IJBPAS, August, 2024, 13(8): 3847-3856

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF CURCUMIN AND ASCORBIC ACID

JADAV M[1*], PITHADIA[2] A AND JHA LL[3]

1: Department of Quality assurance, School of Pharmacy, Parul University, P.O. Limda, Ta. Waghodia – 391760, Gujarat, India

2: Department of Pharmacology, School of Pharmacy, Parul University, P.O. Limda, Ta. Waghodia – 391760, Gujarat, India

3: Department of Pharmaceutics, School of Pharmacy, Parul University, P.O. Limda, Ta. Waghodia – 391760, Gujarat, India

***Corresponding Author: Ms. Jadav Manisha: E Mail: manisha.jadav121112@paruluniversity.ac.in**

Received 15th May 2023; Revised 8th Sept. 2023; Accepted 4th Nov. 2023; Available online 1st Aug. 2024

https://doi.org/10.31032/IJBPAS/2024/13.8.8240

ABSTRACT

Context- A simple, sensitive, accurate and reproducible spectrophotometric method has been developed. The first order derivative approach was used for Simultaneous estimation curcumin and ascorbic acid. Objective- Methanol was employed as a solvent for the development of spectrophotometric method. Materials and methods- For the measurement of Ascorbic acid, the first order derivative absorption at 422.0 nm (zero crossing point of Curcumin) was used, and for the quantification of Curcumin, the first order derivative absorption at 261.0 nm (zero crossing point of Ascorbic acid) was employed. Curcumin and Ascorbic acid linearity was established over concentration ranges of 2.4-7.2 g/ml and 10-30 g/ml, respectively, with correlation coefficients (r^2) of 0.9999 and 0.9987. The % assay for formulation was found to be 99.89%±0.0500 for Curcumin and 100.39%±0.170 for Ascorbic acid by the proposed method. Mean% recoveries of Curcumin and Ascorbic acid is 99.76%-100.44% and100.09%-100.40%, respectively. Results- The approach was found to be repeatable by analyzing the results of Repeatability, Reproducibility and intermediate precision. LOD and LOQ were 0.30

and 0.99 µg mL-1 for Curcumin, 4.2 and 12.6 µg mL-1 for Ascorbic acid. Conclusion- According to the recovery investigation, the approach can be successfully applied to pharmaceutical formulation with no interference from excipients. All of the validation parameters were within acceptable limits.

Keywords: Ascorbic acid, Curcumin, Development, Spectrophotometric, Validation

1. INTRODUCTION

Chemically, curcumin is (1E, 6E) 1,7 bis (4 hydroxy 3methoxyphenyl) 1,6 heptadiene 3,5 dione. It is the main curcuminoid of turmeric. Turmeric is a very popular Indian spice belonging to the family Zingiberaceae (Zingiberaceae). Desmethoxycurcumin and bisdemethoxycurcumin are the other two curcuminoids of turmeric **[1-5]**. Curcuminoids are natural phenols. The yellow color of turmeric is due to curcuminoids. Curcumin is used to maintain a healthy inflammatory response through its effects on cyclooxygenase, prostaglandins, and leukotriene metabolism. Curcumin seems to support healthy cell cycle function while also functioning as an antioxidant **[6, 7]**. It also helps maintain good liver function by supporting the body's natural detoxification system. Curcumin is used for wound healing by inducing transforming growth factor beta, which induces both angiogenesis and the accumulation of extracellular matrix that continues to the remodeling stage of wound healing **[8-10]**.

Ascorbic acid is natural an antioxidant organic compound. It is a white solid that may appear yellowish if not pure. Vitamin C comes in several forms ("vitamin preparations"). Ascorbic acid is one of them **[11]**. The literature discloses the UV and HPLC methods reported for curcumin. Measurements of ascorbic acid using UV and HPLC have also been reported. So far, simultaneous measurements of curcumin and ascorbic acid granule using UV and HPLC have not been described. As a result, in this study, an attempt was made to design and validate a UV-Spectrophotometric approach utilising standard curcumin and ascorbic acid, and then apply it to curcumin and ascorbic acid content estimate in granule dosage form **[12-15]**.

