LT009

GL Sciences Inc.

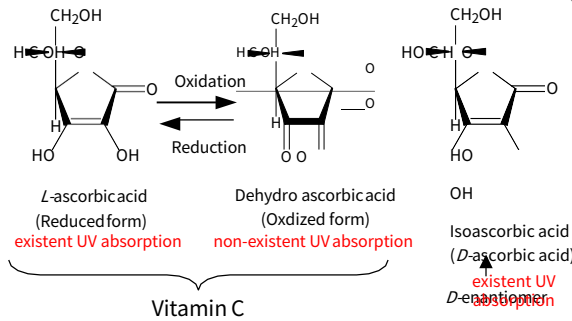
Analysis of Vitamin C in Food by HPLC

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.

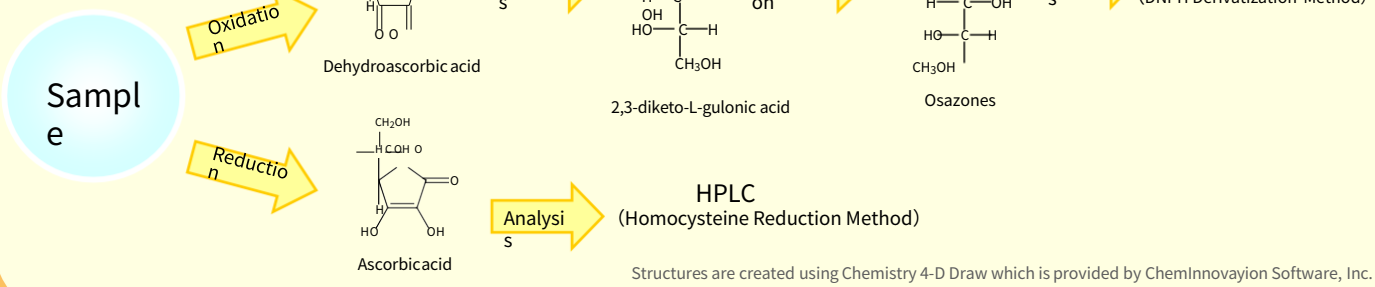
This application was conducted based on the Japanese Food Sanitation Inspection Guideline.

Chemical Structure



Outline

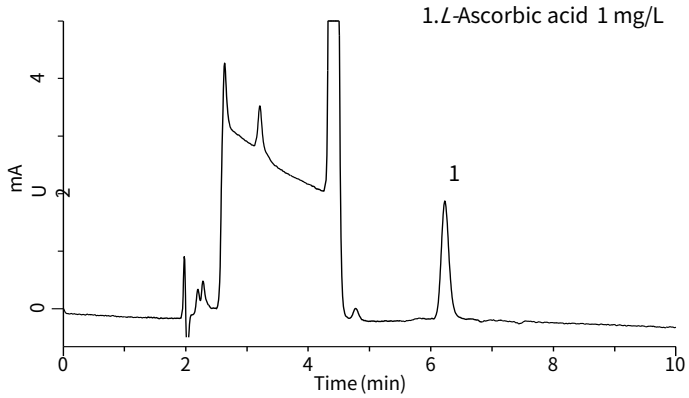
The total amount of Ascorbic acid can be measured by a DNPH Derivatization method.
Simultaneous analysis of Isoascorbic acid and Reduced L-ascorbic acid can be measured by a Homocysteine reudction method.



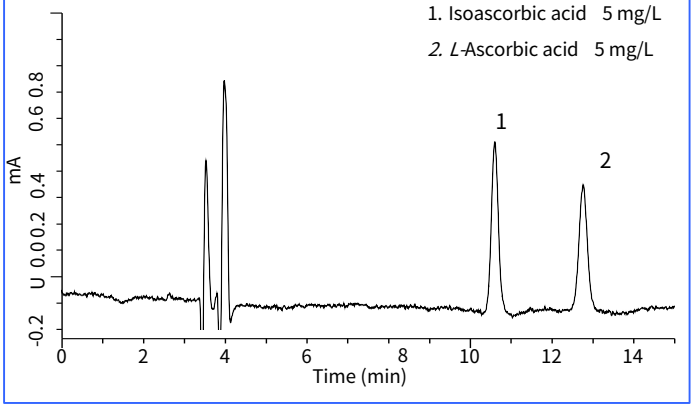
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovayion Software, Inc.

