Supplementary Information for MRMQuant: Automated MRM Data Quantitation for Large-Scale Targeted Metabolomics Analysis

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1. The bile acid dataset for MRMQuant Demonstration

We adopted a dataset extracted from a human aging study to demonstrate the "absolute quantitation using standard curve" experiment type in MRMQuant. The data preparation procedures are provided below. This dataset consisted of 45 testing samples (including five QCs, as listed in Table 1) of 74 bile acids in 53 ion chromatograms (18 of which involved 2–3 isomers). The abbreviated name of the 74 bile acids and other information in the method file is shown in Table 2 and the full name of the 74 bile acids and their corresponding abbreviations is listed in Table 3.

Ultraperformance Liquid Chromatography−Mass Spectrometry (UPLC−MS)

100 μL plasma sample was extracted with 300 μL methanol containing internal standards with liquid-liquid extraction. Sample of mixture was stayed on ice for 30 min and then centrifuged with 12,000 rpm for 30 min at 4 °C for protein precipitation. The supernatant was transferred to sample vial, and analyzed in a LC-MS system (UPLC with Xevo TQ-S MS, Waters, Manchester, UK) with negative electrospray ionization mode with multiple reaction monitoring. The chromatographic separation was achieved on an Acquity BEH C8 reversed-phase column (2.1 × 100-mm i.d., 1.7 μm, Waters Corp., Milford, USA) at 60 °C with mobile phase A (10% acetonitrile with 0.01% formic acid) and mobile phase B (acetonitrile / isopropanol, 1:1, v/v with 0.01% formic acid) and the flow rate was set at 0.6 ml/min. The gradient profile was as follows: keep 10% B for 0.1 min, linear gradient 10–35% B, 9.15 min, 35–85% B, 2.25 min, 85–99% B, 0.5 min, and keep 99% B, 0.5 min. The column was then re-equilibrated for 3 min. The parameters of MSMS were as follows: capillary voltage 1.5 kV; desolvation gas flow 1000 L/h; desolvation temperature 600 °C; source temperature 150 °C, and voltage 60 V. System operation and data acquisition were controlled using Mass Lynx software, and targeted metabolic data were analyzed by TargetLynx (Waters, Milford, USA).

Table 1. Sample name of the 45 testing samples

Sample names	Sample names	Sample names
20220331_a_QC3_std M_01	20220331_Aging plasma_14	20220331_Aging plasma_28
20220331_Aging plasma_01	20220331_Aging plasma_15	20220331_Aging plasma_29
20220331_Aging plasma_02	20220331_Aging plasma_16	20220331_Aging plasma_30
20220331_Aging plasma_03	20220331_Aging plasma_17	20220331_a_QC3_std M_04
20220331_Aging plasma_04	20220331_Aging plasma_18	20220331_Aging plasma_31
20220331_Aging plasma_05	20220331_Aging plasma_19	20220331_Aging plasma_32
20220331_Aging plasma_06	20220331_Aging plasma_20	20220331_Aging plasma_33
20220331_Aging plasma_07	20220331_a_QC3_std M_03	20220331_Aging plasma_34
20220331_Aging plasma_08	20220331_Aging plasma_21	20220331_Aging plasma_35
20220331_Aging plasma_09	20220331_Aging plasma_22	20220331_Aging plasma_36
20220331_Aging plasma_10	20220331_Aging plasma_23	20220331_Aging plasma_37
20220331_a_QC3_std M_02	20220331_Aging plasma_24	20220331_Aging plasma_38
20220331_Aging plasma_11	20220331_Aging plasma_25	20220331_Aging plasma_39
20220331_Aging plasma_12	20220331_Aging plasma_26	20220331_Aging plasma_40
20220331_Aging plasma_13	20220331_Aging plasma_27	20220331_a_QC3_std M_05

Table 2. The 74 bile acid (abbreviated) names and other information in the method file

Compound_Names	RT	RT_tolerance	Precursor_m/z	Product_m/z	Internal_Standard
BA97	3.38	0.1	288.69	96.954	IS71(IS)
BA97x	3.38	0.1	578.25	498.29	IS71(IS)
BA105X	3.89	0.1	296.69	96.954	IS71(IS)
BA105	3.89	0.1	594.24	514.28	IS71(IS)
BA99_BA100	5.43;5.79	0.1	288.69	96.954	IS71(IS)
BA99x_BA100x	5.43;5.79	0.1	578.25	498.29	IS71(IS)
BA96	7.37	0.1	280.69	96.954	IS71(IS)
BA96x	7.37	0.1	562.25	482.29	IS71(IS)
BA01	11.6	0.02	359.29	359.28	IS71(IS)
BA03_BA04	9.96;10.61	0.05	373.27	373.27	IS71(IS)
BA06_BA05	10.37;10.75	0.05	375.29	375.28	IS71(IS)
BA09	9.14	0.1	377.27	377.26	IS71(IS)
BA12_BA10	7.46;7.75	0.05	387.25	387.24	IS71(IS)
BA16	9.43	0.1	389.26	389.25	IS71(IS)
BA17_BA18_BA19	8.01;8.17;8.52	0.05	389.27	389.26	IS71(IS)
BA20	6.91	0.1	391.28	391.27	IS71(IS)
BA32_BA25_BA22	7.7;8.21;8.52	0.05	391.28	391.27	IS71(IS)
BA24_BA23_BA21	10.07;10.22;10.51	0.05	391.28	391.27	IS71(IS)
IS64	10.22	0.1	397.37	397.36	IS71(IS)
BA26	3.52	0.1	401.23	401.22	IS71(IS)
BA28	7.81	0.1	403.25	403.24	IS71(IS)
BA29_BA30	5.82;6.88	0.05	405.26	405.25	IS71(IS)
BMRC20	4.74	0.1	407.28	407.27	IS71(IS)
BA37_BA34	6.19;6.28	0.05	407.28	407.27	IS71(IS)
BA36_BA35	7.39;7.96	0.1	407.28	407.27	IS71(IS)
BA43_BA40	5.63;6.01	0.05	448.31	74	IS71(IS)
BA41_BA42	8.17;8.68	0.1	448.2	74	IS71(IS)
BA39	10.2	0.1	432.31	74	IS71(IS)
BA64	10.19	0.1	455.25	96.953	IS71(IS)
BA44	2.12	0.05	458.25	74	IS71(IS)
BA49	5.28	0.1	464.28	74	IS71(IS)
BA46	6.16	0.1	464.3	74	IS71(IS)
BA47	11.82	0.1	466.3	80	IS71(IS)
IS71(IS)	6.16	0.1	468.43	74.1	IS71(IS)
BA77	6	0.05	471.24	96.953	IS71(IS)
BA75_BA78	8.37;8.66	0.1	471.24	96.953	IS71(IS)
BA48	9.9	0.1	482.29	80	IS71(IS)
BA87	6.42	0.1	487.24	96.953	IS71(IS)
BA50_BA51	5.1;5.37	0.1	498.29	80	IS71(IS)
BA52_BA53	7.57;8.09	0.1	498.29	80	IS71(IS)
IS73	7.57	0.1	502.44	80.03	IS71(IS)
BA54	1.77	0.1	508.24	80	IS71(IS)
BA89x	8.34	0.1	512.27	96.954	IS71(IS)
BA89	8.34	0.1	512.27	432.31	IS71(IS)
BA57	3.53	0.1	514.28	80	IS71(IS)
BA55	4.67	0.1	514.28	80	IS71(IS)
BA59	5.64	0.1	514.28	80	IS71(IS)
BA90x	4.09	0.1	528.26	96.954	IS71(IS)
BA90	4.09	0.1	528.26	448.31	IS71(IS)
BA92x_BA93x	6.34;6.72	0.1	528.26	96.954	IS71(IS)
BA92_BA93	6.34;6.72	0.1	528.26	448.31	IS71(IS)
BA94x	4.67	0.1	544.26	96.954	IS71(IS)
BA94	4.67	0.1	544.26	464.3	IS71(IS)