The term comes from the Greek words a (meaning "no") and scorbutus (scurvy), a disease caused by vitamin C deficiency. Because it is derived from glucose, it can be produced by many animals, but humans need it as a dietary supplement. The chemical formula of ascorbic acid is (R) 5 ((S) 1,2 dihydroxyethyl) 3,4 dihydroxyfuran 2 (5H) on, molar mass 176.12 g mol^{-1}, density 1.65 g / cm^3, melting point 190192 °C, Solubility water about 33g / 100ml. It is a water-soluble vitamin found in a variety of biological and food products (fresh vegetables and fruits, especially citrus

fruits). Ascorbic acid is involved in wound healing and bone formation, as well as collagen formation, iron absorption, and activation of the immune response. It is also a powerful antioxidant that fights diseases caused by free radicals **[16, 17]**.

2. MATERIALS AND METHODS

2.1 Instrument

A double-beam Shimadzu-1780 UV-Visible spectrophotometer, with spectral and width of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

2.1.2 Materials

Curcumin (CUR) standard was purchased from HIMEDIA and Ascorbic acid (AA) was purchased from FINAR Ltd, Ahmedabad, Gujarat, India. AR Grade Methanol was used as solvent and procured from Chiti-Chem corporation, Vadodara, Gujarat.

2.1.3 Method Development

Preparation of Stock Solution: The analysis was carried out using standard stock solutions of CUR (10 µg/ml) and AA (10 µg/ml) prepared in methanol.

2.1.4 Preparation of Sample Solution: 80 mg of sample of was taken and crushed in a motor. From this 80 mg equivalent weight of CUR and AA was transferred into 1000 ml clean dry volumetric flasks and about 1000 mL of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with methanol.

2.1.5 Spectral Characteristics of CUR and AA:

Standard solutions of CUR and AA (10µg/ml, each) were scanned separately in the range of 200-800 nm to determine the maximum absorption for both the drugs. In Zero order spectra CUR showed absorbance maxima at 422nm and AA at 247nm **(Figure 1)**. Solutions of CUR and AA were prepared in methanol by appropriate dilution and spectrum was recorded between 200-800 nm and all zero order spectrums (D0) were converted to first derivative spectrum (D1) using delta lambda 10 and scaling factor 1.0. The overlain first derivative spectrums of CUR and AA at different concentration were recorded. The zero-crossing point (ZCP) of CUR was evaluated from zero order spectrum. Zero order spectra of CUR & AA were overlay and found that at absorption at 422.0 nm for CUR is maximum and zero for AA **(Figure 1)**. The zero-crossing point (ZCP) of AA Cannot be evaluated from zero order spectrum. Hence, the zero-crossing point (ZCP) of AA was evaluated from first order derivative spectrum. First order derivative spectra of CUR & AA were overlay and found that at absorption at 261.0 nm for AA is maximum and Zero for CUR **(Figure 2)**.

2.1.6 The method was validated according to International Conference on Harmonization

guidelines with respect to linearity, Precision, Accuracy, LOD, LOQ and ruggedness **(Table 5 to 8)**.

2.1.7 Preparation of Calibration Curves:

Appropriate dilutions of the standard stock solution were done separately to get 2.4, 3.8, 4.8, 5.7, 7.2 µg/ml of CUR and 10, 15, 20, 25, 30 µg/ml of AA, respectively. The absorption spectra of all solutions were recorded between 200-800 nm. The absorbances were measured at 422.0 nm (λmax of CUR), 261.0 nm (λmax of AA). Beer's lamberts range for CUR and AA were selected and working calibration curves of both the drugs were plotted separately **(Figure 3, 4)**.

2.1.8 Repeatability: Repeatability data, Reproducibility and intermediate precision data of curcumin and ascorbic acid is recorded in **Table 5-8**.

2.1.9 Accuracy (recovery study):

The spiking approach was used to find recovery. The test sample in this procedure has a concentration of 3.8 g/ml for CUR and 15 g/ml for AA. To this standard drug is spiked by adding into the test solution at levels 80, 100 and 120 % of label claim. Standard concentrations of 3.04, 3.8 and 4.56µg/ml of CUR and 12µg/ml, 15µg/ml, 18µg/ml of AA are added to the sample solutions and the absorbance of the three spiked concentrations was recorded. From this absorbance we can determine the amount of drug that can be recovered by the spiking method and results were recorded in the **Table 6**.

