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Application News

Non-Targeted Analysis of Metabolites in Alcoholic Beverages Using LabSolutions Insight™ Profiler

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User Benefits

- Using an LC-QTOFMS system with LabSolutions Insight Profiler non-targeted analysis software enables comprehensive comparative analysis of metabolites in food samples.
- Principal component analysis and volcano plots can be used to detect characteristic features in each sample.
- A variety of functions, including MS/MS spectral library searching, enable easy qualitative analysis of components.

■ Introduction

Metabolomics, a technique that analyzes metabolites in living organisms, is increasingly being applied in the food industry for product development and quality control. A particularly powerful approach is non-targeted metabolomics, which aims to analyze not only known metabolites but also unknown compounds without prior assumptions to reveal unexpected chemical differences between samples. Quadrupole time-of-flight mass spectrometry (QTOFMS), with its accurate-mass capability, is well suited for this purpose. However, the large volume of generated data creates challenges for feature identification and data comparison.

This article presents a case study in which LabSolutions Insight Profiler, a software platform for non-targeted analysis, was used to characterize compounds in beer and beer-like beverages. Peak detection, retention-time alignment, and library searching were performed across multiple datasets. Multivariate analysis revealed compound profiles that likely reflect the differences in raw materials and manufacturing processes, demonstrating the utility of the approach for beverage characterization.

■ LabSolutions Insight Profiler

LabSolutions Insight Profiler is software for batch processing of LC-QTOFMS data that enables non-targeted analysis through an intuitive workflow. It readily supports easy spectral feature detection, alignment, statistical processing, filtering, list screening, MS/MS spectra-based library searching, principal component analysis (PCA), displaying volcano or box plots, and more. Fig. 1 shows the data analysis workflow.

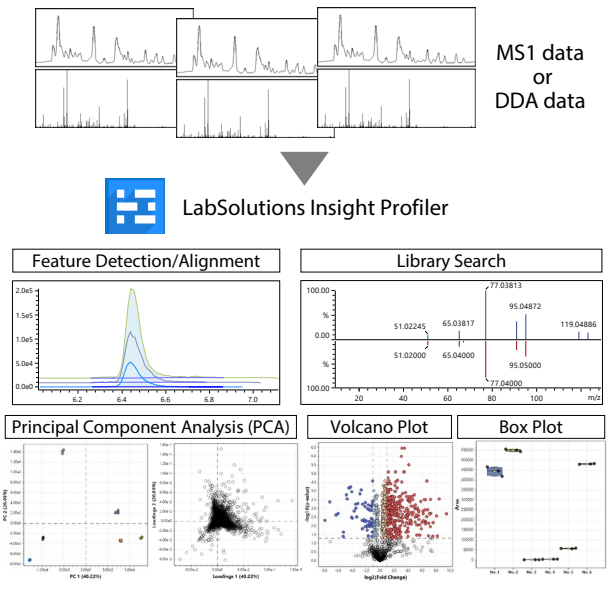

Fig. 1 Analysis Workflow Using LabSolutions Insight Profiler

■ Sample and Pretreatment

Six commercially available beer-type beverages, including beer, low-malt beer, beer-like beverage, and non-alcoholic beer, were used as samples (Table 1). Each sample was pretreated by degassing and then 10-fold dilution with ultrapure water.

Table 1 Sample Information

No	Sample	Description
1	Beer 1	Lager beer (bottom fermentation)
2	Beer 2	Ale beer (top fermentation)
3	Low-malt beer	Purine free
4	Beer-like beverage	Contains soy protein
5	Non-alcoholic beer 1	Made in Japan
6	Non-alcoholic beer 2	Made in Germany

■ Analysis Conditions

Nexera™ X3 UHPLC and LCMS-9030 systems were used as the analytical instruments. The LC method included in the 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 2 shows the analysis conditions.

