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Application News

LCMS™-9030 / LCMS-8060NX High Performance Liquid Chromatograph Mass Spectrometer

New Workflow for Metabolomics Combining LC-QTOFMS and LC-TQMS

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User Benefits

- Realizes a new workflow that seamlessly combines the strengths of two measurement methods.
- Provides highly reliable and easily interpretable results in complex and comprehensive metabolomics analyses.

■ Introduction

Metabolomics utilizing the mass spectrometer (MS) has been a focus of interest in the clinical research and food product development fields in recent years. In metabolomics, the metabolites of amino acids, organic acids, and other low molecular compounds formed by cellular activity are measured comprehensively, and the differences and relationships among multiple sample groups are clarified. As the general workflow, the metabolites are measured inclusively with a high resolution mass spectrometer such as a Quadrupole-Time of Flight (QTOF) MS, a dataset is created from the acquired data, and a multivariate analysis is conducted. This process normally yields an enormous amount of peak information, numbering from several 1000 to several 10000 peaks, but this information also includes peaks with low repeatability or quantitativity, which must be excluded in order to obtain a more reliable dataset (i.e., data preprocessing).

This article introduces a new workflow for metabolomics using a combination of an LC-QTOFMS and a Triple Quadrupole (TQ) MS. As demonstrated here, by conducting a high reliability wide-targeted metabolomics analysis using the information acquired by a non-targeted measurement, it is possible to create a high quality dataset and construct a model by multivariate analysis or machine learning.

■ New Workflow for Metabolomics

Targeted metabolomics by LC-TQMS enables measurement with high sensitivity and high reliability. However, this approach lacks comprehensiveness because it does not measure anything other than metabolites that are included in the analytical method. To measure the metabolites contained in a sample comprehensively by LC-TQMS, a comprehensive measurement by a method corresponding to the sample is necessary.

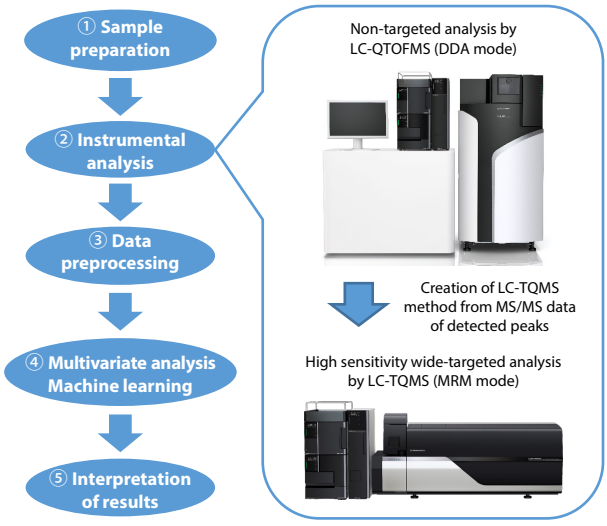


Fig. 1 Workflow Combining LC-QTOFMS and LC-TQMS

Therefore, we conceived the workflow shown in Fig. 1. First, in order to comprehensively acquire the MRMs of the metabolites contained in a sample, non-targeted measurement of the sample is carried out in the DDA (Data Dependent Acquisition) mode of the LC-QTOFMS. When measuring multiple groups of samples, a mixed sample is used in this measurement from the viewpoint of efficiency. Next, an LC-TQMS method is prepared by generating the MRM transitions from all of the acquired MS/MS data. Then, the individual samples are measured by the LC-TQMS using that method. This makes it possible to carry out a comprehensive wide-targeted measurement of all the metabolites in the samples with high sensitivity. In addition, if peaks of interest are discovered by multivariate analysis or machine learning using the acquired data, compound identification and structural estimation of the unknown metabolites are also possible by the LC-QTOFMS data.

