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Overview of Vitamin C Analysis Method during 2000-2020

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ABSTRACT

Ascorbic acid, known as vitamin C is a naturally occurring nutrient present in many foods and an essential nutrient in food products. Several analytical methods have been used to determine ascorbic acid in pharmaceutical preparations, foodstuffs, and biological fluids. Data sources were collected from Sci-Hub, ScienceDirect, Google Scholar, and all available literature during 2000-2020. This review article studied the literature of vitamin C analysis usingUV-Vis spectrophotometry, HPLC, HP-TLC, colorimetry, iodometry, and effective voltammetry. However, UV-Vis spectrophotometry is the most widely used today because it can accurately determine vitamin C levels and is relatively inexpensive.

Keywords: Vitamin CAnalysis, Spectrophotometry, HPLC, HP-TLC, Colorimetry, Iodometric, Voltammetry.

INTRODUCTION

Scurvy is a disease associated with impaired collagen synthesis, which is shown in subcutaneous bleeding and another bleeding, muscle weakness, swollen and tender gums, and tooth loss. Scurvy can be cured by eating fresh fruits and vegetables. The active form of vitamin C is ascorbic acid which functions as a reducing equivalent donor in several important reactions. Ascorbic acid is oxidized to dehydroascorbic acid, which can be a source of vitamins in the body.[1]

Ascorbicacid is one of the essential nutrients in the body. This vitamin is readily oxidized by heat. The need for vitamin C must be met to maintain normal body metabolism. Vitamin C is beneficial for slowing skin aging, maintaining eye health, controlling blood pressure, accelerating wound healing and infection. Vitamin C acts as a catalyst in chemical reactions in the human body, so if this catalyst is unavailable, such as in a vitamin deficiency state, then the body's normal function will be disturbed.[2]

Vitamin C

Ascorbic Acidhas a molecular formula ($C_6H_8O_6$) with a molecular weight of 176.13 and contains not less than 99.0% and not more than 100.5%. The chemical formula as shown in Figure 1.[3]

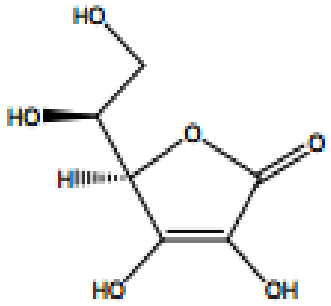

Figure 1. Chemical structure of ascorbic acid.[3]

DATA COLLECTION

In preparing this review article, the technique used was a literature study by searching for sources or literature in primary data or official books and international journals in the last 20 years (2000-2020). In making this review article, a search was carried out using online media with the keyword as follows: Vitamin C analysis, spectrophotometry, HPLC, High-Performance Thin Layer Chromatography (HP-TLC), colorimetry, iodometry, and voltammetry. Data collection was done by searching for information through sci-hub,ScienceDirect, google scholar,and other published and trustworthy journals.

ANALYTICAL METHODS

Many analytical methods have been developed for qualitative and quantitative Vitamin C, such as spectrophotometry, HPLC,TLC,colorimetry, iodometric, and voltammetry.

SPECTROPHOTOMETRY

Several Spectrophotometry methods have been used to analyze vitamin C, asshown in the following table (Table 1).

Table 1.Vitamin C analysis using Spectrophotometry

No	Sample	Solvent	Mode	Wavelength	Concentration	Vitamin C levels	Reference
1	Ascorbic acid and acetaminophen	Water	UV-Vis spectro-photometer	260 and 280 nm.	10^{3}–10^{2}M and 3 × 10^{6}–7.5 × 10^{3}M	0.21690.007 g	[4]
2	Single garlic and multiple	Aquadest	UV-Vis spectro-photometer	250 nm	60 g/ml	Vitamin C level in single garlic for 0 day was 0.28%, 10 days (0.30%), 20 days (0.31%), and 30 days (0.32%), while in multiple garlic for 0 day was 0.27%, 10 days (0.28%), 20 days (0.29%), and 30 days (0.31%)	[5]
3	Banana peel	Aquadest	UV-Vis spectro-photometer	264 nm	40 g/ml	*Barangan* banana peel has an ascorbic acid value of 0.0219 mg/ml, *banten* banana peel of 0.0173 mg/ml, wax banana peel of 0.0172 mg/ml and plantain peel has a value of 0.0253 mg/ml	[6]
4	Copper (II) - neocuproine reagent in beverages and pharmaceuticals	Distilled water	UV-Vis spectro-photometer	450 nm	$8.0\times10^{-6}$and 8.0×10^{-5}M	Ascorbic acid content 3%	[7]
5	Sweet orange peel	Water	SHIMADZU UV-1800®	525 nm	100 g/ml	Extraction with Polar Solvent 1.1350 %, Oxidation with	[8]

