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COLORIMETRIC ESTIMATION OF ASCORBIC ACID FROM DIFFERENT VARITIES OF TOMATOES CULTIVATED IN GUJARAT

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ABSTRACT

Functional roles of ascorbic acid include its use as a nutrition, food additive, antioxidant, reducing agent, stabilizer, modifier, color stabilizer etc. Ascorbic acid has been quantified in fourteen different varieties of tomatoes using UV spectrophotometric method. Ascorbic acid was quantitatively determined according to 2, 6-dichlorophenolindophenol (DCPIP) method. Ascorbic acid showed good linearity in the range of 20-100 µg/ml which was scanned at 515 nm and the method was found linear with r^2 = 0.996. Method was validated according to ICH guidelines for accuracy, precision, range linearity, limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ value were found to be 1.192µg/ml and 3.614µg/ml. The validated UV spectrophotometric method was used for estimation of ascorbic acid in different varieties of tomatoes.

KEYWORDS: Ascorbic acid; Tomatoes; Spectrophotometric, 2, 6-dichlorophenolindophenol (DCPIP), Nutrition.

INTRODUCTION

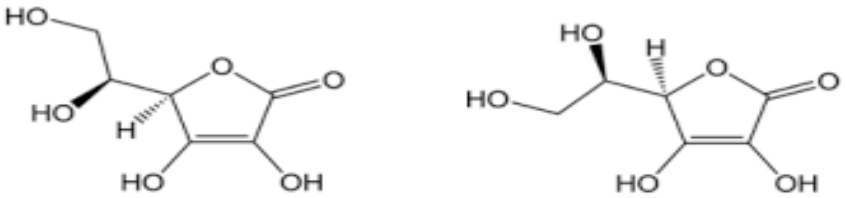
Tomato (*Lycopersicon esculentum* L.) belonging to family solanaceae, is one of the most popular and widely consumed vegetables, worldwide because of its taste, color and high nutritive value and its diversified uses. After potato, tomato is world's largest vegetable crop. But it tops the list of processed vegetables. Because of the increasing demand for the fresh consumption as well as for processing industries, it has taken place as one of the most important vegetables of the world. Worldwide, tomato is consumed in various ways; fresh

tomato in form of salad or as processed tomato products like canned tomato, tomato sauce, tomato juice, tomato ketchup, tomato stews and tomato soup.[1]

It has been suggested from various epidemiological studies that the regular dietary consumption of tomatoes and tomato-based products can be correlated in reduction in the risk of contracting several chronic diseases such as cardiovascular disease and cancer.[2,3,4] This protective positive effect on human health has been mainly attributed to its valuable and rich bioactive components having antioxidant properties.[5]

Ascorbic acid, one of the simplest vitamins, occurs in different concentrations in a variety of natural samples including fruits and vegetables. Chemically, it is related to the C6 sugars, being the aldono-1, 4- lactone of a hexonic acid (L-gluonic acid) and contains an enediol group on C2 and C3. Vitamin C (L-ascorbic acid), fulfils essential metabolic functions in the life of animals and plants (figure 1).[6] It is added to several pharmaceutical products as an essential ingredient, a stabilizer for vitamin B complex, and as an anti-oxidant. Consequent upon its desirable effects, it is widely used in the treatment of certain diseases such as scurvy, anaemia, haemorrhagic disorders etc. It is considered essential for the development and regeneration of muscles, bones, teeth and skin. Also it has been identified as a radical scavenger in vivo. It is familiar molecule because of its antioxidant and cellular reductant properties.[7]

Many methods have been reported for estimation of ascorbic acid like titrimeteric,[8] voltametery,[9] spectrophotometery,[10-16] high performance liquid chromatography,[17-20] and enzymatic[21] etc. But studies regarding quantitation of such phytoconstituents like lycopene in Indian varieties of tomatoes cultivated in Gujarat are not done. Due to large consumption of tomatoes, there is urgent need to assess therapeutically beneficial compounds in the tomatoes and to look for superiority of the breeds based on higher content of medicinally active compounds to promote public health. This paper focuses on quantitative estimation of ascorbic acid in 14 different varieties of tomatoes (Ashoka (Seminis), Ayushman (Seminis), Garv (Seminis), Kedar (Swati), Ratan (Swati), Obama (Swati), Syngenta, Kohenoor (Greenfeild), Syngenta, Badshshah (US Indo), 460 (Crystal), 737 (Crystal), Syngenta) cultivated in Gujarat.



