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Development and Validation of UV spectrophotometric method for the estimation of Curcumin in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract
A rapid, simple, selective and precise UV- Visible Spectrophotometric method has been developed for the determination of Curcumin in bulk forms and solid dosage formulations. The spectrophotometric detection was carried out at an absorption maximum of 421 nm using methanol as solvent. The method was validated for specificity, linearity, accuracy, precision, robustness and ruggedness. The detector response for the Curcumin was linear over the selected concentration range 1 to 7 µg/ml with a correlation coefficient of 0.9995. The accuracy was between 99.1& 101.4%. The precision (R.S.D.) among six sample preparations was 0.39%. The LOD and LOQ are 0.05 and 0.172 µg/ml, respectively. The recovery of curcumin was about 100.4 %. The results demonstrated that the excipients in the commercial tablets did not interfere with the method and can be conveniently employed for routine quality control analysis of Curcumin in bulk drug, marketed tablets and other formulations.

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1. INTRODUCTION

The powdered dry rhizome of the plant *Curcuma longa*, commonly called turmeric, is widely used as a colouring agent and spice in many food items [1]. It contains a wide variety of phytochemicals, including

curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols [2, 3]. Curcumin is the phytochemical that gives a yellow color to turmeric and is now recognized as being responsible for most of the therapeutic effects [4]. Chemically described as (1E, 6E)-1.7-bis (4 hydroxy – 3 methoxyphenyl) – 1,6 Heptadiene-3,5-dione, the aromatic ring systems, which are polyphenols are connected by two α, β – unsaturated carbonyl groups (Fig. 1), while the α,β – unsaturated carbonyl is a good Michael acceptor and undergoes nucleophilic addition [5, 6]. It is hydrophobic in nature and frequently soluble in dimethylsulfoxide, acetone, ethanol, and oils. It has an absorption maximum around 420 nm [7, 8].

Literature survey revealed that a variety of analytical methods viz. HPLC, HPTLC, UV-Visible has been developed for their analysis but in plasma and urine [9, 10]. As the formulations are available without combinations of any drugs, there is a need for coming up with analytical method which is simple, sensitive, rapid and accurate for estimation of Curcumin in pure form and in pharmaceutical preparations [11, 12]. Therefore, the aim of the present work is to develop and validate a method for the analysis by UV-Visible spectrophotometer which is easily adaptable as a routine in quality testing laboratories. This has enabled us to reduce total time of analysis besides taking care of the error caused due to incomplete extraction and use of internal standard.

2. MATERIALS AND METHODS

2.1. Materials

Curcumin was obtained from Loba chieme Mumbai, India as gift sample. Methanol used was of analytical grade and purchased form Merk Chemicals, India. Three formulations collected from market (A, B & C) with drug equivalent to 500 mg curcumin. All the other chemicals and reagents used were of analytical grade.

2.2. Method development

2.2.1. Instrumentation

Spectroscopic analysis was carried out using Double-beam Shimadzu recording UV-Visible Spectrophotometer (Kyoto, Japan) model 1601 with 10 mm path length matched quartz cells was used for analytical purpose.

2.2.2. Standard stock solution

Stock solutions of curcumin containing 10µg/ml were prepared in methanol and its aliquots were transferred in a series of 10 ml volumetric flasks in varying fractions and their volumes were made with methanol to prepare different standard dilutions varying in between 1-7µg ml^{-1}.

2.2.3. Method optimization

2.2.3.1.. Selection and Optimization of Solvent

It is well known that the solvent do exerts a profound influence on the quality and shape of the peak [13]. The choices of solvents for UV method development are: Chloroform, Acetone, Methanol etc. Different of solvents were optimized. Out of which methanol satisfied all the conditions relative to Peak quality & non-interference at the specified wavelength.

2.2.3.2. Selection of Wavelength

The wavelength at which maximum absorption takes place in UV detector is selected for further analysis i.e. 421nm.

