ISSN No:-2456-2165

Development and Validation of a UV/vis Spectrometric Method for Determination of Ascorbic Acid in Pur State (Raw Material) and Dosage Forms

T. Mavanga[a], J. Mankulu[b], M. Mayangi[b], A. Mbenza[b], D. Mana[b], J. Mavar[b], JK. Mbinze [a *]

[a]Laboratory of drugs and health products analysis, Faculty of Pharmaceutical Sciences, University of Kinshasa PO.BOX 212 Kinshasa XI, Democratic Republic of the Congo.

[b]Laboratory of quality control and drugs analysis and diets survey, Faculty of Pharmaceutical Sciences, University of Kinshasa PO.BOX 212 Kinshasa XI, Democratic Republic of the Congo.

***Corresponding Author:** Laboratory of drugs and health products analysis, Faculty of Pharmaceutical Sciences, University of Kinshasa PO.BOX 212 Kinshasa XI, Democratic Republic of the Congo.

Abstract:- A new and economical UV/vis spectrophotometric method using 0.1M hydrochloric acid as a dilution solvent for the estimation of vitamin C pure and in its dosage forms was developed in this work. The linearity study was performed over a range of concentrations from 2 to 12 µg / ml. The maximum absorption wavelength found was 243 nm. Linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, specificity / selectivity and robustness were chosen as criteria for validation of the method according to the requirements of the International Conference of 'Harmonization (ICH). The developed method was used to quantitatively determine vitamin C in its pharmaceutical forms marketed on the local market in the City of Kinshasa, DR Congo. The results obtained in this research demonstrate that the method is exact, precise, reproducible (% RSD <2%), simple, sensitive, robust, selective / specific and rapid.

Keywords:- *Ascorbic Acid, Spectrophotometry, UV/vis, Development, Validation.*

I. INTRODUCTION

Vitamin C or ascorbic acid is the lactone of a hexuronic acid that is closely related to C6 sugars. It is a water-soluble vitamin whose total deficiency causes scurvy (a fatal disease) and the partial deficiency promotes various diseases such as infections, allergies, osteoarthritis, cardiovascular disease, stress and cancer. Ascorbic acid and dehydro-ascorbic acid have the same anti-scurvy properties, and above all represent the two oxidized and reduced forms of a reversible redox system. Ascorbate is a powerful reducing agent capable of trapping and eliminating free radicals (superoxide anion or hydroxyl OH- radical) produced by the metabolic activity of cells, especially during their fight against pathogens. Ascorbate is able to prevent lipid peroxidation induced by oxygen free radicals by promoting the generation of vitamin E. In addition, vitamin C inhibits the formation of oxidation products and the glycation of proteins.

It participates in the hydroxylation of steroid hormones and in the formation of collagen. It is also necessary for the absorption of iron from the gastroduodenal mucosa [1, 2 and 3]. Vitamin C is indicated in clinical circumstances such as fatigue, anorexia, malnutrition, inflammatory digestive pathologies, smoking, alcoholism, severe anemia, dysmyelopoiesis, respiratory ailments, wound healing disorders, dermatological disorders (follicular hyperkeratosis, dull and brittle hair, bruising , haemorrhagic gingivitis), kidney dialysis and if scurvy is suspected [4]. Chemically ascorbic acid is a (5R) -5 - [(1S) -1,2-dihydroxyethyl] -3,4 dihydroxyfuran-2 (5H) -one; whose chemical structure is as follows [5]:

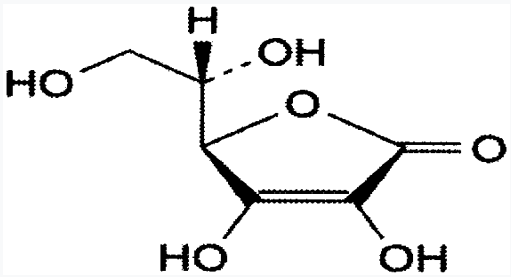

Figure 1. Chemical structure of Ascorbic Acid

For its quantitative analysis, several pharmacopoeias report to us chromatographic methods such as HPLC and volumetric methods (iodometry, cerimetry, etc.) [6, 7]. The first method, which is very expensive, is not accessible in all laboratories in developing countries. Volumetric methods are used less and less nowadays because of their lower sensitivity. The literature reports UV / vis spectrophotometric methods for estimating ascorbic acid; but most of them apply to plant material [8, 9 and 10]. To our current knowledge, a UV/vis spectrophotometric method applied to the pharmaceutical forms of vitamin C, uses the methanol - water mixture (50:50) as dilution solvent [11].

