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A COMPARATIVE ANALYSIS OF CURCUMIN CONTENT IN VARIOUS AYUSH PRODUCTS BY UV-VISIBLE SPECTROPHOTOMETRIC METHOD

Shyni Bernard*, Anjitha T. A., Athulya K. S., Thasni Majeed, V. Shankar Ram and Bindhu R.

College of Pharmaceutical Sciences, Government Medical College, Kottayam, Affiliated to Kerala University of Health Sciences.

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***Corresponding Author**
Dr. Shyni Bernard
College of Pharmaceutical Sciences, Government Medical College, Kottayam, Affiliated to Kerala University of Health Sciences.

ABSTRACT

Background: Curcumin, a pigment from turmeric, is a promising natural products that has been extensively investigated by researchers from both the biological and chemical point of view. Curcumin is freely soluble in methanol, chloroform, ethanol and acetone but practically insoluble in water. To meet the demand for analytical methods of curcumin, several methods such as spectrophotometric, chromatographic, capillary electrophoresis and biosensor techniques have been developed for the quantification. The volatility and reproducibility errors are the disadvantages of spectrophotometric methods using organic solvents. Mobile phase optimization, the broadness of spots, plate-to-plate variations are significant limitations for TLC and HPTLC methods. **Method:** A rapid, simple, selective and precise visible spectrophotometric method has been developed for the determination of curcumin in bulk forms and in tincture formulations. The detection was carried out at an absorption maximum of 487 nm using boric acid and hydrochloric acid reagents and the method was validated for specificity, linearity, accuracy and precision. The detector response for curcumin was linear over the selected concentration range of 10 to 40 µg/ml with a correlation coefficient of 0.9995. The accuracy was between 98.99-100.02%.The precision among six sample preparations was 0.49%. The limit of detection and limit of quantitation are 0.0569 µg/ml and 10 µg/ml, respectively. The recovery of curcumin was about 100.4 %. The results demonstrated that the method is simple and can be conveniently employed for routine quality control analysis of curcumin in bulk drug and Ayush formulations.

KEYWORDS: Curcumin; Spectroscopy; Estimation; Formulations.

INTRODUCTION

In recent years, the demand for Ayush (Ayurveda, Yoga, Unani, Siddha, and Homeopathy) products containing curcumin has increased significantly due to its potential health benefits, including anti-inflammatory, antioxidant, and anti-cancer properties. Due to the advancement in the chemical knowledge of crude drugs, various botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the crude drugs. This will strengthen the regulatory process and minimize quality breach. The importance of turmeric mainly comes from its major phytochemical constituents, called curcuminoids. The assurance of the safety and efficacy of herbal drug requires monitoring of the quality of the herbal medicines. It is recommended that various government agencies should follow a more universal approach to herbal quality by adopting the WHO guidelines and also developing monographs using the various quality parameters such as assay or content of the active constituents and the result should be within the defined limits.[1] Solvent extraction followed by column chromatography has been the most commonly employed method reported for separating curcumin from turmeric, and several polar and non-polar organic solvents have been used, including hexane, ethyl acetate, acetone, methanol, etc. Curcumin is a symmetric molecule, also known as diferuloyl methane. The IUPAC name of curcumin is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. It has three chemical entities in its structure: two aromatic ring systems containing o-methoxy phenolic groups, connected by a seven carbon linker consisting of an α,β-unsaturated β-diketone moiety.[2] The reported spectrophotometric methods of estimation of curcumin uses methanol and ethanol as solvents. Instability and toxicity are the main disadvantages of organic solvents.[3-9] There is a better possibility to develop simple and precise method for the spectrophotometric estimation of curcumin. The present study is new, simple, accurate and sensitive visible spectrophotometric method for the estimation of curcumin.

AIM AND OBJECTIVE

The aim of the present study was **t**o develop a fast simple, accurate and reproducible visible

spectrophotometric method for the estimation of curcumin and to apply this method for determining the curcumin content in various Ayush products available in the local market and also to reduce the hazards of volatile organic solvents.

MATERIALS AND METHOD

Instruments

1. Infra-red analysis was carried out by using Perkin Elmer Spectrum II FT-IR Spectrometer with attenuated total reflection (ATR) contact sampling method.
2. Spectrophotometric analysis was carried out by using a double beam UV-visible Spectrophotometer (Carry100 UV) with 1cm matched quartz cells.

