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Application News

High Performance Liquid Chromatograph LC-2050C 3D

Simultaneous Analysis of Beer Components (Xanthohumol, Humulinones, Iso-α-Acids, α-Acids, and β-Acids) Using an Integrated HPLC System

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User Benefits

- Beer components, xanthohumol and isoxanthohumol are considered to provide health benefits. These two components and bitter taste-related components (humlinones, iso-α-acids, α-acids, and β-acids) can be analyzed simultaneously using LC-2050C 3D, an integrated HPLC.
- Improved reliability for beer component quantitation can be obtained using optimized analytical conditions provide reduced effect from contaminants.

■ Introduction

In recent years, beer manufacturers have been developing beer that not only tastes good but also provides health benefits. Xanthohumol is one of the prenylated flavonoids found in hops and attracting attention as being beneficial for human health because of its functions such as antioxidant, antiinflammatory, and antibacterial properties. During wort boiling, xanthohumol is isomerized to isoxanthohumol, which has been reported to have anticancer and antiviral activity. Hops also contain components related to bitterness such as humulinones, iso-α-acids and β-acids. High-speed simultaneous analysis of the six components in only eight minutes was previously reported in Application News 01-00375A.

However, to reduce column damage when connected to a i-Series LC-2050C 3D high performance liquid chromatograph, an analytical column packed with larger particles compared to those in the column used for the previous Application News was employed in this study. Simultaneous analysis beer components (xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids) using optimized analytical conditions including large-particle packed column to reduce the effects of contaminants is described in this article.

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

Standard solutions were prepared using the reagents in Table 1 according to the procedure in Fig. 1. The standard solution (containing 10 mg/L xanthohumol, 10 mg/L isoxanthohumol, 20 mg/L humulinones, 20 mg/L iso-α-acids, 20 mg/L α-acids, and 12.5 mg/L β-acids) were analyzed with the conditions listed in Tables 2 and 3 to obtain the chromatograms shown in Fig. 2. Because the reagents used to prepare the standard solution contained multiple homologs (Fig. 4), multiple peaks were detected for the humulinones, iso-α-acids, α-acids, and β-acids. Those related peaks were combined into above mentioned groups to create calibration curves. Detection wavelengths were specified based on references 1), 2), 3), and 4) (Table 2).

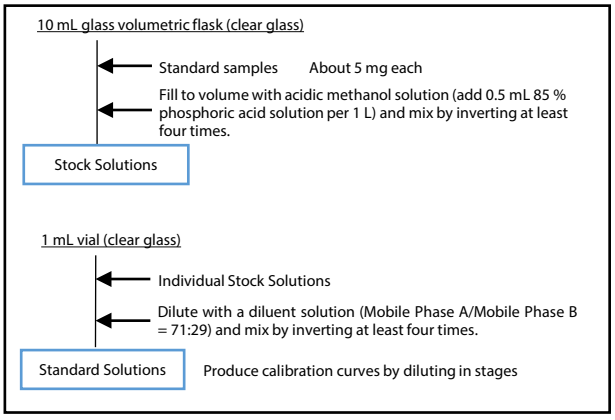


Fig. 1 Standard Solution Preparation

Table 1 Reagents for Preparing Standard Solutions

Reagent	Composition
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	Humulinones 65.6 %

- Reagent source: Xanthohumol purchased from Tokyo Chemical Industry Co., Ltd., (2S)-isoxanthohumol from MedChemexpress Co., Ltd., "DCHA-iso, ICS-I4," "International Calibration Extract 4," and "DCHA-Humulinones, ICS-Hum1" from ASBC or Labor Veritas
- ICS-I4 contains only the trans isomers.

