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Research Paper / Araştırma Makalesi

Validation of an HPLC-UV Method for Simultaneous Analysis of Ascorbic and Oxalic Acids in Beverages

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ABSTRACT

A reversed-phase high-performance liquid chromatography (RP/HPLC-UV) method was developed for the rapid and simultaneous analysis of ascorbic and oxalic acids in both industrial and freshly squeezed beverages. Due to the rapid degradation of ascorbic acid, its stability was also investigated. The analysis was performed using an Inertsil ODS-3 column with a mobile phase consisting of 0.01 mol L^{-1} KH_2PO_4 phosphate buffer at pH 2.3. Detection wavelengths were set at 245 nm for ascorbic acid and 205 nm for oxalic acid. The limits of detection (LOD) were 1.4 mg L^{-1} for ascorbic acid and 1.3 mg L^{-1} for oxalic acid. After 48 hours of opening an industrial fruit juice package, an 18.5% reduction in ascorbic acid content was found. The method demonstrated within-day repeatability (n = 3) of 4.1% for ascorbic acid and 4.6% for oxalic acid. Between-day precision, expressed as relative standard deviation (RSD), was 3.85% for ascorbic acid and 4.33% for oxalic acid, based on 10 replicates (n= 10). The accuracy of the method was validated with an average recovery rate ranging from 93% to 104%.

Keywords: Liquid chromatography, Ascorbic acid, Oxalic acid, Beverage, Fruit juice

İçeceklerde Askorbik ve Oksalik Asitlerin Eşzamanlı Analizi İçin HPLC-UV Yönteminin Validasyonu

ÖZ

Ters faz yüksek performanslı sıvı kromatografisi (RP/HPLC-UV) yöntemi, endüstriyel ve taze sıkılmış içeceklerde askorbik ve oksalik asitlerin hızlı ve eş zamanlı analizi için geliştirilmiştir. Askorbik asidin hızlı bozulması nedeniyle, stabilitesi de araştırılmıştır. Analiz, pH 2.3'te 0.01 mol L^{-1} KH_2PO_4 fosfat tamponundan oluşan hareketli faz ile Inertsil ODS-3 kolon kullanılarak gerçekleştirilmiştir. Tespit dalga boyları askorbik asit için 245 nm ve oksalik asit için 205 nm olarak ayarlanmıştır. Tespit limitleri (LOD), askorbik asit için 1.4 mg L^{-1} ve oksalik asit için 1.3 mg L^{-1} olarak belirlenmiştir. Endüstriyel meyve suyu paketinin açılmasından 48 saat sonra, askorbik asit içeriğinde %18.5'lik bir azalma tespit edilmiştir. Yöntem, gün içi tekrarlanabilirlik (n= 3) açısından askorbik asit için %4.1 ve oksalik asit için %4.6 oranında bir doğruluk göstermiştir. Günler arası hassasiyet, bağıl standart sapma (RSD) olarak ifade edilmiş ve 10 tekrar (n= 10) temelinde askorbik asit için %3.85 ve oksalik asit için %4.33 olarak hesaplanmıştır. Yöntemin doğruluğu, %93 ile %104 arasında değişen ortalama geri kazanım oranı ile doğrulanmıştır.

Anahtar Kelimeler: Sıvı kromatografisi, Askorbik asit, Oksalik asit, İçecek, Meyve suyu

INTRODUCTION

The importance of a healthy and balanced diet is an undisputed fact of life today. Fresh fruit and fruit juice are one of the most important links in the healthy food chain. Organic acids, natural compounds found mainly in fruits, differ in their additive or preservative content between fresh and industrial fruit juices. Fruit juices are an important source of vitamin C and minerals for humans, and their consumption is increasing as ready-made fruit juices become more widely available. Hence, the detection and determination of organic acids in fruit juices are critical for quality and process control. The concentration of organic acids in fruits are important for the taste, color, aroma, stability, nutritional value, and overall quality of fruit juices.

