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UPLC Rapid Quantification of Ascorbic Acid in Several Fruits and Vegetables Extracted using Different Solvents

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Abstract

Ascorbic acid or vitamin C is mostly found in natural products such as fruits and vegetables. Ultra performance liquid chromatography (UPLC) method has been developed to compare the ascorbic acid content in some fresh fruits (apple, orange) and vegetables (carrot, beet, cherry tomato) extracts with two different extraction solvents; i) 9% metaphosphoric acid, ii) 3% citric acid.

The compound has been detected and quantified by the use of UPLC equipped with Photodiode Array (PDA) detector.

The amount of ascorbic acid detected in fruits and vegetables extracts prepared with the two solvents mentioned was different. For the orange extracted using citric acid 3% recorded the highest concentration of ascorbic acid (38.2 mg/100 g FW) higher than with 9% metaphosphoric acid (33.3 mg/100g FW), respectively the extractions of both varieties of apples, carrot, beet and cherry tomato with metaphosphoric extraction solvent recorded the highest values of ascorbic acid: 4.1 mg/100g FW in case of 'Golden', 9.92 mg/100g FW for carrot and 13.56 mg/100g FW for beet.

The results showed that ascorbic acid content was higher by extraction with 9% metaphosphoric acid as compared, by extraction with 3% citric acid.

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Keywords: UPLC, Ascorbic acid, Extraction solvent, Metaphosphoric acid, Citric acid.

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1. Introduction

Vitamin C (ascorbic acid, ascorbate, AA) is a water soluble organic compound involved in many biological processesand is the main vitamin synthesized by plants. Biosynthesis of L-ascorbic acid takes place in the plant tissues as a series of photochemical reactions starting from D-glucose or D-galactose. This process is conducted in mitochondria and partly in microsomal fractions. The content of ascorbic acid (AA) in fruits and vegetables varies depending on the species, variety and agro-pedo-climatic conditions between 3.0 mg/100 g (peanut) and 139 mg/100 g (pepper) (Bădulescu, 2010).

Analysis of AA from specific plant tissue types needs great caution in the use of methods that have been developed (Davey et al., 2000), because AA is oxidized under alkaline conditions. The use of a high ionic strength, acidic extraction solvent is required to block metabolic activity upon disruption of the cell and to precipitate proteins (Fatariah et al., 2015).

L-Ascorbic acid, also known as vitamin C (C_6H_8O) is widely distributed in nature, typically rich in fresh fruits and leafy vegetables such as guava, mango, papaya, cabbage, mustard leaves and spinach (Tee et al., 1997) (Figure 1).

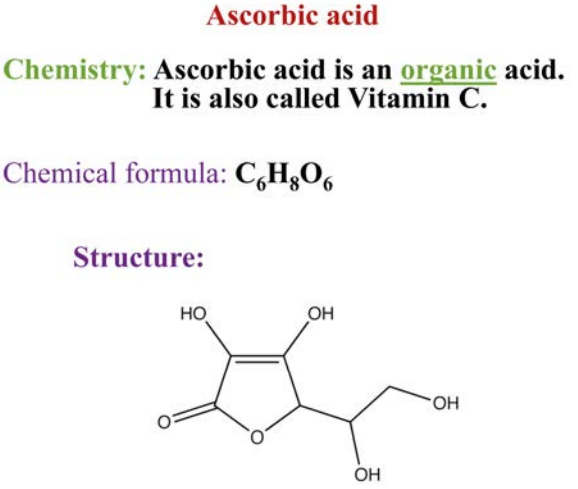

Figure 1. Chemical structure of ascorbic acid

AA is found naturally in a wide range of plants. The human body does not produce vitamin C, therefore the only source of vitamin C for humans is the food based on fruits and vegetables (Pârvu, 2006). As a participant in hydroxylation, the ascorbic acid is necessary for the production of collagen in the connective tissue. These fibres are present throughout the body, providing a stable but flexible structure (Burzo et al., 1999).

There are several methods used to determine AA, one classic method is based on the reduction of the blue dye *2,6 dichlorophenolindophenol* by ascorbic acid (AOAC, 1999). The analysis of AA by Ultra Performance Liquid Chromatography (UPLC), which is a relatively new technique giving new possibilities in liquid chromatography, especially concerning decrease of time and solvent consumption; UPLC chromatographic system is designed in a special way to withstand high system back-pressures (Nováková et al., 2006).