Analysis of Standard Solution

DNPH Derivatization Method



Homocysteine Reduction Method



Analytical Conditions ①

Column	: Inertsil SIL-100A (5µm, 250 x 4.6 mm I.D.)
Mobile Phase	: A) $CH_3COOC_2H_5$ *B)* *n*-Hexane C) CH_3COOH 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 µL

Analytical Conditions ②

Column	: Inertsil NH_2 (5µm, 250 x 4.6 mm I.D.)
Mobile Phase	: A) CH_3CN B) CH_3OH C)0.01M phosphoric Buffer D)0.03% homocystein solution A/B/C/D = 600/30/100/30, v/v/v/v
	: 1.0 mL/min
Flow Rate	: 40 °C
Column Temp.	: PDA 270 nm
Detection	
Injection Volume	: 5 µL

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DNPH Derivatization Method

Pretreatment Conditions

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

Filtration
- Centrifugation 3000 rpm, 10 min
- 0.45 µm Filter

Fractionation
- 2mL Fraction

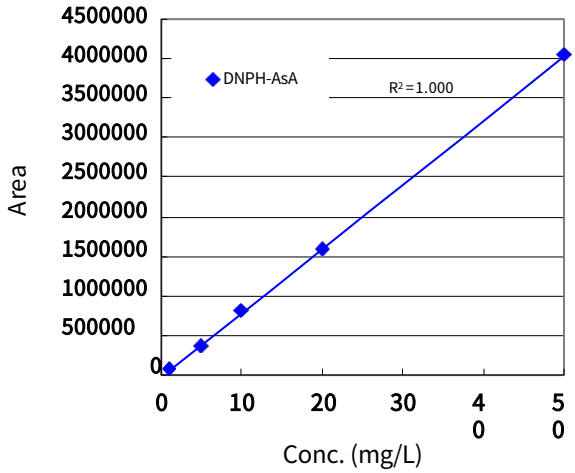
Derivatization
- 5 %Metaphosphoric acid 1 mL
- 2,6-dichloroindophenol 3 drop
- 2 %thiourea・Metaphosphoric acid solution 2 mL
- 2 % 2,4-DNPH・4.5M Sulfuric acid 0.5 mL
- Heating (50°C, 90 min)
- Water cooling

liquid-liquid extraction
- Ethyl acetate 2 mL
- Shake 1 hr

Measurement sample
- Supernatant liquid
- 0.5 mL Fraction
- Dilute to 1 mL with Hexane

Lower layer

Waste

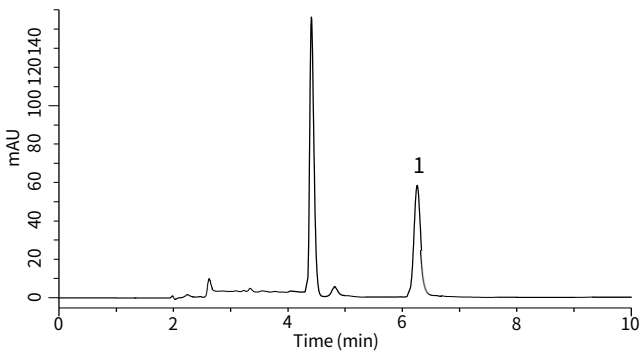


Calibration Curve*1

*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.

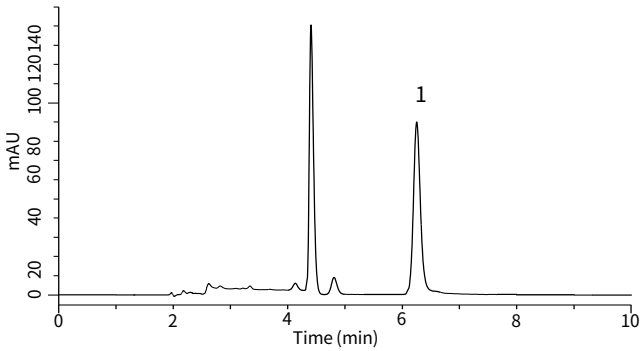
Analysis of food (Analytical Conditions ①)

