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DEVELOPMENT AND VALIDATION OF NEW SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ASCORBIC ACID IN STAR FURIT, ACACIA, URINE AND CREAM FORMULATION

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ABSTRACT

The present study is to develop a new simple and rapid analytical method to estimate ascorbic acid in star fruit, acacia leaves, urine and cream formulation by using UV-visible double beam spectrophotometric method. The developed method will be validated statistically according to ICH guidelines. Spectral and absorbance measurements were made using ELICO-SL 210 UV-Visible double beam spectrophotometer with 10 mm matched quartz cells. Ascorbic acid cream formulation "VITAMIN C FACE SCRUB" containing Ascorbic acid 100 mg was used in the present study. Reagents used are 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. 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%. The percentage 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. **Objective:** To develop a simple, precise, accurate visible spectrophotometric method for the estimation of ascorbic acid in star fruit, acacia, urine and cream formulation

KEYWORDS: Ascorbic acid, Acacia, Star fruit, Urine, UV- visible double beam spectrophotometric method.

Abbrevations: ASA- ascorbic acid, MPA-metaphosphoric acid, DNP -dinitro phenyl hydrazine.

INTRODUCTION

Ascorbic acid chemically is **(**5R)-[(1S)-1,2-Dihydroxy ethyl]-3,4-dihydroxyfuran -2(5H)-one. It is water soluble. It is a white coloured powder. Boiling point of ascorbic acid is 553°c and melting point is 190°c. The density of ascorbic acid is 1.65g/cc. The average mass of ascorbic acid is 176.12Da. The molecular weight of ascorbic acid is 176.12g/mole. The pka value of ascorbic acid is 7.4. It is used to used to treat scurvy. It is important to maintain the health of skin, cartilage, teeth, bone and blood vessels. It also acts as antioxidant. It plays a vital role in collagen formation. Used in Metabolisim of aminoacids and also used for absorption of iron. The present method for the estimation of ascorbic acid in formulations is precise, simple and accurate.

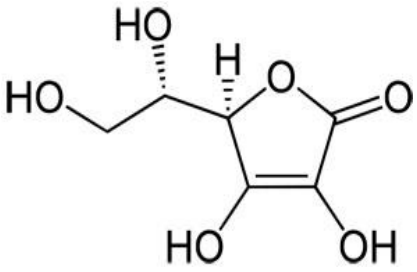

Fig. 1: Structure of ascorbic.

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 cream formulation

"VITAMIN C FACE SCRUB" containing Ascorbic acid 100 mg was used 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

2gms of 2,4 DNP (Di Nitro phenyl hydrazine) dissolved in 4M H_2SO_4 (sulphuric acid) of 100ml.

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_2SO_4

85 ml H_2SO_4 is dissolved in 1000ml water leads to formation of 85% H_2SO_4. It decolourises the solution.

Mfg 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 (100µg/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 100µg/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 5.88µg/ml.

Estimation of Asorbic Acid In Acacia

10gms 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 4.86µg/ml.

Procedure For Estimation of Ascorbic Acid In Cream 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 20min. After the separation of 2 layers collect the organic layer[upperlayer] and evaporate it at temperature of 30-40°c. The obtained residue was extracted by adding 5ml of de-ionised water. Transfer the solution in to volumetric flask and add Metaphosphoric acid-10ml, glacial acetic acid -10 ml, DNP-2ml, Thiourea-1ml, Bromine water -20ml and H_2SO_4 -10ml and 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 calculated from 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 and clear supernatant was collected. To this supernantant 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 30-40°C. The obtained residue was extracted by adding 5ml of de-ionised water. Transfer the solution in to volumetric flask and add Metaphosphoric acid-10ml, glacial acetic acid -10 ml, DNP- 2ml, Thiourea-1ml, Bromine water -20ml and H_2SO_4 -10 ml and make up to 100ml with deionized distilled water. The solution was analysed in 400 -800nm range from absorbance at 415nm was recorded. Concentration of solution was calculated from the slope and intercept values obtained from calibration curves. The maximum absorbance at 415 nm was selected as the wavelength of ascorbic acid in urine sample.

