RESEARCH ARTICLE

Simultaneous Estimation of Curcumin and Vitamin E in Bulk and Cosmeceutical Formulation by UV Spectrophotometry

Shilpa R. Borate[1,2], Atishkumar S. Mundada[2]

[1]*K. K. Wagh College of Pharmacy, Nasik, Maharashtra, India.*
[2]*SNJB's SSDJ College of Pharmacy, Chandwad, Nasik, Maharashtra, India.*

Received: 22th March, 2023; Revised: 12th May, 2023; Accepted: 13th July, 2023; Available Online: 25th September, 2023

ABSTRACT

The goal of present study is to validate the developed assay method per International Council for Harmonisation (ICH) Q2R1 recommendations and create a sensitive, reproducible, and practical method for detecting curcumin and Vitamin E in pure and in cosmeceutical formulations. With the creation and validation of a straightforward, accurate, and repeatable UV spectrophotometric method, curcumin and vitamin E in bulk and cosmeceutical formulation may now be determined simultaneously. The need for a new technique to estimate curcumin and vitamin E in a cosmeceutical formulation has become more pressing due to the lack of a well-described UV analytical method for doing so. Q absorption at 231 and 285 nm were used in the calculation. Vitamin E (16–24 µg/mL) and curcumin (8–12 µg/mL) both behave according to Beer-Lambert's law at the designated wavelengths. The recovery experiments verified the method's adherence to ICH standards for accuracy, precision, and resilience. The method under consideration can be employed to accurately determine the quantities of vitamin E and curcumin present in a cosmeceutical formulation.

Keywords: Curcumin, Vitamin E, Q absorption method, UV spectrophotometry.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.3.30

How to cite this article: Borate SR, Mundada AS. Simultaneous Estimation of Curcumin and Vitamin E in Bulk and Cosmeceutical Formulation by UV Spectrophotometry. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):637-641.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The rhizome of the *Curcuma longa* plant provides curcumin, turmeric's principal bioactive component. Turmeric contains 2 to 8% curcumin, which is primarily responsible for the yellow colour.[1] It possesses antioxidant and anti-inflammatory activity, due to this, curcumin is used in cosmeceuticals. Curcumin exhibits a distinctive biochemical characteristic that renders it notably efficacious in addressing dermatological issues. The compound under consideration functions as a specific phosphorylase kinase (PhK) inhibitor, exhibiting non-competitive characteristics. Following a cutaneous injury, the release of PhK occurs promptly, hence initiating the activation of numerous signaling pathways that subsequently result in tissue inflammation.[2-4] Curcumin's potential anti-aging and skin cancer prevention benefits and its capacity to block photo carcinogenesis in sun-damaged skin may be attributable to its inhibitory effects on these pathways. When the skin barrier is compromised, like in the case of disease or skin injury, curcumin is more readily absorbed when applied topically.[5]

Vitamin E is also a magical ingredient in skin cosmeceuticals. It is an effective antioxidant. It is involved in a variety of physiological functions, preventing cells from damage from oxidation. It also has a significant anti-inflammatory and lipid peroxidation inhibitory effect in the skin. Vitamin E protects skin from the harmful short- and long-term adverse consequences of UV irradiation, such as photoaging and carcinogenesis, as UV light is the main cause of skin tissue's oxidative stress.

Both curcumin and vitamin E have shown promise as ingredients in cosmeceutical formulations. Due to the absence of a documented UV analytical technique for the concurrent determination of curcumin and vitamin E in a cosmeceutical formulation, the development of a novel approach is imperative for the simultaneous analysis of both compounds. This research aimed to develop a new analytical method for determining curcumin and vitamin E concentrations using the Q absorbance technique and a UV spectrophotometer.

Methods

Apparatus

Curcumin was procured from Yucca Enterprises, Mumbai, India. Vitamin E was obtained from GlaxoSmithKline as

**Author for Correspondence: shilpaborate16@gmail.com*

gift sample. The solvents and chemicals utilized were all of analytical grade. The Millipore system gave double-distilled water. The UV spectrophotometric procedure was conducted using a Shimadzu 1800 double-beam UV-visible spectrophotometer equipped with two quartz cells of 1 cm in length.

