Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF CURCUMIN IN BULK AND PHARMACEUTICAL FORMULATION

Kollol Kumar Majumder[1], Jai Bharti Sharma[1], Manish Kumar[1]*, Shailendra Bhatt[1], Vipin Saini[2]

1. *M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana-133207, India.*
2. *M M University, Solan, Himachal Pradesh, India.*

ARTICLE INFO

Received:
03 Nov 2019
Received in revised form:
10 Feb 2020
Accepted:
15 Feb 2020
Available online:
28 Feb 2020

Keywords: Curcumin, Method development, UV spectrophotometer, Validation

ABSTRACT

This study is aimed at developing a simple UV-visible Spectrophotometric method for determination of curcumin in its pure form and pharmaceutical formulations to validate the developed method. Curcumin was estimated using UV-Visible double beam spectrophotometer at absorption maxima of 429 nm in solvent system containing pH 7.4 phosphate buffer and ethanol in mixture (1:1). The analytical parameters like linearity, precision, and accuracy, by following International Conference on Harmonization (ICH) guidelines were determined. The developed method showed linear response for concentration range of 2-10 µg/ml of curcumin and according to Beer's law, a coefficient of correlation of 0.998 was found. The accuracy was between 99.79 and 100.27. The limit of detection (LOD) and limit of quantification (LOQ) were determined for the sensitivity of the method, which were found to be 0.861 µg/ml and 2.872 µg/ml, respectively. The study revealed that the method for estimation of curcumin is linear, accurate, precise and economical and further can be used for testing the pharmaceutical formulations.

Copyright © 2013 - All Rights Reserved - Pharmacophore

To Cite This Article: Kollol Kumar Majumder, Jai Bharti Sharma, Manish Kumar, Shailendra Bhatt, Vipin Saini, (2020), "Development and Validation of Uv-Visible Spectrophotometric Method for the Estimation of Curcumin in Bulk and Pharmaceutical Formulation", ***Pharmacophore, 10(1),*** *115-121.*

Introduction

The dried and fine rhizomes of Curcuma longa L., Zingiberaceae, commonly known as turmeric, are used worldwide as a food-coloring agent. A wide variety of in vitro and in vivo studies confirmed that turmeric extracts have powerful biological properties, such as inflammatory, antibacterial, antidepressant, antidiabetic, antitumor and anticancer properties. The yellow color of turmeric is principally due to the presence of polyphenolic curcuminoids. In commercially-marketed curcumin (turmeric extracts), curcumin available in a mixture of three curcuminoids, typically contains 77% pure curcumin, 17% demethoxycurcumin and 3% bisdemethoxybcurcumin [1-3]. It has been reported that different species of Curcuma have different percentages of curcuminoids. Among the curcuminoids, curcumin has been attained significant attention due to its bioactive potential. Curcumin is an active component that is considered as an antioxidant [4]. It is a yellow-colored polyphenol, extracted from Curcuma longa rhizomes [5]. Pharmacokinetic properties of curcumin indicate that following oral administration, it is poorly absorbed and only traces of the compound are observed within the blood; whereas, most of it is excreted in the face. The anti-inflammatory effect of Curcumin is most likely mediated through its ability to inhibit cyclooxygenase-2(cox-2) and being inducible. Curcumin protects skin by quenching free radicals and reducing inflammation through nuclear factor-KB inhibition. Curcumin treatment also reduces wound healing time, and improves collagen deposition and fibroblast. Curcumin exhibits anticancer properties by inhibiting carcinogen bioactive via suppression of specific cytochrome P450 isozymes, as well as by inducing the activity or expression of phase I carcinogen detoxifying

Corresponding Author: Manish Kumar; M.M. College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana-133207. Email: manish_singh17 @ rediffmail.com

enzymes. Most of the critical reviews are dedicated to the biological activities in vivo and in vitro as well as to the pharmacological effects of curcuminoids and connected plant sources in animal and human body rather than exploring the analytical ways for determination of curcuminoids. Curcumin is readily soluble in methanol, ethanol and oils; but, it has low solubility in the waters and buffers. Several analytical methods were developed for estimation of curcumin using HPLC, UV-visible spectroscopy and HPTLC. The aim of our study is to develop an accurate, specific, repeatable and stability-indicating method for determination of curcumin.