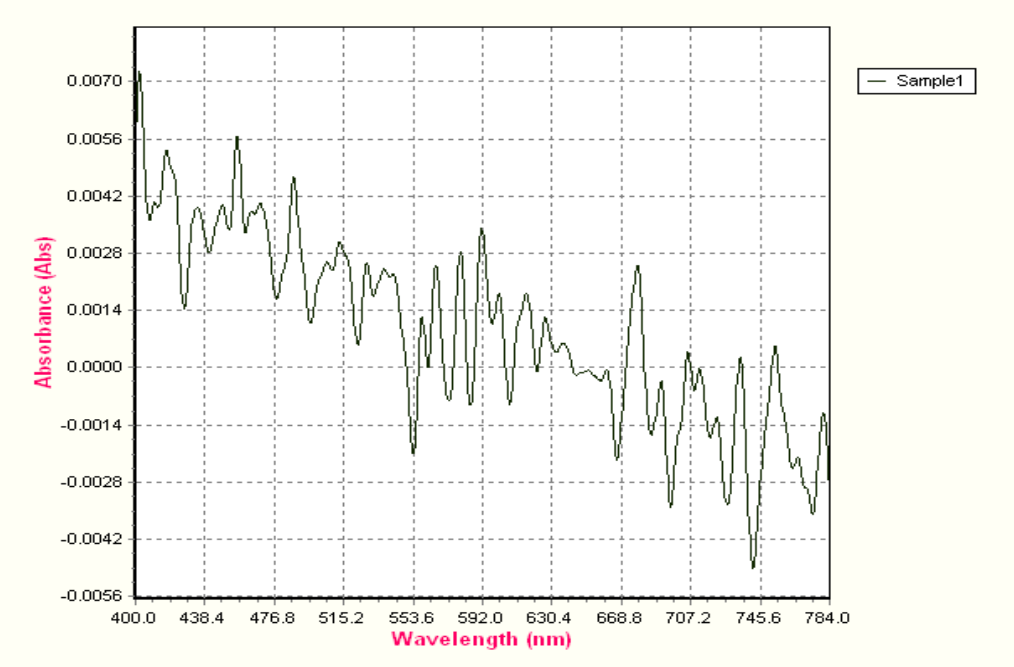

Fig. 2: Spectra of blank.

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.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**

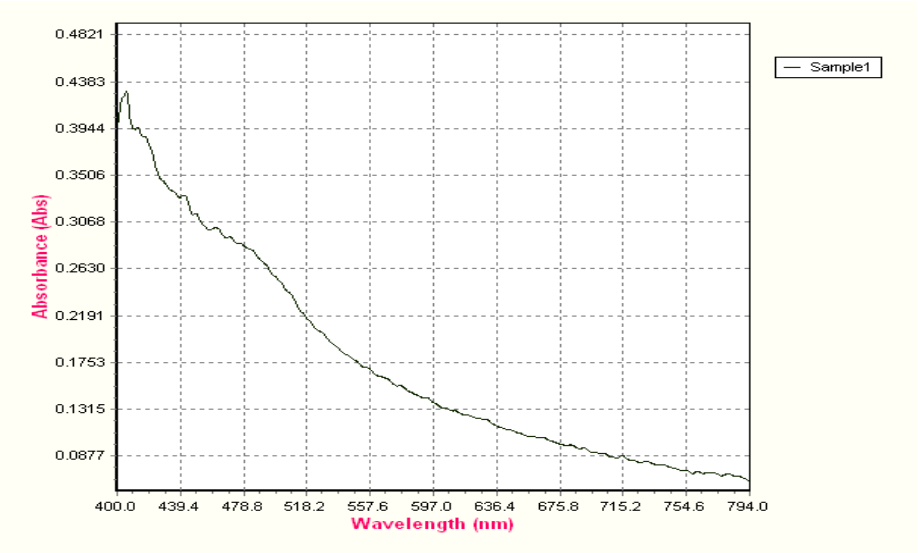

Fig 3: Spectra of ASA of star fruit.

