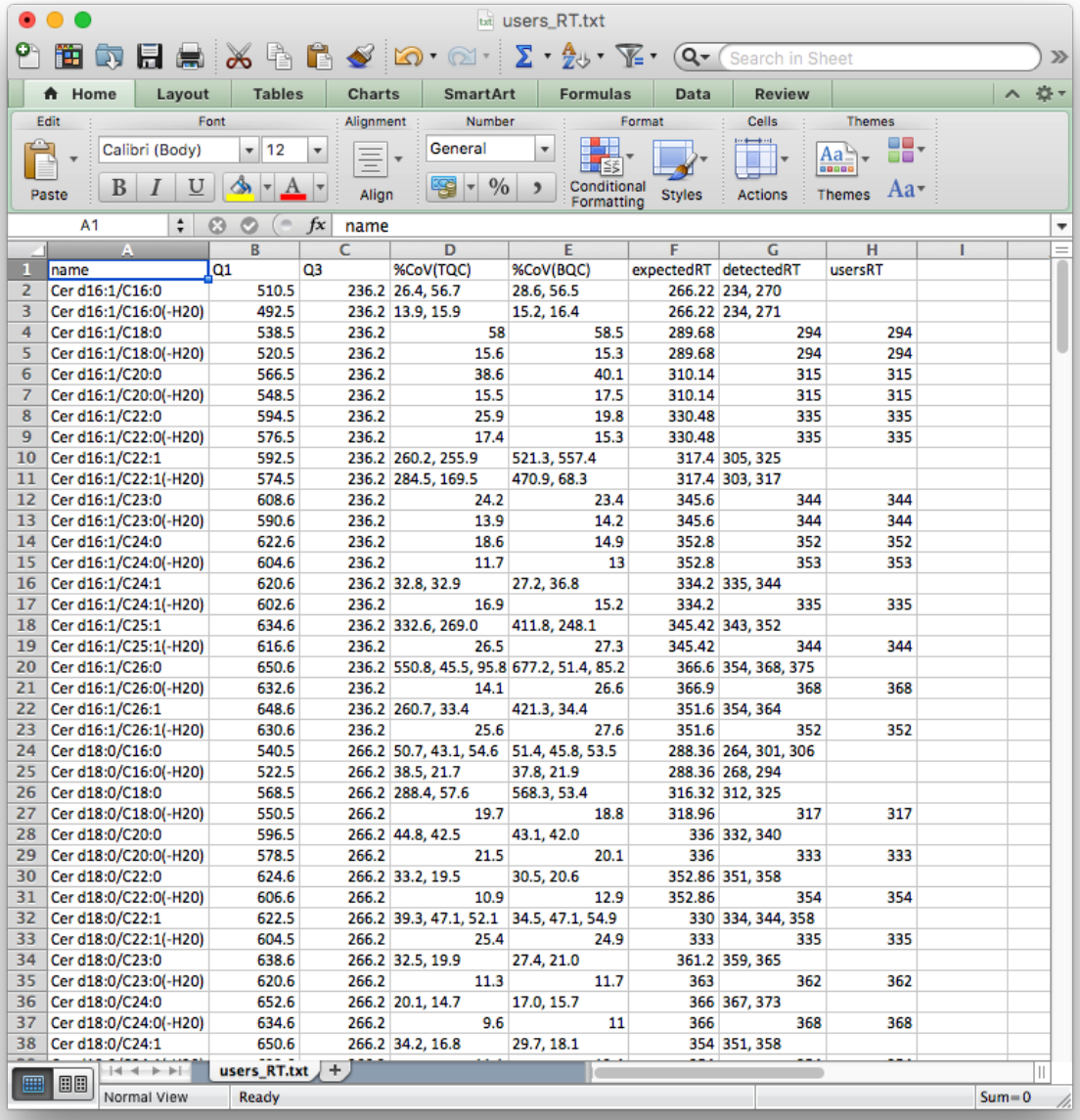
	A	B	C	D	E
1	filename	batchname	type		
2	TQCa-1.mzML	1	TQC		
3	TQCa-2.mzML	1	TQC		
4	TQCa-3.mzML	1	TQC		
5	TQCa-4.mzML	1	TQC		
6	TQCa-5.mzML	1	TQC		
7	PQC#0002.mzML	1	BQC		
8	PQC#0004.mzML	1	BQC		
9	TQCa-6.mzML	1	TQC		
10	58443#0006.mzML	1	Sample		
11	61801#0007.mzML	1	Sample		
12	56079#0008.mzML	1	Sample		
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



