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Application News

High Performance Liquid Chromatograph Nexera™ Series

High Speed Analysis of Xanthohumol in Beer

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User Benefits

- Xanthohumol in beer can be analyzed at high speed.
- Besides xanthohumol, other 6 components such as α acid and β acid can be analyzed in 8 minutes per analysis.
- By using a photodiode array detector, the detection wavelength can be set for each component and measurement can be performed with high sensitivity.

■ Introduction

Xanthohumol is one of the prenylated flavonoids found in hops. It has many functions such as antioxidant, anti-inflammatory, and antibacterial properties, and is attracting attention as being beneficial for human health. During wort boiling, xanthohumol is isomerized to isoxanthohumol. Isoxanthohumol has been reported to have anti-cancer and antiviral activity. Hops also contain ingredients related to bitterness such as humulinones, iso-α-acids and β-acids. Iso-α acids have been reported to be effective in improving cognitive decline.

In this article, xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids are simultaneously analyzed by the high-performance liquid chromatograph Nexera X3, referring to the previously reported Application News (01-00025-EN and L590) and EBC (European Brewery Convention) 9.47.

■ Analysis of the Standard Solutions of Xanthohumol, Isoxanthohumol, Humulinones, Iso-α acids, α-acids, β-acids

A standard solution was prepared (Table 1), and was processed in accordance with Fig. 1. Table 2 shows the analysis conditions, and Fig. 2 shows the chromatogram of the standard solution. The concentrations of each component contained in the standard solution were xanthohumol 10 mg/L, isoxanthohumol 10 mg/L, humulinone 20 mg/L, iso-α-acids 10 mg/L, α-acids 20 mg/L and β-acids 12.5 mg/L. Since the reagent itself for preparing the standard solution contains multiple homologues (Fig. 4), multiple peaks were detected in humulinones, iso-α-acids, α-acids, and β-acids. These peaks were grouped and quantified. The detection wavelength of each component was set to 370 nm for xanthohumol, 280 nm for isoxanthohumol, 270 nm for iso-α-acids and humulinones, and 314 nm for α-acids and β- acids.

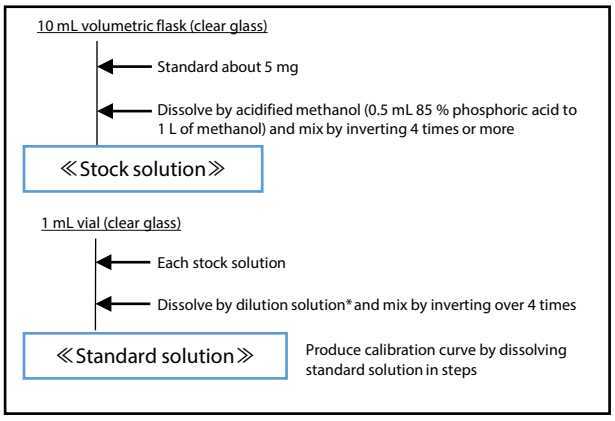

Fig .1 Preparation of Standard Solution

* Mobile Phase A/Mobile Phase B = 1:1

Table 1 Reagents for Standard Solution Preparation

Reagents	Components
Xanthohumol	> 97.0 %
(2S)-Isoxanthohumol	99.77 %
DCHA-iso, ICS-I4	Total Iso-α-acids 65.2 % (Trans isomer only)
International Calibration Extract 4	Cohumunone 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-Hum 1	Humulinonnes 65.6 %

- Procurement: Xanthohumol (Tokyo Chemical Industry Co., Ltd.), (2S) -Isoxanthohumol "DCHA-Iso, ICS-I4," International Calibration Extract 4, "DCHA-Humulinones, ICS-Hum 1" (ASBC or Labor Veritas)
- ICS-I4 contains only the transformer.

