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Determination and Comparison of Vitamin C Content in Fresh Fruit Juices Using HPLC: A Case Study in Phnom Penh, Cambodia

Case Report

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Abstract: Vitamin C, also known as L-ascorbic acid, is a vital micronutrient for human health, involved in immune system function, carnitine and catecholamine metabolism, iron absorption, and collagen production, which helps hold cells together. This study aims to quantify and compare the vitamin C content in six different fruit samples: carrot *(Daucus carota subsp. sativus)*, apple *(Malus domestica)*, guava *(Psidium guajava L. Meer)*, passion fruit *(Passiflora edulis)*, Pursat orange *(Citrus sinensis (L.) Osbeck)*, and fresh sugarcane juice *(Saccharum officinarum)* using High-Performance Liquid Chromatography (HPLC). A standard curve was prepared using L-ascorbic acid solutions at concentrations ranging from 10.0 to 200.0 µg/mL, showing excellent linearity with an R^2 value of 0.9998. Accuracy was expressed as percent recovery, ranging between 80% and 120%, while the %RSD was below 5%, indicating high precision and reliability of the method. Among the tested samples, guava *(Psidium guajava L. Meer)* had the highest vitamin C concentration at 89.054 mg/mL, whereas apple *(Malus domestica)* and carrot *(Daucus carota subsp. sativus)* showed the lowest levels. According to the U.S. Food and Drug Administration (FDA), moderate levels of vitamin C intake, ranging from 30 to 180 mg per day, are estimated to result in absorption efficiencies of vitamin C between 70% and 90%. These findings highlight guava and Pursat orange as rich dietary sources of vitamin C, supporting their potential role in meeting daily nutritional requirements.

1. Introduction

Vitamin C, also known as L-ascorbic acid, is a well-known micronutrient essential for the human body's overall health. Vitamin C, which comes in two primary forms, ascorbic acid (AA) and dehydroascorbic acid (DHA), is a potent antioxidant and an essential cofactor for numerous enzymes. It plays a role in a wide range of biological processes, including the proper operation of the immune system, the metabolism of carnitine and catecholamines, the absorption of iron from food, and the production of collagen (Alberts et al., 2025). The body uses vitamin C to maintain cell integrity by promoting the production of collagen, a connective tissue that binds bones, muscles, and other tissues together. Likewise, vitamin C strengthens blood vessel walls, promotes iron absorption and utilization, facilitates wound healing, aids in bone and tooth development, and functions as an antioxidant (Bellows & Moore, 2012). It must be taken daily through food or supplements since it is a water-soluble vitamin that dissolves in water and is transported to the body's tissues (The Nutrition Source, 2012). As an antioxidant, vitamin C helps shield cells from the damaging effects of free radical molecules, which are generated when the body breaks down food or when exposed to tobacco smoke, radiation from the sun, X-rays, or other

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environmental factors. Free radicals may contribute to cancer, heart disease, and other illnesses. Iron absorption and storage are further benefits of vitamin C (Mayo Clinic Staff, 2023).

Vitamin C taken orally causes tissue and plasma concentrations that are closely regulated by the body. Moderate consumption of 30–180 mg/day results in absorption of about 70%–90% of vitamin C. Ascorbic acid that has been absorbed but not yet metabolized is eliminated in the urine at doses greater than 1 g/day, where absorption drops to less than 50%. Pharmacokinetic studies show that oral dosages of 1.25 g/day ascorbic acid result in mean peak plasma vitamin C concentrations of 135 mmol/L. This is roughly twice as high as the concentrations obtained by ingesting 200–300 mg/day of ascorbic acid from foods high in vitamin C. According to pharmacokinetic modeling, peak plasma concentrations of ascorbic acid would only be 220 μmol/L at dosages as high as 3 g administered every 4 hours (National Institutes of Health, 2021).

