Using the GX-271 ASPEC™ for Automated Extraction of Hop Acids (Iso Alpha Acids) in Beer

Application Note FB0411

Keywords

Gilson GX-271 ASPEC™ with Dual 406 Syringe Module, TRILUTION® LH, Hop Acids, Iso Alpha Acids, Solid Phase Extraction (SPE), Beer, HPLC Analysis, Breweries, MEBAK, European Brewery Convention (EBC)

Introduction

This application note information on Hop Acids Extraction at Bitburger Braugruppe GmbH was provided by Gilson BV, Germany.

Hops (*Humulus lupulus*) are commonly known as the beer component responsible for beer flavor, aroma, and bitterness. The female hops flowers are harvested from a perennial vine, desired for their use in beer production (see Figure 1).

Figure 1. Image of the Climbing Hops Plant (Humulus lupulus) (source: beersmith.com)

Aroma and flavor hops typically do not contribute bitterness to beer, but instead give beer its finish quality. Bittering hops are responsible for the large majority of the bitter flavor of a beer. Bitterness is a necessary beer quality (depending on the specific brew) in order to offset the traditional sweet taste of beer malt.

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Alpha acid is the hop resin responsible for a beer's bitter flavor. It can be isomerized with heat to form iso-alpha acid (see Figure 2). For overall quality and consistency in brew, there is a need to consistently monitor the flavor compounds derived from hops during the production process (in wort) and in the final beer product. Hop acids have a strong UV absorbance at 270 nm. HPLC testing to monitor the amount of iso-alpha acid is described in the German brewery guide (MEBAK) and in the European Brewery Convention (EBC); specified as the degree of bitterness in International Bitterness Units (IBUs).

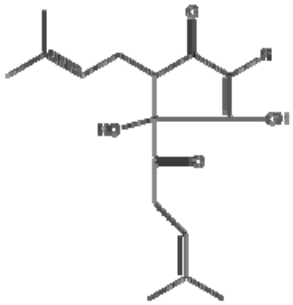


Figure 2. Image of Iso-Alpha Acid (courtesy of Wikipedia.org)

This application automates the removal of particulates and contaminates via Solid Phase Extraction (SPE) using the Gilson GX-271 ASPEC™ with Dual 406 Syringe Pump. SPE is performed prior to HPLC analysis for iso-alpha acid content to extend column life and improve system performance.

Figure 3. Gilson GX-271 ASPEC with Dual 406 Syringe Pump

Materials & Methods

Iso-Alpha Acids SPE Materials:

SPE Cartridges: Phenomenex Strata™ C18-3mL
SPE Solutions:
A: 0.2 mL of Phosphoric Acid in 50 mL Water and 50 mL Methanol
B: 0.2 mL of Phosphoric Acid in 100 mL Water
C: 0.1 mL of phosphoric acid in 90 mL Methanol and 10 mL Water

Iso-Alpha Acids SPE Method:

1. Condition SPE cartridge with 5 mL of Methanol
2. Condition SPE cartridge with 5 mL of Solution A
3. Load 20 mL of acidified sample onto SPE cartridge
4. Wash SPE cartridge with 5 mL of Solution B
5. Elute SPE cartridge with 10 mL Solution C
6. Mix
7. Transfer
8. Transfer

Figure 4. TRILUTION® LH Iso-Alpha Acid Solid Phase Extraction Method Using the GX-271 ASPEC™ with Dual 406 Syringe Pump

Iso-Alpha Acids, Alpha Acids, and Beta Acids - Analytical HPLC Materials:

HPLC System
- Binary Gradient Mobile Phase Pumps
- Column Heater
- Dual UV Detector

Mobile Phase:
- A: Citrate/Phosphate Buffer
- B: 90 Acetonitrile / 10 Water

Column: Phenomenex Gemini™ C18; 250 x 4.6 mm

Iso-Alpha Acids, Alpha Acids, and Beta Acids - Analytical HPLC Method:

Mobile Phase Gradient:

Time (minutes)	Mobile Phase (% B)
0	50
18	60
23	100
40	100
43	50
50	50

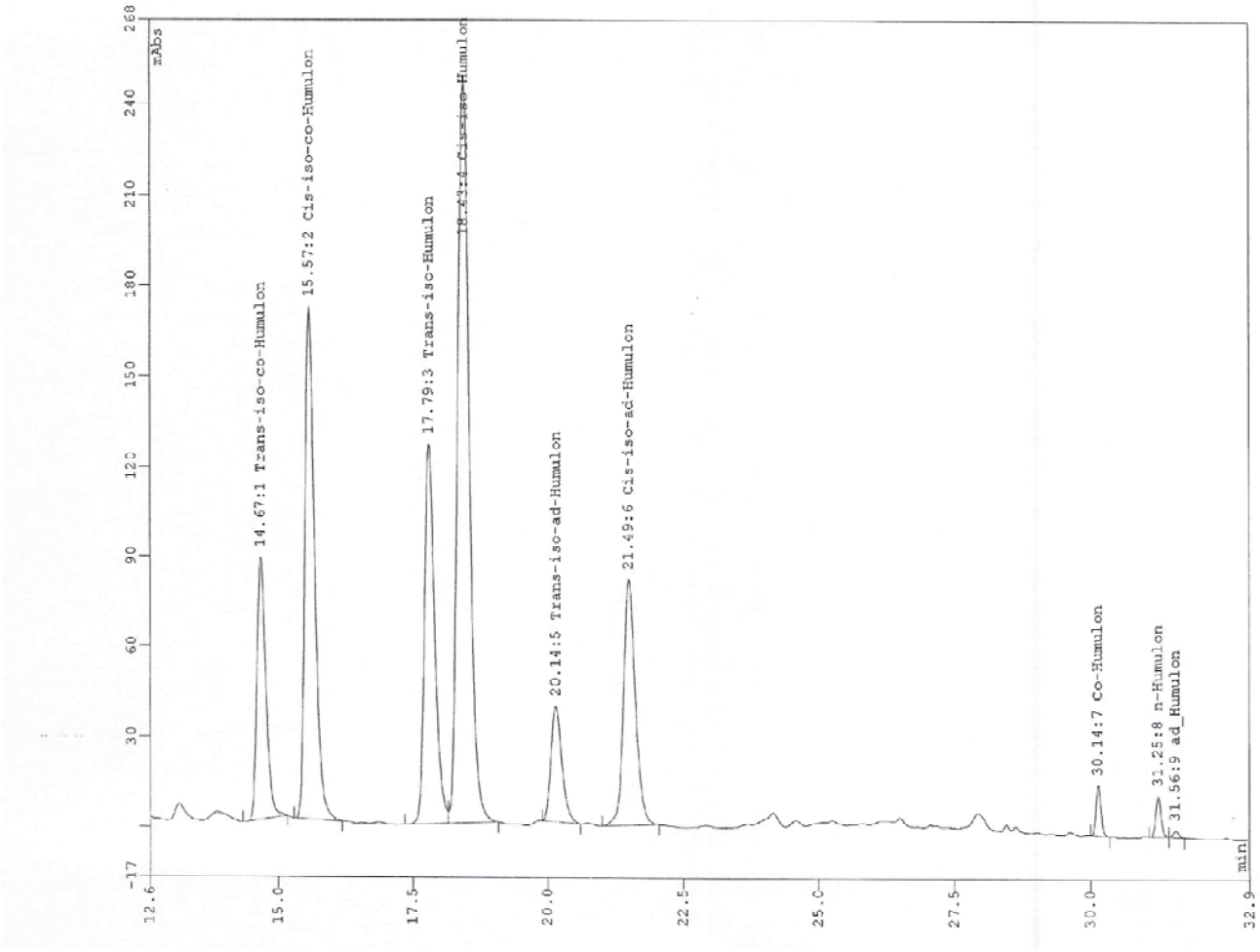
Flow rate: 0.7 ml/min

Column Temperature: 40 °C

Detection: UV 270 nm / 314 nm

Results

Figure 6. Example Chromatogram of Iso-Alpha Acids, Alpha Acids, and Beta Acids from a Beer Sample



Summary

Performing Solid Phase Extraction (SPE) prior to HPLC analysis of Iso-Alpha Acids, Alpha Acids and Beta Acids is necessary to extend column life and remove particulates that could interfere with analysis. The GX-271 ASPEC™ and Dual 406 Syringe Pump allowed for addtional automated liquid handling steps to be performed following the SPE process which added efficiency and eliminated a manual intervention step.

SPE is a requirement prior to other analysis in beer (see below), and enabling automation for these additional analyses using the Gilson solution and flexible TRILUTION LH software creates additional laboratory efficiencies:

- Free Fatty Acids
- Analysis of Sugars
- Mycotoxins (OTA - ZON)
- Resveratrol and Piceid
- Phytoestrogens

References

Determination of iso-alpha-Acids, alpha- and beta-Acids in Isomerized Hop Pellets by HPLC, Biendl, M.; Virant, M.; Varju, P., Journal of the Institute of Brewing Vol. 110, No. 3, 2004, 242-243 - European Brewery Convention