x or X denotes isomer of the corresponding compound

Table 3. The full name of the compounds and their abbreviations

Group	Compound name	Abbreviation
FreeBA	Ursocholanic Acid	BA01
FreeBA	5-Cholenic Acid-3β-ol	BA03
FreeBA	3-Ketocholanic acid	BA04
FreeBA	Lithocholic acid	BA05
FreeBA	Isolithocholic acid	BA06
FreeBA	3a,12a, 23-Nordeoxycholic acid	BA09
FreeBA	9(11), (5β)-Cholenic Acid-3α-ol-12-one	BA10
FreeBA	3,7-Diketocholanic Acid	BA12
FreeBA	5β-Cholenic Acid-7α-ol-3-one	BA16
FreeBA	5α-Cholanic Acid-3α-ol-6-one	BA17
FreeBA	3α-hydroxy-7 ketolithocholic acid	BA18
FreeBA	3α-hydroxy-12 ketolithocholic acid	BA19
FreeBA	Murocholic acid	BA20
FreeBA	Isodeoxycholic acid	BA21
FreeBA	5β-Cholanic Acid-3β, 12α-diol	BA22
FreeBA	Deoxycholic acid	BA23
FreeBA	Chenodeoxycholic acid	BA24
FreeBA	Hyodeoxycholic acid	BA25
FreeBA	3,7,12 Dehydrocholic acid	BA26
FreeBA	3α-hydroxy-6,7-Diketocholanic acid	BA28
FreeBA	5β-Cholanic acid-3α, 6α-diol-7-one	BA29
FreeBA	3-Dehydrocholic acid	BA30
FreeBA	Ursodeoxycholic acid	BA32
FreeBA	β-Muricholic acid	BA34
FreeBA	Cholic acid	BA35
FreeBA	Hyocholic acid	BA36
FreeBA	alpha-Muricholic acid	BA37
FreeBA	Ursocholic acid	BMRC20
FreeBA	Deoxycholic Acid-d6	IS64
GlycoBA	Glycolithocholic acid	BA39
GlycoBA	Glycohyodeoxycholic acid	BA40
GlycoBA	Glycochenodeoxycholic acid	BA41
GlycoBA	Glycodeoxycholic acid	BA42
GlycoBA	Glycoursodeoxycholic acid	BA43
GlycoBA	3,7,12- Glycodehydrocholic acid	BA44
GlycoBA	Glycocholic acid	BA46
GlycoBA	Glycohyocholic acid	BA49
GlycoBA	Glycocholic Acid-d4	IS71
TauroBA	Tauro-ursocholanic Acid	BA47
TauroBA	Taurolithocholic acid	BA48
TauroBA	Tauro-ursodeoxycholic acid	BA50
TauroBA	Taurohyodeoxycholic acid	BA51
TauroBA	Taurochenodeoxycholic acid	BA52
TauroBA	Taurodeoxycholic acid	BA53
TauroBA	3,7,12 Taurodehydrocholic acid	BA54
TauroBA	Taurohyocholic acid sodium salt	BA55
TauroBA	Tauro alpha-Muricholic acid sodium salt	BA57
TauroBA	Taurocholic acid	BA59
TauroBA	Taurochenodeoxycholic Acid-d4	IS73
freeBA-sulfate	lithocholic acid-3-sulfate	BA64
freeBA-sulfate	chenodeoxycholic acid-3-sulfate	BA75
freeBA-sulfate	Ursodeoxycholic acid-3-sulfate	BA77
freeBA-sulfate	Deoxycholic acid-3-sulfate	BA78
freeBA-sulfate	cholic acid-3-sulfate	BA87
GlycoBA-sulfate	Glycolithocholic acid-3-sulfate	BA89
GlycoBA-sulfate	Glycoursodeoxycholic acid-3-sulfate	BA90
GlycoBA-sulfate	Glycochenodeoxychlolic acid-3-sulfate	BA92
GlycoBA-sulfate	Glycodeoxycholic acid-3-sulfate	BA93
GlycoBA-sulfate	Glycocholic acid-3-sulfate	BA94
TauroBA-sulfate	Taurolithocholic acid-3-sulfate	BA96
TauroBA-sulfate	Tauroursodeoxycholic acid-3-sulfate	BA97
TauroBA-sulfate	Taurochenodeoxycholic acid-3-sulfate	BA99
TauroBA-sulfate	Taurodeoxycholic acid-3-sulfate	BA100
TauroBA-sulfate	Taurocholic acid-3-sulfate	BA105

2. The Phospholipid Dataset for Tool Comparisons

We constructed a dataset consisting of eight technical replicates (as shown in Table 4) of a QC sample extracted from a human aging study to compare the quantitation accuracy of five existing tools (MRMKit, MRMQuant, OpenMS, Skyline, TargetLynx) that are capable of performing MRM data quantitation. The data preparation procedures are provided below. Each replicate contained 184 targeted phospholipids from human plasma, some of which were isomers that appeared on the same chromatogram. However, as some tools are incapable of handling multiple compounds in an ion chromatogram, only the first compound was retained in each chromatogram, resulting in 163 out of the original 184 phospholipids in the method file, as listed in Table 5. In addition, the boxplots of normalized abundances of the 163 phospholipids among the 8 QC sample replicates of the five tools are presented in Figure 1, whereas the top-20 compounds that exhibited the highest variation across replicates are listed in Table 6. Finally, some examples of problematic quantitations in similar tools are presented in Figure 7.