						$KMnO_4$0.4995%, and Oxidation with Bromine 0.4817%.	
6	Mangosteen	Distilled water	UV-Vis spectro-photometer	515 nm	100 ug / ml	0.05909 mg / g	[9]
7	Fig and passionfruit	Distilled water	Mini BioSpec Shimadzu UV-Vis	620 nm	8 ug/ml	Figs: 1.244 mg/L, passion fruit: 1.904 mg/L	[10]
8	Vitamin C tablets and syrup	Water	UV-Vis spectro-photometer	600 nm	100 g/ml	2.4%	[11]
9	Moringa leaves	Ethanol 96 %	Shimadzu	570 nm and 450 nm	350 g/ml	7.96 mg/g and 3.31 mg/g	[12]
10	Several types of chili	Aquadest	UV-Vis spectro-photometer	200 nm	2.475 g/ml	Vitamin C content in red curly chili (50 g/100 g) and followed by orange-red jablay chili (38 g/100 g), green cayenne pepper (29 g/100 g), large red chili (22 g/100 g), and large green chili (9 g/100 g)	[13]
11	Kiwi fruit	Distilled water	UV-Vis spectro-photometer	266 nm	40 g/ml	0.351%	[14]
12	Fresh longan fruit and canned longan flesh	Distilled water	UV-Vis spectro-photometer	260 nm	40 g/ml	Fresh longan fruit has an ascorbic acid value of 70.02 mg/ 100 g and canned longan flesh 35.86 mg/ 100 g	[15]
13	Red and white pomegranate	Aquadest	UV-Vis spectro-photometer	570 nm	210 g/ml	Red pomegranate has vitamin C content: 0.24475 mg/g and White pomegranate has value: 0.11577 mg/g	[16]

Vitamin C analysis was carried out at ascorbic acid and acetaminophen samples in dosage form using a UV-Vis dual-beam spectrophotometer and deionized water as solvent. Concentration ranges: 10^3–10^2M for ascorbic acid and 3×10^6–7.5×10^3M for acetaminophen. A mixture of ascorbic acid and acetaminophen was prepared: 1: 1, 1: 2, 1: 3, 2:1, and 3:1. Absorbance values were obtained at 260 and 280 nm, and gain 0.21690.007 g of ascorbic acid. This method is valid and accurate for the determination of ascorbic acid in pharmaceutical formulations.[4]

Spectrophotometry appears to analyze the effect of time variation (days) on single and multiple garlic vitamin C, using aquadest as a solvent. The maximum absorbance is 3.994 at a maximum wavelength of 250 nm. Based on the results of the Anova test, the levels of vitamin C in single black garlic against time variations are as follows: Fcount 898.489 ≥ Ftable7.59. In contrast, the vitamin C levels in multiple black garlic against time variations were Fcount922.562 ≥ FTable 7.59. The analysis found that the % of vitamin C in single garlic for 0 day was 0.28%, 10 days (0.30%), 20 days (0.31%), and 30 days (0.32%), while in multiple garlic for 0 day was 0.27%, 10 days (0.28%), 20 days (0.29%), and 30 days (0.31%). There are significant differences in vitamin C levels of single and multiple black garlic concerning time variations. This shows that this method is useful for determining vitamin C in cooking ingredients.[5]

Determination of vitamin C content of banana peel samples *(Musa paradisiaca)* was analyzed using visible UV spectrophotometry with a wavelength of 264 nm, with distilled water as a solvent. This method is based on a quantitative test. Vitamin C level in the *Barangan* banana peel was 0.0219 mg/ml; for the *Banten*banana peel sample, it was 0.0173 mg/ml, for the wax banana peel sample, it was 0.0172, and for the plantain banana peel sample, it was 0.0253 mg/ml. . Based on the results of the research that has been carried out, it can be concluded that harvest time, climate, soil and differences in growing places affect vitamin C levels. This method was carried out because the analysis using this method has accurate results.[6]
Spectrophotometric methods were used to determine ascorbic acid (AA) levels using copper(II)-neocuproine reagent in beverages and pharmaceuticals. This method is based on the oxidation of AA to dehydroascorbic acid and is measured at 450 nm. Beer's law was observed over the concentration ranges of $8.0 \times 10^{-6}$and 8.0×10^{-5}M. The standard deviation for 90 g of ascorbic acid content was 3%. This fast and inexpensive method for AA testing in complex matrices was developed to determine ascorbic acid in pharmaceutical preparations.[7]

Vitamin C in sweet orange peel extract (*citrus*) using UV-Vis Spectrophotometry (SHIMADZU UV-1800®). The oxidation method with bromine, the oxidation method with $KMnO_4$, and the extraction method with water solvents compare the results of the three ways. It was done by extracting sweet orange peel with 70% ethanol solvent by maceration. The results of the analysis showed that the levels of vitamin C in the extract of sweet orange peel obtained in the oxidation method with bromine, the oxidation method with $KMnO_4$, and the extraction method with polar solvents were 0.4817%, 0.4995%, 1.1350%, respectively.The levels of vitamin C obtained in the oxidation method with bromine and the oxidation method with $KMnO_4$ are not much different. The results of vitamin C levels using the extraction method with polar solvents are not specific for the optimal vitamin C test method because polar solvents affect the extraction process of other compounds in addition to Vitamin C.[8]

Determination of vitamin C levels of mangosteen (*Garcinia mangostana*)fruit samples was analyzed quantitatively using a visible light spectrophotometer, with aquadest as a solventwith a wavelength at 515 nm. The results showed that the mangosteen fruit contained 0.05909 mg/gvitamin C. This indicates that this method helps determine vitamin C levels in fruits.[9]

Analysis of vitamin C content in samples of figs (*Ficus carica* L.)and forest passion fruit (*Passiflora foetida* L.) was performed using visible UV spectrophotometry (Shimadzu BioSpec mini UV-Vis), with aquadest as a solvent. In determining the maximum wavelength of vitamin C, the maximum absorbance is 0.54 at a wavelength of 620 nm. The analysis found that the vitamin C in figs and passion fruit was 1.244 mg/L, 1.904 mg/L. The results of the work evaluation to determine vitamin C by spectrophotometry have high accuracy and precision.[10]

