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Research Article

ESTIMATION OF NARINGENIN CONTENT FROM DIFFERENT VARIETIES OF TOMATOES CULTIVATED IN GUJARAT BY UV SPECTROSCOPIC METHOD

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ABSTRACT

Naringenin is one of the most abundant polyphenols, occurring in skin of tomatoes as aglycone. Naringenin content has been quantified in different varieties of tomatoes by UV Spectroscopic method. Naringenin showed good linearity in the concentration range of 2-12 µg/mL which was scanned at 290 nm and method was found to be linear with $r^2 = 0.998$. 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 0.06 µg/ml and 0.19 µg/ml. The proposed method was found to be accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of Naringenin in different tomato varieties without inference and with good selectivity.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) belonging to family solanaceae, is the world's second largest crop, after potato. It has gained a great popularity and wide consumption worldwide, because of its attractive colour, taste and high nutritive value and its diversified uses. It is consumed in many different ways like fresh salad, tomato juice, processed or cooked like canned tomatoes, ketchup, sauces, stews etc.^[1,2] 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. Various epidemiological studies suggest that the dietary consumption of tomatoes and tomato-based products regularly, can be correlated in reduction in the risk of developing various chronic diseases such as cardiovascular disease and cancer.^[3,4,5] This health benefits are imparted on human beings has been attributed mainly due to presence of valuable and rich bioactive components having antioxidant properties.^[6, 2]

Citrus fruits and tomato (*Lycopersicon esculentum*) are rich and prime sources of naringenin.^[7, 8] Fresh tomatoes, especially tomato skin, also contain naringenin chalcone, which is converted to naringenin during processing to tomato ketchup.

^[9] Naringenin in citrus fruits is principally present in glycosidic forms such as naringenin-7-neohesperidoside (naringin) and naringenin-7-rutinoside (naringinrutin), whereas in tomato, where naringenin is one of the most abundant polyphenols, it is present in the skin as aglycone. The naringenin concentration of tomato is reported to range from 0.8 to 4.2 mg/100 g whole red tomato.^[8, 10]

Many methods for have been reported for estimation of Naringenin like Micellarelectrokinetic chromatographic method,^[11] UV Spectroscopic methods,^[12, 13] High performance liquid chromatographic methods,^[14, 15, 16] mass spectrometric method,^[12, 17] high performance thin layer chromatographic method^[18] and gas chromatographic methods.^[19] But studies regarding quantitation of such phytoconstituents like Naringenin 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 Naringenin in 14 different varieties of tomatoes (Ashoka (Seminis), Ayushman (Seminis), Garv (Seminis), Kedar (Swati), Ratan (Swati), Obama (Swati),

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Syngenta, Kohenoor (Greenfield), Syngenta, Badshshah (US Indo), 460 (Crystal), 737 (Crystal), Syngenta cultivated in Gujarat.

MATERIAL AND METHOD

Instrument and Apparatus: A Shimadzu UV – Vis spectrophotometer (model UV 1800) with matched 1 cm silicacells is used for all spectral and absorbance measurements. Sihmadzu Aux 120(Gottingen, Germany) analytical balance was used. The glasswares used in each procedure were rinsed thoroughly with double-distilled water and dried in dust-free air. Volumetric flasks, pipettes, measuring cylinders, beakers of borosilicate glass were used.

Chemicals and Reagents: Naringenin (TCI Chemicals Gujarat, India) and methanol (Merck specialitiespvt. Ltd., Mumbai).

Estimation of Naringenin content by UV Spectroscopic method

Preparation of standard solution for calibration curve

An accurately weighed standard 10 mg of Naringenin was transferred in 100 mL volumetric flask, dissolved in Methanol and sonicated for 15 minutes. The solution was diluted up to the mark with Methanol to obtain final concentration 100 µg/mL. From this standard stock solution, suitable aliquots were transferred into 10 mL volumetric flask and volume was made up to the mark with Methanol. The absorbance of resulting solutions of final concentration (2-12 µg/mL) was measured at 290nm.