UPLC allows the determination of ascorbic acid in an easy, fast and precise method. UPLC is considered a sensitive, selective and rapid method and therefore suitable for active substance determination; it is also suitable for the evaluation of stability in formulations in the pharmaceutical and cosmetic industries (Marshall et al., 1995). In addition, as pointed in a previous study (Fatariah et al., 2015), liquid chromatography can avoid the problem of non-specific interference and ion-pair (Ke et al., 1994) NH_2 bonded-phase (Silva, 2005) and reverse phase (Franke et al., 2004) techniques. The purpose of this study was to develop a UPLC method for the quantification of ascorbic acid in several fruits and vegetables extracts using different extraction solvents.

2. Materials and methods

2.1. Plant material and experimental design

This research was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest at the Research Centre for the study of quality food products - USAMV Bucharest.

The fruits and vegetables tested included two varieties of apples (*Malus domestica*), orange fruit (*Citrus × sinensis*), cherry tomato (*Solanum lycopersicum* var. *cerasiforme*), red bell pepper (*Capsicum annuum*), carrot (*Daucus carota sub sp. sativus*), lettuce (*Lactuca sativa*), and beet (*Beta vulgaris*).All fruits and vegetable that were tested were brought from the market store.

2.2. Chemicals

Ascorbic acid was purchased from Supelco, USA, metaphosphoric acid and citric acid were purchased from Sigma-Aldrich, Germany.

2.3. Standard preparation

Ascorbic acid (5 mg) was weighed accurately and transferred to 50 ml volumetric flask. The standard was dissolved in 50 ml UPLC grade water to prepare standard stock solution of 50 µg/ml.

2.4. Sample preparation

The technique of extraction was a simulation of the method performed by Giannakourou and Taoukis (2003), with some modifications: fresh fruits and vegetables samples were cut into small pieces. Each 5 g of the fresh fruit-vegetable were homogenized using a mortar adding 15 ml extraction solvent and stored at 4 ºC for 45 min. After extraction time the samples were prefiltered and centrifuged at 7800 rpm for 10 min at 4˚C. Then, the slurry was filtered through Agilent polytetrafluoroethylene (PTFE) filter membrane (0.20 µm pore size) prior to injection into UPLC system (Figure 2). There were two extraction solvents involved in this investigation; i) 9% metaphosphoric acid, ii) 3% citric acid, both of them in triplicates.

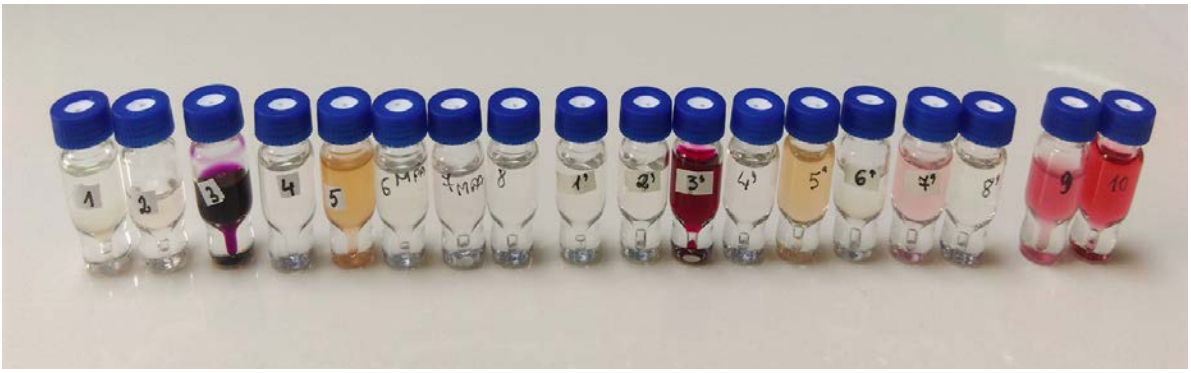

Figure 2. Filtered samples of each fresh fruits and vegetables analysed

2.5. UPLC method preparation

The liquid chromatographic method used for the determination of AA consisted of an isocratic elution procedure. The analyses were carried out on a modular chromatographic system Waters Acquity I Class Ultra

Performance Liquid Chromatography (Waters, USA) equipped with Photodiode Array (PDA) detector and controlled with data acquisition software (Figure 3). The chromatographic system was equipped with an Acquity UPLC HSS T3 1.8µm (2.1 x 100 mm column), using an isocratic mobile phase at a flow rate of 0.45 mL/min. The temperature of the analytical column was kept at 30ºC using a chromatography column oven, while sample temperature was kept at 4°C. Detection wavelength for the UV-visible detector was set at 270 nm. AA peak was identified by comparing its UV-visible spectral characteristics and retention time with a commercial standard of AA. The spectrum (detection wavelengths from 200 to 700 nm) was recorded for the peak identified as AA by retention time, using a visible photodiode array detector. For each sample type, using three replicates, the efficiency of peak separation was checked by the peak purity test carried out at maximum absorbance.

