A UNIQUE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ASCORBIC ACID IN STAR FRUIT, ACACIA, URINE, AND CREAM FORMULA IS DESIGNED AND VALIDATED

[1]K.ANEELA, [2]ANANDARANI.P, [3]THOTAPALLY LALITHA, [4]MANDAM VINAY KUMAR REDDY

[1]Professor, [2,3]Assistant Professor, [4]UG Student, [1,2,3,4]Department of Pharmacy, Brilliant Grammer School Educational Society Group of Institutions-Integrated Campus, Hyderabad, India.

ABSTRACT

The current goal is to provide a novel, quick, and easy analytical technique to measure ascorbic acid in pharmaceutical dose utilising a spectrophotometric technique. It may be the most effective method for assessing the calibre of pharmaceutical formulations. By comparing the samples of urine, serum, acacia leaves, starfruits, and ascorbic acid to a standard ascorbic acid, we may determine the content of ascorbic acid in each. Averroha carambola is the scientific name for star fruit, and its primary chemical components include water, ascorbic acid, sugars, minerals magnesium, phosphorus, and sodium, as well as vitamins thiamin, riboflavin, and niacin. Acacia arabica is the scientific name for acacia, and arabin is one of its main chemical constituents (mixture of calcium, magnesium, potassium salts of Arabic acid). It contain little amount of D-galactose ,L-arabinose, L-rhamnose,and D-gluconic acid .It contain traces of ascorbic acid . It also contain enzyme and peroxidise. Vitamin C in chloroform exhibits an absorption peak at 406.0 nm and in diethyl ether exhibits an absorption peak at 412.0 nm. Linearity of the method was observed in the concentration range of 2-10 µg/mL with correlation co-efficient(r^2=0.9908) . The recovery experiment was carried out at three different levels i.e., 80%, 100% and 120%. Thepercentage recovery was found to be in the range 98.6-99.7 % .The precision of the method was studied as an intra-day and inter-day and repeatability. A low value of % RSD indicated the precision of proposed method.This method can be used for routine quality control analysis of this drug in pharmaceutical dosage forms

Keywords: Ascorbic acid, UV Spectrophotometric method, Method development and method validation.

INTRODUCTION

In addition to acting as an antioxidant in star fruit and acacia, ascorbic acid, generally known as vitamin C, is crucial for maintaining the integrity of blood vessels, skin, cartilage, teeth, and bones. It is essential for the production of collagen and amino acid metabolism. Iron absorption and scurvy are both treated with it. It has the IUPAC name (5R) Dihydroxyethyl ((1S)-1,2) The molecular weight of -3,4-dihydroxyfuran -2(5H)-one is 176.12 g/mole Its 5530C boiling point. It appears as a powder with a white hue. In the body, ascorbic acid undergoes reversible oxidation to dehydro ascorbic acid. These two vitamin types are thought to be crucial in oxidation-reduction processes. Tyrosine metabolism, converting folic acid to folinic acid, carbohydrate metabolism, protein and lipid synthesis, iron metabolism, resistance to infections, and cellular respiration are all affected by the vitamin.

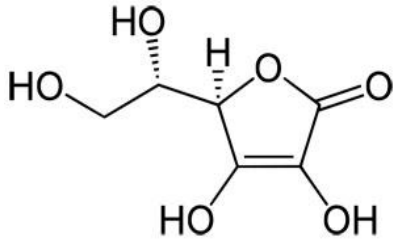

Fig.1: Structure of ascorbicacid

MATERIALS AND METHODS

Instrumentation and reagents

Spectral and absorbance measurements were made using ELICO-SL 210 UV-Visible double beam spectrophotometer with 10 mm matched quartz cells. Ascorbic acid creamformulation "VITAMIN C FACE SCRUB" containing Ascorbic acid 100 mg wasused in the present study. Chloroform (or) diethyl ether, 2, 4 DNP, 10%Thio urea, 85%Sulphuric acid, Metaphosphoric acid (5%, 10%) were of analytical grade acquired from S.D. Fine chemicals, Mumbai.

