

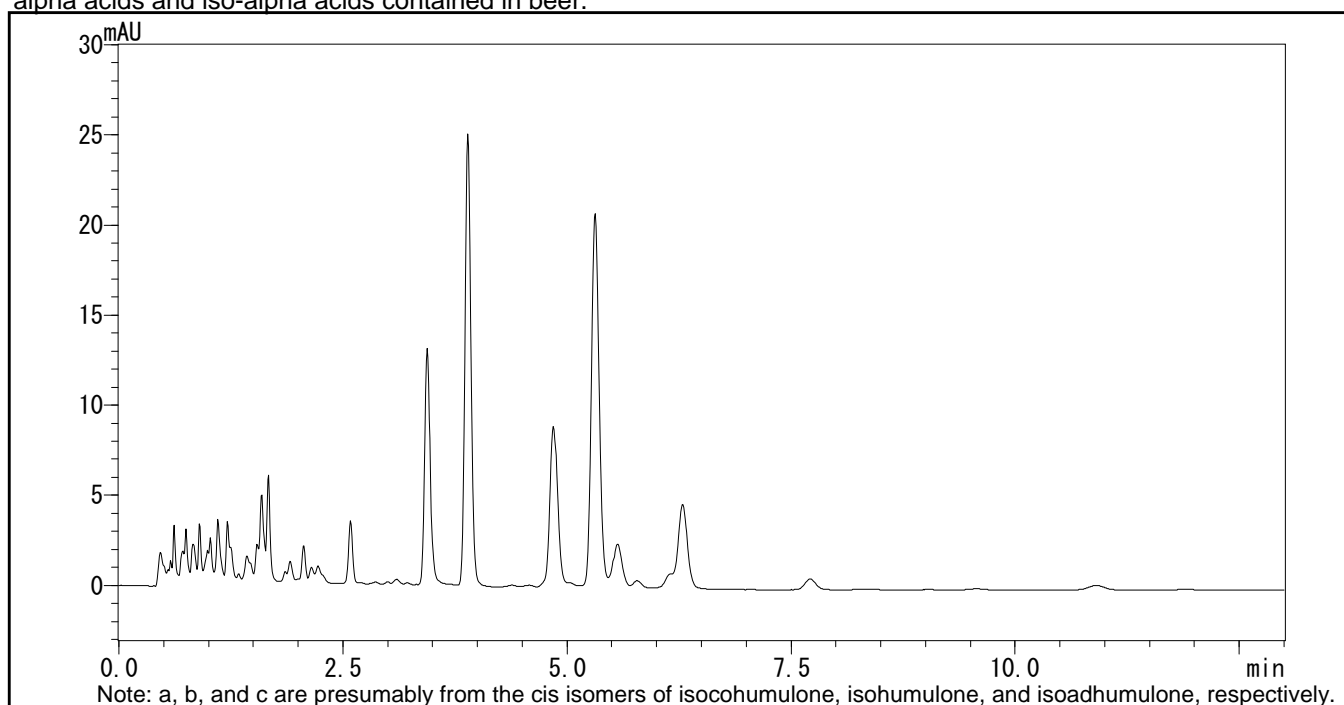
Application Data Sheet

High Performance Liquid Chromatography

No. 73

High Speed Analysis of Alpha Acids and Iso-Alpha Acids in Beer

Iso-alpha acids (isohumulones), which give beer its bitter taste, are generated by the isomerization of alpha acids (humulones) contained in hops during the brewing process. Here we present an example of high speed analysis of alpha acids and iso-alpha acids contained in beer.



Analysis of Alpha Acids and Iso-Alpha Acids in Beer

[Sample Preparation]*

- 1) Place approximately 20 mL of beer in a large conical flask and shake until all carbon dioxide gas has been removed.
- 2) Dispense 10 mL of this solution into a separate container and add 0.5 mL of 6 mol/L hydrochloric acid and 20 mL of 2,2,4-trimethylpentane.
- 3) Shake this solution for 30 minutes.
- 4) Let rest for 15 minutes, collect 12 mL of organic solvent from the supernatant layer, and transfer it to a 50 mL recovery flask.
- 5) Use an evaporator to concentrate and dry under reduced pressure.
- 6) Completely dissolve and mix with methanol for HPLC.
- 7) Filter this through a 0.22 μ m membrane filter, and use it as the analysis sample.

* Methods of Analysis of BCOJ, edited and revised by the Brewery Convention of Japan [Analysis Committee] of the Brewers Association of Japan, and published by the Brewing Society of Japan (2004)

Analytical Conditions

Instrument	: Prominence UFLCXR system
Column	: HALO [®] C18 (150 mm × 3.0 mm <i>i.d.</i>)
Mobile Phase	: A) Water/Methanol/Phosphoric acid(85%)/Triethylamine = 300 mL/700 mL/19.6 g/15.1 g B) Methanol B Conc. 0 % (0 min) → 35% (10 min)
Flow Rate	: 1.1 mL/min
Column Temperature	: 50 °C
Detection	: Absorbance at 330 nm with Semi-micro flow cell
Sample Volume	: 4 μ L