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SERVICES FOR SCIENCE AND EDUCATION

Method Development and Validation of Vitamin C Formulations Using Zeroth Order UV-visible Spectrophotometry

[1]D.S.P.C.N de Silva, [2]P.C.M Nonis, [3,*]U.K Jayasundara
[1]Department of Chemistry, Institute of Chemistry Ceylon, Rajagiriya, Sri Lanka 10100;
[2]Department of Chemistry, University of Bonn, Regina-pacis-Weg 3, 531113 Bonn, Germany;
[3]Department of Chemistry, College of Arts and Science, University of Maine at Presque Isle, Presque Isle ME, USA 04769;
udaya.jayasundara@maine.edu

ABSTRACT

Vitamin C, also known as ascorbic acid (AA), is an antioxidant and one of the essential nutrients and used as a treatment during the COVID-19 pandemic. In this research, an accurate, precise, reproducible, and cost-effective UV-visible spectrophotometric method has been developed based on the International Conference on Harmonization guidelines to determine the AA content of vitamin C in tablet formulations. The method validation study was conducted by evaluating and optimizing parameters such as linearity, range, limit of detection, limit of quantification, repeatability, inter-day precision, accuracy, and stability. A series of standard stock solutions (6 ppm to 18 ppm) was prepared by using HCl as the co-solvent and measuring the absorbance at 242 nm using a double-beam UV-Vis spectrophotometer. The calibration plot of the standard solutions resulted in a linear graph with a correlation coefficient (R2) of 0.9997 which obeyed the Beer's Law in the selected range. The stability studies confirmed that the samples were stable for up to 7 days in the refrigerator conditions and 3 days in room temperature conditions. The accuracy was tested with the spike recovery method which showed the mean recoveries occurred from 91.15% to 105.23%. In addition, sample analysis was performed using the developed method.

Keywords: Beer-Lambert law, repeatability, inter-day precision, ICH Guidelines, Healthy life, and Ascorbic acid.

INTRODUCTION

Health is the wealth. Hence those who maintain healthy lives will have a long-life span as well as be financially stable. To maintain healthy living and well-being, the human body requires a trace amount of substances called vitamins. The body does not constitute vitamins; therefore, vitamin supplements must be provided from the diet or other nutrients. Based on the age and body mass index, the vitamin requirement could be different from person to person. Vitamins are mainly organic compounds that are barely detectable in natural meals and may degrade due to conventional food processing technologies such as pasteurization, thermal treatment, and freezing.[1-4]

Table 1: Physical and chemical properties of AA

Physical properties	Chemical properties
White crystalline powder. [5,6]	Dibasic acid with pKa values of 4.2 and 11.6. [7]
	Both enolic hydroxyl groups can dissociate.

Density: 1.65 g/cm3. [8]	Act as a mild reducing agent and antioxidant.
Soluble in water, ethanol, methanol, glycerol, etc.	Oxidizes to dehydroascorbic acid (DHA) via radical intermediate by losing of the second electron.
Insoluble in diethyl ether, chloroform, benzene, petroleum ether, oils, and fats.	Ascorbate radical is stable and less in reactivity.
Molar mass: 176.12 g/mol. [8]	Available as sodium and calcium salt.
	A nucleophilic attack on ascorbic acid on a proton result in 1,3-diketone.

Among the available vitamins, vitamin C is considered to be an essential nutrient for health. The active ingredient of vitamin C is the ascorbic acid (AA). The IUPAC name of AA is ((5R)-[(1s)-1,2-Dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one) and it has a structure as shown in figure 1. Determination of properties of drug components has become more convenient with the introduction of in-silico studies which support the identification of chemical and physical properties as shown in Table 1.[5-14] The human body requires vitamin C to perform various functions, including wound healing processes ,formation of blood vessels, muscle, cartilage, and collagen in bones.[15] Since the vitamin C is an antioxidant, it acts as a shield to protect human cells from free radicals generated from harmful molecules due to tobacco smoke, x-rays, or other radiations from the sun.[16] These free radicals can lead to cancer, heart disease, and arthritis. In addition, vitamin C is essential for the growth, development, and repair of body tissues. Vitamin C is one of the main nutrients that support for human immune system and absorbs iron.[15]

