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Quantization of ascorbic acid in ayurvedic amla capsule by various analytical techniques

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ABSTRACT

Phyllanthus Emblica, commonly known as amla used in the ayurvedic preparations of Indian system of medicine. As there are no reported methods for this ayurvedic capsule dosage form of amla, so in this present study, an attempt has been made to develop an analytical method in spectroscopy and TLC for the estimation of ascorbic acid in this formulation. Spectrophotometric estimation of ascorbic acid shows the maximum absorbance at 264nm. Beer's law is obeyed in the concentration range of 1-6 µg/ml. Assay method was developed for the ayurvedic formulation, the percentage purity was found to be 97.68 % w/v. The method was validated as per ICH guidelines and results were statistically calculated. Recovery studies were carried out and the percentage recovery was found to be 99.01 - 100.70%w/v, the system was found to be linear and the correlation coefficient (r^2) was found to be 0.9994, method was found to be precise and the relative standard deviation for intraday and interday was found to be 0.122 and 0.163 respectively, detection and quantification limit was found to be 0.3µg/ml and 1µg/ml respectively. TLC method were carried out for identification of ascorbic acid in standard and ayurvedic formulation by using a solvent system of dichloromethane: acetone: GAA (4:3:3 v/v/v) and the R_f value of ascorbic acid was found to be 0.77. The stress degradation studies were performed for both API and Ayurvedic formulation as per ICH guidelines, the degradation was observed in oxidative, photolytic, hydrolytic degradation under acidic, alkaline conditions and dry heat induced studies.

Keywords: Ascorbic acid, Spectrophotometry, TLC, Stress degradation studies.

INTRODUCTION

Amla (syn: *Emblica officinalis, Emblica Myrobalan, Phyllanthus Emblica*) also known as Indian gooseberry. It is a deciduous tree of the family belong to family Euphorbiaceae [1] and this species is native to India and also grows in tropical and subtropical regions including Pakistan, Srilanka, South East Asia, China and Malaysia. The fruits of *Emblica officinalis* are widely used in the Ayurveda [2] and are believed to defense against diseases. Different types of gooseberry species are present world widey, those are *Pereskia aculeate* (syn: Barbados gooseberry**)** belongs to family Cactaceae and it is native to tropical America, *Physalis peruviana* (syn: cape goose berry) belongs to family Solanaceae and it is native to South Africa, *Dovyalis hebecarpa* (syn: Ceylon gooseberry) belongs to family Flacourtiaceae and it is native to Sri Lanka and southern India, *Kiwifruit* (syn: Chinese goose berry) belongs to family Actinidiaceace and it is native to NewZealand, Italy, Chile, Greece and france, *Phyllanthus acidus* (syn: star gooseberry) belongs to family Phyllanthaceae and it is native to South East Asian countries, *Ribes grossularia* (syn: European berry) belongs to family Grossulariaceae and it is native to Europe east to the Caucasus and south to North Africa, *Ribes hirtellum* (syn: American goose berry) belongs to family Melastomaceae and it is native to Northern America.

Vitamin C or ascorbic acid is a water-soluble nutrient and it can be extracted from various fruits and vegetables [3] i.e. Amla, parsley, broccoli, bell peppers, strawberries, oranges, lemon juice, papaya, cauliflower, kale, mustard

greens and Brussels sprouts. The following pharmacological actions have been reported for vitamin c which includes anti-diabetic, anti-oxidant [4], anti-tumour, anti-plasmodial, anti-inflammatory, anti-microbial [5], anti-rheumatic and also possess hepatoprotectivity.

Vitamin C was also used for preventing and treating scurvy, common cold, it is applied to the skin to help with damage from radiation therapy [6]. Additional uses include slowing aging, hardening of the arteries, preventing clots in veins and arteries and heart attack.