PREPARATIONS OF REAGENTS :

1. Preparation of 2,4 DNP:

2,4 DNP (Di Nitro phenyl hydrazine) dissolved in 4M H_2So_4 (sulphuric acid)of 100ml Weigh 2g of 2,4 DNP .Mfg by : Merck life sciences private limited

2. 10% thiourea :

10gms of the Thiourea dissolved in 100 ml distilled water Mfg by : Merck life sciences private limited

3. 85% H_2So4 :

85 ml H2So4 is dissolved in 1000ml water leads to formation of 85% H_2So_4 . It decolourises the solutionMfg by : Standard reagents Hyderabad.

4. 5 % Meta phosphoric acid :

5g of glacial Meta phosphoric sticks are dissolved in 100 ml distilled water.Mfg by : Merck life sciences private limited

5) 10% meta phosphoric acid ;

10g of glacial Metaphosphoric sticks are dissolved in 100ml distilled water .Mfg by : Standard reagents Hyderabad

6) Glacial acetic acid: Mfg by: Merck life sciences private limited

7) Bromine water :

The 20 ml of bromine vial is dissolved in 700 ml of distilled water leads to the formation of bromine water.It impacts red color to the solution

METHOD:

Preparation of standard stock solution (100 µg/ml):

Weighed accurately about 100 mg of Ascorbic acid and transferred it into a 100 mL volumetric flask. The content of the flask was dissolved with little quantity of reagents and volume was made upto the mark with distilled water. From this 1ml of solution is taken and dilute it to 10ml which is *100 µg/ml.*

PROCEDURE FOR ESTIMATION OF ASCORBIC ACID IN STAR FRUIT

10 gms of star fruit was blended . it should be homoginised about 50 ml of 5% metaphoshoric acid and 10% glacial acetic acid solution. It was quantitatively, transferred into 100ml volumetric flask then it was diluted upto the mark by the 5% MPA , 10% glacial acetic acid , sulphuric acid , 2,4-DNP , bromine water , finally make up with distilled water . Then the solution was filtered and clear filtrate was collected this filtrate can be standardized with standard ascorbic acid solution for the estimation of ascorbic acid concentration in the star fruit .The obtained concentration found to be 5.88 µg/ml

ESTIMATION OF ASORBIC ACID IN ACACIA

10 gms of Acacia leaves were blended . it should be homoginised about 50 ml of 5% metaphoshoric acid and 10% glacial acetic acid solution. It was quantitatively,transferred into 100ml volumetric flask then it was diluted upto the mark by the 5% MPA , 10% glacial acetic acid , sulphuric acid , 2,4-DNP , bromine water , finally make up with distilled water . Then the solution was filtered and clear filtrate was collected this filtrate can be standardized with standard ascorbic acid solution for the estimation of ascorbic acid concentration in the acacia leaves .The obtained concentration found to be 4.86 µg/ml.

PROCEDURE FOR ESTIMATION OF ASCORBIC ACID IN GEL FORMULATION:

1g of vitamin c cream was dissolved in de- ionized distilled water and this solution was sonicated for 10 min .After the process sonication add this mixture in separating funnel ,to this add 5ml of carbonate buffer and 15ml of diethyl ether. Now shake this mixture simultaneously for about 20 min .After the separation of 2 layers collect the organic layer[upperlayer] and evaporate it at temperature of 30-40c .the obtained residue was extracted by adding 5ml of de-ionised water . transfer the solution in to volumetric flask andaddMetaphosphoric acid-10ml, glacial acetic acid -10 ml ,DNP-2ml,Thiourea-1ml,Bromine water -20mland H_2SO_4 -10 mland make up to 100ml with de-ionized distilled water. the solution was analysed in 400-800nm range from absorbance at 415nm was recorded.Concentration of solution was calculatedfrom the

slope and intercept values obtained from calibration curves.The maximum absorbance at 406 nm was selected as wavelength of the sample.