Simultaneous determination of vitamin C levels in complex mixtures, such as multivitamins, tablets and syrups, vitamin C tablets and powders, and effervescent tablets were made into powders. The powder was weighed and dissolved in water measured using a visible spectrophotometer at 600 nm oxidized quantitatively. The recovery was good, with a value of 2.4%. This method is simple for the determination of ascorbic acid in pharmaceutical preparations.[11]

Qualitative and quantitative analysis of vitamin C was determined using UV-Vis spectrophotometric method on vitamin C and -carotene samples in Moringa leaf extract (*Moringa oleifera* Lam.). Vitamin C in Moringa leaves was extracted with 96% ethanol; quantitatively, the maximum absorption was at 570 nm. The value obtained by the quantitative test was an average of 7.96 mg/g. While qualitatively using UV-Vis spectrophotometry at a wavelength of 450 nm and received an average value of 3.31 mg/g. This shows that this method is useful in determining vitamin C levels in Moringa leaves.[12] the

Levels of vitamin C contained in several types of chili using UV-Vis spectrophotometry method and quantitative tests. The highest levels of vitamin C were obtained using a wavelength of 200 nm, dissolved in distilled water. Based on the research results that have been done, it can be concluded that when blending chilies, not all seeds are mashed, so only the chili meat is smooth, so it is not detected from the levels of vitamin C in each sample. The analysis found that vitamin C levels in red curly chili (50 g/100 g) followed by orange-red chili pepper (38 g/100 g), green cayenne pepper (29 g/100 g), large red chili (22 g/100 g), and large green chilies (9 g/100 g). This indicates that this method helps determine vitamin C in foodstuffs before and after processing.[13]

Quantitative analysis of vitamin C levels in kiwifruit (*Actinidia deliciosa*)was measured using visible spectrophotometry at 266 nm, centrifuged using distilled water as solvent. The line curve regression equation y = 0.0778+0.106x with a correlation coefficient (*r*) 0.998. From these results, it can be concluded that there is a correlation, which means a positive correlation. The results of the analysis of vitamin C resulted in levels of 0.351%. This method is simple, and it shows that the spectrophotometric method is useful for determining vitamin C in fruits. [14]

Analysis of vitamin C content in fresh longan fruit and canned longan flesh (*Dimocarpus longan* L), with a descriptive method using distilled water as a solvent. Measured using visible spectrophotometry at 260 nm. From the results of the calculations obtained the regression equation y = 0.0508 x + 0.0398 with a correlation coefficient (*r*) = 0.9970. The results showed that vitamin C in fresh longan fruit and canned longan flesh was 70.02 mg/ 100 g and 35.86 mg/ 100 g. The results of the work evaluation to determine vitamin C by spectrophotometry have high accuracy and precision.[15]

Quantitative analysis of vitamin C was determined using the UV-Vis spectrophotometry method on pomegranate (*Punica granatum* L.)red and white samples. This method was carried out using specific reagents; the maximum absorption value of ascorbic acid was obtained at 570 nm. The results showed that the level of vitamin C in red pomegranate flesh was 0.24475 mg/g and pomegranate flesh was 0.11577 mg/g. This indicates that this method is useful in determining vitamin C levels in fruits.[16]

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Several HPLC methods have been used to analyze vitamin C, as shown in the following table (Table 2).

Table 2. Vitamin C analysis using HPLC

No	Sample	Mobile phase	Flow rate	Wavelength	Volume Injection	Column	Analysis Time	Vitamin C levels	Reference
1	Orange	Potassium dihydrogen phosphate in 900 ml water	0, 7 ml/min	254 nm.	5 L	Analytical and diode Hypersil Gold aQ	20 min	Orange content has a value of 0.215-0.718 g/l	[17]
2	Roses	NaH_2PO_4 and acetonitrile (93: 7)	1.2 mL/min	245nm	20 L	Column C18	20 min	417 mg/100 g	[18]
3	Peel and stem of the pineapple	Water and methanol in a ratio (95:5,% v/v)	1 mL/min	261 nm	5 m	Column C18	10 minutes	pineapple stem (121.2 mg/100 g), pineapple peel (212.9 mg/100 g)	[19]
4	Chili	Diethyldithiocarbamic acid, metaphosphoric acid	0.7 mL / min	254nm	20 L	Nucleosil C18 column (4.6 X 250 mm, 5 um)	15 min	63.1–64.9 mg / 100 g	[20]
5	Mango	Isopropanol: acetonitrile: methanol and isocratic elution at 70:20:10	0.8 mL/min	4000-500 nm	10.0 L	C18 monomer column (13 mm, 0.45 m)	10 min	0.9642	[21]

Analysis of vitamin C in oranges in the separated sample test solution. Assessed by reverse phase chromatography at a size of 250 mm × 4.6 mm, 5 m Hypersil Gold aQ Analytical Column particles. The absorbance value of ascorbic acid was obtained at 254 nm. HPLC analysis was carried out with a Thermo Electron System Surveyor, processed by extraction. The mobile phase in this study consisted of a solution of phosphate dissolved with potassium dihydrogen phosphate in 900 ml of water. The mobile phase flow rate was 0.7 ml/min, the injected volume was 5 L, so the yield obtained on the sample was 0.215-0.718 g/L.The choice of this method was madebecause the analysis using this method has good results.[17]

The HPLC method was used to determine ascorbic acid in samples of roses (*Rosa canina* L, wavelength with UV-Visible detection at 245 nm. In the C_{18} protective column, the mobile phase used was a mixture of 0.5% NaH_2PO_4 (pH 2.25) acetonitrile (93: 7). The mobile phase flow rate was 1.2mL min 1, and an injection volume of 20 L was used in the quantitative analysis. Standard solutions and samples were extracted using a yellow flask. The results obtained in the sample on the rose fruit was (417 mg 100 g). This method was carried out because the analysis using this method was sensitive. [18]