Emblica officinalis primarily contains tannins (gallic acid, ellagic acid) [7], 1-O-galloyl-beta-D-glucose, 3, 6-di-O-galloyl-Dglucose, chebulinic acid, quercetin, alkaloids, phenolic compounds, amino acids and carbohydrates [8], chebulagic acid. Its fruit juice contains the highest vitamin C. The principal constituent of ascorbic acid shown in **Fig. 1**

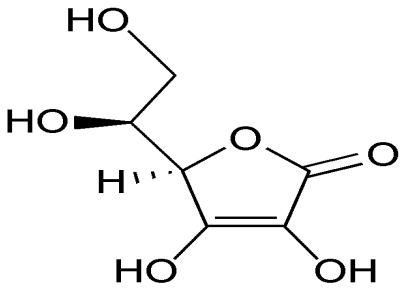

Figure1: Chemical Structure of Ascorbic acid

Mol Formula - $C_6H_8O_6$, Mol Wt - 176.12 g mol

Ascorbic acid is official in Indian [9], British, European and Japanese pharmacopoeias. For the estimation of Ascorbic acid few analytical methods such as UV [10, 11], HPLC [12], TLC [13], GC, HPTLC [14, 15], GC-MS, Capillary electrophoresis [16], Fourier transform infrared spectroscopy (FT-IR),Nuclear Magnetic Resonance (NMR), Nuclear Inductive Resonance (NIR) [17], Differential scanning calorimetry (DSC), Amperometric and voltametric methods were reported. In the present investigation a spectrophotometric method and Thin Layer Chromatographic methods was developed.

MATERIALS AND METHODS

The drug sample Ascorbic acid was obtained from (SDFCL (SD Fine Chem. Limited) Company. All chemicals and reagents were of analytical grade such as Hydrogen peroxide (SDFCL (SD Fine chem. limited), Sodium Hydroxide (Mio chem. Pvt Ltd), and Hydrochloric acid (Merck Chemicals). Amla capsule (The Rising Pharmaceuticals) with 500 mg of label claim obtained from local drug store.

Spectroscopic conditions:
ELICO-SL 244 UV/ VIS double beam spectrophotometer, (spectra treats) with PMT detector, was used for the spectrophotometric estimation of ascorbic acid in API and Ayurvedic preparations**.**

Reagents:
Hydrochloric acid (1N):
An accurately measured volume of 85 ml of Hydrochloric acid was dissolved in 30 ml of water and the final volume was adjusted to 1000 ml with distilled water.

Hydrogen Peroxide (3%):
An accurately measured volume of 3 ml of Hydrogen Peroxide was dissolved in 30 ml of water and the final volume was adjusted to 100 ml with distilled water.

Sodium Hydroxide (1N):
An accurately weighed quantity of 40 g of Sodium Hydroxide was dissolved in 30 ml of water and sonicated for 10 min for proper dissolution and the final volume was made to 1000ml with distilled water.

Preparation of standard:
Accurately weighed 10 mg quantity of API was dissolved in 100 ml of distilled water to give a concentration of 100µg/ml. The final concentration was brought to 1µg/ml by diluting the stock solution with distilled water. This

stock solution is used for further studies. The resulting stock solution was measured from 200 – 400 nm against the corresponding blank.

Assay of Ayurvedic capsules:
Twenty capsules were weighed and the average weights of capsules were calculated. An accurately weighed portion of the powder, equivalent to 100 mg of Ascorbic acid was transferred into a separating funnel and extract with 25 ml of distilled water , filter the extract, and this filtrate was further extracted with 20 ml of ether for 10 min ,after the extraction process, the ether layer was separated into a china dish and evaporate [18] ,the resulting residue was dissolved with 100 ml distilled water in 100ml standard flask to get a stock solution of 1000µg/ml and further dilutions were made to a concentration of 10µg/ml. The resulting solution was measured from 200 – 400 nm against the corresponding blank.

Method Validation:
The method was validated for linearity, accuracy, precision, LOD, LOQ as per the ICH guidelines [19, 20].

Thin Layer Chromatography:
The experiment was performed on a silica gel plate, previously activated at 105^0 C; this activated plate was spotted with standard and ayurvedic formulation. The elution was done by using mobile phase consists of dichloromethane: acetone: GAA (4: 3: 3v/v/v), the eluted spots were detected by using iodine chamber.

STRESS DEGRADATION STUDIES:
The stress degradation studies such as hydrolytic (in acidic & alkali medium), photolytic, oxidative and dry heat induced studies were performed for both API and Ayurvedic formulation as per ICH guidelines.