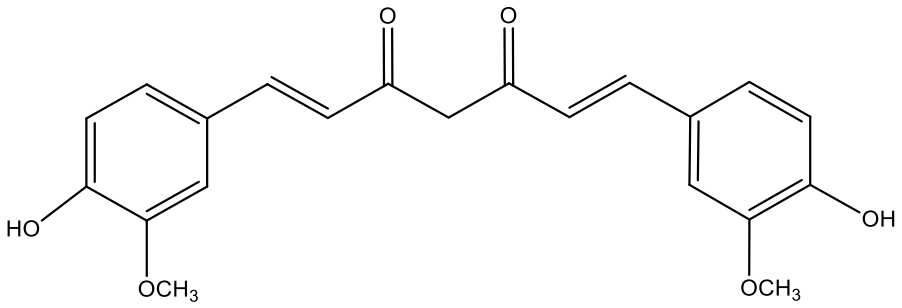

Figure 1: The chemical structure of curcumin

Materials and Method

Materials

Curcumin was obtained as a gift sample from Sanat Products Ltd, Delhi, India. Double beam UV Spectrophotometric (UV 2375 electronics India) with 10mm quartz curettes were used for spectral measurements the later being inexpensive and easy to use [6]. All the opposite chemicals and reagents used were of analytical grade.

Method

Preparation of standard solution

10 mg of curcumin was accurately weighted and transferred into 10 ml volumetric flask. The volumetric flask was filled up to the mark with pH 7.4 phosphate buffer and ethanol (1:1) mixture. The clear solution was obtained by sonicated the solution on bath sonicator.

Determination of maximum wavelength

Curcumin 10 µg/ml solution was scanned in UV spectrophotometer within the wavelength range of 200-800 nm. pH 7.4 phosphate buffer and ethanol (1:1) mixture was used as blank [7].

Preparation of standard calibration curve

The standard calibration curve of curcumin was obtained by measuring the absorbance of curcumin solution in concentration range (2-10 µg/ml) prepared from stock solutions in phosphate buffer pH 7.4 and ethanol at 429nm in triplicate. Calibration curve of curcumin was then plotted with absorbance on y-axis and curcumin concentration on x-axis [7, 8].

Analytical method validation

Validation can be defined as (ICH) establishing documented proof, which provides a high degree of assurance that a selected activity can systematically produce a desired result or product meeting its preset specifications and quality characteristics. The following parameters were evaluated for method validation [8].

Linearity and range

The linearity of the analytical methodology was its ability to take a look at results that are measured directly proportional to analyte concentration. To ascertain the linearity of the projected methodology, 2-10 µg/ml of the standard solution of curcumin was prepared from stock solution and analyzed. All the measurements were performed in triplicate [9].

Precision

Precision studies were carried out to determine the reliability of the projected analytical method. Repeatability was resolute by having six replicates of 4 µg/ml concentration of the same. Therefore, the absorbance was measured intraday and precision study was meted out by preparing drug resolution of 4 µg/ml concentration and analyzing it at 3 completely different times during a day. A similar procedure was followed for 3 completely different days to work reportable as %RSD [10]. The precision result showed an honest reliability but a pair of results of intraday and interday precision studies were measured [10].

Accuracy

Accuracy of the planned technique was determined using recovery studies. The recovery studies were administrated by adding completely different amounts (80%, 100%, and 120%) of the pure curcumin [11, 12].

Ruggedness

Ruggedness was ascertained by closing analyzing 4 µg/ml concentration solutions in pH7.4 phosphate buffer and ethanol (1:1) mixture six times by 2 totally different analysts at 429nm.The results were indicated as %RSD [13].

Robustness

Curcumin 4µg/ml solution was analyzed six times at two different temperatures (room temperature and 20°C) to determine robustness of the method [14].

LOQ and LOD

Limit of detection (LOD) is that the lowest quantity of analyte within the sample that may be detected. Limit of quantification (LOQ) is that the lowest quantities of analyte within the sample that may be quantitatively determined by appropriate precision and accuracy. LOQ and LOD were determined using the subsequent equation:

LOQ-10 s\m

LOD-3 s\m

Where s is the standard deviation of the response and m is the slope of the related calibration curve [15, 16].