Instrument

The experimental setup utilised a Shimadzu-1800 twin-beam UV-visible spectrophotometer from Japan, equipped with a 10 mm quartz cell. The ultrasonicator (12L300H) is a product manufactured by Bio Technics India.

Method development

The solvent selection process involved using various solvents to conduct a solubility study on curcumin and vitamin E. Through this investigation, it was determined that methanol is the appropriate solvent for the creation of a UV-spectroscopic technique.

Standard stock solution preparation

Transferring 10 mg of curcumin into a 50 mL volumetric flask yielded the standard stock solution. After adding 30 mL of methanol to dissolve the curcumin, the volume was brought up to the mark, and the final concentration of curcumin was determined to be 200 µg/mL. To achieve a concentration of 10 µg/mL of curcumin, an additional 2.5 mL of the stock solution was diluted with methanol to a final volume of 50 mL.

A total of 20 mg of vitamin E was measured out and added to a 50 mL volumetric flask. The volume was then brought up to the mark, and 30 mL of methanol was added to aid in the breakdown of curcumin, resulting in a 400 µg/mL vitamin E concentration. Additionally, methanol was added to a concentrated solution of 2.5 mL to bring the total amount to 50 mL. Vitamin E is present in the solution at a 20 µg/mLconcentration.

Determination of λ_{max} of both drugs and isobestic point

The λmax of curcumin (10 µg/mL) and vitamin E (20 µg/mL) were determined by scanning working standard solutions in the UV range of 800–200 nm.

Q absorbance ratio method

The measurement of absorbance is conducted at two specific wavelengths, one of which is an isosbestic point and the other corresponds to the wavelength at which one of the two medications exhibits maximum absorption. The wavelengths 231 (the iso-bestic point) and 285 nm (the maximum of vitamin E) were selected for the Q absorbance equation from the overlapping spectra in (Figure 3). In methanol, five working standard solutions were created with concentrations of 8, 9, 6, 10, 11, and 12 µg/mL for curcumin and 16, 18, 20, 22, and 24 µg/mL for vitamin E. The measurements of absorbance were conducted at two specific wavelengths, namely 231 (the iso-absorptive point) and 285 nm (the maximum absorption wavelength of Vitamin E). Subsequently, the absorptivity coefficients were computed.[6]

The absorbance values of the sample solution containing 10 µg/mL of curcumin and 20 µg/mL of vitamin E were measured at two specific wavelengths: 231 (Iso-absorptive point) and 285 nm (λ_{max} of vitamin E). These values were denoted as A1 and A2, respectively. The ratio of A2 to A1 was then computed.

The relative concentration of two medications in the sample was determined using the following formulae.

$$C_X = [(Q_M - Q_Y)/ (Q_X - Q_Y)] \times A_1/ax_1 \ldots\ldots\ldots\ldots\ldots \text{(i)}$$
$$C_Y = [(Q_M - Q_X)/ (Q_Y - Q_X)] \times A_1/ay_1 \ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

The above formulas were used to get the Q-values and absorptivity for both drugs:

Q_M = Absorbance of sample solution at 285 nm (A_2)/ Absorbance of sample solution at 231 nm (A_1)

Q_X = Absorptivity of curcumin at 285 nm (ax_2)/ Absorptivity of curcumin at 231 nm (ax_1)

Q_Y = Absorptivity of vitamin E at 285nm (ay_2)/ Absorptivity of vitamin E at 231 nm (ay_1)

Where, A)

Where, A_1 and A_2 are absorbance of mixture at 231 nm and 285nm; Qx and Qy are Q values of curcumin and vitamin E, respectively; aX1 and aY1 are absorptivities of curcumin and vitamin E at 231 nm.

