SHIMADZU
Excellence in Science

Application News

High Performance Liquid Chromatograph Nexera™ XR

High Speed Analysis of α-Acids and β-Acids in Hops

A. Morita

User Benefits

- The α-acids and β-acids in hops can be analyzed in approximately 5 min/analysis.
- Analysis at low system back pressure is possible by using the Shim-pack Velox™ column with core shell technology.
- Iso-α-acids can be simultaneously analyzed with α-acid and β-acid in the same instrument setup.

■ Introduction

The hops used as a raw material for beer contain α-acids (humulones) and β-acids (lupulones). The α-acids are converted to iso-α-acids (isohumulones), which are bitterness components of beer, by isomerization in the brewing process. The β-acids are not strongly related to the strength of beer bitterness, but are thought to influence the balance of bitterness. The applications of iso-α-acid other than beer are attracting attention because it has been reported to prevent Alzheimer's disease and improve cognitive decline.

High performance liquid chromatography (HPLC) is generally used in analyses of α-acids and β-acids, and the analysis time usually takes approximately 30 minutes.

This article introduces an example of a high speed analysis of the α-acids and β-acids in hops with a Shimadzu Nexera XR HPLC, referring to EBC (European Brewery Convention) 7.7 and ASBC (American Society of Brewing Chemists) Hops-14. An example of an analysis in which the hop extract was spiked with iso-α-acids is also introduced.

■ Analysis of Standard Solution of α-Acids and β-Acids

Fig. 1 shows the structural formulas of the α-acids and β-acids contained in hops. Both the α-acids and the β-acids consist of three homologues. Here, "International Calibration Extract 4" (purchased from ASBC or Labor Veritas) was used as a reagent when preparing the standard solution. Table 1 shows the reagent composition. In preparing the standard solution, 0.5 g of the reagent was dissolved in methanol to make up a total volume of 100 mL, after which 10 mL of this solution was taken and adjusted to a total volume of 50 mL and then filtered with a membrane filter having a pore size of 0.22 µm. Fig. 2 shows the chromatogram of the standard solution. Because the reagent used in preparing the standard solution itself contained multiple homologues, grouping was done for the α-acids and the β-acids, respectively. Table 2 shows the analysis conditions. It may be noted that the maximum system back pressure in this analysis was approximately 26 MPa. It is possible to conduct analyses at low back pressure, even with methanol, which tends to increase the pressure on the column, by using a Shim-pack Velox analytical column with core shell technology.

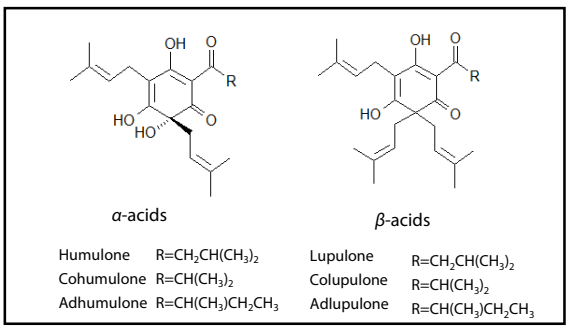

Fig. 1 Structural Formulas of α-Acids and β-Acids

Table 1 Reagent Used in Preparation of Standard Solution

Reagent name	Composition
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%**

Table 2 Analytical Conditions

System	: Nexera XR
Column	: Shim-pack Velox C18 (50 mm × 3.0 mm I.D., 1.8 µm)*1
Mobile phase A	: 10 mmol/L (Sodium) phosphate buffer (pH2.6)+ 0.2 mmol/L ETDA・2Na aq.
Mobile phase B	: Methanol
Flow rate	: 0.7 mL/min
Time program	: B Conc. 80% (0 min)-90% (3 min)-80% (3.01-5 min)
Column temp.	: 40 ˚C
Injection vol.	: 2 µL
Detection	: UV 314 nm (SPD-M40), Standard cell
Vial	: Shimadzu Vials, LC, 1.5 mL Clear Glass*2

*1 P/N: 227-32008-01 *2 P/N: 227-34001-01

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 1L deionized water.

