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Original article

Development and validation of UV Spectrophotometric method for the estimation of Curcumin in cream formulation

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ABSTRACT

Introduction: Turmeric (*Curcuma Longa* Linn.) belongs to Zingiberaceae family, in which major constituents are curcuminoids (approx. 6%) of which curcumin constituents 50–60%. The standard Curcumin and isolated Curcumin creams were prepared by o/w emulsification technique. UV–Visible Spectrophotometric method has been developed for the determination of Curcumin in cream formulations by using pure Curcumin as a biomarker.
Object: Validation of UV Spectrophotometric Method for estimation of Curcumin.
Method: Isolation of curcumin was done by defatting the powder of *C. longa* with n-hexane and extracted with acetone. The (O/W) emulsion-based creams were formulated using, emulsifier and other oil soluble components by incorporating standard curcumin and isolated curcumin separately. The spectrophotometric detection was carried out at an absorption maximum of 422 nm using methanol as solvent. The method was validated for linearity, accuracy, precision, limit of detection, and limit of quantitation.
Result: Significant results have been found, it was found that Curcumin obeys linearity within the concentration range of 1 μg/ml–7 μg/ml and coefficient correlation was found to be 0.999. The proposed method was found to be specific while estimating commercial formulations without interference of excipients.
Conclusion: The developed method was found to be simple, sensitive, accurate, precise, reproducible & the most important cost effective and can be used for routine quality control analysis Curcumin.

1. Introduction

Turmeric (*Curcuma Longa* Linn.) belongs to Zingiberaceae family, an important medicinal plant is found throughout India. It has a widespread occurrence in the tropical areas of Asia to Africa and Australia.[1,2] Major constituents are curcuminoids (approx. 6%) of which curcumin constituents 50–60%, desmethoxycurcumin, bisdemethoxycucumin, essential oil (2–7%) zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols, common phytosterols, fatty acid and polysaccharides A,B,C,D.[3,4] Chemically curcumin is 1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione = diferuloylmethane. Curcumin is the phytochemical that gives a yellow color to turmeric and is responsible for most of the therapeutic effects.[4,5] It is potentially used as pharmacotherapeutic agent acts as an anti-inflammatory, antioxidant, antimutagenic, anti HIV properties and reduces glucose, HDL. Curcumin is very low soluble in water at neutral pH and room temperature which results in decreases its bioavailability.[6,7] It is hydrophobic in nature and frequently soluble in dimethylsulfoxide, acetone, ethanol, and oils. It shows better absorption around 420 nm.[8] Few HPLC, HPTLC methods were reported for determination of Curcumin in cream formulation. Literature survey revealed that no simple UV method has been reported for determination of Curcumin in cream formulation.

2. Material and methods

2.1. Material

Turmeric powder was obtained from S.G. Phyto Pharma Pvt. Ltd. S-53, M.I.D.C., Gokul Shirgaon, Kolhapur – 416237 (Maharashtra), India.

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2.2. Isolation of Curcumin

Hundred grams of powdered drug of *C. Longa* (rhizomes) were packed in soxhlet apparatus and extracted with n-hexane by defatting the drug at temperature 60 °C. Defatted powdered drug was then extracted with acetone at temperature was not exceeding 30 °C. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

2.3. Preparation of cream

The oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated using, emulsifier (Stearic acid 17%) and other oil soluble components (Bees wax 2%, Dimethicone 2%) were dissolved in the oil phase (Part A) and heated to 75 °C. The preservatives and other water soluble components (Bronopol 0.1%, Glycerine 5%) were dissolved in the aqueous phase using distil water q.s. (Part B) and heated to 75 °C. The isolated Curcumin was mixed in triethanolamine (Part C) with continues heating till mixed completely. The Part C was added in Part B till mixed completely. This mixture was then added in portions to the oil phase (Part A) with continuous stirring until cooling of emulsifier took place. The standard Curcumin and placebo cream was prepared by above same procedure.

3. Method development

3.1. Instrument: Double beam UV Visible Spectrophotometer (UV SHIMADZU 1800)

3.1.1. Method

In order to ascertain the wavelength of maximum absorption (λ_{max}) of Curcumin, stock solution of 100 μg/ml was prepared by taking 10 mg of drug in 100 ml of methanol. Different solution of drugs (1 μg/ml, 10 μg/ml and 100 μg/ml) in methanol were scanned using spectrophotometer within the wavelength region of 800–400 nm against methanol as blank. The resulting spectra were shown in Fig. 1 & absorption curve showed characteristic absorption maxima at 422 nm for drug.