Preparation of Sample Solution

Naringenin from various tomato varieties were extracted by adding 30 mL of ethylacetate to 100 gm of tomato, followed by vortex mixing for 3 min and sonicating for 1 min. After that solutions were centrifuged for 5 min. The extraction procedure was repeated and the two organic layers were combined and evaporated. The residue was dissolved into 100mL Methanol and filtered. The resulted solution was used as a sample and absorbance was measured at 290nm.

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 and 1.2 mL) from stock solutions were transferred indifferent 10 mL volumetric flasks and volume was adjusted up to the mark using mobile phase to get concentration of 2-12 µg/mL. Calibration curves were plotted using absorbance versus concentrations, and the regression equation was obtained. Calibration curve was repeated for five times.

Accuracy (% Recovery)

The accuracy of the method was studied by standard addition method. It was determined by calculating recovery of Naringenin using 80, 100, 120 % level. Known amount of standard solutions of Naringenin (0.32, 0.4, 0.48 mL) were added to three different volumetric flask of 10 mL capacity containing 0.4 mL sample stock solution. The volume was

adjusted up to mark. By measuring absorbance of each solution three times, %recovery was calculated with help of regression equation.

Precision

The repeatability of method was checked by analyzing (n = 6) Naringenin solutions (4µg/mL each) and response was recorded. By measuring the responses for 3 different concentration of Naringenin (4, 6 and 8 µg/mL), 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. The absorbance was measured. The % assay values were calculated. The % RSD was reported.

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

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

$$LOD = 3.3 \times (SD/Slope)$$

$$LOQ = 10 \times (SD/Slope)$$

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

Slope = Mean slope of the calibration curve.

Estimation of Naringenin from Different variety of Tomatoes Using Developed Colorimetric Method

These sample solution of Naringenin prepared as per section 2.3. The absorbances of Naringenin in 14 different varieties of tomatoes were compared with that of standard Naringenin. The amount of Naringenin present in 14 different varieties of tomato was analyzed using regression equation.

RESULT AND DISCUSSION

Calibration Curve of Naringenin

Linear correlation was obtained between absorbance and concentration of Naringenin in the range of 2-12 µg/mL. The linearity of the calibration curve was validated by the value of Regression coefficient (R²) as shown in Figure 1

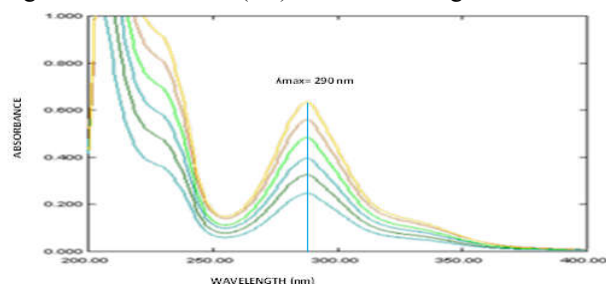


Figure 1 UV Overlay spectra of Naringenin standard solution in range of 2-12µg/mL

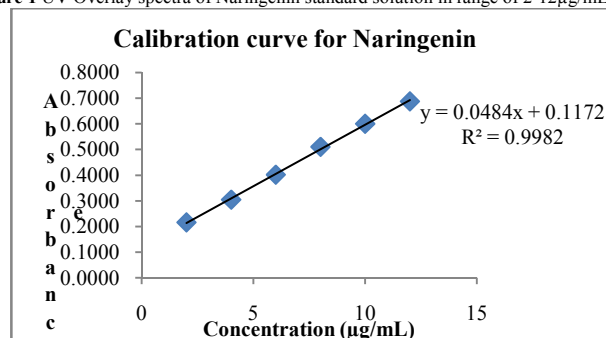


Figure 2 Calibration curve for Naringenin in range of 2-12 µg/mL

Linearity and Range (n=5)

The linearity range for Naringenin was found to be in the range of 2-12 µg/mL. The linearity of the calibration curve was validated by the value of regression coefficients (R^2). The data are indicated in Table: 1

Table 1 Linearity data of Naringenin (n=5)

Conc. (µg/mL)	Absorbance (mean ± SD)	% RSD
2	0.2159 ± 0.0003	0.07
4	0.3041 ± 0.0007	0.07
6	0.4016 ± 0.0005	0.07
8	0.5105 ± 0.0006	0.07
10	0.6006 ± 0.0009	0.07
12	0.6877 ± 0.0005	0.07

Accuracy

Accuracy of the method was confirmed by recovery study of Naringenin at three level of standard addition. Percentage recovery of Naringenin was found to be 98.69-101.54%. The data are indicated in Table: 2.