ISSN No:-2456-2165

Methanol, like so many other organic solvents, is expensive for our laboratories. Another method, colorimetric, involves the diazotization between 2,4-dichloroaniline and ascorbic acid; seems to us to be indirect [12].

The objective of this research was to develop and validate a simple UV/vis spectrophotometric method using 0.1M HCl as dilution solvent, due to its availability in our laboratories and for determination of ascorbic acid in raw material and in its dosage forms according to the recommendations of the International Harmonization Conference [13, 14, 15 and 16].

II. MATERIALS AND METHODS

2.1 Materials and solvents

We used the Thermo Scientific UV/vis spectrophotometer and a precision SV series analytical balance.

The Ascorbic Acid standard was purchased from Shandong Luwei Pharmaceutical CO., LTD (China), Batch number: 190142047, degree of purity: 100.00%. The pharmaceutical forms of ascorbic acid, in particular the tablets (Branded MyVita + Zinc), the solution for injection as well as the syrup (From Phatkin Laboratory) were purchased on the Kinshasa market (DR Congo). Hydrochloric acid (Merck KGaA, Germany) was analytical grade.

2.2. Methods

2.2.1. Preparation of 0.1M hydrochloric acid

0.1M hydrochloric acid was prepared by diluting with deionized water in a 1000 ml flask, 8.5 ml of concentrated hydrochloric acid [17].

2.2.2. Preparation of ascorbic acid stock solution

20 mg of ascorbic acid were dissolved in 100 ml of 0.1M HCl and the volume was adjusted to the mark with the same solvent in a 200 ml volumetric flask in order to obtain a stock solution of 100 µg / ml.

2.2.3. Determination of the maximum absorption wavelength of vitamin C

A suitable aliquot was diluted with 0.1M hydrochloric acid solution to obtain a solution of 10µg / ml. This solution was scanned in a spectrophotometer between 200 and 400 nm. Vitamin C showed maximum absorption at 243 nm. This wavelength was used for the rest of the tests.

2.2.4. Preparation of the solution for method validation

2.2.4.1. Solution for linearity

Adequate dilutions of the vitamin C stock solution were made to obtain solutions of final concentrations equal to 2; 4; 6; 8; 10 and 12 µg / ml.

The respective absorbances were measured at 243 nm along 3 consecutive days in the same week and the regression line was constructed by integrating the absorbances against the respective concentrations. Figure 2 illustrates the regression line obtained in this study [18, 19] (n = 6).

2.2.4.2. Solution for specificity / selectivity

The 0.1M HCl solution and that containing 10 µg of vitamin C were scanned in order to observe a possible interference between the two solutions at the maximum absorption wavelength of the vitamin C solution. It was observed that 0.1M hydrochloric acid did not absorb at this maximum absorption wavelength of the solution containing vitamin C. Figures 3 and 4 illustrate the two spectra [11].

2.2.4.3. Solution of intra and inter-day precision

The intra-day and inter-day precision of the proposed method was determined by analyzing 6 times three different concentrations (4; 8 and 12 µg / ml) of the standard solution of ascorbic acid. The intra-day precision was determined on the same day; while the inter-day precision was determined on three consecutive days of the same week. Absorbances were measured and the mean concentrations found, the relative standard deviations (RSD) and the relative errors were calculated.

