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Quantitative Analysis of Ascorbic Acid (Vitamin C) in Banana by HPLC with Optimised Extraction Method

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❑ Introduction

Ascorbic acid (AA) or vitamin C plays critical roles in the body such as the immune system helping prevent infections, the synthesis of collagens and absorption of iron. The human body does not produce or store AA, so people ingest ascorbic acid daily from diet like fruits and vegetables. Quantitative analysis of ascorbic acid (vitamin C) in fruits, vegetables and various foods becomes essential in health and nutrition sciences, and food processing and quality labelling. Initial method (e.g AOAC 967.21) for quantitative determination of vitamin C was established based on titrimetric method and it was used for vitamin C preparations and juices [1]. HPLC method such as AOAC 2012.22 official method provides accurate measurement of vitamin C in sample like infant and adult formula etc. [2]. However, a common issue faced in HPLC analysis is the oxidation of ascorbic acid (AA) to dehydroascorbic acid (DHAA) during sample preparation [3]. DHAA has poor UV absorbance thus it will be difficult to detect its presence. Therefore, sample preparation prior to analysis must be carried out under acidic condition with presence of reducing reagent to convert DHAA to AA form [4]. This study focuses on optimization of sample preparation for reliable quantitative determination of total vitamin C in banana by HPLC with UV detection.

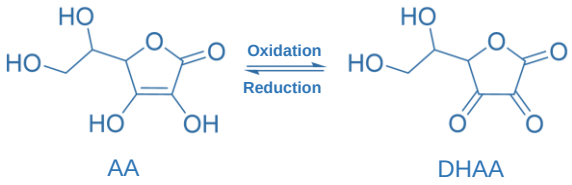

Figure 1: Reversible oxidation of ascorbic acid (AA) to form dehydroascorbic acid (DHAA)

❑ Experimental

Chemicals and standards

Ascorbic acid (AA) solid standard, meta-phosphoric acid (MPA) and Tris-[2-carboxylethyl] phosphine (TCEP) were obtained from Sigma Aldrich. A stock solution of AA at 1000 µg/mL was prepared using a diluent of 0.3% MPA, 0.8% acetic acid and 0.5 mM TCEP. The stock solution was diluted into different working standards using the same diluent to concentrations ranging from 0.1 µg/mL to 10 µg/mL to construct calibration curve.

Sample preparation (banana)

Peeled fresh banana (5g) was sliced into thin pieces and placed into a 50 mL centrifugation tube. Adding 25mL of extraction solution with 5 mM TCEP as reducing reagent, the sample tube was vortexed for 5 min. The sample-solution was then centrifugated at 4ºC for 10 min at 10000 RPM. The clear solution was transferred to another tube and filtered with 0.22 um nylon filter. The obtained solution was diluted 10x with milliQ water for HPLC analysis.

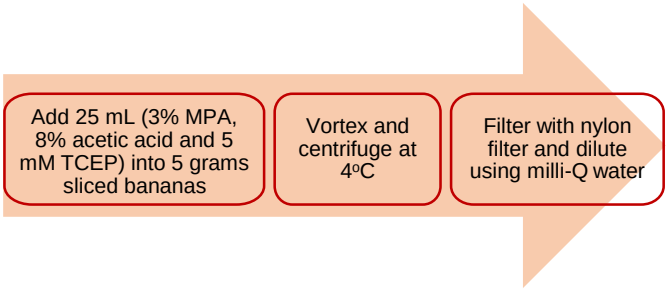

Figure 2: Extraction of ascorbic acid in banana samples with acidic solution including MPA and TCEP (reducing agent).

Analytical conditions

Shimadzu Prominence-*i* Plus HPLC with photodiode array detector (PDA) was used in this analysis. The LC conditions are shown in Table 1.