The analysis was done 3 times with sample solution.[7]

Preparation of calibration curve

A series of 20 mL volumetric flasks were prepared by transferring 1.6, 1.8, 2.0, 2.2, and 2.4 mL of a curcumin standard solution with a 200 µg/mL concentration. This transfer was performed using a validated 1-mL pipette to ensure accuracy. The flasks were then diluted with methanol up to the mark on each flask. The solutions were prepared with concentrations of 8, 9, 10, 11, and 12 µg/mL of curcumin. A series of 20 mL volumetric flasks were prepared by transferring 1.6, 1.8, 2.0, 2.2, and 2.4 mL of a standard solution of vitamin E (400 µg/mL) into each flask using a pipette. The flasks were then diluted with methanol up to the mark. Vitamin E concentrations were observed to be 16, 18, 20, 22, and 24 µg/mL.

Method validation

The validity of the proposed analytical method was confirmed by adhering to the guidelines outlined in ICH Q2 (R1).

Linearity and range

To determine linearity, standard solutions were made in 6 different concentrations. Curcumin and vitamin E showed linearity in the range of 8 to 12 and 16 to 24 µg/mL, respectively. The absorption of curcumin and vitamin E were measured at λ_1 and λ_2 for each solution. The calibration curves of concentration versus absorbance were constructed. By using linear regression analysis, it was shown that absorbance responses to concentrations are linear.[8,9]

Precision

The proposed methods' repeatability, intraday precision, and interlay precision were evaluated. Six replicates of the test solution

containing the active ingredients curcumin (10.01 µg/mL) and vitamin E (20.2 µg/mL) were used to collect results in order to calculate the repeatability precision (intraday and intraday). The results were expressed as %RSD.[10]

Accuracy

The conventional addition technique was employed to conduct recovery investigations. The test solution of curcumin and vitamin E was supplemented with standard concentrations of curcumin (8, 10, and 12 µg/mL) and vitamin E (16, 20, and 24 µg/mL), which corresponded to 80, 100, and 120% of the label claim, respectively. The study was replicated three times, with each replication corresponding to a different level of recovery. Table 2 presents a comprehensive summary of the findings obtained from the recovery investigations.

LoQ and LoD

The limits of detection (LoD) and limits of quantification (LoQ) for the proposed technique were calculated using the calibration curve, yielding the corresponding equations of LoD = 14 3.3*/S and LoQ = 14 10*/S. Let S¼ be the average slope of the six calibration curves and σ ¼ be the standard deviation of the y-intercepts of the regression lines.

Robustness

The ability of the suggested method to endure variations in the method parameters is known as robustness. The influence of a change in extraction time is used to assess the robustness of an analytical method. Robustness was performed by changing the extraction time of the sample by 15 ± 5 minutes.[11]

Assay

A test sample weighing 1 gm was precisely measured and combined with 70 mL of methanol. The mixture was then subjected to sonication for a duration of 15 minutes. The volume was increased to 100 mL using methanol. The test solution underwent filtration and subsequent dilution, with 1-mL of the filtrate being mixed with 10 mL of methanol. The resulting mixture was then subjected to absorbance measurements at 231 and 285 nm wavelengths.[12]

RESULTS

Determination of λ_{max} of both drugs and isobestic point

Curcumin and vitamin E showed λ_{max} 420 and 231 nm, respectively. Both spectra overlayed each other and showed an isobestc point at 285 nm. (Figures 1-3).

Linearity and range

The concentration ranges utilized for determining the linearity of curcumin and vitamin E were 8 to 12 µg/mL and 16 to 24 µg/mL, respectively. Regression analysis observations revealed a significant correlation between absorbance and drug quantity, with the regression equation and R2 values mentioned in Figures 4-7.