The amount of organic acids in fruits and vegetables depends on factors such as the type of fruit, soil, and stress conditions to which the fruit is exposed. Fruit juices contain oxalic acid, an antioxidant and weak organic acid, and ascorbic acid, commonly known as vitamin C. Vitamin C (L-ascorbic acid) is a water-soluble vitamin. Fruit juices are an important source of vitamin C for humans and since ready-made fruit juices are easy to obtain, their use is becoming more widespread [1-5]. It protects our immune system and increases our resistance. Ascorbic acid and calcium ascorbates are used as antioxidants in pharmaceutical preparations and the food industry. They are essential for growth and development, as well as playing a significant role in cell renewal, protection, and defense against oxidative stress [6]. However, ascorbic acid is highly sensitive to various deteriorative factors during food processing and storage, making it one of the most vulnerable vitamins [7]. It is easily lost during food processing, storage, and preparation. Due to its sensitivity to processing, ascorbic acid loss is used as a criterion to assess the negative effects of many food processing techniques. Additionally, oxalic acid, being a weak acid, has the tendency to form stable complexes with calcium in the body, leading to the formation of calcium oxalate precipitates. This not only prevents the absorption of calcium in the human body but also easily initiates the formation of bladder stones. Excess oxalic acid can affect human health [8]. Therefore, the determination of the amount of oxalic acid in some samples, such as fruits, etc. has very important practical importance [9].

It is important to identify the organic acids present in fruits and vegetables. Total acidity, microbial stability, freshness, and other sensory and chemical properties of the matrix are all influenced by the amounts and relative ratios of organic acids. Many methods have been published for the determination of organic acids in foods and beverages such as cheese, tomatoes, green beans, carrots, apples, kiwi, blackberries, currants, fruit juices, grape must, and wine. In particular, methods such as spectroscopy [10,11], electrochemical [12-15], spectrofluorimetry [16,17], high-performance liquid chromatography [18,19] and colorimetry [20] were used to determine ascorbic and oxalic acids.

Among these methods, chromatographic methods are mainly based on GC or HPLC separations and the simultaneous determination of organic acid amounts. There are several methods of analysis of fruit and fruit juices based on GC. Although these methods offer excellent separation and sensitivity, they have disadvantages such as being time-consuming, involving derivatization steps, and the use of toxic derivative markers. Additionally, the high temperature needed for these analyses can lead to sample deterioration. On the other hand, the disadvantage of HPLC methods is based on low separation power and high detection limits. Nevertheless, HPLC separations are effective methods for the separation and quantification of organic acids due to its simplicity and better chromatographic conditions [21].

This study aims to establish a direct RP/HPLC method for the simultaneous analysis of ascorbic and oxalic acids in both industrial and freshly squeezed beverages, such as iced tea and fruit juices, under optimized conditions. Additionally, the levels of ascorbic and oxalic acids present in various beverages are profiled and quantified.

MATERIALS and METHODS

Reagents and Samples

HPLC-grade phosphoric acid and sodium dihydrogen phosphate monohydrate were purchased from Merck (Darmstadt, Germany). The standards of ascorbic and oxalic acids were of analytical purity and were purchased from Sigma-Aldrich. Solutions were prepared and diluted using ultra-pure deionized water with a resistance of 18 MΩ·cm that was acquired from an ultra-pure water system (Human Power I plus Water Purification System).

Fruit juices and cold teas were purchased from local markets in Denizli (Türkiye). The samples analyzed were new-production, 100% fruit juices (orange, cherry, pomegranate, apple, apricot, mixed fruit, and cold tea in 1 L TetraPak packages) without any preservatives or added sugars. Natural orange juice samples were obtained from a local market in Denizli, squeezed in the juice apparatus, and analyzed without delay. All samples were run in triplicate.

Preparation of Standard Solutions

To prepare solutions with concentrations of 1000 mg L^{-1} of ascorbic acid and oxalic acid, 0.05 g of solid ascorbic acid and 0.05 g of solid oxalic acid were weighed and dissolved in an aqueous buffer (pH 2.5). These solutions were prepared in 50 mL volumetric flasks. Working standard solutions were prepared by diluting the appropriate volume of stock solutions with buffer at concentrations between 10-500 mg L^{-1} of ascorbic acid and 15-500 mg L^{-1} of oxalic acid. All standard solutions were stored at 4°C.