Vitamin C can be found naturally in plants, fruits (oranges, lemons, grapefruit, watermelon, papaya, strawberries, mango, pineapple, raspberries, and cherries) tomatoes, green leafy vegetables, etc.[17] However, cooking may convert vitamin C into molecules that the human body does not need.[16,18] According to in vivo studies, gluconolactone oxidase which is the terminal enzyme of biosynthetic pathway of ascorbic acid synthesizing in mammals, is absent in humans. Therefore, vitamin C is unable to be produced and stored in the human body since it is a water-soluble compound, Hence, it is necessary to include vitamin C in the everyday diet.[7] Sometimes healthcare professionals recommend getting vitamin C externally since the vitamin C deficiency may result in scurvy (which causes bleeding gums, teeth loss, and weak tissue growth and wound healing), infections, allergies, osteoarthritis, cardiovascular disease, stress, and cancer.[18] However, vitamin C is commercially available as tablets and syrups as an over-the-counter drug. However, there is no guarantee that vitamin C contains the desired amount of active pharmaceutical ingredient (API). Therefore, it is important to quantitatively analyze whether the label claim is present in vitamin C. The API concentration analysis is a crucial stage in the dose formulation because each patient needs a certain dose based on their age, weight, and health condition. Both overdosing and underdosing are extremely dangerous and sometimes life-threatening.[19] According to the United States Department of Health and Human Services, the recommended daily intake of vitamin C based on age and health conditions is given in Table 2.[18] Similar to other drug substances, vitamin C may have side effects such as nausea, vomiting, and diarrhea, stomach pain or bloating, fatigue, sleepiness, insomnia, a headache, and flushed skin. Taking large doses of vitamin C supplements orally may lead to kidney stones for some persons.[7] Further, taking over 2,000 mg of vitamin C supplements orally per day for an extended period may raise the risk of serious adverse effects.[7,18]

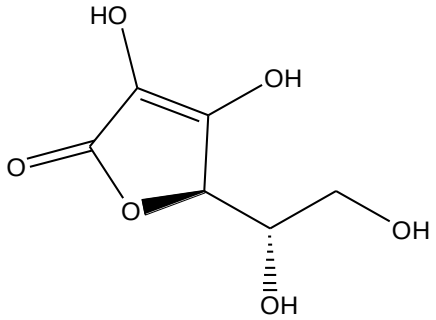

Figure 1: Chemical structure of AA

Vitamin C plays a major role in human skin health by containing a high amount of vitamin C in the skin.[20] According to the early studies vitamin C level of aged and photo-damaged skin is comparatively lesser. Vitamin C protects the human skin from harmful solar radiation and helps the formation of collagen in the skin while giving anti-oxidant protection.[21,22] Recent research discovered that exposure to ultraviolet A (UVA), 320 nm to 390 nm, light may be a major cause of photoaging and severe skin cancers. Sunlight contains more UVA than ultraviolet B (UVB) which is 290 nm to 320 nm, by more than 30 times. [17] The UVB is almost absorbed within the first 0.1 mm of skin; however, UVA can pass through the skin into deeper layers. Further, due to UVA's greater capacity to produce reactive oxygen species than UVB, UVA is particularly harmful. A family of tiny, oxygen-based molecules known as reactive oxygen species either already have an unpaired electron or have the potential to do so. Other environmental irritants in addition to UV light also produce reactive oxygen species (including smoke, pollutants, pesticides, herbicides, heat, and cold). Recent research demonstrates that vitamin C is a good antioxidant for UVA and UVB protection, making it a helpful supplement to sunscreens (but not an alternative for them). [23] Further vitamin C inhibits pigmentation by removing the dark spots of skin. Vitamin C has anti-inflammatory properties that will maintain the skin's oil production.[21,23] Instead there are plenty of benefits that can be obtained by applying vitamin C to the skin such as providing hydration, skin brightness, and reducing redness and under-eye circles. The vitamin C is also used in cosmetics as an ingredient and direct application as well.[24] Therefore, a validated and widely accepted analytical technique should be available to estimate the API content of the vitamin C used by patients. In literature, several chromatographic methods like HPLC and spectrophotometric methods like UV-visible spectrophotometry are reported.[6,8,17,25–28] These methods are time-consuming and costly, and special training is required for the operators. In this study, the applicability of the UV-visible method is tested to determine the API content of vitamin C formulations using 0.1 mol dm-3 HCl as the solvent, and the research was conducted according to the parameters of International Conference on Harmonization (ICH) guidelines. such as linearity, range, the limit of detection (LOD), the limit of quantification (LOQ), precision, accuracy, ruggedness, specificity, and stability.[5,26,29–31]

Table 2: The recommended average daily intake of vitamin C based on age and different health conditions.