Vitamin C can be detected using a variety of innovative and effective methods, such as HPLC (High-performance Liquid Chromatography) (Gazdik et al., 2008), UV spectrophotometry (Rahman Khan et al., 2006), titration (Abe-Matsumoto et al., 2020), Ion pair liquid Chromatography (LC) (Hernández et al., 2006), Cyclic Voltammetry and square Wave voltammetry (Škugor Rončević et al., 2022). The vitamin C content varies significantly among different fruits and vegetables. Carrots (*Daucus carota subsp. sativus*) contain approximately 9.92 mg of vitamin C per 100g fresh weight (FW) (Cotruţ & Bădulescu, 2016), while commercial apples (*Malus domestica*) only have a concentration of about 10 mg of vitamin C per 100 g of fresh weight (Lemmens et al., 2020). In contrast, the level of vitamin C contained in crystal guava (*Psidium guajava L. Meer*) is 20.79 mg/5 g (Yulian et al., 2023). Vitamin C content in passion fruit (*Passiflora edulis*) was 30 mg per 100g (Mandal, 2017). The vitamin C content in Pursat orange (*Citrus sinensis (L.) Osbeck*), which is classified as a sweet orange of the citrus group, was 39.8 μg/mL (Mahawar et al., 2025). The study aims to quantify and compare the vitamin C content in Carrot (*Daucus carota subsp. sativus*), Apple (*Malus domestica*), Guava (*Psidium guajava L. Meer)*, Passion fruit (*Passiflora edulis*), Pursat Orange (*Citrus sinensis (L.) Osbeck*), and fresh Sugarcane juice (*Saccharum officinarum*).

2. Materials and Methods

2.1. Chemicals

All chemicals of HPLC grade were utilized in this experiment. Methanol, vitamin C, and purified distilled water were used as the mobile phase in a ratio (v:v) (5:95). L-ascorbic acid and double-deionized water were used for the preparation of standards. Methanol and vitamin C standards are purchased from the Fisher Scientific company in the United States (USA).

2.2. Standard Curve

A stock solution of vitamin C standard (1.0 mg/mL) was prepared by dissolving 50.0 mg of vitamin C powder in purified distilled water using a 50.0 mL volumetric flask. The purified distilled water was filtered through a 0.22 μm syringe filter before use in the experiment. The standard curve was generated using the working standard solution of L-ascorbic acid, ranging from 10.0 to 200.0 μg/mL.

2.3. Sample Collection

All samples were purchased from the Neak Meas Market, a local market in Phnom Penh, Cambodia. Sugarcane juice (*Saccharum officinarum*) was purchased fresh, without ice, from the market. Other fruit samples, including carrot (*Daucus carota subsp. sativus*), apple (*Malus domestica*), guava (*Psidium guajava L.*

Meer), passion fruit (*Passiflora edulis*), and pursat Orange (*Citrus sinensis (L.) Osbeck*), were randomly chosen from three distinct sellers for each fruit, packed in a polyethylene bag, to avoid exposure to the sunlight and rapidly delivered to the laboratory. The total number of samples was 18, and all samples were analyzed in their fresh condition.

2.4. Vitamin C Determination

All samples were squeezed into juice using the same preparation method, but limited to each type of fruit. Apple (*Malus domestica*) and guava (*Psidium guajava L. Meer*) are directly squeezed after rinsing. At the same time, Carrots (*Daucus carota subsp. sativus*) and oranges (*Citrus sinensis (L.) Osbeck*) were peeled. Passion fruit (*Passiflora edulis*) was cut, and the passion fruit pulp was scooped out before being squeezed. After squeezing the fruit, the extracted juice was transferred into a 15.0 mL Centrifuge tube, centrifuged at 5000 rpm for 30 min, and the resulting supernatant was filtered through filter paper (Najwa & Azrina, 2017). A 1.0 mL aliquot of the filtrate was then transferred into a 10.0 mL volumetric flask, diluted to volume with purified distilled water, and gently inverted three times to ensure thorough mixing. The final solution was filtered using a 0.22 µm syringe filter and transferred into a vial for analysis in HPLC (Shimadzu Corporation, Japan). All experiments were repeated in triplicate.