Sample Preparation

The ready-to-drink fruit juices were directly analyzed. For this purpose, three samples of the same type of fruit juice

(3 L) were homogenized simultaneously. Prior to injection, they were centrifugated at 4,000 rpm for 10 min and filtered through a 0.45 µm membrane filter (Millipore Corporation, France). The mobile phase (2.5 pH buffer solution) was degassed in an ultrasonic bath before use. The prepared fruit juice samples were further diluted 5-fold and then injected into the HPLC (20 µL).

The stability of ascorbic acid concentration over time and the effect of dilution with the mobile phase were also evaluated. For this purpose, the same juice sample was analyzed every 15 min for up to 90 min to detect changes in ascorbic acid concentration.

Instrument and Chromatographic Conditions

A Hettich EBA 20 centrifuge was used for centrifugation of the sample before injection. An ultrasonic bath from Bandelin Sonarex (Berlin, Germany) was used to remove dissolved oxygen from the mobile phase. Chromatographic separations were performed using an HPLC system from Shimadzu (Kyoto, Japan) equipped with the following: LC-20AD pump, SPD-M20A photodiode array detector (DAD), SIL-20A automatic sampler, CTO-20A column oven, and DGU-20A5 degasser. Automatic sampling was performed with an autosampler volume of 20 µL. An Inertsil ODS-3 column (250×4.6 mm; GL Sciences, Japan) was used as the analytical column at 25°C. The mobile phase consisted of a 0.01 mol L^{-1} $NaH_2PO_4.H_2O$ buffer solution (adjusted to pH 2.5 with phosphoric acid) and was eluted isocratically at a flow rate of 0.9 mL min^{-1}.

Standard solutions of ascorbic and oxalic acids were injected separately to determine their retention times and elution order. The results obtained and chromatograms were processed via LabSolutions (Shimadzu).

Analytical Parameters of the Method

The analytical parameters of a method include linear range, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) [22]. Once the method was optimized, the determination of ascorbic acid and oxalic acid in real samples was carried out.

Linearity

Analytes were identified by comparing the UV-visible region spectra of ascorbic and oxalic acids via standard addition. The linearity of the RP/HPLC-UV method was studied by constructing seven-point calibrations for selected organic acids over a wide concentration range. Calibration curves were generated by plotting peak areas against the concentration of the selected organic acids. Correlation coefficients were calculated from the best-fit line. The experimental results were expressed as the mean (X)±standard deviation (SD) of three replicates.

Repeatability and Accuracy

The repeatability and reproducibility of the method were calculated by taking the percent relative standard deviation of the retention times (RSD, %) for six independent samples containing known amounts of ascorbic and oxalic acid, both on the same day (intraday) and on different days (interday).

For the determination of the organic acids studied, the accuracy of the RP/HPLC-UV system was expressed as the relative error term and calculated by ten repeated injections of the solution with standard addition. The concentrations were calculated from the calibration curve equation for each organic acid.

Limit of Detection and Limit of Quantification

The theoretical LOD and LOQ for the analyzed ascorbic acid and oxalic acid were represented by 3.3 times and 10 times the ratio of the standard deviation of the lowest concentration value to the slope of the calibration graph. The equations for calculating LOD and LOQ are provided below [23]:

$$\text{LOD (mg } L^{-1}) = (3.3 \times \text{residual standard deviation of y - intersection of the regression line) / slope} \quad (1)$$

$$\text{LOQ (mg } L^{-1}) = (10 \times \text{residual standard deviation of y - intersection of the regression line) / slope} \quad (2)$$

The content of ascorbic and oxalic acids in the samples was determined by interpolating from the calibration curve, taking into account the dilution factors applied during sample preparation.