Table 2 Analysis Conditions

HPLC Conditions (Nexera X3)	
Column:	Reversed-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, Positive
Mode:	Data dependent acquisition (DDA)
MS Event:	*m/z* 50-1000, 0.1 sec
MS/MS Event:	*m/z* 10-1000, 0.1 sec x5
Collision Energy:	35.0 ± 17.0 V
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

■ Data Analysis

LabSolutions Insight Profiler was used for data analysis. Fig. 2 shows an example of the LabSolutions Insight Profiler analysis screen. Alignment results, chromatograms, and spectra are linked with PCA and volcano plots, facilitating the interpretation of complex datasets. For library searches, the NIST 23 Mass Spectral Library, which contains approximately 50,000 compounds, was used. Scores were calculated based on MS/MS spectral matching.

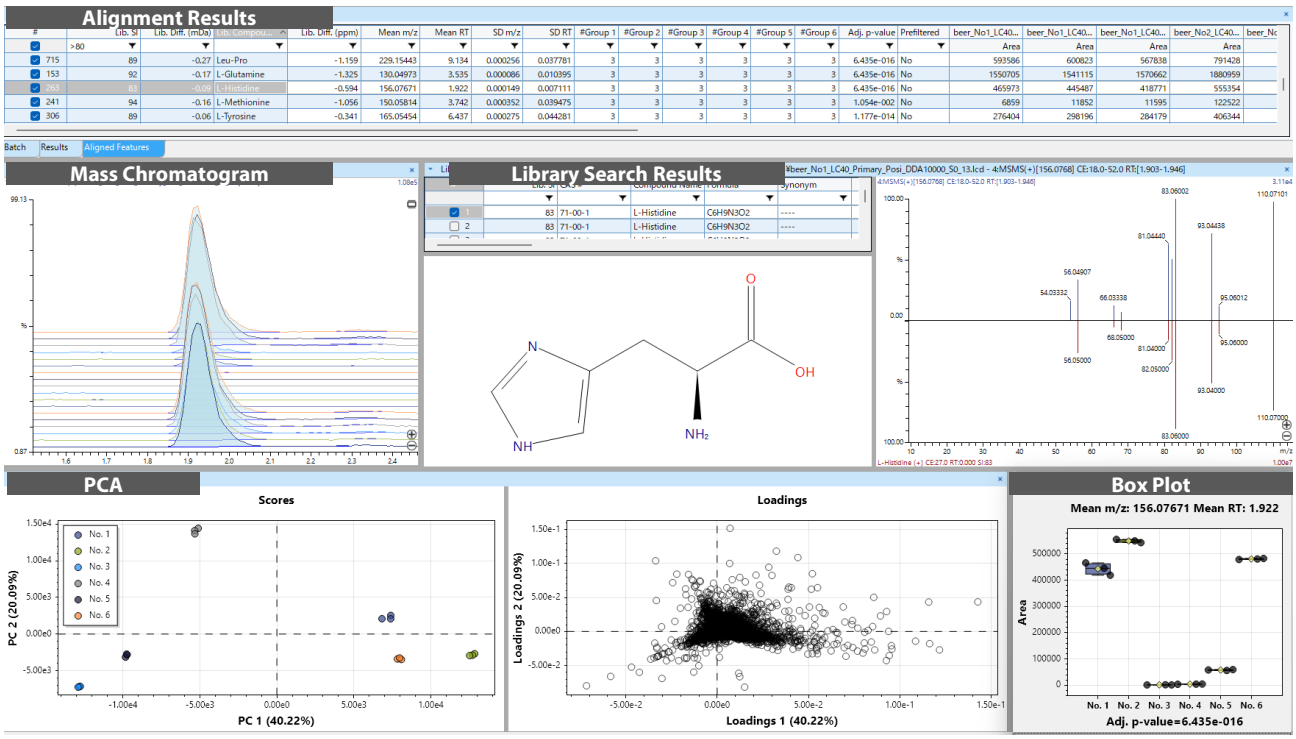

Fig. 2 Example of the LabSolutions Insight Profiler Analysis Screen

■ Results of Alignment and Library Search

The LCMS-9030 system was used to analyze six types of beer and beer-like beverages in three repeat measurements. LabSolutions Insight Profiler detected 4,695 features. A NIST 23 Mass Spectral Library search of the MS/MS spectral features detected 403 compounds with a similarity score of 50 or higher, including 214 compounds with a score of 80 or higher. Fig. 3 shows the library-matched MS/MS spectrum for the feature with an average detected *m/z* of 156.077 and a retention time of 1.9 min, identified as L-Histidine with a similarity score of 83.