2.1.10 Limit of detection and limit of quantification:

Calculating the signal-to-noise ratio (i.e. 3.3 for LOD and 10 for LOQ) using the following formulae indicated by the International Conference on Harmonization (ICH) guideline (Table 8) yielded the drug's limit of detection (LOD) and limit of quantification (LOQ):

$$\mathbf{LOD = 3.3 \text{ and } LOQ = 10 \text{ X } \sigma/S}$$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

2.1.11 Ruggedness: The ruggedness of the method was determined by analysis of samples under a variety of conditions such as change in instrument, Diluent make and effect on the %Assay was studied and summarises in **Table 1**.

3. RESULTS & DISCUSSION

Standard solution of Curcumin and Ascorbic acid were scanned separately in the range of 200-800 nm to determine the maximum absorption for both the drugs **(Figure 1)**. The proposed method for Curcumin was found to be linear in the range of 2.42 µg/ml to 7.2 µg/ml with correlation coefficient (r^2) 0.9999, slope 0.1725 and intercept 0.0077 as shown in **Table 1**. The proposed method for Ascorbic acid was found to be linear in the range of 10 µg/ml to 30 µg/ml with correlation coefficient (r^2) 0.9987, slope -

0.0025 and intercept 0.0134 as shown in **Table 2**. The method was validated in terms of linearity, range, repeatability, precision, accuracy, LOD, LOQ, robustness. The results of all validation parameters are shown in **Table 1** to **Table 7**.

Table 1: Calibration and Regression Analysis Data for Curcumin at 422nm

S. No.	Concentration (µg/ml)	Absorbance at 422.0nm Mean ± S.D (n=3)	%RSD
1	2.4	0.42± 0.001	0.2381
2	3.8	0.672± 0.0006	0.0859
3	4.8	0.839± 0.0006	0.0688
4	5.7	0.998± 0.0015	0.1531
5	7.2	1.252± 0.0021	0.1662
Range		2.4-7.2 µg/ml	
Regression equation (y= mx+c)		y = 0.1725x + 0.0077	
Slope (m)		0.1725	
Intercept(c)		0.0077	
Correlation coefficient		0.9999	

Table 2: Calibration and Regression Analysis Data for Ascorbic Acid at 261nm

S. No	Concentration (µg/ml)	Absorbance at 261.0nm Mean ± S.D (n=3)	%RSD
1	10	-0.012± 0.0000	0.00
2	15	-0.023± 0.0003	1.30
3	20	-0.035± 0.0000	0.00
4	25	-0.048± 0.0000	0.00
5	30	-0.061± 0.0000	0.00
Range		10-30µg/ml	
Regression equation (y= mx+c)		y = -0.0025x + 0.0134	
Slope (m)		-0.0025	
Intercept(c)		0.0134	
Correlation coefficient		0.9987	
Range		10-30µg/ml	

Table 3: Repeatability Data of Curcumin and Ascorbic Acid

S. No	Absorbance	Assay %	Assay %
1	0.075	99.96%	101.02%
2	0.075	100.5%	102.84%
3	0.076	101.65%	101.02%
4	0.076	100.37%	101.02%
5	0.076	101.65%	101.02%
	Mean	100.80%	101.323%
	SD	0.7404	0.7430
	%RSD	0.73	0.73

Table 4: Intermediate Precision: Curcumin

S. No	Absorbance	Assay %
1	0.073	100.9%
2	0.074	102.41%
3	0.073	100.9%
	Mean	101.40
	SD	0.8718
	%RSD	0.86

Table 5: Intermediate Precision: Ascorbic Acid

S. No	Absorbance	Assay %
1	-0.040	99.2 %
2	-0.040	99.2 %
3	-0.040	99.2 %
	Mean	99.200
	SD	0.0000
	%RSD	0.00

Table 6: Accuracy Data for Curcumin and Ascorbic Acid (% Recovery)

Amount of drug present in preanalyzed solution (µg/ml)		Amount of drug spiked (µg/ml)		Total amount (µg/ml)		Total amount of Drug (µg/ml) Mean* ± SD (n=3)		% Recovery	
CUR	AA	CUR	AA	CUR	AA	CUR	AA	CUR	AA
3.8	15	3.04	12	6.84	27	6.87± 0.015	27.03±0.000	100.44%	100.11%
3.8	15	3.8	15	7.6	30	7.62± 0.006	30.12±0.000	100.26%	100.40%
3.8	15	4.56	18	8.36	33	8.34± 0.053	33.03±1.730	99.76%	100.09%