Figure 3. UPLC System Acquity equipped with Photodiode Array (PDA) and fluorescence (FLR) detector

2.6. Data acquisition

Data were acquired and processed using Waters Empower^{TM}2 Software. Results were obtained by comparison with standards and expressed as mg/100 g FW.

2.7. Calibration plots

Solutions of ascorbic acid standard with different concentrations were prepared by dilution of the standard solution. The standard response curve for ascorbic acid was a linear regression at each of five concentrations (0.006 to 0.1 mg/ml). Each solution was chromatographed and the peak areas were measured. Peak areas against the respective concentration for ascorbic acid were then plotted to find the linear range of ascorbic acid.

3. Results and discussions

3.1. Concentrations of ascorbic acid in several fresh fruits and vegetables extracted with two different solvents

The present results showed that the extraction using different solvents recorded different concentrations after quantification was accomplished using UPLC (Table 1).

In the extractions of both varieties of apples, in carrot, beet and cherry tomato the metaphosphoric extraction solvent recorded the highest values of ascorbic acid: 4.1 mg/100g of fresh weight (FW) in case of 'Golden', 9.92 mg/100g FW for carrot and 13.56 mg/100g FW for beet. With some small exceptions the concentration of ascorbic acid using metaphosphoric acid 9% was higher. This indicated that metaphosphoric acid is an efficient solvent in extracting ascorbic acid. Accordingly to Franke et al, 2004, this result gives us the fact that metaphosphoric acid may provide proficient ascorbic acid extraction by preventing oxidation compared to citric acidic solvent.

Table 1. Concentration of ascorbic acid from fresh fruits and vegetables extracts prepared with two different solvents (n = 3).

Samples	Vitamin C mg/100g FW	
	Citric Acid 3%	Metaphosphoric acid 9%
Apple 'Golden'	1.1±0.15	4.1±0.10
Apple 'Jonathan'	1.2±0.17	3.9±0.11
Orange	38.2±0.24	33.3±0.12
Carrot	1.9±0.24	9.92±0.14
Beet	0.3±0.18	13.56±0.9
Lettuce	6.3±0.26	6.72±0.10
Red Bell Pepper	16.19±0.23	15.88±0.15
Cherry tomato	10.02±0.13	16.68±0.08

The orange extracted using citric acid 3% recorded the highest concentration of ascorbic acid (38.2 mg/100 g FW) higher than with 9% metaphosphoric acid (33.3 mg/100g FW), also higher as compared with those determined in the case of the other samples.

These results were slightly different from those reported by Zbynek et al. (2008). Thus, AA amount in oranges (*Citrus aurantium*) varied between 30 and 56 mg/100 g FW and was different in apples (*Malus sp.*), from 11 to 19 mg/100 g FW.

However, comparing the result, the higher values are recorded from adding metaphosphoric acid to the extracting solution and this contributed to ascorbic acid protection during the extraction process, because ascorbic acid is easily oxidized under alkaline conditions. In addition, because the vitamin is easily oxidized during extraction process especially in natural products, adding metaphosphoric acid it was an indispensable measure in the case of vegetable analysis (Campos et al., 2009,)

Fatariah et al. (2015) who quantified ascorbic acid in *Benincasa hispida* fruit samples, revealed the importance of extraction using different solvents. Metaphosphoric acid was recommended as a suitable extraction solvent for ascorbic acid in *B. hispida* fruit, compared to acetic acid and distilled water. Also, Wimalasiri and Wills (1983) have mentioned that auto-oxidation of ascorbic acid by oxygen is greatly decreased by an acidic medium, which is necessary to stabilize ascorbic acid.

From the present study, the mobile phase conditions were optimized, thus, there was no interference from solvent and other compounds. The determination of AA consisted of an isocratic elution procedure was found to be a suitable mobile phase allowing good separation of ascorbic acid at flow rate 0.45 ml/min using Acquity UPLC HSS T3 1.8µm (2.1 x 100 mm column). Under this system, the ascorbic acid in fruits and vegetables extracts and standard was able to be detected. The chromatograms of orange samples using two different solvents are shown in Figure 4.