Age	Male/mg	Female/mg
0-6 months	40	40
7-12 months	50	50
1-3 years	15	15

4-8 years	25	25
9-13 years	45	45
14-18 years	75	65
19+ years	90	75
Pregnant		80 – 85
Lactating mothers		115 – 120

UV-visible spectrophotometry is one of the commonly employed analytical techniques in the analysis of pharmaceutical tablet formulations which follows the Beer-Lambert law.[26,32] The UV-Vis spectrophotometry relies on the measurement of the attenuation of electromagnetic radiation by an absorbing substance. This radiation's spectral range is roughly 190 to 800 nm, and it differs from related radiation in terms of energy ranges and the type of excitation it undergoes. This attenuation is caused by interferences, scattering, absorption, or reflection. However, precise measurements of attenuation can be made by merely considering the absorbance. Within certain bounds, the absorbance is proportional to the analyte concentration to be measured as well as to the distance of the light as it travels through the sample during irradiation. The above relationship between concentration and absorbance is given by the Beer-Lambert law, which is written as,

$$\boldsymbol{A = \in cl}$$

Where,

- A = Absorbance or optical density
- C = concentration of the solute in a solution is usually given in units of moles per Liter (mol L^{-1})
- l=path length of radiation through the sample (cm)
- ∈=molar absorptivity

To ensure the accuracy and precision of the data produced by a certain and acceptable, analytical method validation is necessary. This is important to make judgments based on the facts acquired from the analysis. Several elements can affect accuracy. Validating the techniques employed to obtain those results is crucial to the accuracy of analytical results. The method used to conduct the analysis is a significant additional component that can have an impact on the outcomes. The accuracy and precision of the results can be impacted by how a sample is prepared, how measurements are taken, and how data is handled. To make sure that these factors do not hurt the results, it is crucial to check the analytical approach once more. The selection of a method is one of the most significant variables that might impact the outcomes of an analysis. Even when measuring the same thing, different methods can produce different findings. For this reason, it's crucial to pick the best strategy for the task and to validate it before applying it to decision-making. Finally, to make sure that analytical procedures are appropriate for the task at hand, they must be validated. In other words, they must be proven to be able to deliver accurate and precise findings for the particular issue that they are being used to address. Without validation, it is impossible to know whether an analytical method is appropriate for the goal for which it was developed. Today, several internationally famous organizations provide recommendations on method validation and related research such as the Codex Committee on Methods of Analysis and Sampling (CCMAS), the European Committee for

Normalization (CEN), Cooperation on International Traceability in Analytical Chemistry (CITAC), European Cooperation for Accreditation (EA), United States Food and Drug Administration (FDA) and International Conference on Harmonization (ICH), etc.[26,33–35] The present research was conducted according to the ICH guidelines which is a unique endeavor that has brought together pharmaceutical trade associations and drug regulatory bodies from Europe, Japan, and the US to talk about the technical and scientific facets of medical product registration. The significance and advantages of ICH, which was introduced in 1990, are now becoming clear to regulators. Companies and regulatory bodies are no longer required to put together and review separate submissions for each location thanks to the unified submission standards and format that ICH has developed. As more nations adopt the ICH principles, we foresee further advantages, such as promoting effective review procedures and, ultimately, a uniform regulatory language that will ease further interactions between international drug regulatory bodies.[26,30,33,36] The developed method in this research is accurate, precise, reproducible, and cost-effective.

EXPERIMENTAL

Materials and Methods

Chemicals. A standard sample of Ascorbic Acid (AA) was obtained from the State Pharmaceutical Manufacturing Corporation (SPMC), Rathmalana, Sri Lanka. The hydrochloric acid (HCl) was purchased from the Research-lab fine chem industries, Mumbai, India. Test samples of 500 mg, 250 mg, and 100 mg tablets manufactured in different countries were purchased from local pharmacies in different areas of Western Province, Sri Lanka.

Apparatus and Instruments

A double-beam UV spectrophotometer (HITAACHI-U2910) located in the Institute of Chemistry Ceylon (IChemC) laboratory was used to measure the absorbance of all prepared samples. Preparation of the Solvent. Analytical Graded HCl (assay: 35.4%, concentration: 11.45 mol dm-3) was used to prepare 0.1 M HCl solution. About 1000 mL of 0.1 mol dm-3 HCl solution was prepared by transferring 8.7 mL of Analytical graded HCl in a 1000 mL volumetric flask.