Table 1: LC conditions of ascorbic acid analysis

Column	Shim-pack GIST AQ-C18, 150mm × 3.0 mm, 3µm particle size
Flow Rate	0.7 mL/min
Mobile Phase	A: 20mmol/L NaH_2PO_4 and 2mmol/L sodium 1-hexanesulfonic acid, (pH 2.5) B: Acetonitrile
Elution Mode	Gradient program: 0~2min (0%B) ➔ 2.5~3.5min (50%B) ➔ ➔ 3.51min (0%B) ➔ 12min (stop)
Oven Temp.	40 ºC
Inj. Volume	10 µL
Detector	Photodiode array detector (245 nm)

❑ Results and Discussion

Optimization of extraction solvent

Ascorbic acid is more stable in acidic condition. Reducing agent is also needed to convert DHAA back to AA. In this study, a combination of 3% MPA, 8% acetic acid and 5 mM TCEP was found to give reproducible and stable quantitation results of vitamin C in banana. Therefore, this mixed aqueous solution was used as extraction solution of banana sample.

HPLC method and calibration curve

An optimized HPLC method with UV detection (245 nm) was established using ascorbic acid standards prepared in the diluent described above. Ascorbic acid appears at 1.913 min as a sharp peak without interference in standard and actual samples as shown in Figure 2.

A linear calibration curve for the range of 0.1 µg/mL – 10 µg/mL was established with r2 greater than 0.9999. (Figure 4). Each calibrant was injected twice and the average peak area was used to plot the calibration curve. The limit of detection (LOD) and limit of quantitation (LOQ) are calculated from the lowest calibration (0.1 µg/mL) to be 0.065 µg/mL and 0.02 µg/mL, respectively. Repeatability of retention time and peak area (n=6) were evaluated with ascorbic acid standard sample of 1 µg/mL. The %RSD values of RT and peak area are 0.024% and 0.35%, respectively.

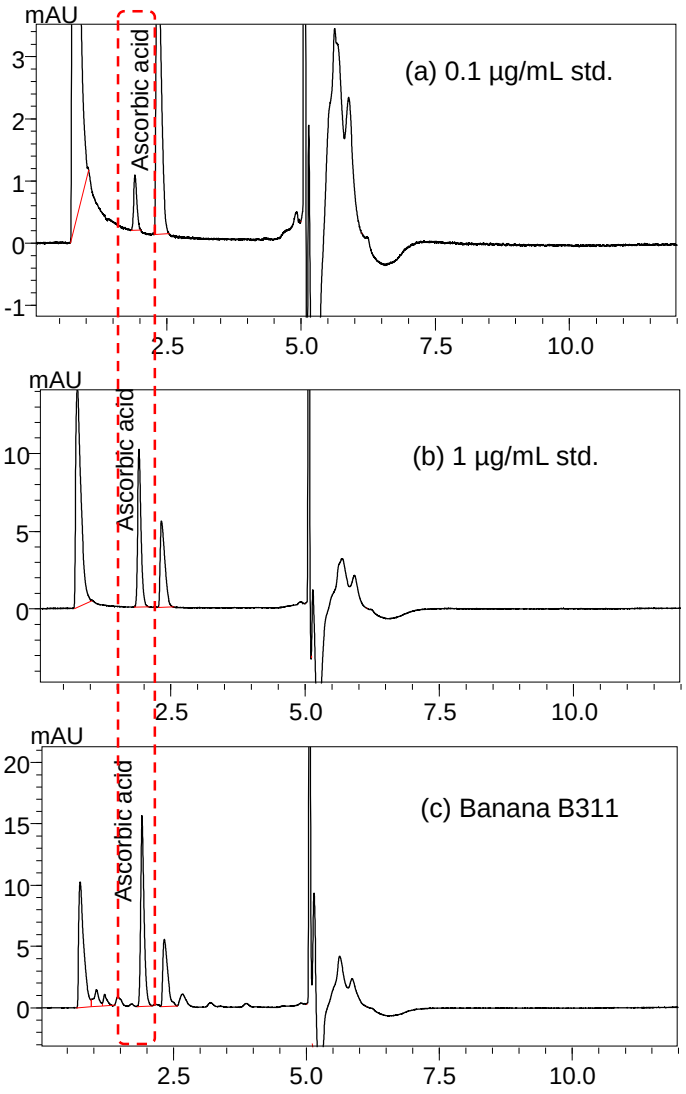

Figure 3: Chromatograms of ascorbic acid in standard 0.1 µg/mL (a), standard 1 µg/mL (b) and banana sample extract (c).

