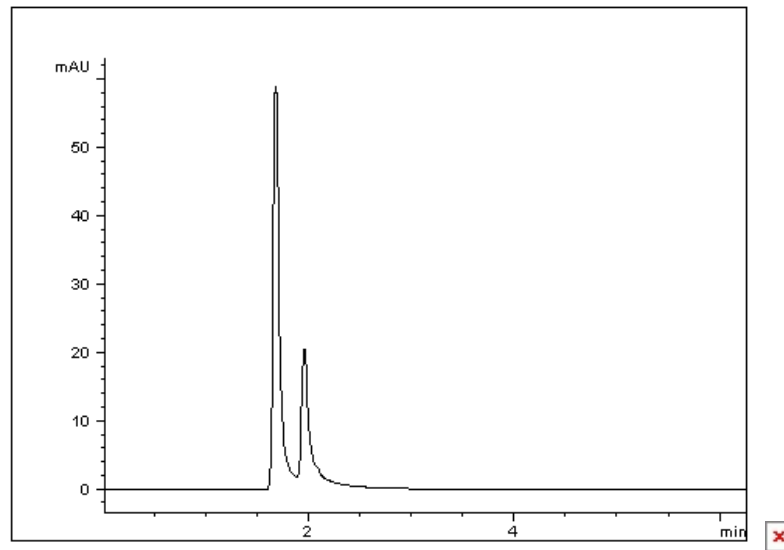


D and L Ascorbic acid Separation Analyzed by HPLC- AppNote

An Analysis of Vitamin C

Click [HERE](#) for Column Ordering Information.

This simple Method shows good separation between two Enantiomers as well as Retention, considering these Acids both have a - 1.6 log p. This Method is Robust as results were verified with three different HPLCs and two separate Columns, all showing excellent Resolution of these two Acids.





Peaks:

1. D- Isoascorbic acid
2. L- Ascorbic acid

Method Conditions

Column: Cogent Diamond Hydride™, 4μm, 100Å

Catalog No.: 70000-10P

Dimensions: 4.6mm x 100mm

Mobile Phase: 98% Acetonitrile 2% DI Water / 0.1% Formic Acid

Flow Rate: 1.0mL / minute

Injection Volume: 1uL

Detection: UV 254nm

Injection vol.: 1μL

Sample Preparation: D- Isoascorbic Acid and L- Ascorbic Acid in 1.0 mg/mL in diluent of 50% Acetonitrile / 50% DI Water (v/v)

t₀: 1.20 Minutes

K₁: 0.39

K₂: 0.62

α: 1.59

Note: Ascorbic acid exists as two enantiomers (mirror-image isomers), commonly denoted "l" (for "levo") and "d" (for "dextro"). The l isomer is the one most often



encountered and occurs naturally in many foods, and is one form of Vitamin C, an essential nutrient for many animals. Deficiency of Vitamin C causes scurvy. Vitamin C is used as a food additive and a dietary supplement for its antioxidant properties. The "d" form can be made via chemical synthesis but has no significant biological role.

Capacity Factor - Relative Retention $k = (t_R - t_0)/t_0$
 $\alpha = K_2/K_1$

