Automatic Precolumn Derivatization of Amino Acids and Analysis by Fast LC using the Agilent 1290 Infinity LC System

Application Note

Food

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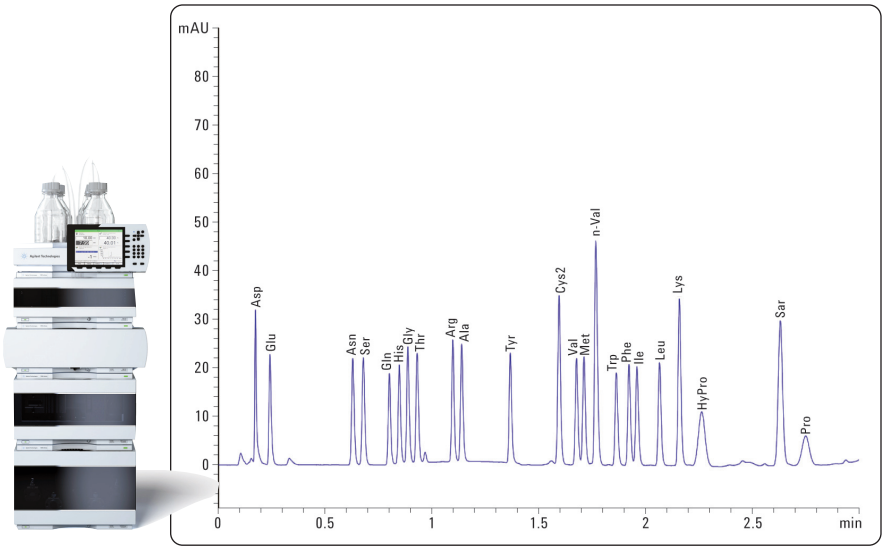

Abstract

The Agilent 1290 Infinity LC system offers a fast, highly sensitive means of analyzing water soluble amino acids in food products. When coupled with automated, precolumn derivatization, a method can be developed that provides rapid, accurate results required in protein chemistry and food analysis fields. This Application Note describes the analysis of amino acids in energy drinks on the Agilent 1290 Infinity LC System.

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Introduction

Amino acid analysis is an important application area in protein chemistry and food analysis. Many different applications exist in this field. Automated precolumn derivatization followed by reverse phase LC has become a useful procedure for efficient analysis, since it is an easy and simple to use technique. Recently, fast LC methods employing column particle sizes below 2 µm help to increase sample throughput and productivity. In this application, we used the Agilent 1290 Infinity LC System with automated precolumn derivatization for fast and sensitive analysis of amino acids.

Experimental

- o-phthalaldehyde (OPA) for Class 1 Amino Acids (nonpolar and neutral)
- 9-fluorenylmethyl chloroformate (FMOC) for Class 2 Amino Acids (polar and neutral)

Derivatization was executed on the autosampler to prevent tedious manual procedures. Reagents were put in vials on the autosampler in the following order:

vial 1: Borate buffer (Agilent p/n 5061-3339),

vial 3: OPA (Agilent p/n 5061-3335),

vial 4: FMOC (Agilent p/n 5061-3337),

vial 5 : Injection dilution (Same as in Agilent app.note 5990-4547EN. That is, 100 mL of mobile phase A + 0.25 mL of H_3PO_4)

The following injector program was created. See Table 1.

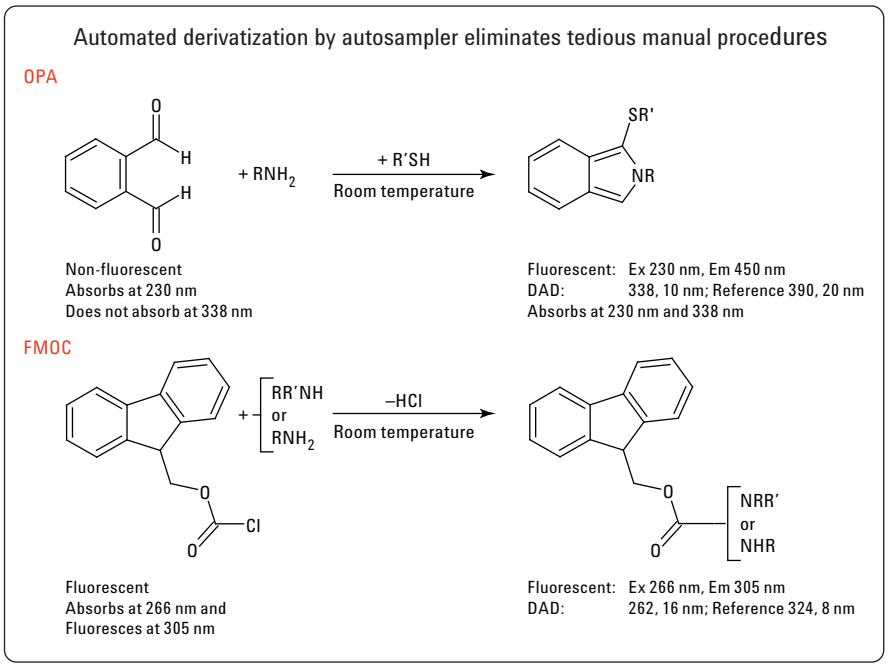

Figure 1
Amino acid derivatization reaction.

