



15

chapter

Vitamin C Determination by Indophenol Method

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15.1 INTRODUCTION

15.1.1 Background

Vitamin C is an essential nutrient in the diet, but is easily reduced or destroyed by exposure to heat and oxygen during processing, packaging, and storage of food. The instability of vitamin C makes it more difficult to ensure an accurate listing of vitamin C content on the nutrition label.

The official method of analysis for vitamin C determination of juices is the 2,6-dichloroindophenol titrimetric method (AOAC Method 967.21). While this method is not official for other types of food products, it is sometimes used as a rapid, quality control test for a variety of food products, rather than the more time-consuming microfluorometric method (AOAC Method 984.26). The procedure outlined below is from AOAC Method 967.21.

15.1.2 Reading Assignment

AOAC International. 2016. *Official Methods of Analysis*, 20th ed., (On-line). Method 976.21, AOAC International, Rockville, MD.

Pegg, R.B., and Eitenmiller, R.R. 2017. Vitamin analysis. Ch. 20, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

15.1.3 Objective

Determine the vitamin C content of various orange juice products using the indicator dye 2,6-dichloroindophenol in a titration method.

15.1.4 Principle of Method

Ascorbic acid reduces the indicator dye to a colorless solution. At the endpoint of titrating an ascorbic acid-containing sample with dye, excess unreduced dye is a rose-pink color in the acid solution. The titer of the dye can be determined using a standard ascorbic acid solution. Food samples in solution then can be titrated with the dye and the volume for the titration used to calculate the ascorbic acid content.

15.1.5 Chemicals

	CAS no.	Hazards
Acetic acid (CH ₃ COOH)	64-19-7	Corrosive
Ascorbic acid	50-81-7	
2,6-Dichloroindophenol (DCIP) (sodium salt)	620-45-1	
Metaphosphoric acid (HPO ₃)	37267-86-0	Corrosive
Sodium bicarbonate (NaHCO ₃)	144-55-8	

15.1.6 Reagents

(**It is recommended that samples be prepared by the laboratory assistant before class.)

- Ascorbic acid standard solution (prepare only at time of use)
 - Accurately weigh (on an analytical balance) approximately 50 mg ascorbic acid [preferably US Pharmacopeia (USP) Ascorbic Acid Reference Standard]. Record this weight. Transfer to a 50-mL volumetric flask. Dilute to volume *immediately before use* with the metaphosphoric acid-acetic acid solution (see below for preparation of this solution).
- Indophenol solution – dye
 - To 50 mL deionized distilled (dd) water in a 150-mL beaker, add and stir to dissolve 42 mg sodium bicarbonate, and then add and stir to dissolve 50 mg 2,6-dichloroindophenol sodium salt. Dilute mixture to 200 mL with dd water. Filter through fluted filter paper into an amber bottle. Close the bottle with a stopper or lid and store refrigerated until used.
- Metaphosphoric acid-acetic acid solution
 - To a 250-mL beaker, add 100 mL dd water then 20 mL acetic acid. Add and stir to dissolve 7.5 g metaphosphoric acid. Dilute mixture to 250 mL with distilled water. Filter through fluted filter paper into a bottle. Close the bottle with a stopper or lid and store refrigerated until used.
- Orange juice samples**
 - Use products processed and packaged in various ways (e.g., canned, reconstituted frozen concentrate, fresh squeezed, not-from-concentrate). Filter juices through cheesecloth to avoid problems with pulp when pipetting. Record from the nutrition label for each product the percent of the Daily Value for vitamin C.

15.1.7 Hazards, Precautions, and Waste Disposal

Preparation of reagents involves corrosives. Use appropriate eye and skin protection. Otherwise, adhere to normal laboratory safety procedures. Waste likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

15.1.8 Supplies

(Used by students)

- Beaker, 250 mL
- 2 Bottles, glass, 200–250 mL, one amber and one clear, both with lids or stoppers
- Buret, 50 or 25 mL

- 9 Erlenmeyer flasks, 50 mL (or 125 mL)
- Fluted filter paper, two pieces
- Funnel, approx. 6–9 cm diameter (to hold filter paper)
- Funnel, approx. 2–3 cm diameter (to fill buret)
- 2 Glass stirring rods
- Graduated cylinder, 25 mL
- Graduated cylinder, 100 mL
- Pipette bulb or pump
- Ring stand
- 3 Spatulas
- Volumetric flask, 50 mL
- Volumetric flask, 200 mL
- Volumetric flask, 250 mL
- 2 Volumetric pipettes, 2 mL
- Volumetric pipette, 5 mL
- Volumetric pipette, 7 mL
- Volumetric pipette, 10 or 20 mL
- Weighing boats or paper

15.1.9 Equipment

- Analytical balance

15.1.10 Notes

The instructor may want to assign one or two types of orange juice samples to each student (or lab group) for analysis, rather than having all students analyze all types of orange juice samples. Quantities of supplies and reagents specified are adequate for each student (or lab group) to standardize the dye and analyze one type of orange juice sample in triplicate.

15.2 PROCEDURE

(Instructions are given for analysis in triplicate.)

15.2.1 Standardization of Dye

1. Pipette 5 mL metaphosphoric acid-acetic acid solution into each of three 50-mL Erlenmeyer flasks.
2. Add 2.0 mL ascorbic acid standard solution to each flask.
3. Using a funnel, fill the buret with the indophenol solution (dye) and record the initial buret reading.
4. Place the Erlenmeyer flask under the tip of the buret. Slowly add indophenol solution to standard ascorbic acid solution until a light but distinct rose-pink color persists for >5 s (takes about 15–17 mL). Swirl the flask as you add the indophenol solution.
5. Note final buret reading and calculate the volume of dye used.
6. Repeat Steps 3–5 for the other two standard samples. Record the initial and final buret

readings and calculate the volume of dye used for each sample.