ESTIMATION OF ASCORBIC ACID IN URINE:

Procedure for estimation of ascorbic acid in urine sample:

6ml of urine sample was collected and centrifuge it for 20 min .After the process of centrifugation the sample was filtered andclear supernatant was collected.To this supernantent solution add known amount[0.5g] of ascorbic acid was spiked .the sample was transferd into separating funnel , to this add 5ml of carbonate buffer and 15ml of diethyl ether. Now shake this mixture simultaneously for about 20 min .After the separation of 2 layers collect the organic layer[upperlayer] and evaporate it at temperature of 30-40c .the obtained residue was extracted by adding 5ml of de-ionised water transfer the solution in to volumetric flask and addMetaphosphoric acid-10ml, glacial acetic acid -10 ml ,DNP-2ml,Thiourea-1ml,Bromine water -20mland H_2SO_4 -10 mland make up to 100ml with de-ionized distilled water. the solution was analysed in 400 -800nm range from absorbance at 415nm was recorded.Concentration of solution was calculatedfrom the slope and intercept values obtained from calibration curves. The maximum absorbance at 415 nm was selected as thewavelengh of ascorbic acid in urine sample. sample.

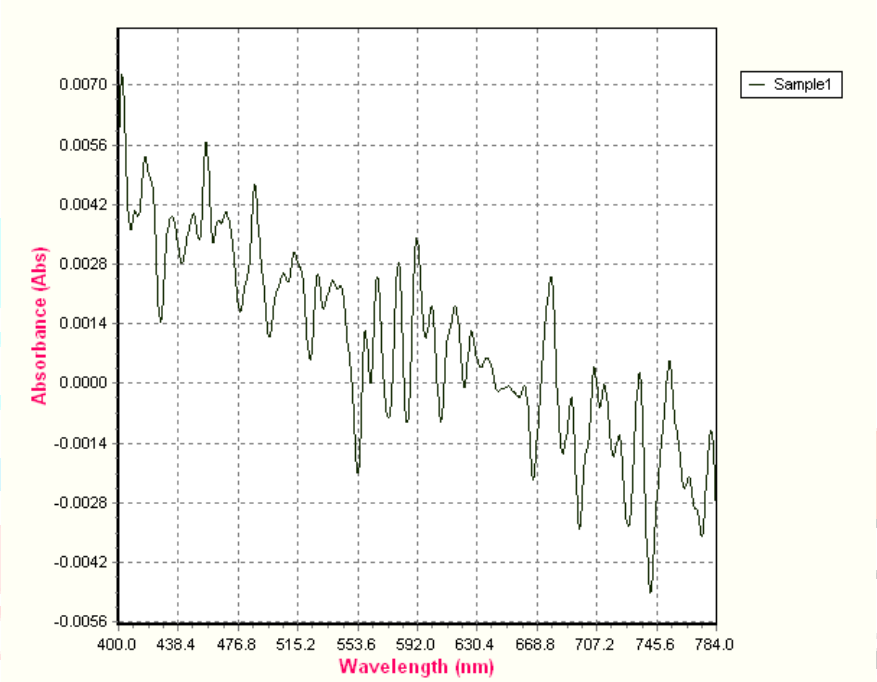

Fig 2 spectra of blank

Procedure for calibration curve

Aliquots of 2, 4, 6, 8, 10ml of 100 µg/ml Ascorbic acid standard solution were accurately transferred into a series of 10 mL volumetric flasks and volume was made up to the mark with diethyl ether. The absorbance of the resulting solution was measured at 412nm against blank. The absorbance spectrum was shown in Fig. 5.2. A calibration graph for Ascorbic acid was plotted by taking concentration of drug on x-axis and absorbance on y-axis.