Table 2 Analytical Conditions

System	:	Nexera X3
Column	:	Shim-pack™ Velox C18 (50 mm × 3.0 mm I.D., 1.8 µm)*1
Mobile Phase A*2	:	10 mmol/L (sodium) phosphate buffer (pH2.6) + 0.2 mmol/L EDTA•2Na aq.
Mobile Phase B	:	Methanol
Flow Rate	:	0.7 mL/min
Time Program	:	B Conc. 50 % (0 min) - 90 % (6 min) - 90 % (7 min) - 50 % (7.01-8 min)
Column Temp.	:	40 °C
Injection Vol.	:	5 µL
Detection	:	PDA (SPD-M40), Standard cell
Vial	:	Shimadzu Vials, LC, 1.5 mL Clear Glass*3

*1 P/N: 227-32008-01
*2 Mobile phase A: Sodium dihydrogen phosphate dihydrate 5 mmol (1.5619 g) and Phosphoric acid (85 %, 14.7 mol/L) 5 mmol (0.68 mL) and EDTA•2Na 148.47 mg are dissolved in 2 L deionized water.
*3 P/N: 227-34001-01

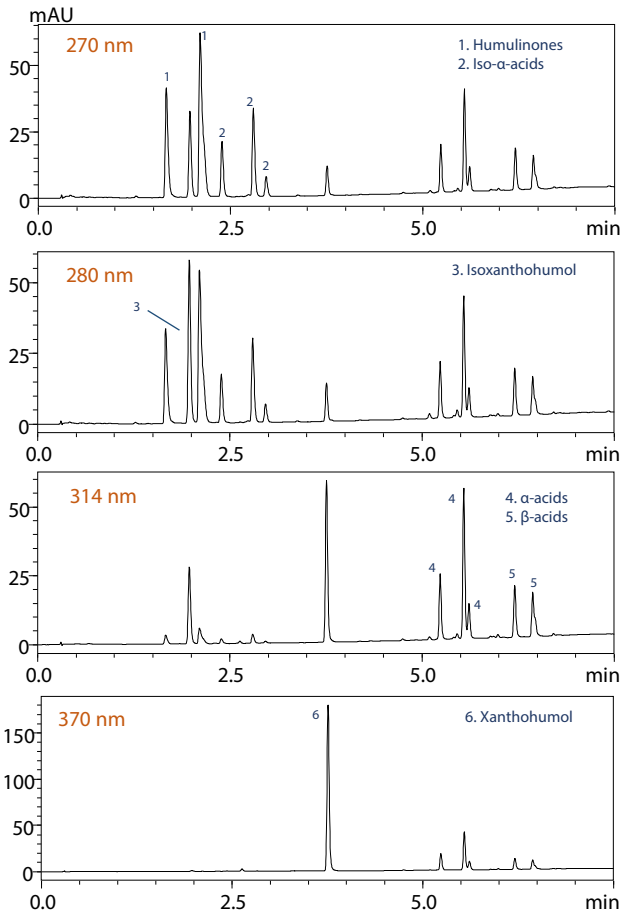

Fig. 2 Chromatograms of the Standard Solution

■ Beer Analysis

Seven types of beer were processed with reference to EBC 9.47. Fig. 3 shows the pretreatment method. Figs. 5 to 11 show the chromatograms when each sample was measured. Five-point calibration curves were prepared and each component was quantified. Table 3 shows the respective calibration curve concentration ranges and coefficients of determination. All of the coefficients of determination obtained were greater than 0.999. The quantitative results in Table 4 show the concentration contained in beer. In addition to the Trans isomer, a peak presumed to be the Cis isomer was detected in the iso-α-acid, and these were combined and quantified.

Spike and recovery tests and reproducibility tests were conducted using beers 2, 4 and 7. In the spike and recovery test, the recovery rate was calculated from the difference of the average value of the samples in which the standard solution was added and the pretreatment shown in Fig. 3 was performed 6 times, and in which the standard solution was not added and the pretreatment was performed 3 times (Table 5). In the reproducibility test, Table 6 shows the relative standard deviations of the peak areas of the 6 beer samples to which the standard solution had been added.

