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Comprehensive metabolomics of wine using LC-QTOFMS and LC-TQMS; Novel workflow to transfer analytical method from LC-QTOFMS to LC-TQMS

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1. Overview

Novel workflow to transfer analytical method from LC-QTOFMS to LC-TQMS was developed for comprehensive metabolomics.

2. Introduction

Recently, increasing attention has been devoted to metabolomics using MS in the medical and food industry. In general, metabolomics is performed by high-resolution mass spectrometer such as quadrupole time-of-flight (QTOF)MS. Although QTOFMS can comprehensively analyze metabolites, the sensitivity, dynamic range, and quantification of QTOFMS are inferior to those of triple quadrupole (TQ)MS. Therefore, it is effective to use both QTOFMS and TQMS for more comprehensive and accurate metabolomics. In this study, we comprehensively analyzed hydrophilic compounds in wines using LC-QTOFMS and LC-TQMS. For easy method transfer from LC-QTOFMS to LC-TQMS, a tool to automatically generate SRM transitions for LC-TQMS from MS/MS data that is acquired by Data Dependent Acquisition (DDA) mode of LC-QTOFMS was developed.

3. Methods

Three types of red wine were purchased from the market for evaluation. The portion was centrifuged at 14,000 g for 15 min, the supernatant was diluted 10-fold (QTOFMS) or 100-fold (TQMS) with water, and filtrated. The LC-MS analysis was performed using a Nexera™ X3 system coupled with a QTOF type LCMS-9030 or TQ type LCMS-8060 mass spectrometer (Shimadzu Corporation, Japan). Separation was achieved on Discovery®HS F5-3 column (Sigma-Aldrich, U.S.A.).

Analytical Conditions

HPLC conditions for metabolomics (Nexera X3 system)

Column: Discovery HS F5-3 (150 mmL. × 2.1 mmI.D., 3.0 µm)
Mobile phase   A: 0.1% Formic acid / water
                B: 0.1% Formic acid / acetonitrile
Flow rate: 0.25 mL/min
Time program: B conc. 0% (0 - 2 min) - 25% (5 min) - 35% (11 min) – 95% (15 - 20 min) - 0% (20.1 - 25 min)
Injection vol.:   3 µL
Column temp.:   40°C

MS conditions (LCMS-9030 : QTOFMS)

Ionization: Positive or negative , DDA MS/MS mode
DL temp.:   250°C   HB temp.:   400°C
Interface temp.: 300°C   Nebulizing gas: 3.0 L/min
Drying gas: 10 L/min
Heating gas: 10 L/min

MS conditions (LCMS-8060 : TQMS)

Ionization: Positive, MRM mode
DL temp.:   250°C   HB temp.:   400°C
Interface temp.: 300°C   Nebulizing gas: 3.0 L/min
Drying gas: 10 L/min
Heating gas: 10 L/min

4. Results

4-1. Accurate MS analysis of Metabolites in red wine (QTOFMS)

