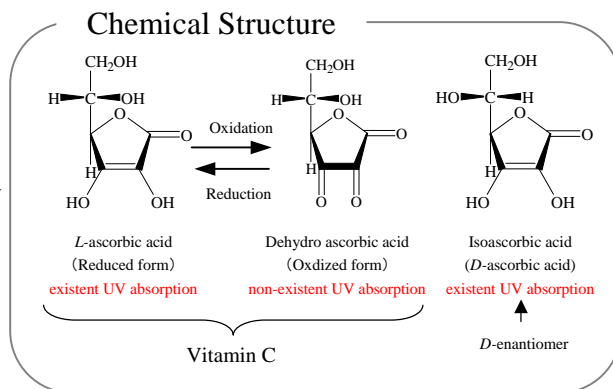


This is an application data of analyzing L-ascorbic acid and Dehydroascorbic acid, which are known to have a Vitamin C activity and Isoascorbic acid by HPLC using PDA.

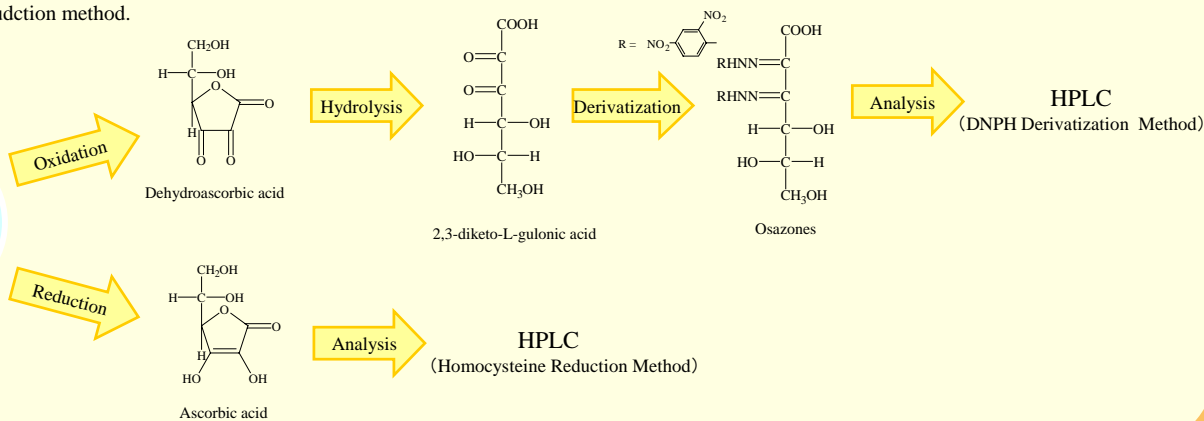
Dehydroascorbic acid is a Vitamin C compound like Ascorbic acid. Dehydroascorbic acid (DHAsA) is an oxidized form of Ascorbic acid (AsA). AsA can be detected by an UV Detector, but DHAsA can not. Therefore, it is necessary to convert the structure of the compound to make it detected by an UV Detector analyzing the total amount of Vitamin C. Also, there is an isomer of AsA known as Isoascorbic acid (ErA), which is a food additive.

This application was conducted based on the Japanese Food Sanitation Inspection Guideline.



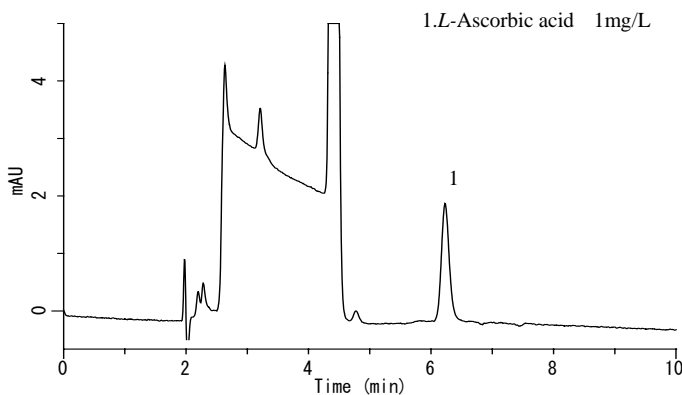
Outline

The total amount of Ascorbic acid can be measured by a DNPH Derivatization method. Simultaneous analysis of Isoascorbic acid and Reduced L-ascorbic acid can be measured by a Homocysteine reduction method.