Step	Description
Draw	Draw 0.7 µL from vial 1 with default speed using default offset
Draw	Draw 0.3 µL from sample with default speed
Mix	Mix 1 µL from washport with maximum speed for 5 times
Draw	Draw 0.2 µL from vial 3 with default speed using default offset
Mix	Mix 1.2 µL from washport with maximum speed for 5 times
Mix	Mix 1.2 µL from washport with maximum speed for 5 times
Wait	Wait 0.1 min
Draw	Draw 0.2 µL from vial 4 with default speed using default offset
Mix	Mix 1.4 µL from washport with maximum speed for 5 times
Mix	Mix 1.4 µL from washport with maximum speed for 5 times
Wait	Wait 0.2 min
Draw	Draw 8 µL from vial 5 with default speed using default offset
Mix	Mix 5 µL from washport with maximum speed for 5 times
Mix	Mix 5 µL from washport with maximum speed for 5 times
Wait	Wait 0.1 min
Inject	inject

Table 1.
Injector Program on the Agilent 1290 Infinity LC system.

For separation, the following chromatographic method was used:

Method	
Column:	Agilent ZORBAX Eclipse Plus RRHD C18 (2.1 mm × 50 mm, 1.8 µm)
Solvent A:	10 mM disodium phosphate +10 mM sodium tetraborate (pH 8.2)
Solvent B:	ACN/MeOH/H_2O(45:45:10)
Gradient:	0.1–3 min/ 3-3-58 (%B)
Detection :	UV 338 nm (Pro UV262 nm)
Flow:	1 mL/min
Column temperature:	40 °C

First, four concentrations (90, 225, 450 and 900 pmol/µL) of an amino acid standard were run. Secondly, five injections of an isotonic drink containing five concentrations of the standard were run. The respective chromatograms are displayed in Figure 2, 3 and 4.

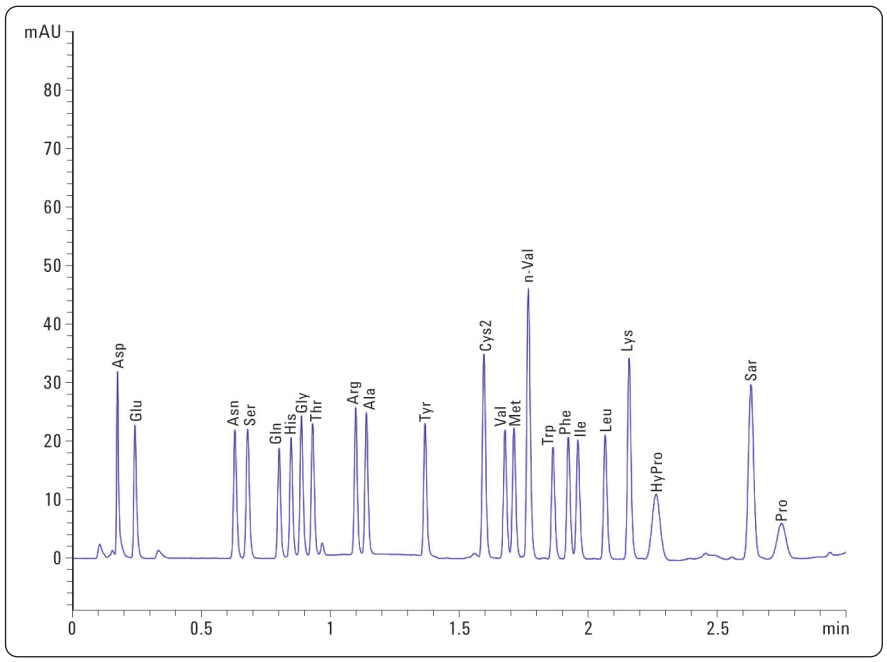

Figure 2
Chromatogram of amino acids standard (250 pmol/µL).

