Identification of Oxidation Products of L-Ascorbic Acid by HPLC

Application Note

Author

Linda Lloyd
Agilent Technologies, Inc.

Introduction

Ascorbic acid is a water soluble sugar acid with antioxidant properties. The L-isomer of ascorbic acid is commonly known as vitamin C and is found naturally in fruits and vegetables. It is also added to fruit juices and other processed products as an antioxidant. Vitamin C is an essential nutrient in the human diet in the manufacture of collagen. In humans, absence of the vitamin leads to scurvy, a deficiency disease.

The acid has strong reducing power but when oxidized is converted to several compounds that do not have the same antiscorbutic or reducing properties. Given the importance of the vitamin in human health and its widespread use as an antioxidant in processed foods, study of its degradation products is merited. This note describes aspects of the rate of degradation of L-ascorbic acid and the nature of some of its degradation products using PLRP-S columns. PLRP-S is a rigid macroporous styrene/divinylbenzene HPLC phase with outstanding chemical and physical stability. The high surface area of the 100Å pore size enables retention of water soluble solutes.

Agilent Technologies

The aim of this study was to develop a rapid HPLC method for the quantitative and qualitative analysis of L-ascorbic acid.

Materials and Reagents

Reference samples: commercial L-ascorbic acid, oxalic acid, L-dehydroascorbic acid (DHAA) dimer.

Conditions

Columns: 2 x PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Eluent: 0.2 M NaH_2PO_4, pH 2.14
Flow rate: 0.5 mL/min
Inj Vol: 20 µL
Detector: UV, 268 and 220 nm

Results and Discussion

Reference materials

The retention of reference compounds is shown in Figure 1. L-ascorbic acid was well resolved from its possible degradation products.

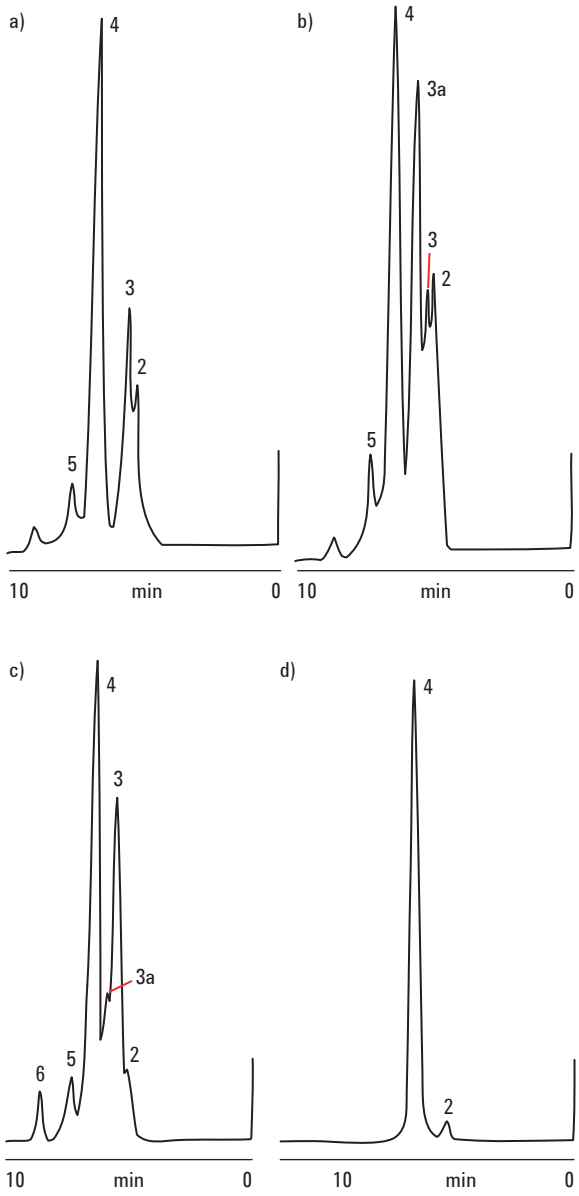
Bromine oxidation of L-ascorbic acid

HPLC of freshly prepared solutions of L-ascorbic acid treated with bromine produced the curves shown in Figure 2. Peak 1 is a high response at 220 nm, whereas peak 2 is a smaller response later identified as DHAA monomer with a free side chain. Peak height intensity was proportional to the amount of bromine added. Further addition of bromine was made to a cold solution of L-ascorbic acid (2 °C) to control degradation of DHAA. Homocysteine was then added and the reduction reaction went ahead at room temperature.

