

Simultaneous Analysis of Beer Components (Xanthohumol, Isoxanthohumol, Humulinones, Iso- α -Acids, α -Acids, and β -Acids)

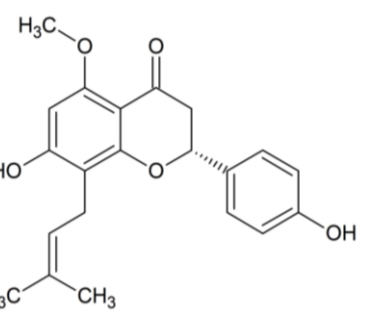
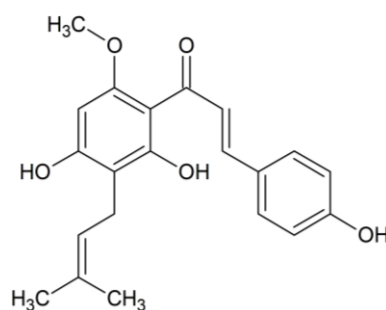
Mizuki Hayakawa¹, Hidetoshi Terada²

1 Shimadzu Corporation, Kanagawa, Japan

2 Shimadzu Corporation, Kyoto, Japan

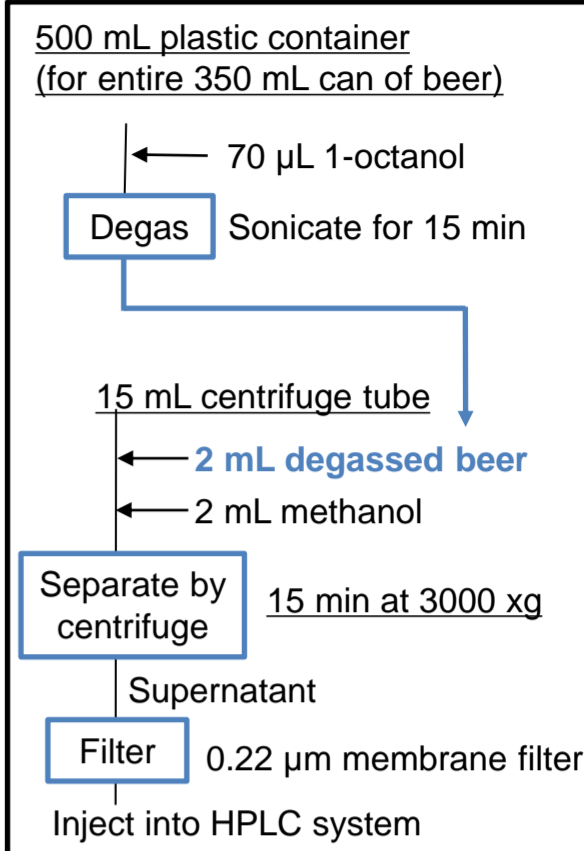
1. Introduction

In recent years, beer manufacturers have been developing beer that not only tastes good but also provides health benefits. Xanthohumol (Fig. 1) 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 [1]. During wort boiling, xanthohumol is isomerized to isoxanthohumol (Fig. 2), which has been reported to have anticancer and antiviral activity [1][2]. Hops also contain components related to bitterness such as humulinones, iso- α -acids and β -acids. Especially iso- α -acids are reported to be effective for the functional decline due to dementia [3]. The European Brewery Convention (EBC) and the American Society of Brewing Chemists (ASBC), two measure related organizations issue beer analysis methods. However, xanthohumol and isoxanthohumol are not described. In this paper, we report simultaneous analysis of xanthohumol, isoxanthohumol, humulinones, iso- α -acids, α -acids, and β -acids with HPLC referring to EBC 9.47 [4].



2-2. 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 2). The pretreatment method is shown in Fig. 5. The chromatograms from real sample analyses are shown in Fig. 6. The concentrations of the respective components in each beer are shown in Table 3. 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 [7]. 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 4. 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. 5, samples were spiked with standard solution and then the remaining steps were repeated three times (Table 5).