Precision

The precision of the proposed method was quantified by expressing it in terms of relative standard deviation (%RSD)

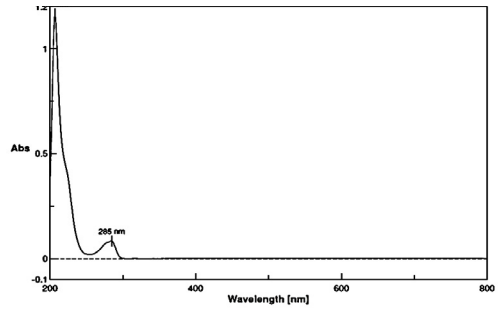

Figure 1: UV spectrum of vitamin E

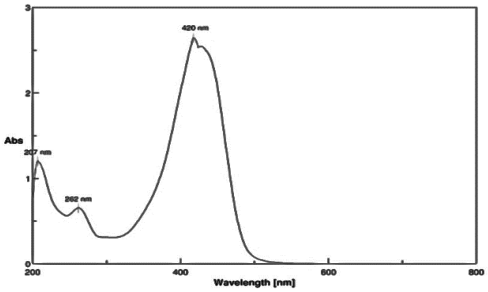

Figure 2:UV spectrum of curcumin

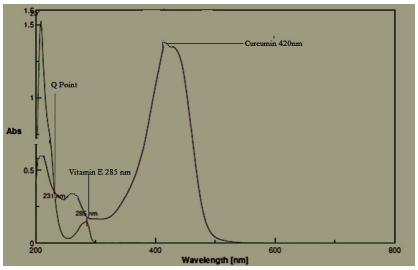

Figure 3: Overlay spectrum of curcumin and vitamin E with isobesticpoint

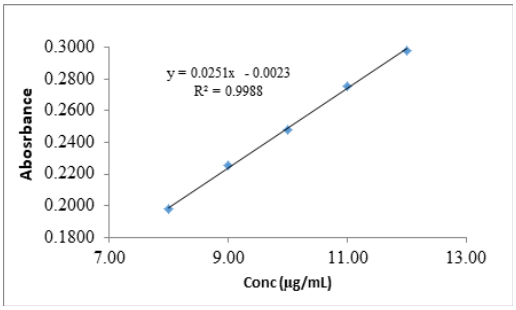

Figure 4: Linearity of curcumin at 231 nm

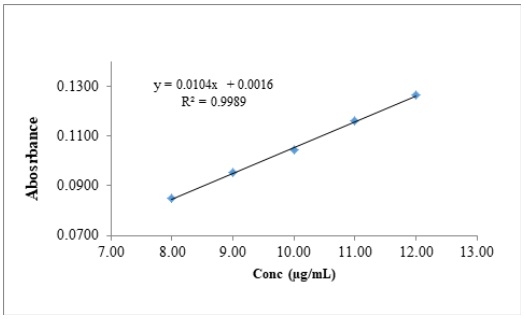

Figure 5: Linearity of curcumin at 285 nm

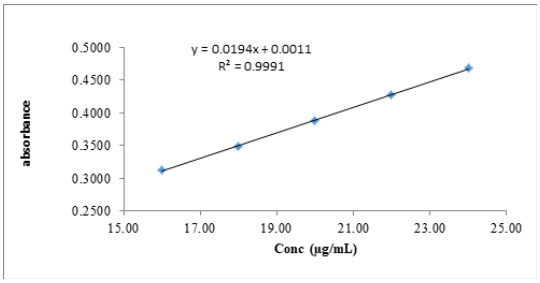

Figure 6: Linearity of vitamin E at 231 nm

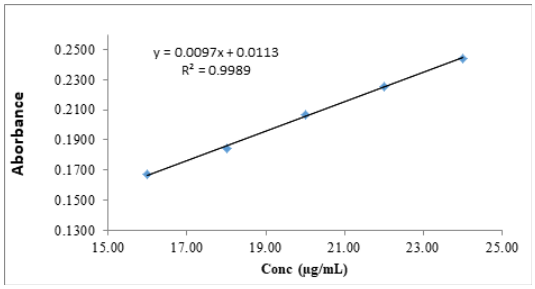

Figure 7: Linearity of vitamin E at 285 nm

Table 1: Repeatability, intra-day and inter-day precision

Precision	*Conc.of Test solution(µg/mL)*		*Absorbance ± S.D*		*% Recovered*		*% RSD*	
	Cur.	*Vit.E*	*231 nm*	*285 nm*	*Cur.*	*Vit. E*	*Cur.*	*Vit. E*
Repeatability	10.01	20.02	0.6366 ± 0.004	0.3106 ± 0.0009	99.13	100.01	1.351	1.063
Intraday	10.01	20.02	0.63664 ± 0.0018	0.3104 ± 0.0011	100.48	99.23	1.593	1.212
Interday	10.01	20.02	0.6361 ± 0.016	0.310383 ± 0.007	98.02	99.45	1.655	1.039

