

## APPLICATIONS

## HPLC-UV Analysis of Vitamin C from Tablets and Soft-Gels

### Method Status: Scientifically Valid per cGMPs for Dietary Supplements

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#### Introduction

The verification of label claim data in nutraceutical formulations has come under scrutiny by the FDA. Manufacturers and contract testing labs alike are looking for accurate and scientifically valid methods that are suitable for use with different formulations. The complex nature of nutraceuticals and botanicals often requires long analysis and difficult sample cleanup steps to resolve matrix interferences.

There were two primary goals of this project: 1) to determine an accurate and reproducible means of analyzing Vitamin C and 2) demonstrate the suitability for analysis of two commercially available formulations of Vitamin C.

#### Therapeutic Use Overview

Vitamin C (Ascorbic Acid) is a water soluble vitamin and an important nutrient essential for human life. Vitamin C is required for the synthesis of collagen in the human body and a deficiency causes scurvy. Widely available in capsule, tablet and drink mixes for nutritional supplementation, Vitamin C is also an antioxidant and has had historic interest for a wide variety of therapeutic uses such as immune system support.

#### Experimental

HPLC analysis was performed using an Agilent® 1100 LC System (Agilent Technologies, Inc., Palo Alto, CA, USA). The system was optimized in order to reduce dead volume and improve performance including increasing the UV scan rate, changing the injector needle seat, re-plumbing the system with red PEEKsil™ tubing (SGE), and using a semi-micro flow cell. The Gemini® 3 µm C18 150 x 4.6 mm HPLC column was from Phenomenex, Torrance, CA. All chromatographic conditions are specified on **Figure 1**.

The Vitamin C reference material was provided by ChromaDex®, Inc. The formulated products, a tablet and a soft-gel, were purchased from a local health food store.

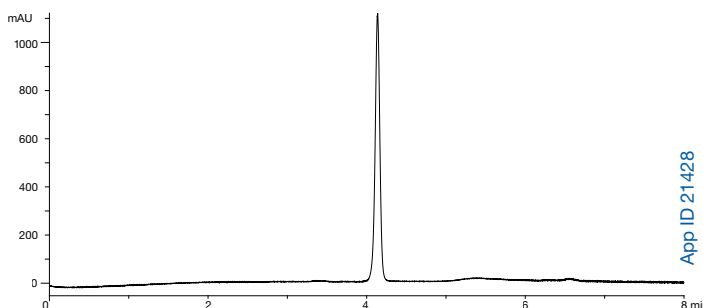
The tablets and soft-gels were prepared by placing 2 tablets or soft-gels into a 1 L volumetric flask with a stir bar. 200 mL THF and approximately 300 mL of Extraction Buffer (see Preparation of Extraction Buffer) were added and the solution was allowed to stir loosely capped for 1 hour on low heat. After 1 hour, the solution was removed from the heat and was cooled to room temperature. The solution was then brought to a final volume of 1L with Extraction Buffer. Prior to analysis, sample was filtered with a Phenex™ 0.45 µm PTFE syringe filter (Phenomenex part no. AF0-3102-12).

#### Preparation of Extraction Buffer

1. Weigh 100 mg of DL-dithiothreitol into a 1 L volumetric flask
2. Add 900 mL of Milli-Q water and mix well
3. Add 1 mL of H<sub>3</sub>PO<sub>4</sub> and dilute to volume of 1 L with Milli-Q water
4. Add a stir bar and add 100 mg of EDTA
5. Stir until dissolved

*Extraction buffer is good for 1 week after preparation*

**Figure 1.**  
Vitamin C Reference Standard (1000 µg/mL) Using a Gemini® 3 µm C18 Column



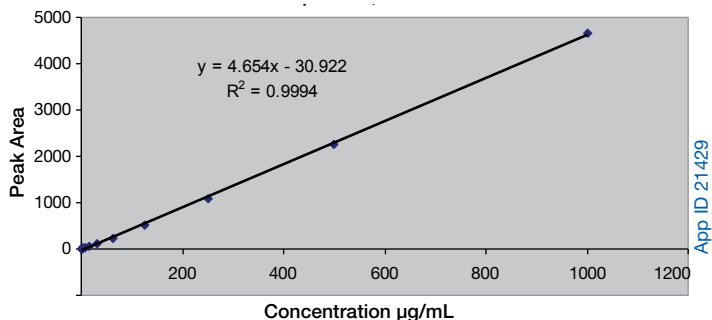
**Column:** Gemini 3 µm C18  
**Dimensions:** 150 x 4.6 mm  
**Part No.:** 00F-4439-E0  
**Mobile Phase:** A: 0.1 % Formic acid in Water  
                   B: Methanol  
**Flow Rate:** 600 µL/min  
**Gradient:**