For comprehensive and qualitative analysis of hydrophilic metabolites in three red wines (a bottled red wine, a boxed red wine, and a nonalcoholic red wine), these wines were mixed. Hydrophilic metabolites in the mixture were comprehensively analyzed by LC-QTOFMS (DDA mode). Figure 1 shows the MS chromatograms of red wine mixture. As a result of the analysis, 12,522 and 8,283 compounds were detected in positive mode and in negative mode, respectively. Among them, MS/MS data of 1,443 and 759 compounds were obtained in positive mode and in negative mode, respectively.
 Each compound can be confirmed by empirical formula prediction analysis and database search. The mass accuracy of typical metabolite: Tryptophan shown in Figure 2 was within 1 mDa of theoretical mass values for both MS and MS/MS.
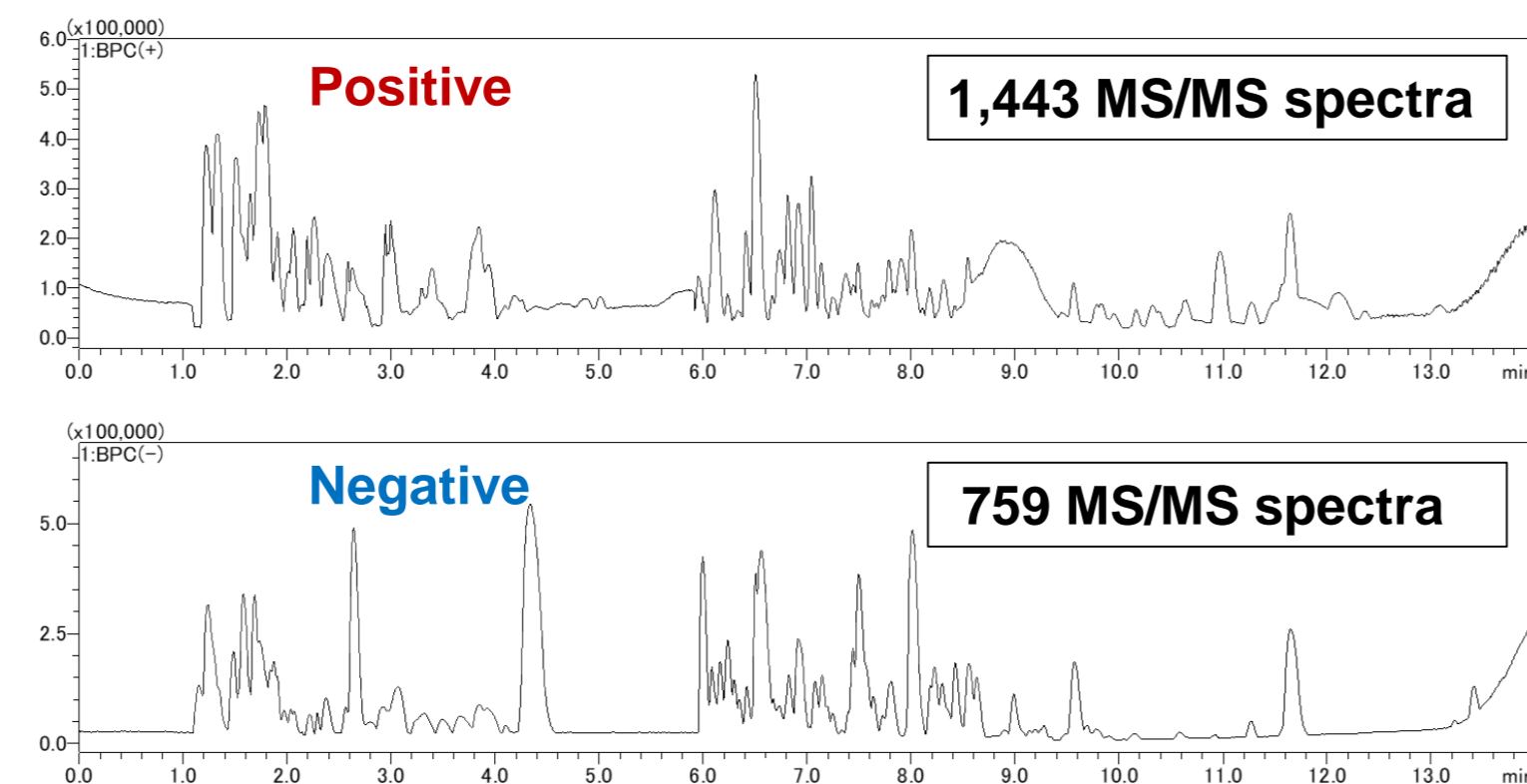

Figure 1   MS chromatogram of red wine (QTOFMS)

