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Application News

LCMS-2050 High Performance Liquid Chromatograph Mass Spectrometer

Food Metabolomics of Alcoholic Beverage Using Single-Quadrupole Mass Spectrometer —Oligosaccharide and Polysaccharide Profiling—

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User Benefits

- A single quadrupole LC/MS system allows for highly sensitive analysis of oligosaccharides at sub-nanogram levels.
- The wide mass range of the LCMS-2050 system allows for comprehensive profiling of oligosaccharides and polysaccharides in alcoholic beverages.
- The Multi-omic Analysis Package allows even inexperienced users to perform multivariate analyses with ease.

■ Introduction

Food metabolomics is the application of metabolomic techniques for food. In recent years, food metabolomics has begun to be used for a variety of purposes, including food quality assessment, predicting food quality, improving manufacturing and storage processes, and evaluating functional properties. Although food contains a huge number of metabolites, many of the metabolites linked to flavor, quality, and functional properties have been identified by prior research. Because of this, food metabolomics normally targets pre-determined components of interest. Focusing only on these important components and performing an exhaustive analysis of them is an efficient approach for achieving useful results. Application News 01-00334-EN describes a case of food metabolomics in which a single quadrupole LC/MS system was used to perform targeted analysis of mainly amino acid, organic acid, and nucleic acid metabolites.

This Application News describes an example in which a single quadrupole LC/MS system was used to perform a comprehensive analysis and profiling of oligosaccharide and polysaccharide components. Compared to triple quadrupole LC/MS systems, single quadrupole LC/MS systems are cheaper and offer easier parameter configurations so even users inexperienced in mass spectrometry can perform metabolomic analyses with ease.

■ Samples and Sample Preparation

Table 1 shows a lager beer (beer 1), an ale beer (beer 2), one low-malt beer (*happoshu*), one new genre beer (*daisan*), and two non-alcoholic beers that were analyzed. Samples were prepared by diluting each beverage by a factor of 10 with ultrapure water.

Table 1 Sample Details

Sample	Description
Beer 1	Lager beer (bottom fermentation)
Beer 2	Ale beer (top fermentation)
Low-malt beer (*happoshu*)	Sugar and purine free
New genre beer (*daisan*)	Uses soy protein as a raw material
Non-alcoholic beer 1	Sugar and purine-free (made in Japan)
Non-alcoholic beer 2	Made in Germany

■ Analytical Conditions

Analysis was performed using the Nexera™ XR HPLC system and the LCMS-2050 single quadrupole mass spectrometer (Fig. 1). The LCMS-2050 single quadrupole mass spectrometer is compact but affords excellent ease of use and performance. The LCMS-2050 is equipped with a heated DUIS™ dual ion source that combines the benefits of electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI), and it supports a mass range of *m/z* 2 to 2000. These features are especially useful in metabolomics, which typically analyzes metabolites with a wide range of physical properties.

Fig. 1 Nexera™ XR and LCMS-2050

HPLC and MS analytical conditions are shown in Table 2. This analysis targeted maltooligosaccharides and polysaccharides that are likely to be found in beer, and selected ion monitoring (SIM) mode was configured for simultaneous analysis of molecules with up to 40 monosaccharide units. Components with molecular weights of 1500 or higher were analyzed as multivalent ions due to their measurable mass range and sensitivity considerations.

Table 2 Analytical Conditions

HPLC Conditions (Nexera XR)	
Column:	Shodex Asahipak NH2P-40 3E (250 mm × 3.0 mm I.D., 4.0 µm)
Flowrate:	0.3 mL/min
Mobile Phases:	A) 2.5 mmol/L Ammonium bicarbonate aq. B) 25 mmol/L Ammonium bicarbonate aq. /Acetonitrile=10:90
Time Program:	70 %B (0 min)→40 %B (25 min) →70 %B (25.01-30 min)
Mixer:	20 µL
Column Temp.:	40 °C
Injection Volume:	5 µL
MS Conditions (LCMS-2050)	
Ionization:	ESI/APCI (DUIS), Negative mode
Mode:	SIM (40 Events)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	400 °C
DL Temp.:	150 °C

■ Analysis of a Mixed Standard Solution

Fig. 2 shows the mass chromatogram for a 10 mg/L mixture of maltooligosaccharides (G1 to G10 mix, BC-GM, oligosaccharide analysis standard, Senshu Scientific Co., Ltd.). As well as oligosaccharides DP1 to DP10, polysaccharides were detected with up to 24 monosaccharide units. Using the highly sensitive LCMS-2050 as a detector enabled the detection of oligosaccharides and polysaccharides present in very small amounts not otherwise detectable by LC.

