⟨580⟩ VITAMIN C ASSAY

INTRODUCTION

The following procedures are provided for analysis of different forms of vitamin C as ascorbic acid ($C_6H_8O_6$), sodium ascorbate ($C_6H_7NaO_6$), and calcium ascorbate dihydrate ($C_{12}H_{14}CaO_{12} \cdot 2H_2O$) or their mixtures in finished dosage forms, as Capsules, Tablets, or Oral Solutions.

METHOD I—TITRIMETRIC METHOD

• **PROCEDURE**

Unless specified in the individual monographs, proceed as follows.

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to 100 mg of ascorbic acid, to a 200-mL volumetric flask, and add 75 mL of metaphosphoric–acetic acids TS. Insert a stopper into the flask, and shake by mechanical means for 30 min. Dilute with water to volume.

Sample solution for Oral Solutions: Transfer a volume of Oral Solution equivalent to 50 mg of ascorbic acid, previously diluted with water if necessary, to a 100-mL volumetric flask. Add 20 mL of metaphosphoric–acetic acid TS, dilute with water to volume, and mix.

Sample solution for Tablets: Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent of 100 mg of ascorbic acid, to a 200-mL volumetric flask, and add 75 mL of metaphosphoric–acetic acids TS. Insert a stopper into the flask, and shake by mechanical means for 30 min. Dilute with water to volume.

Blank: A mixture of 5.5 mL of metaphosphoric–acetic acids TS and 15 mL of water.

Titrimetric system

(See *Titrimetry* ⟨541⟩.)

Mode: Direct titration

Titrant: Standard dichlorophenol–indophenol solution VS

Endpoint detection: Visual

Analysis: Transfer a portion of the *Sample solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid into a 50-mL conical flask, and add 5 mL of metaphosphoric–acetic acids TS. Titrate with *Titrant* to a rose-pink color that persists for at least 5 s. Correct for the volume of *Titrant* consumed by the *Blank*.

Calculate the percentage of ascorbic acid ($C_6H_8O_6$) in the portion of sample taken:

$$\text{Result} = \{[(V_S - V_B) \times F]/W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample solution* (mL)
V_B = *Titrant* volume consumed by the *Blank*
F = concentration of *Titrant* in terms of its equivalent of ascorbic acid (mg/mL)
W = nominal amount of ascorbic acid taken for *Analysis* (mg)

METHOD II—CHROMATOGRAPHIC METHOD

• **PROCEDURE 1**

Unless specified in the individual monographs, the *Diluent*, *Standard solution*, and *Sample solutions* are prepared as follows. [NOTE—Protect samples from air, light, and heat.]

Buffer: 2.04 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

Mobile phase: *Buffer*

Diluent: 0.56 g of edetate disodium dihydrate and 2.04 g of monobasic potassium phosphate per 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

Standard solution: 0.25 mg/mL of USP Ascorbic Acid RS in *Diluent*

Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to about 25 mg of ascorbic acid, into a 100-mL volumetric flask. Add 60 mL of *Diluent*, shake mechanically for 15 min, dilute with *Diluent* to volume, mix well, and pass through a membrane filter of 0.45-µm pore size, discarding the first 4 mL.

Sample solution for Oral Solutions: Dilute an accurately measured volume of Oral Solution with *Diluent* to obtain a solution with a concentration of 0.25 mg/mL of ascorbic acid. Mix carefully.

Sample solution for Tablets: Transfer a portion from NLT 20 finely powdered Tablets, nominally equivalent to about 25 mg of ascorbic acid, into a 100-mL volumetric flask. Add 60 mL of *Diluent*, shake mechanically for 15 min, dilute with *Diluent* to volume, mix well, and pass through a membrane filter of 0.45-µm pore size, discarding the first 4 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1
Flow rate: 1.0 mL/min
Injection volume: 5 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%
Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of vitamin C, as ascorbic acid ($C_6H_8O_6$), in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of ascorbic acid from the *Sample solution*
r_S = peak area of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

• **PROCEDURE 2**
Unless specified in the individual monographs, the *Diluent*, *Standard solution*, and *Sample solutions* are prepared as follows. [NOTE—Protect samples from air, light, and heat. All prepared samples must be analyzed within 4 h.]
Buffer: 7.8 g/L of sodium dihydrogen phosphate dihydrate in water. Adjust with phosphoric acid to a pH of 2.5.
Mobile phase: *Buffer* and methanol. See *Table 1* for gradient.

Table 1

Time (min)	Buffer (%)	Methanol (%)
0	100	0
3	100	0
5	0	100
6	50	50
7	100	0
10	100	0

Diluent: Dissolve 73 g of metaphosphoric acid in 800 mL of water, add 84 mL of glacial acetic acid, and dilute with water to 1000 mL.
Standard stock solution: 1 mg/mL of USP Ascorbic Acid RS in *Diluent*. [NOTE—Sonicate with intermittent shaking to help dissolve, if necessary. Prepare fresh every time.]
Standard solution: Dilute *Standard stock solution* with *Diluent* to obtain a solution containing 0.05 mg/mL of USP Ascorbic Acid RS.
Sample solution for Capsules: Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to about 25 mg of ascorbic acid, into a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, sonicate for 15 min, and centrifuge at about 2000 rpm for 5 min. Quantitatively dilute the clear supernatant with *Diluent* to obtain a solution containing 0.05 mg/mL of ascorbic acid. Mix and pass through a membrane filter of 0.45-µm pore size.
Sample solution for Oral Solutions: Dilute an accurately measured volume of Oral Solution with *Diluent* to obtain a solution with a concentration of 0.05 mg/mL of ascorbic acid. Mix carefully.
Sample solution for Tablets: Transfer a portion from NLT 20 ground Tablets, nominally equivalent to about 25 mg of ascorbic acid, into a 50-mL centrifuge tube. Add 25.0 mL of *Diluent*, sonicate for 15 min, and centrifuge at about 2000 rpm for 5 min. Quantitatively dilute the clear supernatant with *Diluent* to obtain a solution containing 0.05 mg/mL of ascorbic acid. Mix and pass through a membrane filter of 0.45-µm pore size.
Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 245 nm
Column: 4.6-mm × 15-cm; 3.5-µm packing L7
Flow rate: 0.8 mL/min
Injection volume: 10 µL
System suitability
Sample: *Standard solution*
Suitability requirements
Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of vitamin C, as ascorbic acid ($C_6H_8O_6$), in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of ascorbic acid from the *Sample solution*
r_S = peak area of ascorbic acid from the *Standard solution*
C_S = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)
C_U = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** ⟨11⟩

  USP Ascorbic Acid RS