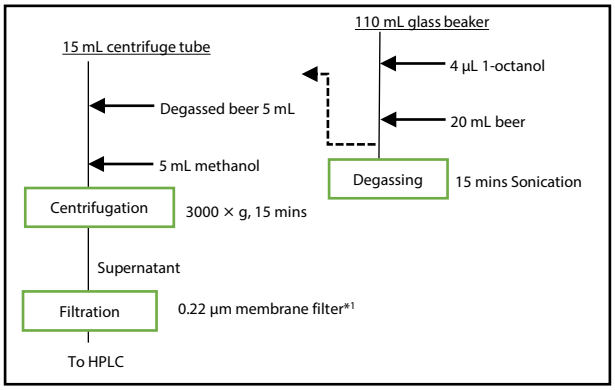

Fig. 3 Pretreatment Method for Beer

*1 P/N: GLCTD-HPTFE1322

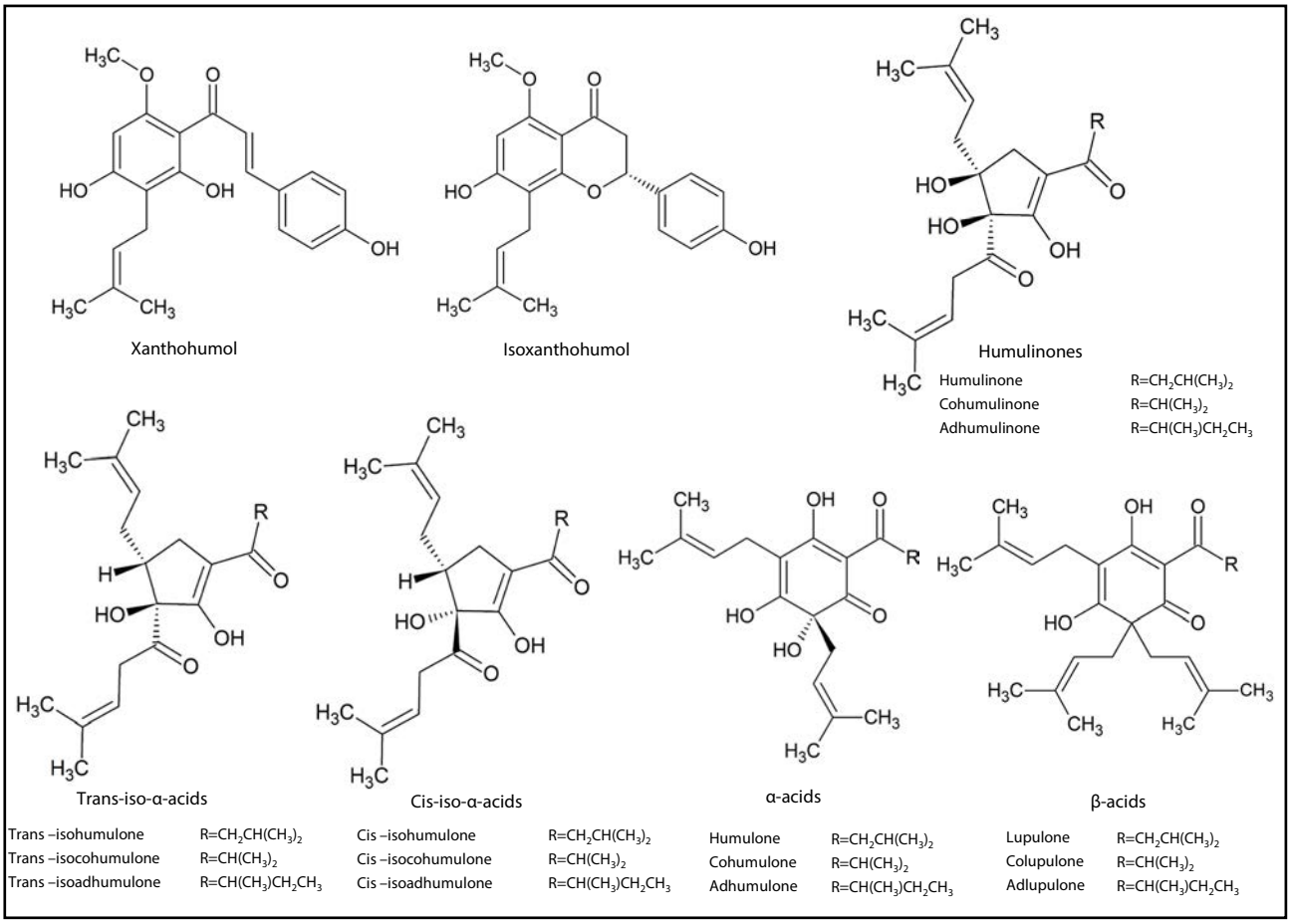

Fig. 4 Chemical Structures of Xanthohumol, Isoxanthohumol, Humulinones, Iso-α-acids, α acids and β acids