Reagents and Chemicals

1. Methanol solution analytical grade supplied from Ajanta Pharma, Mumbai
2. Boric acid analytical grade was obtained from Intas Pharmaceuticals, Ahmedabad
3. Hydrochloric acid, analytical grade was obtained from Lupin Limited, Mumbai
4. Curcumin standard was the gift sample from Innopolis Bio Innovations Private Limited, Cochin India.
5. Commercially available curcumin containing Ayush products were purchased from corresponding pharmacies:-Haridrakhandam -100g, Batch No. YFJD, Manufacturing Date 7/20-24, expiry Date 6/20-27 Nagarjuna Herbal Concentrates Ltd. Haridrakhandam -100g Batch No. 531072, Manufacturing Date 7/20-24, expiry Date 6/20-27 Vaidyaratnam P. S Warier's Aryavaidya sala, Kottakal. Curcuma longa, batch no. 3131IN47012A, manufacture date :9/2021, expiry date:1/2026, from Dr. Reckeweg & Co. GmbH. Curcuma longa mother tincture 30ml (batch no:0543623, Mfg. date: Mar 20, expiry date: Feb 25) manufactured by Dr. Willmar Schwabe India Pvt limited. SBL 30 ml(batch no:, Mfg date: Feb 20, Exp date: March 25) manufactured by SBL Pvt. Limited.

Methodology adopted

1.Preliminary qualitative tests for Curcumin
2.Preparation of standard solution and development of colored complex
3.Study of spectral characteristics of Curcumin – Boric acid colored complex.
4.Preparation of calibration curve
5.Statistical evaluation of calibration plot
6.Stability profile of the coloured complex
7.Validation of the proposed Method
- Accuracy
- Precision
- Detection limit (LOD)
- Quantitation limit (LOQ)
- Linearity
- Range

8. Determination of Curcumin in various Ayush products.
9. Recovery studies
10. Comparison of the proposed method with a reported method.

1. Preliminary qualitative tests for Curcumin

The qualitative tests were performed by observing the color changes in response to different pH levels.

2. Preparation of standard solution

Curcumin standard solution of concentration ranging from 10- 50 µg/ml were prepared and the red coloured complex was developed using 1 ml 6% boric acid and 2ml of 2N HCl.

3. Study of spectral characteristics of coloured complex

Carry-100 double beam UV-Visible spectrophotometer was used for scanning the red coloured complex from 400-800 nm after enabling blank correction in the above region. An absorption band ranging from 450-700 nm was observed with maximum absorption at 487 nm.

4. Preparation of calibration curve

The colour complex was developed using the drug concentrations ranging from 10-40µg/ml. The absorbance of each solution was measured at 487nm with blank.

5. Statistical evaluation of calibration plot

The data in table (1) was used to derive a regression equation of the absorbance (Y) on the concentration (X) by the principle of least squares.
The equation is as follows
$Y = a x + b$
$Y=0.03127x +0.0126$
Correlation coefficient was found to be 0.999592.

6. Stability Profile

Stability of absorbance is of major importance in spectrophotometric measurements. The period over which a steady absorbance value at 487 nm, of the coloured complex was investigated using three different concentrations of 20, 30 and 40 µg/ml. The absorbance values were measured at 15 min intervals for a period of 1 hour.

7. Validation of the proposed Method[10]

a. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted as reference value and the value found. Accuracy was evaluated by carrying out a recovery study and the method was found to be accurate.

b. Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple sampling of the same homogenous sample under prescribed conditions. Precision may be considered at three levels:

repeatability, intermediate precision and reproducibility. Repeatability expresses the precision under the same operating conditions over a short interval of time. The precision of an analytical procedure is usually expressed as the standard deviation of a series of measurements. The reproducibility of the method was studied using three different concentrations of colored complex (10, 20 and 30µg/ml) which were prepared from stock solution A. The absorbance was measured at 487 nm using reagent blank. The absorbance was measured two more times for each concentration and their mean values were calculated.

The intraday and interday precision study was carried out by estimating the corresponding responses three times on the same day and on three different days (1st, 2nd and 5th day) for three different concentrations (10, 20 and 30 µg/ml) and the results are reported in terms of relative standard deviation.

c. Detection limit (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

d. Quantitation Limit (LOQ)

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.

e. Linearity

The linearity of an analytical procedure is its ability, within a given range to obtain test results that are directly proportional to the concentration of analyte in the sample.

f. Range

The range of an analytical procedure is the interval between smallest and largest concentration that maintains a linear relationship between the concentration and the response of the method.

