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LCMS-9030 High Performance Liquid Chromatograph Mass Spectrometer

Application News

Screening Analysis of Metabolites in Red Wine

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User Benefits

- The LCMS-9030 is ideal for screening metabolites in food.
- LabSolutions Insight Explore™ can rapidly screen for metabolites.
- Given the compound name and composition formula information, any compound can be specified as a screening target.

■ Introduction

Metabolomics, a technology for comprehensively analyzing all metabolites in living organisms, has attracted attention in recent years. It is also used in food research to evaluate taste, quality, and nutritional value. High-resolution mass spectrometers are frequently used for metabolomics, but the data analysis process becomes a bottleneck for research because of the large amount of data.

This article describes an example of a screening analysis of metabolites in red wine using an LCMS-9030 quadrupole time-of-flight (Q-TOF) mass spectrometer (Fig. 1). The Screen function in LabSolutions Insight Explore was used to predict metabolites in the wine sample. By preparing a list of candidate compounds for precursor ions, samples can quickly be screened for metabolites.

Fig. 1 Nexera™ X3 and LCMS-9030

■ Analytical Conditions

Nexera™ X3 UHPLC and LCMS-9030 systems were used as the analytical instruments. The LC method included in "LC/MS/MS Method Package for Primary Metabolites" was used as the method. The data-dependent acquisition (DDA) mode was used to simultaneously acquire precursor *m/z* and MS/MS data. Table 1 shows the analytical conditions.

Table 1 Analytical Conditions

[HPLC conditions] (Nexera X3)	
Column:	Reverse-phase column
Column Oven:	40 °C
Solvent A:	0.1 % Formic acid in water
Solvent B:	0.1 % Formic acid in acetonitrile
Mode:	Gradient elution
Flowrate:	0.25 mL/min
Injection Volume:	3 µL
[MS conditions] (LCMS-9030)	
Ionization:	ESI, negative
Mode:	Data dependent acquisition (DDA)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	10.0 L/min
Heating Gas Flow:	10.0 L/min
DL Temp.:	250 °C
Block Heater Temp.:	400 °C
Interface Temp.:	300 °C
CID Gas Pressure:	230 kPa

■ Metabolite Screening Workflow

The workflow for metabolite screening is shown in Fig. 2. First, a list of candidate compounds for screening was prepared. In this case, the list was created based on the primary metabolites contained in the "Exact Mass Database for Endogenous Metabolites." That database contains retention time and exact mass information for metabolites previously included in LC/MS/MS series method packages. In addition, some metabolites were also added to this list. As long as the compound name and composition formula information is available, any compound can be easily specified as a screening target. Then the LCMS-9030 system was used to acquire data and extract peaks using the Analyze function in LabSolutions Insight Explore. Finally, the Screen function was used to load the prepared list and screen for metabolites.

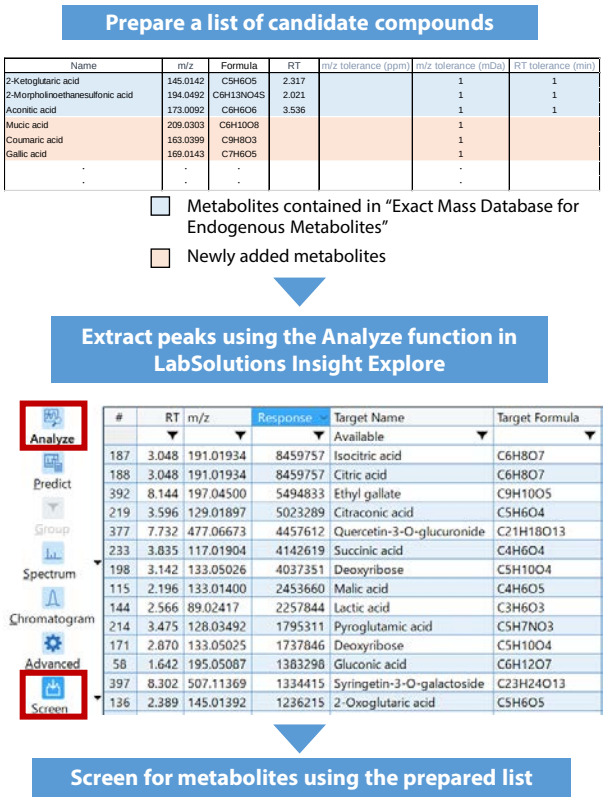

Fig. 2 Metabolite Screening Workflow

■ Sample Pretreatment

Five types of red wines from different origins were mixed in equal amounts for use as the sample. For pretreatment, the wine mixture was centrifuged (12,000 rpm, 5 min, 4 °C) and the supernatant was diluted 10-fold with ultrapure water.

■ Comprehensive Analysis of Metabolites in Wine by LCMS-9030

When red wine was analyzed in the negative mode, a base peak chromatogram was obtained as shown in Fig. 3.