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

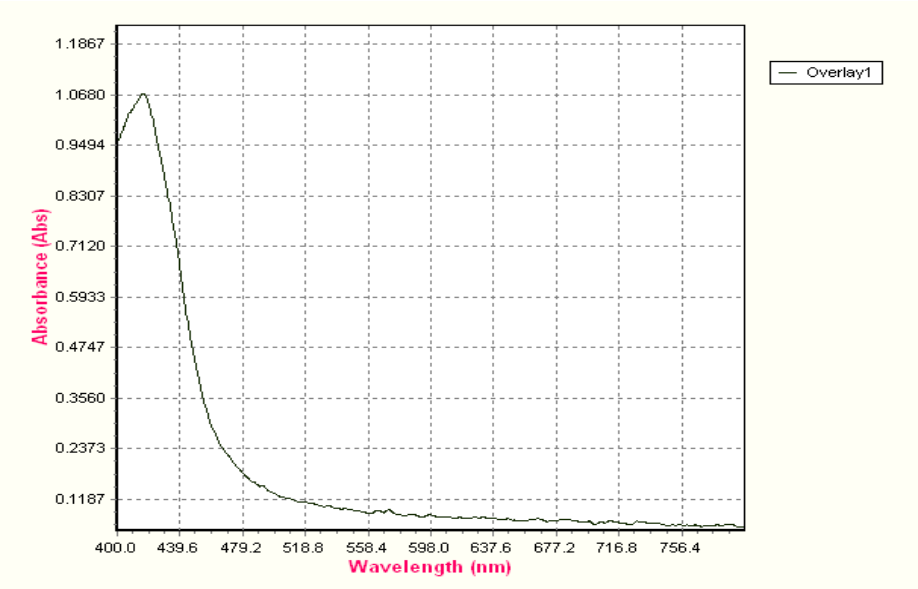

Fig. 4: spetra of ASA of acacia leaves.

- **Determination of λ_{max} of ascorbic acid in urine sample at 406 nm**

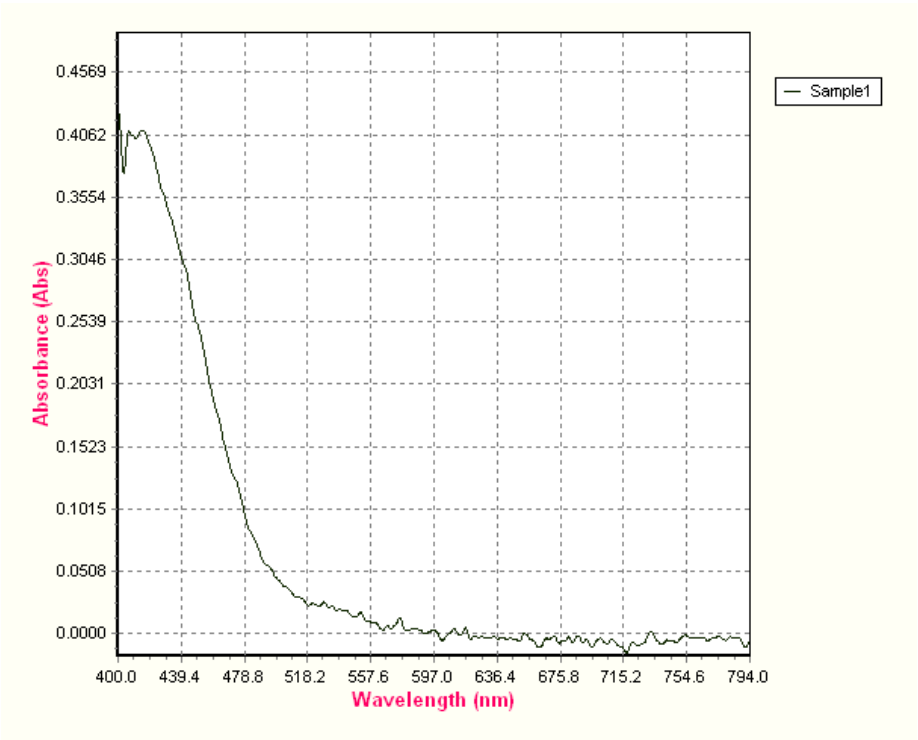

Fig. 5: spectra of ASA in urine.