Table 7: Ruggedness Study

Parameter change	Original	Changed conditions
Instrument Laboratory Day	UV 1800 series (shimadzu)	UV 1800 series (shimadzu)
Diluent (Methanol)	STC	HIMEDIA
%Assay for AA	100.39%	100.56%
%Assay for curcumin	99.89%	100.12%

Table 8: Summary of Validation Parameter

Parameters	Curcumin	Ascorbic acid
Wavelength selected (nm)	422	261
Linearity range (µg/ml)	2.4–7.2 µg/ml	10-30µg/ml
Regression equation (y=mx+c)	y = 0.1725x + 0.0077	y = -0.0025x + 0.0134
Recovery 80%	100.44%	100.11%
100%	100.26%	100.40%
120%	99.76%	100.09%
Slope (m)	0.1725	-0.0025
Intercept (c)	0.0077	0.0134
Limit of Detection (µg/ml)	0.30 µg/ml	4.2 µg/ml
Limit of Quantation (µg/ml)	0.99 µg/ml	12.6 µg/ml

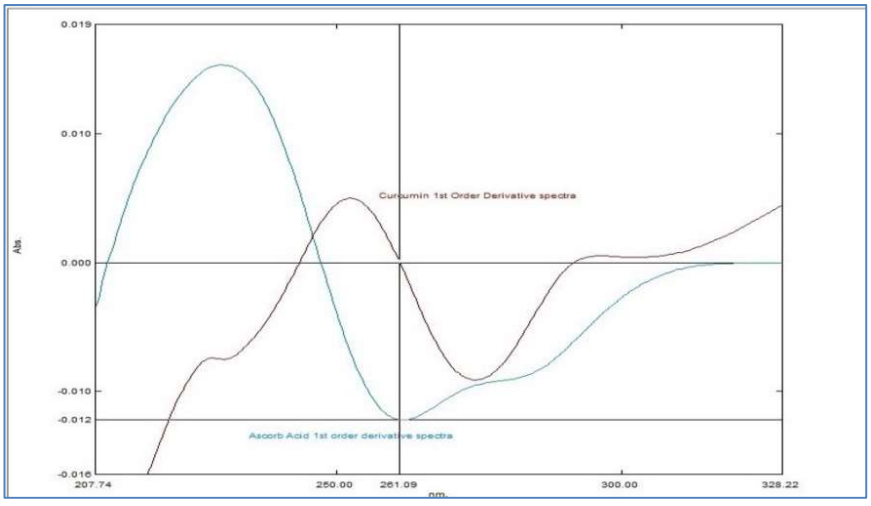

Figure 1: Zero order UV Spectra of Curcumin (grey) & Ascorbic acid (pink) (10µg/ml) in methanol

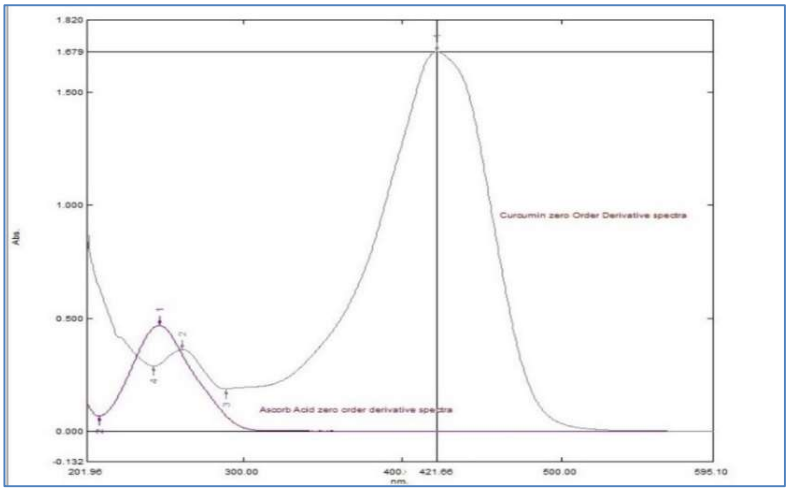

Figure 2: First order UV Spectra of Curcumin (red) & Ascorbic acid (blue) (10µg/ml) in methanol

