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Chemical Evaluation of *Rosa canina* Fruit to Determine Ascorbic Acid Content

ELHAM BAHER, NASER MONTAZERI*, ZAHRA BARAMI and KHALIL PURSHAMSIAN

Department of Chemistry, Tonekabon Branch, Islamic Azad University, Tonekabon (Iran).
*Corresponding author: E-mail:montazer50@toniau.ac.ir

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ABSTRACT

In this study, the fruits of rose hip (*Rosa canina L*.) were assayed for the composition of ascorbic acid (AA); the method of high performance liquid chromatography (HPLC) was used for detecting of ascorbic acid. The results show that the Ascorbic acid content was 447.132 mg per 100 g^{-1} fresh fruit. Since it is well known that ascorbic acid is a sensitive and thermo-labile compound, and its extraction with high yield and without any decomposition has been a matter of difficulty and labor-intensive for the analysts, so the percolation method that was used in this project, because of coefficient time to reach equilibrium and without having any heating process, has efficient mass transfer from marc. Due to accepted recovery of the method (99±2% and the RSD for AA 1.92%.), it seems that this method of AA determination is suitable for quantitative analysis of AA in dog rose and other similar samples.

Key words: Rose hip (*Rosa canina L*.), Ascorbic acid (AA), High performance liquid chromatography (HPLC), Percolator, Propylene glycol, Calibration curve plot.

INTRODUCTION

Rosa canina (commonly known as the dog rose) is a variable scrambling rose species native to Europe, Asia, the Middle East and North America[1]. The dried fruit was used in folk medicine for digestive problems, urinary tract and kidney disorders, rheumatism, gout, colds, and febrile conditions[2-6]. The fruit is noted for its high vitamin C level and is used to make syrup, tea and marmalade[7]. It has been grown or encouraged in the wild for the production of vitaminC, from its fruit (often as rose-hip syrup), especially during conditions of scarcity or during wartime.

The ascorbic acid (vitamin C) is an essential nutrient, involved in the production of certain substances that allow the transmission of nervous influx, and the functions that facilitate the Fe absorption at the digestive tract level[8].

Several methods have been developed for the estimation of ascorbic acid levels in different samples. Nowadays, high-performance liquid chromatography (HPLC) [9-11], with various detection methods has been the most used technique for the analysis of ascorbic acid in different samples. The amount of ascorbic acid has been reported in dog

rose samples using conventional extraction method[12,13].

The purpose of this work, is ascorbic acid content evaluation of R. canina fruit and pericarps

MATERIAL AND METHODS

Chemically materials

L-ascorbic acid, acetonitrile and orthophosphoric acid, (HPLC-Grade) that were bought from Merck (Darmastdt, Germany). Milli-Q system (Millipore, Bedford, USA) was used.

Plant materials

Dog rose (R. canina L.) fully ripeness fruits were collected from the kandelus mountains (Noshahr, Iran) in September of 2010. The fruit were dried in dark and dry place at room temperature.

Instrumentation

The HPLC system consists of Waters liquid chromatography (Milford, MA, USA) equipped with a 600E multisolvent delivery system, an in-line degasser, a manual injection with 20 mL loop (Rheodyne 7125), and Waters 2487 dual l absorbance detector.

Extraction with percolation method

In this method, a percolator (a narrow, cone-shaped vessel open at both ends) was generally used. The powdered material was fed into the percolator along with a suitable solvent (propylene glycol). The material was left in contact with the solvent for 8-10 hour until equilibrium of the active principle was achieved. The solvent extract, known as *miscella*, was taken out from the bottom discharge valve of the percolator. Fresh solvent was added into the percolator and the miscella was drained out after acquiring equilibrium. Overall, the plant material was washed four to five times until it gets exhausted. All washes from the percolator are pooled and concentrated.

A circulation pump that continuously circulates the miscella back to the top of the percolator gives a better mass transfer rate and reduces the equilibrium time considerably.

HPLC analysis

The HPLC method used for the determination of AA consisted of an isocratic elution procedure with UV -Visible detection at 245 nm. Separations were carried out on a 5 mm RP C18 column of 250mm–4.6mm (Spherical, Optimals ODS-H, Capital HPLC) fitted with a 5 mm RP C18 guard column of 20mm–4.6mm (Spherical, Optimals ODS-H, Capital HPLC, UK). The mobile phase employed was a mixture of 0.5% NaH_2PO_4 (pH 2.25 with H_3PO_4)-acetonitrile (93:7). Flow rate of the mobile phase was 1.2mLmin_1 and an injection volume of 20 mL was used in quantitative analysis.