Table 2 Results of recovery study of Naringenin

% Added Level	Conc. (µg/mL)	Sample Conc. (µg/mL)	Total Amount Recovered (µg/mL)	Mean Absorbance ± SD	% Recovery	%RSD
80	3.2	4	7.2	0.4628 ± 0.0003	101.54	0.93
100	4	4	8	0.5005 ± 0.0009	100.71	0.56
120	4.8	4	8.8	0.5348 ± 0.0003	98.69	0.82

Precision**Repeatability**

The data for repeatability of Naringenin are indicated in Table 3. The %RSD of Naringenin was found to be 0.21.

Intraday and Interday Precision

The data for Intraday and Interday precision of Naringenin are indicated in Table 4. % RSD for intraday precision was found to be 0.39 and for interday precision was found to be 0.42.

Table 3 Results of repeatability of Naringenin (n=6)

Naringenin Conc. (µg/mL)	1	2	3	4	5	6	SD	%RSD
4	0.3005	0.3005	0.3005	0.3005	0.3005	0.3005	0.0006	0.21

Table 4 Results of Interday and Intraday Precision of Naringenin

Naringenin Conc. (µg/mL)	Intra-day precision			Inter-day precision		
	Mean Absorbance ± SD	Mean Conc. ± SD	% RSD	Mean Absorbance ± SD	Mean Conc. ± SD	% RSD
4	0.31 ± 0.0006	4.01 ± 0.013	0.21	0.3010 ± 0.0004	4.00 ± 0.015	0.23
6	0.40 ± 0.0005	5.88 ± 0.010	0.12	0.4016 ± 0.0004	6.08 ± 0.011	0.14
8	0.50 ± 0.002	7.97 ± 0.05	0.51	0.6009 ± 0.0006	7.99 ± 0.05	0.54
Mean %RSD		0.39				0.42

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 9.3.4. The

Limit of Detection (LOD) was found to be 0.06 and Limit of Quantification (LOQ) was found to be 0.19.

Estimation of Naringenin from Different variety of Tomatoes Using Developed Colorimetric Method

Naringenin in different tomato varieties was estimated according to procedure stated in section 2.5. Results are stated in Table: 5.

Table 5 Estimation of Naringenin in 14 different varieties of tomatoes

Sr. no.	Tomato Varieties	Average Absorbance (n = 3)	Conc. Found (mg/100 gm) ± SD
1	Ashoka (Seminis)	0.2570	2.89 ± 0.03
2	Ayushman (Seminis)	0.1542	0.77 ± 0.01
3	Garv (Seminis)	0.2174	2.07 ± 0.02
4	Kedar (Swati)	0.1834	1.37 ± 0.02
5	Ratan (Swati)	0.1753	1.20 ± 0.02
6	Obama (Swati)	0.3128	4.04 ± 0.03
7	Abhinav (Syngenta)	0.1237	0.13 ± 0.01
8	Kohenoora (Greenfield)	0.1651	0.99 ± 0.01
9	1004 (Syngenta)	0.2167	2.06 ± 0.02
10	Badshah (US Indo)	0.3641	5.10 ± 0.01
11	460 (Crystal)	0.2229	2.18 ± 0.03
12	737 (Crystal)	0.1853	1.41 ± 0.02
13	1057 (Syngenta)	0.1504	0.69 ± 0.02
14	Avinash (Syngenta)	0.1370	0.41 ± 0.01

CONCLUSION

The variety 'Badshah (US Indo)' was found to have the highest Naringenin content 5.10 ± 0.01 mg/100 gm by UV method which is cultivated in high altitude and in cold climate, and this may be the reason for the high content of Naringenin. Avinash (Syngenta) is shown to have lesser Naringenin content of 0.41 ± 0.01 mg/100 gm by UV method. The variation in the Naringenin content in different tomato varieties may be due to the different cultivation conditions like temperature and light intensity which have been reported in some studies. Thus, it can be concluded that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully for the estimation of Naringenin in different tomato varieties without inference and with good selectivity.

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