■ Automatic Generation of MRM Transitions

For easy method transfer from the LC-QTOFMS to the LC-TQMS, we developed a tool (supporting LabSolutions LCMS Ver. 5.97 and higher) which automatically generates MRM transitions for use with the LC-TQMS from all MS/MS data acquired in the DDA mode of the LC-QTOFMS. As shown in Fig. 2, by using this tool, it is possible to select the fragment ions with the highest intensity from the MS/MS spectrum originating from each peak acquired in the DDA mode of the LC-QTOFMS, and generate MRM transitions for use with the LC-TQMS.

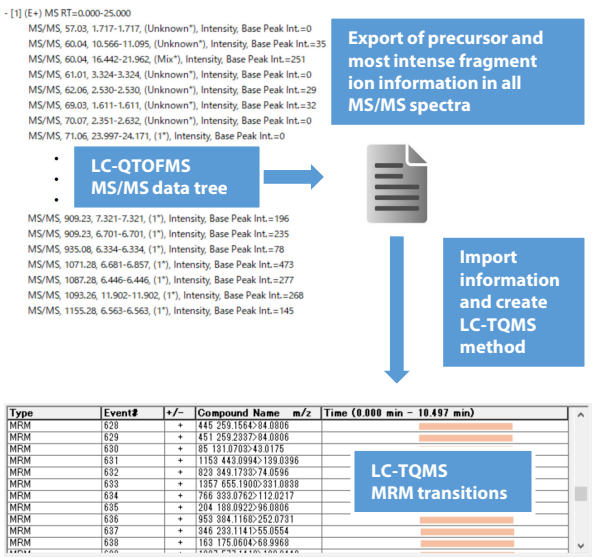


Fig. 2 Automatic Generation of MRM Transitions for LC-TQMS

■ Comprehensive Acquisition of MRMs of Metabolites by LC-QTOFMS (LCMS-9030)

The samples used here were three types commercially-available red wine (bottled wine, boxed wine, nonalcoholic wine). In order to acquire the MRMs of the hydrophilic metabolites contained in each wine efficiently and comprehensively, a mixed sample of these red wines was used as the analysis sample. The analysis sample was centrifuged for 5 min at 12000 rpm, and the supernatant was diluted 10× with ultrapure water and used in the LC/MS analysis. Based on the conditions of the Exact Mass Database for Endogenous Metabolites (Method Package) for LC-QTOFMS, the sample was measured with the LC-QTOFMS (DDA mode) under the analysis conditions in Tables 1 and 2. Fig. 3 shows the MS chromatogram of the mixed red wine sample. MS/MS data with sufficient peak intensity were acquired for 1443 peaks in the positive mode and 759 peaks in the negative mode.

Table 1 HPLC Analysis Conditions (Nexera™ X3 System)

Column	: Reversed-phase column
Mobile Phase A	: 0.1 % Formic acid in water
Mobile Phase B	: 0.1 % Formic acid in acetonitrile
Mode	: Gradient elution
Flow Rate	: 0.25 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 3 µL

Table 2 MS Analysis Conditions (LCMS-9030)

Ionization	: ESI (Positive or negative, DDA MS/MS mode)
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10 L/min
Heating gas flow	: 10 L/min
DL temp	: 250 ˚C
Block heater temp.	: 400 ˚C
Interface temp.	: 300 ˚C

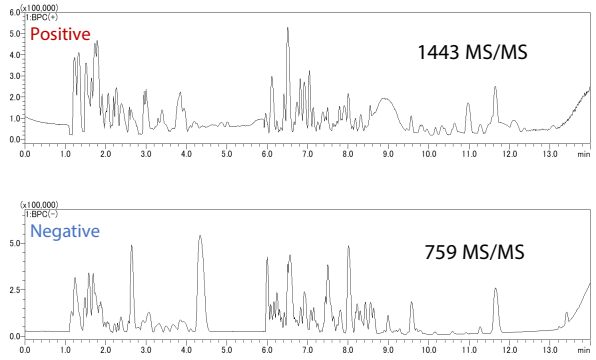
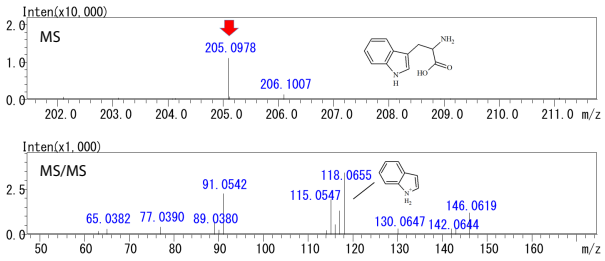


