Analysis of Amino Acids in Beer using HPLC with Online Derivatization

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Food

Abstract

Both primary and secondary amino acids were analyzed in one run.

The amino acid composition of proteins can be used to determine the origin of meat products and thus to detect adulteration of foodstuffs. Detection of potentially toxic amino acids is also possible through such analysis. Through the use of chiral stationary phases as column material, D and L forms of amino acids can be separated and quantified.

HPLC in combination with automated online derivatization is now a well-accepted method for detecting amino acids owing to its short analysis time and relatively simple sample preparation.

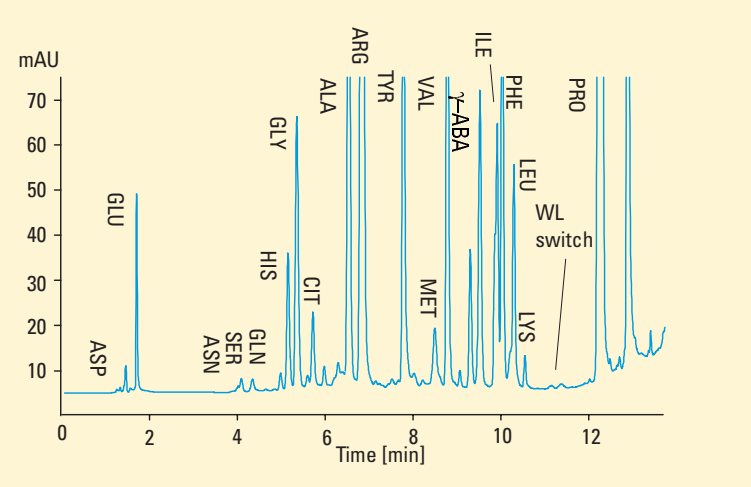

Figure 1
Analysis of amino acids in beer after online derivatization

Conditions

Column 200 ˘ 2.1 mm Hypersil ODS, 5 µm
Mobile phase A = 0.03M sodium acetate pH = 7.2 + 0.5% THF
B = 0.1M sodium acetate/ ACN (1:4)
Gradient
at 0 min 0% B at 0.45 ml/min flow rate
at 9 min 30% B
at 11 min 50% B at 0.8 ml/min flow rate
at 13 min 50% B
at 14 min 100% B at 0.45 ml/min flow rate
at 14.1 min at 0.45 ml/min flow rate
at 14.2 min at 0.8 ml/min flow rate
at 17.9 min at 0.8 ml/min flow rate
at 18.0 min at 0.45 ml/min flow rate
at 18 min 100% B; at 19 min 0% B
Post time 4 min
Flow rate 0.45 ml/min
Column compartment 40 ºC
Injection vol 1 µl standard
Detector UV -DAD 338 nm and 266 nm
Fluorescence
Excitation wavelength: 230 nm
Emission wavelength: 450 nm at 11.5 min
Excitation wavelength: 266 nm
Emission wavelength: 310 nm
Slit width excitation: 2 mm (25 nm)
Slit width emission 1: 4 mm (50 nm)
Slit width emission 2: 4 mm (50 nm)
Photomultiplier gain: 12
Cut-off filter: 280 nm
Lamp: 55 Hz (always on)
Response time: 4 s

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Sample preparation

Hydrolyzation with HCl or enzymatic hydrolysis is used to break protein bonds.

Chromatographic conditions

The HPLC method presented here was used in the analysis of secondary and primary amino acids in beer with precolumn derivatization and fluorescence detection.[1]

HPLC method performance

Limit of detection
5 pmol

Repeatability of
RT over 6 runs <1 %
areas over 6 runs <5 %

Linearity
1 pmol to 4 nmol

References

1.
R. Schuster, "Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivatization and HPLC", *J. Chromatogr.*, **1988**, 431, 271–284.

Conditions

Injector program for online derivatization

1. Draw 3.0 µl from vial 2 (borate buffer)
2. Draw 1.0 µl from vial 0 (OPA reagent)
3. Draw 0.0 µl from vial 100 (water)
4. Draw 1.0 µl from sample
5. Draw 0.0 µl from vial 100 (water)
6. Mix 7.0 µl (6 cycles)
7. Draw 1.0 from vial 1 FMOC reagent
8. Draw 0.0 µl from vial 100 (water)
9. Mix 8.0 µl (3 cycles)
10. Inject

Equipment

Agilent 1100 Series

- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector
- fluorescence detector

Agilent ChemStation + software

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