2.2.4.4. Solution for the correctness of the method

The accuracy of the method was calculated by estimating the recovery rate at three concentration levels (80%; 100% and 120%) by the standard addition method: an amount of powder equivalent to 20 mg of ascorbic acid (MyVita + Zinc) obtained from 20 tablets finely ground using mortar and pestle, was exactly weighed and transferred into a 200 ml volumetric flask containing 100 ml of 0.1 M hydrochloric acid. The mixture was stirred in an ultrasonic bath for 5 minutes, and then the volume was adjusted to the mark with 0.1N HCl. After mixing and filtration using Whattmann filter paper (No. 41), 5 ml of pre-analyzed filtrate was transferred to three different 50 ml volumetric flasks in which the ascorbic acid concentration was fortified with 4, respectively; 5 and 6 ml of standard solution and the volume were adjusted to the mark with 0.1N HCl finally to obtain spiked solutions of respective concentrations of 80%; 100% and 120%. Absorbances were measured. The recovery rates were calculated using the following formula:

$$\% = C_f - C \times \frac{100}{C_a}$$

Where C_f is the concentration of the fortified sample, C as the concentration of the unfortified sample and C_a, the concentration of the added substance [12].

2.2.4.5. Solution for the robustness of the method

The robustness of the method was determined by performing 6 measurements of the absorbances of the stock solution containing ascorbic acid at lengths of plus or minus 243 nm ± 5 [20, 21, 22 and 23].

ISSN No:-2456-2165

2.2.4.6. Solution for limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the proposed method were calculated on the basis of the data obtained from the study of linearity: the slope of the regression line and the standard deviation were calculated. The limit of detection (LOD) and the limit of quantification (LOQ) were determined according to the following mathematical relationships [10] [11] [16]

$$LOD = \frac{S_b \times 3}{S}$$

$$LOQ = \frac{S_b \times 10}{S}$$

Where, S_b is the standard deviation on the intercept; S, the slope of the regression line.

2.2.4.7. Solution for the determination of vitamin C

To measure the vitamin C in the tablets, 20 My Vita + Zinc tablets were weighed one by one and their average weight was determined; then all of the tablets were reduced to impalpable particles using mortar and pestle. An aliquot of this powder was dissolved and then diluted with 0.1M hydrochloric acid to finally obtain a solution of 10 µg / ml. For syrup and solution for injection of ascorbic acid; adequate volumes were respectively diluted with 0.1M hydrochloric acid so as to obtain solutions of the same concentration as that obtained in the case of tablets. Absorbances were measured at 243 nm. Table 5 shows the statistical dosage parameters of vitamin C in its pharmaceutical forms.

III. 3. Results and discussion

3.1. Linearity

The proposed method showed good linearity for a series of ascorbic acid concentrations ranging from 2-12 µg / ml in 0.1 M hydrochloric acid, used as a solvent. The equation of the regression line calculated as shown in Fig. 1 was: Y= 0,0555x avec R^2= 0, 9999

The analytical parameters of the ascorbic acid linearity study are cosigned in Table 1.

Table 1. Analytical parameters of the linearity of ascorbic acid (n = 6)

Parameters	Ascorbic Acid
λmax(nm)	243
Limit of Beer(µg/ml) detection	2-12
Linear regression Y= bx +a	Y= 0,0555x
Coefficient of determination (R^2)	0,9999
LOD	0,6738
LOQ	2,2461

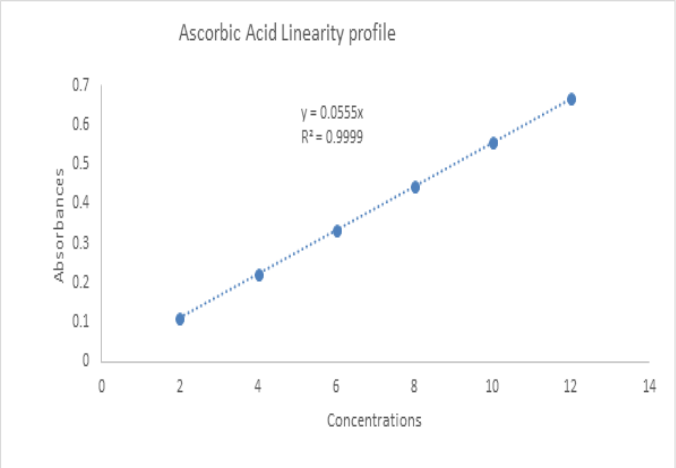

Figure 2. Ascorbic Acid Regression Line

3.2. Limit of detection (LOD) and limit of quantification (LOQ) of the method

The limit of detection (LOD) and limit of quantification (LOQ) calculated from the linearity study data were equal to 0.6738 µg / ml and 2.2461 µg / ml, respectively. The results are shown in Table 2.

