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Qualitative Analysis of Curcumin in Marketed dosage form by using UV spectroscopy

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ABSTRACT:
Curcuma longa (Turmeric) is Indian rhizomatous medicinal plant from the family Zingiberaceae. Curcumin, Demethoxycurcumin (DMC), and Bisdemethoxycurcumin (BDMC) are the most constituents of the turmeric and are collectively referred to as curcuminoids. Curcuminoids has number of medicinal uses such as anti-inflammatory, anti – HIV, antitumour, antiviral, anticancer, antifungal and antiparasitic. Different analytical methods are developed in recent year for the standard control analysis of curcuminoids in turmeric extract including UV-Visible Spectrophotometry. While the first component curcumin from curcuminoids remains lacking for its analytical method development along side validation. Therefore, within the present study, an easy UV visible method was developed and validated consistent with international conference harmonization (ICH) guidelines for the quantitative estimation of curcumin in marketed formulation.

Keywords: Curcuminoids, curcuma longa, marketed dosage form, UV visible spectroscopy

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]. Phe 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]. Phe 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
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2.1Material and Methods Material

2.2 Method

Preparation of standard solution of Curcumin

Curcumin 10mg was accurately weighed and transferred in a 100ml volumetric flask. Methanol was added upto the mark to obtain a concentration of 100µg/ml of Stock solution. From Stock solution the solutions were withdrawn and diluted to 10ml with methanol to obtain concentrations of 5,10,15,20,25,30µg/ml, respectively.

Determination of maximum wavelength by UV Visible Spectroscopy

Curcumin 5 µg/ml solution was scanned in UV spectrophotometer in the range of 200-800nm methanol was used as blank. Wavelength corresponding to maximum absorbance of curcumin in methanol was observed at 421nm.

Preparation of standard calibration curve of Curcumin

The standard calibration curve of curcumin was obtained by measuring the absorbance of curcumin solution in concentration range (5-30µg/ml) prepared from stock solutions in methanol at 421 nm in triplicate. Calibration curve of curcumin was then plotted with absorbance on y-axis and curcumin concentration on x-axis

Preparation of test solution

1 mg of Curcuma longa extract was accurately weighed and transferred into 100 ml volumetric flask. Methanol was added up to the mark and the resulting solution were used for analysis.

2.3. Method validation

Validation of the method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1). And accordingly the parameters evaluated were:

2.3.1. Sensitivity

Sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). Series of concentrations of drug solutions (0.01–8 µg/ml) were used and analyzed to determine LOD and LOQ. LOD and LOQ were experimentally verified by diluting known concentration of Curcumin until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations

2.3.2. Specificity and selectivity

Three different marketed tablets of Curcumin of concentration 4 µg/ml were prepared in methanol and 4 µg ml-1 of standard Curcumin were analyzed by the proposed method. The estimated amounts of marketed formulation were compared with that of pure Curcumin solution of the same strength.

2.3.3. Linearity and range

Seven different concentrations (1-7 µg/ml) of Curcumin were prepared in methanol from a fresh stock of 10 µg/ml. Least square regression analysis was done for the obtained data.

2.3.4. Accuracy

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In standard analysis method, three different concentrations of the standard Curcumin in methanol were prepared (2.5 µg/ml, 4 µg/ml and 5.5 µg/ml) from independent stock solutions and their strengths were estimated by the standard curve. Standard addition method was followed to support the accuracy by adding separately three different standard concentrations of Curcumin (0.5 µg/ml, 1 µg/ml and 1.5 µg/ml) to a pre-analyzed Curcumin solution of 4 µg/ml and analyzing them again in the same way. The accuracy was reported as % recovery ± (% confidence interval) with % relative error on the base of actual and estimated concentrations.

2.3.5. Precision

Repeatability was done by analyzing three different concentrations of Curcumin(2.5µg ml-1, 4 µg ml-1 and 5.5 µg ml-1) in methanol in six let on a single day. Intermediate precision was done by analyzing the same three concentrations on three different days in six let (drug was found stable for three days). Reproducibility was determined by analyzing three different concentrations of Curcumin (2.5 µg ml-1, 4 µg ml-1 and 5.5 µg ml-1) in six let on different UV spectrophotometers (Double beam UV Spectrophotometer and two different Shimadzu double beam Spectrophotometers in different labs). % Relative standard deviation, standard deviation and confidence interval of the estimated concentrations based on standard curve were reported for each set of data.

2.3.6. Robustness

Robustness of the proposed method was also determined by changing the λ max of the analysis (λ max 420 nm) by ± 1.0 nm. % Mean recovery (± % confidence interval) as well as % relative error was reported.

2.3.7. Use of above method for marketed formulations

The content of Curcumin in tablets (labelled claim: 500 mg per tablet) were determined by powdering twenty tablets and powder equivalent to 10 mg of Curcumin was weighed. The drug from the powder was extracted with methanol. For complete extraction of the drug, it was sonicated for 30 min and volume was made up to 100 ml. The resulting solution was centrifuged at 2500 rpm for 10 min and supernatant was analyzed for drug content.

Three different marketed tablets of Curcumin were used to prepare three independent stocks of Curcumin in methanol of 500 µg ml^{-1} concentration. These three stocks were used individually to prepare three different concentrations of Curcumin (2.5 µg ml-1, 4 µg ml-1 and 5.5 µg ml-1). The prepared solutions were assayed by the proposed method. The % assay values with % confidence intervals are reported.

