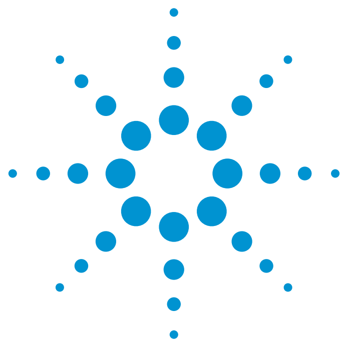


Automated Precolumn Derivatization for the Enantioseparation of Amino Acids Using the Agilent 1290 Infinity II LC

Application Note

Food Testing & Agriculture

Author

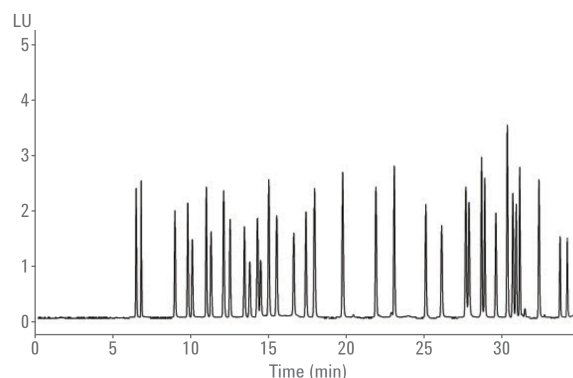
Hiroki Kumagai

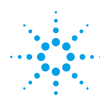
Agilent Technologies Japan Ltd.

Tokyo, Japan

Abstract

High-resolution and highly sensitive enantioseparation of amino acids was performed with the Agilent 1290 Infinity II LC equipped with a fluorescence detector (FLD) and an Agilent Poroshell HPH-C18 column. The method shown in this Application Note is a fully automated precolumn derivatization using *o*-phtalaldehyde (OPA) combined with N-isobutyryl-L-cysteine. This method can be run simply and easily, and showed sensitive and accurate performance.





Agilent Technologies

Introduction

D-amino acid occurs naturally, have a different physiology, and taste different from L-amino acids[1,2]. The enantioseparation of amino acids has been performed primarily using two techniques: one uses a chiral-selective stationary phase, the other separates the diastereomer using reversed-phase chromatography. The enantioseparation of amino acids using the corresponding diastereomeric derivates is superior to the chiral-selective stationary phase when many amino acids must be analyzed simultaneously. There are many reports regarding enantioseparation using the corresponding diastereomers. Among various enantioseparation methods based on diastereomer derivatization, using *o*-phtalaldehyde (OPA) with N-acyl-cysteine has been widely investigated[3]. In this method, a fluorescent diastereomer was formed during the derivatization process (Figure 1).

Although the simultaneous separation of diastereomers from proteinogenic amino acids requires complete separation of over 30 peaks for high-precision analysis, recent advances in the field of UHPLC technology and reversed-phase column packings provide a high resolution and precision for the enantioseparation of amino acids. This Application Note shows an easy, sensitive, and reproducible automated precolumn derivatization method for the enantioseparation of amino acids using an Agilent 1290 Infinity II LC coupled with an Agilent Poroshell HPH-C18 column.

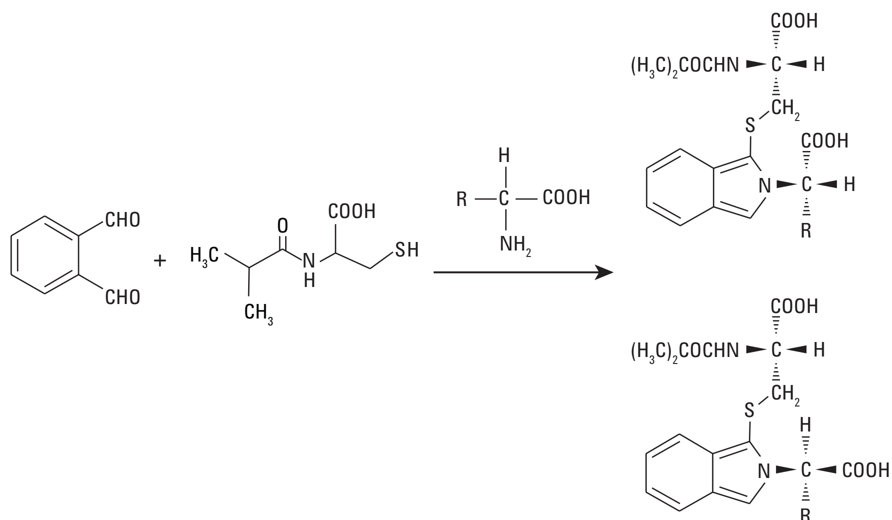


Figure 1. Derivatization scheme.