1. Hydrolytic degradation under acidic conditions:
Hydrolytic degradation studies was performed by taking 2ml (100µg/ml) of stock solution, to this 1ml of 1N HCL was added and volume was made to 10ml with water, kept at normal conditions for 90min. 5ml of the above solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

2. Hydrolytic degradation under alkaline condition:
Hydrolytic degradation studies was performed by taking 2ml (100µg/ml) of stock solution, to this 1ml of 1N NaOH was added and volume was made upto 10ml with water kept at normal conditions for 90min, from this 5ml of solution was pipetted out into 10ml flask and the volume was adjusted with distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

3. Dry heat induced degradation:
Dry heat induced degradation study was performed by taking drug in Petri plate and subjected to a temperature of 70^oC for 48 hrs. After 48 hrs 10mg of the drug was taken and diluted with the distilled water such that to get a final concentration of 5µg/ml, Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

4. Oxidative degradation:
Oxidative degradation studies were performed by taking 1.5 ml (100µg/ml) of stock and to this 1ml of 3% hydrogen peroxide was added and volume is made up to the mark of 10ml of the flask and kept at room temperature for 15 min. A blank solution was prepared with 1 ml of 3% w/v hydrogen peroxide into a 10 ml flask and volume is made upto the mark. The resulting solution was stored over night. Both solutions are boiled to remove excess of hydrogen peroxide. Solutions are kept for 15 min and then diluted to a concentration of 5 µg/ml. Keeping distilled water as a blank , the resulting solution was scanned from 200-400nm.

5. Photolytic degradation:
A photolytic degradation study was performed by exposing the sample to near UV light for 30 minutes in a UV chamber. After UV exposure 10 mg of substance was taken and the final dilution was made to get a concentration of 5µg/ml using distilled water. Keeping distilled water as a blank, the resulting solution was scanned from 200-400nm.

RESULTS AND DISCUSSION

Selection of λ max:
Accurately weighed 10 mg quantity of API was dissolved in 10 ml of distilled water to give a concentration of 1000µg/ml. The resulting stock solution was scanned in the range of 200 – 400 nm against the corresponding blank, λ max of standard Ascorbic acid was found to be 264nm, the UV spectrum was shown in **Fig.2**

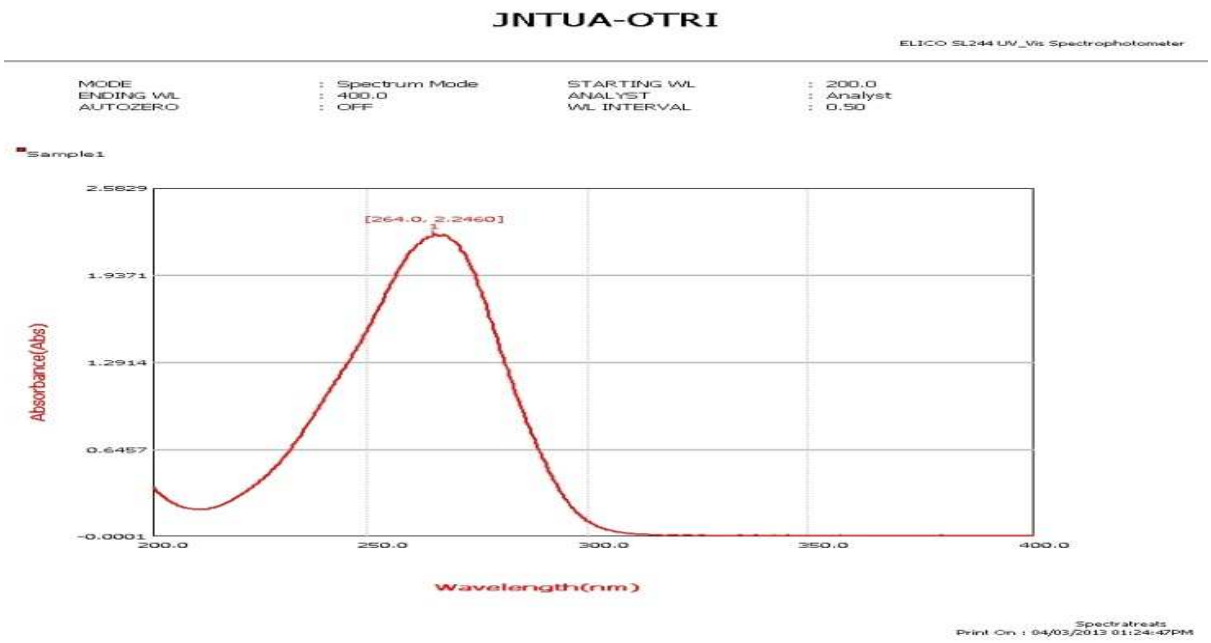

Figure 2: UV Spectrum of Ascorbic acid

Assay of Ayurvedic capsule:

From the above assay procedure, the resulting solution was scanned in the range of 200–400 nm, λ max of Ascorbic acid from capsule was found to be 264.5nm which was shown in **Fig. 3**. Absorbance was measured at 264nm against the corresponding blank and the percentage purity was found to be was 97.68% w/v.

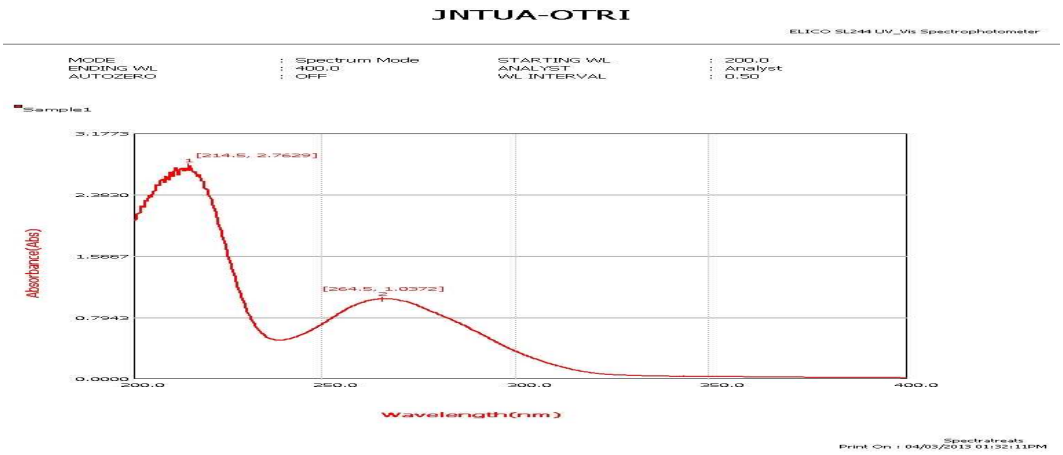

Figure 3: UV Spectrum of Extracted Ascorbic acid from capsule

Method validation:

The method was validated for linearity, accuracy, precision, LOD, LOQ as per the ICH guidelines. The linearity was observed to obey the Beer's- Lamberts law in concentration ranging from 1-6µg/ml. The linearity plot plotted with concentration against absorbance is showed in **Fig.4 and Table.1**, correlation coefficient (r^2) was found to be. The intraday and interday precision studies were performed for six repeated absorbance of same homogenous solution having the concentration of 1µg/ml and the percentage relative standard deviation was found to be 0.122 and 0.163 respectively and the observations are shown in **Table.2,3**, percentage recovery studies was performed for the 80% 100% and 120% respectively, percentage recovery of ascorbic acid was found to be in between 99.01 – 100.70 %w/v and the observations are shown in **Table.4**, detection limit and quantification limit was found to be 0.3µg/ml and 1µg/ml. The result table of the method development and validations was summarized in **Table.5**.