Ultraperformance Liquid Chromatography−Mass Spectrometry (UPLC−MS)

For lipid extraction, 10 μl of the human plasma sample was added to a new microtube. Then, 470 μl of IPA and 20 μl IS solution (Avanti 330709 10 ul; Sigma 605581 5 μg/mL 10ul) and vortexed for 1 minute. The mixture was left at room temperature for 30 min to allow for protein precipitation. The sample was then centrifuged at 12000 ×g for 30 minutes at 4°C, 400 ul of the supernatant was collected, and add 400 ul acetonitrile/water (1:1, v/v). After vortexed for 1 minute, the sample was further diluted by adding 900 μL IPA/ACN/H_2O=2/1/1 mixture with 100 ul sample. The analysis was conducted using Waters ultra-high-performance liquid chromatography coupled with Waters Xevo TQ-S system (Waters, Milford, MA, USA) in positive-ion electrospray ionization (ESI) mode. Chromatographic separation was performed on a Waters ACQUITY BEH C18 column (2.1 mm × 100 mm × 1.7 μm). Column temperature was maintained at 60°C. The mobile phase A was acetonitrile/water (40:60, v/v) with 10 mM ammonium formate, while the mobile phase B was isopropanol/acetonitrile (90:10, v/v) with 10 mM ammonium formate. The flow rate was 0.45 mL/min, and the solvent gradient was as follows: 0–10 min, 40–99% solvent B; 10–10.1 min, 99–40% solvent B; 10.1–12 min, 40% solvent B. The capillary voltage was set at 1000 V for ESI positive ion and 500 V for ESI negative ion. The sampling cone voltage was set at 30 V. The desolvation gas flow rate was set at 1000 L/h, and the cone gas flow was maintained at 150 L/h. The desolvation and source temperatures were set at 500°C and 150°C, respectively. A mixture of all samples, called the Mix QC sample, was prepared and analyzed after every 10th sample during the analytical runs.

Table 4. Name of the eight replicates

Sample Name
20200426_plate2_QC3_A1
20200426_plate2_QC3_A2
20200426_plate2_QC3_A3
20200426_plate2_QC3_A4
20200426_plate2_QC3_A5
20200426_plate2_QC3_A6
20200426_plate2_QC3_A7
20200426_plate2_QC3_A8

Table 5. The 163 phospholipid names and other information in the method file.

Compound Names	RT	RT tolerance	Precursor m/z	Product m/z	Internal Standard	Concentration
LPE 16:0	1.6	0.2	454.2928	313.2928	LPE 18:1 (d7)	1
LPE 18:2	1.34	0.2	478.3085	337.3085	LPE 18:1 (d7)	1
LPE 18:1	1.7	0.2	480.3085	339.3085	LPE 18:1 (d7)	1
LPE 18:0	2.2	0.2	482.3241	341.3241	LPE 18:1 (d7)	1
LPE 18:1 (d7)	1.68	0.2	487.3085	346.3085	LPE 18:1 (d7)	1
LPE 20:4	1.29	0.2	502.2928	361.2928	LPE 18:1 (d7)	1
LPE 22:6	1.23	0.2	526.2928	385.2928	LPE 18:1 (d7)	1
LPC 14:0	1.08	0.2	468.3085	184.07	LPC 18:1 (d7)	45
LPC(P-16:0/0:0)	1.72	0.2	480.3449	104.08	LPC 18:1 (d7)	45
LPC(O-16:0/0:0)	1.78	0.2	482.3605	104.08	LPC 18:1 (d7)	45
LPC 15:0	1.29	0.2	482.3605	184.07	LPC 18:1 (d7)	45
LPC 16:1	1.16	0.2	494.3241	184.07	LPC 18:1 (d7)	45
LPC 16:0	1.53	0.2	496.3398	184.07	LPC 18:1 (d7)	45
LPC 17:1	1.38	0.2	508.3398	184.07	LPC 18:1 (d7)	45
LPC 17:0	1.82	0.2	510.3554	184.07	LPC 18:1 (d7)	45
LPC 18:2	1.28	0.2	520.3398	184.07	LPC 18:1 (d7)	45
LPC 18:1	1.62	0.2	522.3554	184.07	LPC 18:1 (d7)	45
LPC 18:1 (d7)	1.61	0.2	529.3054	184.07	LPC 18:1 (d7)	45
LPC 20:5	1.05	0.2	542.3241	184.07	LPC 18:1 (d7)	45
LPC 20:4	1.24	0.2	544.3398	184.07	LPC 18:1 (d7)	45
LPC 20:3	1.43	0.2	546.3554	184.07	LPC 18:1 (d7)	45
LPC 20:2	1.75	0.2	548.3711	184.07	LPC 18:1 (d7)	45
LPC 22:6	1.18	0.2	568.3398	184.07	LPC 18:1 (d7)	45
LPC 22:5	1.45	0.1	570.3398	184.07	LPC 18:1 (d7)	45
LPC(P-18:0/0:0)	2.37	0.2	508.3398	104.08	LPC 18:1 (d7)	45
LPC(O-18:0/0:0)	2.43	0.2	510.3554	104.08	LPC 18:1 (d7)	45
LPC 18:0	2.13	0.2	524.3711	184.07	LPC 18:1 (d7)	45
LPC 19:0	2.46	0.2	538.38	184.07	LPC 18:1 (d7)	45
LPC 20:1	2.2	0.2	550.3867	184.07	LPC 18:1 (d7)	45
LPC 20:0	2.82	0.2	552.4024	184.07	LPC 18:1 (d7)	45
LPC 22:0	3.54	0.2	580.43	184.07	LPC 18:1 (d7)	45
LPC 24:0	4.24	0.2	608.47	184.07	LPC 18:1 (d7)	45
SM C12:0	3.75	0.2	647.52	184.07	SM C18:1 (d9)	40
SM C13:0	4.09	0.2	661.53	184.07	SM C18:1 (d9)	40
SM C14:1	3.87	0.2	673.54	184.07	SM C18:1 (d9)	40
SM C14:0	4.43	0.2	675.55	184.07	SM C18:1 (d9)	40
Dihydro SM C14:0	4.66	0.12	677.53	184.07	SM C18:1 (d9)	40
SM C16:1	4.53	0.2	701.59	184.07	SM C18:1 (d9)	40
CE 18:3	8.22	0.2	664.58	369.35	CE 18:1 (d7)	530
CE 18:2	8.41	0.2	666.59	369.35	CE 18:1 (d7)	530
CE 18:1 (d7)	8.62	0.2	675.61	369.35	CE 18:1 (d7)	530
CE 20:5	8.07	0.2	688.58	369.35	CE 18:1 (d7)	530
CE 20:4	8.29	0.2	690.59	369.35	CE 18:1 (d7)	530
CE 20:3	8.45	0.15	692.61	369.35	CE 18:1 (d7)	530
CE 22:6	8.17	0.2	714.59	369.35	CE 18:1 (d7)	530
PC 28:1	4.43	0.2	676.46	184.07	PC 33:1 (d7)	210
PC 32:2	4.71	0.2	730.5538	184.07	PC 33:1 (d7)	210
PC 32:1	5.18	0.2	732.5538	184.07	PC 33:1 (d7)	210
PC 32:0	5.62	0.2	734.5694	184.07	PC 33:1 (d7)	210
PC 33:1 (d7)	5.4	0.2	753.54	184.07	PC 33:1 (d7)	210
PC 34:4	4.64	0.2	754.55	184.07	PC 33:1 (d7)	210
PC 34:3a	4.79	0.05	756.5538	184.07	PC 33:1 (d7)	210
PC 34:2	5.3	0.2	758.5694	184.07	PC 33:1 (d7)	210
PC 34:1	5.65	0.2	760.5851	184.07	PC 33:1 (d7)	210
PC 36:6	4.5	0.2	778.54	184.07	PC 33:1 (d7)	210
PC 36:5	4.84	0.2	780.553	184.07	PC 33:1 (d7)	210
PC 36:4a	4.91	0.15	782.5694	184.07	PC 33:1 (d7)	210
PC 36:3a	5.35	0.05	784.5851	184.07	PC 33:1 (d7)	210
PC 38:7	4.6	0.05	804.55	184.07	PC 33:1 (d7)	210