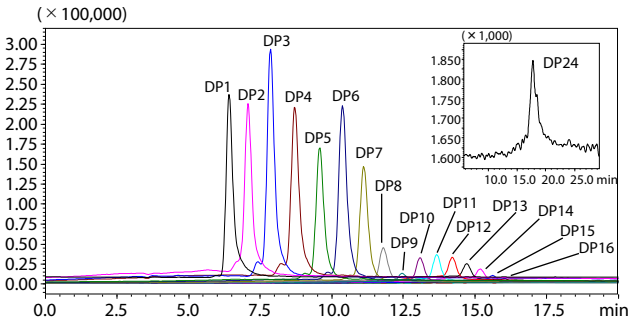


Fig. 2 Mass Chromatogram of Maltooligosaccharide Mixture

Calibration curves were prepared for glucose and maltooligosaccharides DP2 to DP7 from the mixed standard. All calibration curves showed good linearity with a coefficient of determination r^2 of 0.995 or higher. Figs. 3 and 4 give typical examples, showing calibration curves and the mass chromatogram for the lowest data point on the calibration curves for maltotriose and maltopentaose. Table 3 shows the concentration range, coefficient of determination, and repeatability (%RSD, n = 6) of the lowest data point for all calibration curves. Using a single quadrupole LC/MS system as a detector enabled a highly sensitive analysis of components at sub-nanogram levels that would otherwise be difficult when using a refractive index detector (RID), evaporative light scattering detector (ELSD), or charged aerosol detector (CAD).

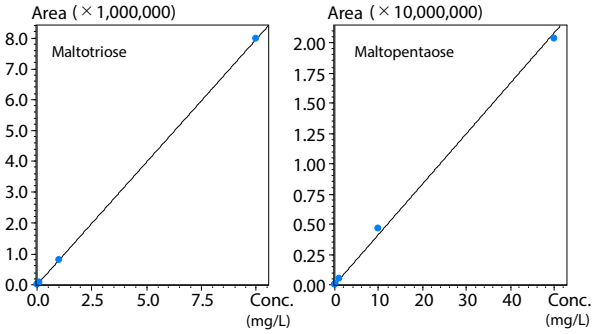


Fig. 3 Calibration Curves

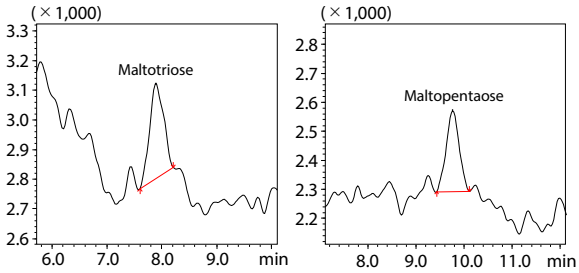


Fig. 4 Mass Chromatogram of Lowest Data Point in Calibration Curve

Table 3 Concentration Range, Coefficient of Determination (r^2), and Repeatability of Calibration Curves

Compound	Conc. Range (mg/L)	r^2	%RSD (R.T.)	%RSD (Peak area)
Glucose	0.05-100	0.998	0.25	4.49
Maltose	0.05-10	0.999	0.20	5.09
Maltotriose	0.01-10	0.999	0.40	8.33
Maltotetraose	0.01-50	0.995	0.36	6.46
Maltopentaose	0.01-50	0.997	0.52	5.66
Maltohexaose	0.05-50	0.997	0.23	4.35
Maltoheptaose	0.05-100	0.997	0.37	6.65

■ Analysis of Beers

The number of oligosaccharides and polysaccharides detected in each sample is shown in Table 4. Almost no oligosaccharides or polysaccharides were detected in the low-malt beer and non-alcoholic beer 1, which are both labeled sugar-free. By contrast, maltose, other maltooligosaccharides, and polysaccharides believed to be glucose polymers were detected in high numbers in beer 1, beer 2, and non-alcoholic beer 2. Fig. 5 shows the mass chromatogram for beer 2. Polysaccharides with up to 36 monosaccharide units (mean molecular weight: 5855.09) were detected as trivalent ions (*m/z* 1949.63). Polysaccharides with molecular weights as high as this were detected thanks to the LCMS-2050 system, which is a compact, single quadrupole LC/MS system with a wide mass range.