RESULTS

- Determination of λ_{max} of ascorbic acid in star fruit at 408 nm

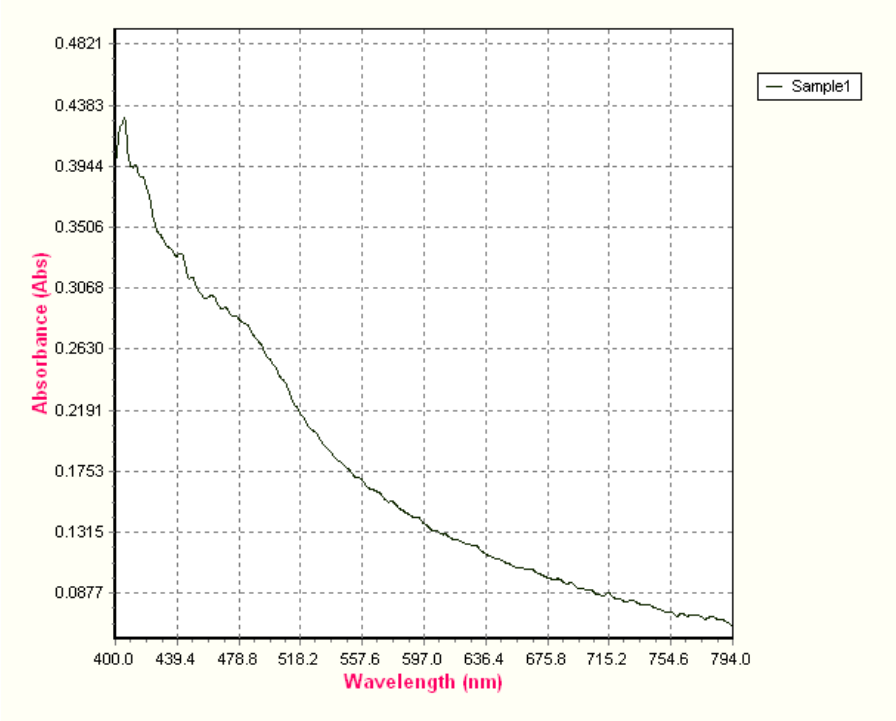

Fig:3 Spectra of ASA of star fruit

Determination of λ_{max} of ascorbic acid in acacia leaves at 410 nm

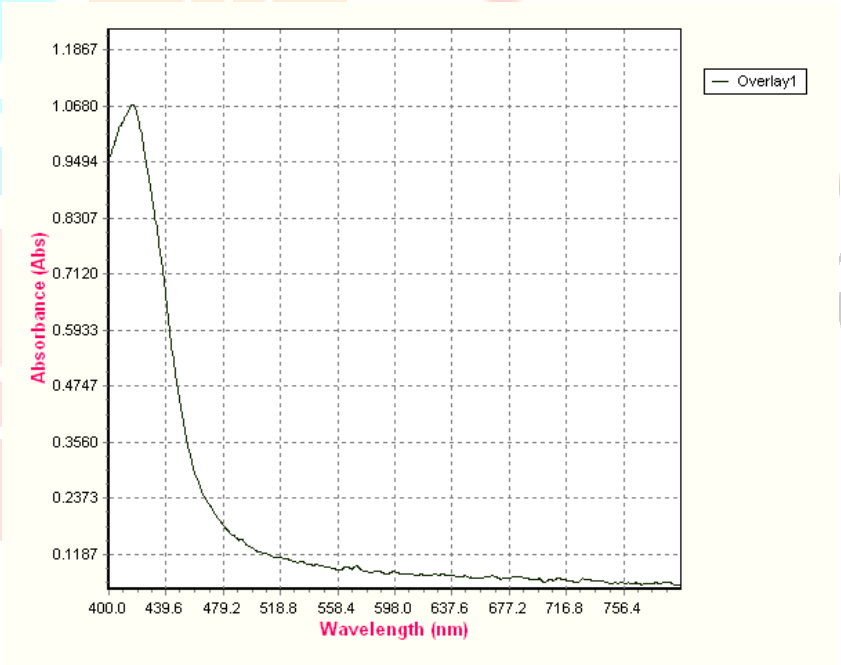

Fig 4:spectra of ASA of acacia leaves

Determination of λ_{max} of ascorbic acid in Urine sample at 406 nm

