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Application News

No. L590

Nexera™ XR High Performance Liquid Chromatograph

High Speed Analysis of Iso-α-Acids, α-Acids, and Humulinones in Beer and Usage of Multi-Data Report

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User Benefits

- Iso-α-acids, α-acids and humulinones in beer can be analyzed simultaneously in about 5 minutes by using high speed analysis conditions.
- The multi-data report creation function of LabSolutions can automatically output quantitative values and graphs without manual data transcription.

■ Introduction

Iso-α-acids are bittering components in beer which are formed by heating and isomerization of the α-acids contained in hops in the brewing process. Humulinones are also bittering components, and are formed by peroxidation of α-acids.

International Bitterness Units (IBU) are generally used in measurements of the bitterness value. The IBU value is calculated based on the results of solvent extraction of the bittering components in beer and spectrophotometric measurement of absorption at 275 nm, which is near the maximum wavelength of iso-α-acids. Although this is a simple method, overestimation is possible if the sample includes substances that have ultraviolet (UV) absorption at 275 nm. A high performance liquid chromatograph (HPLC) measures the absorption after separation with a column, so each component can be quantified more accurately.

This article introduces a simultaneous analysis of iso-α-acids, α-acids, and humulinones in five types of beer using a Nexera XR high performance liquid chromatograph, referring to EBC 9.47 (EBC: European Brewery Convention). IBU was also measured with a UV-1900i UV-Vis spectrophotometer and compared with the results of the HPLC. Use of high speed separation conditions and the multi-data report creation function of LabSolutions are also introduced.

■ Analysis of Standard Solution of Iso-α-Acids, α-Acids, and Humulinones

Fig. 1 shows the structural formulas of the iso-α-acids, α-acids, and humulinones contained in beer, each of which has three homologues. In addition, iso-α-acids also include cis-trans isomer. The reagents used in formulation of the standard solution were "DCHA-Iso, ICS-I4," "International Calibration Extract 4," and "DCHA-Humulinones, ICS-Hum1." Table 1 shows the compositions of the respective reagents. The calibration solution is prepared by dissolving the reagents in methanol containing phosphoric acid, and the standard solution is then prepared by diluting the calibration solution with the mobile phase (Fig. 4). Fig. 2 and Fig. 3 show the chromatogram of the standard solution obtained by an analysis conforming to EBC 9.47, and an analysis under the high speed analysis conditions, respectively. Because the reagents used to prepare the standard solution contain multiple homologues, grouping of iso-α-acids, α-acids, and humulinones were carried out here.

Under the EBC-compliant conditions, a pH meter is necessary in preparation of the mobile phase and one analysis requires 45 min, but under the high speed analysis conditions, a pH meter is not necessary and the analysis can be completed in only 5 min, reducing the analysis time by about 90%. Under the high speed analysis conditions, EDTA is added to the mobile phase to improve the peak shape of the iso-α-acids.