Recovery Study

The accuracy of the method was calculated as the recovery efficiency by adding known amounts of the studied organic acids. For this purpose, Ascorbic acid and oxalic acid were spiked into freshly squeezed orange juice at concentrations of 20 mg L^{-1} , 25 mg L^{-1}, 50 mg L^{-1}, and 100 mg L^{-1} prior to extraction. Analyte addition was repeated three times.

Stability of Ascorbic Acid

The oxidation of ascorbic acid during sample preparation affects the measurement results. The stability of ascorbic acid was tested in freshly squeezed orange juice obtained immediately after squeezing or, in the case of commercially available orange juice samples, immediately after opening the package. Both samples were diluted 5-fold with the mobile phase and filtered through a 0.45 µm membrane filter. Prior to sample preparation, both orange juices were stored at 5 °C.

RESULTS and DISCUSSION

Optimization of the RP/HPLC-UV Method

RP/HPLC coupled with UV detection is a common technique used for the determination of organic acids. Separating and quantifying organic acids using high-performance liquid chromatography is challenging because these acids have similar chemical structures and spectral properties.

In the RP/HPLC-UV method, a UV region was scanned to determine a common wavelength for ascorbic acid and oxalic acid. Since the chemical structures of the organic acids being studied are different, they exhibited maximum absorption at different wavelengths. Ascorbic acid was determined at a maximum absorbance wavelength of 245 nm, while oxalic acid was at 205 nm. However, the acceptable common wavelength for both ascorbic and oxalic acids was determined to be 220 nm.

Generally, when C18 is used as the packing material, it can be operated within the pH range of 2 to 7. When the pH is ≤ 2.00, protonation can occur, which may increase solubility. However, this condition is limited by column efficiency. On the other hand, when the pH is ≥ 7, the siloxane material that makes up the column packing can undergo hydrolysis, causing the column packing to degrade.

Most organic acids have relatively low and similar pKa values. This limits the pH values that can be used for chromatographic separation [24]. An acidic eluent (pH=1.5-2.5) is necessary to keep organic acids protonated. This ensures the best interaction between organic acids and the stationary phase, resulting in optimal separation. The solubility performance of ascorbic and organic acids was tested within a pH range of 2-3. For the simultaneous determination of ascorbic and oxalic acids with optimal separation and peak shape, a mobile phase consisting of a 0.02 mol L^{-1} $NaH_2PO_4.H_2O$ buffer solution was used at a flow rate of 1 mL min^{-1}.

In Figure 1, the standard solution of ascorbic acid, ascorbic acid peaks in ready-to-drink orange juice and apple juice are superimposed, and in Figure 2, the standard solution of oxalic acid, oxalic acid peaks in ready-to-drink orange juice and apple juice are superimposed. The peaks are seen overlapped on top of each other.

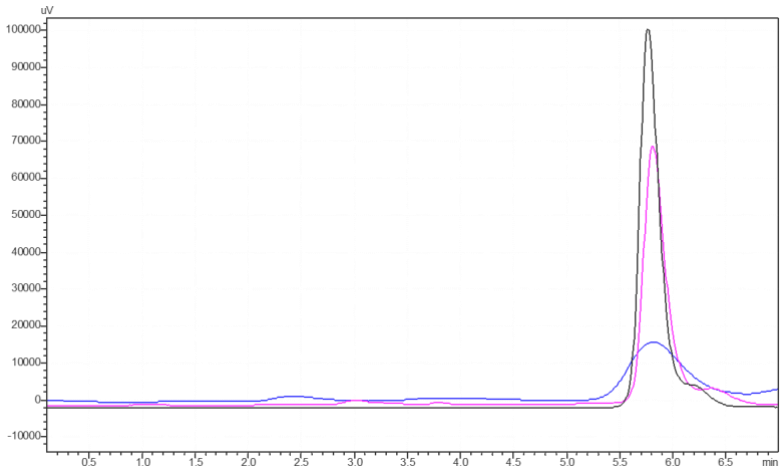

Figure 1. Chromatogram of the standard solution of the ascorbic acid (black), ready-to-drink orange juice (pink), and ready-to-drink apple juice (blue) at 245 nm