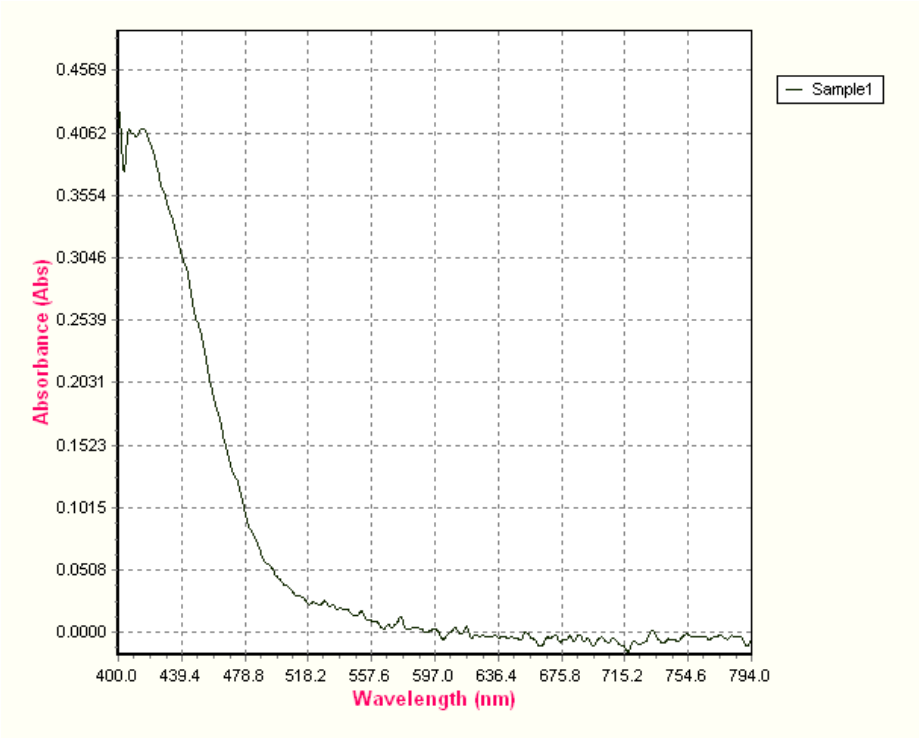

Fig 5: Spectra of ASA in urine

Determination of λ_{max} of ascorbic acid in standard ascorbic acid at 412 nm

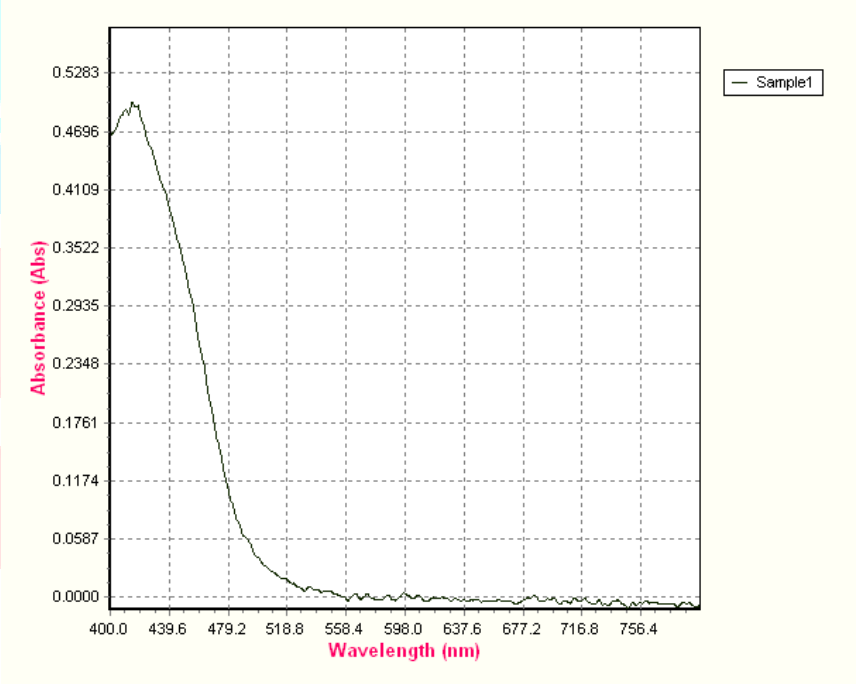

Fig 6: Spectra of ASA in standard ascorbic acid

Determination of λ_{max} of ascorbic acid in cream sample at 412nm.