2.5. HPLC Condition

To quantify the vitamin C contents in samples, HPLC (Shimadzu Corporation, Japan) was used. HPLC condition was adopted from Najwa & Azrina (2017). The analysis was performed on C18 (4.6 mm × 250 mm, 5 µm) using a mobile phase of methanol-water (5:95, v/v), an injection volume of 20 µL, a flow rate of 1 mL/min, a temperature of 25 °C, and a wavelength of 254 nm.

2.6. Statistical Analysis

All experiments were done in triplicate. The data are reported as mean ± standard deviation (SD), and the mean vitamin C concentrations in samples were compared using one-way ANOVA and the Turkey comparison test, with a 95% confidence interval and p-value <0.05. The data analysis was assessed using Minitab software version 21.2.0.0.

3. Results and Discussion

3.1. Standard Curve

A standard calibration curve for Vitamin C was established by plotting the peak area against known concentrations ranging from 10.0 to 200.0 µg/mL. The resulting linear regression equation was $y = 5.8042x + 16.587$, with a high correlation coefficient ($R^2 = 0.9998$), indicating excellent linearity, as R^2 values above 0.995 are considered optimal (Harris, 2010). The result demonstrated a strong relationship between peak area and concentration, and a calibration curve suitable for determining the Vitamin C content in unknown samples.

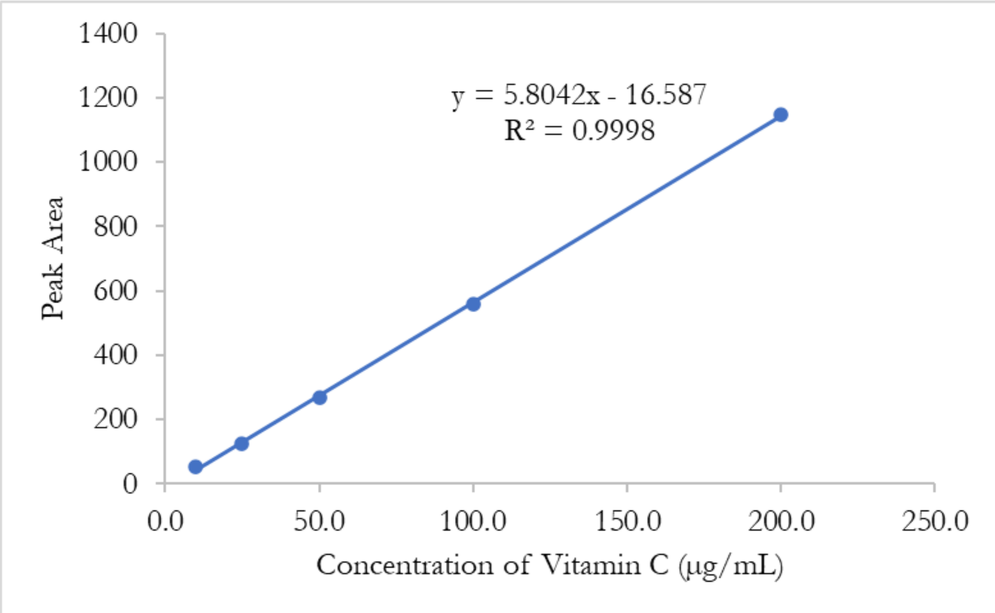

Figure 1. The standard curve of Vitamin C from 10.0 to 200.0 µg/mL

3.2 Method Validation

3.2.1 Limit of Detection and Limit of Quantification

The lowest concentration of an analyte in the sample that can be detected is known as the limit of detection (LOD). The lowest concentration of an analyte in a sample that can be identified with acceptable precision and accuracy is known as the limit of quantification (LOQ). Table 1 shows the LOD and LOQ values as 0.3689 µg/mL and 1.2295 µg/mL, respectively. Equations 1 and 2 are used for LOD and LOQ calculation (Kalra, 2011).

$$\text{LOD} = \frac{3 \times SD}{m} \quad (1)$$

$$\text{LOQ} = \frac{10 \times SD}{m} \quad (2)$$

where m is the slope obtained from the plot of the standard curve, and SD is the standard deviation of the lowest concentration (10 µg/mL) used in the standard curve, which was run 10 times (N=10).