Analysis of Standard Solution

DNPH Derivatization Method



Analytical Conditions ①

Column : Inertsil SIL-100A
(5 μ m, 250 x 4.6 mm I.D.)

Mobile Phase : A) CH₃COOC₂H₅
B) n-Hexane
C) CH₃COOH
A/B/C = 50/40/10, v/v/v

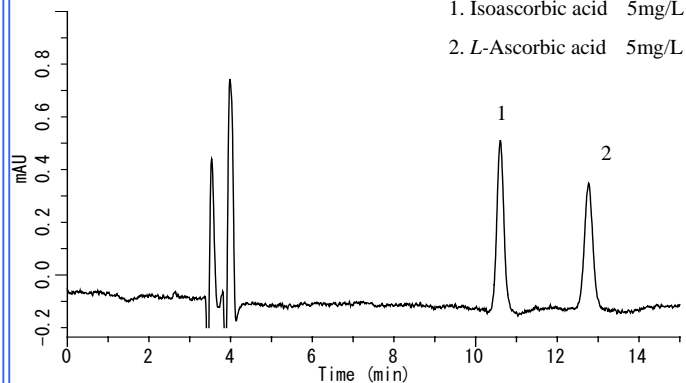
Flow Rate : 1.5 mL/min

Column Temp. : 40 °C

Detection : PDA 495 nm

Injection Volume : 20 μ L

Homocysteine Reduction Method



Analytical Conditions ②

Column : Inertsil NH₂
(5 μ m, 250 x 4.6 mm I.D.)

Mobile Phase : A) CH₃CN
B) CH₃OH
C) 0.01M phosphoric Buffer
D) 0.03% homocystein solution
A/B/C/D = 600/30/100/30, v/v/v/v

Flow Rate : 1.0 mL/min

Column Temp. : 40 °C

Detection : PDA 270 nm

Injection Volume : 5 μ L

DNPH Derivatization Method

Pretreatment Conditions

Sample

- 5g
- 5% Metaphosphoric acid 30mL
- grinding extraction
- Dilute to 50mL with 5% Metaphosphoric acid

Filtration

- Centrifugation 3000rpm, 10min
- 0.45 μ m Filter

Fractionation

- 2mL Fraction

Derivatization

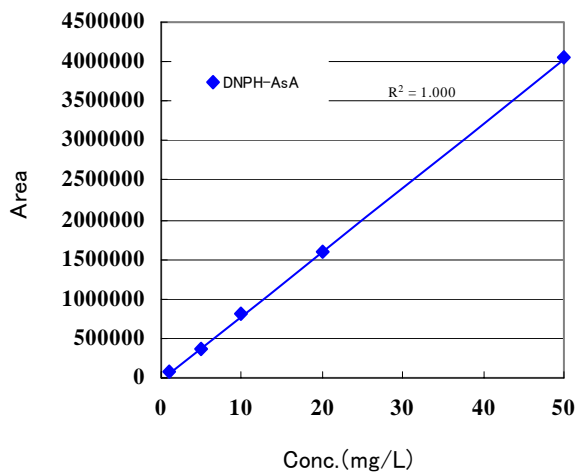
- 5% Metaphosphoric acid 1mL
- 2,6-dichloroindophenol 3 drop
- 2% thiourea • Metaphosphoric acid solution 2mL
- 2% 2,4-DNPH • 4.5M Sulfuric acid 0.5mL
- Heating (50°C, 90min)
- Water cooling

liquid-liquid extraction

- Ethyl acetate 2mL
- Shake 1hr

Measurement sample

- Supernatant liquid
 - 0.5mL Fraction
 - Dilute to 1mL with Hexane
- Lower layer
Waste