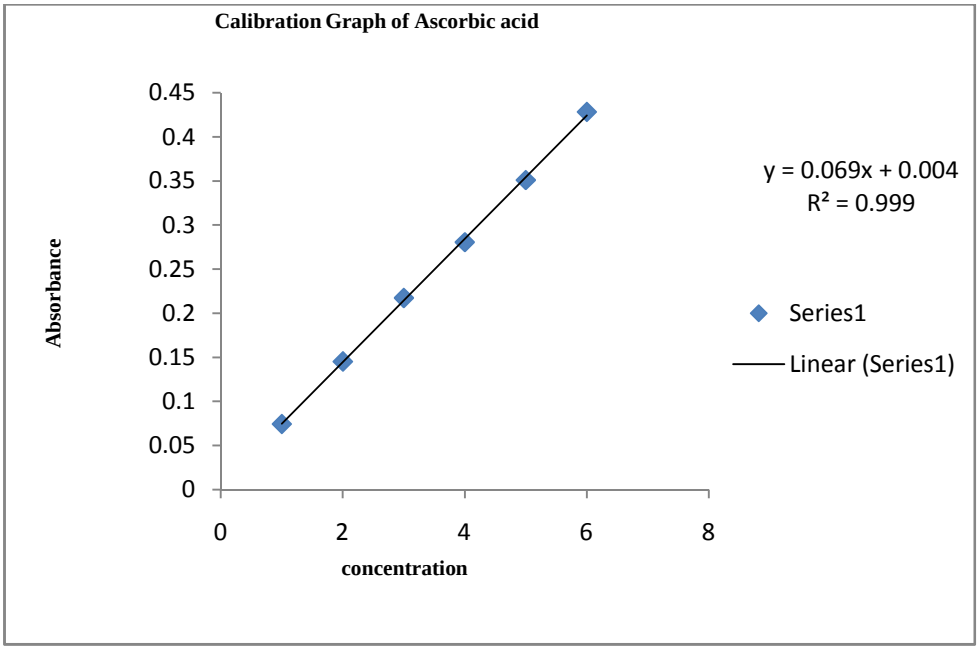

Fig2: Calibration graph of Ascorbic acid

Table 1: Showing the linearity studies for Ascorbic acid

S.NO	Concentration (in µg / ml)	Absorbance
1	1	0.0747
2	2	0.1455
3	3	0.2175
4	4	0.2807
5	5	0.3701
6	6	0.4283
		Correlation coefficient r^2 = 0.9994

Table 2: Showing the intraday precision studies of Ascorbic acid

S.NO	Concentration (in µg/ml)	Absorbance
1	1	0.0140
2	1	0.0154
3	1	0.0168
4	1	0.0194
5	1	0.0167
6	1	0.0144
		Mean = 0.0161
		S .D = 0.001976
		%RSD = 0.122

Table 3: Showing the interday precision studies of Ascorbic acid

S.NO	Concentration (in µg / ml)	Absorbance
1	1	0.0148
2	1	0.0193
3	1	0.0242
4	1	0.0208
5	1	0.0235
6	1	0.0208
		Mean = 0.0205
		S.D = 0.00337
		%RSD = 0 .163

Table 4: Showing the accuracy studies of Ascorbic acid

S.NO	Level of Recovery	Amount of Standard Added(mg)	Absorbance	Amount of Recovery(mg)	%Recovery	Mean
1	80%	1237.5	0.0705	1225.3	99.01	
		1237.5	0.0710	1238.9	100.11	99.09%
		1237.5	0.0699	1214.9	98.17	
2	100%	990	0.0708	984.43	99.43	
		990	0.0717	992.78	100.70	100.13%
		990	0.0714	992.75	100.26	
3	120%	825	0.0729	830.36	100.65	
		825	0.0721	821.25	99.54	100.41%
		825	0.0732	833.72	101.06	

Table 5: Optical density characterization of Ascorbic acid

Parameter	Observation
λ max(nm)	264nm
Beer's law limits (µg/ml)	1 - 6 µg/ml
Molar absorptivity (Liter/mole^{-1}cm^{-1})	72
Correlation Coefficient (r^2)	0.9994
Regression Equation (Y)*	Y = 0.0699X + 0.0048
Slope (b)	0.0699
Intercept(a)	0.0048
Intraday %RSD	0.122
Interday %RSD	0.163
LOD	0.3 µg/ml
LOQ	1 µg/ml

Stress degradation studies:

The stress degradation studies were performed for both API and Ayurvedic formulation as per the ICH guidelines by subjecting the drug to different conditions that stimulate the drug degradation. Further the spectrum of the drug was analysed from 200-400 nm for the degraded changes. The observations are shown in the **Table.6** and the spectrums are shown in **Fig.5 - 9.**