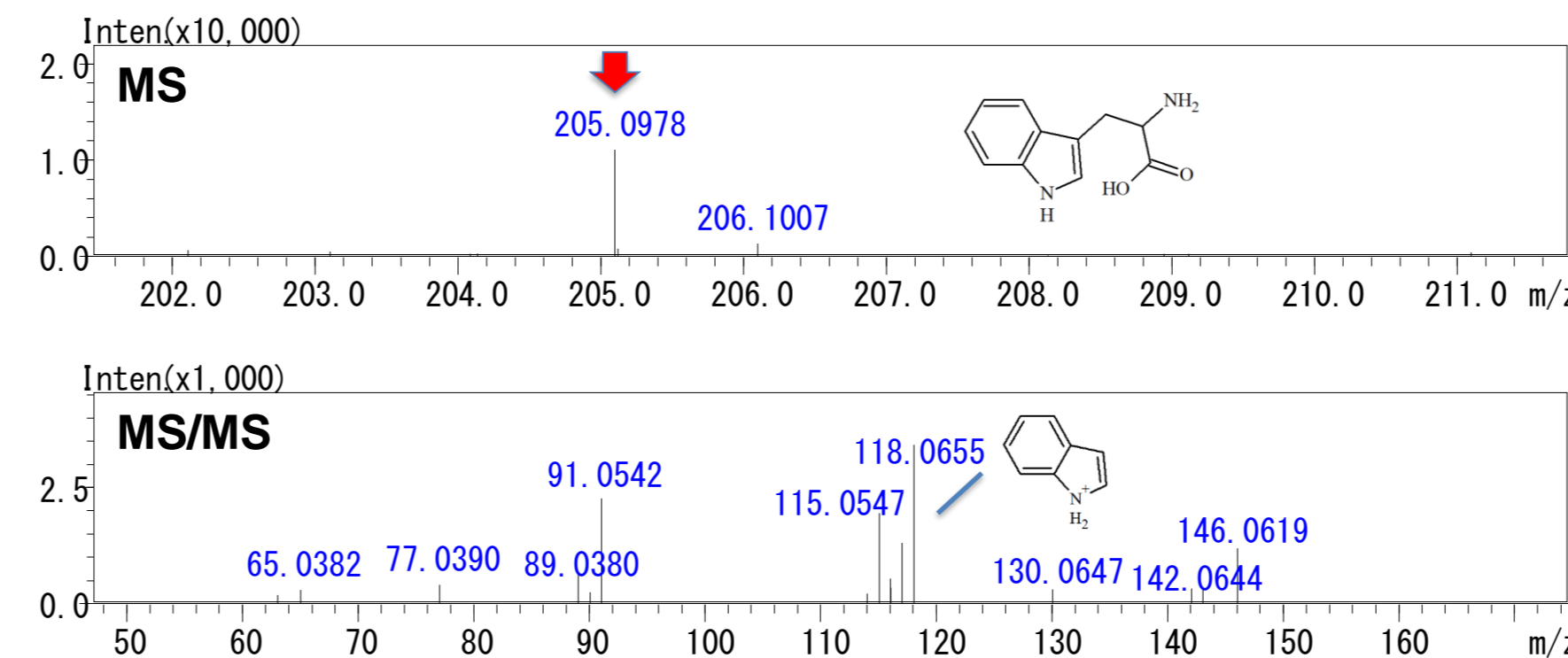| Compound	RT (min)	Ion type		Theoretical m/z	Observed m/z	Accuracy (mDa)
Tryptophan C$_{11}$H$_{12}$N$_2$O$_2$	9.43	MS	[M+H]⁺	205.0972	205.0978	0.6
		MS/MS	C$_8$H$_8$N(+)	118.0651	118.0655	0.4

Figure 2   Mass accuracy of tryptophan data

4-2. Automatic generation of SRM transitions for LC-TQMS from MS/MS data by QTOFMS