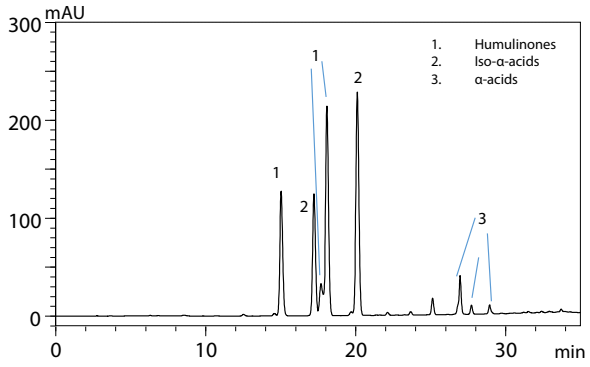

Fig. 2 Chromatogram of Standard Solution Under EBC 9.47-Compliant Conditions

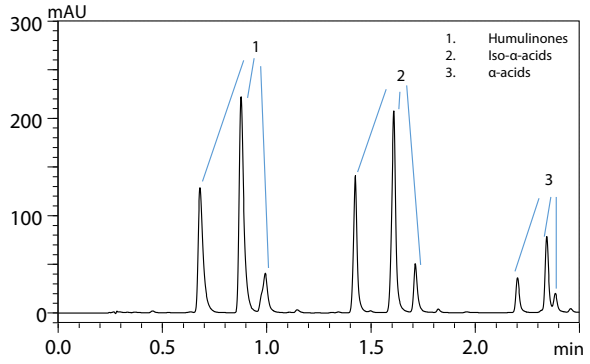

Fig. 3 Chromatogram of Standard Solution Under High Speed Analysis Conditions

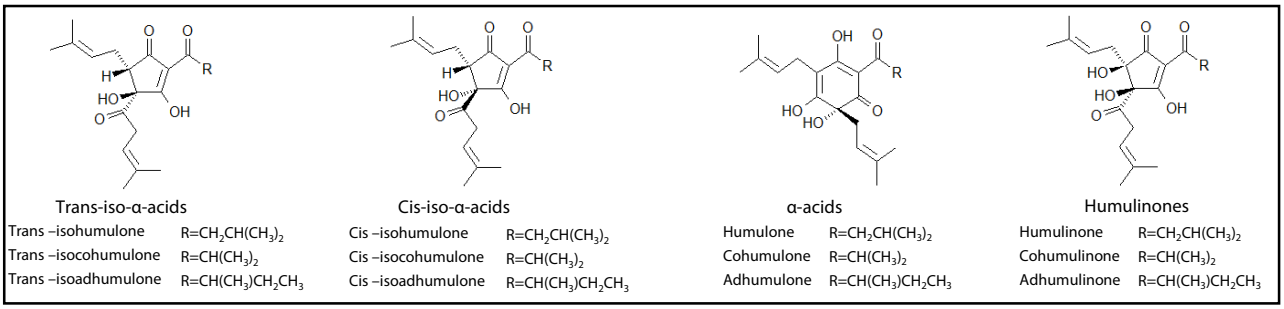

Fig. 1 Structural Formulas of Iso-α-Acids, α-Acids, and Humulinones

Table 1 Reagents Used in Preparation of Standard Solution

Reagent name	Composition
DCHA-Iso, ICS-I4	Total Iso-α-acids 65.2% (Trans isomer only)
International Calibration Extract 4	Cohumulone 10.98% N+adhumulone 31.60% **Total α-acids 42.58%**
	Colupulone 13.02% N+adlupulone 13.52% **Total β-acids 26.54%**
DCHA-Humulinones, ICS-Hum1	Humulinones 65.6%

All reagents were manufactured by the American Society of Brewing Chemists (ASBC) or Labor Veritas. ICS-I4 contains only trans isomers.

Because the β acids (colupulone, lupulone and adlupulone) contained in the standard solution elute after the α-acids, a rinsing process using a solvent with higher elution strength (Mobile Phase B) was added to both analysis conditions.

Table 2 EBC 9.47-Compliant Analysis Conditions

System	: Nexera XR
Column	: Shim-pack™ GIST C8 (250 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase A	: Acetonitrile/1% citric acid buffer(pH7.0)=30:70
Mobile Phase B	: Methanol
Flow Rate	: 1.0 mL/min
Time program	: B Conc. 15%(0-5min)-80%(30-33 min)-15%(35-45 min)
Column Temp.	: 35 ˚C
Injection Vol.	: 50 µL
Detection	: UV 270 nm
Vial	: Shimadzu Vials, LC, 1.5 mL Clear Glass *2

* 1 P/N: 227-30173-09 , *2 P/N: 227-34001-01

1% citric acid buffer(pH7.0): 10.9 g citric acid monohydrate (analytical grade) is dissolved in about 950 ml deionized water. The pH is adjusted to 7.0 with 45% KOH and deionized water added to make up to 1000 mL. The solution is filtered through a 0.45 µm filter.

