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Kinetic Spectrophotometric Determination of Ascorbic Acid by Using Phenanthroline Agent in *Eruca sativa*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Ascorbic acid is used for treatment in hypovitaminosis, nutritional deficiency, scurvy, gingivitis, Barlow and Basedow diseases. In this study, a simple and sensitive kinetic spectrophotometric method was developed for the determination of ascorbic acid in *Eruca sativa*. The rate of formation of the product formed as a result of the reaction of ascorbic acid with iron (III) sulfate and phenanthroline reagents was determined by measuring the absorbance at 510 nm wavelength at 5 minute intervals for 60 minutes. The measurement curve prepared from standard ascorbic acid solution is linear in the concentration range of 4.0-6.0 μg/mL. The regression equation obtained from the graph of logarithms of molar concentrations versus logarithms of slopes was found as log (rate) = 2.5721 logC - 9.461 (r=0.9999) according to the initial rate method. The developed method was applied to *Eruca sativa* samples and the amount of ascorbic acid in fresh leaves was found to be 72.80 mg/100 g.

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Keywords: Kinetic spectrophotometry; ascorbic acid; phenanthroline; Eruca sativa.

1. INTRODUCTION

Ascorbic acid (vitamin C), one of the water-soluble vitamins, is the vitamin that the organism most needs (Fig. 1). It was extracted from the adrenal glands, as well as green peppers and cabbage by Szent-György between 1928 and 1932, and named "hexuronic acid". L-ascorbic acid is a very strong monoacid. The pH of its solution at 1% concentration is 2.8, and the pH of 10% solution is 2. In addition, it is a colorless, odorless substance that dissolves at 190 - 192 ^{o} C, dissolves in 1 g in 3 mL, and tastes sour like citric acid. The specific optical conversion in water is [α]D20 = +23^{o}. Very soluble in alcohol, slightly soluble in acetone, insoluble in benzene, ether, chloroform and oils. On the other hand, ascorbic acid has the ability to retain oxygen and is an important antioxidant for foods with properties such as discoloration and loss of natural odor. Only humans, monkeys and guinea pigs cannot make ascorbic acid in their bodies and they have to take it from outside. This is because; Although they have three of the four enzymes required for the synthesis of ascorbic acid, they do not have the enzyme L-gulonolactant oxidase. In ascorbic acid deficiency, the body's resistance to microbe diseases decreases and the complete deficiency of the vitamin causes scurvy, bleeding occurs first on the gums and then on the skin. It is an essential vitamin for tissue respiration [1].

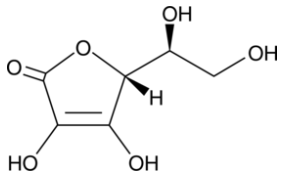

Fig. 1. Chemical formula of ascorbic acid

Ascorbic acid; It is used in many medicines, for enriching foods and beverages with vitamins. The daily need for vitamin C varies between 75 mg for men, 70 mg for women, and 30 to 100 mg for children according to their age. 1 international unit of vitamin C (M.U.) is 0.05 mg of pure ascorbic acid. The amounts of foods containing 1 mg of vitamin C, corresponding to 20 MU, are as follows: 1.8 g strawberries, 24 g apples, 5 g tomatoes, 33 g cucumbers, 4.1 g spinach, 0.6 g red peppers, 2 g cauliflower, 28 g women milk, 5.2 g asparagus, 10–80 g apricot, 27 g horseradish, 12 g cherries, 18 g melon, 4.8 g liver, 0.7 g parsley, 10 g banana, 2.3 g lemon, 2.3 g orange, 3 g fresh corn, 80 g milk, 15 g onions, 14 g lettuce, 12.5 g green beans, 8 g potatoes and 28.5 g grapes [2].

Quantification of ascorbic acid in various vegetables and fruits was made by spectrophotometric [3,4], flow injection chemiluminescence [5,6], high performance liquid chromatographic [7-9], electrophoretic [10] and electrochemical [11-13] methods. In addition, ascorbic acid determination was performed kinetically in tomatoes, green peppers and oranges [14] by kinetic spectrofluorimetric method and in various vegetables by electrokinetic capillary chromatography method [15].

In the literature review, few studies were found in which the kinetic spectrophotometric method was used for the determination of the amount of ascorbic acid in fruits and vegetables, and in these studies, the amount of ascorbic acid was determined in melon, watermelon, parsley, potato and coriander [16,17]. However, according to the literature, the amount of ascorbic acid in the arugula (*Eruca sativa*) plant, whose fresh leaves are used as salad, has been determined by some analytical methods [18,19], but it has not yet been determined by the kinetic spectrophotometric method. Therefore, we aimed to develop a simple and sensitive kinetic spectrophotometric method for the quantification of ascorbic acid in fresh arugula leaves.