Result and Discussion

The projected methodology provides a straightforward, accurate, economical and convenient methodology for the analysis of curcumin using UV Spectrophotometric. The strategy was found to be to be less than two RSD for interday and intraday categories. The strategy was also found to be rugged and robust as the RSD values were found to be less than two. The limit of detection and limit of quantification of the projected methodology was found to be 0.861 µg/ml and 2.872 µg/ml indicating that the method developed is sensitive. The results of assay obtained were found to be in smart agreement with the tagged claim, indicating the absence of interference of the excipients.

Determination of maximum wavelength

The wavelength like most absorbance in pH7.4 phosphate buffer and ethanol (1:1) mixture was found at 429 (Figure 2).

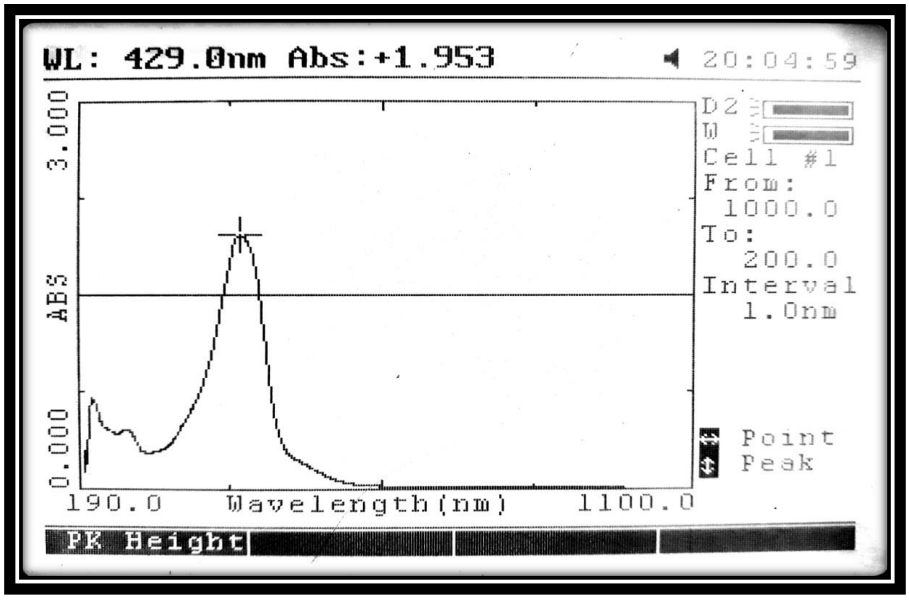

Figure 2: UV Spectrum of curcumin in phosphate buffer pH 7.4 and ethanol

Preparation of standard calibration curve

The calibration plot for curcumin was found to be linear with a coefficient of correlation of 0.998 as shown in (Figure 3).

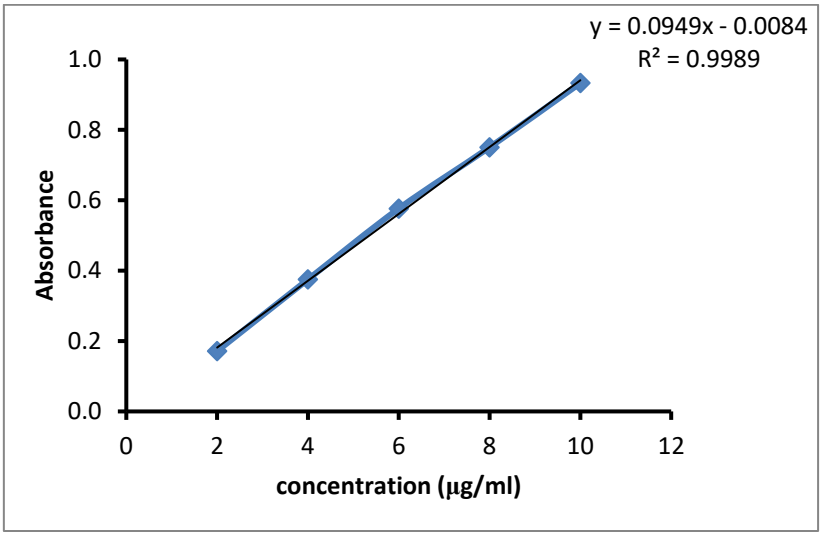

Figure 3: Calibration curve of curcumin in phosphate buffer pH 7.4 at 429nm

Analytical method validation

The method was validated for many parameters like one-dimensionality, accuracy, precision, ruggedness, robustness, limitof detection (LOD), and limit of quantification (LOQ) in line with ICH guidelines [17].