Diluted solution of standard solution*3: Acetonitrile/1% citric acid buffer(pH7.0)=50:50(v/v)

Table 3 High Speed Analysis Conditions

System	: Nexera XR
Column	: Shim-pack Velox™ C18 (50 mm × 3.0 mm I.D., 1.8 µm)*4
Mobile Phase A	: 10 mmol/L (Sodium) phosphate buffer (pH2.6)+0.2 mmol/L ETDA・2Na aq.
Mobile Phase B	: Acetonitrile
Flow Rate	: 0.8 mL/min
Time program	: B Conc. 40%(0 min)-90%(2.1-3.5 min)-40%(3.51-5 min)
Column Temp.	: 40 ˚C
Injection Vol.	: 5 µL
Detection	: UV 270 nm
Vial	: Shimadzu Vials, LC, 1.5 mL Clear Glass *2

*4 P/N: 227-32008-01

Mobile phase A: Sodium dihydrogen phosphate dihydrate 5 mmol (0.78 g) and Phosphoric acid (85%, 14.7 mol/L) 5 mmol (0.34 mL) and EDTA・2Na 0.074 g are dissolved in 1 L deionized water.

Diluted solution of standard solution*5: mobile phase A / mobile phase B =50:50(v/v)

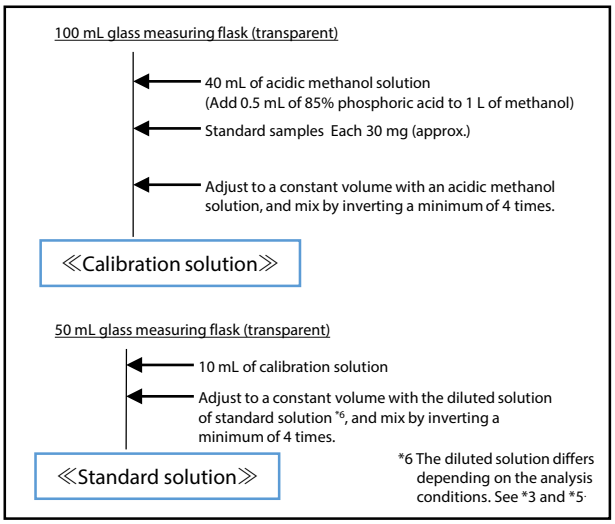

Fig. 4 Standard Solution Preparation Method

■ Repeatability of Standard Solution of Iso-α-Acids, α-Acids, and Humulinones

Table 4 shows the relative standard deviation (%RSD) of the peak area in five repeated analyses of the standard solution prepared according to the procedure in Fig. 4. Satisfactory results were obtained under both analysis conditions, as the relative standard deviation was no more than 1%, indicating that system performance is stable.

Table 4 Relative Standard Deviation (%RSD) of Peak Area of Standard Solution in 5 Repeated Analyses

Analytical condition	Humulinones	Iso-α-acids	α-acids
Based on EBC 9.47	0.04	0.19	0.28
High speed analysis	0.03	0.03	0.03

■ Analysis of Beer Samples

Samples of five commercially-available beers were prepared referring to EBC 9.47. Table 5 shows the details of each sample, and Fig. 5 shows the sample preparation procedure. Fig. 6 shows the calculation method for quantitative values in accordance with EBC 9.47, and Fig. 7 shows the chromatograms when each of the samples was measured under the EBC 9.47 conditions and the high speed analysis conditions.