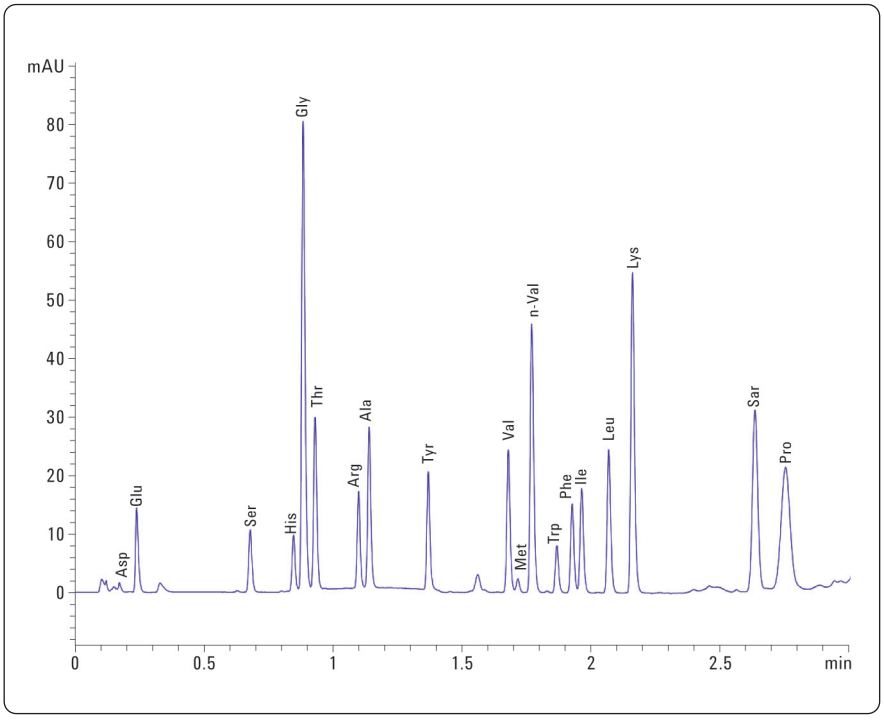

Figure 3
Chromatogram of amino acids in an isotonic sports drink (10x dilution)
The internal standard (5 times dilution) was added to the sample.

Conclusion

The Agilent 1290 Infinity LC system employs sub to micron columns for fast chromatographic analysis. In this application it could be shown that a complex sample containing 22 amino acids could be separated by the Agilent 1290 Infinity LC system in less than three minutes with optimum separation power.

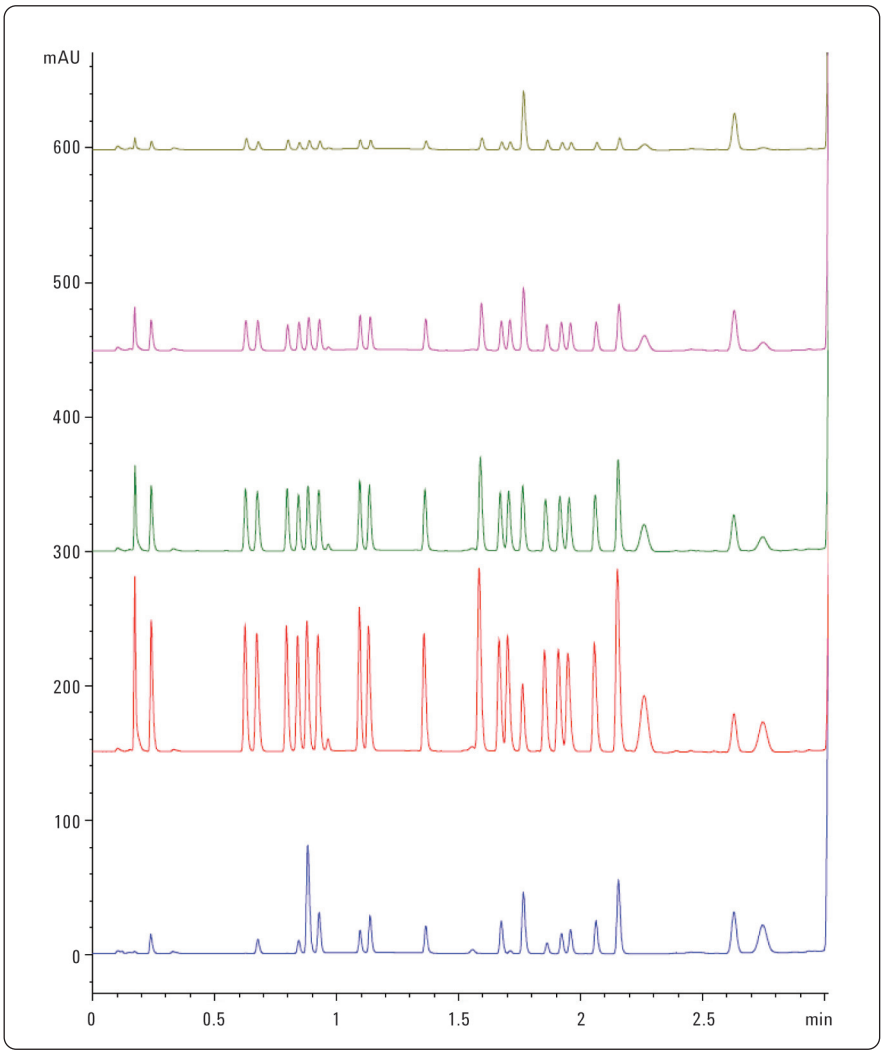

Figure 4
Overlay.pdf is a chromatogram of a four point calibration of standard solution and isotonic drink (90 pmol/µL standard, 225 pmol/µL standard, 450 pmol/µL, 900 pmol/µL standard, sample).

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