8. Estimation of Curcumin in various Ayush products

Curcumin Mother Tinctures were diluted to obtain concentration of 20 and 30µg/ml and the coloured complex were developed.

Haridrakhandam Churnam *was* weighed and extracted and diluted the extracts to obtain concentration of 20 and 30µg/ml and the coloured complex were developed.

9. Recovery studies

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of Curcumin in the pre analyzed samples. This parameter was evaluated by the recovery studies at concentration levels of 50%, 100%, and 150% of curcumin which consisted of adding known amounts of curcumin reference materials to the samples.

10. Comparison of the proposed method with reported method[11]

The proposed method was compared with other reported UV spectrophotometric method. The results were also compared statistically by student t-test and by the variance ratio F-test with those obtained by official method at 95% confidence level.

RESULTS

The chemical structure and the Infrared spectrum of curcumin R.S is furnished in “Fig.1” and “Fig .2” respectively. The method development started with the qualitative test for curcumin.

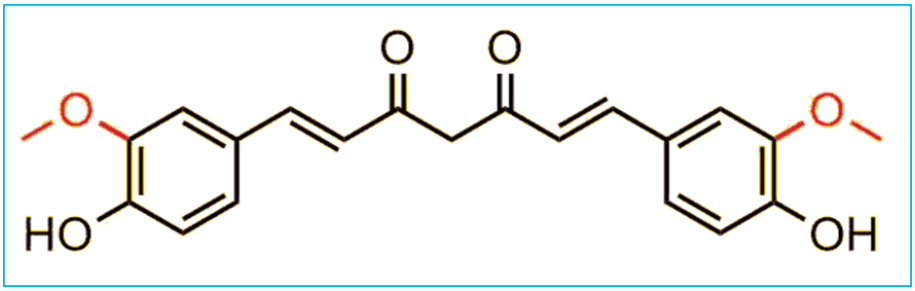

Fig. 1: chemical structure of curcumin.

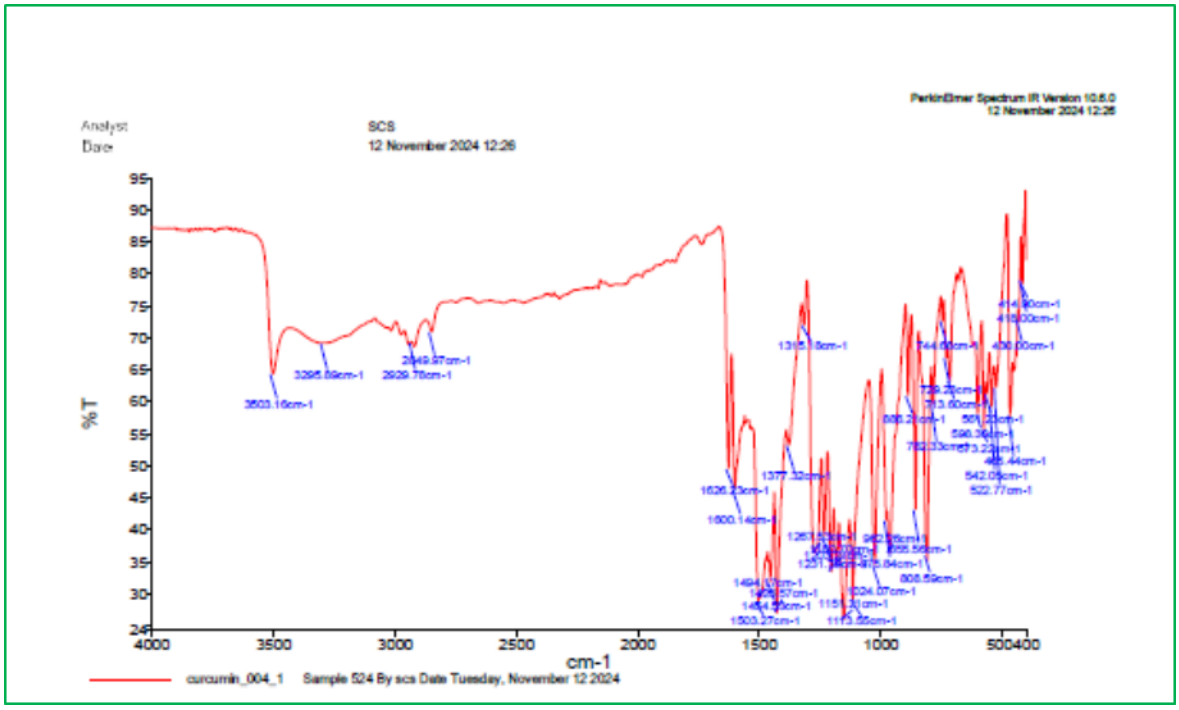

Fig. 2: FTIR spectrum of Curcumin Sample.