Table 2: Recovery study

Level (%)	*Absorbance + S.D n = 3*		*Recovered conc. (µg/mL)*		*Curcumin %Recovery n = 3*		*Vitamin E %Recovery n=3*	
	231 NM	*285 NM*	*Cur.*	*Vit. E*	*Mean*	*% RSD*	*Mean*	*% RSD*
80	0.5160 ± 0.0015	0.252 ± 0.0008	7.99	16.28	99.5	0.251	100.74	0.647
100	0.6411 ± 0.0013	0.3127 ± 0.0005	10.07	20.07	98.69	0.198	99.46	0.691
120	0.7693 ± 0.001	0.3754 ± 0.0006	12.02	24.16	99.59	0.362	99.68	0.313

Table 3: Sensitivity study

Drug Name	*231 NM*		*285 NM*	
	LoD (µg/mL)	*LoQ (µg/mL)*	*LoD (µg/mL)*	*LoQ (µg/mL)*
Curcumin	0.19	0.57	0.17	0.52
Vitamin E	0.32	0.96	0.35	1.06

Table 4: Robustness study

Parameter	*%Recovered*		*%Difference*	
	Cur.	*Vit. E*	*Cur.*	*Vit. E*
15 – 5 minutes	99.65	99.95	0.52	0.06
15 +5 minutes	98.65	100.50	0.48	0.49

Table 5: Analysis of cosmeceuticals

Particulars	*Label Claim in mg*		*Recovered amount in mg*		*%Assay ± S.D (n=3)*	
	Cur.	Vit. E	Cur.	Vit. E	Cur.	Vit. E
Formulation	10	20	10.082	19.843	100.82 0.3402	99.22 0.0564

values. The percentage relative standard deviation (RSD) for repeatability, inter-day, and intra-day was calculated. The RSD values that were found to be less than 2 indicate the accuracy of this approach (Table 1 and Table 2).

Accuracy

The recovery study was carried out in triplicate at 80, 100, and 120%. The recovery efficiency of the approach was assessed using the standard addition method. The results showed that the recovery rates for curcumin and vitamin E varied between 98.69 and 99.59, and 99.46 and 100.74%, respectively. These findings suggest that the method exhibits high accuracy (Table 2).

LoD and LoQ

LoD and LoQ values of the curcumin and vitamin E at 231 and 285 nm were found to be as mentioned in Table 3.

Robustness

To assess the robustness, curcumin and vitamin E were used as working concentrations of 10 and 20 µg/mL, respectively. The robustness of the approach was assessed by varying the extraction duration within a range of ± 5 minutes (Table 4).

Assay

The entrapment efficiency of curcumin and vitamin E was found to be 100.82 ± 0.3402 and 99.22 ± 0.0564, respectively (Table 5).

Summary of UV method development and validation parameters mentioned in Table 6.

DISCUSSION

UV spectrophotometer readings of curcumin and vitamin E were taken simultaneously utilizing the tried and true Q absorption ratio technique. Curcumin (8–12 µg/mL) and vitamin E (16–24 µg/mL) exhibited Beer's law behavior at 231 and 285 nm, respectively, at 8–12 g/mL concentrations. It was shown that Curcumin and Vitamin E had a strong linear association, with a correlation coefficient close to 1.00 indicating this. The average percent recoveries for curcumin