L-Ascorbic Acid D-Ascorbic acid

Figure 1: Structure of Ascorbic Acid.

MATERIAL AND METHOD

Apparatus: A Shimadzu UV – VIS spectrophotometer (model UV – 1800) with matched 1 cm silica cells is used for all spectral and absorbance measurements.

The glassware use in each procedure rinsed thoroughly with double-distilled water and dried in dust-free air.

Reagents: 3% (w/v) metaphosphoric acid standard solution, indophenol standard solution DCPIP (2, 6- dichlorophenolindophenol), Ascorbic acid standard solution.

Preparation of standard solution for calibration curve: Ascorbic acid was quantitatively determined according to 2, 6- dichlorophenolindophenol (DCPIP) method. Accuretely weighed 100 mg of standard ascorbic acid was dissolved in in 100 ml 3% (w/v) metaphosphoric acid (1000µg/mL). From this stock solution, suitable aliquots were taken and 3mL of 0.2 mM DCPIP was added. The resulting solutions of final concentration (20-100 µg/mL) measured absorbance of resulting solutions immediately after mixing for 15 sec at 515 nm.

Preparation of Sample Solution: Each of the freeze-dried tomato samples (1 g) was extracted with 20 mL of 3% (w/v) metaphosphoric acid followed by shaking at 300 rpm for 30 min. The extract was centrifuged at 4000 rpm for 10 min. The supernatant was collected and used for further analysis. In 1 mL of sample extract, 3 mL of 0.2 mM DCPIP was added and measured immediately after mixing for 15 sec at 515 nm. The results were expressed in mg ascorbic acid per 100 g dry weigh (mg/ 100 g DW).

Method Validation: The method was validated according to the International Conference on Harmonization guidelines for validation of analytical procedures Q2 (R1) (ICH, 1996).

Linearity and Range

Aliquots (0.2, 0.4, 0.6, 0.8 and 1 mL) from stock solutions were transferred in different 10 mL volumetric flasks and volume was adjusted up to the mark using mobile phase to obtain concentration of 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL. Calibration curves were plotted using peak areas versus concentrations, and the regression equation was obtained. Calibration curve was repeated for five times.

Accuracy (% Recovery)

Standard addition method was used to study the accuracy of the method. It was determined by calculating recovery of ascorbic acid using 80, 100, 120% level. Known amount of standard Solutions of ascorbic acid (3.2, 4.0, 4.8 mL) were added to three different volumetric flask of 10 mL capacity containing 4 mL sample stock solution. The volume was adjusted up to mark. Percentage recovery was calculated by measuring absorbance of each solution three times and % recovery was calculated with help of regression equation.

Precision

The repeatability of method was checked by analyzing (n = 6) ascorbic acid solutions (40 µg/mL each) and response was recorded.

The intra-day (3 times on the same day) and inter-day (3 different days over a period of 1 week) precisions of the proposed method was checked by measuring the responses for 3 different concentration of ascorbic acid (20, 60 and 100 µg/mL). The absorbance was measured. The % assay values were calculated. The % RSD was reported.

LOD and LOQ

The Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by the equation,

LOD = 3.3 x (SD/Slope)

LOQ = 10 x (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the calibration curve.

Slope = Mean slope of the calibration curve.