Table 5 Types of Measurement Samples

Sample	Beer type	Country of manufacture
Beer Ⅰ	Lager	Japan
Beer Ⅱ	Lager	USA
Beer Ⅲ	Lager	Italy
Beer Ⅳ	Ale	Japan
Beer Ⅴ	IPA (Indian Pale Ale)	Japan

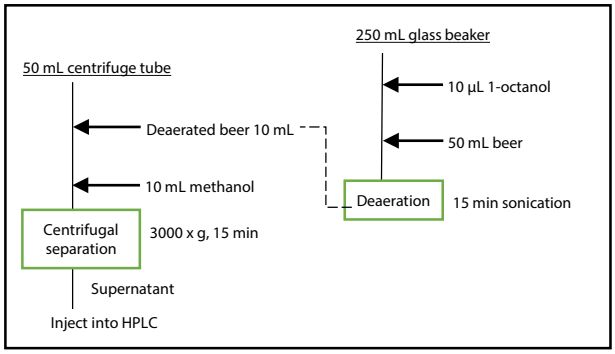

Fig. 5 Beer Sample Preparation Method

$$F = A_{std} \times 50 \times 100 / (V_{std} \times V_{inj} \times M_{std} \times C_{std} / 100)$$

where:
F = response factor (average of 4 injections) (area per mg)
A_{std} = total area of the peaks representing a particular type of iso-α-acids in the standard
M_{std} = weight of international calibration standard in mg
C_{std} = concentration of a particular type of iso-α-acids in the international calibration standard in % (m/m)
V_{std} = volume of stock standard solution in mL diluted at 50 mL (working standard solution)
V_{inj} = volume injected in mL

$$Cs = As \times D \times 1000 / (V_{inj} \times F)$$

where:
Cs = concentration of a particular type of iso-α-acids in the beer expressed as mg/L
As = total area of the peaks representing a particular type of iso-α-acids in the sample (average of 2 injections)
F = response factor in area per mg
D = dilution factor = 2 x 0.967 = 1.934 where 0.967 is due to the volume change when mixing methanol and beer 1:1 by volume.

Fig. 6 Quantitative Value Calculation Method

2* indicates a trans isomer of iso-α-acid, and 2 indicates a cis isomer.