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

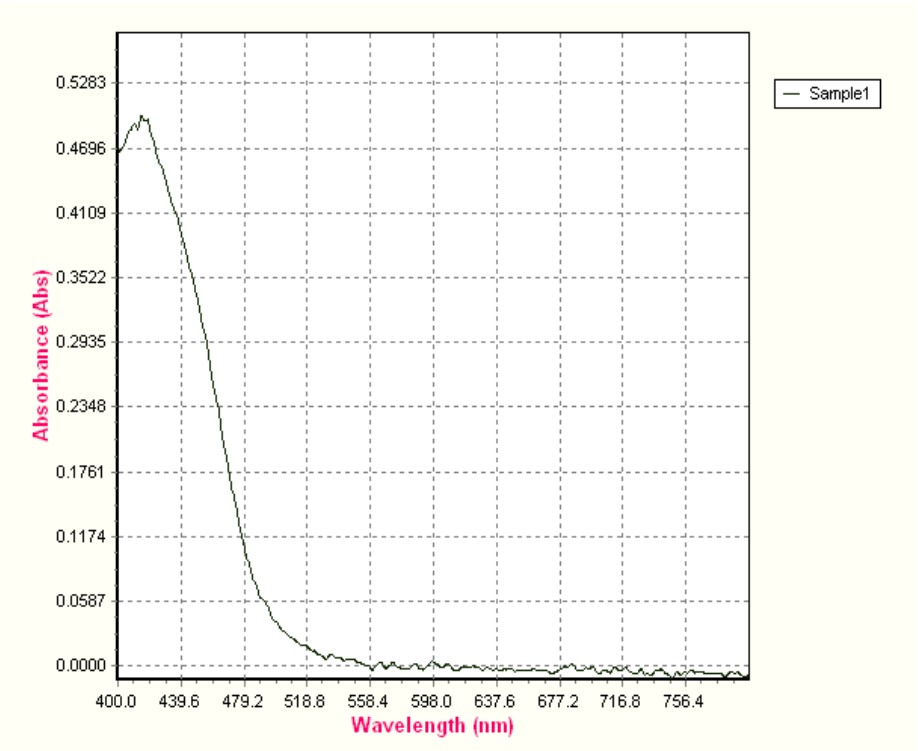

Fig. 6: Spectra of ASA in standard ascorbic acid.

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

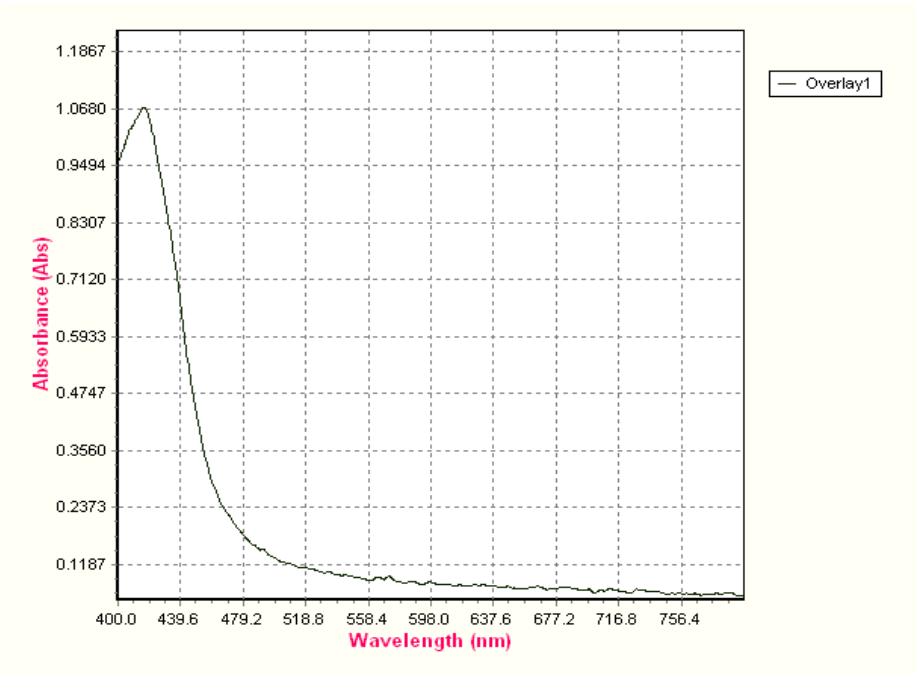

Fig. 7: spectra of ASA in cream formulation.

LINERITY

Table 1: Calibration data table for ascorbic acid.

S.No	Conc.(µg/ml)	Absorbance
1	10	0.3069
2	20	0.3412
3	30	0.3778
4	40	0.4047
5	50	0.4288

Calibration curve of ascorbic acid

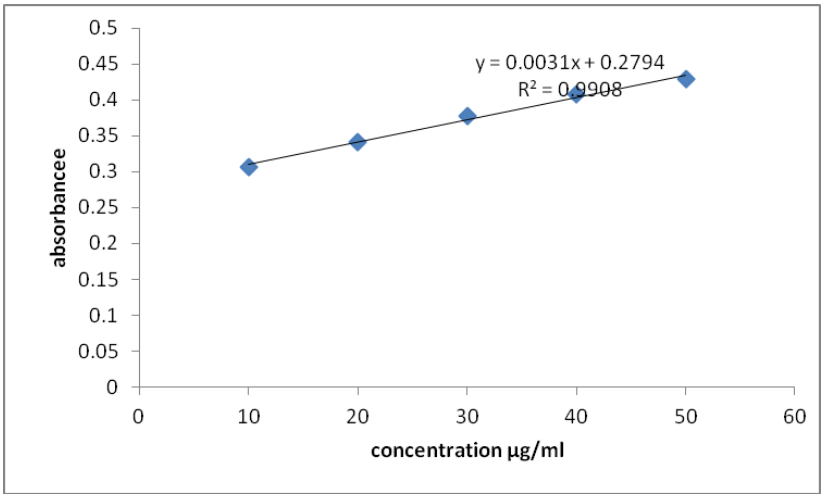

Fig. 8: calibration curve of ascorbic acid.