Tea leaf

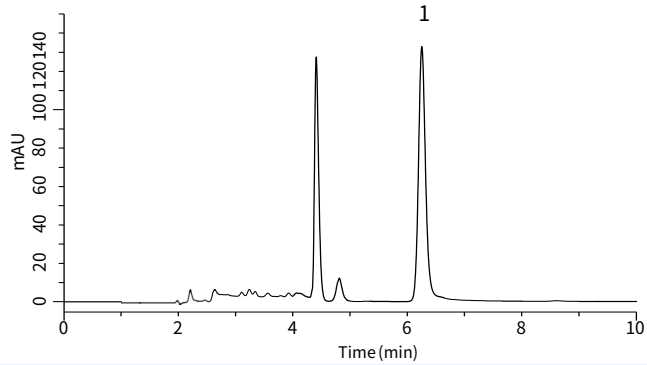


Sausage

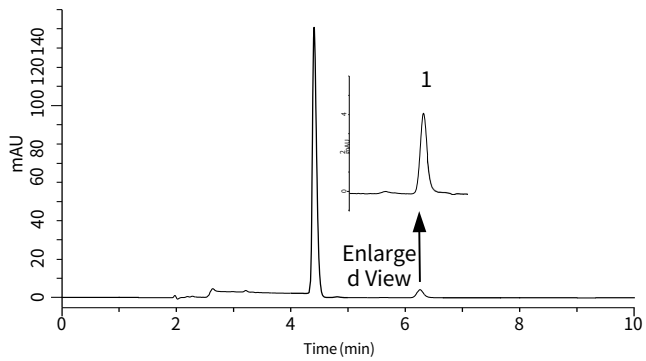
1. *L*-Ascorbic acid

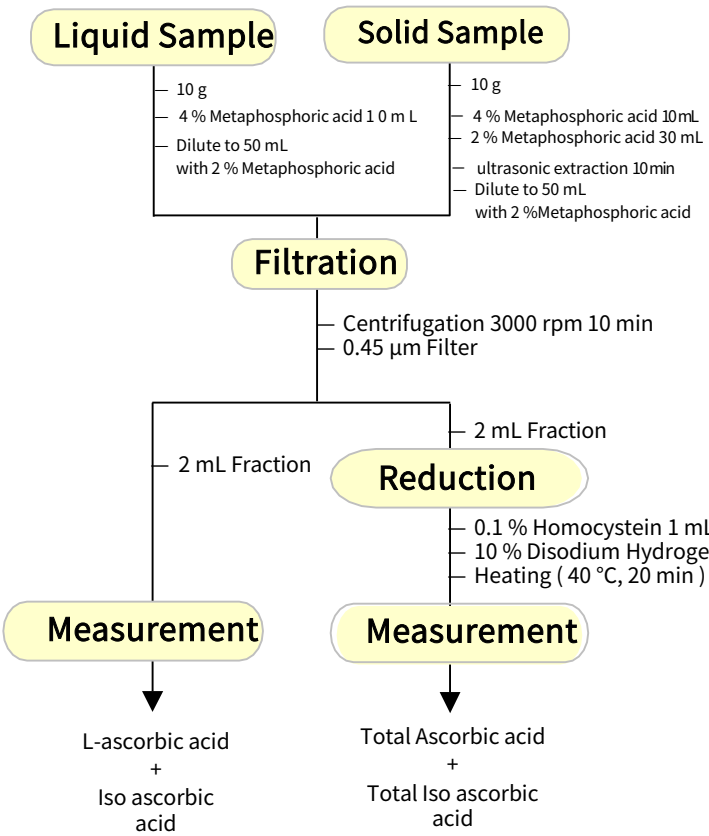


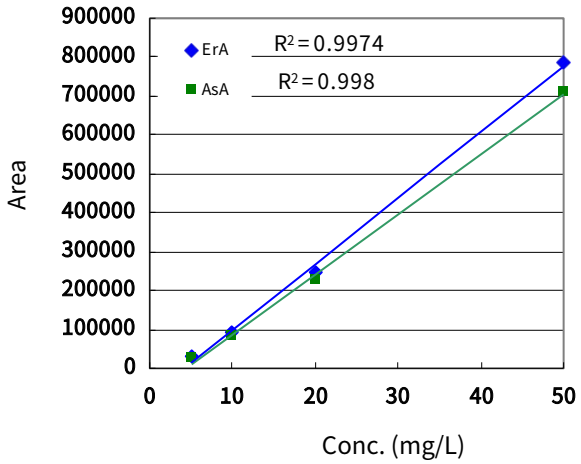
Baby formula



Spinach

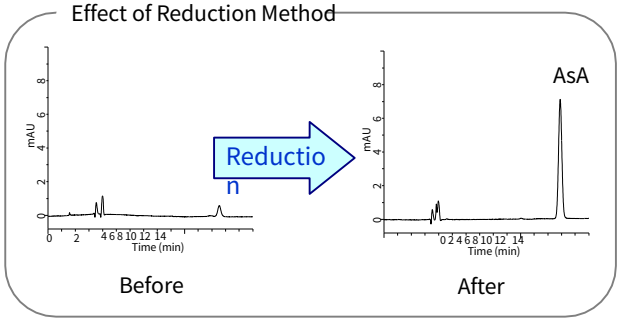






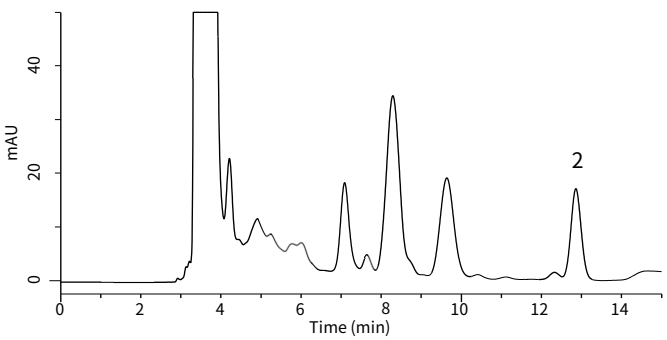
Calibration Curve*2

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



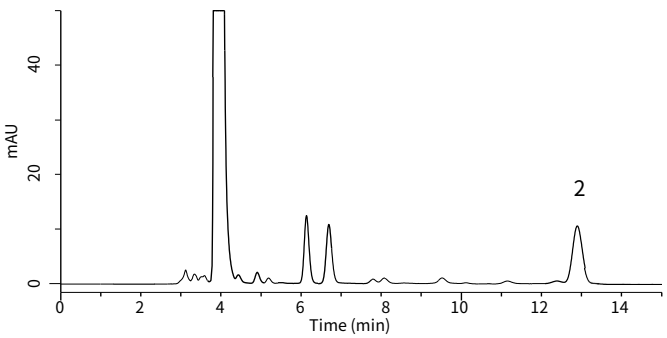
Analysis of food (Analytical Conditions ②)

Tea leaf