Vitamin C analysis using the High-Performance Liquid Chromatography method on the pineapple (*Ananas comosus*) liquidby-product fraction and hot water extract were assessed through HPLC-DAD (diode array detector).The separation was carried out in a C_{18} inverted phase column; the mobile phase was a mixture of water and methanol (95:5% v/v), with a 1 mL/min flow rate. With the AA detection wavelength at 261 nm. The analysis results showed that the samples obtained in the skin and stem of the pineapple were 121.2 (mg/100 g) and 212.9 (mg/100 g). Therefore, it can be concluded that the HPLC method is valid for use in analyzing vitamin C in the fruit processing industry.[19]

The HPLC method carried out the kinetics of vitamin C in the extraction process of selected pepper (*Capsicum annum*L.). Determination of vitamin C in test samples using High-Performance Liquid Chromatography was done under the following conditions: flow rate 0.7 mL/min; injection volume 20

L; wavelength 254 nm; Visible UV detector; Nucleosil C18 column (4.6 X 250 mm, 5 um); and analysis time of 15 minutes. The results showed very high vitamin levels from pepper raw materials (63.1–64.9 mg/100 g on a wet basis). The results of the analysis that the HPLC method is valid for determining vitamin C.[20]

Development and validation were carried out according to the field method used to test ascorbic acid in mangoes by the HPLC method. Determination of vitamin C of test samples using High-Performance Liquid Chromatography was done under the following conditions: flow rate 0.8 mL/min; injection volume 10.0 L; on a C_{18} monomer column with isopropanol:acetonitrile:methanol mobile phase and isocratic elution at 70:20:10; and analysis time of 10 minutes. The results showed that vitamin levels were formed from four known concentrations: y = 313531x - 12.164, R^2 = 0.9642. This indicates that this method is useful for determining vitamin C levels in foods before and after processing.[21]

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HP-TLC)

Several HP-TLC has been used to analyze vitamin C, as shown in the following table (Table 3).

Table 3.Vitamin C analysis using HP-TLC

No	Sample	Mobile phase	Wavelength	Injection volume	Rf value	Vitamin C levels	Reference
1	Dragon fruit	Ethanol: acetic acid (9.5: 0.5)	254 nm	2 l	Rf = 0.64	31.21564 ± 2.58116 ppm	[22]
2	Herb	Water, ethanol: glacial acetic acid: toluene (5.5: 1:1.5) ethyl acetate: toluene: acetone (4.5: 4:1)	254 nm	3 l	Rf = 0.74 ± 0.1	98.5631-101.916%	[23]
3	Amla	Ethanol: Acetic Acid (9.5: 0.5 v/v)	254 nm	-	Rf = 0.76 ± 0.03	607.5 mg/100 gm	[24]

Qualitative analysis of vitamin C determined by separation on purple dragon fruit (*Hylocereus polyrhizus*)using High-Performance Thin Layer Chromatography (HPTLC)-densitometry with silica gel 60 F_{254} as stationary phase. Dragon fruit was extracted using methanol by the maceration method, and the maceration extract was concentrated by boiling using a rotary evaporator. Separation of vitamin C using ethanol: acetic acid (9.5: 0.5) as the mobile phase.The staining using UV light with a wavelength of 254 nm and an Rf of 0.64. The content of vitamin C in dragon fruit was 31.21564 ± 2.58116 ppm. The above analysis shows that the method developed in this study is valid for analyzing vitamin C in the dragon fruit flesh.[22]

Water was selected as the solvent to prepare the standard solution to analyze gallic acid and ascorbic acid in herbal medicine. The quantitative estimation of gallic acid and ascorbic acid was carried out at a wavelength of 254 nm, separately on the stationary phase of TLC silica gel 60 F_{254}plates of aluminum (plate size 10 cm x 10 cm, layer thickness 0.2 mm). Ascorbic acid showed an R*f* value of 0.74 ± 0.1 using ethanol: glacial acetic acid: toluene (5.5: 1: 1.5) and gallic acid showed an R*f* value of 0.54 ± 0.1, using ethyl acetate: toluene: acetone (4.5: 4:1) as the mobile phase, the value of ascorbic acid contained in herbal medicine was 98.5631-101.916%.The results of this study are simple, precise, and accurate methods for the quantitative estimation of ascorbic acid.[23]

Determination of vitamin C in amla fruit was carried out by thin-layer chromatography (TLC) and qualitative tests. This method was carried out on an aluminum spleen coated with TLC with 60 GF silica gel as a stationary phase, and ethanol: acetic acid (9.5:0.5 v/v) as the mobile phase Rf value of 0.76 ± 0.03. Quantitative analysis was carried out in absorbance at 254 nm. Linearity regression analysis for calibration showed *r* = 0.992 and 0.986 area and peak height in the concentration range of 0.5-5.0 g. The level of vitamin C in fresh amla is 607.5 mg/100 gm, and this method is effective and efficient in identifyingvitamin C in medicinalingredients. [24]

COLORIMETRY

Several colorimetry methods have been used to analyze vitamin C, as shown in the following table (Table 4).