2. MATERIALS AND METHODS

2.1 Chemicals and Samples

Ascorbic acid was obtained from Deva (Istanbul, Turkey) pharmaceutical company, $Fe_2(SO_4)_3$, phenanthroline, metaphosphoric acid and acetic acid were obtained from Merck (Darmstadt, Germany). Fresh arugula leaves were purchased commercially from a grocery store.

2.2 Instrument

Glass and quartz cuvettes were used with Shimadzu UV-visible spectrophotometer (Tokyo, Japan) device for measurements.

2.3 Standard Solutions

The stock solution of ascorbic acid at a concentration of 1 mg/mL was prepared in 3%

metaphosphoric acid solution (weight/volume) containing 8% CH_3COOH. To prepare standard solutions with a concentration of 20.0, 22.5, 25.0, 27.5, 30.0 µg/mL from the stock solution, appropriate amounts were taken and diluted with the same solvent.

2.4 Procedure

1 mL of each of the standard solutions was taken and 2 mL of 0.010 M $Fe_2(SO_4)_3$ solution and 2 mL of 0.010 M phenanthroline solution were added, and the final concentrations were adjusted as 4.0, 4.5, 5.0, 5.5 and 6.0 µg/mL (linear concentration range). Absorbance was measured at 510 nm for 60 min at 5 min intervals (n=6).

2.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The smallest value of the calibration curve was accepted as LOQ value and the LOD concentration was calculated by dividing this value by 3.3. Then the LOD solution was prepared and measured.

2.6 Accuracy and Precision

Recovery of the method was evaluated at three different concentration levels. The precision was evaluated in terms of repeatability by performing intraday and inter day analysis.

2.7 Sample Assay

For the determination of ascorbic acid in arugula leaves, 5 g of fresh arugula leaves were chopped, mixed with 50 mL of 3% metaphosphoric acid solution containing 8% CH_3COOH in a magnetic stirrer for 45 minutes and filtered with blue banded filter paper. 350 µL of filtrate was diluted to 1 mL with the same solvent, then 2 mL of 0.010 M $Fe_2(SO_4)_3$ solution and 2 mL of 0.010 M phenanthroline solution were added. Absorbance was measured at 510 nm for 60 min at 5 min intervals.

3. RESULTS AND DISCUSSION

The solution of ascorbic acid in water showed maximum absorption at 264 nm. The UV spectrum is given in Fig. 2. The product formed as a result of the reaction of ascorbic acid with $Fe_2(SO_4)_3$ and phenanthroline showed maximum absorption at 510 nm. The UV spectrum of the product is given in Fig. 3.

Ascorbic acid reduced Fe(III) to Fe(II) and Fe(II) formed a colored compound with the phenanthroline indicator, and the increase in absorbance lasted for 60 min. The graph of absorbance versus time is given in Fig. 4. In order to obtain a highly accurate graph, it was considered sufficient to make the measurements every 5 minutes, and the absorbances of the solutions at 4.0, 4.5, 5.0, 5.5 and 6.0 µg/mL concentrations (linear range) were measured at 5 minute intervals for 60 minutes. Absorbance graphs against time were obtained from the measurements of each solution, and the slopes of the linear parts of the graphs were calculated. The regression equation obtained from the graph of logarithms of molar concentrations versus logarithms of slopes (Fig. 5) was found to be:

log (rate) = 2.5721 logC - 9.461 (r=0.9999)
by using the initial rate method [20].

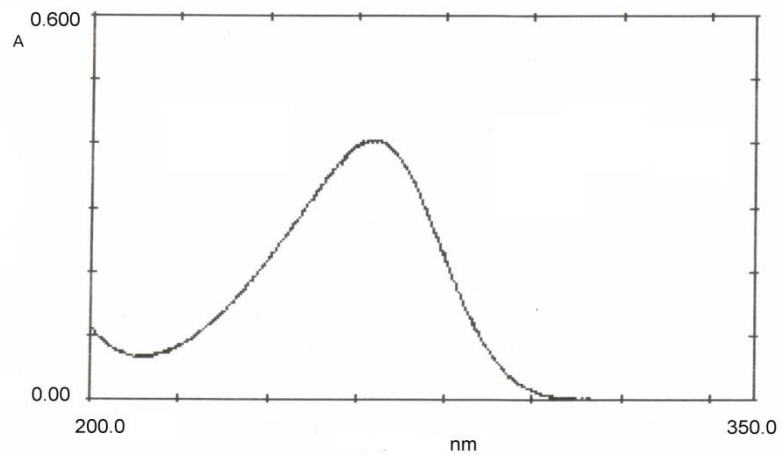

Fig. 2. UV-absorption spectrum of ascorbic acid (1 mg/mL)