PC 38:6a	4.87	0.1	806.5694	184.07	PC 33:1 (d7)	210
PC 38:5a	5.28	0.1	808.5851	184.07	PC 33:1 (d7)	210
PC 40:8	4.69	0.2	830.5694	184.07	PC 33:1 (d7)	210
PC 40:7	5.13	0.2	832.5694	184.07	PC 33:1 (d7)	210
PC 40:6	5.62	0.2	834.5694	184.07	PC 33:1 (d7)	210
SM C15:0	4.77	0.1	689.56	184.07	SM C18:1 (d9)	40
SM C16:0	5.08	0.2	703.57	184.07	SM C18:1 (d9)	40
Dihydro SM C16:0	5.28	0.15	705.59	184.07	SM C18:1 (d9)	40
SM C17:0	5.32	0.2	717.59	184.07	SM C18:1 (d9)	40
SM C18:1	5.13	0.2	729.59	184.07	SM C18:1 (d9)	40
SM C18:0	5.61	0.2	731.61	184.07	SM C18:1 (d9)	40
SM C18:1 (d9)	5.15	0.2	738.54	184.07	SM C18:1 (d9)	40
SM C20:1	5.67	0.1	757.64	184.07	SM C18:1 (d9)	40
PCe 30:0	5.41	0.2	692.53	184.07	PCP 36:1 (d9)	10
PCp 32:0	5.85	0.2	718.54	184.07	PCP 36:1 (d9)	10
PCe 32:0	5.91	0.2	720.54	184.07	PCP 36:1 (d9)	10
PCp 34:2	5.51	0.2	742.62	184.07	PCP 36:1 (d9)	10
PCe 34:2	5.62	0.1	744.62	184.07	PCP 36:1 (d9)	10
PCe 34:1	5.95	0.2	746.62	184.07	PCP 36:1 (d9)	10
PCe 36:5	5.2	0.1	766.57	184.07	PCP 36:1 (d9)	10
PCe 36:4	5.54	0.2	768.59	184.07	PCP 36:1 (d9)	10
PCe 36:3	5.75	0.05	770.61	184.07	PCP 36:1 (d9)	10
PCe 38:6	5.42	0.05	792.59	184.07	PCP 36:1 (d9)	10
PCe 38:5	5.58	0.1	794.61	184.07	PCP 36:1 (d9)	10
PCe 40:6	5.62	0.1	820.62	184.07	PCP 36:1 (d9)	10
PEp 34:2	5.64	0.2	700.5276	364.2	PE 33:1 (d7)	0.7
PE 33:1 (d7)	5.51	0.2	711.48	570.5381	PE 33:1 (d7)	7
PE 34:2	5.4	0.2	716.5225	575.5225	PE 33:1 (d7)	7
PEp 36:5	5.23	0.2	722.51	364.2	PE 33:1 (d7)	0.7
PEp 36:4	5.57	0.2	724.5276	364.2	PE 33:1 (d7)	0.7
PEp 36:3	5.69	0.2	726.5276	390.2	PE 33:1 (d7)	0.7
PE 36:4	5.33	0.2	740.5225	599.5225	PE 33:1 (d7)	7
PE 36:3	5.44	0.2	742.5381	601.5381	PE 33:1 (d7)	7
PEp 38:6	5.43	0.2	748.5276	364.2	PE 33:1 (d7)	0.7
PEp 38:5	5.61	0.2	750.5432	390.2	PE 33:1 (d7)	0.7
PE 38:6	5.2	0.2	764.5222	623.5225	PE 33:1 (d7)	7
PE 38:5	5.4	0.1	766.5381	625.5381	PE 33:1 (d7)	7
PEp 40:7	5.48	0.2	774.5432	390.2	PE 33:1 (d7)	0.7
PE 40:7	5.24	0.2	790.5381	649.5381	PE 33:1 (d7)	7
PEp 34:1	6.03	0.2	702.5432	364.2	PE 33:1 (d7)	0.7
PE 34:1	5.76	0.2	718.5381	577.5381	PE 33:1 (d7)	7
PEp 36:2	6.12	0.2	728.5589	392.2	PE 33:1 (d7)	0.7
PEp 36:1	6.45	0.2	730.5745	392.2	PE 33:1 (d7)	0.7
PEP 36:1 (d9)	6.46	0.2	739.63	392.2	PEP 36:1 (d9)	0.7
PE 36:2	5.89	0.2	744.5538	603.5538	PE 33:1 (d7)	7
PE 36:1	6.27	0.1	746.5694	605.5694	PE 33:1 (d7)	7
PEp 38:4	6.05	0.2	752.5582	392.2	PE 33:1 (d7)	0.7
PE 38:4	5.83	0.2	768.5538	627.5538	PE 33:1 (d7)	7
PE 38:3	6.04	0.1	770.55	629.55	PE 33:1 (d7)	7
PEp 40:6	5.93	0.2	776.5589	392.2	PE 33:1 (d7)	0.7
PEp 40:4	6.47	0.2	780.59	420.2	PE 33:1 (d7)	0.7
PE 40:6	5.7	0.2	792.5532	651.5538	PE 33:1 (d7)	7
PE 40:5	5.87	0.1	794.5694	653.5694	PE 33:1 (d7)	7
Dihydro SM C18:0	5.83	0.1	733.62	184.07	SM C18:1 (d9)	40
SM C19:0	5.91	0.1	745.63	184.07	SM C18:1 (d9)	40
SM C20:0	6.12	0.1	759.64	184.07	SM C18:1 (d9)	40
Dihydro SM C20:0	6.28	0.1	761.65	184.07	SM C18:1 (d9)	40
SM C21:0	6.35	0.1	773.66	184.07	SM C18:1 (d9)	40
SM C22:2	5.71	0.1	783.64	184.07	SM C18:1 (d9)	40
SM C22:1	6.18	0.1	785.65	184.07	SM C18:1 (d9)	40
SM C22:0	6.56	0.1	787.67	184.07	SM C18:1 (d9)	40