Table 6: Summary of the method validation parameters

Sr.no	*Validation Parameter*	*Q Absorption method*			
		Curcumin		*Vitamin E*	
		231 nm	285 nm	231 nm	285 nm
1	Specificity	Specific		Specific	
2	Linearity range	8–12 µg/mL		16–24 µg/mL	
3	Linearity equation	y = 0.0251x - 0.0023	y = 0.0104x + 0.0016	y = 0.0194x + 0.0011	y = 0.0097x + 0.0113
4	Correlation coefficient	0.9988	0.9989	0.9991	0.9989
5	Precision	%RSD (n=5)			
	Repeatability	1.351		1.063	
	Intraday	1.593		1.212	
	Interday	1.655		1.039	
6	Accuracy	99.50-99.59		99.68-100.74	
7	Robustness	%Difference			
		0.48-0.52		0.06–0.4999.78–100.04	
8	LoD (µg/mL)	0.19	0.17	0.32	0.35
9	LoQ (µg/mL)	0.57 µg/mL	0.52	0.96	1.06
10	%Assay	100.82 ± 0.3402		99.22 ± 0.0564	

and vitamin E were 99.50–99.59 and 99.78–100.04%, respectively. Consistent analysis of these medications in cosmeceutical formulation was possible using the proposed technique. It has been proven reliable in accordance with ICH standards.[13]

CONCLUSION

The approach that has been developed exhibits characteristics of simplicity, rapidity, and validation in terms of linearity, accuracy, precision, specificity, and repeatability. This method has shown success in the routine simultaneous estimate of both drugs in bulk and cosmeceutical formulations.

REFERENCES

1. Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. The molecular targets and therapeutic uses of curcumin in health and disease. 2007 Jan 1:453-70.
2. Heng MC. Curcumin targeted signaling pathways: basis for anti-photoaging and anti-carcinogenic therapy. International Journal of Dermatology. 2010 Jun;49(6):608-22.
3. Reddy S, Aggarwal BB. Curcumin is a non-competitive and selective inhibitor of phosphorylase kinase. FEBS letters. 1994 Mar 14;341(1):19-22.
4. Heng MC. Wound healing in adult skin: aiming for perfect regeneration. International journal of dermatology. 2011 Sep;50(9):1058-66.
5. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. Molecular pharmaceutics. 2007 Dec 3;4(6):807-18.
6. Singh G, Kumar D, Sharma D, Singh M, Kaur S. Q-Absorbance ratio spectrophotometric method for the simultaneous estimation of prednisolone and 5-Amino salicylic acid in tablet dosage form. Journal of applied pharmaceutical science. 2012 Jul 30;2(7):222-6.
7. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, Part II. CBS Publications and Distributors, New Delhi. 1997;1:275-320.
8. Borman P, Elder D. Q2 (R1) validation of analytical procedures: text and methodology. ICH quality guidelines: an implementation guide. 2017 Sep 27:127-66.
9. Farouk M, Abd Elaziz O, Tawakkol SM, Hemdan A, Shehata MA. Comparative study between univariate spectrophotometry and multivariate calibration as analytical tools for quantitation of Benazepril alone and in combination with Amlodipine. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014 Apr 5;123:473-81.
10. Joshi VG, Geetha M. Development and Validation of UV-spectrophotometric Method for the Estimation of Wintergreen Oil in Pharmaceutical Formulation. Indian Journal of Pharmaceutical Education & Research. 2023 Apr 1;57(2).
11. Srilatha D, Nasare M, Nagasandhya B, Prasad V, Diwan P. Development and validation of UV spectrophotometric method for simultaneous estimation of hesperidin and diosmin in the pharmaceutical dosage form. International Scholarly Research Notices. 2013;2013.
12. Urmi KF, Nawaz MS, Islam SA. Analytical quality by design approach to RP-HPLC method development and validation for simultaneous estimation of esomeprazole and naproxen in modified-release dosage form. Future Journal of Pharmaceutical Sciences. 2022 Dec;8:1-6.
13. Mujawar T, Ahmad S, Tajane P, Sable V, Gaikwad A, Tare H. Development and validation of ultraviolet spectrophotometric method for Saquinavir estimation in bulk and dosage form. Multidiscip. Sci. J. 2023;5(2):2023020.