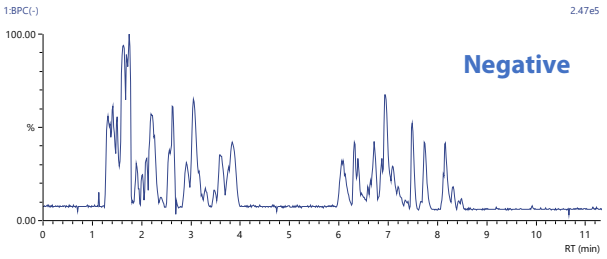

Fig. 3 Base Peak Chromatogram of Red Wine Sample

Using the Analyze function in LabSolutions Insight Explore, 455 peaks were extracted. Using a prepared *m/z* list for about 500 compounds, metabolites were screened with a mass error of 1 mDa. As a result, 90 of the 455 peaks were labeled with a candidate compound name. In the negative mode, many organic acids were detected, such as citric acid, succinic acid, and gallic acid. Some flavonoids such as quercetin 3-O-glucuronide were also detected.

■ Verification of Screened Compounds

The following shows fragment assignment results for gallic acid (retention time 6.385 min, *m/z* 169.01390) and the confirmation results using the standard. As shown in Fig. 4, gallic acid was predicted with high mass accuracy (error -0.35 mDa).

#	RT	m/z	Response	Target Name	Target Formula	Target m/z	Mass Error (mDa)
				Available			
119	2.225	191.01936	1159690	Citric acid	C6H8O7	191.01970	-0.34
43	1.600	209.02996	1120974	Mucic acid	C6H10O8	209.03029	-0.33
269	6.385	169.01390	936758	Gallic acid	C7H6O5	169.01425	-0.35
374	7.669	189.07634	900018	Hydroxysuberic acid	C8H14O5	189.07660	-0.26
175	2.901	129.01896	881913	Citraconic acid	C5H6O4	129.01933	-0.37

Fig. 4 Wine Screening Results

When an online search (Assign function) was conducted using the ChemSpider database for the composition formula $C_7H_6O_5$, gallic acid was found as a top candidate compound. The results of automatic assignment of MS/MS fragments are shown in Fig. 5 and the predicted fragments are shown in Fig. 6.

#	m/z	Intensity	Formulae...	Charge	Pred. m/z	+/- mDa	+/- ppm
14	125.02392	4199	C6H5O3	[-H]-	125.02442	-0.50	-3.999
6	79.01856	1788	C5H3O••	[-H]-	79.01894	-0.38	-4.809
5	79.01856	1788	C5H3O	- / [-H]-	79.01894	-0.38	-4.809
13	124.01611	773	C6H4O3•	[-H]-	124.01659	-0.48	-3.870
4	69.03491	469	C4H5O	- / [-H]-	69.03459	0.32	4.635

Fig. 5 Results of Automatic Assignment of MS/MS Fragments

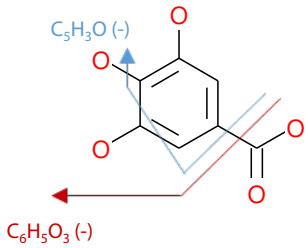

Fig. 6 Predicted Fragments of Gallic Acid

Finally, results from analyzing a gallic acid standard are shown. The retention time of the gallic acid standard on the extracted ion chromatogram is consistent with that of the predicted compounds in red wine (Fig. 7). The MS/MS spectrum pattern also matched (Fig. 8).

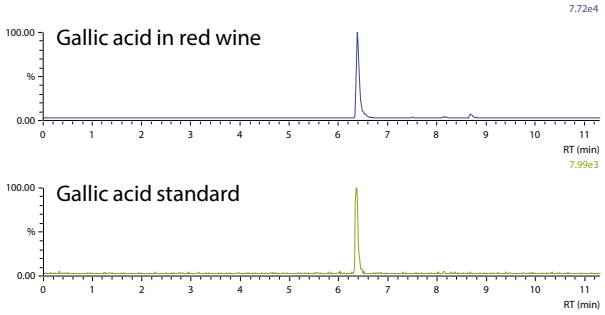

Fig. 7 Extracted Ion Chromatogram

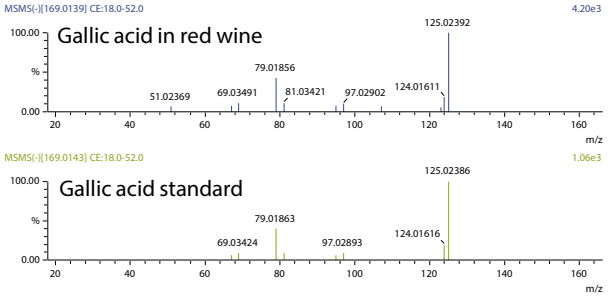

Fig. 8 MS/MS Spectrum

Screening results were verified by analyzing the standard as described above.

■ Conclusion

In this study, red wine was analyzed with an LCMS-9030 quadrupole time-of-flight mass spectrometer to screen for metabolites related to functionality and taste. By preparing a list of candidate compounds for precursor ions, 90 compounds were predicted from the negative mode data. In addition, the screening results for gallic acid were verified using the standard. Gallic acid is a phenolic antioxidant that is said to have anticancer benefits. Also, the organic acids detected in this analysis are closely related to the taste of wine.

This workflow enables rapid metabolite screening. Any compound with known name and composition formula information can be easily specified as a screening target.

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