SM C23:1a	6.31	0.02	799.68	184.07	SM C18:1 (d9)	40
SM C24:3	5.77	0.1	809.67	184.07	SM C18:1 (d9)	40
SM C24:2	6.16	0.1	811.68	184.07	SM C18:1 (d9)	40
SM C24:1	6.5	0.2	813.69	184.07	SM C18:1 (d9)	40
SM C26:2	6.58	0.1	839.71	184.07	SM C18:1 (d9)	40
PCe 34:0	6.4	0.1	748.62	184.07	PCP 36:1 (d9)	10
PCe 36:2	6.1	0.1	772.62	184.07	PCP 36:1 (d9)	10
PCe 36:1a	6.35	0.04	774.62	184.07	PCP 36:1 (d9)	10
PCP 36:1 (d9)	6.33	0.2	781.68	184.07	PCP 36:1 (d9)	10
PCe 38:4	6.03	0.1	796.62	184.07	PCP 36:1 (d9)	10
PCe 38:3	6.22	0.1	798.64	184.07	PCP 36:1 (d9)	10
PCe 38:2	6.39	0.1	800.65	184.07	PCP 36:1 (d9)	10
PCe 40:5	6.03	0.2	822.61	184.07	PCP 36:1 (d9)	10
PCe 40:4	6.44	0.2	824.62	184.07	PCP 36:1 (d9)	10
PCe 40:3	6.5	0.2	826.64	184.07	PCP 36:1 (d9)	10
PCe 42:6	6.09	0.1	848.64	184.07	PCP 36:1 (d9)	10
PCe 42:5	6.42	0.2	850.64	184.07	PCP 36:1 (d9)	10
PCe 44:6	6.47	0.2	876.65	184.07	PCP 36:1 (d9)	10
PC 34:0	6.13	0.1	762.6007	184.07	PC 33:1 (d7)	210
PC 36:2	5.78	0.2	786.6007	184.07	PC 33:1 (d7)	210
PC 36:1a	6.17	0.1	788.6164	184.07	PC 33:1 (d7)	210
PC 36:0	6.7	0.1	790.63	184.07	PC 33:1 (d7)	210
PC 38:4	5.72	0.2	810.6007	184.07	PC 33:1 (d7)	210
PC 38:3a	5.95	0.1	812.6007	184.07	PC 33:1 (d7)	210
PC 38:2a	6.23	0.1	814.6007	184.07	PC 33:1 (d7)	210
PC 38:0	6.94	0.1	818.67	184.07	PC 33:1 (d7)	210
PC 40:5a	5.77	0.1	836.5694	184.07	PC 33:1 (d7)	210
PC 40:4a	6.08	0.1	838.57	184.07	PC 33:1 (d7)	210
PC 40:3ab	6.58	0.1	840.57	184.07	PC 33:1 (d7)	210
PC 40:2	6.67	0.05	842.66	184.07	PC 33:1 (d7)	210
PCe 36:0	6.77	0.1	776.62	184.07	PCP 36:1 (d9)	10
PCe 38:1	6.72	0.2	802.67	184.07	PCP 36:1 (d9)	10
PCe 42:4	6.55	0.1	852.65	184.07	PCP 36:1 (d9)	10
PCe 42:3	6.84	0.2	854.67	184.07	PCP 36:1 (d9)	10
PCe 42:2a	7.16	0.05	856.68	184.07	PCP 36:1 (d9)	10
PCe 44:5	6.79	0.2	878.67	184.07	PCP 36:1 (d9)	10
PCe 44:4	7.19	0.1	880.68	184.07	PCP 36:1 (d9)	10
Dihydro SM C22:0	6.7	0.08	789.68	184.07	SM C18:1 (d9)	40
SM C23:0	6.72	0.2	801.69	184.07	SM C18:1 (d9)	40
SM C24:0	6.93	0.2	815.7	184.07	SM C18:1 (d9)	40
Dihydro SM C24:0	7.07	0.08	817.72	184.07	SM C18:1 (d9)	40
SM C26:1	6.93	0.2	841.72	184.07	SM C18:1 (d9)	40
SM C26:0	7.29	0.2	843.73	184.07	SM C18:1 (d9)	40

Table 6. The top-20 compounds that have the highest standard deviation in the computed abundance using different tools

MRMkit	Skyline	TargetLynx	OpenMS	MRMquant
PEp34_1	PC 34:3a	PC 32:2	LPC 15:0	PEp 34:1
PE40_7	SM C23:1a	PC 34:1	PCe 36:0	PEP 36:1 (d9)
PE38_3	Dihydro SM C24:0	PC 40:6	PC 40:8	PE 40:7
PEP36_1_d9	PEp 34:1	PCp 34:2	LPC 20:1	PE 38:3
PE40_5	LPC 22:0	PEp 38:5	PCe 34:0	PE 40:5
PEp36_3	PE 40:7	Dihydro SM C18:0	SM C23:0	PEp 36:3
PEp36_1	PEp 36:5	SM C22:0	PCe 36:5	PEp 40:4
PEp34_2	PE 36:3	SM C23:1	PC 38:5a	PEp 36:5
PEp36_5	PE 38:3	PC 36:1a	PC 33:1 (d7)	PEp 36:1
PEp40_4	PEP 36:1 (d9)	PEp 34:1	SM C16:0	PEp 34:2
PE34_1	LPC 22:5	PE 40:7	PCe 42:5	PE 36:3
PEp38_5	PE 40:5	PE 40:5	LPC 18:2	PE 38:5
PE36_3	PEp 36:3	PEp 36:5	PC 34:2	LPE 18:1
LPE18_1	SM C24:3	PE 38:3	PEp 36:1	LPC 22:5

PE38_5	PEp 36:1	PEp 34:2	SM C15:0	PE 34:1
LPC22_5	PEp 34:2	PEp 40:4	PCe 42:4	PEp 38:5
PE40_6	PCe 42:4	CE 20:3	SM C14:0	PEp 40:6
SMC23_1a	PEp 40:4	PEp 36:3	PC 36:2	PE 40:6
CE20_4	PE 38:5	PEp 36:1	PC 40:6	PEp 36:4
PC40_2	LPE 18:1	LPE 18:1	SM C24:2	Dihydro SM C24:0