Table 4 Numbers of Detected Compounds

Beer 1	Beer 2	Low-malt beer	New genre beer	Non-alcoholic beer 1	Non-alcoholic beer 2
36	36	4	36	15	36

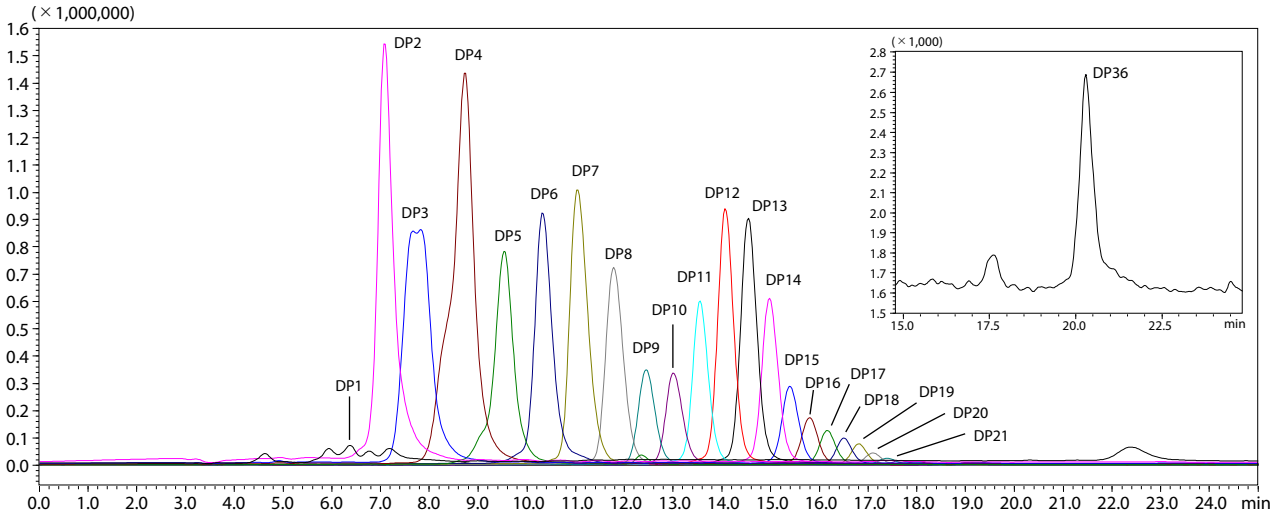


Fig. 5 Mass Chromatogram of Beer 2

The peak areas of each component were used to perform principal component analysis with the Multi-omics Analysis Package (Fig. 6). The Multi-omics Analysis Package is metabolic engineering software that can automatically display large volumes of data from metabolomic, proteomic, and fluxomic analysis on metabolic pathway maps and perform a variety of multivariate analyses. The streamlined workflow and data visualization of the Multi-omics Analysis Package facilitates intuitive understanding of data and is especially useful for drug discoveries, diagnostics, bioengineering, and other fields of life science research. The Multi-omics Analysis Package has data analysis gadgets (software tools) and data processing gadgets linked to each data analysis gadget that are used together in a manner that resembles a single integrated piece of software.

The results of the principal component analysis are shown in Fig. 7. The score plot in Fig. 7 (a) shows that beer 1 and beer 2 are clustered near to each other, as are low-malt beer and non-alcoholic beer 1, indicating similar characteristics in their components. Non-alcoholic beer 2 is located away from the other samples, indicating characteristics different from the other samples. The loading plot in Fig. 7 (b) shows a large number of oligosaccharides and polysaccharides plotted on the left side for the first principal component (PC1). This suggests the first principal component axis represents the percentage of malt in the raw materials. Using principal component analysis in this way provides an easy means of differentiating between samples and understanding the characteristics of each sample.