Linearity and Range

The linearity of the calibration curve was validated by the value of correlation coefficients (r^2). The value of correlation coefficient for curcumin was found to be 0.998 as shown in Table 1.

Table 1: Linearity Table of Curcumin

Concentration µg/ml	Absorbance	
2	0.171	
4	0.375	Y= 0.094x- 0.008
6	0.576	R^2=0.998
8	0.750	
10	0.933	

Precision

The intraday and interday were performed at totally different days with not much distinction among them. The result shows that the proposed methodology is consistent. The precision results are represented in (Tables 2, 3, 4). The proportion of relative variance was also calculated.

Table 2: Precision results showing repeatability

Concentration (µg/ml)	Absorbance	Statistical Analysis
4	0.370	
4	0.381	
4	0.369	Mean-0.373
4	0.372	SD-0.005715
4	0.366	%RSD-1.53
4	0.378	

Table 3: Intraday Precision

Concentration (µg/ml)	Absorbance 1(11:00am)	Absorbance 2(1:00pm)	Absorbance 3(3:00pm)	Average %RSD
4	0.370	0.371	0.375	
4	0.381	0.369	0.378	
4	0.369	0.379	0.367	
4	0.372	0.370	0.372	1.475
4	0.366	0.376	0.383	
4	0.378	0.381	0.370	
%RSD	1.534	1.348	1.544	

Table 4: Interday Precision

Concentration (µg/ml)	Absorbance 1(DAY1)	Absorbance 2(DAY 2)	Absorbance 3(DAY 3)	Average %RSD
4	0.374	0.371	0.376	
4	0.383	0.370	0.377	
4	0.371	0.375	0.370	
4	0.375	0.374	0.375	1.097
4	0.377	0.368	0.380	
4	0.369	0.379	0.373	
%RSD	1.312	1.065	0.914	

Accuracy

Accuracy of the planned method was established by usual addition and also the recovery was found to be within the range of 98-101.30 (Table 5).

Table 5: Accuracy reading of curcumin

Labeled claim	Level addition	Amount of drug added	Average % recovery
10	80%	8	100.27±0.23
10	100%	10	99.79±0.19
10	120%	12	100.18±0.26

Ruggedness

Ruggedness was ascertained by carrying out the assay with constant condition on completely different days, by different analysts, different instrument and different time. The check results were found at the range of 99-101% as shown in (Table 6)

Table 6: Result of ruggedness

Concentration (µg/ml) Analyst 1	Absorbance	Statistical Analysis
4	0.370	
4	0.381	Mean-0.373
4	0.369	SD-0.005715
4	0.372	%RSD-1.53
4	0.366	
4	0.378	
Analyst 2		
4	0.371	
4	0.376	Mean-0.375
4	0.369	SD-0.00405
4	0.378	%RSD-1.079918
4	0.377	
4	0.379	

Robustness:

Robustness was firm by closing the assay throughout modification wavelength. The sharp RSD was found to be not more than 2% which was within the limit as shown in (Table 7)

Table 7: Results showing robustness

Concentration (µg/ml)	Absorbance	Statistical Analysis
Temp.-19°C		
4	0.370	
4	0.381	Mean-0.373
4	0.369	SD-0.005715
4	0.372	%RSD-1.53
4	0.366	
4	0.378	
Temp.-30°C		
4	0.370	

4	0.379	Mean-0.376
4	0.377	SD-0.005692
4	0.368	%RSD-1.514
4	0.382	
4	0.380	

LOQ AND LOD:
LOQ and LOD values were found to be 0.0861 and 2.872 µg/ml, respectively.

Table 8: Optical characteristics [9, 15]

Parameter	Result
Absorption maxima	429
Beers law range	2-12 µg/ml
Correlation coefficient	0.998
Regression equation	0.094x-0.008
Slope	0.094x
Intercept	0.008
Accuracy	98-101.5%
Precision	Intraday 1.475,interday1.097
LOD µg/ml	0.861 µg/ml
LOQ µg/ml	2.872 µg/ml

Conclusion

The proposed UV Spectrophotometric method can considered sample, fast and economical which is also applied in many studies such as [18]. The method is valid compliance with ICH guidelines and appropriate for estimation of curcumin with excellent accuracy, precision and linearity.