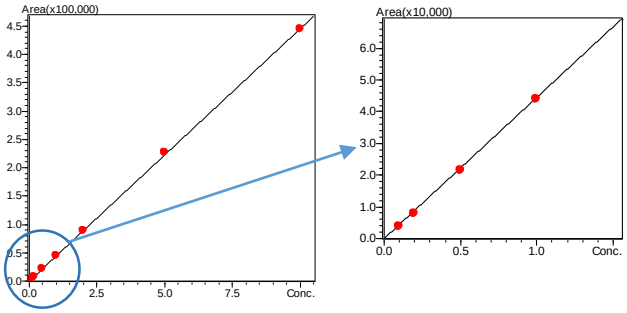

Figure 4: Calibration curve of ascorbic acid (0.1~10 µg/mL)

Recovery of ascorbic acid

Recovery of the established method was investigated with banana as the matrix. Ascorbic acid was spiked in banana at a concentration of 5mg/100g. Both spiked samples and non-spiked samples were analysed. Duplicate samples were prepared and each sample was injected thrice to HPLC. The average results obtained were used to calculate recovery. Good recovery of 107.7% was obtained.

Ascorbic acid content in banana samples

The method established was applied to quantify ascorbic acid content in three banana samples labelled as D119, S903 and B311. The results are shown in Table 2. The contents (5.3~8.0mg / 100g) measured are essentially in accordance with the reference content of vitamin C in banana (8.7mg / 100g) [5].

Table 2: Quantitation results of three banana samples

Banana Sample	Total Vitamin C (mg / 100g)
D119	6.0
S903	5.3
B311	8.0

Stability of quantitation method

Stability of the HPLC method in terms of analysis time after sample preparation was evaluated, as it is a critical factor for obtaining accurate and reliable quantitative results in routine use.

The evaluation was performed by checking the peak area of ascorbic acid in banana extract over a period of time after samples being prepared. As shown in Figure 5, the peak area variation over 10 hours is very small, indicating the excellent stability and reproducibility

of the method for quantitation of vitamin C in banana using a aqueous mixture of 3% MPA, 8% acetic acid and 5 mM TCEP as solvent for extraction of ascorbic acid from banana.

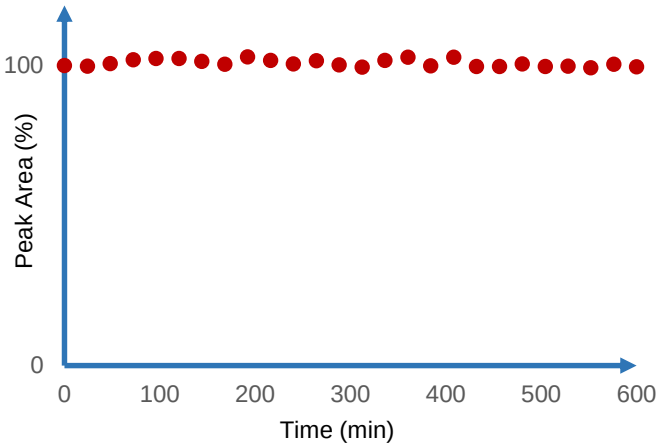

Figure 5: Peak area variation of ascorbic acid in banana extract over 10 hours by consecutive injections per 24 min.

❑ Conclusion

A HPLC method with optimized extraction procedure was established for quantitative determination of vitamin C in banana. An aqueous mixture consisting of 3% MPA, 8% acetic acid and 5 mM TCEP was used as solvent for extraction of vitamin C from banana. The presence of TECP functions as reducing reagent to convert DHAA to AA. The method was found to be stable in quantitation of vitamin C in banana in 10 hours after the sample preparation. Three banana samples were analysed and their vitamin C content range at 5.3~8.0 mg / 100g.

❑ Reference

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2. Official Methods of Analysis of AOAC INTERNATIONAL (2016) 20th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 2012.22
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5. http://www.dietandfitnesstoday.com/vitamin-c-in-a-banana.php

Shimadzu Prominence-i Plus Integrated HPLC/UHPLC system

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