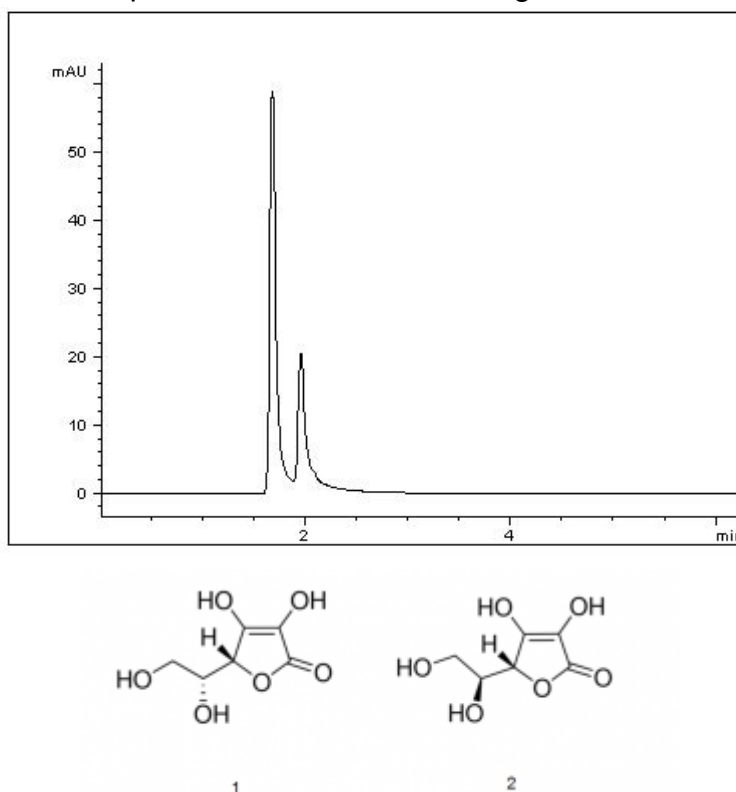


## Ascorbic Acid D and L Isomer Separation by HPLC- AppNote

### 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)

**t<sub>0</sub>:** 1.20 Minutes

**K<sub>1</sub>:** 0.39

**K<sub>2</sub>:** 0.62

**α:** 1.59

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*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  $k = (t_R - t_0)/t_0$

$\alpha = K_2/K_1$

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