Time (min)	% B
0	5
5	80
5.1	5
8	5

**Injection Volume:** 1 µL  
**Temperature:** 22 °C  
**Detection:** UV @ 245 nm



**Figure 2.**  
Representative Calibration Curve for Vitamin C from 2 to 1000 µg/mL



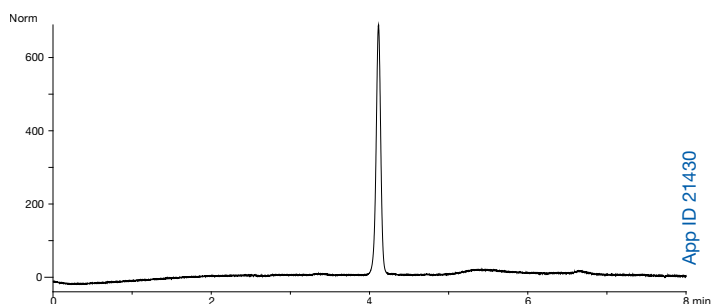
**Table 1.**  
Calibration Curve from a Gemini® 3 µm C18 Column Based on ChromaDex® Reference Materials

Compound	LOD		LOQ	
Vitamin C	1.95 µg/mL	S/N = 5.5	3.91 µg/mL	S/N = 13.9

**Table 2.**  
Determination of Method Accuracy from 20 Repeated Injections of Vitamin C Reference Standard (1 mg/mL, 1 µL injection)

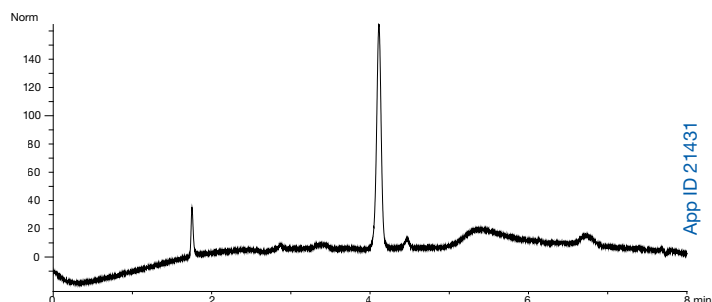
	Area
Average	5050
STDEV	99.71
% CV	1.97

**Figure 3.**  
Analysis of Commercial Tablet Formulation Using a Gemini 3 µm C18 Column



Running conditions can be found in **Figure 1**.

**Figure 4.**  
Analysis of Commercial Soft-Gel Formulation Using a Gemini 3 µm C18 Column



Running conditions can be found in **Figure 1**.

**Table 3.**  
Experimental Results as Compared to Label Claims

	Label Claim Vitamin C (mg) (per serving)	Experimental Results Vitamin C (mg) (per serving) n = 3	Recovery (%)	RSD (%)
Tablet	250	298	116	3.74
Soft-Gel	250	221	88	0.69

## Results and Discussion

When analyzing botanicals and nutraceuticals, the separation of standards can often be misleading since the plant extract can contain many other endogenous components that could lead to poor results. To demonstrate specificity of the new method, Vitamin C reference materials were run using our proposed method and no coelutions were observed (**Figure 1**).

Having demonstrated that the method provided acceptable results, we performed experiments to determine linearity, accuracy, range, and limit of quantitation (LOQ) using the Gemini HPLC column. The method was shown to be linear over a range of 2 to 1000 µg/mL (**Figure 2**). The LOD was determined to be 1.95 µg/mL while the LOQ was determined to be 3.91 µg/mL (**Table 1**). Accuracy and precision were determined at the 1 mg/mL level and found to be less than 2 % CV (**Table 2**).

The final experiment was to analyze commercially available formulations and determine if the results obtained were similar to label claims. To ensure that we properly tested our new assay, we attempted to choose difficult formulations such as tablets and soft-gel pills.

Analysis of a formulated product obtained results that were slightly higher than label claim for the tablets and slightly lower than label claim for the soft-gels (**Table 3**). Chromatographic separation for each formulation is depicted in **Figures 3** and **4**.

## Conclusion

Analysis of Vitamin C reference materials provided results that were consistent with the supplied certificate of analysis. Analysis of formulated products resulted in values that were slightly higher than label claims for tablets and slightly lower than label claims for soft-gel pills. Providing standardized reference methods for analysis is the first step to ensuring quality in nutraceutical products.

## Scientifically Valid

Section 21CFR111.320 of cGMPs for Dietary Supplements requires you to "identify and use an appropriate scientifically valid method for each established specification for which testing or examination is required to determine whether the specification is met". The FDA does not elaborate on what is considered a scientifically valid method in the cGMPs. ChromaDex has defined scientifically valid as a method that meets minimum linearity, precision, sensitivity and range requirements. These requirements are outlined in an FDA laboratory document, ORA LABORATORY PROCEDURE Food and Drug Administration, ORA-LAB.5.4.5. This laboratory guidance document defines minimal performance attributes for selected methods of analysis and has been applied by ChromaDex to the selection of methods that are fit for purpose in the dietary supplements industry. According to the above definition, the method detailed in this document is considered scientifically valid as application to the cGMP requirements. Product specific, full method validations according to AOAC guidelines can be applied to customer samples upon request, to further document method performance in specific samples and matrices.