2.3. Method validation

Validation of the method was carried out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) (ICH, 2005) [14]. 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) [15]. Series of concentrations of drug solutions (0.01–7 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 [16].

2.3.2. Specificity and selectivity

Three different marketed tablets of Curcumin of concentration 4 µg ml^{-1} 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 (1-7µg ml^{-1}) of Curcumin were prepared in methanol from a fresh stock of 10 µg ml^{-1}.Least square regression analysis was done for the obtained data.

2.3.4. Accuracy

In standard analysis method, three different concentrations of the standard Curcumin in methanol were prepared (2.5 µg ml^{-1}, 4 µg ml^{-1}& 5.5 µg ml^{-1}) 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}, 1 µg ml^{-1} and 1.5 µg ml^{-1}) to a pre-analyzed Curcumin solution of 4 µg ml^{-1} 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 (One Cintra 5 double beam UV Spectrophotometer and two different Shimadzu 1601 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 (Hanif et al., 1997). 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.

3. RESULTS AND DISCUSSION

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine estimation of Curcumin in bulk drug and pharmaceutical dosage forms.

3.1. Analysis of the Drug

Melting point of curcumin was found to be 183°C. Drug was freely soluble in methanol, chloroform, ethanol, acetone and practically insoluble in water. Spectral scan – λmax of curcumin was found to be at 421 nm.

3.2. Analytical Method Validation Parameters

The method was validated as per ICH guidelines (Q2 (R1).

3.2.1. Linearity and Range

Linearity of an analytical method is its ability, within a given range, to obtain test results that are directly, or through a mathematical transformation, proportional to concentration of analyte [17]. Good linear correlations were obtained between absorbance and concentration in the selected range of 1 – 7 µg/ml. Characteristic parameters are Slope ± S.D. 0.1265 ± 0.15, Intercept ± S.D. 0.0174 ± 0.27, regression coefficient of 0.9991 and correlation coefficient of 0.9995 between the standard drug concentration and corresponding mean absorbance show a good linearity of standard curve (Table 1).

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 [18]. 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 [19, 20]. 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 (Table 2).

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 preanalyzed samples solutions [21, 22].

The % recovery for the standard analysis and reference analysis method for all the three concentration levels ranged from 99.1% to 101.4% 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 =0.05µg/ml

LOQ=10 σ / S = 0.1724µg/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.

3.2.7. Application of the Validated UV-Visible Spectrophotometer Method on the Marketed Formulation

The marketed tablet formulations were analyzed by the proposed method. In accordance with ICH guidelines the assay values for all these formulations were found to be ranging in between 99.89 to 100.19 with a maximum % confidence interval of ± 0.11 (Table 3).

4. 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. Method was successfully validated as per ICH guidelines and can be conveniently employed for routine quality control analysis of Curcumin in bulk drug, marketed tablets and other formulations without any interference from excipients. The method was comparable to the existing methods in all respects, which analyze the drugs but in plasma.

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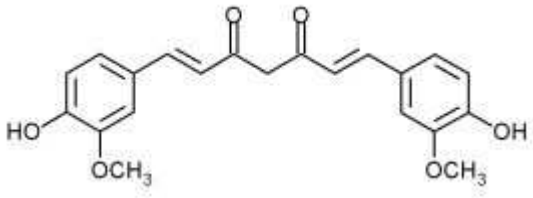

Figure 1: Structure of Curcumin.

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

Validation parameters	Result obtained
λ_{max}	421 nm
Beer's law range (µg ml^{-1})	1-7
Slope ± SD	0.1265±0.15
Intercept ±SD	0.0174±0.27
Correlation coefficient	0.9995
Accuracy	99.1-101.4 %
Precision (%RSD)	0.39
LOD (µg ml^{-1})	0.05
LOQ (µg ml^{-1})	0.1724

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

Amount of drug injected (µg /ml)	Amount of drug detected (µg, mean ± SD)	%RSD
Intraday (n = 5)		
2.5	2.48 (0.012)	0.492
4.0	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
4.0	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 [a]	%RSD [b]	% 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

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