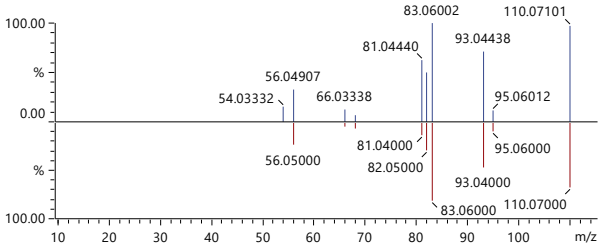

Fig. 3 Example of Library-Matched MS/MS Spectrum (L-Histidine, SI=83)

■ Principal Component Analysis of 6 Types of Beer

Fig. 4 shows the results of principal component analysis for peak area values observed among the samples. Principal component analysis is a technique that reduces dimensionality by transforming data into new, mutually orthogonal principal components while minimizing information loss. Pareto scaling was used for data scaling. On the score plot, Beer 1 (No. 1), Beer 2 (No. 2), and Non-alcoholic beer 2 (No. 6) were plotted close together. Low-malt beer (No. 3) and Non-alcoholic beer 1 (No. 5) were also plotted close together. Beer-like beverage (No. 4) was plotted in a different position from the other samples along the PC2 axis.

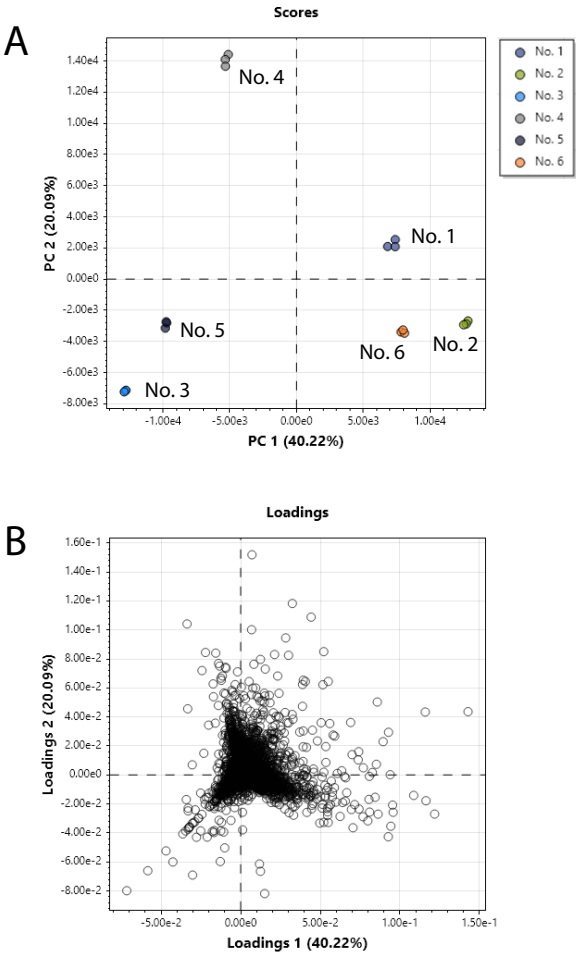

Fig. 4 Results of Principal Component Analysis
(A) Score Plot, (B) Loading Plot

■ Characteristic Compounds in Samples

Fig. 5 shows the area values of certain compounds that were characteristic among the samples. Amino acids (histidine, phenylalanine, tyrosine, and tryptophan) and nucleic acid metabolites (cytidine and guanosine) were detected in large quantities in samples No. 1, 2, and 6. An alkaloid (hordenine), thought to be derived from barley, was also detected. The detection of similar characteristic compounds in samples No. 1, 2, and 6 is presumed to be due to common raw materials and fermentation processes used in their manufacturing methods. Although No. 6 is a non-alcoholic beer, it is manufactured using beer ingredients and a fermentation method that