3.3. Intra and inter-day precision

The intra and inter-day precision was determined by measuring 6 times the absorbances of three concentrations of the standard solution (2; 8 and 12 µg / ml). The method exhibited good precision. The calculated statistical parameters are shown in Table 2.

Table 2. Statistical data of the intra-day and inter-day precision of the method (n = 6).

Introduced Concentrations (µg/ml)	4	8	12
Intra-day Concentrations found (µg/ml)	3,9478	7,9856	11,9784
% found	98,6960	99,8201	99,8200
Relative Bias (%)	-1,305	- 0,1800	-0,18
Standard Deviation	0,00187	0,00212	0,0333
RSD (%)	0,8542	0,4777	0,4621
Inter-day Concentrations found (µg/ml)	3,9688	8,0035	12,0203
% found	99,2206	100,044	100,1698
Relative Bias (%)	-0,78	0,0437	0,1691
Standard Deviation	0,0017	0,0017	0,0032
RSD (%)	0,787	0,787	0,485

ISSN No:-2456-2165

3.4. Accuracy of the method

The accuracy of the method was determined by the metered additions method at three concentration levels (80; 100 and 120%). As shown in Table 3; the recovery rates and their averages, the SDs and % RSDs calculated at all three concentration levels; prove the correctness of the developed method.

Table 3. Method Accuracy (n = 6).

N^{O}	My Vita Tablets (µg/ml)	% standard added	Standard recovered(µg/ml)	% of recovery	Means	SD	%RSD
1	10,1348	80 80 80 80 80 80	8,300 8,264 8,156 8,264 8,174 8,138	103,75 103, 30 101,95 103,30 102,18 101,73	102,69	0,8437	0,8216
2	10,1348	100 100 100 100 100 100	10,224 10,314 10,242 10,272 10,182 10,170	102,24 103,14 102,24 102,72 101,18 101,70	102,19	0,6997	0,6848
3	10,1348	120 120 120 120 120 120	12,185 12,155 12,113 12,089 12,209 12,167	101,54 101,29 100,94 100,74 101,74 101,39	101,27	0,3737	0,3690

3.5. Specified / Selectivity

By comparing spectra of the ascorbic acid solution and 0.1M HCl solution used as a blank, we observed that the latter did not absorb at the maximum absorption wavelength of the ascorbic acid solution (λm = 243nm); which proves that there was no interference between the two solutions. And the specificity as well as the selectivity of the method was thus validated. Figures 2 and 3 respectively illustrate the spectra of vitamin C and that of 0.1M HCl.