## ChromaDex Ordering Information Phytochemical Reference Standards

Description	Quantity	Part No.
ASCORBIC ACID, L-(+)-(VITAMIN C)(P)	250 mg	ASB-00111020-250
ASCORBIC ACID, L-(+)-(VITAMIN C)(P)	1 g	ASB-00111020-001
ASCORBIC ACID, L-(+)-(VITAMIN C)(P)	5 g	ASB-00111020-005
ASCORBIC ACID, L-(+)-(VITAMIN C)(P)	10 g	ASB-00111020-010
ASCORBIC ACID, L-(+)-(VITAMIN C)(SH)	1 g	ASB-00111022-001
ASCORBIC ACID, L-(+)-(VITAMIN C)(RG)	1 g	ASB-00111021-001
VITAMIN STANDARDS KIT: WATER SOLUBLE	10 x 250 mg	KIT-000022828-250
VITAMIN STANDARDS KIT: WATER SOLUBLE	10 x 1 g	KIT-000022828-001
VITAMIN STANDARDS KIT: COMPLETE	19 x 250 mg	KIT-000022830-250
VITAMIN STANDARDS KIT: COMPLETE	19 x 1 g	KIT-000022830-001

See reverse for ChromaDex contact information

## Phenomenex Ordering Information

### Gemini® HPLC Columns

3 µm Microbore, Minibore and Narrow Bore Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	50 x 1.0	20 x 2.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0*
C18	00B-4439-A0	00M-4439-B0	00A-4439-B0	00B-4439-B0	00D-4439-B0	00F-4439-B0	00B-4439-Y0	00D-4439-Y0	00F-4439-Y0	AJ0-7596

for ID: 2.0-3.0 mm

3 µm Analytical Columns (mm)						SecurityGuard Cartridges (mm)	
Phases	20 x 4.0	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
C18	00M-4439-D0	00A-4439-E0	00B-4439-E0	00D-4439-E0	00F-4439-E0	00G-4439-E0	AJ0-7597

for ID: 3.2-8.0 mm

5 µm Minibore and Narrow Bore Columns (mm)									SecurityGuard Cartridges (mm)	
Phases	30 x 2.0	50 x 2.0	150 x 2.0	250 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	250 x 3.0	4 x 2.0*	
C18	00A-4435-B0	00B-4435-B0	00F-4435-B0	00G-4435-B0	00B-4435-Y0	00D-4435-Y0	00F-4435-Y0	00G-4435-Y0	AJ0-7596	

for ID: 2.0-3.0 mm

5 µm Analytical Columns (mm)						SecurityGuard Cartridges (mm)	
Phases	30 x 4.6	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*	
C18	00A-4435-E0	00B-4435-E0	00D-4435-E0	00F-4435-E0	00G-4435-E0	AJ0-7597	

for ID: 3.2-8.0 mm

5 µm Semi-Prep Columns (mm)		SecurityGuard Cartridges (mm)	
Phases	150 x 10	250 x 10	10 x 10*
C18	00F-4435-N0	00G-4435-N0	AJ0-7598

for ID: 9-16 mm

5 µm Axia™ Packed Preparative Columns (mm)								SecurityGuard Cartridges (mm)	
Phases	50 x 21.2	100 x 21.2	150 x 21.2	250 x 21.2	50 x 30	75 x 30	15 x 21.2**	15 x 30.0*	
C18	00B-4435-P0-AX	00D-4435-P0-AX	00F-4435-P0-AX	00G-4435-P0-AX	00B-4435-U0-AX	00C-4435-U0-AX	AJ0-7846	AJ0-8308	

for ID: 18-29 mm      30-49 mm

5 µm Axia Packed Preparative Columns (mm) continued					SecurityGuard Cartridges (mm)	
Phases	100 x 30	150 x 30	250 x 30	50 x 50	15 x 30.0*	
C18	00D-4435-U0-AX	00F-4435-U0-AX	00G-4435-U0-AX	00B-4435-V0-AX	AJ0-8308	

for ID: 30-49 mm

Other phases available, contact your Phenomenex technical consultant.

See reverse for Phenomenex contact information

\*SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

\*\*SemiPrep SecurityGuard Cartridges require holder, Part No.: AJ0-7220

\*\*\*PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223

◆PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277

## guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

## APPLICATIONS



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Gemini is patented by Phenomenex. U.S. Patent No. 7,563,367

Axia is patented by Phenomenex. U.S. Patent No. 7,674,383

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362

**CAUTION:** this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

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