suppresses alcohol production, resulting in a similar profile to No. 1 and 2. No. 3 showed high levels of umami-related compounds (glutamic acid) and the flavor stabilizer (triethyl citrate). An antioxidant (vitamin C) was detected in samples No. 3 and 5. Since samples No. 3 and 5 are produced by blending raw materials, their compound profiles are considered to differ from those of beer. Isoflavones (daidzin and genistein) were detected in No. 4. Since No. 4 is manufactured using soybeans as a raw material, the detected compounds are presumably derived from soybeans.

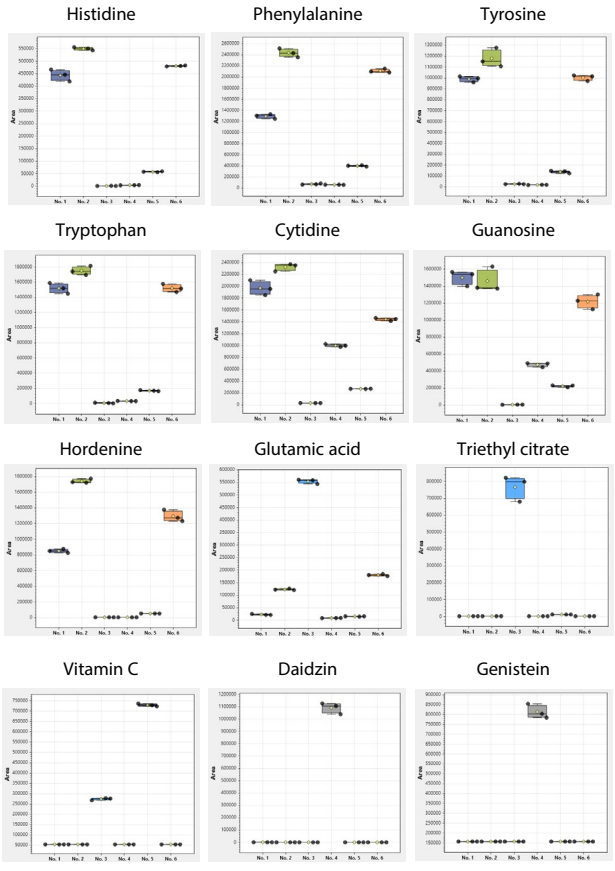

Fig. 5 Box Plots of Characteristic Compounds Among Samples
(Y-Axis: Area Value, n = 3)

■ Comparison of Two Beers Using a Volcano Plot

Fig. 6 shows the results of a volcano plot analysis comparing Beer 1 (No. 1) and Beer 2 (No. 2). A volcano plot is a scatter plot that simultaneously visualizes the magnitude of change (fold change) and statistical significance (p-value). In this analysis, the thresholds were set at a fold change of at least 2 and $p < 0.05$.

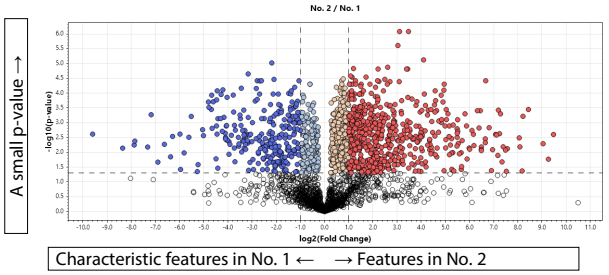

Fig. 6 Volcano Plot of No. 1 and No. 2

Fig. 7 shows the area values of specific compounds that exhibited large fold changes and significant differences among the samples. Sample No. 1 contained higher levels of adenosine, the methyl group donor S-adenosyl-methionine, and the hop-derived polyphenol xanthohumol compared to No. 2. In contrast, No. 2 showed higher levels of adenine and hypoxanthine, which are related to purine metabolism, as well as the sulfur-containing amino acid methionine.