* Different colors in the table represent the same compound in different tools

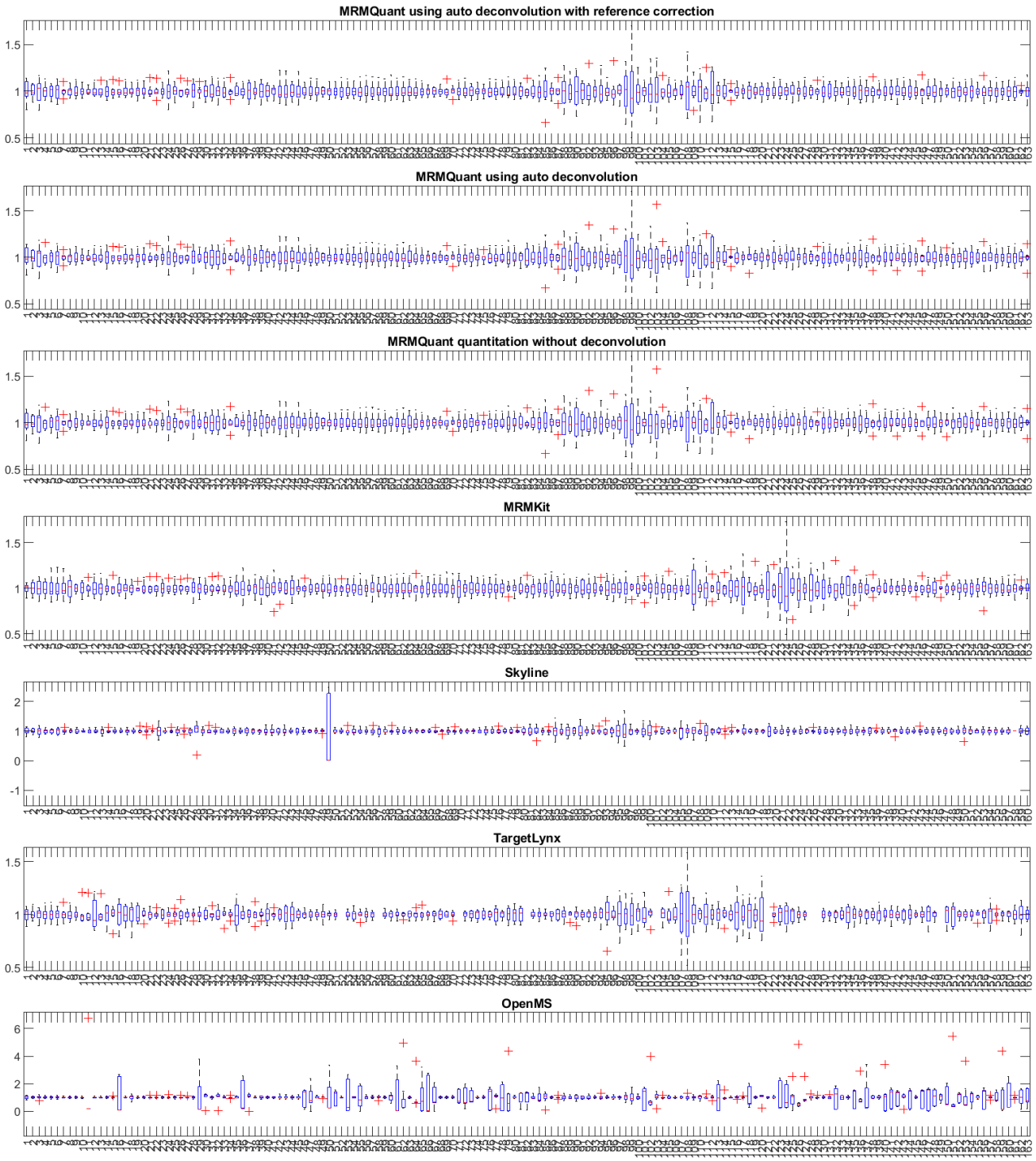


Figure 1. The boxplots of normalized abundances of the 163 phospholipids among the 8 QC samples of the 5 tools.