Experimental

Instrumentation

An Agilent 1290 Infinity II LC was used with the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) equipped with a 40 µL loop and sample cooler (Option #100)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1260 Infinity Fluorescence Detector Spectra (G1321B)

Standard solutions

L- and D-amino acids were bought from Sigma-Aldrich (St. Louis, USA) and Peptide Institute (Osaka, Japan).

A 5 nmol/µL stock solution of each amino acid was prepared from the racemic amino acids except Glu and Ile. All amino acids were dissolved in 0.1 N HCl except Asn, Gln, and Trp. Asn, Gln, and Trp were dissolved in water. Mixture solutions were prepared from the individual stock solutions by diluting with water.

Sample preparation

One milliliter of each vinegar was filtered using a 0.22-µm centrifugal membrane filter; the filtrate was then diluted 50-fold with water.

Derivatization reagent

The derivatization solution was 260 mM N-isobutyryl-L-cysteine (IBLC)/170 mM *o*-phtalaldehyde (OPA) in borate buffer (p/n 5061-3339).

Derivatization using an injector program (Table 2)

Location 1: Borate buffer (p/n 5061-3339)

Location 2: Derivatization reagent described above

Location 3: Injection dilution (0.1 % acetic acid)

Method parameters

Table 1. Chromatographic conditions for the analysis of enantiomeric amino acids.

Parameter	Value
Column	Agilent Poroshell HPH-C18, 3.0 × 150 mm, 2.7 µm (p/n 693975-502)
Mobile phase	A) 50 mM sodium acetate (pH 6.0) B) Acetonitrile/methanol/water 45/45/10
Flow rate	0.7 mL/min
Gradient Pump	0 to 2.0 minutes, 4 %B 2.0 to 4.0 minutes, 10 %B 4.0 to 15 minutes, 20 %B 15 to 27 minutes, 35 %B 27 to 35 minutes, 50 %B 35 to 37 minutes, 100 %B 37 to 42 minutes, 100 %B
Post time	10 minutes at 4 %B
Column temperature	30 °C
Injection	See injector program
Needle wash	40 °C
Detection	Ex. 230 nm, Ex. 450 nm

Table 2. Injector program for amino acid derivatization.

Step	Mode	Action
1	Draw	Draw 2.5 µL from location 1 with default speed
2	Draw	Draw 0.5 µL from sample
3	Wash	Wash needle in flush port with S1 for 3 seconds with 100 µL /min
4	Mix	Mix 3.0 µL from air at maximum speed 10 times
5	Wait	Wait 0.5 minutes
6	Draw	Draw 0.25 from location 2 with 100 µL/min speed
7	Mix	Mix 3.25 µL from air at maximum speed 20 times
8	Wait	Wait 0.5 minutes
9	Draw	Draw 15 µL from location 3 with default speed
10	Mix	Mix 20 µL from air at maximum speed 10 times
11	Wait	Wait 0.1 minutes
12	Inject	Injection
13	Wait	Wait 0.5 minutes
14	Valve	Switch valve to **Bypass**

Results and Discussion

This Application Note shows the enantioseparation of amino acids using automated precolumn derivatization. Figure 2 shows the analysis of primary proteinogenic amino acids. The separation of the derivatized amino acids took 35 minutes. Since only OPA as the derivatization reagent was used, secondary amino acids, such as Pro, were not detected by this method. In addition, Cys was also not detected because IBLC was used as thiol.

The retention time and peak area precision (n = 6) was less than 0.1 and 3.0 %, respectively. The detection limits (signal-to-noise = 3) were 0.04–0.15 pmol/µL.