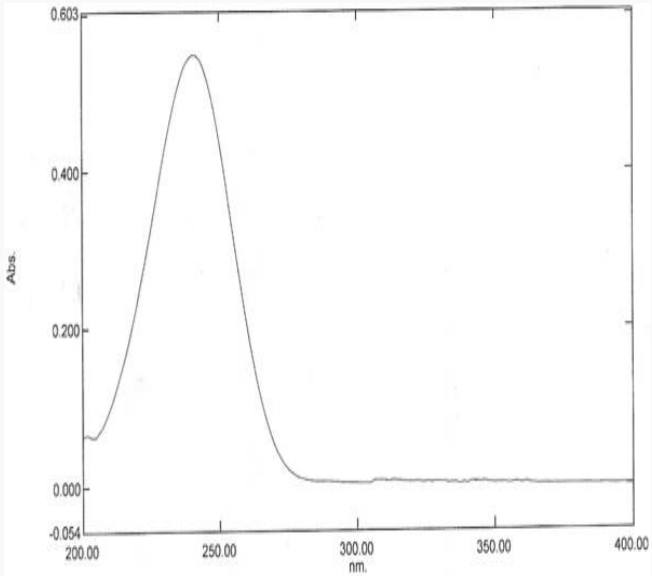

Figure 3. Ascorbic Acid solution spectrum

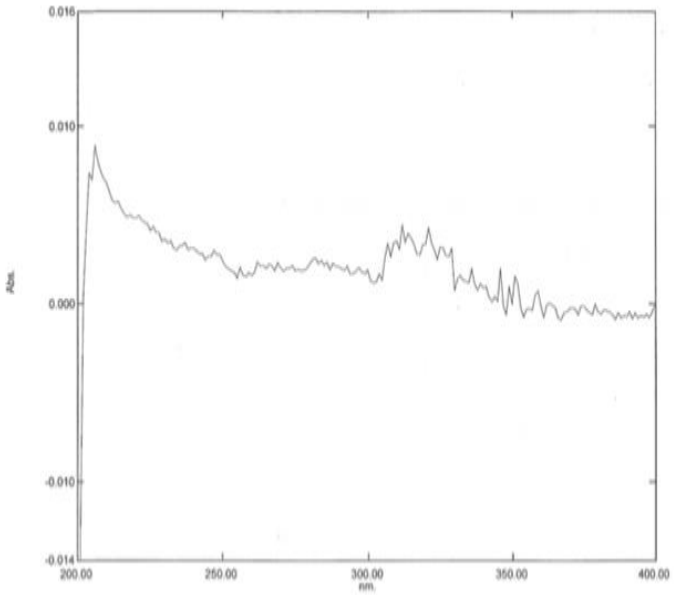

Figure 4. 0,1M Hydrochloride acid spectrum

3.6. Robustness of the method

The robustness of the method was calculated by deliberately changing the maximum absorption length to plus or minus 5nm (λmax ± 5nm). In all% RSD remained below 2%. The statistical data of this test are reported in Table 4.

Table 4. Statistics of the robustness of the method (n = 6).

Sr.Nº	λ±5nm(243)	Conc.(µg/ml	Absorbances	Means	SD	RSD	%RSD
1.	243	12	0,697				
2.	243	12	0,701				
3.	243	12	0,697				
4.	243	12	0,696	0,696	0,002598	0,0036348	0,363448
5.	243	12	0,694				
6.	243	12	0,695				
1.	238	12	0,674				
2.	238	12	0,678				
3.	238	12	0,674	0,6745	0,001760	0,0026103	0,261035
4.	238	12	0,674			5	
5.	238	12	0,673				
6.	238	12	0,674				
1.	248	12	0,649				
2.	248	12	0,649				
3.	248	12	0,647	0,647	0,001414	0,0021858	0,21858
4.	248	12	0,647				
5.	248	12	0,646				
6.	248	12	0,646				

3.7. Dosage of pharmaceutical forms by the developed method

Three pharmaceutical forms were analyzed at three repetitions by the developed method. The results obtained are presented in Table 5.

Table 5. Dosage of pharmaceutical forms of vitamin C by the developed method (n = 3).

Dosage forms	Introduced Concentration (µg/ml)	Found Concentrations (µg/ml)	Found Concentration (%)	SD	RSD	%RSD
MyVita+Zinc Tablets	10	10,1348	101,348	0,6797	0,0067	0,670
Vitamine C injectable	10	10,2158	102,158	0,6489	0,006352	0,6352
Vitamine C Sirop(Phatkin)	10	10,0119	100,119	0,374379	0,003739	0,3739

IV. CONCLUSION

In this study, we developed a UV / vis spectrophotometric method for the estimation of pure ascorbic acid and its dosage forms without 0.1 M HCl used as dilution solvent; does not interfere with the maximum absorption wavelength of ascorbic acid solution. The solvent and unsophisticated equipment used in this study are accessible in our laboratories.

The method has been shown to be fast, simple, sensitive, selective, exact, precise, reproducible, robust and economical. Thus it can be recommended for the routine analytical control of vitamin C pure and in its pharmaceutical forms.