Beer 1

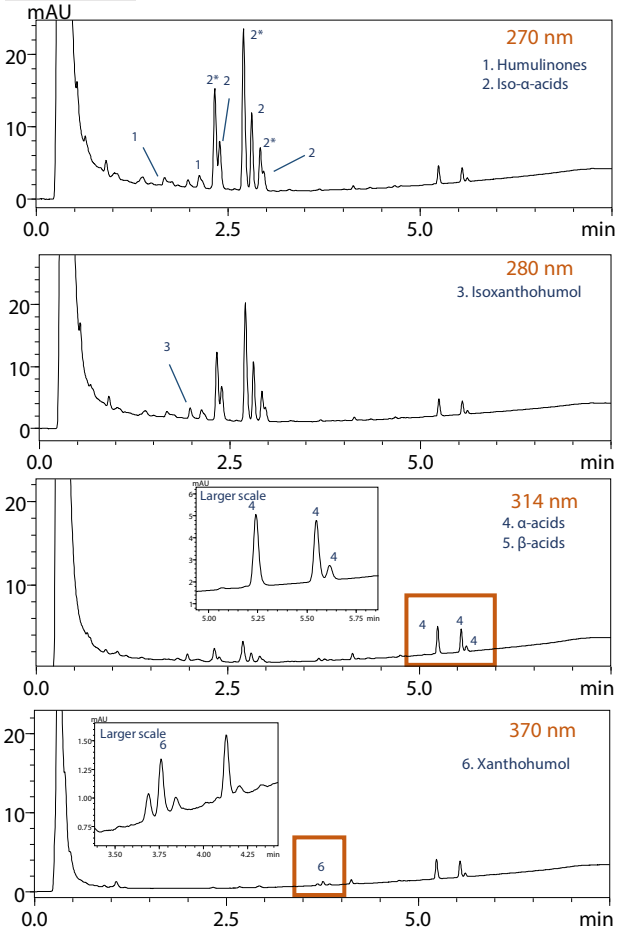

Fig. 5 Chromatograms of Beer 1

Beer 2

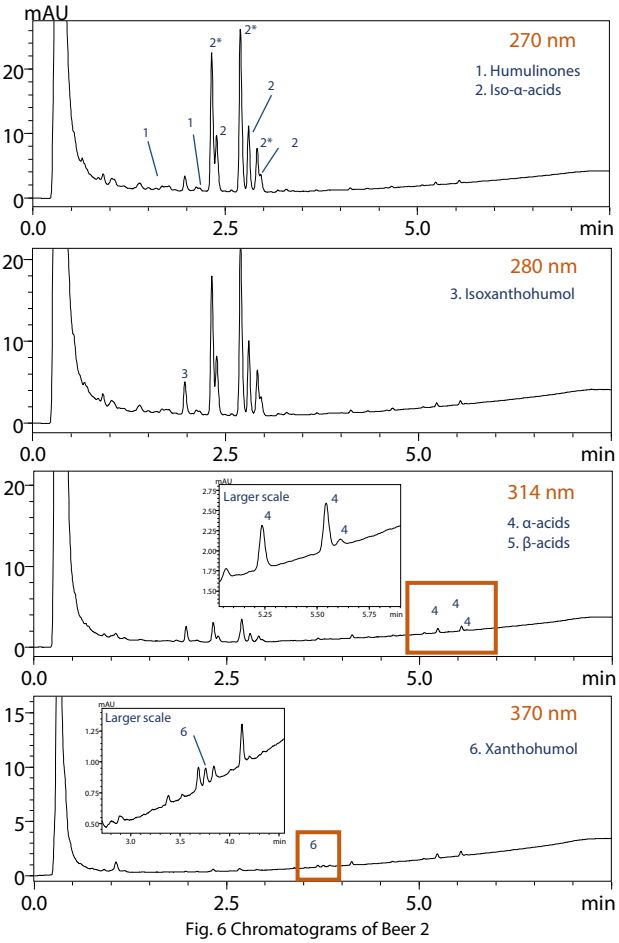

Fig. 6 Chromatograms of Beer 2

Beer 3

2 is assumed to be the trans iso-α acid, and 2* is assumed to be the cis iso-α acid.