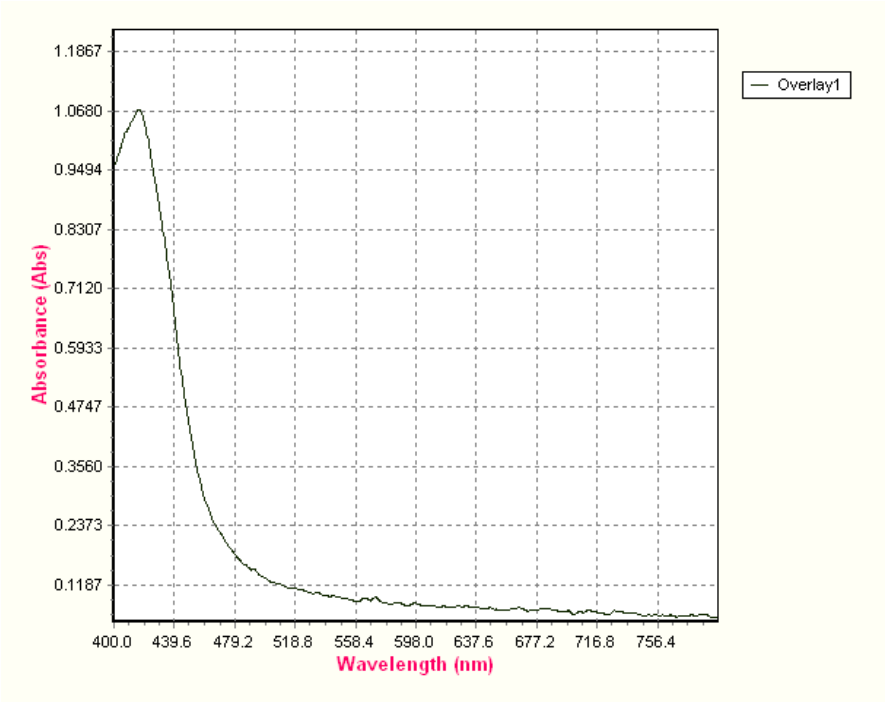

Fig 7:Spectra of ASA in cream formulation

Linearity:

Table 1: Calibration data table for ascorbic acid

S.No	Con. (µg/mL)	Absorbance
1	10	0.3069
2	20	0.3412
3	30	0.3778
4	40	0.4074
5	50	0.4288

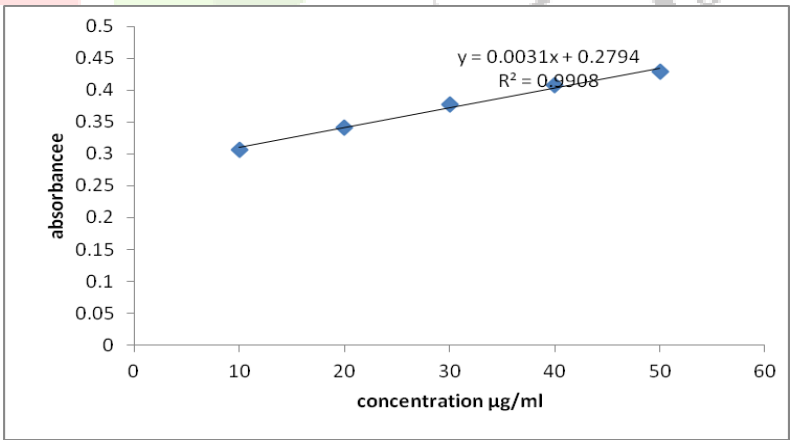

Fig 8:Calibration curve of ascorbic acid

ESTIMATION OF ASCORBIC ACID IN CREAM FORMULATION:

Marketed Ascorbic acid formulations containing 100 mg of Ascorbic acid was analyzed by this method. From the cream, an amount equivalent to 100 mg of Ascorbic acid was weighed and transferred to 100 ml volumetric flask. The contents of the flask were dissolved in diethyl ether/chloroform with the aid of ultrasonication for 20 min. The solution was filtered through Whatmann filter paper no 41. The filter paper was washed with diethyl ether/chloroform. The washings were added to the filtrate and the final volume was made up to 100 ml diethyl ether/chloroform with to obtain concentration of 100 µg/ml. From the above solution 3 mL was transferred to 10 mL volumetric flasks a concentration of 30 µg/ml. The absorbance of the final sample corresponding to 30 µg/mL was recorded against the blank at 412.0 nm. The amount of drug in pharmaceutical formulation was calculated from calibration curve.