Table 4.Vitamin C analysis using colorimetry

No	Sample	Solvent	Temperature	Wavelength	Analysis time	Vitamin C levels	Reference
1	Thiram	Distilled water	-	527nm	6 min	<3.7%	[25]
2	Gold Nanoparticles	Water	4°C	3nm	20 min	0.9976	[26]
3	Tablets and orange juice	Water	40°C	652 nm	15 min	5.2%	[27]
4	AA in tablets and beverages.	Deionized water	70 °C	420 nm	30 seconds	2.34%	[28]
5	Gabapentin	Distilled water	-	390 nm and 531 nm	30 min	3	[29]
6	Copper	Water	40°C	425 nm	20 min	3	[30]

Identification of Vitamin C in thiram samples has been developed using a colorimetric method synthesized by reducing $HAuCl_4$ with acid ascorbate in aqueous micellar media as a solvent. The proposed method determines thiram in the range of 2.0 x 10^{-7}- 1.0 x 10^{-5}mol $L^{-1}$with a detection limit of 1.7 x 10^{-7}mol L^{-1}. Identification of vitamin C using UV 527nm, analysis time of 6 minutes obtained results <3.7%. Therefore, it can be concluded that the thiram sample tested positive for vitamin C. This method is effective and efficient in identifying vitamin C in medicinal ingredients. [25]

Analysis of ascorbic acid by alkyne-azide click reaction using gold samples using colorimetric method and water as solvent. Ascorbic acid with different concentrations and incubated for 20 minutes at room temperature 4 °C , with ascorbic acid concentration is linear in the range of 4.4 x 10^{-9}M to 3.0 x 10^{-8}M, and the regression equation is 0.078 + 0 0.0037,The value of ascorbic acid was 0.9976. Ascorbic acid can rapidly induce aggregation of activated gold nanoparticles, resulting in a red to purple (or pink) color change. It can be concluded that the proposed method is sensitive to detect vitamins at a basic level.[26]

Colorimetric methods were used for the determination of vitamin C in tabletscommercial and orange juice. Simultaneously in the mixture, water as a solvent, using UV 652 nm, quantitative assays with different concentrations and incubated for 15 minutes at 40°C. There was a difference in concentrations of 4.02 mM and 0.088 g L^{-1} commercial tablets in this study. The recovery for AA ranged from 95.1% to 104.1%, with the relative standard deviation (RSD) ascorbic acid being 5.2%.The proposed method is sensitive to detect vitamin C in pharmaceutical preparations ann beverage ingredients.[27]

Analysis of ascorbic acid was used to test for AA in tablets and beverages. Different concentrations with the standard addition method for measurements at a wavelength of 420 nm,incubatefor 30 seconds. To catalyze the oxidation of the peroxidase substrate, which produces a green product in the presence of H_2O_2, the visible color fades. Standard solutions of AA (0.05, 0.1, and 0.2 mmol/L) AA in tablets and recovery drinks ranged between 95% and 106%, with the relative standard deviation (RSD) below 2.34%. This method is sensitive and rapid for the determination of ascorbic acid in pharmaceutical formulations.[28]

The colorimetry method was developed and validatedto determine vitamin C in Gabapentin (GP) samples. This method is based on coupling GP with ascorbic acid to give colors that have maximum analytical usefulness. The analysis shows a goodcorrelation (not less than 0.999) in the 12-60 g/mL concentration range. Detection and quantification limits were 0.73 g/ml and 1.69 g/ml, 2.43 g/ml and 5.65 g/ml at 390nm and 531nm, respectively. Average recoveries for the commercial preparation (Gabapentin capsules 400mg) were 100.06 ± 1.02% and 102.83 ± 5.49%; n = 3. This method is simple, accurate, precise, and can be successfully applied to the analysis of ascorbic acid in pharmaceutical preparations.[29]

The kinetics of vitamin C in making enzyme copper samples was systematically carried out by a colorimetric method. With exploration process, and incubated for 20 minutes at 40°C, with a fluorimetric ascorbic acid sensor developed, the concentration of AA was linear within 0.5−30 M (regression equation) as follows: $F = 8.090C + 4.173$ (R^2= 0.991)) LOD was 0.144 M (S/N=3). The colorimetric sensor detection limit was 0.89(MS/N=3), that AA was oxidized to dehydroascorbic acid (DHAA) specifically. So it can be concluded that these two methods can detectaccurately in vitamin C testing.[30]

IODOMETRY

Several iodometry methods have been used for vitamin C, as shown in the following table (Table 5).

Table 5.Vitamin C analysis using iodometry

No.	Sample	Titrant	Temperature	Analysis time	Vitamin C levels	Reference
1	Fruit	Distilled water	-	-	Concentration of Papaya (1673.018 ± 136.1096 mg / 100 mL), Oranges (141.34 ± 22.07 mg / 100 mL), Lemon (199.8133 ± 126.5819 mg/100 mL), Mango (1104.459 ± 204.5954 mg/100 mL) and Tomato (542.002 ± 101.55 mg/100 mL)	**[31]**
2	Apples, orange, pineapple,and watermelon	Water	-	-	Apple used as control (7.94 ± 0.13mg/100ml). The highest vitamin C content was found in oranges (10.13±0.10mg/100ml) higher than apples, followed by pineapples (6.40±0.18mg/100ml). However, watermelon had the lowest amount of vitamin C (4.08±0.12mg/100ml).	[32]
3	Oranges, watermelon, and cashew nut	Distilled	25 ^{o}C	12 hours	Orange has an ascorbic acid value of 43.80 mg/cm^{3}; watermelon contained27.30 mg /cm^{3}, cashew nut included the highest ascorbic acid with 213.62 mg/cm^{3}	[33]
4	Ascorbic acid intablets	Water	-	25 minutes	The content of ascorbic acid per tablet in both drugs was 173.84 mg	[34]
5	Watermelon, strawberry pomegranate, green beans, red pepper, mint leaves, cabbage, spinach, and pumpkin	Aquadest	-	-	100.8%	[35]
6	Vitamin C in gold-based poly(dimethylsiloxane) (PDMS)	Water	120^{o}C	4 hours	0.023 to 7.00 mM and 30-100 mM	[36]
7	Fruits	Distilled	-	-	Tangerine has a value of ascorbic acid, 98.851mg/100mL, Papaya 90.041mg/100g, orange, 75.000mg /100mL, wine, 70 345 mg/100mL, lime, 44.138mg/100mL, banana 17.356mg/100g, and pineapple have a value of 14.036mg/100g	[37]