Table 2 Analytical Conditions

System:	i-Series LC-2050C 3D
Column:	Shim-pack Velox™ Biphenyl (100 mm × 3.0 mm I.D., 2.7 µm) *1
Mobile Phase A*2:	10 mmol/L (Sodium) phosphate buffer (pH2.6) + 0.2 mmol/L EDTA・2Na aq.
Mobile Phase B:	Acetonitrile
Flow Rate:	0.7 mL/min
Column Temp.:	40 °C
Injection Vol.:	20 µL
Detection:	Xanthohumol: 370 nm Isoxanthohumol: 280 nm Iso-α-acids and Humulinones: 270 nm α-acids and β-acids: 314 nm
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*3

*1 P/N: 227-32016-03
*2 Mobile phase A: Sodium dihydrogen phosphate dihydrate 5 mmol (1.56 g) and phosphoric acid (85%, 14.7 mol/L) 5 mmol (0.68 mL) and EDTA・2Na 148 mg are dissolved in 2 L deionized water.
*3 P/N: 227-34001-01

Table 3 Time Program

Time (min)	B.conc
00.00	29
17.00	31
17.25	50
29.00	58
29.01	95
32.00	95
32.01	29
35.00	29

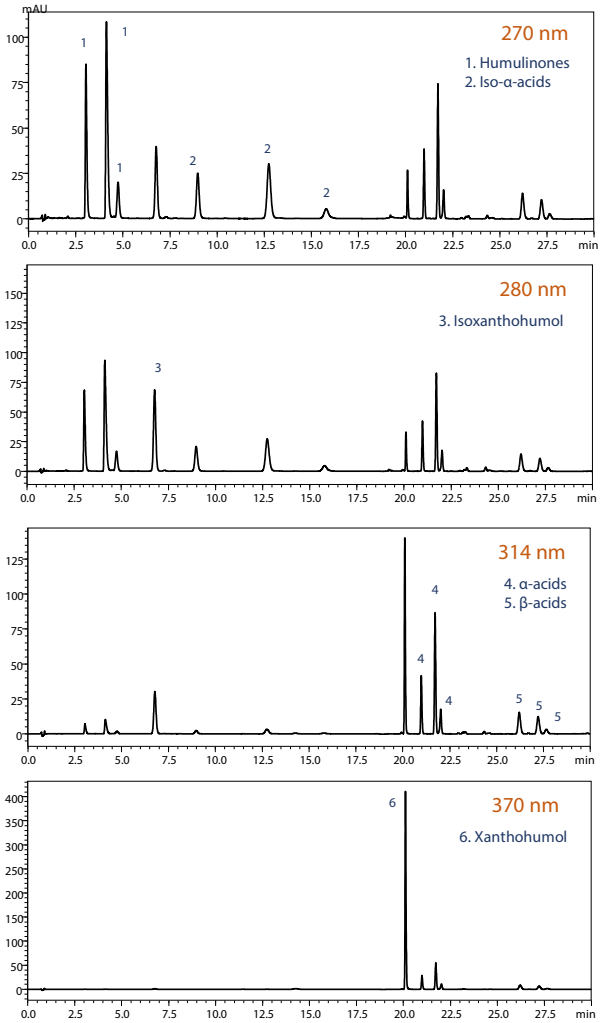
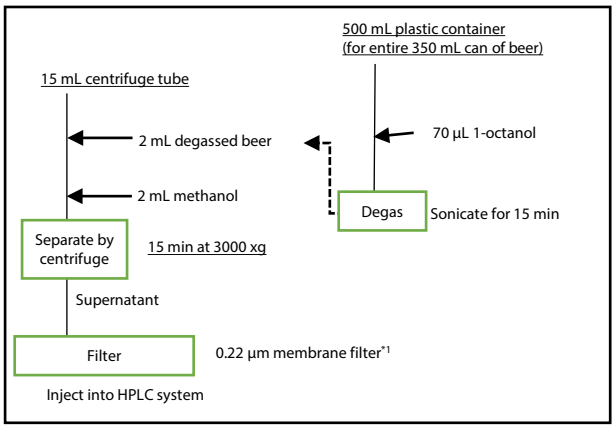


Fig. 2 Chromatograms of Standard Solutions

■ Beer Analysis

The five-level calibration curves created for six types of target components achieved the coefficients of determination over 0.999 and exhibited excellent linearity (Table 4). Three types of beer were pretreated based on the method described in Application News 01-00375A and then analyzed. The pretreatment method is shown in Fig. 3. The chromatograms from real sample analyses are shown in Fig. 5 to 7. The concentrations of the respective components in each beer are shown in Table 5. The concentrations are the totals of related *cis*- and *trans*-isomers because the peaks for presumably the *cis*-isomers of iso-α-acids were detected.