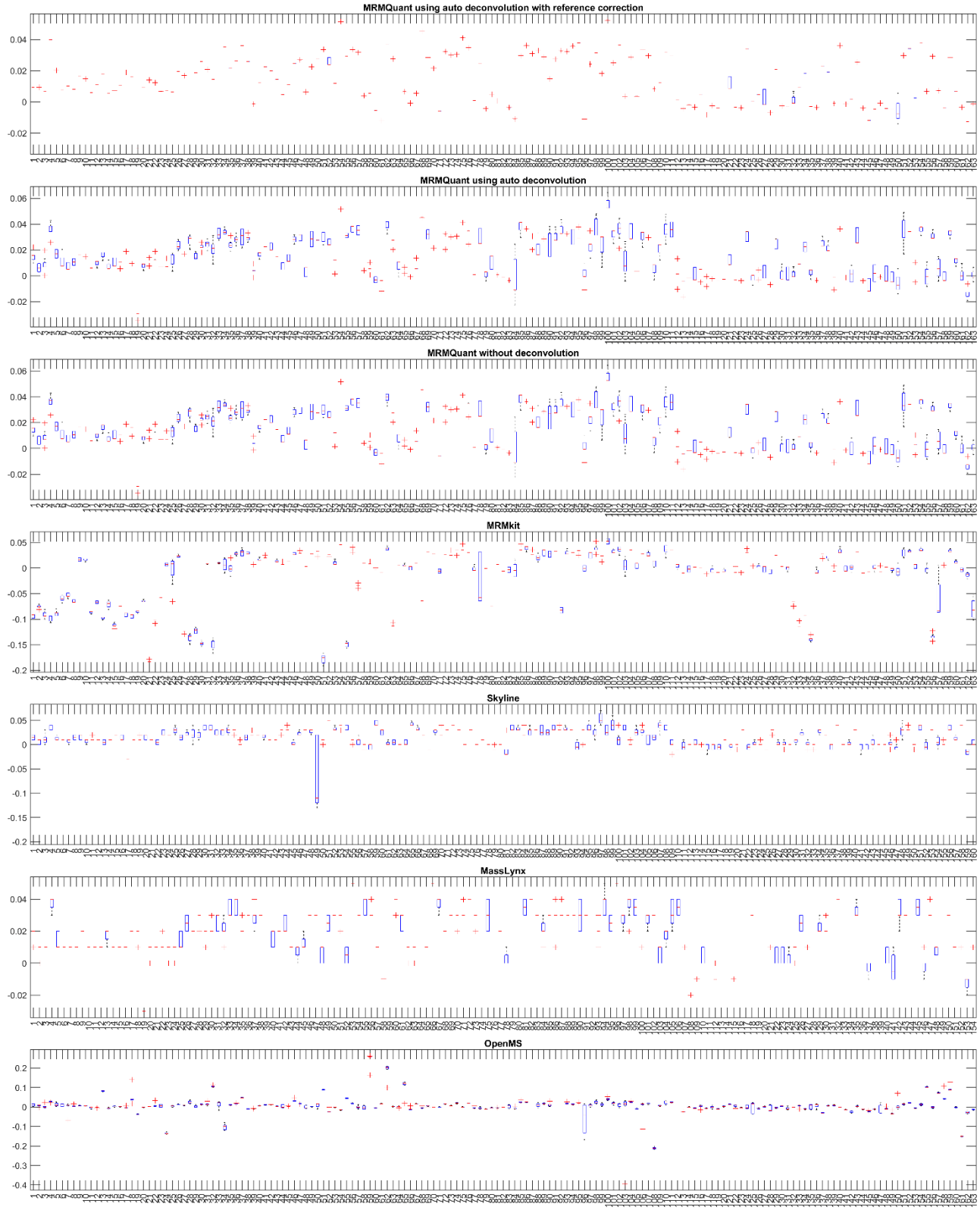
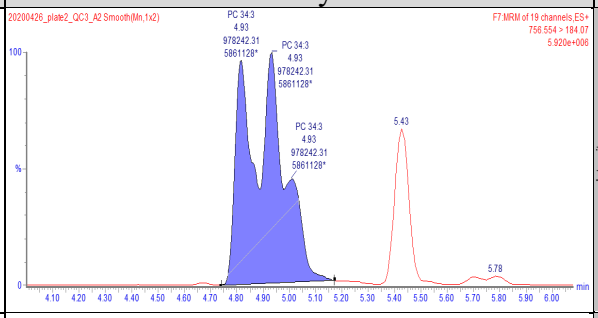
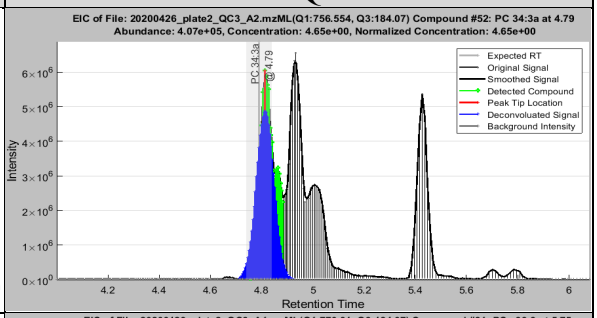
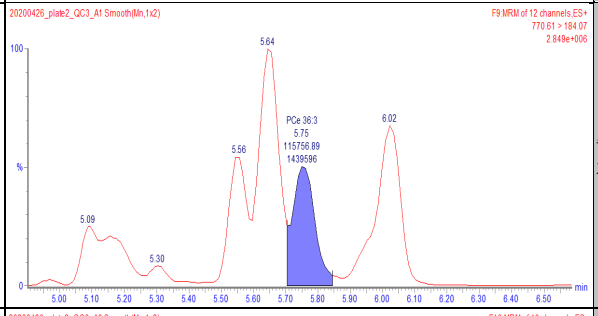
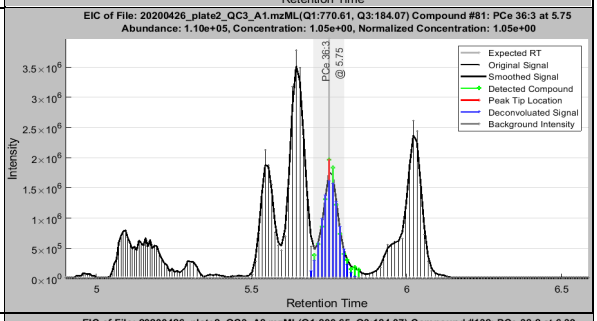
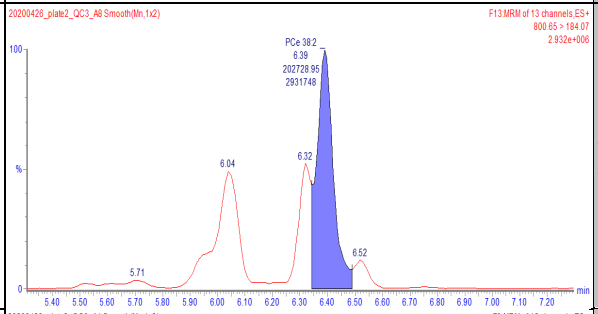
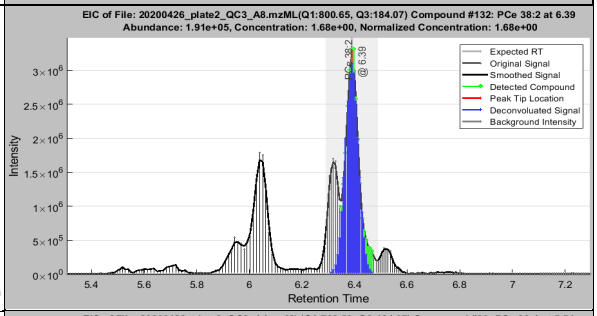
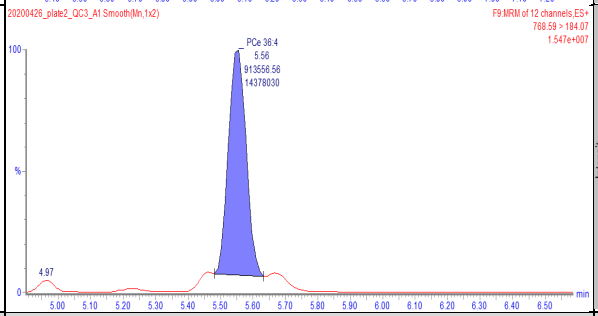
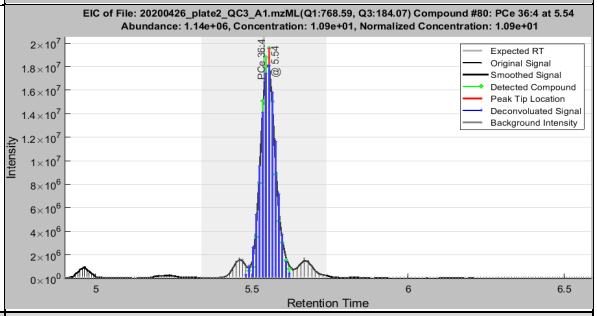
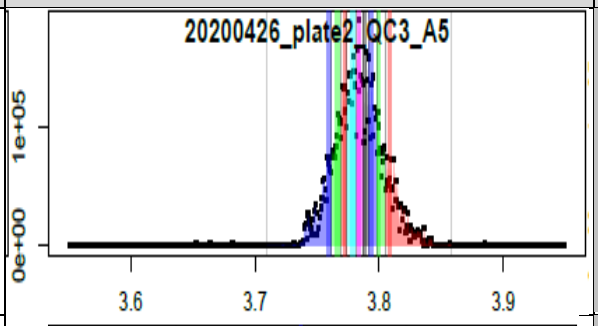
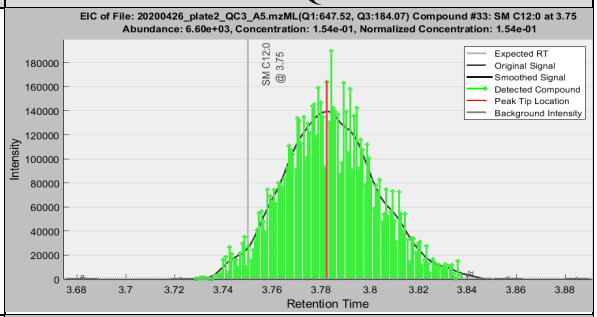
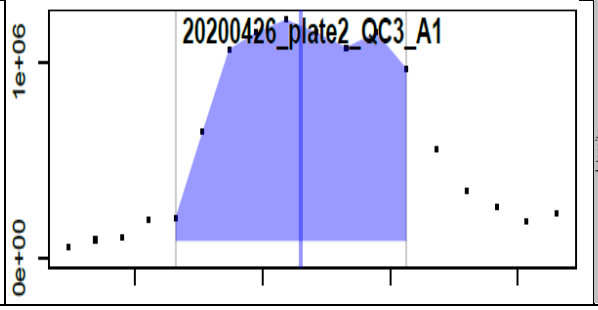
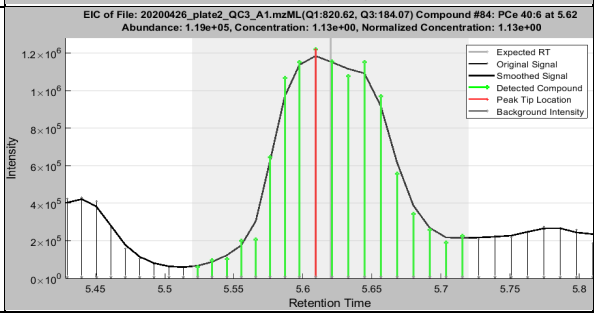
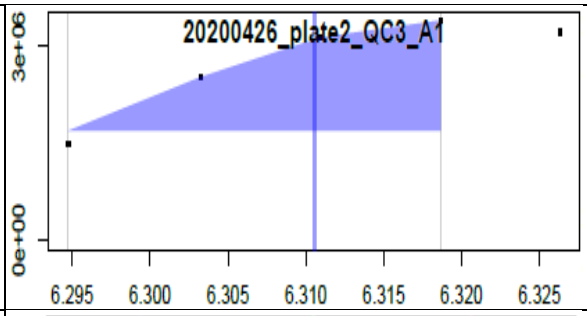
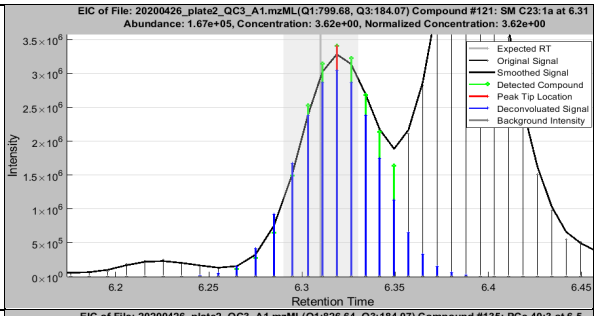
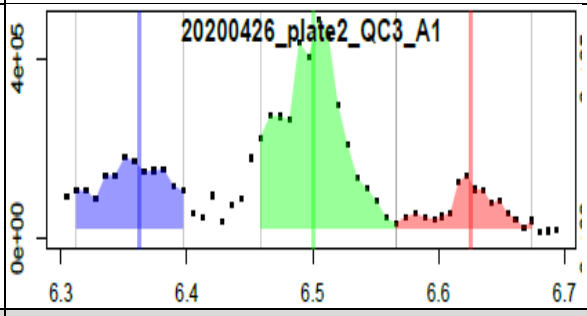
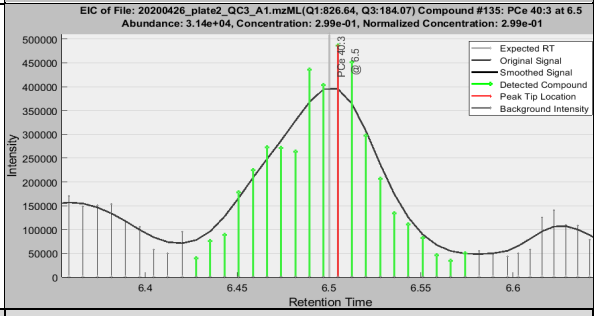
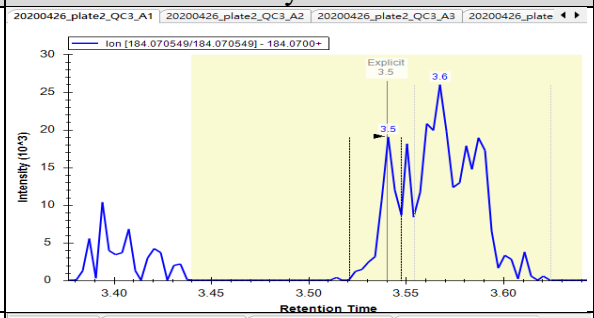
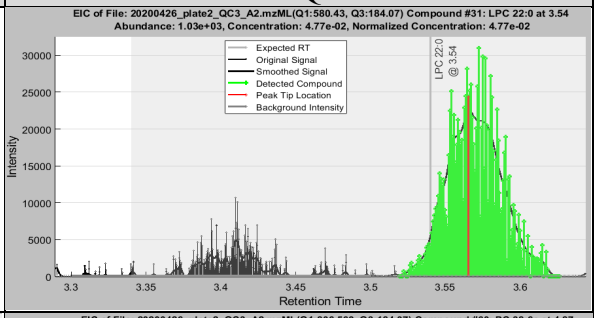
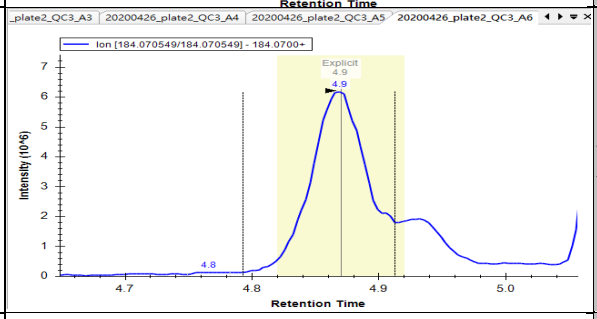