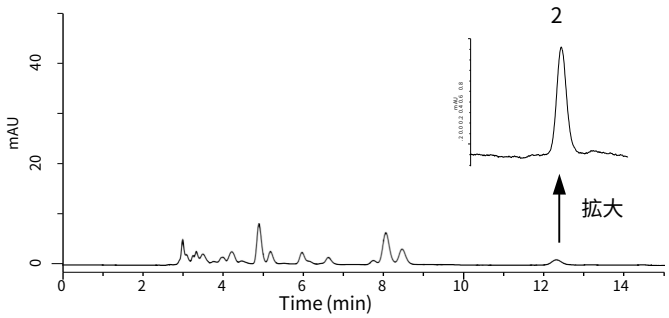


Sausage

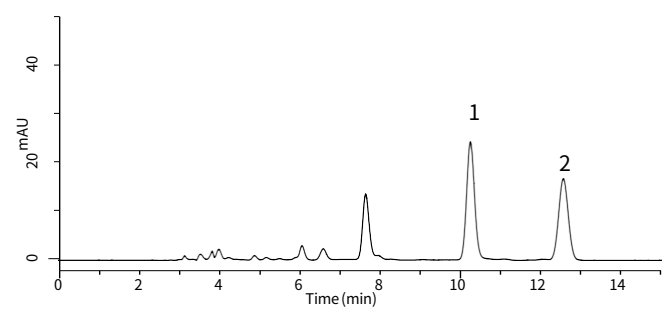
1. Iso ascorbic acid
2. L-Ascorbic acid



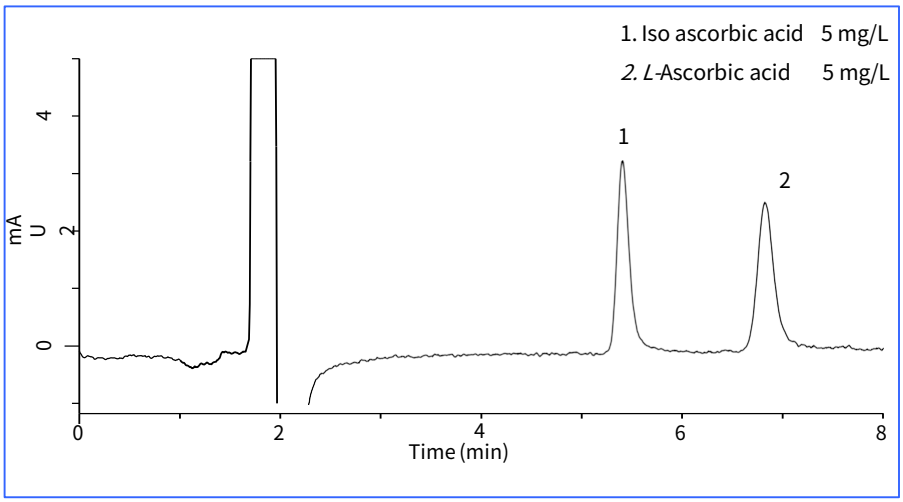
Beer



Fish sausage



Modified analytical conditions by Homocysteine Reduction Method



Analytical Conditions ③

Column	: Inertsil NH_2 (5 µm, 250 x 4.6 mm I.D.)
Mobile Phase	: A) CH_3CN B) H_2O C) CH_3COOH 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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