To test reproducibility, the steps after degassing were repeated six times. The relative standard deviation for peak area values of the six components from six times repeated analyses for three samples are listed in Table 6. During beer pretreatment, 1-octanol was added to samples as an antifoaming agent in order to reduce sampling errors caused by beer bubbles. Beer 1 was also used for spike and recovery testing. After the degassing step shown in Fig. 3, samples were spiked with standard solution and then the remaining steps were repeated three times. The spike and recovery test results are listed in Table 7.



*1 P/N: GLCTD-HPTFE1322

Fig. 3 Beer Pretreatment Method

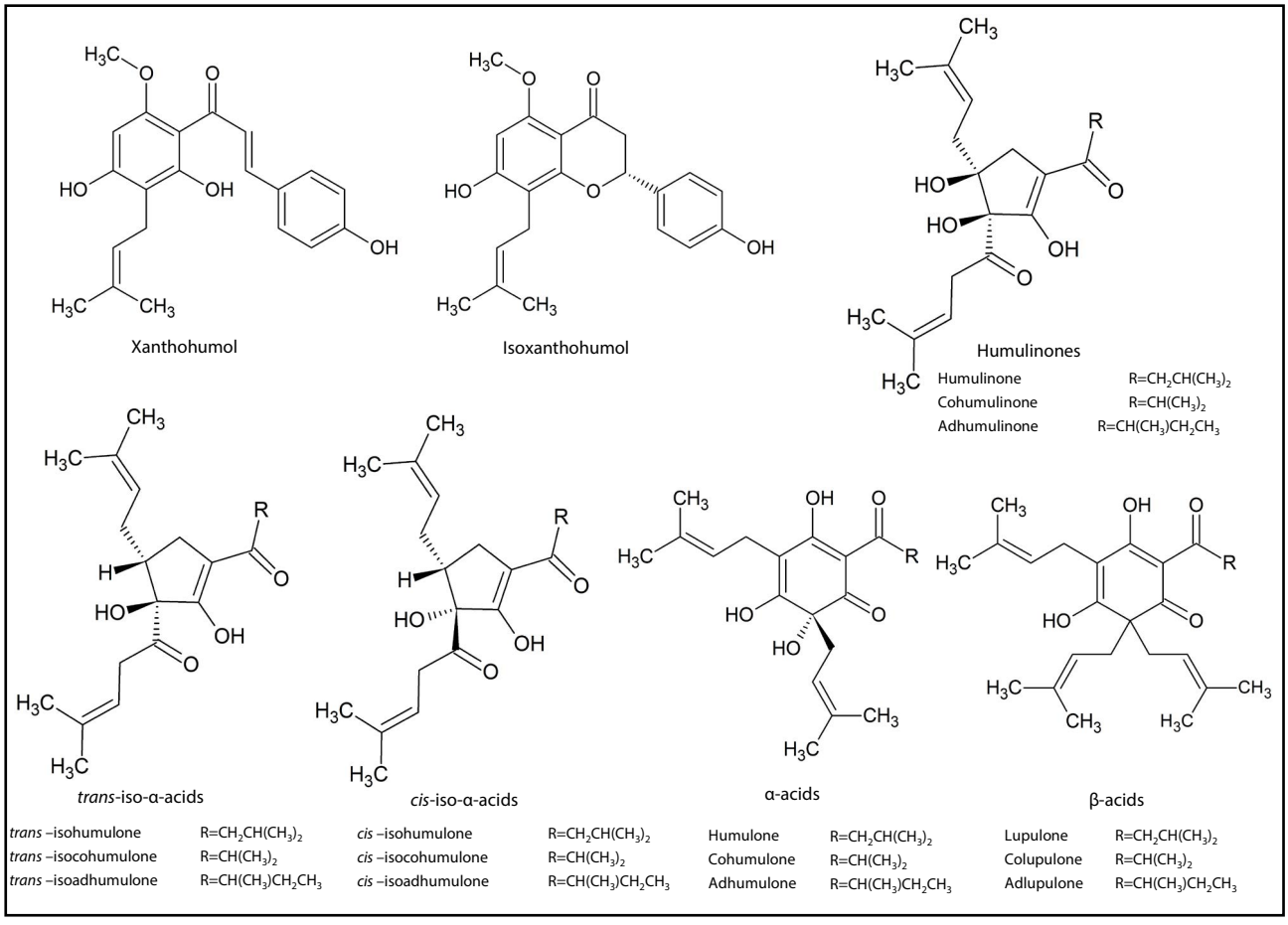


Fig. 4 Structural Formulas of Xanthohumol, Isoxanthohumol, Humulinones, Iso-α-Acid, α-Acid, and β-Acid