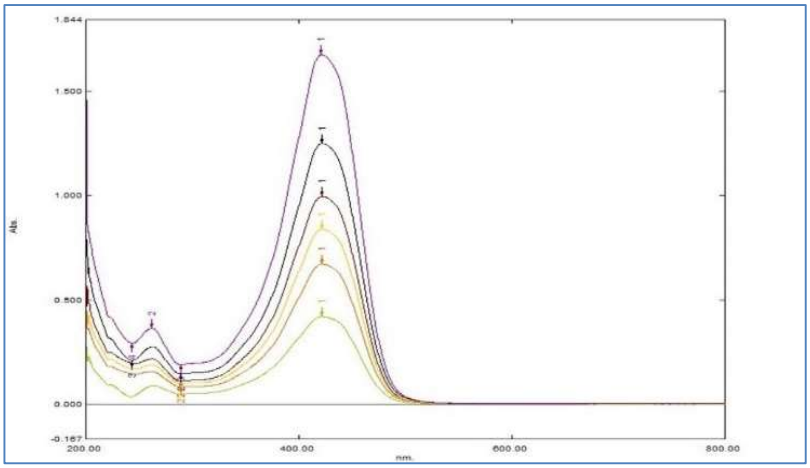

Figure 3: Overlain Spectra (Zero order) of Curcumin at 422nm

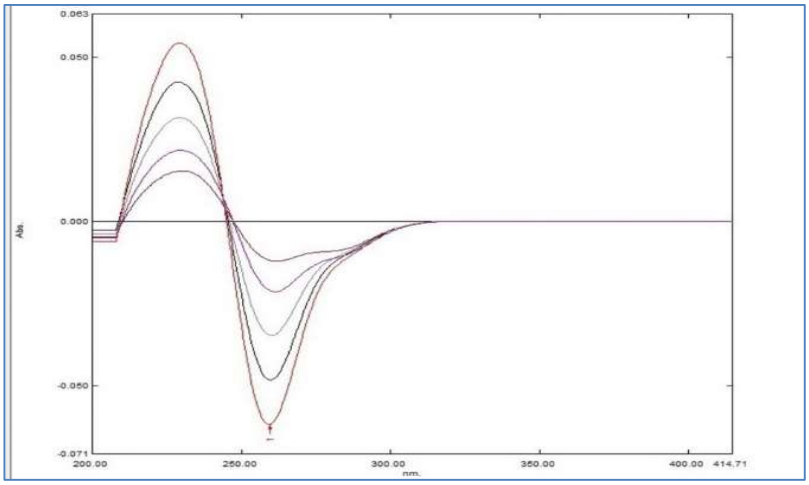

Figure 4: Overlain Spectra (1st order derivative) of Ascorbic acid at 261nm

The results of interday and intraday precision and the results of method precision and intermediate precision (% RSD less than 2), indicate that the proposed method is precise for the analysis of drug. The accuracy was determined by performing recovery studies by standard spiking method. Values of recovery ± SD greater than 98.0% indicate that the proposed method is accurate for the analysis of drug. Limit of detection and limit of Quantitation were found to be 0.30 µg/ml and 4.2 µg/ml respectively for curcumin. Limit of detection and limit of Quantitation were found to be 0.99 µg/ml and 12.6 µg/ml respectively for Ascorbic Acid. Robustness of the method was determined by changing some of the operating conditions results are shown that the proposed method is robust. Method was also applied to the determination of curcumin and Ascorbic acid in granule dosage form. Percentage recovery results **(Table 6)** Indicates that the process is unaffected by the excipients used in the formulation.

The method allows for the simultaneous analysis of CUR and AA in a simple and accurate manner. The wavelengths chosen for analysis in the first order derivative approach were 422.0 nm (max of CUR) and 261.0 nm (max of AA). Low LOD and LOQ readings suggested that the proposed approach is sensitive. Recovery studies were used to verify the accuracy of the proposed procedures. By this method, the percent recovery for CUR and AA was found to be 100.26 % for CUR and 100.40 % percent for AA respectively. The proposed method could be employed for routine quality control of Curcumin and Ascorbic acid in combined granule formulation. According to ICH requirements, this exposition work included the creation and validation of a UV-Spectrophotometric method, first order derivative approach. Curcumin and ascorbic acid in bulk and pharmaceutical dosage form can be successfully analysed using the established method for routine quality control analysis. This approach is highly sensitive, reproducible, fast, and specific, and it allows for selective drug quantification without interference from bulk. This method of analysis was found to be straightforward, repeatable, accurate, and precise.