Acknowledgement

The authors are grateful to Prof. (Dr.) Manish Kumar and all staff members of MM college of Pharmacy, Mullana for providing us the facilities for carrying out this research work.

References

1. Kadam PV, Yadav KN, Bhingare CL, Patil MJ. Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. Journal of Pharmacognosy and Phytochemistry. 2018;7(5):1913-8.
2. Rohman A. Analysis of curcuminoids in food and pharmaceutical products. International Food Research Journal. 2012; 19(1): 19-27.
3. Sheikh S, Asghar S, Ahmad S. Development of HPTLC method and its validation for the estimation of curcuminoids from polyherbal mouth ulcer gel formulation. J Pharm. 2013 Jan;3:29-34.
4. Rosidi A. The difference of Curcumin and Antioxidant activity in Curcuma Xanthorrhiza at different regions. Journal of Advanced Pharmacy Education & Research| Jan-Mar. 2020;10(1):15.
5. Alnahdi HS. The Possible Ameliorative Mechanisms of Curcumin and/or Coenzyme Q10 Against Hyperthyroidism Induced Liver Damage in Rats. Entomology and Applied Science Letters. 2018 Mar 3;5(1):7-16.
6. Tsvetkova D, Ivanova S. Estimation of validation parameters of UV-Spectrophotometric method for analysis of Valsartan. Journal of Advanced Pharmacy Education & Research| Jul-Sep. 2018;8(3).
7. Yoshasri, J., Venkata Suresh, P., Ramarao. Stability indicating RP-HPLC Determination of curcumin in vicco turmeric cream and kasturi turmeric churna. International Journal of Development Research. 2017; 7(8): 14692-14714.
8. 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. Int J. Pharma and Drug. Anal. 2017; 5(5): 193-197.
9. Singh A, Avupati VR. Development and validation of UV-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulations. Journal of Young Pharmacists. 2017 Oct 1;9(4):491.

10. Holkar VV, Nirmal DR, Mistry SK. Development and validation of UV spectrophotometric method for the estimation of curcumin in bulk and pharmaceutical formulation. J. Pharm. Pharm. Sci. 2015;4(5):1456-63.
11. Wichitnithad W, Jongaroonngamsang N, Pummangura S, Rojsitthisak P. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochemical Analysis. 2009 Jul;20(4):314-9.
12. ICH Harmonized Tripartite Guidelines, Validation of analytical procedures: text & bcmethodology, Q2 (R), Nov 2005.
13. Kadam PV, Bhingare CL, Nikam RY, Pawar SA. Development and validation of UV spectrophotometric method for the estimation of curcumin in cream formulation. Pharmaceutical Methods. 2013 Nov 1;4(2):43-5.
14. Savale SA. Simultaneous Determination of Curcumin and Gefitinib in Pure Form by Using UV Spectrophotometric Method. Hygeia: journal for drugs and medicines. 2017 Jul;9(1):1-8.
15. Raja Narender. P. Pavan Kalyan. Formulation and evaluation of bilayered tablet of candisertan cilexetil. International Journal of Pharmacy & Technology march 2016.
16. Halima O. A, Aneesh T. P, Reshma Ghosh, Nathasha. R. Thomas. Development and validation of UV Spectrophotometric method for the estimation of asenapine maleate in bulk and pharmaceutical formulation, Scholars Research Library Der Pharma Chemica, 2012; 4(2): 644-649.
17. Yuvraj, D.D., Sandip Mohan, H., Somnath Devidas, B. Development and validation of UV-Spectrophotometric method for estimation of metformin in bulk tablet dosage form. International journal of pharmaceutical education and research. 2017.
18. Mazi, C., Karaderi, S., Arıöz, F. Determination of UV-Vis Spectrophotometric Method of Metal Complexes Stoichiometry between Cu (II) and Zn (II) with Vilazodone Hydrochloride. International Journal of Pharmaceutical Research & Allied Sciences, 2018; 7(3):146-152.