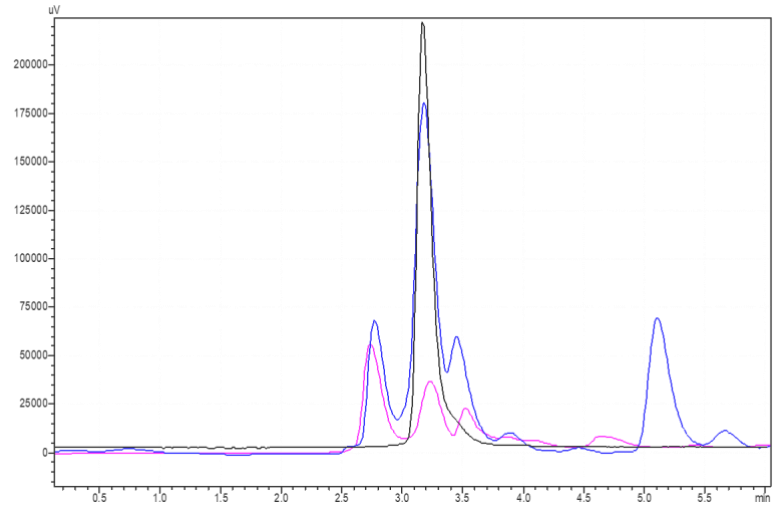

Figure 2. Chromatogram of the standard solution of the oxalic acid (black), ready-to-drink orange juice (pink), and ready-to-drink apple juice (blue) at 205 nm

According to Figures 1 and 2, the analysis time required for the separation of ascorbic and oxalic acids using an ODS column at 1 mL min^{-1} was less than 6.5 min.

Evidently, the method provides good selectivity and resolution for the simultaneous determination of ascorbic acid and oxalic acid.

Method Validation

Linearity, LODs, LOQs, Reproducibility and Accuracy

In the RP/HPLC-UV method, the linear range of the calibration curve was established for ascorbic acid within the concentrations of 10, 20, 30, 50, 100, 250, and 500 mg L^{-1}, and for oxalic acid within the concentrations of 15, 30, 50, 100, 250, and 500 mg L^{-1}. Linearity was evaluated based on the calibration curves. Each standard was injected three times to assess repeatability. The calibration curves for ascorbic and oxalic acids were linear based on a high correlation coefficient (R^2). The method exhibits a wide linear range.

The repeatability of the RP/HPLC-UV method for retention times, both intraday and interday, was obtained through repeated injections of standard solutions at 250 mg L^{-1}. The calculated LOD and LOQ, retention times (t_R), and %RSD of retention times for the method are provided in Table 1. The intraday and interday %RSD for retention times were less than 4.6%. As seen in Table 1, the LOQ for each acid has a good correlation coefficient ($R^2 \geq 0.997$). Coupled with good stability and repeatability values, these data indicate that the applied method can be reliably used for the simultaneous analysis of ascorbic and oxalic acids in fruit juices. Using a test method that conforms to the guidelines stated in internationally accepted guidance publications allowed the procedure to be validated (Eurachem 2014).

Table 1. Linear calibration equation, range, LOD, LOQ, and precision of retention time (t_R) of standard organic acids for the RP/HPLC-UV method

Organic Acids	Analytical Parameters							
	Linear Range (mg L^{-1})	Calibration Equation	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})	t_R (min)	%RSD of t_R	
							Intra day	Inter day
Ascorbic	10-500	y= 65603x-184517	0.995	1.4	4.2	6.2	4.6	4.3
Oxalic	15-500	y= 27906x+31434	0.997	1.3	3.9	3.1	4.1	3.8

The accuracy of the RP/HPLC-UV method was verified by analyzing standard solutions of ascorbic and oxalic acids with concentrations of three different ppm values within the calibration graph range of 10-500 mg L^{-1}. The concentration of the standard solutions was calculated using a calibration curve (Table 2). The relative error was found to be less than %5.