4. CONCLUSION

The developed spectrophotometric method for the simultaneous estimation of curcumin and ascorbic acid is accurate, precise, robust, and cost-effective. It can be successfully applied for routine analysis and quality control of curcumin and ascorbic acid formulations in the pharmaceutical industry. The method provides a valuable tool for assessing the combined therapeutic potential of curcumin and ascorbic acid and can contribute to the development of novel formulations and dosage forms in the future.

5. ACKNOWLEDGEMENTS

The authors are grateful to Gujarat Council on Science and Technology (GUJCOST) Department of Science and Technology, Gandhinagar, Gujarat, India for providing the financial support.

6. REFERENCES

[1] Sharma k, Agrawal SS, Gupta M. Development and Validation of UV spectrophotometric method for the estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms. International Journal of Drug Development and Research, 4, 2012, 375-380.

[2] Tsvtkova D, Ivanova S. Estimation of validation parameters of UV-Spectrophotometric method for analysis of valsartan. Journal of advance pharmacy education and research, 8, 2018, 37-42.

[3] Wenming Z, Frank M, Alexander M. Development and application of a novel UV method for the analysis of ascorbic acid. Journal of Pharmaceutical and Biomedical Analysis, 2005, 36, 1107-1111.

[4] Rahman MM, Khan MMR, Hosain MM Analysis of Vitamin C (ascorbic acid) Contents in various fruits and vegetables by UV-spectrophotometry. Bangladesh Journal of Scientific Industrial Research, 42, 2007, 417-424.

[5] Priyadarsini KI. The chemistry of curcumin: From extraction to therapeutic agent. Molecules, 19, 2014, 20091–20112.

[6] Shah BH, Nawaz Z, Pertani SA, et al. Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acid-mediated platelet aggregation through inhibition of thromboxane formation and Ca2+ signalling. Biochem Pharmacol, 58, 1999, 1167-1172.

[7] Wilson JX. Regulation of vitamin C transport. Annu. Rev. Nutr, 25, 2005, 105–125.

[8] Mingyue Li, Grace Gar-Lee Yue, Stephen Kwok-Wing Tsui, Kwok-Pui Fung, Clara Bik-San Lau. Turmeric extract, with absorbable curcumin, has potent anti-metastatic effect in vitro and in vivo, 46, 2018, 131-141.

[9] Shailendra SS, Zaranappa, Chaluvaraju KC, Sarvesh, Nagesh P. Validated UV-Spectrophotometric method for simultaneous analysis of aceclofenac and pentoprazole in bulk and pharmaceutical dosage forms. Journal of Pharmacy and Chemistry, 9, 2015, 13-19.

[10] Naveed S, Shabana NZ, Fatima Q, Nimra W, Safeena N, Simple UV Spectrophotometric assay of new formulation gentamycin. J App Pharm, 6, 2014, 407-10.

[11] Hazra K, Kumar R, Sarkar BK, Chowdary YA, Devgan M, Ramaiah M, UV-Visible Spectrophotometric Estimation of Curcumin in Nano formulation. International Journal of Pharmacognosy, 2, 2015, 127-30.

[12] Anal PD, Shuchi D, UV Spectroscopic method for Determination of Vitamin C (Ascorbic acid) Content in Different Fruits in South Gujarat Region. International Journal of Environmental sciences and Natural resources. 2019, 21.

[13] Devang Y, Shelat, Sanjeev R, Acharya. Ur-ca-thione: a novel curcumin concoction with enhanced water solubility and brain bio-availability International Journal of Pharmacy and Pharmaceutical Sciences. 8, 2016, 265-270.

[14] Kamboj PC, Pharmaceutical Analysis. 2nd edition. New Delhi. Vallabh Publication; Volume I. 2003, 1-3.

[15] Willard H, Metrit LL, Dean JA, Settle FA, Instrumental methods of analysis. 7th edition. New Delhi: CBS publishers and distributors. 1986, 11.

[16] Sethi PD, Quantitative analysis of pharmaceutical formulation. 1st edn. New Delhi: Sathish Kumar Jain for CBS publishers and distributors. 2001; 3- 12.

[17] ICH guidelines, validation of analytical procedure: Methodology Q2B; I.C.H. Harmonized Tripartite Guidelines, 1996.