Fig. 3 MS Chromatogram of Mixed Red Wine Sample (LC-QTOFMS)

Because extremely high mass accuracy in both MS and MS/MS can be obtained with the LCMS-9030, this instrument is a powerful tool for identification of unknown compounds detected in metabolomics measurements. Fig. 4 shows the MS and MS/MS spectrum, and mass accuracy of the representative metabolite tryptophan. The molecular associated ions in MS and MS/MS were assigned with high mass accuracy.



Compound	RT (min)	Ion type		Theoretical mass *m/z*	Observed mass *m/z*	Accuracy (mDa)
Tryptophan $C_{11}H_{12}N_2O_2$	9.43	MS	$[M+H]^+$	205.0972	205.0978	0.6
		MS/MS	$C_8H_8N(+)$	118.0651	118.0655	0.4

Fig. 4 Mass Accuracy in LC-QTOFMS Data for Tryptophan

■ High Sensitivity Analysis of Metabolites by LC-TQMS (LCMS-8060NX)

Based on the MS/MS data acquired with the LC-QTOFMS, an MRM method (positive mode, 1443 MRM transitions) for use with the LC-TQMS was created by the procedure in Fig. 2, and LC-TQMS analyses were carried out for the three types of red wine using the LCMS-8060NX. With the LCMS-8060NX, which supports high speed analysis, more than 1000 MRM events can be set in an analysis, and an ultrahigh sensitivity wide-targeted metabolomics measurement is possible. As samples for the LC/MS analysis, each wine was centrifuged for 5 min at 12000 rpm, and the supernatant was diluted 100x with ultrapure water. A PCA (Principal Component Analysis) and one-way ANOVA (one-way analysis of variance) analyses of the acquired data were conducted using the Traverse MS software of Reifycs Inc. As shown in Fig. 5, the three types of red wine were clearly classified by the PCA. Fig. 6 shows an example of a comparison of the peak intensities in metabolites (p-value < 0.05) in which it was judged that a significant difference existed for the three types of red wine.

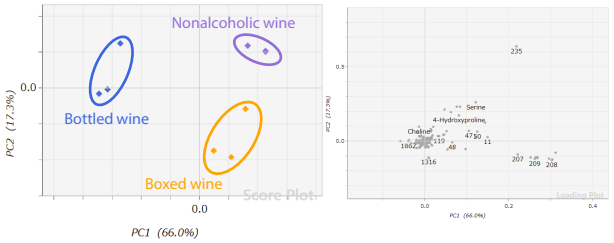


Fig. 5 Score Plots and Loading Plots in PCA

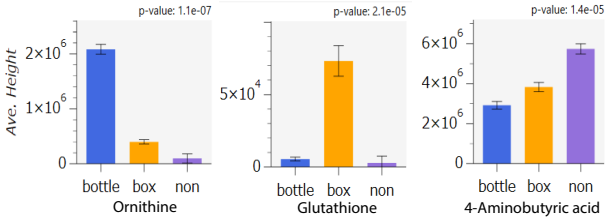


Fig. 6 Results of ANOVA Assay of Metabolites in 3 Types of Red Wine

■ Conclusion

Comprehensive and highly reliable metabolomics measurements are possible by using a combination of LC-QTOFMS and LC-TQMS. Accompanying the progress of AI and machine learning, the necessity of metabolomics utilizing highly reliable data will continue to increase in the future. The new workflow described here will be one effective technique for addressing this need.

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