REFERENCES

[1]. Leboulanger J., Vitamin C, In: vitamins. Biochemistry-Mode of action-Therapeutic interest, F. Hoffmann-La Roche et Cie Ed, Neuilly / Seine, 1984: 151-163.
[2]. Nguyen-Khoa T., Vitamin C, In: Kamoun P, Fréjaville J.P., Guide to laboratory examinations, 4th Ed. Médecine-Sciences Flammarion, Paris 2002: 1373-1375.
[3]. Gilland JC, Le Moel G. Vitamin C. In Bioforma 2007 medical biology training notebook; No. 38: 176-189.
[4]. Le Moel G, Fain O. Vitamin C Deficiency and Scurvy. In Bioforma 2007 medical biology training book; No. 38: 345-353.
[5]. European Pharmacopoeia-6th Edition
[6]. Britsh Pharmacopoeia 2012 7th Edition
[7]. USP 30-NF25 2007
[8]. MM Rahman Khan, MMRahman, MS Islam and SA Begum, A Simple UV-spectrophotometric Method for the Determination of Vitamin C Content in various Fruits and Vegetables at Sylhet Area in Banglandesh, Journal of Biological Sciences 6 (2): 388- 392, 2006
[9]. Anal Parinal Dessai. UV Spectroscopic Method for Determination of Vitamin C (Ascorbic Acid) Content in Different Fruits in South Gujarat Region. Int. J About Sci Nat Res. 2019; 21 (1): 556055. DOI: 10.19080 / IJESNR. 2019. 21.556056
[10]. Kapur, A. Haskovic, A. , Copra-Janicijevic, A., Klepo, L., Topocagic, A., Tahirovic, I., Sofic, E .; Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables; Bulletin of the Chemists and Tecnologists of Bosnia and Herzegovina 2012, 38,39-42

[11]. Yogesh P Pancham, Girish B and Shailendra Suryawanshi Sanjav, UV-Spectrphotometric method for quantification of ascorbic acid in bulk powder, The Pharma Innovation Journal 2020; 9 (5): 05-08
[12]. Ahmed Z. Mohamed Hussein; Spectrophotometric Determination of Ascorbic acid in Aqueous Solutions Pharmaceuticals formulations, Journal of Al-Nahrain Universty vol. 16 (3), September, 2013, pp. 65-71.
[13]. ICH, Harmonized Tripartite Guideline, Validation of Analytical Procedures, Text and Methodology Q2 (R1). (2004).
[14]. ICH guidance, Validation of analytical method: definition and terminology. International Conference on Harmonization, Q2A: Geneva.
[15]. ICH guidance, validation of analytical Procedures. Methology. International Conference on Harmonization, Q2B: Geneva.
[16]. https://www.pharmaguideline.com/2010/12/analytical-method validation.html. 22/05/2019
[17]. British Pharmacopoeia 2008
[18]. Ashutosh Kar, Pharmaceutical Drug Analysis-2005, PP. 83-85.
[19]. Jean Toullec and Monique Mottet, Quantitative chemical analysis of Vogel, pp. 142-147; 662-665
[20]. Dange YD, Honmane SM, Bhinge SD, Salunkhe VR, Jadge DR. Development and validation of UV-Spectrophotometric Method for Estimation of Metformin in Bulk and tablet Dosage Form. Indian J of Pharmaceutical Education and Research. 2017; 51 (4S): S754-S60.
[21]. Durgesh Rameshlal Parakh, Moreshwar P. Patil, Sandeep S. Sonawane, Chetan P. Jai. Development and validation of spectrophotometric method for estimation of mebendazole in bulk and pharmaceutical formulation, World Journal of pharmaceutical Research, Volume 4, issue 7, 2223-2235, June 2015.
[22]. Jain Nilesh (2010) Spectrophotometric Method Development and validation for Quantitative Estimation of Amlodipine Besilate in Bulk Drug and their Dosage Forms by Using Hydrotropic Agent. Eurasian J Anal Chem 5 (3): 212-217.
[23]. Gajjar Anuradha K, Shah VD (2010) Simultaneous UV-spectrophometric Estimation of rosuvastatin and ezetimibe in their combined dosage forms. International of pharmacy and pharmaceutical Sciences 2 (1): 131-138.