Table 1. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Vitamin C Standard Solution (µg/mL)	10.0
N	10
SD	0.7090
LOD (µg/mL)	0.3689
LOQ (µg/mL)	1.2295

3.2.2 Accuracy and Precision

To demonstrate the accuracy and precision of the method, the recovery percentage (% Recovery) and relative standard deviation (% RSD) were evaluated through the fortification of samples with vitamin C at two concentrations (50.0 and 200.0 μg/mL). Spiked samples were prepared and immediately injected into the HPLC for analysis. All experiments were run in triplicate. The European Commission Decision 2022/657/EC (2002) states that a recovery percentage between 80 and 120% and a relative standard deviation of no more than 20% ensure the method's accuracy and precision (European Commission, 2002). Table 2 shows the %Recovery was 95.772 - 100.037%, which was between 80 - 120%, while %RSD was less than 5%, which displays the reliability for accurate and precise quantification of vitamin C in fruit juice samples. The formula for calculating %Recovery is provided in Equation 3.

$$\%Recovery = \frac{Cspiked - Cunspiked}{Cadded} \times 100\% \quad (3)$$

Where: C_{spiked} is the concentration found in the sample after fortification.
$C_{unspiked}$ is the concentration found in the sample before fortification.
C_{added} is the concentration used for fortification.
The formula for calculating %RSD is provided in Equation 4.

$$\%RSD = \frac{SD}{x} \times 100\% \quad (4)$$

Where: SD is the standard deviation of each sample fortification.
X is the mean of each sample fortification level.

Table 2. Recovery Percentage (%Recovery) and Relative Standard Deviation (%RSD)

Spike Concentration (μg/mL)	Concentration (μg/mL) (Mean ± SD)	%Recovery	%RSD
Unspiked	17.980 ± 1.569	-	-
50.0	67.999 ± 3.434	100.037	3.433
200.0	209.525 ± 4.405	95.772	4.600

3.3 Determination of Vitamin C in Samples

The vitamin C contents in fresh Fruit samples such as Apple (*Malus domestica*), Carrot (*Daucus carota subsp. sativus)*, Sugarcane juice (*Saccharum officinarum*), Pursat orange (*Citrus sinensis (L.) Osbeck*), Passion fruit (*Passiflora edulis*), and Guava (*Psidium guajava L. Meer)* were presented in mean ± standard deviation (SD) as shown in Table 1. Among the tested samples, guava (*Psidium guajava L. Meer*) exhibited the highest mean concentration of vitamin C at 89.054 mg/mL. Figure 2 shows the chromatogram of vitamin C in the guava sample. In contrast, the lowest concentrations were observed in apple (*Malus domestica*) and carrot (*Daucus carota subsp. sativus*), with mean values of 3.966 mg/mL and 4.363 mg/mL, respectively. Intermediate levels of vitamin C were found in sugarcane juice (*Saccharum officinarum*) and passion fruit (*Passiflora edulis*), with concentrations of 18.089 mg/mL and 17.516 mg/mL, respectively.

Table 3. The Concentration of Vitamin C (mg/mL) in Fruit Samples

Fruit Sample		Concentration of Vitamin C (mg/mL)
Common Name	Scientific Name	Mean ± SD
Apple	*Malus domestica*	$3.966^{d} \pm 0.706$
Carrot	*Daucus carota subsp. sativus*	$4.363^{d} \pm 0.900$
Sugarcane	*Saccharum officinarum*	$18.089^{c} \pm 0.760$
Pursat orange	*Citrus sinensis (L.) Osbeck*	$33.324^{b} \pm 2.794$
Passion fruit	*Passiflora edulis*	$17.516^{c} \pm 0.620$
Guava	*Psidium guajava L. Meer*	$89.054^{a} \pm 2.344$

Note: Data are presented as the mean values; SD means standard deviation. Values with similar letters refer to "not significantly different", where a, b, c, and d refer to "significantly different" ($p < 0.05$) with the following order: a > b > c > d (Minitab 21.2.0.0 ANOVA one-way test with Tukey comparison method).