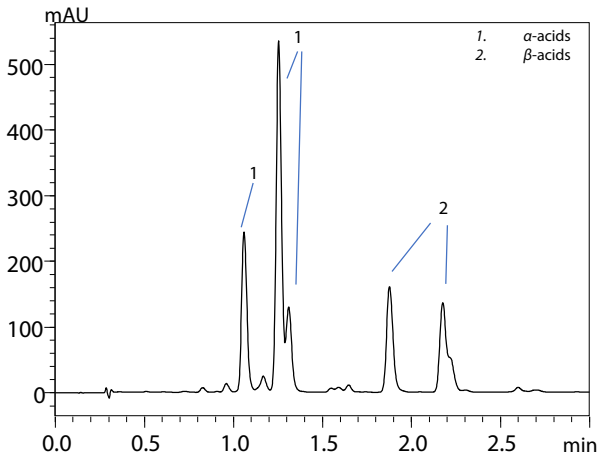

Fig. 2 Chromatogram of Standard Solution

■ Analysis of α-Acids and β-Acids in Hops

Solvent extraction and analysis were conducted using two types of commercial hop pellets. Fig. 3 shows the sample preparation protocol, and Fig. 4 and Fig. 5 show the respective chromatograms.

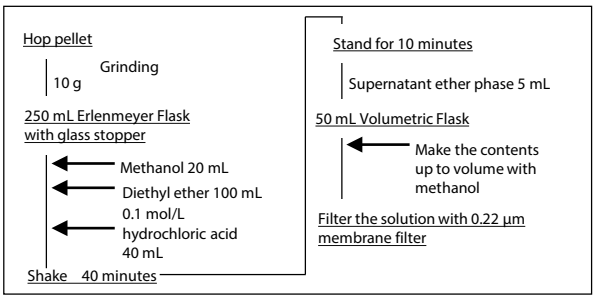

Fig. 3 Sample Preparation Protocol

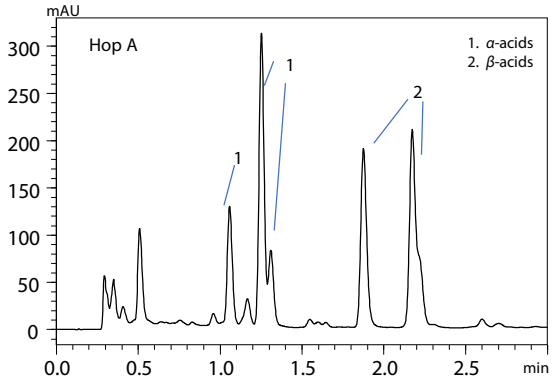

Fig. 4 Chromatogram of Hop A

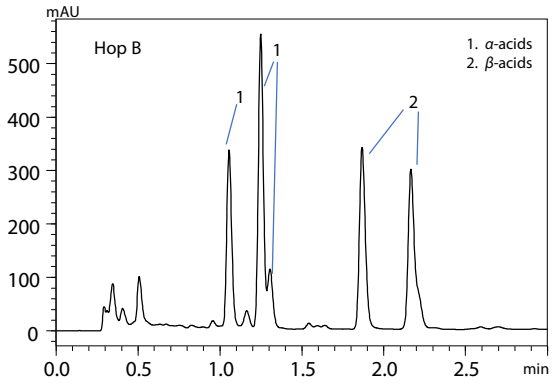

Fig. 5 Chromatogram of Hop B

$$C_i=(DF \times M_{CS} \times C_{iC} \times A_i)/(M_s \times A_{ic})$$

where:
C_i = concentration of component i in the sample expressed as percent by weight
DF = dilution factor, DF = 2 for hops and hop powder products
M_{cs} = weight of the calibration standard in g
C_{ic} = concentration of component i in the calibration standard expressed as percent by weight
A_i = peak area of component i from the sample run (average)
M_s = weight of the sample in g
A_{ic} = peak area of component i from the calibration run (average)

Fig. 6 Quantitative Value Calculation Method

The sample preparation shown in Fig. 3 was conducted 6 times, and the quantitative values were calculated by the formula in Fig. 6. Standard addition was carried out by spiking the solvent before shaking with a 1/10 concentration of the standard solution, and a spike-and-recovery test was conducted. Table 3 shows the results. The quantitative values and recovery rates indicate the average values when the analysis was repeated 6 times.