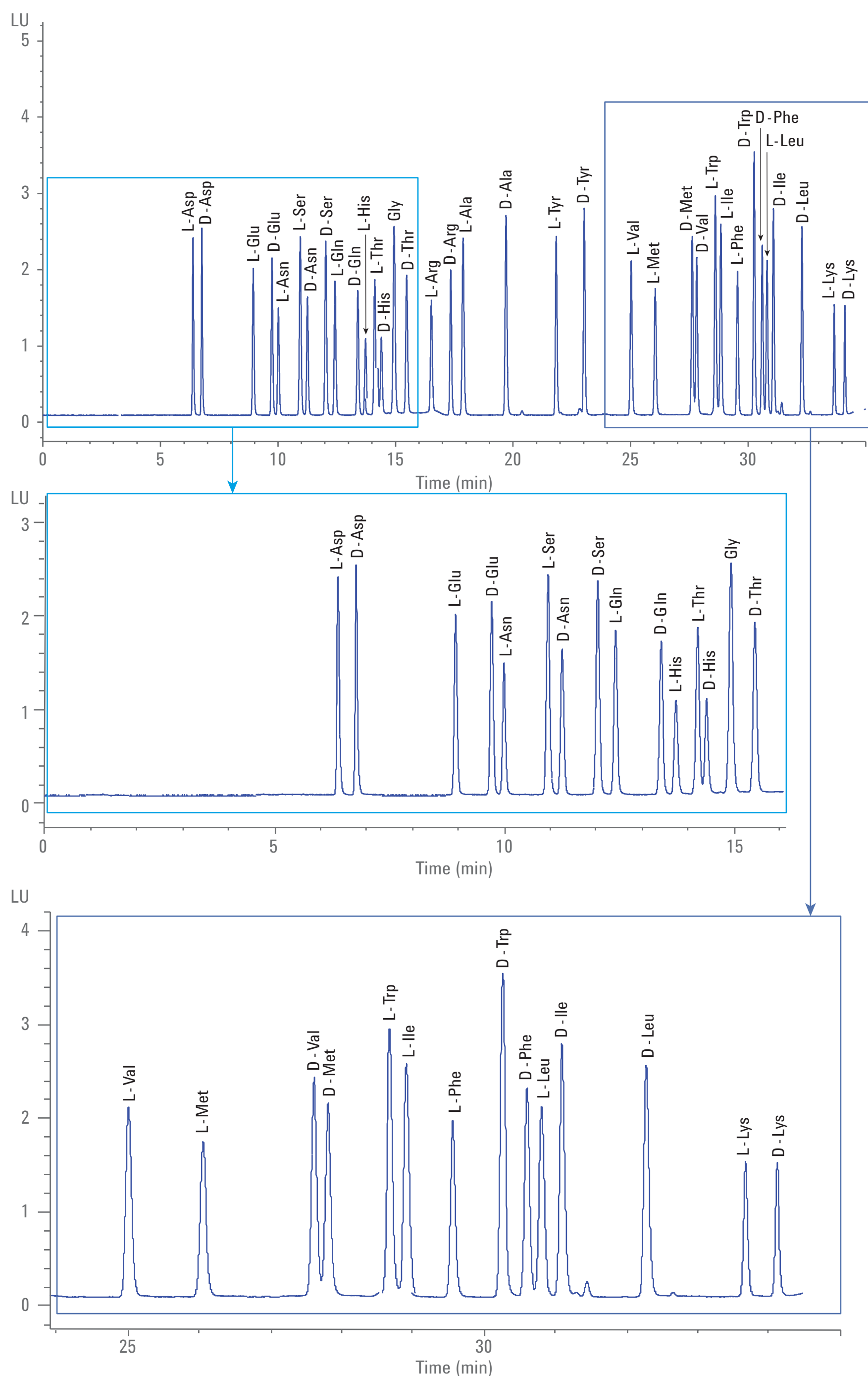


Figure 2. Analysis of standard solution containing 10 pmol/µL amino acids.

In the next step, the derivatization method was applied to real-life samples. Three different vinegars were analyzed: Kurozu (a kind of rice vinegar), common rice vinegar, and balsamic vinegar. The enantioseparation of amino acids from the three different vinegars can be seen in Figure 3.

Kurozu shows a higher variety of amino acids with a partially higher abundance than common rice vinegar or balsamic vinegar. D-amino acids were detected in all three vinegars, but for Kurozu, the range of D-amino acids was larger than for the others. These findings may be attributed to the difference of materials and fermentation processes.

Conclusion

This Application Note demonstrates the separation of D- and L-amino acids using an Agilent 1290 Infinity II LC equipped with a fluorescence detector (FLD). Online precolumn derivatization with OPA and IBLC enabled the separation of D- and L-amino acids on an Agilent Poroshell HPH-C18 column, followed by fluorescence detection.

The method was tested on a standard mix of various amino acids, with excellent chromatographic performance regarding retention time, area precision, and limit of detection. In addition, three different vinegars were analyzed with the established method, and differences in the presence of D- and L-amino acids were detected.