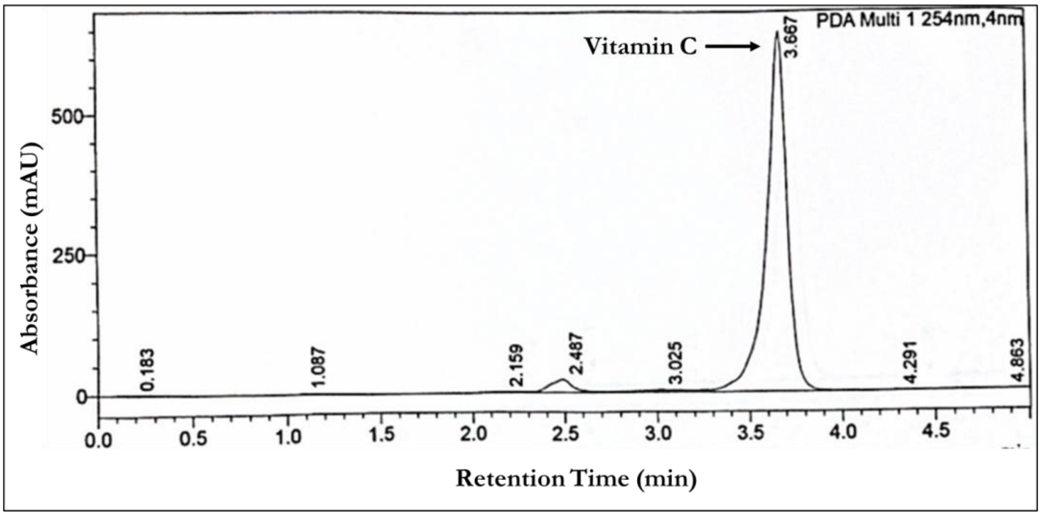

Figure 2. The Chromatography of Vitamin C in the Guava Sample

According to the U.S. Food and Drug Administration (FDA), the recommended daily intake of vitamin C is 90 mg for adults and children aged four and above. For pregnant and lactating women, the requirement increases to 120 mg/day (FDA, 2018). At moderate intake levels ranging from 30 to 180 mg per day, the absorption efficiency of vitamin C is estimated to be between 70% and 90% (National Institutes of Health, 2021). Based on these guidelines, the present findings indicate that guava and Pursat oranges serve as rich dietary sources of vitamin C.

4. Conclusion

This study successfully quantified and compared the vitamin C content in six different fruit samples, such as carrot (*Daucus carota subsp. sativus)*, apple *(Malus domestica)*, guava *(Psidium guajava L. Meer)*, passion fruit *(Passiflora edulis)*, Pursat orange *(Citrus sinensis (L.) Osbeck)*, and fresh sugarcane juice *(Saccharum officinarum),* using HPLC. All samples were bought from the local market in Phnom Penh, Cambodia, and randomly selected from three different sellers. The method demonstrated excellent linearity (R^2 = 0.9998), precision (%RSD < 5%), and acceptable recovery rates (95.772–100.037%), confirming its reliability and validity for vitamin C analysis. Among the fruits tested, guava exhibited the highest vitamin C concentration (89.054 mg/mL), followed by Pursat orange (33.324 mg/mL). In contrast, apple (3.966 mg/mL) and carrot (4.363 mg/mL) had the lowest levels. Guava and Pursat orange are excellent dietary sources of vitamin C, suggesting they can help people meet their daily nutritional needs. While the study successfully quantified vitamin C levels, it is essential to consider that the concentration can vary depending on factors such as the fruit's age, growing conditions, and storage time. The FDA recommends a daily intake of 90 mg of vitamin C for adults and children over four. Vitamin C is a water-soluble vitamin that the body does not store, so it must be replenished daily through a balanced diet or supplements.

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Data availability: Not applicable

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Conflicts of interest: Authors declare no conflicts of interest.

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