Standard Preparation

A 50 mg sample of vitamin C standard was weighed accurately and transferred into a 250 mL volumetric flask carefully. After adding about half the volume, 0.1 M HCl, the solution was homogenized for 2 minutes at 7200 rpm to improve solubility. The resulting solution was topped up to the mark with 0.1 M HCl to obtain the 200-ppm vitamin C standard solution.

Determination of Maximum Absorption Wavelength of AA

Wavelength selection was performed using 0.1 mol dm^{-3} HCl as the blank measuring absorbance in the wavelength range from 200 nm to 400 nm using a UV-Vis spectrophotometer. The maximum absorbance of the AA was observed at 242 nm which is the wavelength maxima (λ_{max}) as shown in figure 2.

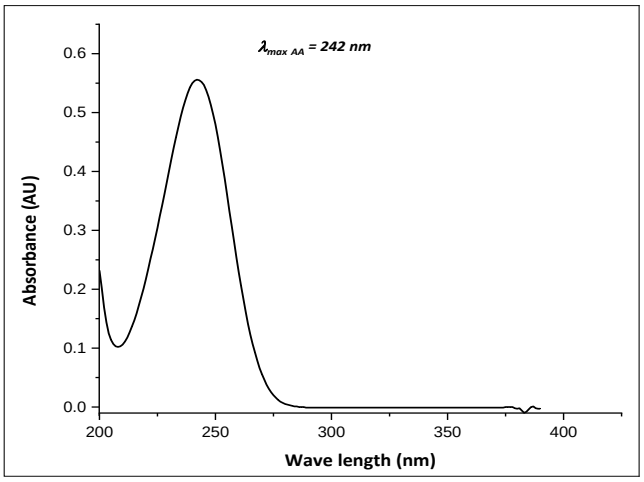

Figure 2: Absorption spectrum of AA

Preparation of Standard Calibration Curve

To determine the reproducibility of the observation, the above procedure was triplicated, and the five-point standard calibration curve was constructed by diluting the 200-ppm vitamin C stock solutions for the range 6 ppm to 18 ppm by measuring the absorbance at 242 nm which was determined at the wavelength selection.

Statistical Analysis

Microsoft Office Excel and Origin Pro 9.0 packages were used to do all the statistical analysis and structures were drawn using the ChemDraw ultra 7.0 software package.

RESULTS AND DISCUSSION

Method Validation

The method validation was conducted according to the ICH guidelines. A complete method validation was done using standard AA samples including the parameters of specificity, linearity, precision, accuracy, stability, range, limit of detection (LOD), and limit of quantification (LOQ).

Specificity:

The ability to analyze an analyte clearly in the presence of components that could be anticipated to be present is known as specificity which was determined by comparing the absorption spectrum of dilution solvent (0.1 mol dm-3 HCl) and the solution containing AA scanned between the wavelength range from 200 to 400 nm and determined for interference of any absorbance at 242 nm.

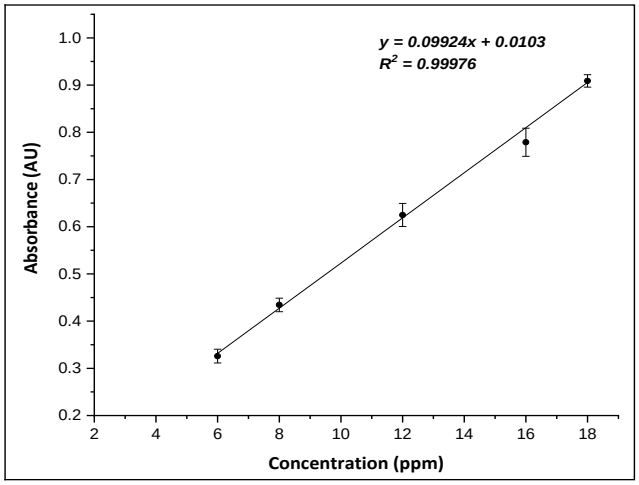

Figure 3: Standard calibration curve of AA (the error bars indicate the standard deviation of the absorbance values)

Linearity:

The ability of an analytical process to produce test results that are directly proportional to the concentration of analyte in the sample is known as linearity. Concentration of 200 ppm AA stock solution was prepared by transferring 50.00 mg of accurately ascorbic acid into the 250 mL volumetric flask using 0.1 mol dm^{-3} HCl as the dilution solvent and before it was made up to the mark, homogenized for 2 minutes in 7200 rpm. From this stock solution, a five-point calibration curve was prepared for the concentration range 6 ppm to 18 ppm. The resulting solution was prepared in triplicates, and the absorbance was measured at 242 nm. As shown in figure 3 the calibration curve was observed to be linear in the selected concentration range. The linear regression equation and the correlation coefficient were determined.