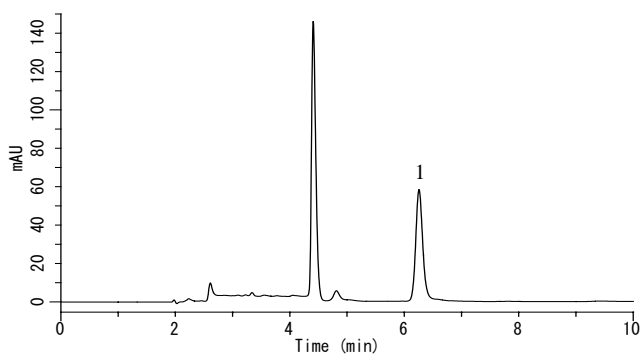


Calibration Curve※1

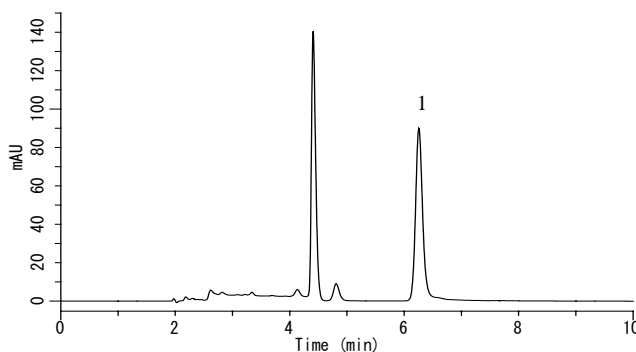
※1: The calibration sample was prepared by diluting L-Ascorbic acid in steps and pretreating it. The concentration described above is the concentration after diluting the sample.

Analysis of food (Analytical Conditions ①)

Tea leaf

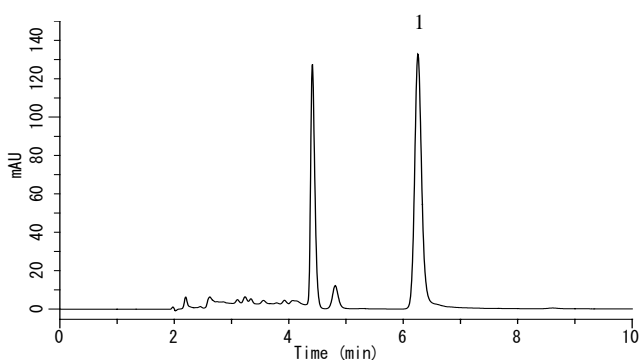


Sausage

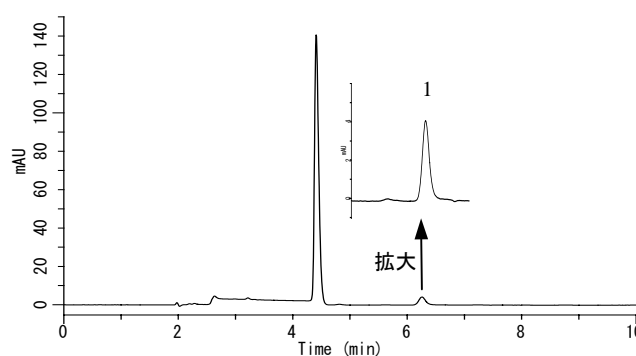


1. L-Ascorbic acid

Baby formula



Spinach



Homocysteine Reduction Method

Pretreatment Conditions

Liquid Sample

10g
4% Metaphosphoric acid 10mL
Dilute to 50mL
with 2% Metaphosphoric acid

Solid Sample

10g
4% Metaphosphoric acid 10mL
2% Metaphosphoric acid 30mL
ultrasonic extraction 10min
Dilute to 50mL
with 2% Metaphosphoric acid