An iodometric method of identifying vitamin C was carried out using fruit samples and deionized water as a titrant. This adopted method was applied to test the vitamin C content. These results showed that the ascorbic acid content in each fruit were: Papaya (1673.018 ± 136.1096 mg/100 mL), Orange (141.34 ± 22.07 mg/100 mL), Lemon (199,8133 ± 126.5819 mg/100 mL), Mango (1104.459 ± 204.5954 mg/100 mL) and Tomato (542.002 ± 101.55 mg/100 mL). This method found significant differences between samples of ripeness and regional varieties of fruits, and this method is valid for identifying vitamin C in fruits.[31]

Vitamin C analysis using samples on four commercial fruit apples (*Malus domestica*), Orange (*Citrus sinensis*), Pineapple (*Ananas comosus*), and Watermelon (*Citrullus lanatus*).The iodometric titration method is based on an oxidation-reduction reaction. The analysis results showed that the levels of vitamin C studied in apple samples were used as a control (7.94± 0.13mg/100ml). The highest vitamin C content was found in oranges (10.13± 0.10mg/100ml), higher than apples,followed by pineapple (6.40±0.18mg/100ml). However, watermelon had the lowest amount of vitamin C (4.08±0.12mg/100ml). There was a significant difference in vitamin C content among the fruits; there was a significant difference ($p < 0.05$) of the respective vitamin C levels in the fruit samples. This shows that this method helps determine Vitamin C in beverage ingredients and after processing. [32]

Analysis of ascorbic acid on three fruits, namely oranges, watermelon, and cashew nuts, using the iodometric titration method of water as the titrant. This method is based on a redox oxidation reaction. Theanalysis results show that the vitamin C in oranges is 43.80 mg/cm^3, melon water contains 27.30 mg/cm^3, cashew nuts have the highest ascorbic acid with 213.62 mg/cm^3. This method is easy, safe, and fast for fruits analysis. [33]

Ascorbic acid analysis of water-soluble vitamins by the Iodometric titration methodin ascorbic acid with quantitative analysis. Standard Deviation (SD) = 0.525357. Ascorbic acid content per tablet in both drugs was 173.84 mg, within the normal range, and there was a significant difference ($P < 0.001$) of each ascorbic acid in the samples. Based on the research results, it can be concluded that the titration method was successfully used quantitatively.[34]

The iodometric method (IM)was used to develop estimates of ascorbic acid from fresh fruit and vegetables watermelon, strawberry pomegranate, green beans, red pepper, mint leaves, cabbage, spinach, and pumpkin. The efficiency of IM was compared with the standard dye method (Dm) using the titration technique, with distilled water as the titrant. Colored samples containing a wide range (0.5 - 3.0 mg), consistent recovery from 98.03 to 100.8%, showed that there was no significant difference ($p < 0.05$) between each determinant with both methods, which means the suggested method is very accurate.[35]

Quantitative analysis of vitamin C in gold-based poly(dimethylsiloxane) (PDMS) for ascorbic acid detection using the iodometric method. Water was deionized asthe titrant, stored at room temperature of 120^{o}C for 4 hours. The analysis results showed that the ascorbic acid levels studied in the samples ranged between 0.023-7.00 mM and 30-100 mM, respectively. The detection limit was 0.008 mM (S/N = 3). This method is simple and flexible for quantitative detection in assays for vitamin C.[36]

An iodine titration carried the determination of ascorbic acid with some fruits using iodine added to the starch solution, reacting to produce a purple color. The results showed that tangerines had ascorbic acid values, 98.851mg/100mL, papaya 90.041mg/100g, oranges, 75.000mg/100mL, grapes, 70.345mg/100mL, lime, 44.138mg/100mL, banana 17.356mg/100g, and pineapple has a value of 14.036mg/100g. The results of the work evaluation to determine vitamin C using the iodometric method are suggested to be very accurate.[37]

VOLTAMMETRY

Several voltammetry methods have been used for vitamin C, as shown in the following table (Table 6).

Table 6.Vitamin C analysis using voltammetry

No.	Sample	Solvent	Concentration	Peak Current	Vitamin C levels	Reference
1	Fruits	Water	1-5 mmol	580 Mv	The ascorbic acid values in green bell peppers were (182.34 mg/100g), long peppers (138.54 mg/100g), red bell peppers (125.59 mg/100g), tangelo (68.82 mg/100 cm^3), grapes (68.82 mg/100 cm^3), oranges (64 mg/100 cm^3), limes (56.57 mg/100 cm^3), papaya (55.8 mg/100 cm^3), cherries (54.86 mg/100g), and guava (51.02 mg/100g)	[38]
2	Acids (AA), dopamine (DA), and uric acid (UA)	Water	4.2×10^{-10} mol	100 mV	4.0-792.0 mol/L AA, 0.2-45.8 mol/ DA L, and 0.06 to 166.0 mol / L UA	.[39]