	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

Figure 3: **users_RT** file



	A	B	C	D	E	F	G	H	I
1	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	266.22	234, 270		
3	Cer d16:1/C16:0(-H20)	492.5	236.2	13.9, 15.9	15.2, 16.4	266.22	234, 271		
4	Cer d16:1/C18:0	538.5	236.2	58	58.5	289.68	294	294	
5	Cer d16:1/C18:0(-H20)	520.5	236.2	15.6	15.3	289.68	294	294	
6	Cer d16:1/C20:0	566.5	236.2	38.6	40.1	310.14	315	315	
7	Cer d16:1/C20:0(-H20)	548.5	236.2	15.5	17.5	310.14	315	315	
8	Cer d16:1/C22:0	594.5	236.2	25.9	19.8	330.48	335	335	
9	Cer d16:1/C22:0(-H20)	576.5	236.2	17.4	15.3	330.48	335	335	
10	Cer d16:1/C22:1	592.5	236.2	260.2, 255.9	521.3, 557.4	317.4	305, 325		
11	Cer d16:1/C22:1(-H20)	574.5	236.2	284.5, 169.5	470.9, 68.3	317.4	303, 317		
12	Cer d16:1/C23:0	608.6	236.2	24.2	23.4	345.6	344	344	
13	Cer d16:1/C23:0(-H20)	590.6	236.2	13.9	14.2	345.6	344	344	
14	Cer d16:1/C24:0	622.6	236.2	18.6	14.9	352.8	352	352	
15	Cer d16:1/C24:0(-H20)	604.6	236.2	11.7	13	352.8	353	353	
16	Cer d16:1/C24:1	620.6	236.2	32.8, 32.9	27.2, 36.8	334.2	335, 344		
17	Cer d16:1/C24:1(-H20)	602.6	236.2	16.9	15.2	334.2	335	335	
18	Cer d16:1/C25:1	634.6	236.2	332.6, 269.0	411.8, 248.1	345.42	343, 352		
19	Cer d16:1/C25:1(-H20)	616.6	236.2	26.5	27.3	345.42	344	344	
20	Cer d16:1/C26:0	650.6	236.2	550.8, 45.5, 95.8	677.2, 51.4, 85.2	366.6	354, 368, 375		
21	Cer d16:1/C26:0(-H20)	632.6	236.2	14.1	26.6	366.9	368	368	
22	Cer d16:1/C26:1	648.6	236.2	260.7, 33.4	421.3, 34.4	351.6	354, 364		
23	Cer d16:1/C26:1(-H20)	630.6	236.2	25.6	27.6	351.6	352	352	
24	Cer d18:0/C16:0	540.5	266.2	50.7, 43.1, 54.6	51.4, 45.8, 53.5	288.36	264, 301, 306		
25	Cer d18:0/C16:0(-H20)	522.5	266.2	38.5, 21.7	37.8, 21.9	288.36	268, 294		
26	Cer d18:0/C18:0	568.5	266.2	288.4, 57.6	568.3, 53.4	316.32	312, 325		
27	Cer d18:0/C18:0(-H20)	550.5	266.2	19.7	18.8	318.96	317	317	
28	Cer d18:0/C20:0	596.5	266.2	44.8, 42.5	43.1, 42.0	336	332, 340		
29	Cer d18:0/C20:0(-H20)	578.5	266.2	21.5	20.1	336	333	333	
30	Cer d18:0/C22:0	624.6	266.2	33.2, 19.5	30.5, 20.6	352.86	351, 358		
31	Cer d18:0/C22:0(-H20)	606.6	266.2	10.9	12.9	352.86	354	354	
32	Cer d18:0/C22:1	622.5	266.2	39.3, 47.1, 52.1	34.5, 47.1, 54.9	330	334, 344, 358		
33	Cer d18:0/C22:1(-H20)	604.5	266.2	25.4	24.9	333	335	335	
34	Cer d18:0/C23:0	638.6	266.2	32.5, 19.9	27.4, 21.0	361.2	359, 365		
35	Cer d18:0/C23:0(-H20)	620.6	266.2	11.3	11.7	363	362	362	
36	Cer d18:0/C24:0	652.6	266.2	20.1, 14.7	17.0, 15.7	366	367, 373		
37	Cer d18:0/C24:0(-H20)	634.6	266.2	9.6	11	366	368	368	
38	Cer d18:0/C24:1	650.6	266.2	34.2, 16.8	29.7, 18.1	354	351, 358		

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.

5 Example "param.txt"

```
mzML_files
*.mzML

batch_info
batchinfo.txt

length_of_ion_chromatogram
4 40

transition_list
SP2 MRM list.txt

ISTD_trace_all
1

batch_correction
1
```

6 Copyrights