2. Experiments and Results

2-1. Analysis of Standard Solution of Xanthohumol, Isoxanthohumol, Humulinones, Iso- α -Acids, α -Acids, and β -Acids

Standard solutions were prepared according to the procedure in Fig. 3. The standard solution were analyzed with the conditions listed in Tables 1 to obtain the chromatograms shown in Fig. 4. Because the reagents used to prepare the standard solution contained multiple homologs, 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.

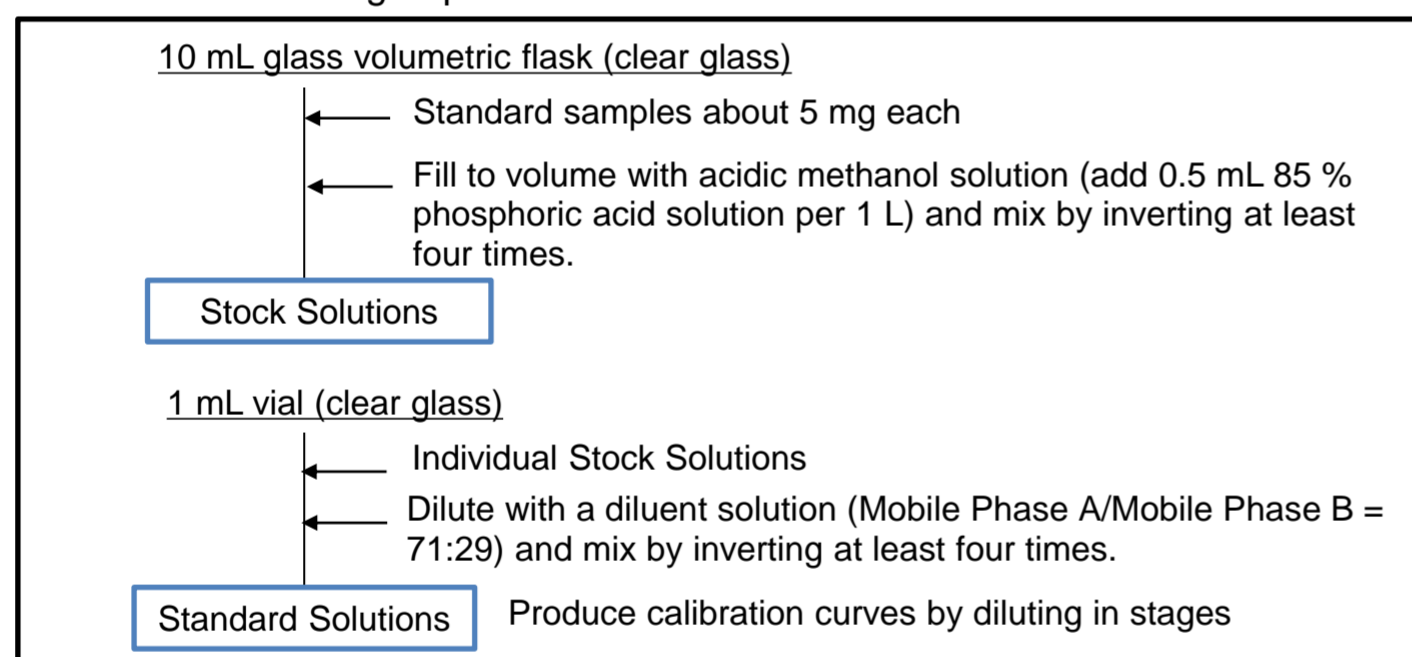


Fig. 3 Standard Solution Preparation

Table 1 Analytical Conditions

System:	i-Series LC-2050C 3D
Column:	Shim-pack Velox™ Biphenyl (100 mm × 3.0 mm I.D., 2.7 μ m)
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
Time Program:	B.Conc 29% (0 min)-31% (17 min)-50% (17.25 min)-58% (29 min)-95%(29.01 min-32 min)-29% (32.01-35 min)
Column Temp.:	40 °C
Injection Vol.:	20 μ L
Detection:	Xanthohumol: 370 nm [5]/ Isoxanthohumol: 280 nm [1]/ Iso- α -acids and Humulinones: 270 nm [6]/ α -acids and β -acids: 314 nm [3]