Table 3: LOD and LOQ values of AA

m	Sy	b	LOD (ppm)	LOQ (ppm)
0.047494	0.003726	0.04881	0.252	0.763

Range:

The gap between the upper and lower levels of analyte concentrations including those boundary concentrations in the sample for which it has been shown that the analytical procedure has a sufficient level of precision, accuracy, and linearity is known to be the range. According to Figure 3, the lower concentration limit was 6 ppm, while 18 ppm was the upper concentration limit. Therefore, the range used to validate the method is acceptable.

LOD and LOQ:

The lowest amount of analyte in a sample that can be detected but not always quantitated as an accurate value is the LOD of a particular analytical method while LOQ is the lowest amount of analyte in a sample that can be quantitatively measured with enough precision and accuracy. The corresponding LOD and LOQ values for the AA were determined from the generated standard calibration curve using Equations 1-3 and Figure 3. The results are shown in Table 3.

$$LOD = \frac{(3.3)\ S_y}{b} \quad (1)[19]$$

$$LOQ = \frac{(10)\ S_y}{b} \quad (2)[19]$$

$$\sigma_y \approx S_y = \sqrt{\frac{\sum_{i=1}^{N} d^2}{N-2}} = \sqrt{\frac{\sum_{i=1}^{N}[y_i-(mx_i+b)]^2}{N-2}} \quad (3)[19]$$

- σ_y=S_y=residual standard deviation of the regression line
- m=slope of the calibration curve
- xi=concentration of each point
- N=Number of values in the data set
- y_i=Average absorbance of each point
- b=Intercept of the calibration curve

Precision. Precision data were analyzed for repeatability and inter-day precision.

Repeatability:
Repeatability also known as the Intra- assay precision is the precision within the identical test conditions during short time intervals which was determined by measuring the absorbance of AA samples at 242 nm of six replicates for each of the three concentrations (6 ppm, 12 ppm, and 18 ppm). The standard deviation (SD) and percent relative standard deviation (%RSD) of the absorbance of the test samples as shown in Table 4 were calculated using equations (4) and (5) respectively.19 Since the %RSD value for the AA was below 2% as shown in Table 3, it can be concluded that the data satisfied the ICH guidelines.

Table 4: Repeatability data of AA (% RSD of the absorbance at 242 nm was calculated for each of six replicates of the three concentrations)

Sample Number	Concentration (ppm)		
	6	12	18
1	0.356	0.589	0.911
2	0.321	0.649	0.908
3	0.325	0.63	0.898
4	0.332	0.644	0.889
5	0.315	0.642	0.904
6	0.343	0.62	0.902
Average	0.332	0.629	0.902
SD	0.006	0.008	0.003
%RSD	1.706	1.361	0.323

$$SD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2} \quad (4)[19]$$

$$\%RSD = \frac{(SD)\ 100}{Average} \quad (5)[19]$$

$\bar{x}$=Mean of all values in the data set.

Inter-day Precision:

Inter-day precision was evaluated by measuring the absorbance of AA samples at 242 nm of six replicates for each of the three concentrations (6 ppm, 12 ppm, and 18 ppm) in three consecutive days. According to the results as shown in Table 5, the percent relative standard deviation (%RSD) values observed below 2% for inter-day precision and it can be concluded that the data satisfied the ICH guidelines.

Accuracy:

Accuracy is the measure of the closeness of the experimental value to the actual amount of the substance in the sample. Accuracy was evaluated by conducting a standard addition method at the recovery levels of 50%, 100%, and 150% concentrations as shown in Table 5. The standard solutions (100 ppm) were spiked with the test sample solutions (100 ppm) and corresponding absorbance values were measured at 242 nm. To do the accuracy test, seven brands of AA were used and as shown in column 4 of Table 6, the recovery percentages were calculated using the obtained data. The equation 6 was used for the calculations of the recovery. Since the recovery percentages of each of the tablet brands were observed in between 90% and 110%, it is concluded that the accuracy of the method was satisfied with the ICH guidelines.[19]

$$\%\ Recovery = \frac{Amount\ found\ (\ amount\ recoverd)100}{Amount\ added\ (\ total\ amount\ present)} \quad (6)$$