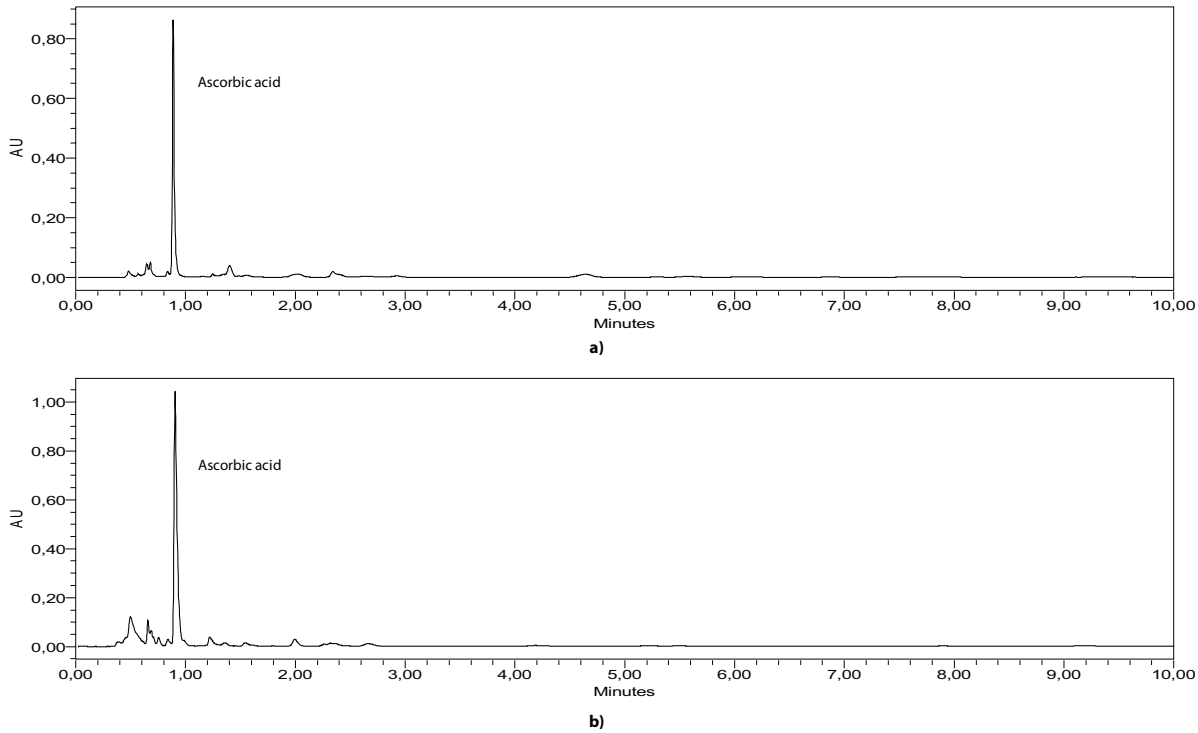

Figure 4. Chromatograms of orange fruit extracts a) with 3% citric acid and b) with 9% metaphosphoric acid

Using this method and this system, as shown from chromatograms, the ascorbic acid was clearly eluted with retention time at 0.885 min and at 0.992 min, respectively.

4. Conclusions

AA is the least stable of all vitamins, being an antioxidant that can be easily oxidized during processing and storage. Quantifying ascorbic acid amount in natural samples, because of its sensitivity, can be difficult, that is why an appropriate extraction solvent may play an important role in determining ascorbic acid.

By using UPLC technique for determination of ascorbic acid in fresh fruits and vegetables extracted with different solvents, we found in case of carrot, both varieties of apples, beet and cherry tomato extractions, that using 9% metaphosphoric extraction solvent were recorded the highest values of ascorbic acid.

The concentration amount of AA analysed using both solvents in the case of red bell pepper samples was appropriate, with comparable results. In the case of orange samples the acid citric 3% solvent generated higher amount of AA, probably due to the already existent content in citric acid in the fruit.

Comparing with CA 3%, MPA 9% used has a better efficiency in extraction results probably due to reduced degradation in processing of the samples. Simultaneously, this difference may be due to UPLC parameters used during analysis, incompatibility of extraction mediums or different types of samples. Ultra Performance Liquid Chromatography coupled with Photodiode Array (PDA) detector is a fast, accurate and suitable analytical instrument for sensitive ascorbic acid determination.

This developed method, focused on fruits and vegetables could be extended to other type of food commodities namely, processed foods, nevertheless a very important aspect should be considered that AA determination of different varieties of fruits and vegetables, cultivated in different technology practices and storage technology, must be taken into consideration the analyse, both sample preparation and determination.

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