Estimation of Ascorbic Acid from Different variety of Tomatoes Using Developed Colorimetric Method

The sample solution of ascorbic acid prepared as per section 2.4. The absorbances of ascorbic acid in 14 different varieties of tomatoes were compared with that of standard ascorbic acid. The amount of ascorbic acid present in 14 different varieties of tomato was analysed using regression equation.

RESULTS AND DISCUSSION

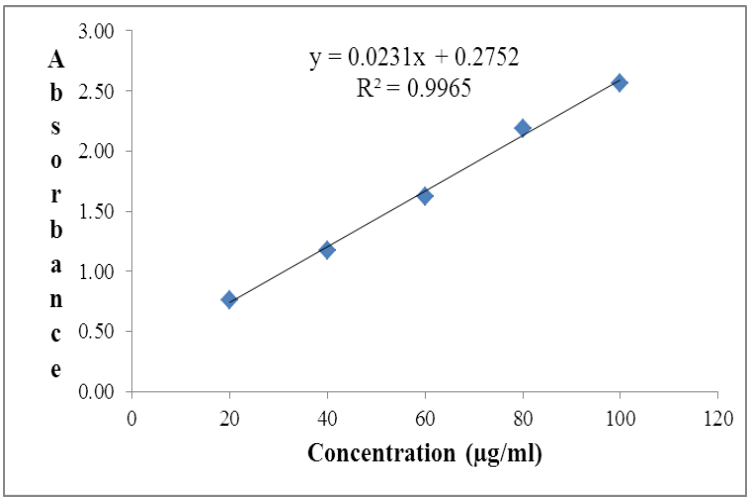


Figure 2: Calibration curve for Ascorbic Acid in range of 20-100 µg/ml.

Linearity and Range (n=5)

The linearity range for Ascorbic acid was found to be in the range of 20-100µg/mL. The linearity of the calibration curve was validated by the value of correlation coefficients of the regression (r). The data are indicated in Table: 1.

Table 1: Linearity data of ascorbic acid (n=5).

Conc. (µg/mL)	Absorbance (mean ± SD)	% RSD
20	0.76 ± 0.01	1.09
40	1.17 ± 0.01	0.88
60	1.62 ± 0.01	0.72
80	2.19 ± 0.01	0.46
100	2.57 ± 0.02	0.66

Accuracy

Accuracy of the method was confirmed by recovery study of lycopene at three level of standard addition. Percentage recovery of Ascorbic acid was found to be 97.50-103.72%. The data are indicated in Table: 2.

Table 2: Results of recovery study.

% Level	Conc. Added (µg/mL)	Sample Conc. (µg/mL)	Total Amount Recovered (µg/mL)	Mean Absorbance • ± SD	% Recovery	%RSD
80	20	40	58.50	1.627 ± 0.02	97.50842	0.93
100	40	40	82.97	2.192 ± 0.01	103.7229	0.56
120	60	40	99.21	2.567 ± 0.02	99.21212	0.82

Precision

1. **Repeatability:** The data for repeatability of Ascorbic acid are indicated in Table 3. The %RSD of Ascorbic acid was found to be 1.09.
2. **Intraday and Interday Presicion:** The data for Intraday and Interday precision of Ascorbic acid are indicated in Table 4. %RSD for intraday precision was found to be 0.29 and for interday precision was found to be 0.52.

Table 3: Results of repeatability (n=6).

Ascorbic acid Conc. (µg/mL)	1	2	3	4	5	6	SD	% RSD
60	1.64	1.62	1.67	1.65	1.65	1.63	0.02	1.09

Table 4: Results of Interday and Intraday Precision.

	Intra-day precision			Inter-day precision		
Ascorbic acid Conc. (µg/mL)	**Mean Absorbance ± SD**	**Mean Conc ± SD**	**% RSD**	**Mean Absorbance ± SD**	**Mean Conc ± SD**	**% RSD**
20	0.76 ± 0.005	20.98 ± 0.22	0.69	0.76 ± 0.012	21.13 ± 0.24	1.66
60	1.62 ± 0.008	58.21 ± 0.34	0.49	1.63 ± 0.018	58.64± 0.74	1.10
100	2.57± 0.021	99.34 ± 0.91	0.82	2.59 ± 0.024	100.20± 0.90	0.95
Mean %RSD	0.66			1.23		

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as per formula stated in 2.5.4 The Limit of Detection (LOD) was found to be 1.19 and Limit of Quantification (LOQ) was found to be 3.61.