Table 5: Inter-day precision data of AA

Concentration (ppm)	Average absorbance (AU)	%RSD
6	0.321 ± 0.004	1.327
12	0.618 ± 0.005	0.821
18	0.884 ± 0.005	0.585

Table 6: Accuracy data of vitamin C

Tablet brand	Tablet Concentration (ppm)	% Recovery Level	Recovery Percentage %	Mean Recovery %	%RSD
	5	50	96.35		
A	5	100	96.25	96.53 ± 0.32	0.33
	5	150	96.98		
	5	50	93.53		
B	5	100	92.95	93.23 ± 0.24	0.25
	5	150	93.21		
	5	50	91.35		
C	5	100	91.12	91.15 ± 0.15	0.16
	5	150	90.98		
	5	50	105.13		
D	5	100	104.98	105.23 ± 0.26	0.25
	5	150	105.59		
	5	50	109.41		
E	5	100	107.81	108.45 ± 0.69	0.64
	5	150	108.13		
	5	50	97.52		
F	5	100	97.52	97.09 ± 0.61	0.63

	5	150	96.23		
	5	50	92.56		
G	5	100	92.15	93.01 ± 0.54	0.58
	5	150	94.32		

Stability:

As shown in Table 7, 6 ppm, 12 ppm and 18 ppm of AA samples were prepared in triplicates using three 200 ppm AA stock solutions and, the absorbance of each sample was measured at 242 nm in 24, 48, 72 hours, 7 days and 14 days of time intervals to determine stability. The stability test was conducted separately for room temperature (27℃ – 30℃) conditions and refrigerator conditions (10℃ – 15℃). From the absorbance values, it can be concluded that the samples were stable for up to 3 days at room temperature and 7 days at refrigerator conditions by meeting the 10% acceptance criteria of ICH guidelines.[29]

Table 7: Stability data of vitamin C (Room temperature:27℃ – 30℃ and Refrigerator conditions: 10℃ – 15℃)

Temperature conditions	Concentration (ppm)	Initial Absorbance (AU)	Percent Stability (%)				
			24 hours	48 hours	72 hours	7 days	14 days
Room Temperature	6	0.353	99.14	98.2	90.9	74.14	56.8
	12	0.646	99.6	99.3	95.8	82.9	72.9
	18	0.915	99.8	99.6	93.8	89.3	79.8
Refrigerator conditions	6	0.371	99.7	99.4	98.9	91.9	87.3
	12	0.657	99.6	99.3	98.7	92.9	83.8
	18	0.938	99.8	99.6	98.8	94.9	87.6

Statistical Analysis:

According to the data of Table 6, it can be concluded that AA was stable at room temperature condition up to 3 days while the refrigerator conditions for up to 7 days since they met the acceptance criteria. Therefore, AA had higher stability in refrigerator conditions than at room temperature; however, there was no stability after 14 days in the media it was prepared.

Market Sample Analysis:

Seven different brands of AA manufactured in different countries were purchased from different pharmacies in the western province of Sri Lanka. These tablets were analyzed according to the developed method in this research, and as shown in Table 8, the percentages of active ingredients were calculated. tablets were purchased in different dosages as shown in the second column of table 8. Brand A and C were 100 mg tablets. Their API contents were obtained as 106 ± 1.06 mg and 81. 07 ± 1.27 mg respectively as shown in the 3rd column of table 8. The obtained API content of tablet D was 190.47 ± 1.08 mg even though the label claimed was 250 mg. As shown in the 4th column the % API content of brand D was 76.19%. rest of the tablet brands which are B, E, F, and G were 500 mg tablets. The obtained API contents of those tablet brands were 412.18 ± 0.52 mg, 421.15 ± 2.06 mg, and 453.50 ± o.25 mg respectively. The percentage API contents of 500 mg tablets varied from 82.43% to 90.7 % as shown in column 8. Brand A and brand G contained the highest amount of active ingredient, which is 106.06% and 90% respectively, while brand D, contains the lowest percentage of active ingredient. Therefore, it can be concluded that the brand A and G would be the best

compared to all other brands tested. Among the selected tablets brand G and A did not contain any flavors but others contained different flavors such as orange, strawberry, etc. Further, the brand G was an Australian brand.