RESULT AND DISCUSSION:

A rapid, simple, selective and precise UV-Visible Spectrophotometric method has been developed for the determination of marketed dosage form of curcumin. The spectrophotometric detection was carried out at an absorption maximum of 426nm using methanol as solvent. The method was validated for various validation parameters The linearity range was found to be 5-30µg/ml with a correlation coefficient of 0.9352. The accuracy was found to be within limit respectively. The results demonstrated that the method can be conveniently employed for marketed dosage form of quality control analysis of Curcumin**.** The projected methodology provides a straightforward, accurate, economical and convenient methodology for the analysis of curcumin using UV Spectrophotometric. Method development During method development phase, the use of a few milliliters of ethanol as solvent resulted in considerable outcome in UV analysis. Hence, the solvent was optimized to ethanol. The main reason for the selection of ethanol as solvent for developing UV method was based on its biodegradable and ecofriendly (not dangerous for the environment) properties. It is one of the most commonly used solvent in chromatographic separation of curcuminoids. the absorption range of curcuminoids at 400-600 nm. The proposed UV-spectrophotometric method was found to be specific and selective for assay of curcumin the optimized solvent was ethanol and wavelength of maximum absorption (λmax) of curcumin was appeared at 426 nm

Absorbance of UV

Concentration(µg/ml),	Absorbance
5	0.023
10	0.032
15	0.125

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20	0.142
25	0180
30	0.235

3.2.2. Precision

The precision of an analytical method expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment whereas Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. Repeatability (intraday) was assessed by analyzing these three different Concentrations (2.5, 4.0, 5.5 µg/ml), three times a day. Intermediate precision (Interday) was established by analyzing these three different concentrations(2.5, 4.0, 5.5 µg/ml), three times a day for at least three different days.The Standard Deviation, % RSD and Confidence Interval for the intra-assay precision, intermediate precision and reproducibility for all the three concentration levels were found below 0.018, 0.495, ± 0.014 & 0.016, 0.570, ± 0.013 and 0.09, 100.006, ± 0.072 respectively. The data indicated above showed an excellent intraday precision, intermediate precision and reproducibility of the proposed method.

3.2.3. Accuracy

Accuracy of an analytical method is the closeness of test results to true value. It was determined by the application of analytical procedure to recovery studies, where known amount of standard is spiked in reanalyzed samples solutions. The % recovery for the standard analysis and reference analysis method for all the three concentration levels ranged from 98.9% to 102.4% with confidence interval ranging from ± 0.090 to ± 0.190 showing that any small change in the drug concentration can be accurately determined with high accuracy. The results obtained form the standard addition and reference analysis method ware also found supporting the accuracy of the proposed method.

3.2.4. Specificity

The presence of excipients in formulation does not interfere with the drug peak. Therefore, the proposed method was found specific and selective for the drug.

3.2.5. LOD/LOQ

LOD and LOQ were calculated according to the formulae:

LOD=3.3 σ / S =0.07µg/ml
LOQ=10 σ / S = 0.1652µg/ml

3.2.6. Robustness

The variation in the λ max within limits ± 1.0 nm brought % recovery lying in between 99.0 to 99.7 with a maximum % confidence interval of ± 0.009, indicating it to be a sufficiently robust method.

Table 1: Results of validation parameters obtained by the developed method

Validation Parameters	Results obtained
λ max	421nm
Beer's law range (µg $ml^{-1)}$	1-8
Slope ±SD	0.125±0.17
Intercept ±SD	0.0168±0.25
Correlation coefficient	0.9357
Accuracy	98.9-102.1
Precision (%RSD)	0.37
LOD(µg $ml^{-1)}$	0.07
LOQ(µg $ml^{-1)}$	0.1652

Table 2: Intra- and interprecision studies (n = 3).

Amount of drug injected (µg /ml)	injected (µg /ml) Amount of drug detected (µg, mean ± SD	%RSD
Intraday (n = 5)		

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2.5	2.48(0.012)	0.492
4	4.00(0.016)	0.350
5.5	5.49(0.013)	0.235
Intraday (n = 5)		
2.5	2.49(0.012)	0.434
4	4.00(0.014)	0.398
5.5	5.49(0.016)	0.289

Table 3: Formulation study data for three different formulations

Sr.no.	Brand name	Amount labeled	Amount found	SD	%RSD	%Recovery
1	A	500	499.46	0.084	0.017	99.89
2	B	500	500.01	0.064	0.013	100.00
3	C	500	500.96	0.140	0.028	100.19

CONCLUSION

The analytical method developed on UV-Visible Spectrophotometer was simple, reliable, accurate and reproducible. The method eliminates extraction steps thus reduce analytical time, cost and minimize the extraction errors. Low cost, faster speed, satisfactory precision and good specificity, to assess unequivocally the analyte in the presence of components, which may be expected to be present, are the main features of this method. the above results of UV-Vis spectrophotometric data it is visible that the ethanol extract have the nearest almost same λmax value if we compared with the pure curcumin.. Turmeric is commonly known for its medicinal values in the Indian traditional systems of medicine. Turmeric has been used traditionally in "ayurvedic medicine" as an antiseptic, wound healing, and anti inflammatory compounds.

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