Table 2. Accuracy of the RP/HPLC-UV method for the determination of organic acids

Organic Acids	Concentration (mg L^{-1})	Calculated Concentration (mg L^{-1})	Relative Error (%)
Ascorbic	10	10.4±1.2	4.0
	30	29.5±2.3	1.6
	100	97.5±3.4	2.5
Oxalic	20	20.9±1.6	4.8
	30	29.4±2.5	2.0
	100	98.5±3.2	1.6

Recovery

The accuracy of the RP/HPLC-UV method for the determination of ascorbic and oxalic acids was also evaluated by calculating the recovery according to the standard addition method to eliminate possible matrix effects. Initially, the amount of ascorbic and oxalic acids in freshly squeezed orange juice was determined. After finding the initial amount, the solution was diluted 5 times, and the addition method was then performed. 20, 25, 50, and 100 mg L^{-1} ascorbic acid were added to the diluted juice. The recovery results obtained are given in Table 3. According to Table 4, recovery values in the range of 81-102% in apple juice and 81-103% in orange juice were obtained for the studied organic acids. The low recovery value might be due to the sample preparation. This step included filtration and solid-phase extraction. These values indicate that the accuracy of the applied analytical method is highly efficient and applicable for the simultaneous determination of ascorbic and oxalic acids in various industrial and freshly squeezed fruit juices.

Table 3. Recovery of ascorbic acid added to fresh orange juice

Initial Content (mg L^{-1})	Added (mg L^{-1})	Found (mg L^{-1})	Recovery±Standard Deviation (%)
35.0±1.6	25	55.5±2.5	82±2.2
	50	85.7±3.2	101±1.5
	100	127.0±4.1	92±1.7

Table 4. Recovery of organic acids added to industrial apple and orange juices

Juice	Organic Acid	Initial Content (mg L^{-1})	Added (mg L^{-1})	Found (mg L^{-1})	Recovery±Standard Deviation (%)
Apple	Ascorbic	4.0±0.6	20	22.0±1.7	90.2±2.1
			50	53.1±2.7	98.3±1.7
			100	106.0±3.1	102.0±1.3
	Oxalic	72.0±1.3	20	88.3±2.4	81.7±2.2
			50	121.1±2.5	98.1±1.4
			100	174.5±4.9	102.5±1.6
Orange	Ascorbic	26.7±1.2	20	44.5±1.5	89.2±2.4
			50	74.1±1.3	94.8±1.7
			100	130.1±4.1	103.3±1.4
	Oxalic	12.7±1.4	20	29.1±1.5	81.7±2.3
			50	64.1±1.6	102.7±1.7
			100	113.0±3.9	100.3±1.3

Stability Results for Ascorbic Acid

The stability of ascorbic acid was also examined in freshly squeezed and industrial orange juice samples at room temperature after dilution with the mobile phase. The label information on the industrial orange juice recommended consumption within 3 days of opening the package. In the case of the chosen industrial orange juice, the ascorbic acid concentration was intermittently analyzed during the first 48 hours after the package was opened (Figure 2). The ascorbic acid concentration decreased by 1.5% after 4 hours of opening the package, by 9% after 24 hours, and by 18.5% after 48 hours. Thus, after opening the package of industrial orange juice and diluting the sample, it was observed that there was no significant decrease in ascorbic acid concentration 48 hours later (2 days) compared to the initial concentration ($p < 0.05$). Therefore, it is possible to accurately analyze orange juice without significant ascorbic acid loss for at least 48 hours after opening the package. Similarly, fresh-squeezed orange juice was also monitored for a decrease in ascorbic acid concentration for 48 hours after dilution with the mobile phase, and the results were comparable to those obtained with industrial juice (Figure 3).