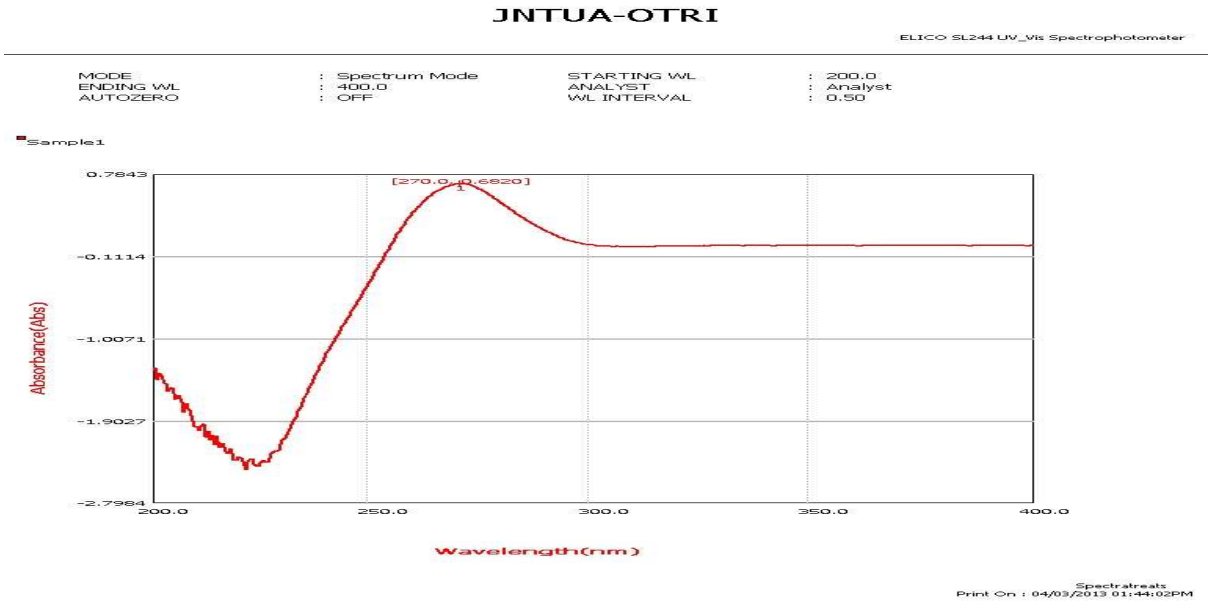

Figure 5: Oxidative degradation

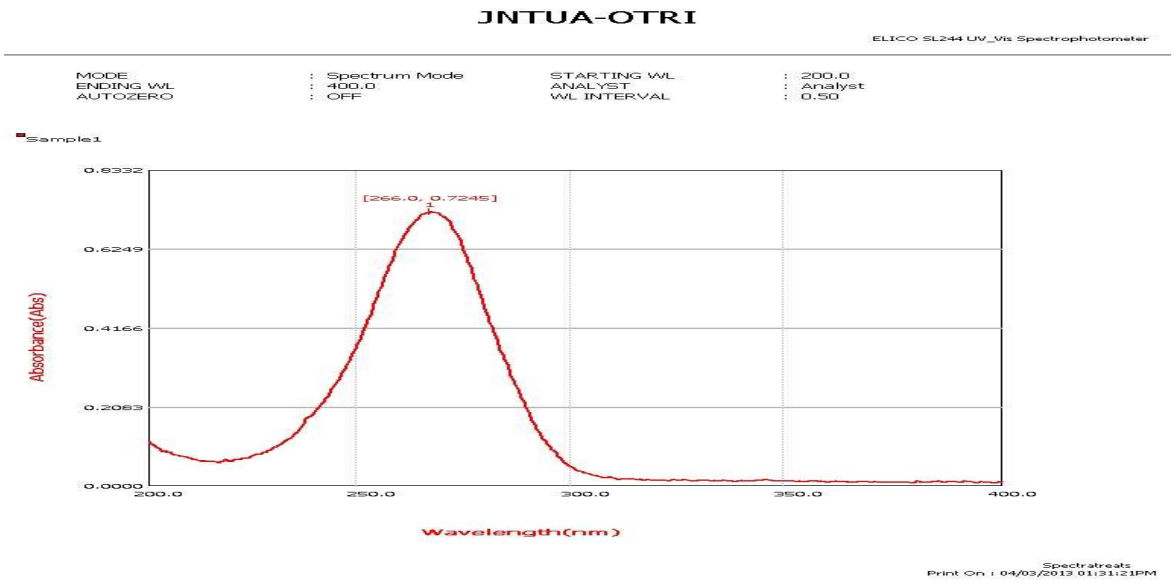

Figure 6: Photolytic degradation

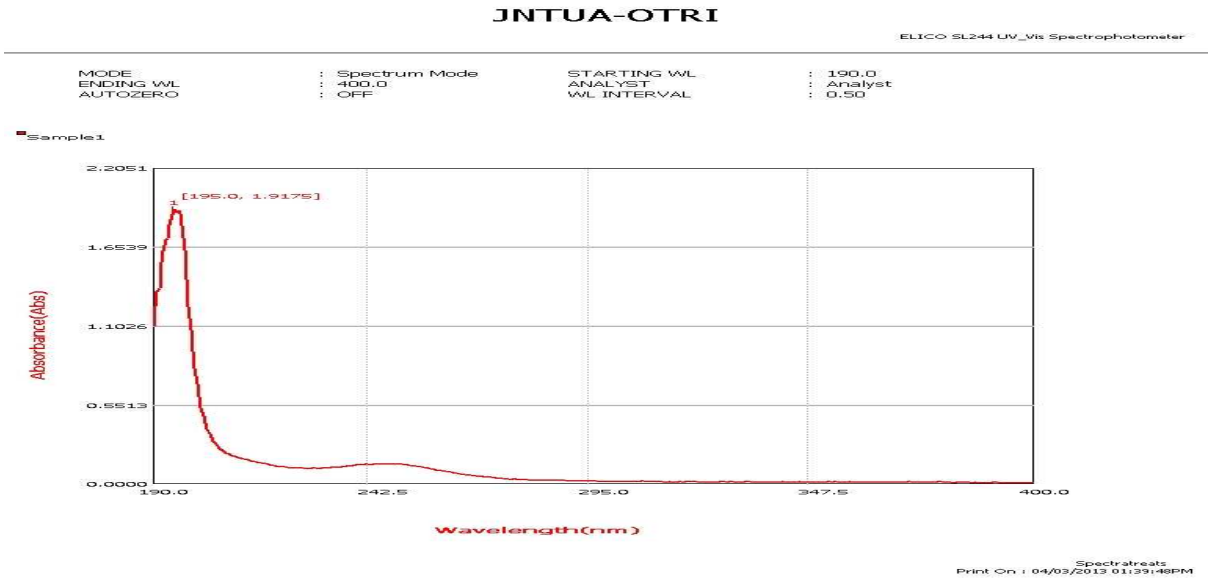

Figure 7: Hydrolytic degradation under acidic conditions

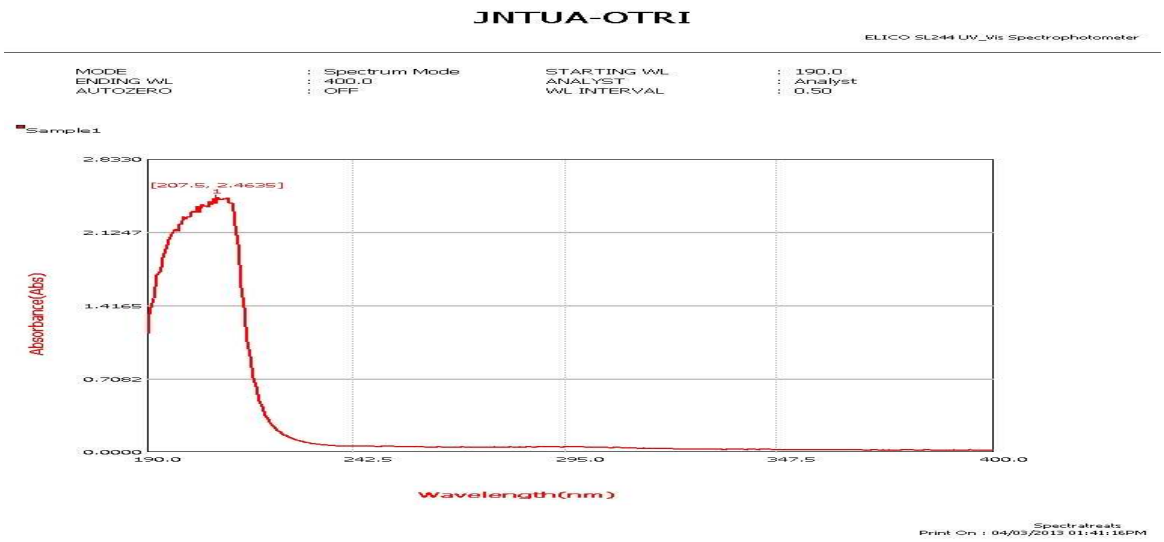

Figure 8: Hydrolytic degradation under alkali conditions

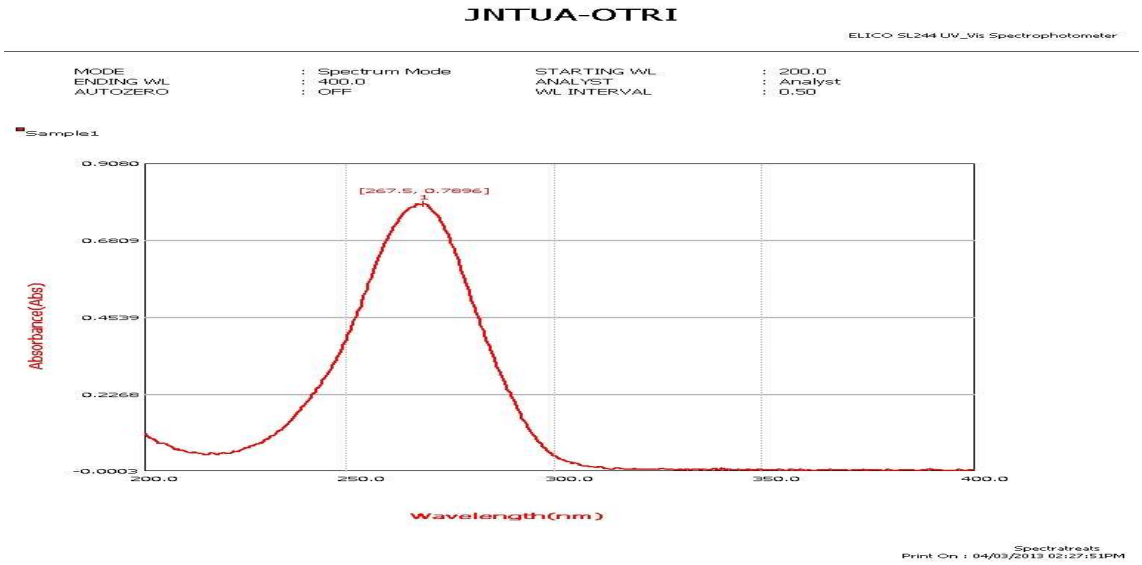

Figure 9: Dry heat degradation

Table 6: Showing the Stress Degradation Studies of Ascorbic acid

S.NO	STRESS DEGRDATION STUDIES	TIME PERIOD	STANDARD	SAMPLE
1	Hydrolytic degradation under acidic conditions	90min	Degradation was Observed	Degradation was Observed
2	Hydrolytic degradation under alkali conditions	90min	Degradation was Observed	Degradation was Observed