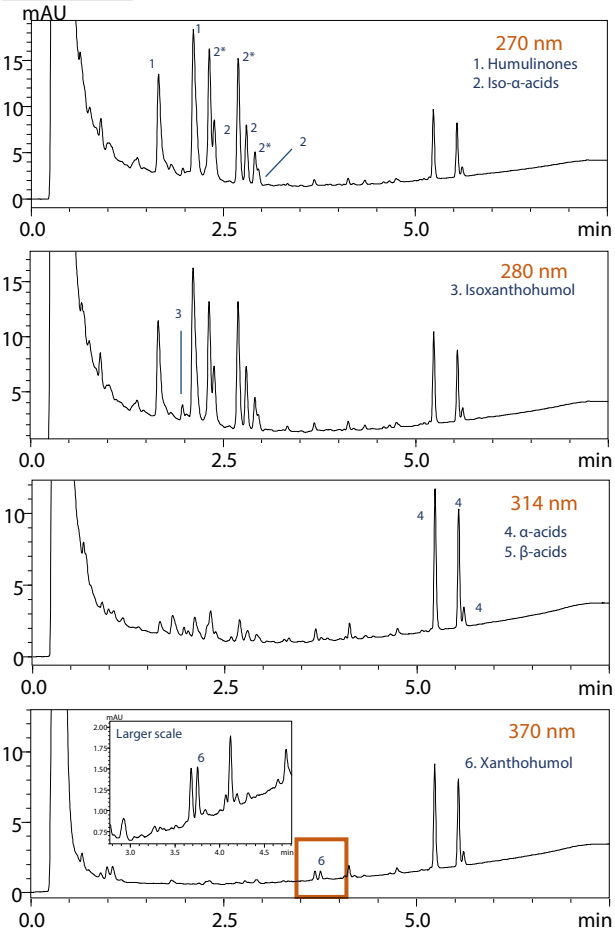

Fig. 7 Chromatograms of Beer 3

Beer 4

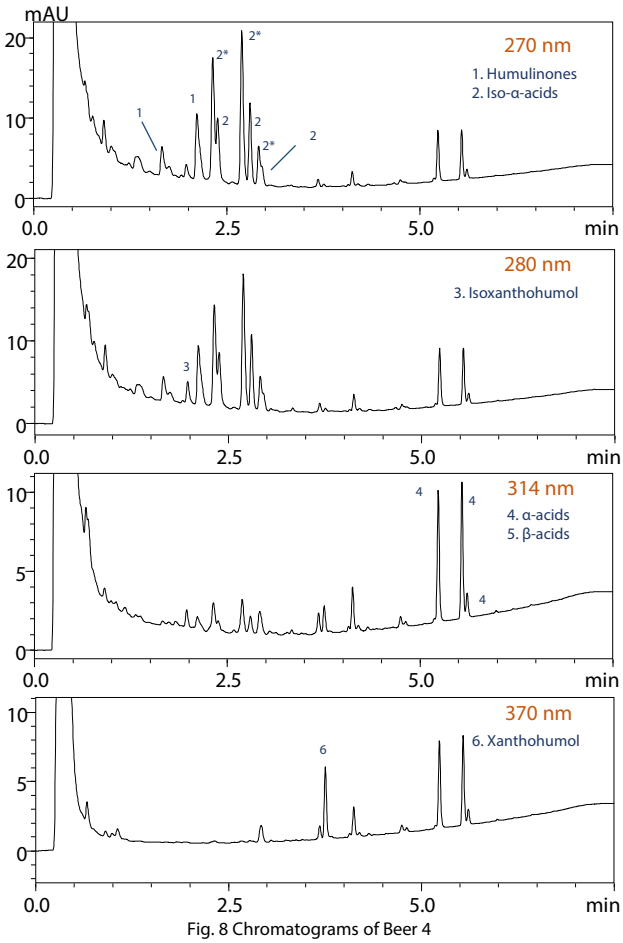

Fig. 8 Chromatograms of Beer 4

Beer 5

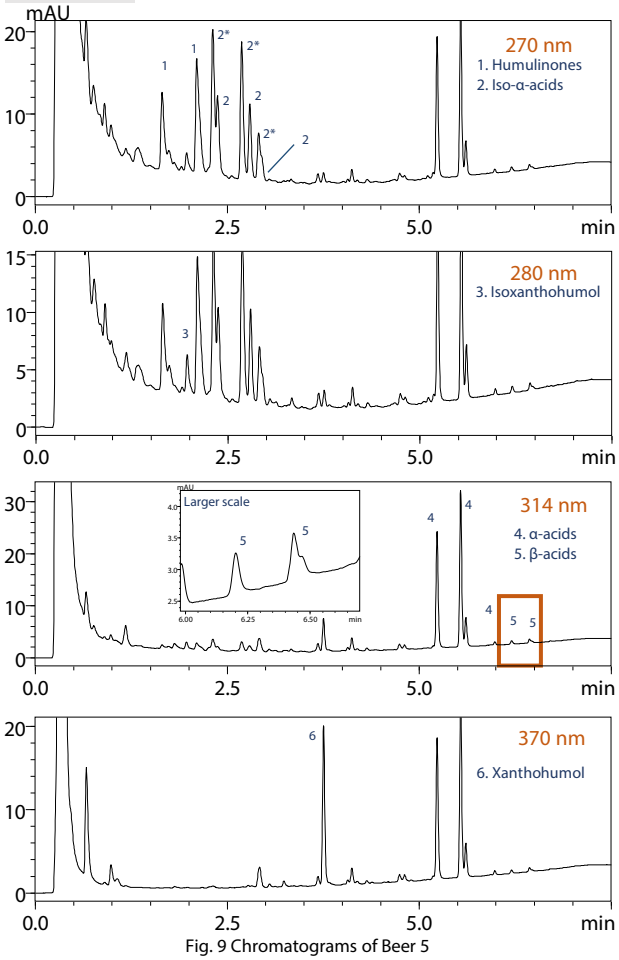