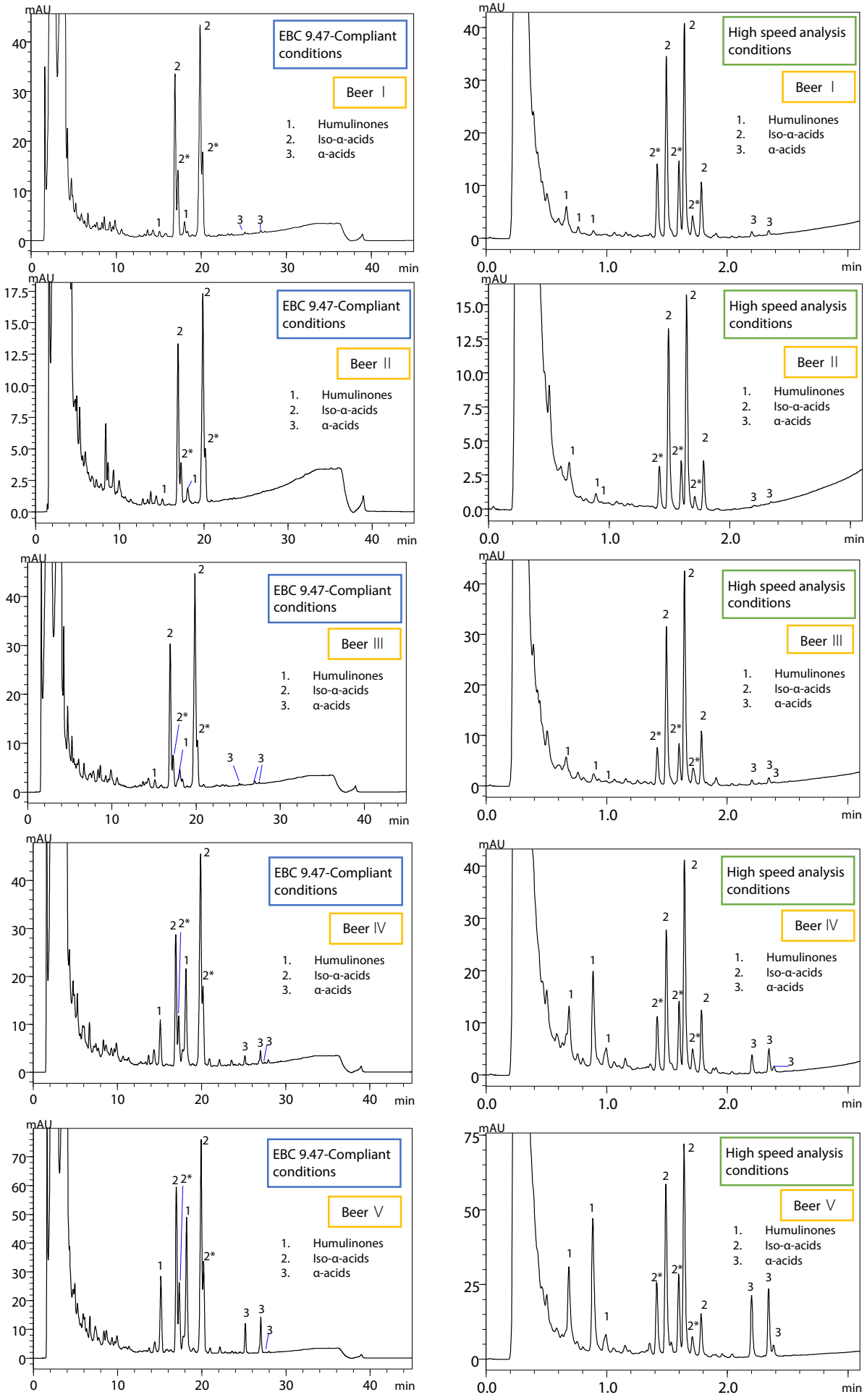

Fig. 7 Chromatograms of Beer Sample Solutions

Table 6 Quantitative Analysis Results
(EBC 9.47-Compliant Conditions)

Unit: mg/L

Sample	Humulinones	Iso-α-acids	α-acids
Beer Ⅰ	1.0	23.0	0.7
Beer Ⅱ	0.4	8.0	0.0
Beer Ⅲ	1.5	19.4	1.1
Beer Ⅳ	7.5	22.1	4.3
Beer Ⅴ	17.1	40.1	18.6

Table 7 Quantitative Analysis Results
(High Speed Analysis Conditions)

Unit: mg/L

Sample	Humulinones	Iso-α-acids	α-acids
Beer Ⅰ	1.2	21.0	0.7
Beer Ⅱ	0.6	7.0	0.1
Beer Ⅲ	1.3	18.2	0.9
Beer Ⅳ	6.4	19.7	3.6
Beer Ⅴ	14.9	36.8	18.7

Table 8 Results of Measurement by UV-1900i

Sample	IBU
Beer Ⅰ	16.3
Beer Ⅱ	5.1
Beer Ⅲ	14.1
Beer Ⅳ	23.6
Beer Ⅴ	50.5

The iso-α-acids, α-acids, and humulinones in the beers were quantified in accordance with the formulas shown in Fig. 6. Table 6 and Table 7 show the results under the respective analysis conditions. Substantially the same quantitative values were obtained under both conditions. Furthermore, Table 8 shows the results of measurements of the IBU values with a UV-1900i UV-Vis spectrophotometer in accordance with Methods of the ASBC, Method Beer-23. Details of the IBU measurement conditions may be found in Application News No. A622.

A larger IBU value was obtained for Beer V, which is an IPA (Indian Pale Ale). A distinctive feature of IPA beers is a unique bitter taste obtained by using a large amount of hops. Because hops are also added to the boiled and cooled wort (mixture of malt extract and water before fermentation), a higher level of α-acids and humulinones in comparison with other beers is thought to be a factor in the distinctive flavor of IPAs.

The sample preparation shown in Fig. 5 was carried out five times by standard addition of the same concentration as in the standard solution, and the recovery rate was calculated. Table 9 shows the recovery rate, and Table 10 shows the relative standard deviation of the peak areas of the five spiked samples.