The temperature of analytical column was kept constant at 25 ºC. The calibration curve and quantitative evaluations were accomplished at 245 nm. Standard solutions and extracts were filtered through a prefilter and then a 0.45 mm millipore membrane before their injection. To prevent the loss of AA, standard solutions and extracted samples were protected from light using amber flasks.

Quantitation was performed by comparing the chromatographic peak area with that of the external standard. The calibration curve was plotted in the concentration range of 0.1–500 mg L^{-1} and based on a 12-point calibration.

RESULTS AND DISCUSSION

Standard solutions

For purpose of AA calibration curve plot (the AA peak area against the AA concentration) 12 levels of standard AA concentrations were considered. The response of AA over a concentration range of 0.1–500 mg L^{-1} was linear (Y = 61.003X+1.596) with a regression coefficient (R2) of 0.999. The limit of quantitation (LOQ) and detection (LOD) were 0.10 and 0.11 mg L^{-1}, respectively, the RSD values for repeatability (n = 4) were 0.72%.

Quantification of AA in samples

According to the results, the AA content was obtained 447.132 mg per 100 g_{-1} fresh weight for this perculating method. Ascorbic acid of different rose species was reported to amount to 106–2712 mg/100 g in the studies conducted in different agro-

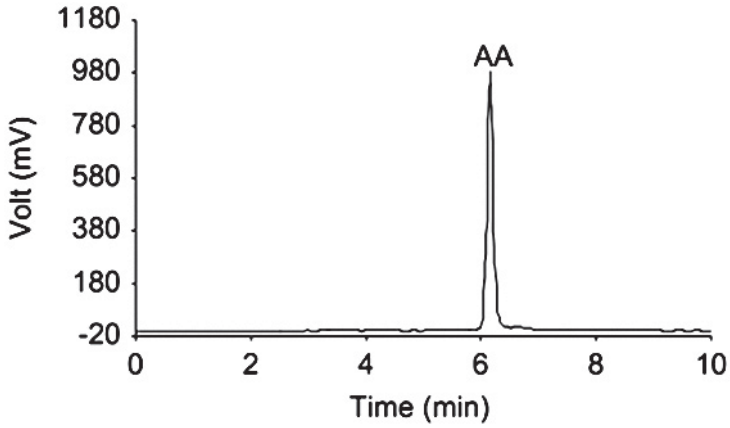

Fig. 1. Typical Chromatograms obtained at 245 nm HPLC condition: how rate 1.2mLmin^{-1}, isocratic, mobile phase was a mixture of phosphate buffer (0.5%)-acetonitril (93:7). Ascorbic acid retention time (6.20 min)

climatic regions of Turkey[14-17]. The differences in ascorbic acid contents might result from the variations in altitude, species, variety, ecological factors, and harvest time[18,19]. It is well known that ascorbic acid is a sensitive and thermo-labile compound, so its extraction with high yield and without any decomposition has been a matter of difficulty and labor-intensive for the analysts. This type of percolation method for sample preparation, because of coefficient time to reach equilibrium and without any heating, has efficient mass transfer from marc The ascorbic acid amount found in this study for fully ripe dog rose was higher than that obtained by Bozan for dog rose (48–114.3 mg per 100 g), The difference in type of samples, grinding of samples, maturity of samples, and other such conditions can cause different results between our laboratory and the Bozan laboratory. The extract was not contained interfering substances. Fig. 1 shows the chromatograms for fully ripe dog rose extract that obtained by perculating sample preparation procedure. The obtained chromatograms reveal the absence of interfering substances.

In order to evaluate the matrix effect on the accuracy of analysis, a recovery test was carried out. AA was added to dog rose samples at two different concentration levels (200 and 500 mg of AA) and analyzed in triplicate using the two sample preparation methods. The mean recovery for AA with this preparation method was 99±2% and the RSD for AA 1.92%. Due to accepted recovery of the method, it seems that this method of AA determination is suitable for quantitative analysis of AA in dog rose and other similar samples.

CONCLUSIONS

If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. Since the ascorbic acid is a sensitive and thermo-labile compound, so its extraction with high yield and without any decomposition has been a problem for the analysts, This type of percolation method without any decomposition were appropriated for this analysis. Also the proposed LC method has good resolution from other contaminants or the solvent peak, and this proposed method can be used to quantify vitamin C in another variety of fruits In conclusion, it would be possible to derive benefit from rose hip fruits, in food and food additive sectors, as well as from using their flowers for the production of rose oil, rose water, rose concrete, and rose absolute.

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