Estimation of Ascorbic Acid from Different variety of Tomatoes Using Developed Colorimetric Method

Ascorbic acid in different tomato varieties was estimated according to procedure stated in section 2.5.5. Results are stated in Table: 5.

Table 5: Estimation of Ascorbic Acid in 14 different varieties of tomatoes.

Sr. No.	Tomato variety	Absorbance			In 1 ml aliquot	In 20 ml extract	Ascorbic acid (mg/100g DW) (Content ± SD)
		Extract 1	Extract 2	Extract 3			
1	Ashoka (Seminis)	0.2756	0.2757	0.2756	0.018	0.37	37.51 ± 0.00005
2	Ayushman (Seminis)	0.2757	0.2756	0.2758	0.021	0.43	43.29 ± 0.0001
3	Garv (Seminis)	0.2759	0.2758	0.2758	0.027	0.54	54.83 ± 0.00005
4	Kedar (Swati)	0.276	0.2759	0.276	0.033	0.66	66.37 ± 0.00005
6	Obama (Swati)	0.2753	0.276	0.276	0.024	0.49	49.06 ± 0.0004
7	Abhinav (Syngenta)	0.2754	0.2756	0.2755	0.012	0.25	25.97 ± 0.0001
8	Kohenoor (Greenfeild)	0.2757	0.2755	0.2756	0.017	0.34	34.63 ± 0.0001
9	1004 (Syngenta)	0.2757	0.2757	0.2758	0.023	0.46	46.17 ± 0.00005
10	Badshshah (US Indo)	0.276	0.2761	0.276	0.036	0.72	72.15 ± 0.00005
11	460 (Crystal)	0.2756	0.2752	0.2758	0.014	0.28	28.86 ± 0.0003
12	737 (Crystal)	0.2756	0.2756	0.2755	0.015	0.31	31.74 ± 0.00005
13	1057 (Syngenta)	0.2757	0.2758	0.2756	0.021	0.43	43.29 ± 0.0001
14	Avinash (Syngenta)	0.2756	0.2757	0.2757	0.020	0.40	40.40 ± 0.00005

Table 6: Summary of validation parameters of Ascorbic acid in different varieties of tomatoes.

Sr. No.	Parameters	Results
1	Linearity (µg/mL)	20–100 µg/mL
2	Linear regression equation	y = 0.0231x + 0.2752
3	Regression coefficient (R^2)	0.9965
4	Accuracy	99.86-100.09%
5	Precision (% RSD) Repeatability Intraday	1.64 0.76-2.67
6	Limit of Detection (µg/mL)	1.19
7	Limit of Quantification (µg/mL)	3.61

CONCLUSION

Ascorbic acid is a vital antioxidant found in fresh tomato fruits. The variety ‘Badshshah (US Indo)’ and ‘kedar (Swati)’ were found to have the highest ascorbic acid content 72.15 ± 0.005 and 66.37 ± 0.00005 mg/100 g, which grow in high altitude and in cold climate, which may be the reason for the high content of ascorbic acid. ‘Abhinav (Sygenta)’ and ‘460 (Crystal)’ is shown to have lesser ascorbic acid content of 25.97 ± 0.0001 and 28.86 ± 0.0003 mg/100 g as they are developed for temperate regions. The variation in the ascorbic acid content in tomato may also be due to the growing conditions like temperature and light intensity which have been reported in earlier studies. All these factors leads to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective and rapid and can

be applied successfully for the estimation of ascorbic acid in bulk drug and in tomato varieties without inference and with good selectivity.

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