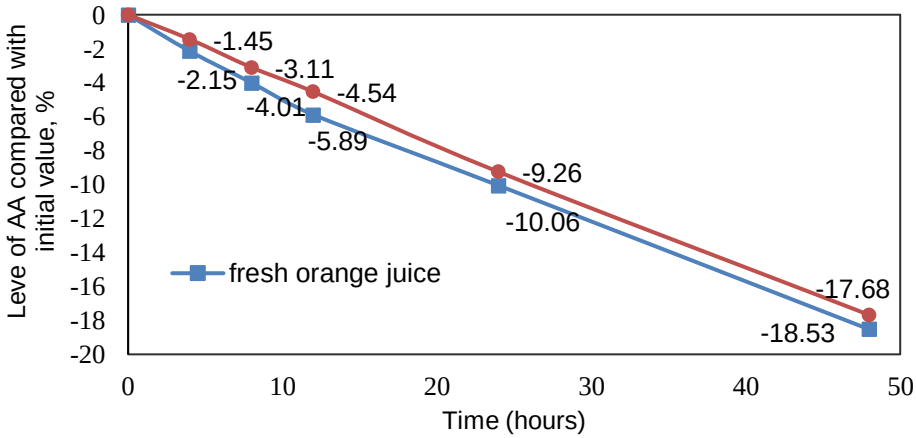

Figure 3. Stability of ascorbic acid in freshly squeezed and industrial orange juice at room temperature

Real Sample Analysis

The proposed method was applied to determine ascorbic and oxalic acids in various brands of beverages purchased from the local market (Table 5).

According to Table 5, ascorbic acid concentration was highest in orange juice (133.5±5.07 mg L^{-1}) and oxalic acid concentration was highest in apple juice (360±4.28 mg L^{-1}). Both ascorbic and oxalic acids were found in the fruit juices studied. The recommended daily allowance (RDA) of vitamin C is 100-120 mg day^{-1} [1]. RDA is the recommended daily intake. It is the minimum amount needed daily in nutrients that varies depending on age, gender, and body weight.

When daily vitamin C intake is adequate and regular, the risks of heart disease, cancer, and stroke are reduced. The daily amount of vitamin C that should be taken from the diet varies between 35-100 mg depending on various factors. This amount is estimated to be 35 mg for infants, 60 mg for adults, and 100 mg for nursing mothers [1]. According to the Food and Agriculture Organization, the RDI value of ascorbic acid for adults is recommended as 45 mg [25]. According to some data in the literature, the concentration of ascorbic acid in orange juice was found to be 32±1.2 mg 100 mL^{-1} [26], 344.3 mg L^{-1} [27], 490 mg L^{-1} [28]. Accordingly, the concentration of ascorbic acid in orange juice in our study (133.5±0.07 mg L^{-1}) is lower than the literature data. We can think that this may be due

to the origin of the fruit and/or the difference in temperature conditions during storage and transportation after the fruit has become juice. The concentration of ascorbic acid in the studied orange juice does not meet the daily intake of one glass of orange juice (200 mL, 26.8 mg). Therefore, it may be recommended to drink at least two glasses of orange juice per day. Freshly squeezed orange juice had a higher concentration of ascorbic acid (35 mg L^{-1}) than industrial orange juice. This means that consuming freshly squeezed orange juice will provide the body with a higher intake of vitamin C than industrial orange juice. So freshly squeezed orange juice should be consumed. In addition to orange juice, ascorbic acid concentration in cold tea was also found to be high (98.5±2.2 mg L^{-1}). The concentration of ascorbic acid in other fruit juices studied was about one-tenth of the concentration in orange juice and was low.

Table 5. Concentrations of ascorbic and oxalic acids in various beverages

Beverage Type	Ascorbic Acid Concentration (X±S.D, mg L^{-1})	Oxalic Acid Concentration (X±S.D, mg L^{-1})
Orange	133.5±5.1	63.5±2.6
Cherry	15.0±1.8	271.0±5.1
Pomegranate	13.0±1.2	172.0±3.7
Apple	20.0±1.1	360.0±4.3
Apricot	12.5±1.1	220.0±3.5
Mix	22.5±1.2	274.0±4.4
Ice Tea	98.5±2.2	44.0±1.4

Table 6 gives a summary of the literature studies with similar analysis methods to our study. Each study has advantages over the other in terms of LOD, LOQ, and recovery values in terms of analysis method. In the developed method, ascorbic acid and oxalic acid were determined simultaneously. This method is of more practical importance, especially for the simultaneous determination of trace levels of oxalic acid with ascorbic acid.