7. Prepare blanks: Pipette 7.0 mL metaphosphoric acid-acetic acid solution into each of three 50-mL Erlenmeyer flasks. Add to each flask a volume of distilled water approximately equal to the volume of dye used above (i.e., average volume of dye used to titrate three standard samples).
8. Titrate the blanks in the same way as Steps 3–5 above. Record initial and final buret readings for each titration of the blank, and then calculate the volume of dye used.

15.2.2 Analysis of Juice Samples

1. Pipet into each of three 50-mL Erlenmeyer flasks 5 mL metaphosphoric acid-acetic acid solution and 2 mL orange juice.
2. Titrate each sample with the indophenol dye solution (as you did in Sect. 15.2.1, Steps 3–5) until a light but distinct rose-pink color persists for >5 s.
3. Record the initial and final readings and calculate the difference to determine the amount of dye used for each titration.

15.3 DATA AND CALCULATIONS

15.3.1 Data

	Rep	Buret start (mL)	Buret end (mL)	Vol. titrant (mL)
Ascorbic acid standards	1			
	2			
	3			
				$\bar{X} =$
Blank	1			
	2			
	3			
				$\bar{X} =$
Sample	1			
	2			
	3			

15.3.2 Calculations

1. Using the data obtained in standardization of the dye, calculate the titer using the following formula:

$$\text{Titer} = F = \frac{\text{mg ascorbic acid in volume of standard solution titrated**}}{\left(\begin{array}{c} \text{average mL} \\ \text{dye used to} \\ \text{titrate standards} \end{array} \right) - \left(\begin{array}{c} \text{average mL} \\ \text{dye used to} \\ \text{titrate blank} \end{array} \right)}$$

**mg ascorbic acid in volume of standard solution titrated:

$$= (\text{mg of ascorbic acid} / 50\text{mL}) \times 2\text{mL}$$

- Calculate the ascorbic acid content of the juice sample in mg/mL, using the equation that follows and the volume of titrant for each of your replicates. Calculate the mean and standard deviation of the ascorbic acid content for your juice (in mg/mL). Obtain from other lab members the mean ascorbic acid content (in mg/mL) for other types of juice. Use these mean values for each type of juice to express the vitamin C content of the juice samples as milligrams ascorbic acid/100 mL and as milligrams ascorbic acid/8 fl. oz. (29.56 mL/fl. oz.).

$$\text{mg ascorbic acid/mL} = (X - B) \times (F / E) \times (V / Y)$$

where:

X = mL for sample titration

B = average mL for sample blank titration

F = titer of dye

(= mg ascorbic acid equivalent to 1.0 mL indophenol standard solution)

E = mL assayed (=2 mL)

V = volume of initial assay solution (=7 mL)

Y = volume of sample aliquot titrated (=7 mL)

Ascorbic acid (AA) content for replicates of orange juice sample:

Replicate	mg AA/ml
1	
2	
3	
	\bar{X} =
	SD =

Summary of ascorbic acid (AA) content of orange juice samples:

Sample identity	mg AA/mL	mg AA/100 mL	mg AA/8 fl. oz.
1			
2			
3			
4			

Example calculation:

Weight of ascorbic acid used = 50.2 mg

Average volume of titrant used:

Ascorbic acid standards = 15.5 mL

Blanks = 0.10 mL

Volume of titrant used for orange juice sample = 7.1 mL

$$\text{Titer} = F = \frac{[(50.2\text{mg} / 50\text{ mL}) \times 2\text{mL}]}{(15.5\text{mL} - 0.10\text{ mL})}$$

$$= 0.130\text{mg} / \text{mL}$$

$$\text{mg ascorbic acid} / \text{mL} = (7.1\text{mL} - 0.10\text{ mL})$$

$$\times (0.130\text{mg} / 2\text{mL}) \times (7\text{ mL} / 7\text{mL})$$

$$= 0.455\text{mg} / \text{mL}$$

$$0.455\text{mg} / \text{mL} = 45.4\text{mg} / 100\text{mL}$$

$$0.455\text{mg ascorbic acid} / \text{mL juice} \times 29.56\text{ mL} / \text{fl.oz.} \times 8\text{fl.oz.}$$

$$= 107.6\text{mL ascorbic acid} / 8\text{fl.oz.}$$

15.4 QUESTIONS

- By comparing results obtained for various orange juice products, did heat and/or oxygen exposure during processing and storage of the samples analyzed seem to affect the vitamin C content?
- How do results you have available for the juice samples analyzed compare to (1) values listed on the nutrition label for the same juice product and (2) values in the US Department of Agriculture Nutrient Database for Standard Reference (web address: <http://ndb.nal.usda.gov/>)? For the nutrition label values, convert percent of Daily Value to mg/8 fl. oz., given that the Daily Value for vitamin C is 90 mg. Why might the vitamin C content determined for a specific orange juice product not match the value as calculated from percent of Daily Value on the nutrition label?

Ascorbic acid content of orange juices (mg AA/8 fl. oz.):

Sample identity	Lab values	USDA database	Nutrition label
1			
2			
3			
4			

- Why was it necessary to standardize the indophenol solution?
- Why was it necessary to titrate blank samples?
- Why might the vitamin C content as determined by this method be underestimated in the case of the heat-processed juice samples?

RESOURCE MATERIALS

AOAC International (2016) Official methods of analysis, 20th edn. (On-line). AOAC International, Rockville, MD

Pegg RB, Eitenmiller RR (2017) Vitamin analysis. Ch. 20. In: Nielsen SS (ed) Food analysis, 5th edn. Springer, New York