Table 9 Results of Spike-and-Recovery Test
(High Speed Analysis Conditions, Average of n=5)

Unit: %

Sample	Humulinones	Iso-α-acids	α-acids
Beer Ⅰ	108	105	103
Beer Ⅱ	115	115	111
Beer Ⅲ	112	109	95
Beer Ⅳ	105	103	94
Beer Ⅴ	106	104	103

Table 10 Relative Standard Deviation of Peak Area of Spiked Samples
(High Speed Analysis Conditions, Average of n=5)

Unit: %

Sample	Humulinones	Iso-α-acids	α-acids
Beer Ⅰ	1.69	1.60	2.12
Beer Ⅱ	1.07	1.48	1.45
Beer Ⅲ	2.60	1.54	3.27
Beer Ⅳ	1.60	1.83	1.63
Beer Ⅴ	0.93	0.85	0.80

■ Use of Multi-Data Report *7

Multi-data report is an optional function of LabSolutions™ DB and CS which enables highly flexible report creation similar to that with spreadsheet programs. Reports can be prepared simultaneously with the completion of analysis by linkage with the analysis schedule. This function not only reduces the time required for manual report creation, but also prevents transcription errors.

A template incorporating the calculation formulas for the quantitative analysis shown in Fig. 6 was prepared, and a multi-data report was created from the measurement results of the iso-α-acids, α-acids, and humulinones in the beers. Because graphs can also be created simultaneously from the quantitative results, the percentage of bittering components can be judged at a glance (Fig. 8).

*7 Multi-data report is an optional function of LabSolutions DB and CS.

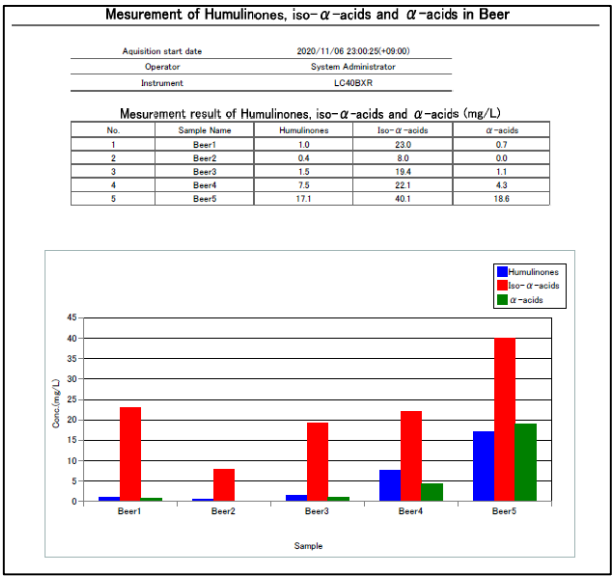

Fig. 8 Automatic Calculation of Iso-α-Acids, α-Acids, and Humulinones in Beer Samples by Multi-Data Report

■ Conclusion

This article introduced an example of an analysis of iso-α-acids, α-acids, and humulinones in five beer samples by the Nexera XR HPLC system. Analyses were performed under two conditions (EBC 9.47-compliant, high speed analysis conditions), and substantially the same quantitative values were obtained. The analysis time can be shortened by about 90% under the high speed analysis conditions, making it possible to complete one analysis in only 5 minutes. The IBU values were also measured with a UV-1900i and compared with the results by the HPLC. As a result, a high IBU value was obtained for the IPA (Indian Pale Ale). More accurate quantitative analysis is possible by employing the HPLC. Use of the multi-data report function of LabSolutions enabled automatic creation of graphs and lists of quantitative values without transcribing complex calculation formulas to a spreadsheet program.

<References>

(1) American Society of Brewing Chemists, ASBC Methods of Analysis, Beer-23
(2) European Brewery Convention, ANALYTICA EBC 9.47

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