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Determination of Ascorbic Acid in Vitamin C (Tablets) by High-Performance Liquid Chromatography

MOHAMED H.S. AHMIDA
Department of Nutrition, Faculty of Public Heath, Al-Arab Medical University, Benghazi, Libya
E-mail: hamzasol@yahoo.com

The objective of the present study is to investigate suitable technique for the determination of ascorbic acid. A sensitive HPLC-UV method is developed for fast and simple quantification of ascorbic acid (vitamin C) in different vitamin C supplements. The method use a Haisil C_{18} column and for the stationary phase. HPLC grade water is brought to pH 2.2 with sulphuric acid: methanol (80:20) for the mobile phase. The flow rate is 1.0 mL/min and UV detection at 243 nm. The limit of detection is estimated at 0.42 ppm. The method showed precision values (relative standard deviation, RSD %) at 1.79 % for effervecense tablet and 2.19 % for conventional tablet. The results obtained are in good agreement with those found by the AOAC official titrimetry method, the accuracy (relative error, RE %) are 1.67 % and to 1.41 % for effervescence and conventional tablets, respectively.

Key Words: Ascorbic acid, Vitamin C, RP-HPLC, Pharmaceutical formulation, Effervescence tablets, Conventional tablets.

INTRODUCTION

Antioxidants play a vital role in scavenging active oxygen species and protect the cells from oxidative damage. Ascorbic acid (vitamin C, Fig. 1) is a primary antioxidant. It acts as cofactor in the hydroxylation of collagen and has a pH regulator properties. It is considered as useful clinical parameter related cardiovascular disease and cancer incidence[1-10]. The importance of ascorbic acid in medicine and food science, is the basis of our interest in determination of ascorbic acid. There are several analytical methods for ascorbic acid quantification based mostly on its antioxidant characteristics[11]. The most common method for determination of ascorbic acid is the oxidative titration with 2,6-dichlorophenol-indophenol[12]. It is a rapid method, but its application is difficult in coloured solutions, due to interference of other oxidizing agents[13]. The high-performance liquid chromatography (HPLC) technique deserves an increasing interest mainly due to its rapidity, selectivity and specificity proprieties and required less sample preparation[14,15]. Hence, the present study describes a quick highly sensitive method for quantification of vitamin C in different vitamin C supplements using isocratic reversed phase liquid chromatography.

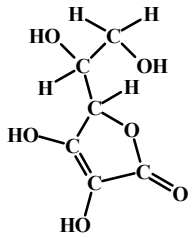

Fig. 1. Ascorbic acid

EXPERIMENTAL

L-ascorbic acid standard was obtained from E. Merck, Darmstadt, Germany, sulfuric acid and methanol, from Sigma, Saint Louis, MO, USA. Water was purified by reverse osmosis and then passed through charcoal and deionizing filters.

Tablets containing ascorbic acid: Vitamin C® conventional tablets and vitascorbol® Effervescent tablets, all labeled to contain 500 mg each were obtained from commercial sources.

Five vitamin C tablets were weighed and pulverized to fine powder, then transfer an accurately weighed amount of the powder equivalent to 40 mg ascorbic acid into a 100 mL volumetric flask and dissolved in the solvent prepared previously (80 % sulfuric acid buffer and 20 % methanol) at pH 2.2 to yield ascorbic acid concentration of 400 ppm. An aliquot of the solution was filtered through a 0.45 µm Gelman filter before dilution by 1: 20 and analyzed immediately.

The HPLC system consisted of a cecil dual piston CE 4100 pump, a variable wavelength UV CE 4201 detector (set at 243 nm), Haisil C_{18} 100 Å column (25 cm × 4.6 mm i.d.) (Higgins Analytical Lnc). Injections of 20 µL were made and vitamin C was eluted isocratically at room temperature in 3.18 ± 0.20 min at a flow rate of 1.0 mL/min with a solvent consisting of HPLC grade water is brought to pH 2.2 with sulphuric acid:methanol (80:20) (pH 2.2). The mobile phase was filtered by passing through a 0.22 µm membrane filter (Millipore, Bradford, MA, USA). The mobile phase was degassed by pumping pure helium gas into the solvent reservoir.

Ascorbic acid concentration in an extract was determined by comparing the area corresponding to the ascorbic acid of the extract with the area-concentration relationship from a standard curve prepared with pure ascorbic acid,. The content of the tablets is calculated from the calibration curve or using the corresponding regression equation.

RESULTS AND DISCUSSION

Fig. 2 shows the chromatogram of the standard solution of ascorbic acid under the described chromatographic conditions. This chromatogram revealed that ascorbic acid gave well-defined peak at retention time t_R 3.18 min. The mobile phase was chosen after several trials with various proportions of methanol at different pH values using H_2SO_4.