A coloured complex developed by the addition of boric acid 1.0 ml and 2N HCl 2ml showed sharp peak with absorption maxima at 487nm. The over lay visible spectrum is shown in Fig:3. The volume of boric acid and 2N HCl were optimized as 1.0ml and 2ml respectively by using different reagent volumes and observing the peak sharpness.

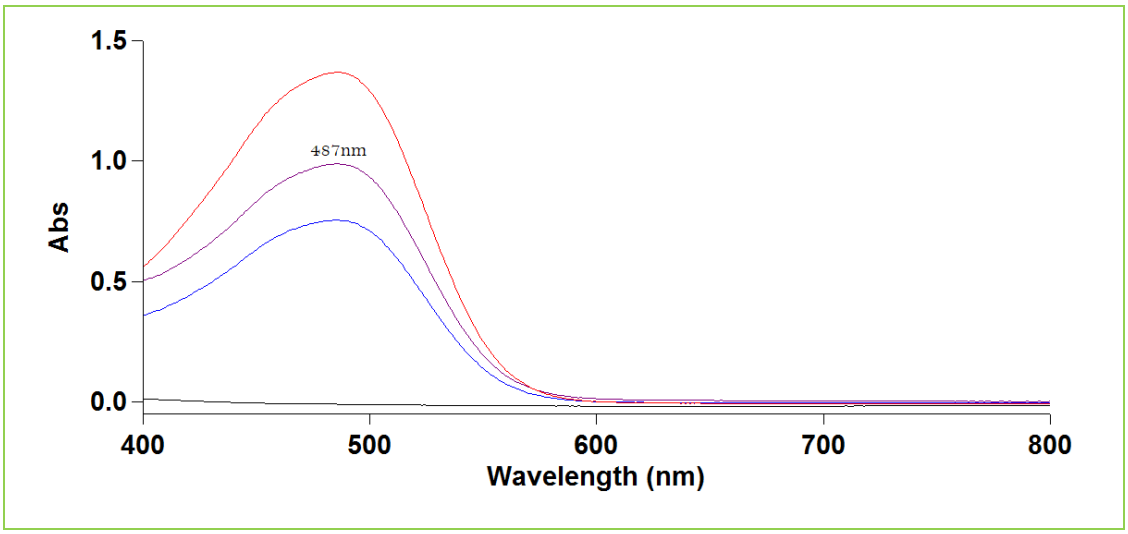

Fig. 3: Visible absorption spectrum of coloured complex.

The calibration curves were constructed by plotting absorbance versus concentration and the regression equation and absorptivity coefficient were calculated. Good linearity was observed in the concentration range of 10-40 µg/ml, The data is furnished in Table. 1 and Fig: 4.

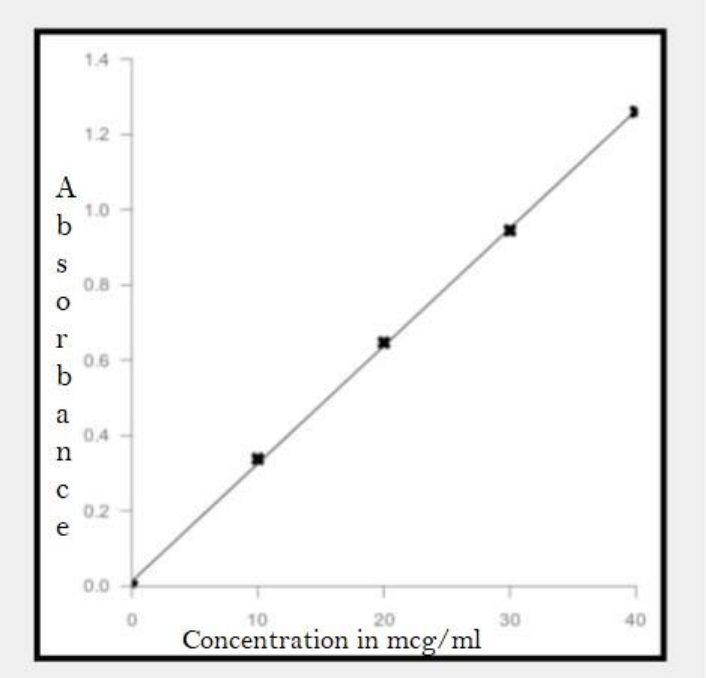

Fig. 4: Beer's Law plot of coloured complex.

