ChromaDex®

Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

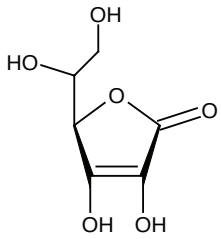
0032 - Ascorbic Acid by HPLC

Chemical Name: *3-Oxo-L-gulofuranolactone; L-3-ketothreohexuronic acid; 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol*

Common Names: L-Ascorbic acid, Vitamin C

Molecular Weight: 176.13

Chemical Formula: $C_6H_8O_6$



Solubility:

Highly water soluble, moderately soluble in alcohol and glycerol. Insoluble in ether, chloroform, benzene, and other nonpolar organic solvents.

Other Physical/Chemical Data:

pKa_1 = 4.17, pKa_2 = 11.57
UV max = 245 nm (acidic solution, ε = 12,200), 265 nm (neutral solution, ε = 16,600)
[α]D = +20.5° to +21.5° at 25oC (c = 1)
Melting point = 190°C to 192°C (decomposition)

Uses:

Prevention and treatment of vitamin C deficiency; as an antimicrobial and antioxidant in food products.

Modes of Action:

Ascorbic acid is an effective reducing agent. It helps maintain the activity of the enzyme prolyl hydroxylase, which is necessary for the synthesis of collagen, by keeping the iron atom at its active site in the ferrous state. Ascorbic acid also acts as a free-radical scavenger within cells.

Methods of Analysis:

Many methods have been published for the analysis of ascorbic acid in dietary supplements and foods. These methods include reversed-phase and ion-exchange chromatography, spectrophotometry, titrimetry, and fluorometry.

Method 1:

Most analytical methods for ascorbic acid focus on food, beverages, tissue, and plasma rather than dietary supplements. Nevertheless, these methods can often be adapted easily to the analysis of dietary supplements.

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Sample & Standard Preparation:

Because of its high solubility in water, ascorbic acid is usually extracted with or dissolved in an aqueous medium. Unfortunately, ascorbic acid is readily oxidized in aqueous solution to dehydroascorbic acid, and it can also be hydrolyzed to 2,3-diketo-L-gulonic acid. Dehydroascorbic acid can be converted back to ascorbic acid by reducing agents; however, the degradation of ascorbic acid to 2,3-diketo-L-gulonic acid is not reversible. The presence of metals in solution increases the rate of oxidative degradation,[1] and heat, light, and basic pH also increase the degradation rate. Therefore, any sample and standard preparation technique should be developed with the goal of minimizing degradation. Low actinic glassware should be used, and the solution pH should be kept acidic. Often metaphosphoric acid is added to the diluent to maintain an acidic pH; it also slows down metal-catalyzed oxidation. Reducing agents and edetic acid (EDTA) can be added to solutions of ascorbic acid to minimize degradation during analysis. Dithiothreitol,[2] homocysteine,[3] L-cysteine,[4] and tris[2-carboxyethyl]phosphine[5] have all been used as reductants to prevent oxidation of ascorbic acid.

Chromatography:

Lykkesfeldt[5] used reversed-phase HPLC with ion-pairing and electrochemical detection to assay ascorbic acid in biological samples. Tris[2-carboxyethyl]phosphine (0.25 mM) was used as a reducing agent in the standard and sample solutions.

Column: Phenomenex Luna C18, 3µm, 150 × 4.6 mm, equipped with SecurityGuard C18 column.
Mobile phase: Solvent A = 100 mM NaH2PO4, 100 mM sodium acetate monohydrate, 1.0 mM EDTA, and 0.189 mM *n*-dodecyltrimethylammonium chloride in water; solvent B = 36.6 M tetraoctylammonium bromide; pH was adjusted to 5.4 with phosphoric acid; 75%A/25%B was used.
Flow rate: 0.6 mL/minute
Column temperature: 30°C
Injection volume: 10 µL
Detection: Coulometric, dual cell (–200 mV and +300 mV)

Validation Data:

Linearity: 5 to 100 µM ascorbic acid, r^2>0.99.
Accuracy: 102.1% recovery of spiked plasma compared to linearity standards.
Precision: <1% RSD (six spiked samples)
Selectivity: Peak identification was achieved by quantitative oxidation of ascorbic acid with scorbic acid oxidase.
Ruggedness: Between-days coefficient of variation less than 3.5%.
Robustness: Not determined
LOD/LOQ: Not determined

References:

1. Tannenbaum SR, Young VR. Vitamins and minerals. In: Fennema OR, ed. *Food chemistry*. New York: Marcel Dekker; 1985:477.

2. Doner LW, Hicks KB. High-performance liquid chromatographic separation of ascorbic acid, erythorbic acid, dehydroascorbic acid, dehydroerythorbic acid, diketogulonic acid, and diketogluconic acid. *Anal Biochem*. 1981;115:225–30.

3. Rose RC, Nahrwold DL. Quantitative analysis of ascorbic acid and dehydroascorbic acid by high-performance liquid chromatography. *Anal Biochem*. 1981;114:140–5.

4. Iwase H, Ono I. Determination of ascorbic acid and dehydroascorbic acid in juices by high-performance liquid chromatography with electrochemical detection using L-cysteine as precolumn reluctant. *J Chromatogr A*. 1993;654:215–20.

5. Lykkesfeldt J. Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable reduction with tris[2-carboxyethyl]phosphine hydrochloride. *Anal Biochem*. 2000;282:89–93.

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