TQMS has better sensitivity, dynamic range, and quantitation than QTOFMS. So, a tool to automatically generate SRM transitions for LC-TQMS from all MS/MS data that is acquired by DDA mode of QTOFMS was developed. At this time, the fragment ion with the highest intensity in each MS/MS spectra is selected. Figure 3 shows the procedure for automatic generation of SRM transitions.
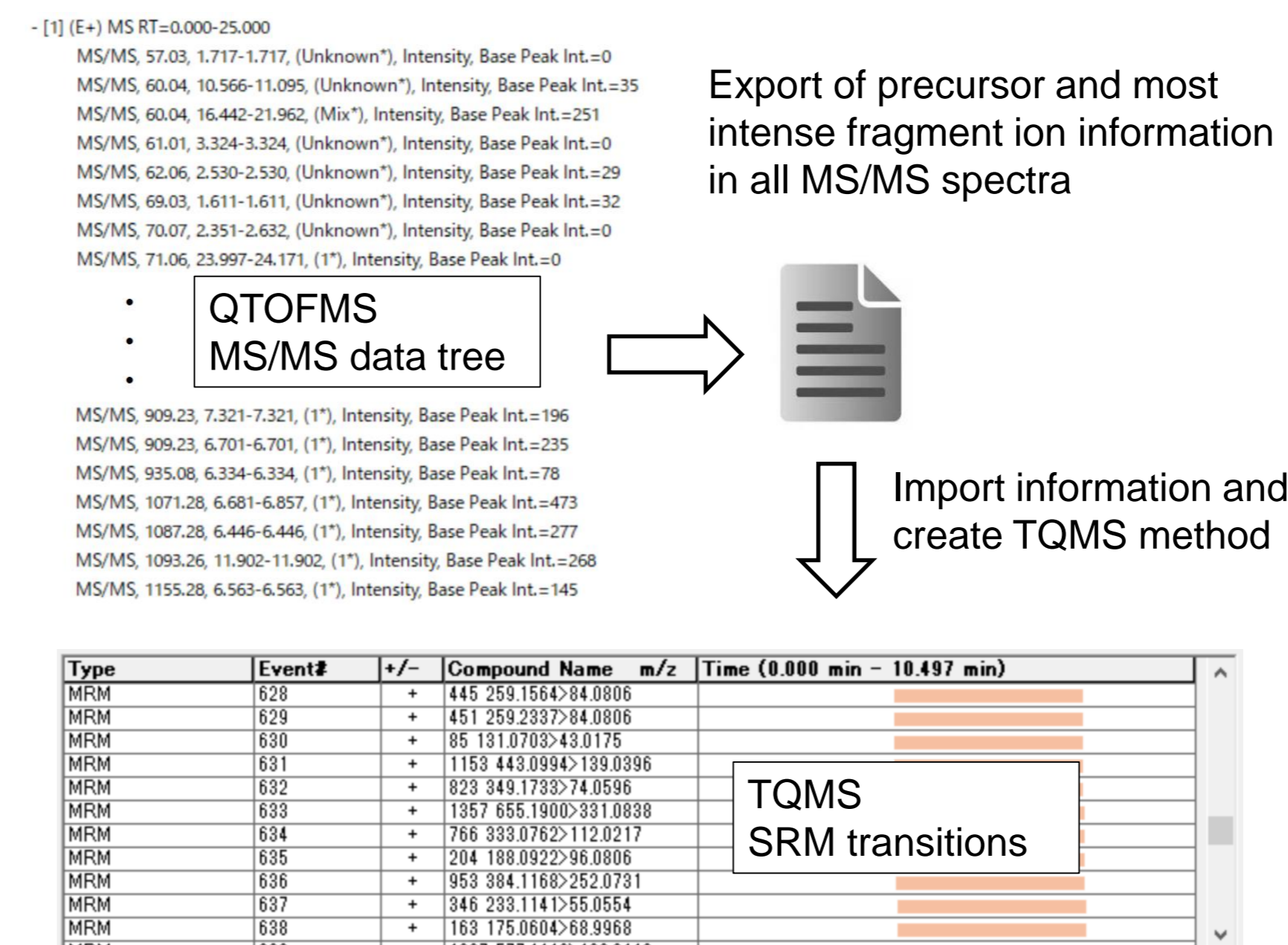

Figure 3   Procedure for automatic generation of SRM transitions

4-3. Data comparison of red wine samples (TQMS)