The present study involves a colorimetric reaction where boric acid reacts with curcumin in acidic solution, producing a red-coloured complex (Fig.5) that can be measured spectrophotometrically to quantify the amount of curcumin present in the sample. Due to its special chemical structure, curcumin can be an excellent chelating agent (the chemical binding process of metal ions).[12] When combined with boric acid, its esters form a complex called rosocyanine.

Fig. 5: Curcumin boric acid colour complex.

Table 1: Data for Beers Law Plot.

SI No:	Concentration of Curcumin in final solution (µg/ml)	Absorbance at 487 nm
1	10	0.338
2	20	0.647
3	30	0.945
4	40	1.260
Inference: Beer's law is obeyed from 10-40 µg/ml.		

Table.2. gives the optical parameters for the calibration plot. The LOD and LOQ were found to be 0.0569µg/ml and 10µg/ml respectively.

Table 2: Optical Characteristics of Beer's law plot (n=3)

Parameters	
Linearity range	10-40µg/ml
Y= aX+b	Y=0.03127x +0.0126
Molar absorptivity	1.0385×10^{4} .L/mol/cm.
Limit of detection	0.0569 µg/ml
Limit of quantification	10µg/ml
Regression coefficient (r)	0.999592
Slope	0.03127

The data of stability study is shown in Table. 3 which reveals that the coloured complex is stable over a period of 1 hr.

Table 3: Data of stability profile of coloured complex.

S.No.	**Concentration Curcumin (µg/ml)**	**Absorbance at 487 nm at 15 minutes intervals**				
		2min.	**15min.**	**30min.**	**45 min.**	**60 min.**
1	20	0.647	0.649	0.648	0.645	0.646
2	30	0.945	0.946	0.944	0.946	0.945
3	40	1.260	1.258	1.258	1.259	1.259

The intra-day and inter-day precision studies shown in Table.4 and 5 were found to be less than 2 indicating the precision of the proposed method.

Table 4: Results of intra-day precision study.

SI No.	**Concentration (µg/ml)**	**Absorbance at 487nm**			**RSD,%**
		0 hr	**1.5 hr**	**3hr**	
1	10	0.336	0.275	0.279	0.48
2	20	0.648	0.646	0.647	0.55
3	30	0.945	0.943	0.944	0.20

Table 5: Results of inter-day precision study.

SI No.	**Concentration (µg/ml)**	**Absorbance at 487 nm**			**RSD,%**
		1st day	**2nd day**	**5th day**	
1	10	0.338	0.274	0.274	0.48
2	20	0.648	0.646	0.650	0.41
3	30	0.945	0.947	0.946	0.36

The results of estimation of curcumin content in various Ayush products showed that the percentage of curcumin content was in the range of 98.51-99.12% (Table.6.).

Table 6: Result of estimation of Curcumin in various brand of Ayush products

Brand	**Concentration µg/ml**	**Absorbance at 487 nm**	**% purity**
Curcumin Mother Tincture			
Brand A	20	0.628	98.51
	30	0.959	
Brand B	20	0.621	99.12
	30	0.991	
Brand C	20	0.624	99.10
	30	0.998	
Haridrakhandam Churnam			
Brand A	20	0.614	98.99
	30	0.918	
Brand B	20	0.610	99.05
	30	0.910	

Accuracy of the proposed method was ascertained by recovery studies and the results were expressed as percentage recovery in Table.7 and were found in the range of 98.99-100.02%. The low values of standard deviation and coefficient of variance are indicating the accuracy of the method.

Table 7: Results of recovery studies.