Filtration

Centrifugation 3000rpm 10min
0.45 μ m Filter

2mL Fraction

2mL Fraction

Reduction

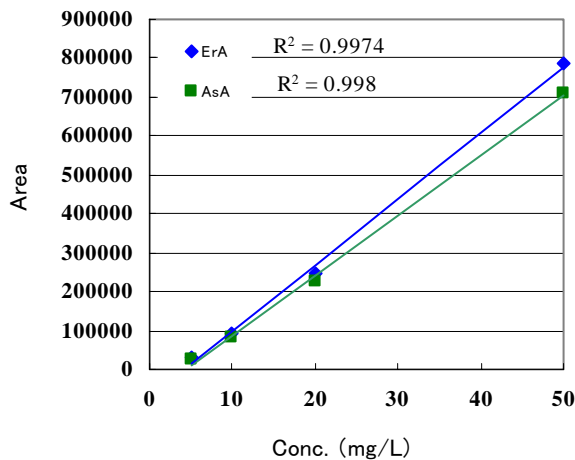
0.1% Homocystein 1mL
10% Disodium Hydrogen Phosphate 1mL
Heating (40°C, 20min)

Measurement Sample

L-ascorbic acid
+
Iso ascorbic acid

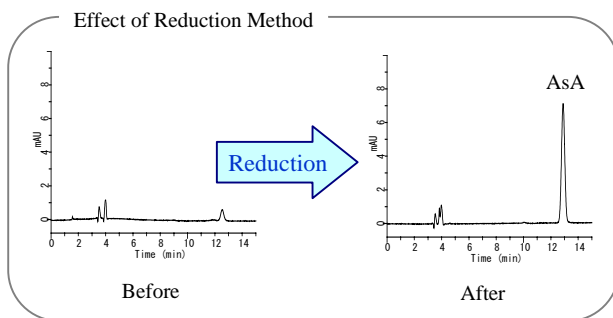
Measurement Sample

Total Ascorbic acid
+
Total Iso ascorbic acid



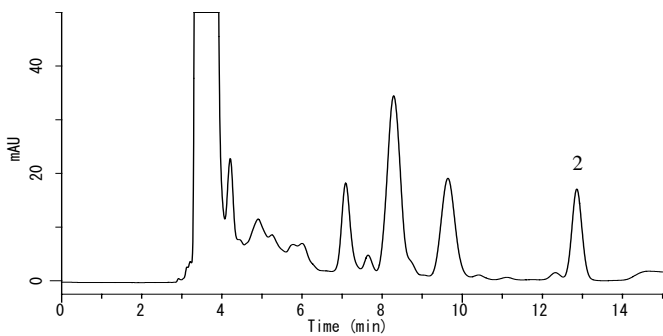
Calibration Curve^{※2}

※2: On this figure, standard solution is diluted by 2% Metaphosphoric acid.

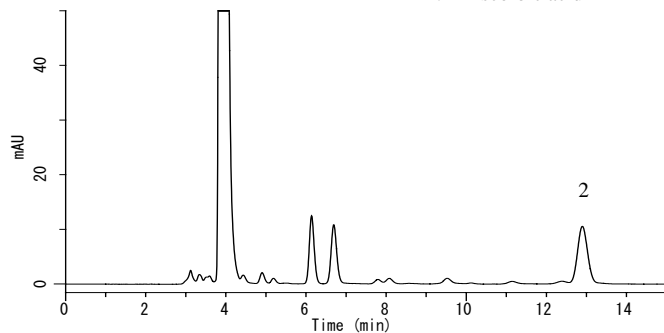


Analysis of food (Analytical Conditions ②)