Three red wines were analyzed with the developed LC-TQMS method (positive mode, 1,443 SRM transitions). Principal component analysis (PCA) and the t-test by Traverse MS™ software (Reifycs Inc., Japan) were conducted to find the difference of three red wines using the TQMS 3 data sets. As shown in Figure 4, three red wines were successfully classified. Figure 5 shows a comparison of peak height for three representative metabolites of red wine. The bottled wine contained more ornithine than boxed wine and nonalcoholic wine. The boxed wine contained more glutathione than bottled wine and nonalcoholic wine. The nonalcoholic wine contained a little more 4-aminobutyric acid than alcoholic wines.
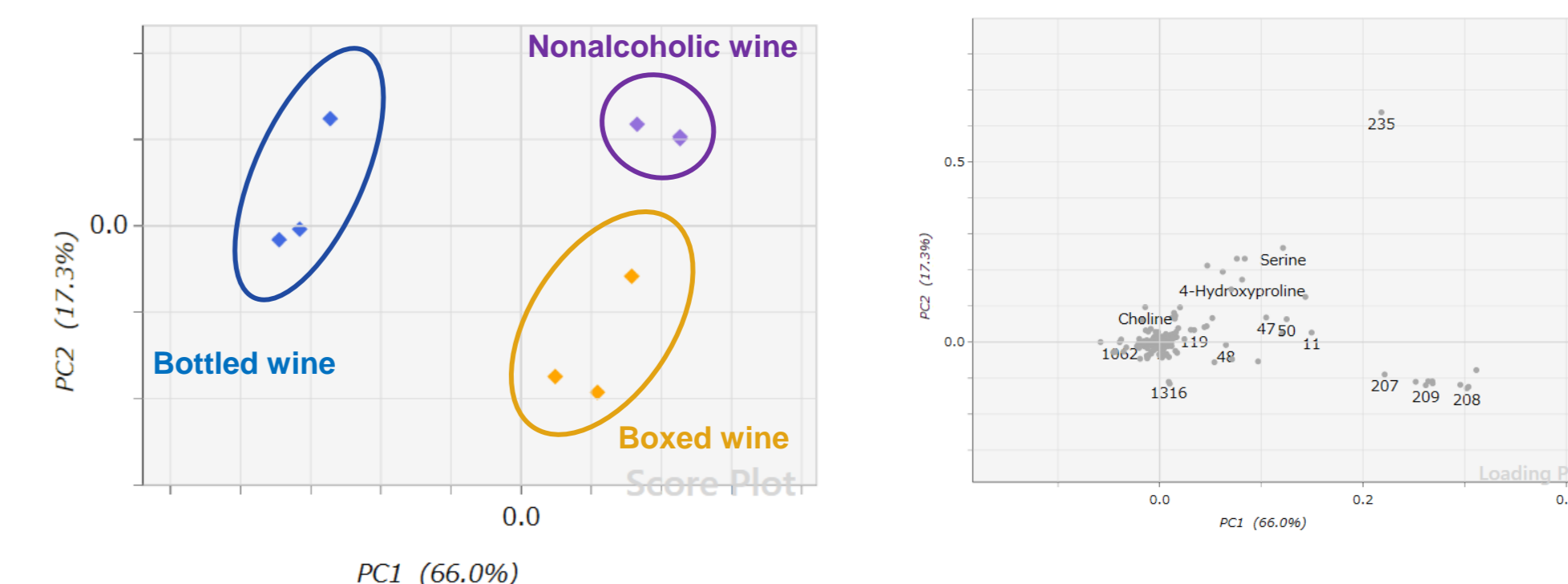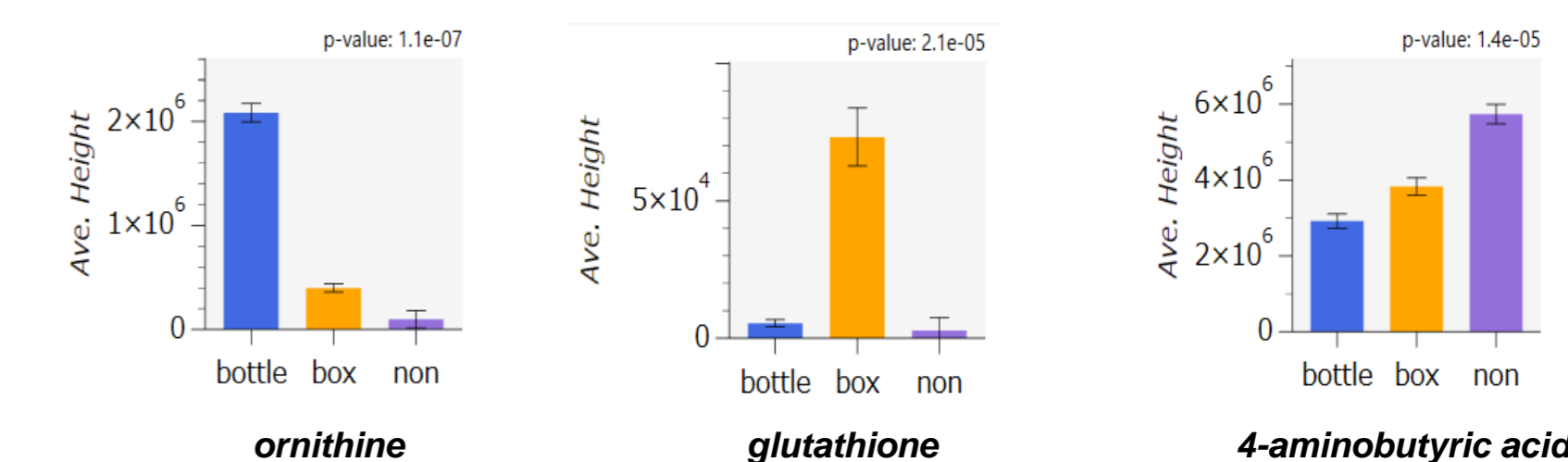

Figure 4   Score plot and loading plot

Figure 5   Comparison of peak height of metabolites among three red wines

5. Conclusions

✓ A tool was developed to automatically generate LC-TQMS SRM transitions from DDA MS/MS data acquired in LC-QTOFMS DDA mode.

✓ Over 1,000 SRM events can be analyzed with a TQMS single run.

✓ It is effective to use both QTOFMS and TQMS for more comprehensive and accurate metabolomics.