Fig. 9 Chromatograms of Beer 5

Beer 6

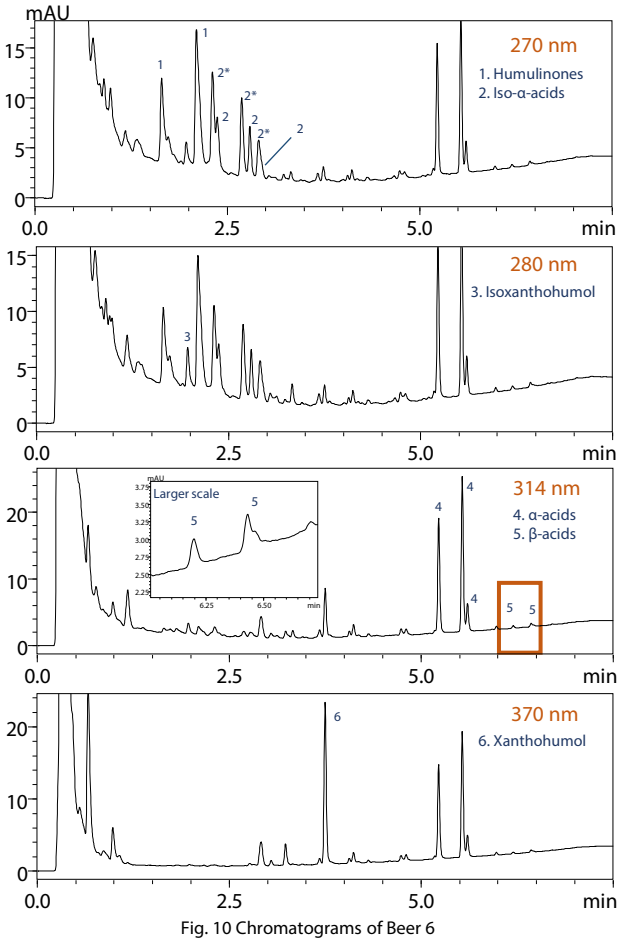

Fig. 10 Chromatograms of Beer 6

Beer 7

2 is assumed to be the trans iso-α acid, and 2* is assumed to be the cis iso-α acid.