3	Dopamine and ascorbic acid	Distilled water	$1x10^{-4}$ mol	227mV	7.75 uA	[40]
4	Simultaneous acetaminophen and ascorbic acid	Distilled water	-	210 mV	0.2 to 150.0 m and 5.0 to 700.0 M	.[41]
5	Food and pharmaceutical preparations	Distilled water	-	960 and 1000 mV	0.0158 A/µM	.[42]
6	Paracetamol tablet mixture, DA , AA and UA	Ultrapure water	2.0×10^{-10}/M 1.5×10^{-5}M	56.9 mV	99.0%	[43]
7	Carbon paste cop- per(II) phosphate	Distilled water	0.5 -2.4 mmol L^{-1}	365 mV	2.0×10^{-5}/ 3.2×10^{-3}mol L^{-1}	.[44]
8	AA,acetaminophen (AC), and isoniazid (INZ)	Water	$1 \times 10^{7}$to 1×10^{4}M (AC) $1 \times 10^{6}$to 1×10^{4}M (AA and INZ)	100 mV	97.3 -102.6%	.[45]
9	Riboflavin and ascorbic acid (Capsule)	Water	1×10^{-3} M	50 mV	Riboflavin 9.88 ± 0.25 AA 146.6 ± 2.9	[46]
10	Quercetin	Water	1.0×10^{12}–1.0×10^{11} M	200 to 600 mV	100.00%	[47]
11	Enzyme alkaline phosphatase (ALP)	HCl	4.0 mol/L	650 mV	2.92	[48]
12	Catechol	Water	1mM	204 and 145 Mv	97.13% - 102.43%	[49]
13	Ascorbic acid (AA) and caffeine (CAF)	Distilled water	10-500 M	202 mV and 1402 mV	AA: 0.141mg/ml CAF: 0.215 mg/ml	[50]

Determination of vitamin C levels in some fruit samples was done by titrimetric methods using N-bromosuccinimide and cyclic voltammetry. Deionized water as solvent, anodic peak current at 580 Mv with ascorbic acid concentration more than 1-5 mmol. The analysis found that the highest vitamin C was found in green bell peppers (182.34 mg/100g), long peppers (138.54 mg/100g), red bell peppers (125.59 mg/100g), tangelo (68.82 mg/100 cm^3), grapes (68.82 mg/100 cm^3), oranges (64 mg/100 cm^3), limes (56.57 mg/100 cm^3), papaya (55.8 mg/100 cm^3), cherries (54.86 mg/100g), and guava (51.02 mg/100g). The results of the work evaluation to determine vitamin C by voltammetry show that it has high accuracy and precision.[38]

The simultaneous kinetics of vitamin C acid (AA), dopamine (DA), and uric acid (UA) was carried out by a modified voltammetric method, with detection limits of 1.79 mol/L AA, 0.07 mol/L DA, and 0.021 mol/L UA obtained at pH 3.0, peak current 100 mV, concentration 4.2×10^{-10} mol. These results indicate that the AA levels studied in the sample ranged from 4.0-792.0 mol/L AA, 0.2-45.8 mol/L DA, and 0.06-166.0 mol/L UA. This indicates that this method is highly selective for determining vitamin C in pharmaceutical preparations.[39]

Determination of vitamin C in pharmaceutical formulations using cadmium oxide (nanoparticles wereCdO) and carbon was carried out by a modified voltammetric method. This method has good selectivity and can be applied for direct voltammetric determination of vitamin C using the precipitation method, distilled water as solvent. Exhibiting an oxidation peak potential at 227mV, this method shows that the peak current increases rapidly in the CdO-modified CPE nanoparticles having a value of 7.75 A, which provides evidence that CdO nanoparticles having a large surface area have a high electrochemical response to AA and dopamine.[40]

Voltammetric methods were developed and validatedto simultaneously determine acetaminophen (APAP) and ascorbic acid (by using the electro-oxidation reaction. The development results showed that the levels of APAP and AA studied were in the range from 0.2 to 150.0 M (R^2= 0.998) and 5.0 to 700.0 M (R^2= 0.992), respectively. Lower detection limits were found to be 0.12 M for APAP and 3.0 M for AA. So, it can be concluded that the voltammetric method is fast and straightforward for use in the development of vitamin C in pharmaceutical and biological samples.[41]

Vitamin C kinetics in determining ascorbic acid in food and pharmaceutical samples from water-based solutions of the modified Voltammetric method. This method is based on an electro-oxidation reaction, which shows that the nanostructure modified electrode can efficiently enhance the electrocatalytic oxidation of ascorbic acid—distilled water asa solvent, peak currents around 960 and 1000 mV, pH 7.0. The results of this study indicate that the levels of AA studied were in the range from 0.08 to 380.0 M to ascorbic acid with sensitivity, and the detection limit is as low as 0.04 M. This method is fast, selective, sensitive in the determination of vitamin C in food and pharmaceutical preparations.[42]

Determination of vitamin C in a mixture of tablets: paracetamol (PAR), ascorbate (AA), dopamine (DA), and uric acid (UA) was performed by a modified voltammetric method. This method is based on an oxidation-reduction reaction, ultrapure water as a solvent, and pH 7. The results show that the peak currents are proportional to the concentrations of PAR with a dynamic linear range of 2.0×10^{-10}M 1.5×10^{-5}M, and the detection limit was 9.0×10^{-11} M and recovered, the mean value was 99.0%. This method has been successfully applied to analyze pharmaceutical preparation mixtures.[43]