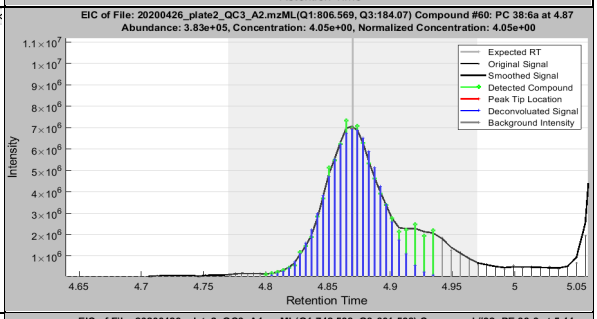
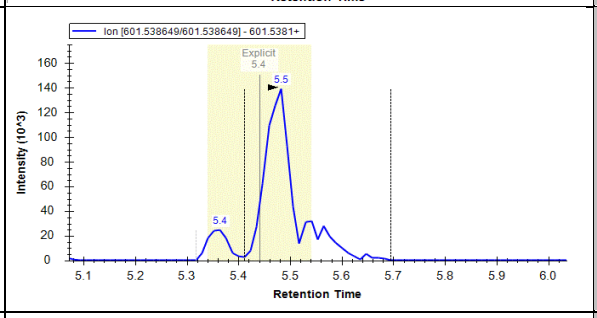
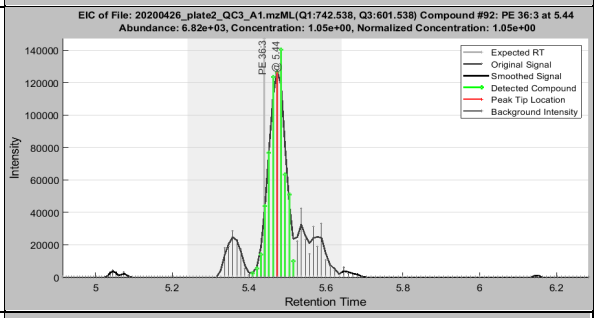


Figure 2. The boxplots of RT differences of the 163 phospholipids among the 8 QC samples of the 5 tools.

Table 7. Some examples of problematic quantitations in similar tools

Problem	MassLynx	MRMQuant
Overestimation for a co-eluted peak		
Underestimation for a co-eluted peak		
Underestimation for a co-eluted peak		
Inaccurate background estimation		
Problem	**MRMKit**	**MRMQuant**
Ambiguous peak area estimation		
Underestimation for a peak		

Problem	Skyline	MRMQuant
Inaccurate peak area estimation		
Ambiguous peak area estimation		
Underestimation for a noisy peak		
Underestimation for a co-eluted peak		
Overestimation for a co-eluted peak		
Incorrect peak area identification