Effect of homocysteine on DHAA

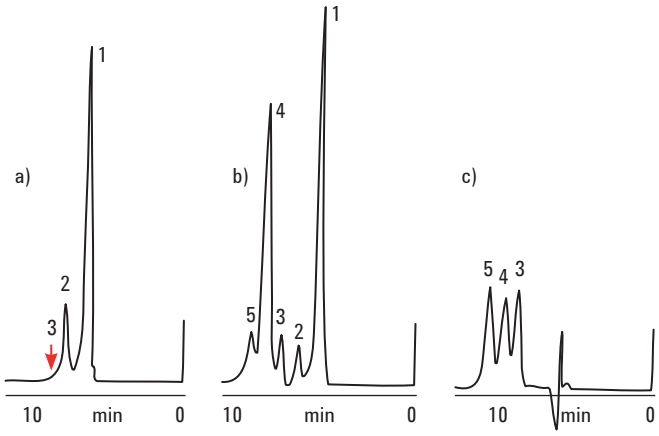
The reaction of homocysteine with DHAA was examined to distinguish the DHAA peak from surrounding peaks in the commercial samples (Figure 3). *Inter alia*, L-ascorbic acid (peak 4a) increased very rapidly after reaction with homocysteine, and peaks 4 and 6 represent monomeric forms of DHAA.

The complete data set and analysis is available in Kennedy *et al.* (1989).



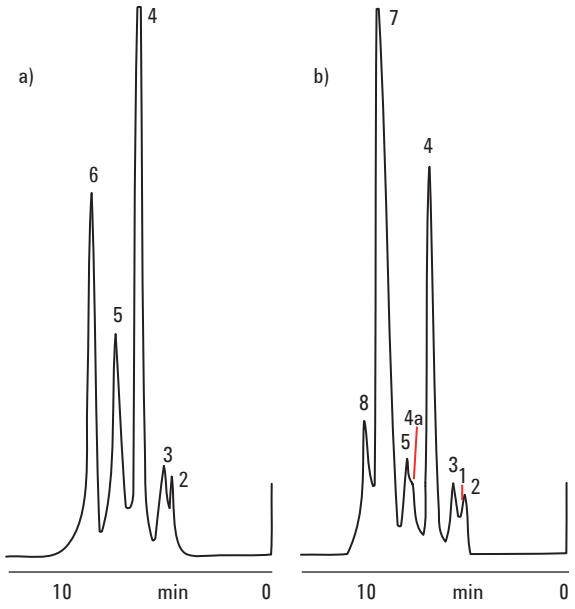
Peak identification
2. oxalic acid
3. L-threonic acid
3a. L-2,3-diketoglutaric acid
4. DHAA monomer in 1,4-lactone form
5. unstable component of DHAA
6. DHAA bicyclic hydrated monomer

Figure 1. HPLC separation of a) L-dehydroascorbic acid solution spiked with b) potassium L-2,3-diketoglutaric acid and c) calcium threonate reference materials. d) is L-dehydroascorbic acid produced by Dietz's method, at 220 nm (0.2 AUFS).



Peak identification
1. oxalic acid
2. DHAA monomer in the 1,4-lactone form
3. L-ascorbic acid
4. Hcys 1
5. Hcys 2

Figure 2. HPLC separation of a) bromine-oxidized L-ascorbic acid, at 220 nm (0.2 AUFS), and b) after addition of homocysteine (1:1), at 220 nm (0.1 AUFS) and c) at 268 nm (0.02 AUFS).



Peak identification
1. unidentified
2. oxalic acid
3. threonic acid
4. DHAA monomer in the 1,4-lactone form
4a. L-ascorbic acid
5. unidentified
6. DHAA monomer in the bicyclic form
7. Hcys 1
8. Hcys 8

Figure 3. HPLC separation of L-dehydroascorbic acid solution a) before and b) after reduction reaction with homocysteine, at 220 nm (0.05 AUFS), using optimized conditions.

Conclusion

PLRP-S columns successfully revealed the identities of some degradation products of vitamin C in the development of a qualitative and quantitative HPLC method for the fast analysis of L-ascorbic acid.

Reference

Kennedy, JF, White, CA, Warner, FP, Lloyd, LL and Rivera, ZS (1989) The identification and analysis of the oxidation products of L-ascorbic acid by HPLC C. *J. Micronut. Anal.*, 5, 91-109.

www.agilent.com/chem

Published in UK, May 20, 2011
5990-8217EN

Agilent Technologies