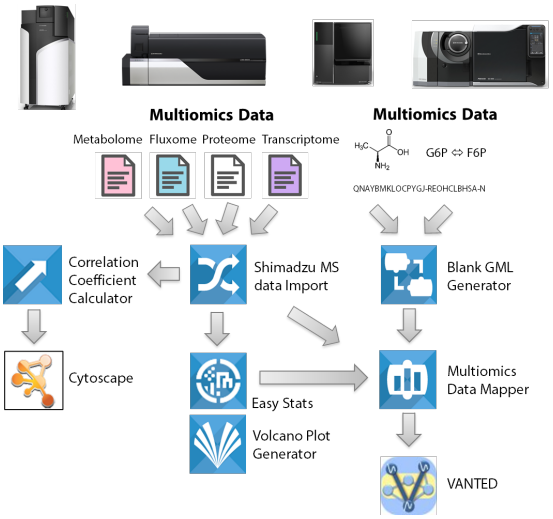


Fig. 6 Multi-omics Analysis Package

Use this QR code to access the introduction and the instructional videos for Multi-Omics Analysis Package
https://www.shimadzu.com/labcamp/multiomics5.html

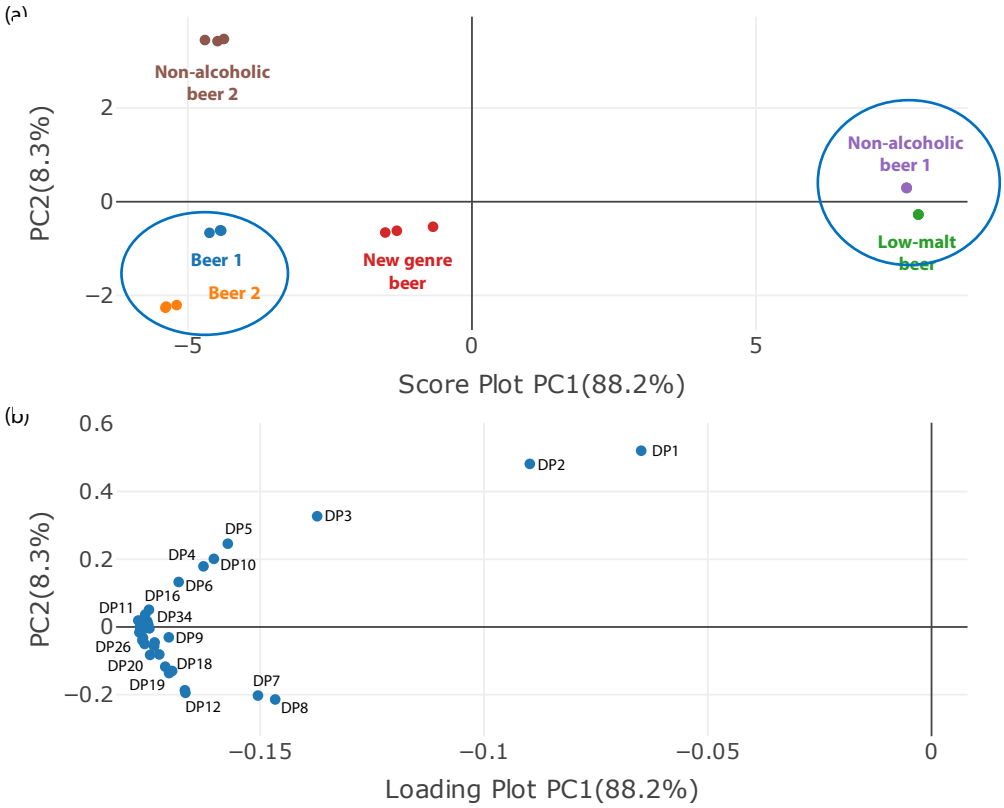


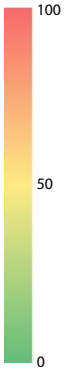
Fig. 7 Results of Principal Component Analysis
(a) Score Plot, (b) Loading Plot

A heat map showing relative peak areas of each oligosaccharide and polysaccharide was also created based on 100 as the maximum peak area of a given component in any sample (Table 5). The map shows that beer 1 and beer 2 contain large amounts of oligosaccharides and polysaccharides that are probably derived from malt, while non-alcoholic beer 1 and non-alcoholic beer 2 show vastly different characteristics despite being the same type of beverage. Non-alcoholic beer 1, which was made in Japan, uses wort as a flavoring and is not fermented.