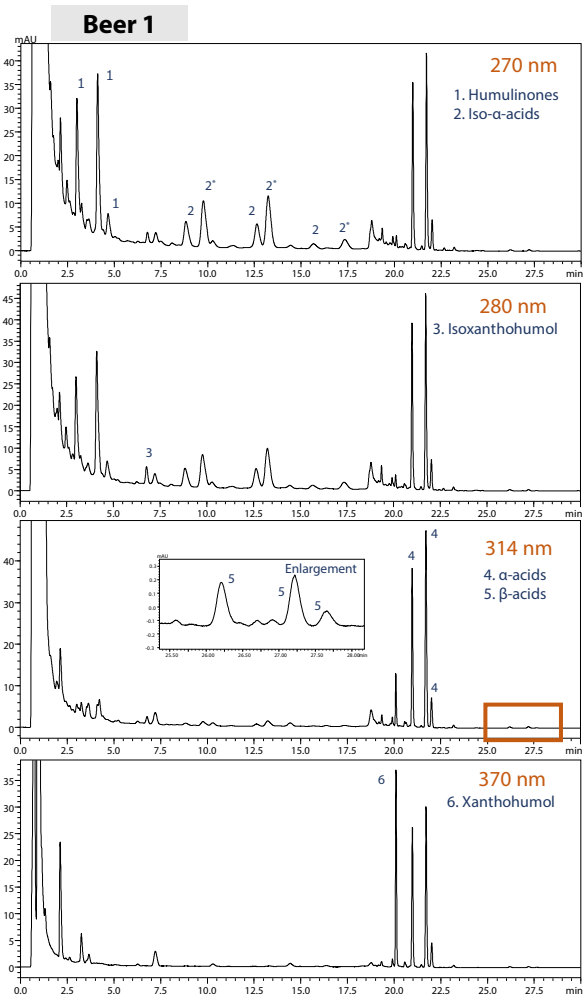


Fig. 5 Chromatograms from Beer 1

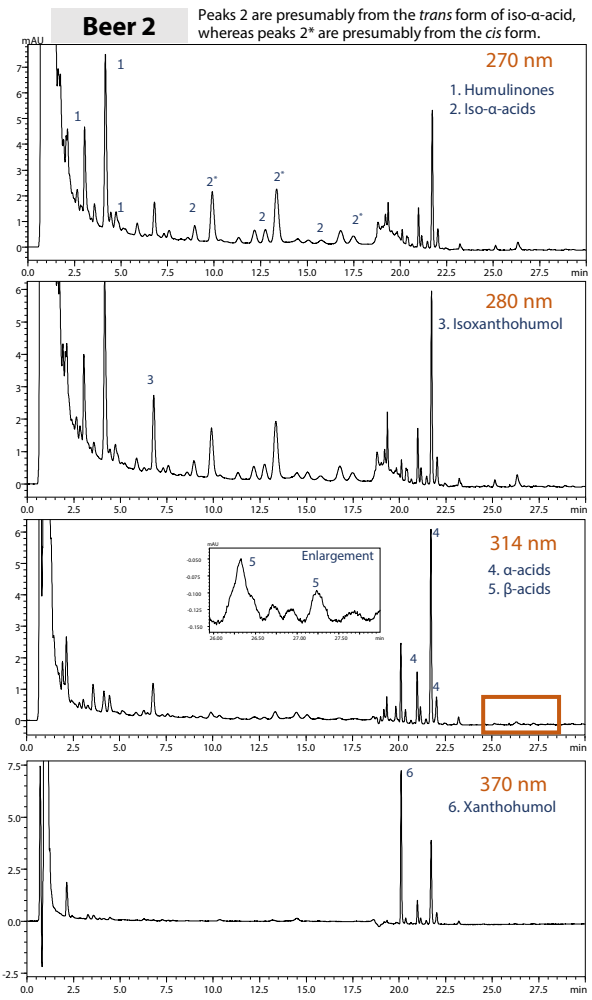


Fig. 6 Chromatograms from Beer 2

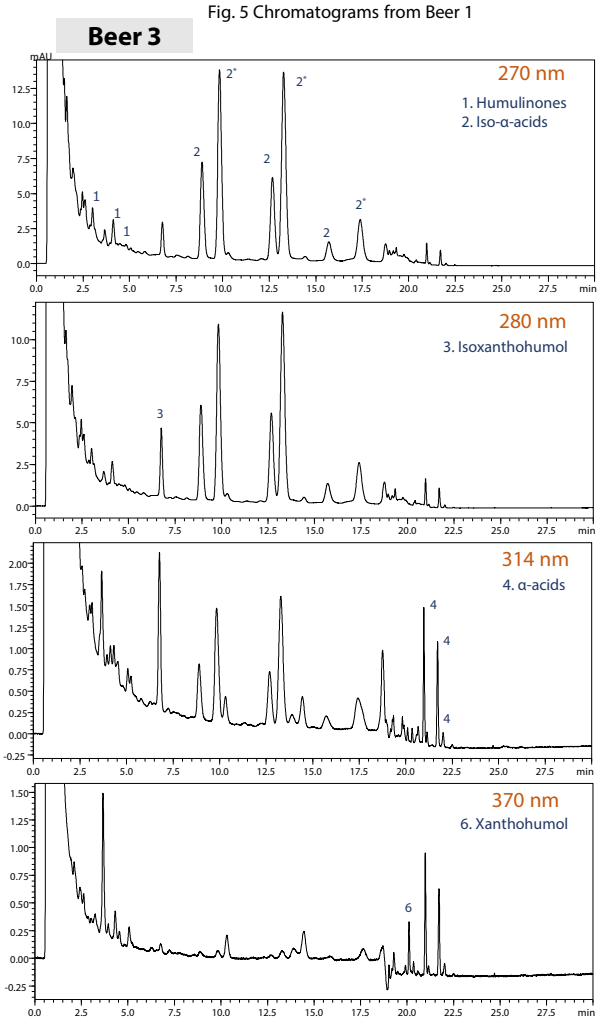


Fig. 7 Chromatograms from Beer 3

Table 4 Calibration Curve Ranges and Coefficients of determination

Compound	Conc. Range(mg/L)	r^2
Xanthohumol	0.016 to 1.000	0.9999
Isoxanthohumol	0.250 to 10.000	0.9998
Humloinones	0.250 to 10.000	1.0000
Iso-α-acids	0.500 to 20.000	0.9998
α-acids	0.500 to 20.000	0.9995
β-acids	0.039 to 1.250	0.9991

Table 5 Concentrations obtained

Unit: mg/L

Sample	Xanthohumol	Isoxanthohumol	Humulinones	Iso-α-acids	α-acids	β-acids
Beer1	1.77	1.07	14.61	29.02	25.16	2.37
Beer2	0.36	0.71	2.18	4.30	3.04	0.29
Beer3	0.01*	1.26	0.84	34.72	1.34	N.D.

* Extrapolated calibration result

Table 6 Reproducibility Test Results (%RSD, n = 6)

	Beer 1	Beer 2	Beer 3
Xanthohumol	3.31	1.33	1.73
Isoxanthohumol	2.90	0.38	1.35
Humlinones	2.72	0.84	2.18
Iso-α-acids	2.71	0.76	1.37
α-acids	3.40	0.62	2.11
β-acids	3.28	0.67	-

Table 7 Spike-Recovery Test Results
(Beer 1, average of n = 3)

Unit: %

	Recovery rate
Xanthohumol	96
Isoxanthohumol	98
Humlinones	92
Iso-α-acids	111
α-acids	97
β-acids	103

■ Summary

This article describes analyzing concentrations of xanthohumol, isoxanthohumol, humulinones, iso-α-acids, α-acids, and β-acids in beer using the i-Series LC-2050C 3D HPLC system. Simultaneous analysis was enabled by improving the separation between target components and contaminants.

Reference Information
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) Dieudonné Nimubona et al. “An approximate shelf life prediction of elaborated lager beer in terms of degradation of its iso-α-acids”. Journal of Food Engineering, 138-143, Nov 2012

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