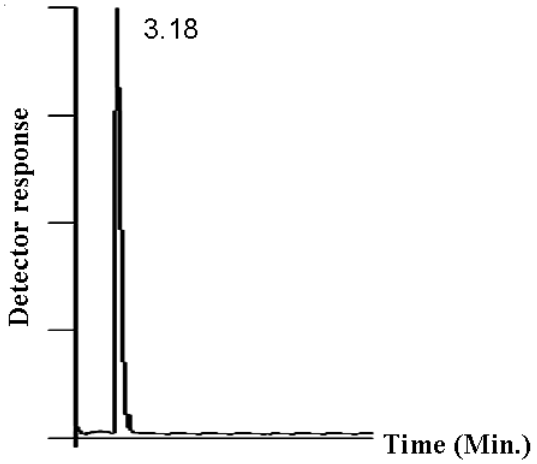

Fig. 2. Typical chromatogram of ascorbic acid (20 ppm) under the HPLC-UV described conditions, A series of standard solutions were prepared from the stock solution of ascorbic acid

A calibration graph of ascorbic acid concentration against area under the peak is plotted as shown in Fig. 3. The standard curve was linear over the range from 1.00 ppm to 80.00 ppm. A typical regression analysis of this plot resulted in a calibration equation a = 2.4C + 0.68, where a = area under the peak, C = concentration of ascorbic acid in ppm with a correlation coefficient of (R^2) 0.999.

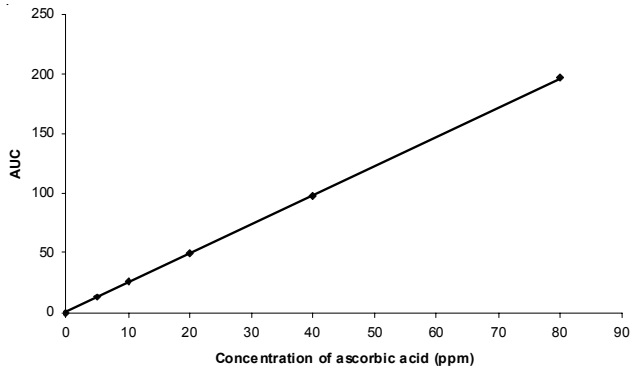

Fig. 3. Calibration curve of ascorbic acid, the linear regression equation a = 2.4C + 0.68 (R^2 = 0.999)

Limit of detection (LOD) for this method was determined from repeated analyses of standard ascorbic acid solution at low concentrations. The lowest concentration could be measured and reported with 99 % confidence (three S.D.) was taken as the limit of detection. The LOD was found 0.42 ppm. Limit of quantification (LOQ) was calculated as 10 times the standard deviation of replicates. The limit of quantification was found 1.40 ppm[16,17].

The proposed method is applied for the determination of ascorbic acid in conventional and effervescence tablets. To assess the repeatability of the method, a single concentration of ascorbic acid in tablets (20 ppm) was prepared and analyzed for 6 different days (five replicate each day). Then the precision was determined by calculating the relative standard deviation (RSD %) for the replicate measurements and accuracy was calculated by assessing the agreement between the amount of ascorbic acid measured by the proposed HPLC method and AOAC method[12] (relative error RE %). These values are reported in Table-1.

TABLE-1
ASCORBIC ACID CONCENTRATION IN PHARMACEUTICAL FORMULATION AS DETERMINED BY THE PROPOSED AND REFERENCE (AOAC) METHODS

Samples Nominal amount (mg tablet^{-1})	Amount found (mg tablet^{-1})	
	Proposed method	AOAC[12]
Effervecense tablet (500)		
Mean ± SD	502[a] ± 9	494[b] ± 12
RSD %	1.79	2.43
RE %[3]	1.67	
Recovery (%)	100.4	98.8
Conventional tablet (500)		
Mean ± SD	503[a] ± 11	496[b] ± 11
RSD %	2.19	2.22
RE %	1.41	
Recovery (%)[c]	100.6	99.2

[a]Mean = average of 30 values (5 replicate per day, each replicate was analyzed for 6 different days); [b]Mean = average of six values, [c]Recovery (%) = [Amount found by proposed method/ Amount found by official method] × 100.

Conclusion

A rapid, precise and selective HPLC-UV method using a single isocratic system is developed for the determination of ascorbic acid in conventional and effervecense tablets. The method is suitable for the quantification of ascorbic acid in pharmaceutical preparation. Present results suggest that a simple sample treatments enable using of this method for routine determination of ascorbic acid in other different pharmaceutical dosage forms (such as syrup and injections) and food products (tomato paste) with good accuracy.

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