Table 2: Estimation of Ascorbic acid

Methods	Assay Sample	Labeled found (mg)	Amount found (mg)	% purity ±SD
Method	Vitamin-C face scrub	100 mg	99.8	99.8%

Table 3: Optical Characteristics of Ascorbic acid

S.No	Parameter	Method
1.	Absorption maximum(nm)	412
2.	Linearity Range (µg/ml)	2-10
3.	Regression Equation	Y=0.003x+0.26y
4.	Slope(a)	4.86
5.	Intercept(a)	0.017
6.	Correlation coefficient (r_2)	0.9908

PRECISION

The precision of analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed condition. The system precision was analysed by six different solutions of same concentration and absorbances were noted. The result was indicated by % RSD. The results are shown in Table 5.4. Repeatability or Intra-day precision was investigated on six replicate sample solutions on the same day. Inter-day precision was assessed by analyzing newly prepared sample solutions in triplicate over three consecutive days. Both inter day and intraday precision was expressed as % RSD. The low value of % RSD indicates the high precision of the method. **Table 4: Results of Intraday precision for method**

Sample	Con. taken (µg/ mL)	Con. found (µg/mL)	% RSD
Star fruit	30	5.86	± 0.05
Acacia	30	4.88	± 0.05
Urine	30	99.8	± 0.05
Vitamin C cream	30	99.8	± 0.05

ACCURACY

Accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% of targeted drug concentration in which the amount of marketed formulation was kept constant and the amount of pure drug was varied. Solutions were prepared in triplicates and accuracy was indicated by % recovery.

Table 5: Accuracy results of method

S.No	% Level of Recovery	Intial amount present (µg/ml)	Amount of standard added (µg/ml)	Total Amount present (µg/ml)	Total Amount Recovered (µg/ml)	% Recovery± S.D
1	80	30	24	54	53.98	99.86±0.78
2	100	30	30	60	60.76	100.12±0.12
3	120	30	36	66	65.92	99.42±0.65

ROBUSTNESS :

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied +2 nm. For changes of conditions, the sample was assayed in triplicates.

Table 6 : Results of Robustness Study (Method A)

Formulation	Amount of drug taken from cream (mg)	At 412 nm (n=3) % Assay ± % RSD
Ascorbic acid	500	99.8 ± 0.05

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

Limit of detection (LOD) and Limit of quantitation (LOQ) were determined by using the formula based on standard deviation of the response and the slope. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the equations LOD = 3 σ /S and LOQ= 10 σ /S, where σ is standard deviation of intercept, S is slope of the line .

SPECIFICITY

The selectivity of analytical method is its ability to measure accurately and specifically the analyte of intrest in the presence of componenets that may be expected to be present in sample matrix. If an analytical procedure was able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method became a selective indicates the sensitivity of the method value of percent RSD indicated the precision of proposed method interfere in this method the recovery experiment of was carried out at 3 different levels that is 80%, 100% and 120% the percentage recovery was found to be in the range of 98.6 to 98.7. the precision of method was studied as an intra-day repeatability. A low value of % RSDindicated the precision of proposed method.

DISCUSSION

The present study was carried out to develop simple and sensitive spectrophotometric method for determination of VITAMIN C cream. The method is a UV –Visible spectrophotometric method in which the absorption spectrum of the drug Vitamin C in chloroform exhibits an absorption peak at 406.0 nm and in diethyl ether exhibits an absorption peak at 412.0 nm.Linearity of the method was observed in the concentration range of 2-10 µg/mL with correlation coefficient(r^2=0.9908) .The proposed method was applied to pharmaceutical formulation and percent amount of drug estimated was found in good agreement with the label claim. The excipients used in the pharmaceutical preparation do not interfere in this analysis. The recovery experiment was carried out at three different levels i.e., 80%, 100% and 120%. Thepercentage recovery was found to be in the range 98.6-99.7 % .The precision of the method was studied as an intra-day and inter-day and repeatability. A low value of % RSD indicated the precision of proposed method.

CONCLUSION

The proposed Visible Spectrophotometric method for the estimation of Ascorbic acid in star fruit, Acacia ,Urine sample , and Cream formulation was found to be simple, precise, rapid,accurate and involved easy sample preparation. The linearity, reproducibility and recovery data confirms no major interference due to excipients in the cream in the assay determination so this method can be used for routine quality control analysis of this drug in pharmaceutical dosage forms.