Tea leaf

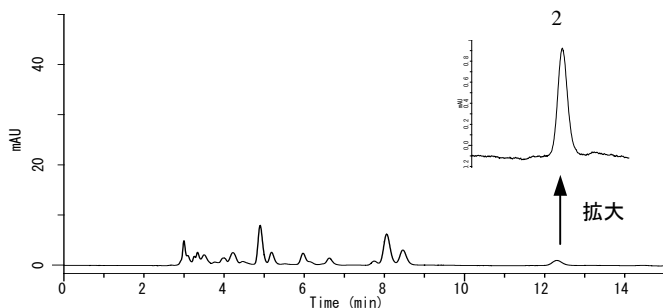


Sausage

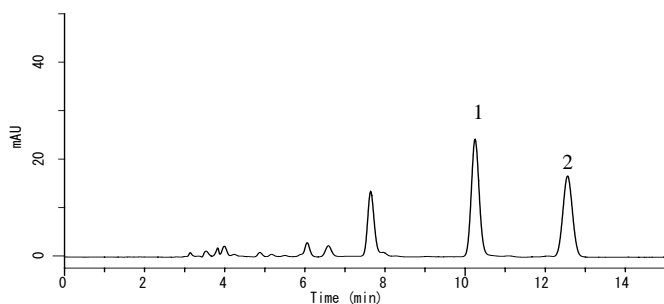


1. Iso ascorbic acid
2. L-Ascorbic acid

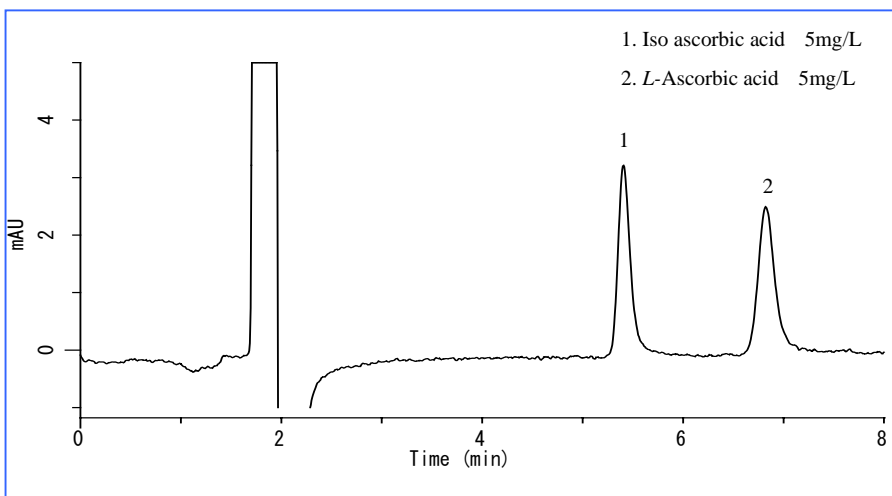
Beer



Fish sausage



Modified analytical conditions by Homocysteine Reduction Method



Analytical Conditions ③

Column : Inertsil NH₂
(5 μm, 250 x 4.6 mm I.D.)

Mobile Phase : A) CH₃CN
B) H₂O
C) CH₃COOH
A/B/C = 87/11/2, v/v/v

Flow Rate : 2.0 mL/min

Column Temp. : 40 °C

Detection : PDA 243 nm

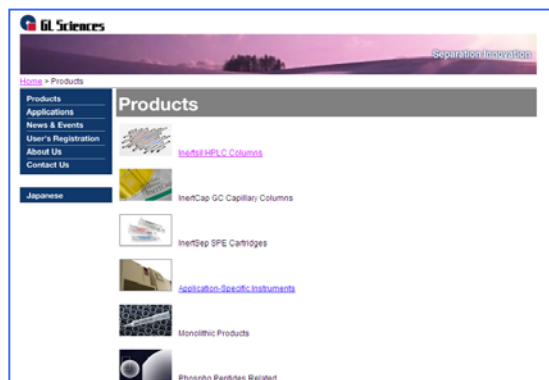
Injection Volume : 20 μL

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