The kinetics of ascorbic acid in pharmaceutical formulations with carbon paste electrodes modified with copper(II) phosphate was carried out using a voltammetric method. Determination of vitamin C in the test sample using titrimetry under the following conditions: Peak current 365 mV, pH 6.7 with a concentration of 0.5-2.4 mmol L^{-1} with a detection limit of 1.0 × 10-5 mol L^{-1}. The analysis results showed that theAA levels in the sample ranged from $2.0 \times 10^{-5}$to 3.2×10^{-3}mol L^{-1}. The method used offers very good linearity with (r = 0.9998; n = 10) with a 95% confidence level. It can be concluded that the voltammetric method is valid for the determination of ascorbic acid in pharmaceutical formulations.[44]

The simultaneous determination of ascorbic acid (AA) acetaminophen (AC) and isoniazid (INZ) was analyzed using modified voltammetry. This method is based on the oxidation reaction, water as solvent. AC concentration in the range $1 \times 10^{7}$to 1×10^{4}M, with a detection limit of 5×10^{-8}M (S/N = 3), (R^2 = 0.9989), Linear range of $1 \times 10^{6}$to 1×10^{4}M for both AA and INZ with detection limits of 3×10^{7}M AA and 5×10^{7}M INZ,AA (R^2 = 0.9995) and INZ (R^2 = 0.9981). Recovery was good, with values in the 97.3–102.6% range. This method is simple, has high sensitivity, and has excellent reproducibility in commercial drugs.[45]

Vitamin C analysis was performed on riboflavin and ascorbic acid in surfactant media simultaneously using voltammetry on a carbon paste electrode based on a redox reaction and deionized water as solvent. Showing the peak oxidation potential at 50 mV, a scan speed of 20 mVs^{-1} was used to record the peak potential or peak current and a concentration of 1×10^{-3} M. The analysis found the vitamin C content Riboflavin 9.88 ± 0.25 AA 146.6 ± 2.9. This method is valid and accurate for determining vitamin C in multivitamin pharmaceutical preparations that wed satisfactory results.[46]

Determination of vitamin C in pharmaceutical formulations on quercetin (QR) samples using square wave voltammetry (SWV) on modified glass carbon electrodes (GCE). Plain water as a solvent, oxidation peak 200 to 600 mV, the solution was prepared from the supernatant by dilution using 0.1 M acetate buffer (pH 5.5). The deveAccordingInternational Conference on Harmonization (ICH) and pro, the developed method was validated, linear, sensitive, specific, precise, and accurate. The QR concentration range was 1.0×10^{12}–1.0×10^{11} M with a detection limit (S/N = 3) 3.0×10^{13} M under optimal conditions. Recovery ranged between 98.10% and 102.30% with RSD < 2.00, with values approaching 100.00%. This method has been successfully applied for QR determination in pharmaceutical preparations.[47]

Vitamin C analysis using samples of alkaline phosphatase (ALP) and ascorbicacid 2-phosphate (AAP) was performed using a voltammetric method. This method is applied to the electrochemical test of the enzyme alkaline phosphatase (ALP) applied to a water-soluble preparation. In alkaline buffer solution, the product of ALP enzymatic hydrolysis of AAP is ascorbic acid, and standard AA was measured by the DPV method in a linear range from

10.0 to 1000.0 mol/L with a detection limit of 8.0 mol/L. Optimal conditions for the enzymatic reaction ALP and voltammetry detection optimized. Under optimal conditions, the calibration curve for the ALP assay showed a linear range from 0.4 to 2000.0 U/L with a detection limit of 0.3 U/L. The results showed that vitamin C was 2.92. This indicates that this method has been successfully applied to determine vitamin C levels in commercial pharmaceutical preparations.[48]

The voltammetric method was used for the electrochemical determination of catechol based on electrochemical oxidation with molded graphite electrodes. For electrochemical determination, catechol showed one anodic peak at 204 and 145 MV, respectively, in a phosphate buffer (pH 6). Effects of different experimental and instrumental parameters such as; type of electrolyte support, pH of the solution, applied potential, and scan speed. The peak oxidation current shows a linear range from $1x10^{-6}$to $1x10^{-4}$M with a correlation coefficient of 0.999. Lower detection limit $2.9x10^{-7}$ M, quantitative recovery 97.13% - 102.43% . The above analysis illustrates that the method developed for determining catechol in the presence of ascorbic acid has the potential for effective simultaneous quantitative analysis used for vitamin C testing.[49]

Determination of ascorbic acid (AA) and caffeine (CAF) by square wave voltammetry (SWV) using a modified glass carbon electrode (GCE). This method is based on the oxidation reaction between AA, and CAF distilled water as solvent shows peak potential at 202 mV and 1402 mV naked GCE. The concentration range was 10-500 M,while the detection limits were 1.0×10^{2} M and 3.52×10^{-3}M, respectively.The correlation coefficients were 0.992 and 0.995, respectively. The analysis found that vitamin C in AA and CAF A was 0.141mg/ml ± 0.0027 and 0.215 mg/ml ± 0.0018, respectively. The results of the work evaluation to determine vitamin C by voltammetry are faster and more accurate for the determination of vitamin C in pharmaceutical preparations. [50]

CONCLUSION

Several analytical methods have been developed to determine vitamin C, including spectrophotometry, high-performance liquid chromatography (HPLC), high-performancethin-layer chromatography (HP-TLC) colorimetry, iodometry, and voltammetry methods. In the analysis of several studies that have been conducted, the spectrophotometric method is more widely used. This can be due to UV-vis the spectrophotometry method can simply determine vitamin C levels, which is cheaper.

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