Curcumin Mother Tincture	**Curcumin added /spiked**	**Total concentration found**	**% recovery of pure drug * (Mean±S.D) (n=3)**	**%RSD**
Brand A	50mg	99.99	99.99 ±0.429	0.48
	100 mg	150.08	100.02 ± 0.126	0.36
	150mg	200.02	100.00 ± 0.305	0.28
Brand B	50mg	99.97	99.99±0.201	0.48
	100 mg	150.18	100.02±0.210	0.36
	150mg	200.22	100.02±0.230	0.28
Brand C	50mg	99.93	99.19±0.201	0.48
	50mg	150.10	100.12±0.210	0.36
	100 mg	200.14	100.16±0.230	0.38
Haridrakhandam Churnam				
Brand A	50mg	99.97	99.97 ±0.429	0.66
	100 mg	150.18	100.12 ± 0.126	0.38
	150mg	200.04	100.20 ± 0.305	0.58
Brand B	50mg	99.73	99.89 ±0.429	0.36
	100 mg	150.02	100.12 ± 0.126	0.38
	150mg	200.14	100.10 ± 0.305	0.58

The results were compared by a reported method and the student ‘t test’ and ‘F value’ showed no significant difference between the assay value of various products, the data is furnished in table.8

Table 8: Comparison of the results by Proposed (Method A) and reported UV spectrophotometric method (Method B).

Parameters	**Method A**	**Method B**
Reagent	6% boric acid and 2N HCl	Methanol
Detection wavelength	487nm	421 nm
Correlation coefficient	0.9995	0.9997
Linearity	10-40µg/ml	5-25 µg/ml
Assay of curcumin	99.10	99.02
Recovery (%)	98.99-100.02%	97.80 -99.95 %

DISCUSSION

The method showed good repeatability and recovery with relative standard deviation less than 2. The method was validated in accordance with the requirements of ICH guidelines and statistically proved by ‘t’ test and corresponding ‘F’ values. The developed method show significant concern regarding accuracy due to the formation of a colored complex which is more stable as compared to the absorbance values obtained in UV spectrophotometric measurements using ethanol and methanol as solvents, the volatility of the solvents is interfering the absorbance measurement and the peaks of the absorption spectra obtained were also not sharp.

CONCLUSIONS

In Summary, the method discussed in the present work provides a simple, accurate, economical and convenient method for the estimation of curcumin by visible spectrophotometry. Hence the developed method is better than the reported method using methanol as solvent in terms of reproducibility.

REFERENCES

1. Bagchi A: Extraction of Curcumin. *Journal of Environmental Science- Toxicology and Food Technology*, 2012; 1(3): 01-16.
2. Priyadarsini, K.I. Chemical and structural features influencing the biological activity of curcumin. Curr. *Pharm, Des.* 2013; 19: 2093–2100.
3. Rafi, M. et al. UV-Vis spectroscopy and chemometrics as a tool for identification and discrimination of four Curcuma species. International Food Research Journal, 2 April 2018; 5(2): 643-648.
4. Ashwinder Singh and Vasudeva Rao Avupati, Development and validation of uv-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulation, J Young Pharm, 2017; 9(4): 491-495.
5. Warule Pooja S., Patel Vipul P., Gosavi Seema A. Development and validation of uv spectrophotometric method for the estimation of curcumin in an ayurvedic formulation Haridrakhand.

International Journal of Pharmaceutics and Drug Analysis, May 25, 2017.
6. Bruno Fonse, Reversed phase high-performance liquid chromatography (HPLC) method for determination of in situ gelling curcumin-loaded liquid crystals in in vitro performance tests, Arabian Journal of Chemistry, 2017; 10(7): 1029-1037.
7. Hazra et al., Uv-visible spectrophotometric nanoformulation estimation of curcumin in nanoformulation. IJP, 2015; 2(3): 127-130.
8. R D Jangle, B N Thorat, Reversed-phase High-performance Liquid Chromatography Method for Analysis of Curcuminoids and Curcuminoid-loaded Liposome Formulation. Indian J Pharm Sci, 2013 Jan-Feb; 75(1): 60–66.
9. Vijay Salunkhe et al. Simultaneous estimation of curcumin and quercetin in Ayurvedic Proprietary medicine by U.V. spectrophotometry, International Journal of Research in Ayurveda and Pharmacy, March 2012; 3(2): 267-271.
10. International Conference on Harmonization. ICH. Topic Q2B, Validation of Analytical Procedures: Methodology, Proceedings of the Commission of the European Communities. Rockville, 1996; 1-10.
11. Kalyan Hazra et al. UV-Visible Spectrophotometric estimation of curcumin in nanoformulation: International Journal of Pharmacognosy, 2015; 2(3): 127-130.
12. Bellamy LJ, Spicer GS, Strickland JDH. Compounds of curcumin and boric acid. Part III. Infra-red studies of rosocyanin and allied compounds. J Chem Soc, 1952; 4653–4656.