Table 8: Market sample analysis for API contents

Brand	Label claim (mg)	Mass of API (mg)	% API content
A	100	106.06 ± 1.06	106.06
B	500	412.18 ± 0.52	82.43
C	100	81.07 ± 1.27	81.07
D	250	190.47 ± 1.08	76.19
E	500	421.15 ± 2.06	84.23
F	500	415.75 ± 2.16	83.15
G	500	453.50 ± 0.25	90.7

CONCLUSION

The UV-visible spectrophotometric method was developed for the determination of vitamin C concentrations in commercially available tablets in pharmacies according to the ICH guidelines. According to the test sample analysis the results have indicated the significant deviation of the API content of the flavoured vitamin C tablets (Brand B, C, D, E, and F). Brand A and G were best among the selected tablet brands. The results demonstrated that the method was validated, as each of the measured parameters has met the acceptance criteria of the ICH guidelines. The developed analytical method was validated and found to be simple, sensitive, rapid, linear, accurate, and precise per ICH guidelines. It can be concluded that the developed analytical method can be used for quantitative analysis of the active pharmaceutical ingredient (API) content of vitamin C.

References

1. Tomas, M., Jafari, S. M, Influence of Food Processing Operations on Vitamins. Encyclopedia of Food Chemistry, Elsevier, 2019: p. 129-139 (https://doi.org/10.1016/B978-0-08-100596-5.21661-2)

2. Mieszczakowska-Frąc, M., Celejewska, K., Płocharski, W, Impact of Innovative Technologies on the Content of Vitamin C and Its Bioavailability from Processed Fruit and Vegetable Products. Antioxidants, 2021. 10(1): p.54 (https://doi.org/10.3390/antiox10010054)

3. Yadav,S., Arora, S.K., Vats, S, Vitamins and Minerals: A Review on Processing Losses and Strategies to Control It. Concepts Dev. Agron, 2023, 12(2): p 1178 (http://dx.doi.org/10.31031/mcda.2023.12.000783)

4. H. Hamishehkar., et al. Vitamins, Are They Safe? Adv. Pharm. Bull. 2016, 6(4): p 467 (https://doi.org/10.15171/apb.2016.061)

5. Mavanga,T., et al. Development and Validation of a UV/vis Spectrometric Method for Determination of Ascorbic Acid in Pur State (Raw Material) and Dosage Forms. Int. J. Innov. Sci. Res. Technol. 2021, 6(4): p 696 (https://ijisrt.com/assets/upload/files/IJISRT21APR349.pdf)

6. Bonde, S., et al. Determination of vitamin -c from formulated drug by hplc and uv/vis-spectrophotometry techniques. Int. J. Anal., Pharm. Biomed. Sci. 2019, 4 (5): p 7

7. Levine,M., Ebenuwa, I., Violet, P. C, Vitamin C. Elsevier, 2020, p 241-262 (https://doi.org/10.1016/B978-0-12-805378-2.00018-8)

8. Hussein,A.Z.M, Spectrophotometric Determination of Ascorbic acid in Aqueous Solutions and in Pharmaceuticals formulations. J. Al-Nahrain Univ. Sci.2013, 16(3): p 65-71 (http://dx.doi.org/10.22401/JNUS.16.3.08)

9. Lushchekina,S.V., et al, Supercomputer Modeling of Dual-Site Acetylcholinesterase (AChE) Inhibition. Supercomput. Front. Innov.2018,5(4):p 89-97 (https://doi.org/10.14529/jsfi180410)

10. Fan, J., Fu, A., Zhang, L, Progress in molecular docking. Quant. Biol,2019,7(2): p 83-89 (https://doi.org/10.1007/s40484-019-0172-y)

11. Deshapriya,U.S., et al, In silico study for prediction of novel bioactivities of the endophytic fungal alkaloid, mycoleptodiscin B for human targets. Mol. Graph. Model. 2020,102 (https://doi.org/10.1016/j.jmgm.2020.107767)

12. Ascorbic Acid.National Center for Biotechnology Information (PubChem), https://pubchem.ncbi.nlm.nih.gov/compound/Ascorbic-Acid ,(accessed 16, April, 2023).