Table 3 Quantitation Results and Recovery Rates (N=6)

	Concentration (%)		Relative standard deviation of concentration (%RSD)		Addition recovery rate (%)		Relative standard deviation of recovery rate (%RSD)	
Sample	α-acids	β-acids	α-acids	β-acids	α-acids	β-acids	α-acids	β-acids
Hop A	5.1	14.3	1.4	1.2	98	90	1.1	1.0
Hop B	9.5	24.0	1.4	1.3	102	97	2.4	2.4

■ Simultaneous Analysis of Iso-α-Acids, α-Acids, and β-Acids

Here, an analysis was performed by spiking the extract of Hop A prepared in accordance with Fig. 3 with 200 mg/L of iso-α-acids. Regarding the iso-α acid standard solution preparation method, please refer to Application News L590. The wavelength of 270 nm was added to the detection wavelength of the photodiode array detector in Table 2, and the gradient time program was changed to B Conc. 60% (0 min) - 100% (3 min) - 60% (3.01 - 5 min). Fig. 7 and Fig. 8 show the chromatograms for the unspiked sample and spiked sample, respectively. A satisfactory result of 99.7% was obtained for the recovery rate of the iso-α-acids.

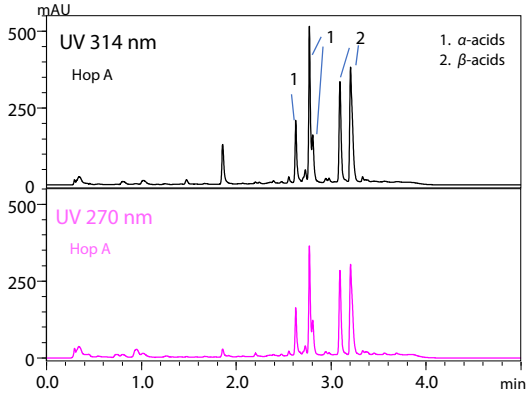

Fig. 7 Chromatogram of Hop A (Not Spiked)

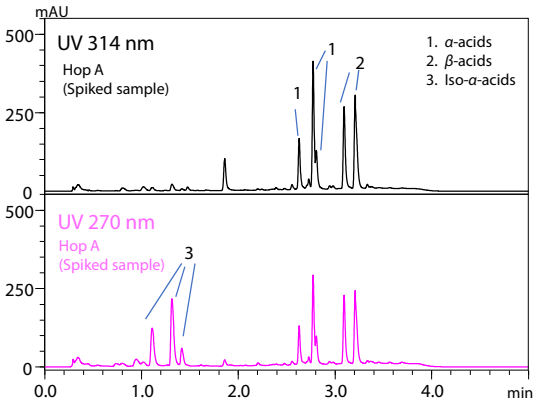

Fig. 8 Chromatogram of Hop A (Spiked with Iso-α-Acids)

■ Conclusion

This article introduced an example of a high speed analysis of the α-acids and β-acids in hops using a Nexera XR. The α-acids and β-acids were extracted from hop pellets referring to EBC and ASBC, and their quantitative values were calculated. A simultaneous analysis of iso-α-acids in addition to the α-acids and β-acids was also possible by changing the gradient time program and adding an appropriate detection wavelength.

<References>
(1) American Society of Brewing Chemists, ASBC Methods of Analysis, Hops-14
(2) European Brewery Convention, EBC ANALYTICA, 7.7

01-00025-EN First Edition: May 2021

Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/