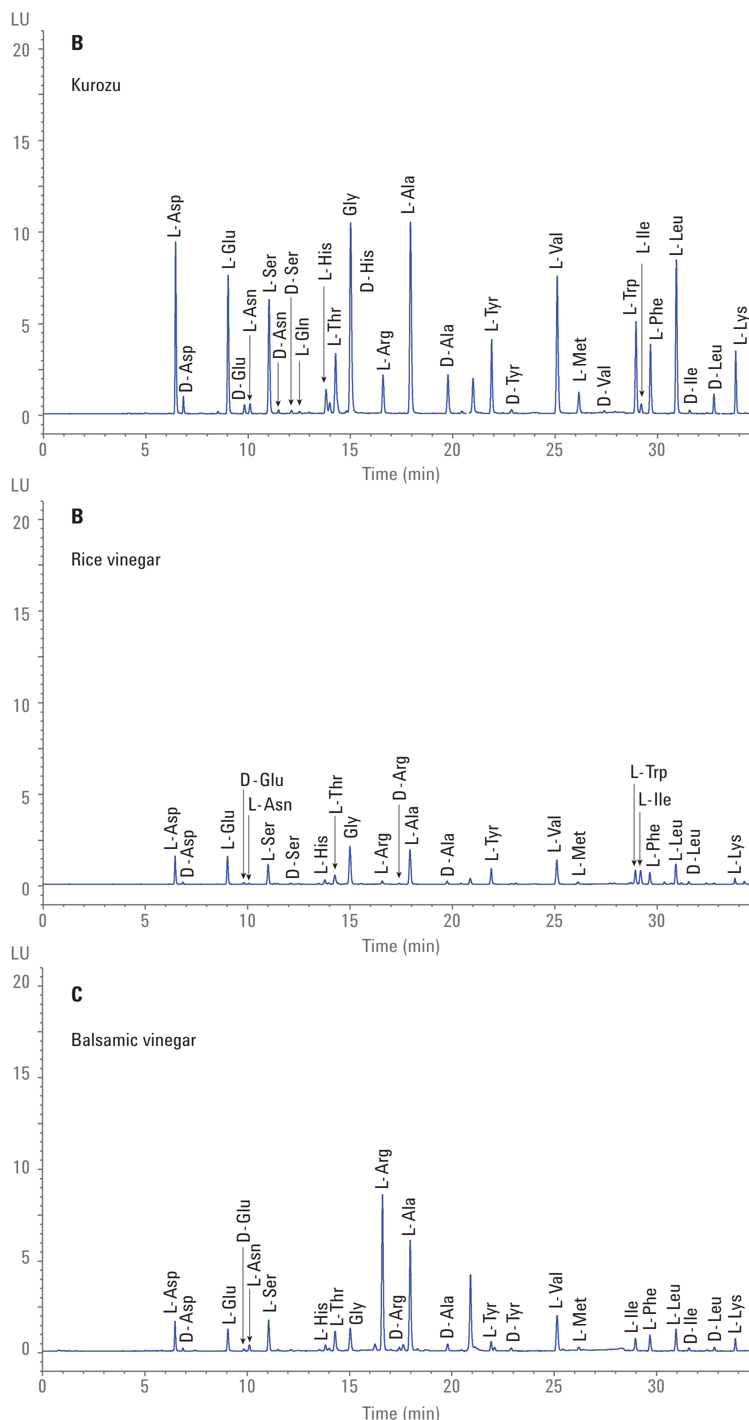


Figure 3. The enantioseparation of amino acids in various vinegars.

References

1. Inoue, Y.; *et al.* Effect of D-Amino Acids as Taste Modifiers in Fermented Foods. *Trace Nutrients Research* **2013**, *31*, 59.

2. Ilisz, I.: *et al.* Recent advances in the direct and indirect liquid chromatographic enantioseparation of amino acids and related compounds: a review. *J. Pharm. Biomed. Anal.* **2012**, *69*, 28.

3. Brückner, H.; *et al.* Liquid chromatographic determination of amino acid enantiomers by determination with *o*-phthaldialdehyd and chiral thiols – Applications with reference to food science. *J. Chromatogra. A* **1995**, *697*, 229.

www.agilent.com/chem

This information is subject to change without notice.

© Agilent Technologies, Inc., 2017

Published in the USA, September 1, 2017
5991-8329EN

Agilent Technologies