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 100µg/ml. From the above solution 3 mL was transferred to 10 mL volumetric flasks a concentration of 30µg/ml. The absorbance of the final sample corresponding to 30µg/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 RSD
Method	Vitamin- C face scrub	100 mg	99.8	99.8 %

Table 3: Optical Characteristics of Ascorbic Acid.

s.no	Parameters	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 Intra-Day Precision For Method.

Sample	Conc. taken (µg/ml)	Conc. Found (µg/ml)	% RSD
Starfruit	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 concentration 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. Solution were prepared in triplicates and accuracy was indicated by % recovery.

Table 5: Accuracy results of method.

S.No	% level of recovery	Initial amount present (µg/ml)	Amount of standard added (ug/ml)	Total amount present (µg/ml)	Total amount recovered (µ/ml)	% recovery ± SD
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 mg	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 interest in the presence of components 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, indicates 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 % 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 interferences due to excipients in the cream in assay determination so, this method can be used for the routine quality control analysis of this drug in pharmaceutical dosage forms.

REFERENCES

1. BRAD BURY J.H. and U. SINGH: Ascorbic acid and dehydro ascorbic acid content of tropical route crops from the south pacific. J. Fd Sci., 1986; 51: 975: 978, 987.
2. 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, 2005; 65(5): 126-1232.
3. S.S.kumar and S.S.narayanan, "ampherometric sensor for the determination of ascorbic acid based on cobalt hexacyaoferrate modified electrode fabricated through a new route," chemical and pharmaceutical bulletin, 2006; 54(7): 963-967.
4. 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 "analytica chimica acta., 2001; 431(2): 239-247.
5. H.J. Kimm and I.K. KIM "analysis of ascorbic acid by ion exclusion chromatography with electrochemical detection" journal of food science, 1988; 53(5): 1525-1527.
6. L.campanella E. martini, G.Rita and M.Tomasetti, "antioxidant capacity of dry vegetable

extracts checked by voltamteric methods, "journal of food, agriculture and environment, 4(1): 135-1342006.

7. Rahman, K.M.N., M.M. Rahman, M.S.Islam and S.A.Begum, a simple UV spectrophotometric method for the determination of vitamin C content in various fruits and vegetables contents at syehed area in bangladesh, journal of biological science, 2006; 6(2): 388-392.
8. Shepard, A.B., S.C. Nichols and A.braitawatte. Moisture induced solid phase degradation of L.Ascorbic acid part-1: A kinetic study using tristeimulus colorimetry and a quantitative HPLC assay. Talanta, 1999; 48(3): 585-593.
9. Zerbin, K.M.L. Rooney and J.Vermue, The vitamin C content of orange juice packed in a oxygen scavenger material. Food chemistry, 2003; 82(3): 387-395.
10. Versari A., A. Mattioli, G.P. Parpinello and S.Galassi, rapid analysis of ascorbic acid, iso-ascorbic acid in fruit juice by capillary electrophoresis. Food control, 2004; 15(5): 355- 358.
11. Silva, F.O., Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography food control, 2005; 16(1): 55-58.
12. Campanella et al. determined the antioxidant capacity of dry vesicle extracts (expressed as mg of ascorbic acid equivalence) by cyclic Volta metric performed at a glassy carbon working electrode.
13. Rahman et al. any analytical methods were used for ascorbic acid determination including titrimetric spectrophotometry and chromatography, 2006.
14. Shepard et al. 1999 many analytical methods were used for ascorbic acid determination including titrimetry, volumetry, potentiometry as an analytical techniques.
15. Zebrine et al., 2003 similarly chromatography were also used for the determination of ascorbic acid from different species of citrus fruits.
16. Versari et al. similarly capillary electrophoresis were also used for the determination of ascorbic acid from different species of citrus fruits, 2004.
17. Silva et al. similarly chromatography were also used in the determination of ascorbic acid from different species of citrus fruits, 2005.