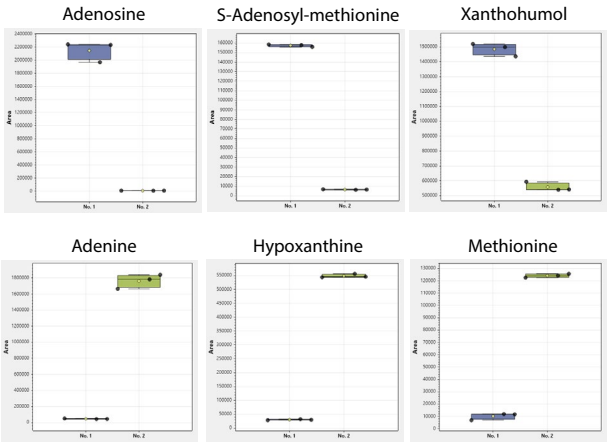

Fig. 7 Box plots of Characteristic Compounds Among Samples
(Y-Axis: Area Value, n = 3)

■ Qualitative Analysis of Detected Features

For the detected spectral features, compositions could be predicted based on accurate mass and isotopic patterns, as well as online searches using the PubChem or ChemSpider databases. For the compounds found in search results, fragment ions in MS/MS spectra can be automatically assigned using the assign function. Fig. 8 shows the composition prediction and ChemSpider database search results for a spectral feature with an average *m/z* of 285.076 and a retention time of 10.8 min.

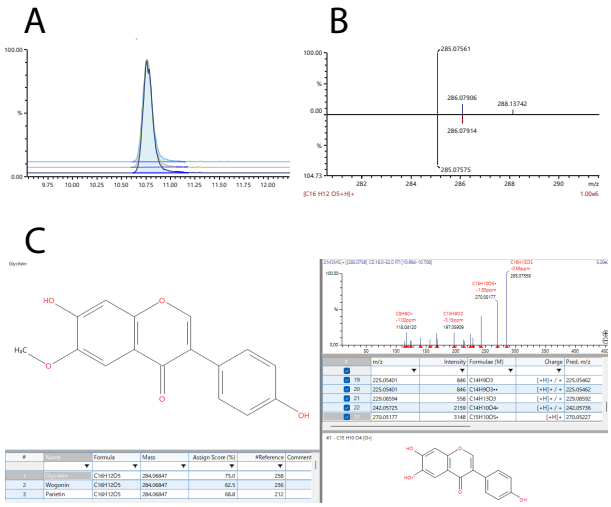

Fig. 8 Qualitative Analysis of Detected Features
(A) Aligned Chromatograms, (B) Composition Prediction Based on MS Spectrum, and (C) ChemSpider Database Search Results

As a result of composition prediction based on the MS spectrum, $[C_{16}H_{12}O_5 + H]^+$ was identified with a score of 99.8. A compound search using the ChemSpider database revealed Glycitein as the compound with the most references. Furthermore, after performing the Assign operation, MS/MS fragments could be assigned with a score of 75. The spectral feature was detected only in the beer-like beverage (No. 4) made from soybeans, suggesting that it reflects the type of raw material.

■ Conclusion

In this study, compounds in various beer and beer-like beverages were analyzed by non-targeted analysis using an LCMS-9030 quadrupole time-of-flight mass spectrometer system. Analyzing compounds characteristic of each sample with LabSolutions Insight Profiler software confirmed that the resulting profiles reflected differences in raw materials and manufacturing methods.

LabSolutions Insight Profiler enables efficient non-targeted analysis by processing LC-QTOFMS data with an intuitive workflow. This workflow allows for objective evaluation of the taste, quality, and nutritional value of food products. Furthermore, this method is applicable not only to the analysis of compounds in beverages but also to non-targeted analysis of other types of samples.

01-01241-EN First Edition: Jul. 2026

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