Table 2 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

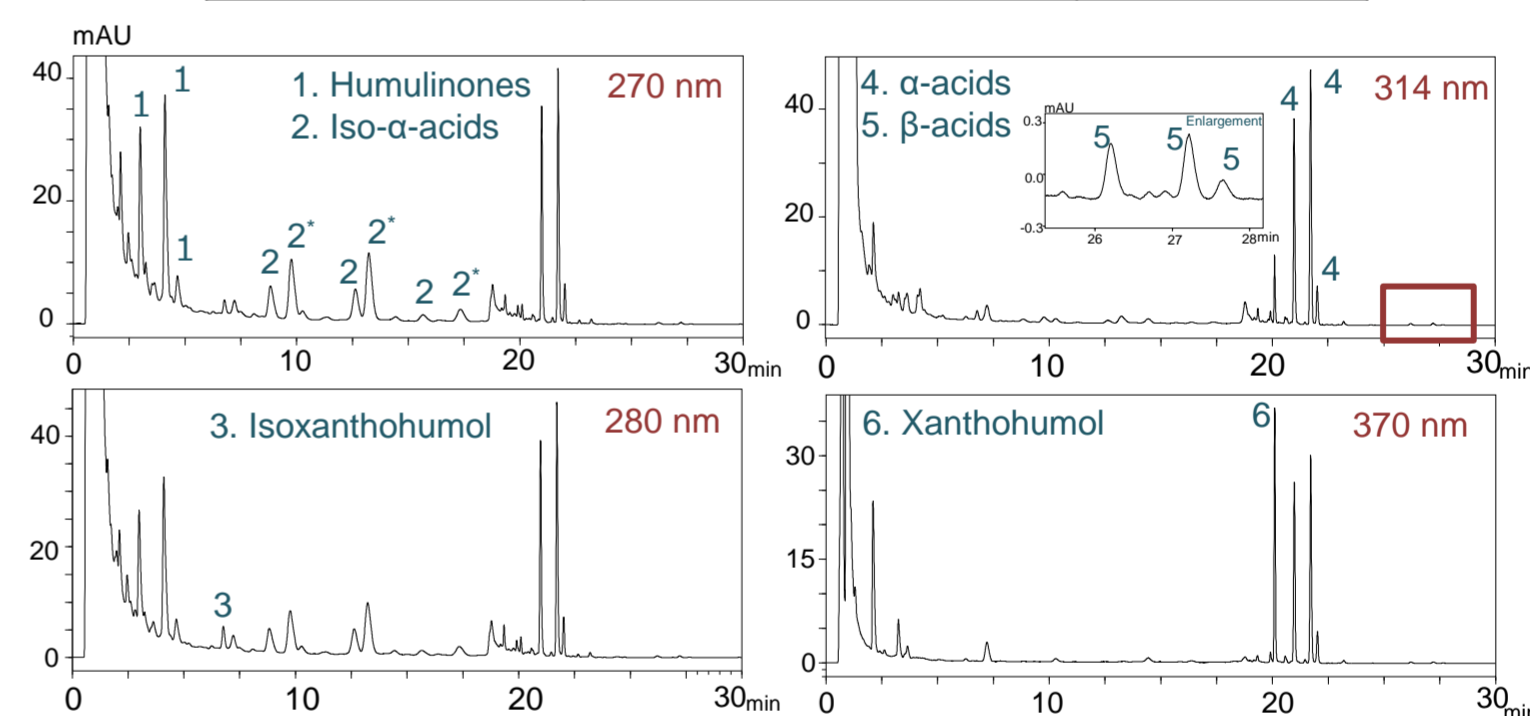


Fig. 6 Chromatograms from Beer 1

Peaks 2 are presumably from the *trans* form of iso- α -acid, whereas peaks 2* are presumably from the *cis* form.

Table 3 Concentrations obtained

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.

Table 4 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 5 Spike-Recovery Test Results (Beer 1, average of n = 3) Unit: mg/L

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

3. Conclusions

This poster 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. Improved reliability for beer component quantitation can be obtained using optimized analytical conditions provide reduced effect from contaminants.