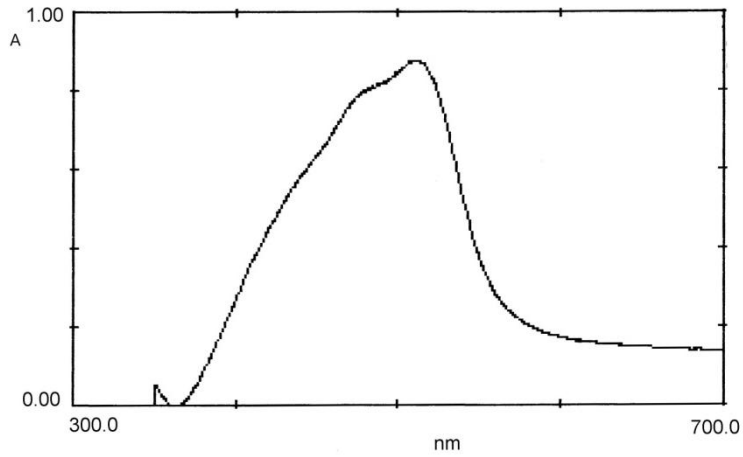

Fig. 3. UV-absorption spectrum of the product formed as a result of the reaction of ascorbic acid with $Fe_2(SO_4)_3$ and phenanthroline (4 µg/mL)

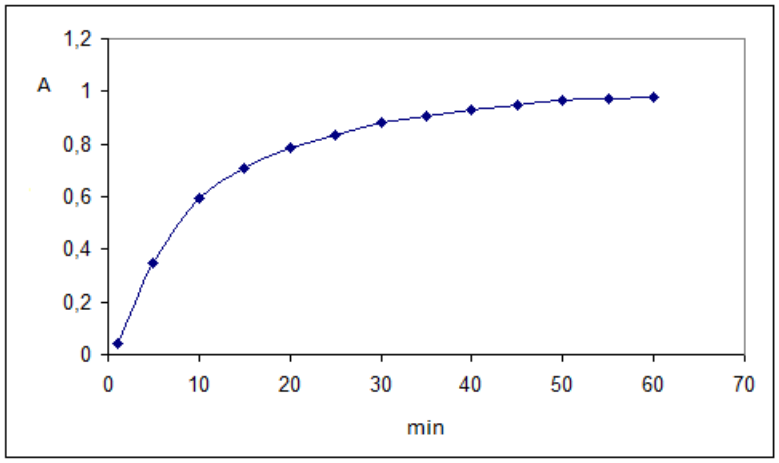

Fig. 4. The plot of absorbance versus time of the reaction of ascorbic acid with $Fe_2(SO_4)_3$ and phenanthroline (5 µg/mL)

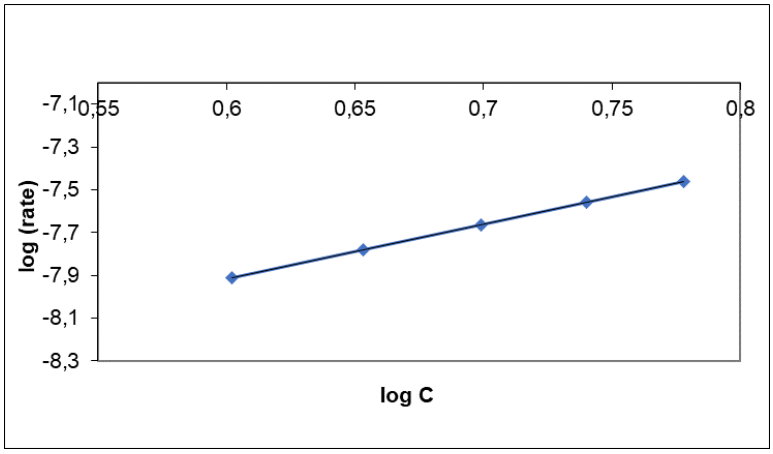

Fig. 5. Calibration graph for the initial rate method

The limit of quantification (LOQ) was accepted as 4.0 mg/mL from the calibration curve and the limit of detection (LOD) was calculated as1.2 mg/mL. In accuracy and precision studies RSD% values were found below 2.0.

The highest efficiency in the extraction of ascorbic acid from arugula leaves was obtained with 3% metaphosphoric acid solution containing 8% CH_3COOH by stirring for 45 minutes after experimenting with mixing times of 15, 30, 45 and 60 minutes The developed method was applied to fresh arugula leaves. It was determined that 100 grams of fresh leaves contain 72.80 mg of ascorbic acid. Standard deviation (±0.05) and relative standard deviation (± % 0.06) were also calculated.

4. CONCLUSION

Ascorbic acid was determined by a simple and sensitive kinetic spectrophotometric method in fresh arugula leaves for the first time in the literature. In the continuation of the study, the amount of ascorbic acid in some other vegetables will also be determined with this developed and validated method.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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