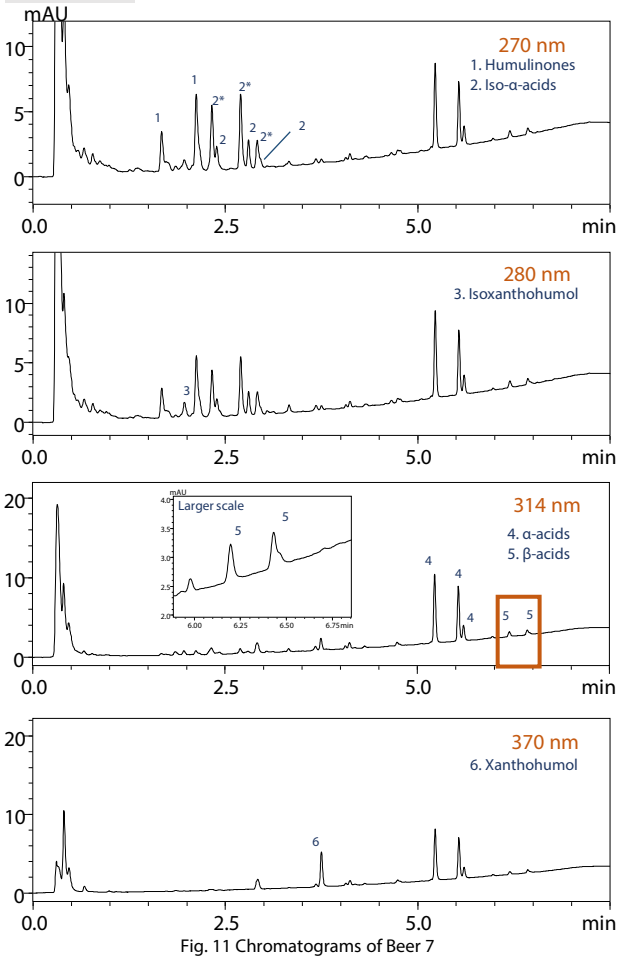

Fig. 11 Chromatograms of Beer 7

Table 3 Concentration Range and Coefficient of Determination for Calibration Curve

Compound	Conc. range (mg/L)	r^2
Xanthohumol	0.016–0.250	0.9991
	0.125–2.500	0.9999
Isoxanthohumol	0.125–2.500	0.9998
Humloinones	0.250–5.000	0.9999
	1.000–20.000	1.0000
Iso-α-acids	0.500–10.000	0.9996
α-acids	0.500–10.000	0.9990
	1.000–20.000	0.9994
β-acids	0.019–0.312	0.9996
	0.039–0.623	0.9999

■ Conclusion

In this article, an example of analysis of xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids in beer with Nexera X3 was introduced. In addition to being able to perform rapid analysis within 8 minutes per analysis, the detection wavelength could be optimized by using a PDA detector, and appropriate sensitivity could be ensured. This method is expected to improve work efficiency.

[Reference]
1) European Brewery Convention, EBC ANALYTICA, 7.15
2) European Brewery Convention, EBC ANALYTICA, 9.47
3) European Brewery Convention, EBC ANALYTICA, 9.50
4) Vázquez Loureiro P et al. "Determination of Xanthohumol in Hops, Food Supplements and Beers by HPLC", foods. 2019
5) Anna Katarzyna Żołnierczyk et al. "Isoxanthohumol — Biologically active hop flavonoid", Fitoterapia. Pages 71-82. Jun 2015
6) Táborský, J et al. "A study of dynamics of bitter acids and xanthohumol in hop pellets during storage". Agronomy Research.16(S2), 1509-1516, 2018
7) A. Gahr et al. "The Stability of Bitter Substances in Beer During the Ageing Process". Brewing Science. Dec 2020
8) Dieudonné Nimubona et al. "An approximate shelf life prediction of elaborated lager beer in terms of degradation of its iso-a-acids". Journal of Food Engineering, 138-143, Nov 2012

Table 4 Concentrations of Each Component Contained in Beer Unit: mg/L

Sample	Xanthohumol	Isoxanthohumol	Humulinones	Iso-α-acids	α-acids	β-acids
Beer 1	0.052	0.640	1.284	21.016*	3.858	0.018*
Beer 2	0.012*	1.432	0.586	25.328*	1.392	0.006*
Beer 3	0.064	0.402	12.126	16.704	9.646	0.012*
Beer 4	0.594	1.030	5.226	21.730*	9.188	0.122
Beer 5	2.092	1.326	10.456	23.150*	26.280	1.120
Beer 6	2.458	1.300	10.752	13.216	20.412	0.708
Beer 7	0.534	0.502	3.348	5.974	8.684	0.906

* Calibration curve extrapolation value

Table 5 Results of Spike and Recovery Test (Average: n = 6) Unit: %

	Beer 2	Beer 4	Beer 7
Xanthohumol	95	104	103
Isoxanthohumol	104	111	107
Humloinones	102	109	108
Iso-α-acids	98	113	106
α-acids	91	103	91
β-acids	96	94	103

Table 6 Results of Reproducibility Test (Average: n = 6) Unit: %

	Beer 2	Beer 4	Beer 7
Xanthohumol	8.609	4.923	2.143
Isoxanthohumol	5.751	5.166	3.711
Humloinones	7.740	4.843	2.570
Iso-α-acids	1.431	2.962	2.627
α-acids	5.914	5.253	2.190
β-acids	8.021	7.937	1.648

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