Reference

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- [2] Anna Katarzyna Żolnierczyk et al. "Isoxanthohumol — Biologically active hop flavonoid", Fitoterapia. Pages 71-82. Jun 2015
- [3] Azusa Morita, "High Speed Analysis of α -Acids and β -Acids in Hops", Shimadzu Co., 01-00025-EN
- [4] European Brewery Convention, EBC ANALYTICA, 9.47
- [5] European Brewery Convention, EBC ANALYTICA, 7.15
- [6] European Brewery Convention, EBC ANALYTICA, 9.50
- [7] 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

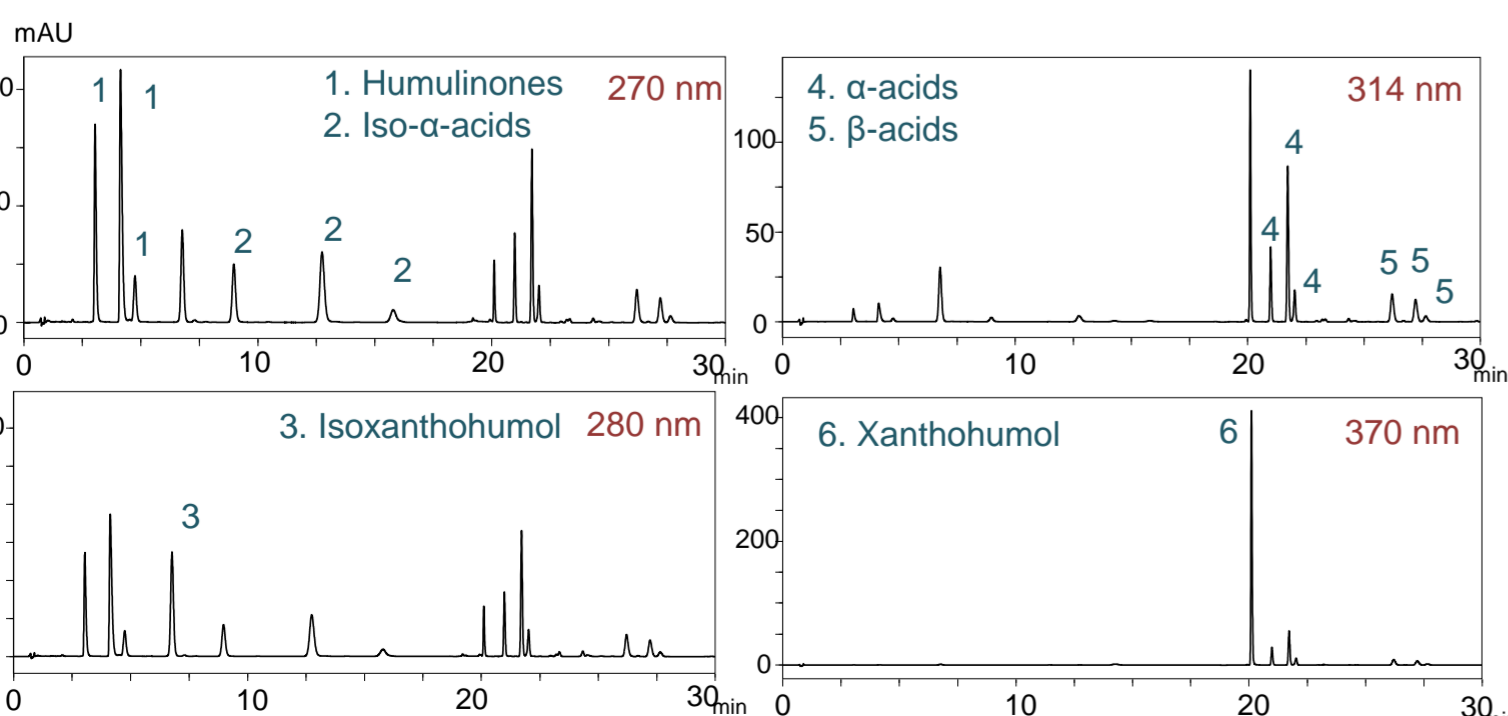


Fig. 4 Chromatograms of Standard Solutions
(10 mg/L xanthohumol, 10 mg/L isoxanthohumol, 20 mg/L humulinones, 20 mg/L iso- α -acids, 20 mg/L α -acids, and 12.5 mg/L β -acid)