Non-alcoholic beer 2, which was made in Germany, uses the same raw materials as normal beer and is fermented by a method that reduces alcohol production. This production process is likely the reason for the large amounts of oligosaccharides and polysaccharides present in non-alcoholic beer 2, which are believed to be derived from malt. Non-alcoholic beer 2 also contains large amounts of DP1 (glucose) and DP2 (maltose), which may also be due to a production process that reduces alcohol fermentation, thereby leaves glucose and maltose unconverted in the solution.

Table 5 Relative Amounts of Oligosaccharides and Polysaccharides in Each Sample

	Beer 1	Beer 2	Low-malt beer	New genre beer	Non-alcoholic beer 1	Non-alcoholic beer 2
DP1	4.0	2.6	1.5	3.9	3.9	100
DP2	25.2	22.2	0.2	31.9	24.3	100
DP3	75.8	31.1	0.3	51.8	15.1	100
DP4	95.2	56.4	0.2	57.3	3.8	100
DP5	79.5	47.5	N.D.	47.1	2.2	100
DP6	82.0	69.0	N.D.	77.6	2.5	100
DP7	73.0	94.3	N.D.	100	1.9	49.6
DP8	70.8	90.6	N.D.	100	1.6	45.1
DP9	72.7	100	N.D.	87.2	1.8	86.7
DP10	53.2	64.3	N.D.	64.6	1.3	100
DP11	94.6	100	N.D.	68.1	1.0	98.2
DP12	80.3	100	N.D.	55.3	0.6	51.6
DP13	71.8	100	N.D.	55.9	0.5	52.6
DP14	68.0	100	N.D.	61.3	0.4	65.6
DP15	73.1	100	N.D.	60.2	0.3	90.1
DP16	83.9	99.1	N.D.	52.5	N.D.	100
DP17	95.0	100	N.D.	46.2	N.D.	80.5
DP18	88.0	100	N.D.	44.1	N.D.	62.4
DP19	80.4	100	N.D.	46.8	N.D.	61.2
DP20	80.3	100	N.D.	54.2	N.D.	72.6
DP21	83.8	100	N.D.	57.9	N.D.	91.4
DP22	91.4	100	N.D.	54.3	N.D.	99.3
DP23	95.0	100	N.D.	48.5	N.D.	89.8
DP24	95.8	100	N.D.	48.7	N.D.	78.6
DP25	95.2	100	N.D.	47.9	N.D.	73.5
DP26	98.5	100	N.D.	72.8	N.D.	92.1
DP27	88.1	100	N.D.	68.5	N.D.	95.5
DP28	93.0	100	N.D.	61.6	N.D.	92.8
DP29	95.8	100	N.D.	57.9	N.D.	81.7
DP30	92.6	100	N.D.	58.0	N.D.	83.5
DP31	95.7	100	N.D.	60.1	N.D.	85.8
DP32	100	98.7	N.D.	70.5	N.D.	92.2
DP33	100	94.9	N.D.	77.8	N.D.	92.5
DP34	100	90.4	N.D.	78.9	N.D.	94.5
DP35	100	98.0	N.D.	80.1	N.D.	95.7
DP36	98.7	100	N.D.	79.1	N.D.	97.3



■ Conclusion

This Application News describes an example of food metabolomics in which a single quadrupole LC/MS system was used to profile oligosaccharides and polysaccharides present in beers and non-alcoholic beers. Using the single quadrupole LC/MS system enabled a highly sensitive analysis of oligosaccharides and polysaccharides. Comprehensive food metabolomics-based analysis can be achieved by combining a metabolomic analysis that principally targets amino acid, organic acid, and nucleic acid metabolites (Application News 01-00334-EN) with the profiling of oligosaccharides and polysaccharides as described in this Application News.

Triple quadrupole LC/MS systems are often used in targeted metabolomics, but targeted metabolomics can also be performed using single quadrupole LC/MS systems. Compared to triple quadrupole LC/MS systems, single quadrupole LC/MS systems are cheaper and offer simpler parameter configurations so even users inexperienced in mass spectrometry can operate the systems with ease. The adoption of single quadrupole LC/MS systems in food metabolomics promises further technological advancements and product developments in the food sector.

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