3	Photolytic degradation	30min	Degradation was Observed	Degradation was Observed
4	Oxidative degradation	15min	Degradation was Observed	Degradation was Observed
5	Dry heat induced degradation	48 hr	Degradation was Observed	Degradation was Observed

TLC studies:

From the TLC studies, it was observed that the elution of gallic acid along with ascorbic acid in capsule within mobile phase consist of Dichloromethane: Acetone: GAA and(4: 3: 3v/v/v) and the R_f value of ascorbic acid and gallic acid was found to be 0.77 and 0.84 respectively. TLC Plate is shown in **Fig.10**.

Figure 10: TLC studies of ascorbic acid
Dichloromethane: Acetone: GAA (4: 3: 3)
***A** – Ascorbic acid **C** – Ayurvedic Formulation **G** – Gallic acid*

CONCLUSION

Literature's reveals that both ascorbic acid and gallic acid have similar pharmacological activities in common and these two active principles gives an synergistic responsible, commonly see in most of the citrus families and these ingredients are been frequently used in traditional system of medicine as Vitamin C. In this present investigation an attempt was made to estimate ascorbic acid present in ayurvedic formulation of amla (Amla Capsules marketed by "The Rising Pharmaceuticals", Hyderabad). Hence the present work was to identify and quantify the ascorbic acid in the presence of gallic acid in ayurvedic herbal formulation.

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