13. SwissADME, http://www.swissadme.ch/index.php ,(accessed 16, April ,2023).

14. Banerjee, P., et al, ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Res.2018,46(2): p 257-263 (https://doi.org/10.1093%2Fnar%2Fgky318)

15. Medical news today, https://www.medicalnewstoday.com/articles/219352 ,(accessed April 16, 2023).

16. WebMD, https://www.webmd.com/diet/features/the-benefits-of-vitamin-c ,(accessed April 16,2023).

17. Khan, M.M.R., et al, A Simple UV-spectrophotometric Method for the Determination of Vitamin C Content in Various Fruits and Vegetables at Sylhet Area in Bangladesh. J. Biol. Sci. 2006,6(2): p 388-392 (https://doi.org/10.3923/jbs.2006.388.392)

18. Ordman, A.R, Vitamin C twice a day enhances health.Health.2010,2(8): p 819-823 (http://dx.doi.org/10.4236/health.2010.28123)

19. Jayasundara,U.K., Herath, H.M.M.B., Kaushalya,P.V.N, Method Development, Validation, and Concentration Determination of Metformin Hydrochloride and Atorvastatin Calcium Using UV-Visible Spectrophotometry. J Anal Bioanal Tech.2021,12(2): p 2155 https://www.omicsonline.org/open-access/method-development-validation-and-concentration-determination-of-metformin-hydrochloride-and-atorvastatin-calcium-using-uvvisible-115091.html

20. Dulińska-Molak,I., et al, Determining the effectiveness of vitamin C in skin care by atomic force microscope. Microsc. Res. Tech.2019, 82(9): p 1430-1437 (https://doi.org/10.1002/jemt.23295)

21. Pullar,J.M., Carr, A.C., Vissers, M.C.M, The Roles of Vitamin C in Skin Health. Nutrients.2017,9(8):p 866 (https://doi.org/10.3390/nu9080866)

22. Pinnell, S.R., Madey, D.L, Topical Vitamin C in Skin Care. Aesthet Surg J. 1998, 18(6): p 468-470 (https://doi.org/10.1016/s1090-820x(98)70085-8)

23. Al-Niaimi, F., and Chiang, N. Y.Z, Topical Vitamin C and the Skin: Mechanisms of Action and Clinical Applications. J Clin Aesthet Dermatol. 2017,10(7): p 14-17 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5605218/)

24. Healthline, https://www.healthline.com/health/beauty-skin-care/vitamin-c-serum-benefits , (accessed April 27, 2023).

25. Mitić, S.S., et al, Rapid and Reliable HPLC Method for the Determination of Vitamin C in Pharmaceutical Samples. Trop. J. Pharm. Res. 2011,10 :p 105 (https://doi.org/10.4314/tjpr.v10i1.66549)

26. Behera,S., et al, UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. J. Anal. Bioanal. Tech. 2012, 3 (https://www.omicsonline.org/uv-visible-spectrophotometric-method-development-and-validation-of-assay-of-paracetamol-tablet-formulation-2155-9872.1000151.php?aid=9714)

27. K. A. Gaidhani, K.A., et al, Lyophilization / freeze drying – a review. World J. Pharm. Res.2015,4: p 516 (https://wjpr.net/abstract_show/3298)

28. A. Selimović,A., et al, Direct spectrophotometric determination of L-ascorbic acid in pharmaceutical preparations using sodium oxalate as a stabilizer. Int. J. Basic Appl. Sci.2011,.11(2): p 106-109

29. ICH Q2(R2): Validation of Analytical Procedures (2022)

30. ICH - Q14: Analytical Procedure Development (2022)

31. ICH Q2 (R1): Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry (2021)

32. Passos, M.L.C., Saraiva, M. L. M. F. S, Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies. Meas. 2019,135: p 896-904 (https://doi.org/10.1016/j.measurement.2018.12.045)

33. Molzon,J.A., et al, The Value and Benefits of the International Conference on Harmonisation to Drug Regulatory Authorities: Advancing Harmonization for Better Public Health. Clin. Pharmacol. Ther.2011, 89(4): P 503-512 (https://doi.org/10.1038/clpt.2011.10)

34. Harmonized Guidelines for the In-House Validation of Methods of Analysis (2001)

35. Jurado,F., et al,A review of the Accreditation Bodies and Processes in Europe. A vision from the Engineering. Frontiers in Education, 2005. FIE '05. Proceedings 35th Annual Conference, (2005), Indianopolis, IN, USA, Frontiers in Education, Indianapolis, 2005, F2D. (https://doi.org/10.1109/FIE.2005.1612047)

36. Pancham,Y.p., Girish,B., Sanjay, S.S, UV-Spectrophotometric method for quantification of ascorbic acid in bulk powder. Pharma Innov. J. 2020, 9(5): p 5-8 (https://www.thepharmajournal.com/archives/?year=2020&vol=9&issue=5&ArticleId=4617)