REFERENCES

1. Dick EC, Mink KA, Olander D, Schult PA, Jennings LC, Inhorn, and SL. Amelioration of rhinovirus type 16 (RV 16) colds in ascorbic acid supplemented volunteers. 30th ICAAC Proceedings 1990;
2. Riordan HRD, Jackson JA, Schultz M. Case study: high-dose Intravenous vitamin C in the treatment of a patient withAdenocarcinoma of the kidney. J Ortho Med 1990; 5:5-7.
3. Tamayo C, Richardson MA. Vitamin C as a cancer treatment:State of the science and recommendations for research. AlternTher Health Med 2003;9(3):94-101.
4. Holmes HN, Campbell K. The Determination of Vitamin C in Urine. Journal of Laboratory and Clinical Medicine 1939;24:1293-1296.
5. Expert Group on Vitamins and Minerals. UK government update Paper EVM/99/21/P. 1999.
6. Food and Nutrition Board (RDA Committee). Dietary Reference Intakes for Vitamin C, Selenium and Carotenoids. The National Academy of Science, United States. 2000.
7. Kallner A, Hartmann D, Horning D. Steady-state turnover and Body pool of ascorbic acid in man. Am J ClinNutr 1979; 32:530–539.
8. Blanchard J, Tozer TN, Rowland M. Pharmacokinetic Perspectives on megadosis of ascorbic acid. Am J ClinNutr 1997; 66:1165-71.
9. Cathcart RF. Vitamin C – the non-toxic, nonrate-limited,Antioxidant free radical scavenger. Medical Hypothesis. 1985,18:61-77.
10. Yung S, Mayersohn M, Robinson JB. Ascorbic acid elimination In humans after intravenous administration. Journal of Pharmaceutical Science 1978;67:1491-2.
11. BRAD BURY J.H. and U. SINGH (1986): Ascorbic acid and dehydro ascorbic acid content of tropical route crops from the south pacific . J. FdSci ., 51:975, 978, 987
12. K.Guclu K. Sozgen, E. Tutem, M. Ozyurek, and R.Apak, “spectrphotometric determination of ascorbic acid using copper(ii)-Neocuproine reagent in beverages and pharmaceuticals” Talanta vol.65, no.5, pp.126-1232,205
13. S.S.kumar and S.S.narayanan, “ ampherometric sensor for the determination of ascorbic acid based on cobalt hexacyaoferrate modified electrode fabriacted through a new route,” chemical and pharmaceutical bulletin, vol.54 no.7,pp.963-967,2006
14. P.J.O’Connell, C.Gormally, M.Pravda, and G.G. guilbault, “ developement of an ampherometric L-ascorbic acid (vitamin C) sensor based on electropolymerised aniline for pharmaceutical and food analysis “ analyticachimicaacta., vol.431 no.2,pp.239-247,2001
15. H.J.Kimm and I.K. KIM “ analysis of ascorbic acid by ion exclusion chromatography with electrochemical detection” journal of food science ,vol,53,no.5,pp.1525-1527,1988
16. L.campanella E. martini, G.Rita and M.Tomasetti, “antioxidant capacity of dry vegetablr extracts checked by voltamteric methods, “ journal of food, agriculture and environment, vol.4,no.1,pp.135-1342006
17. Rahman, K.M.N., M.M. Rahman, M.S.Islam and S.A.Begum, 2006 a simple UV spectrophotometric method for the detrmination of vitamin C content in various fruits and vegetables contents at syehedd area in bangladesh, journal of iological science 6(2:388-392)
18. Shephard, A.B., S.C. Nichols and A.braitawatte 1999. moisture induced solid phase degradation of L.Ascorbic acid part-1: a kinetic study using tristeimuluscolorimetry and a quantitative hplc assay. Talanta, 48(3): 585-593
19. Zerbin, K.M.L.Rooney and J.Vermue, 2003. the vitamin C content of orange juice packed in a oxygen scavenger material. Food chemistry, 82 (3):387-395
20. Versari A., A.Mattioli, G.P.Parpinello and S.Galassi, 2004. rapid analysis of ascorbic acid iso ascorbic acid in fruit juice by capillary electrophorosis. Food control, 15(5):355-358
21. Silva, F.O.,2005. Total ascorbic acid determination in fresh squeezed orange juice by gas chromatograophy food control,16(1)55-58