Table 6. Comparison of the described method with other methods in the literature

Compound	t_R[1]	Range (ppm)	Mobile Phase	FR[2]	IV[3]	WL[4]	Column Type	%R	LOQ (ppm)	LOD (ppm)	RsDr (%)	RsDt (%)	Ref.
Ascorbic Acid	4.3	1-100	%0.1 Formic acid	1.0	10	245	ODS-3						[29]
Ascorbic Acid	4.2	2-100	Phosphate buffer pH:2.2	1.0	20	245	ODS-4	93	2.00	0.50	0,9	4.5	[27]
Oxalic Acid	2.2	5-80	Phosphate buffer pH:2.2	1.0	20	210	ODS-4	105	5.00	1.00	4.9	5.4	
Ascorbic Acid	4.2	6-330	Phosphate buffer pH:2.6	0.5	20	250	RP-C18		0.03	0.10	1.9	3.8	[26]
Ascorbic Acid	5.3	1-215	Phosphate buffer/ACN pH:4.75	1.2	20	205	S5NH2	94		0.18	0.9	4.0	[30]
Oxalic Acid	11.9	15-150	Sulfuric acid pH:2.5/MeOH	0.35	20	215	VP-ODS	85		0.94	5.2	4.5	[31]
Ascorbic Acid	14.5	10-100	Sulfuric acid pH:2.5/MeOH	0.35	20	215	VP-ODS	106		5.17	0.3	1.6	
Ascorbic Acid	8.3	1-100	Aqueous sulfuric acid pH:2.2	0.6	20	210	ODS		1.05	0.34	1.0	1.2	[24]
Oxalic Acid	5.7	0,1-100	Aqueous sulfuric acid pH:2.2	0.6	20	210	ODS		4.08	1.34	0.2	1.4	
Ascorbic Acid	6.2	10-500	Phosphate buffer pH:2.5	1.0	20	245	ODS-3	93	4.20	1.40	4.1	3.9	This Study
Oxalic Acid	3.1	15-500	Phosphate buffer pH:2.5	1.0	20	205	ODS-3	104	3.90	1.30	4.6	4.3	

[1]Retention time (min), [2]Flow rate (mL min^{-1}), [3]Injection volume (microliter), [4]Wavelength (nm)

CONCLUSIONS

The present study describes the simultaneous analysis of ascorbic and oxalic acids in various fruit juices using RP/HPLC-UV. The linearity of the method is very high over a wide concentration range (R^2 0.997-1.002). The relative error is less than 5%. The wide linear range obtained allows the determination of ascorbic and oxalic acids in more samples with the current method. The theoretical LOD and LOQ were 1.4 mg L^{-1} and 4.2 mg L^{-1} for ascorbic acid and 1.3 mg L^{-1} and 3.9 mg L^{-1} for oxalic acid, respectively. Extraction recoveries were between 82-103.3% for ascorbic acid and 81.7-102.5% for oxalic acid. The proposed method was applied to a real sample. Ascorbic acid was highest in orange juice and oxalic acid was highest in apple juice. Ascorbic acid was found in higher concentrations in freshly-squeezed orange juice than in industrial orange juice. But even this value (35 mg) is lower than the RDI for ascorbic acid for adults (45 mg), according to the Food and Agriculture Organization. In this case, drinking 2 glasses (400 mL) of freshly-squeezed orange juice can be recommended to get enough vitamin C daily. The developed method provides an easy, simple, and acceptable solution for the accurate, reliable, and precise simultaneous determination of ascorbic and oxalic acids in a variety of fruit juices with a wide linear range and interchangeable LOD and LOQ values. The RP/HPLC-UV method used offers convenience with uncomplicated sample preparation. This method is suitable for high-throughput analysis of multiple samples.

DISCLOSURE STATEMENT

The authors report no conflict of interest.

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