3.1.2. Preparation of standard stock solution

Accurately weighed 1 mg of standard curcumin was dissolved in 100 ml of methanol (standard stock solution). From this standard stock solutions (10 μg/ml), prepare the aliquots of different concentration by suitable dilutions varying in between 1 and 7 μg/ml using methanol. These diluted solutions were analyzed for Linearity, Accuracy, Precision, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

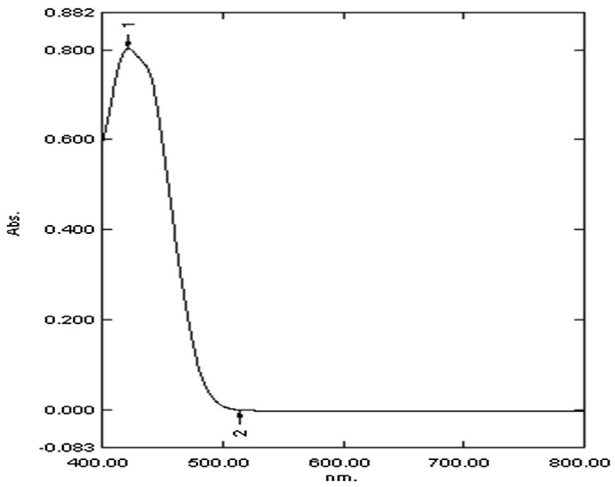

Fig. 1. UV Spectrum of Curcumin in methanol.

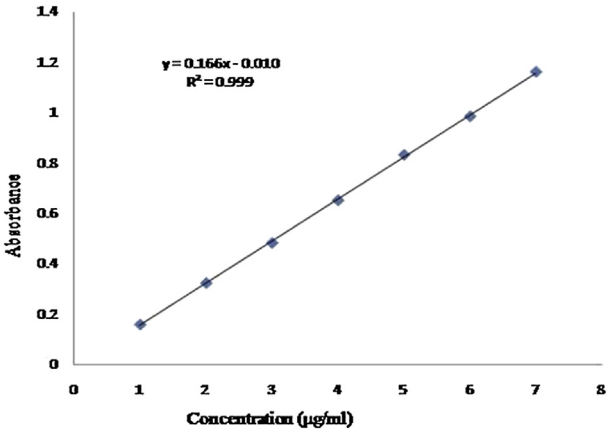

Fig. 2. Standard curve of Curcumin.

3.1.3. Linearity

The linearity of this method was determined at concentration levels ranging between 1 μg/ml and 7 μg/ml. The plot of absorbance v/s concentration (Fig. 2) of Curcumin was found to be linear in the range in Table 1 Beer's law was obeyed over this concentration range.[9]

3.1.4. Precision

The precision of the method was assessed by repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was determined by analyzing 5 μ/ml of Curcumin for three times within the day and average % RSD was calculated. Inter-day precision was determined by analyzing the same concentration of solutions for three days and average % RSD was calculated.[9]

3.1.5. Accuracy

Accuracy is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of Curcumin with three different concentrations of 1 μg/ml, 5 μg/ml, and 7 μg/ml in Table 3.[9]

3.1.6. LOD & LOQ

LOD ($k = 3.3$) and LOQ ($k = 10$) of the method were established according to ICH definitions. LOD and LOQ of method are reported in Table 2. In this study, LOD and LOQ were based on the standard

Table 1
Data for Standard curve of Curcumin.

Concentration (μg/ml)	Absorbance at 422 nm
1	0.160
2	0.324
3	0.483
4	0.651
5	0.831
6	0.983
7	1.151

Table 2
Validation parameters.

Parameter	Results		
λ_{max} (nm)	422		
Beer's law range (μg ml^{-1})	1–7		
Correlation coefficient	0.999		
Accuracy	99.1–101.4%		
Precision (% RSD)	0.22		
LOD (μg ml^{-1})	0.28		
LOQ (μg ml^{-1})	0.87		
Precision (% RSD)	Concentration 5 μg/ml	Intra-day (% RSD) 0.22	Inter-day (% RSD) 0.25

deviation of the response and the slope of the corresponding curve using the following equations:

$$\text{LOD} = 3.3\ S/M; \quad \text{LOQ} = 10\ S/M$$

where S is the standard deviation of the absorbance of the sample and M is the slope of the calibrations curve.[9]

3.2. Absorptivity determination of Curcumin by UV Visible Spectrophotometric method

Accurately weighed 0.1 g of Curcumin was taken and dissolved it in 25 ml of methanol. After complete dissolution, Filter through Whatman no.41 filter paper. Wash the filter paper with methanol, transfer the filtrate to 100 ml volumetric flask and make up the volume with methanol. From the resulting solution pipette out 10 ml, transfer it to 100 ml volumetric flask and make up the volume with methanol. Measure the absorbance at 422 nm in 1 cm cell using methanol as blank.

$$\text{Absorptivity of Curcumin}(A) = 0.49/L \times 0.0025$$

3.3. Determination of percent Curcumin in sample (isolated curcumin) by UV Visible Spectrophotometric method

Accurately weighed 0.05 g, 0.1 g and 0.2 g of Vacuum dried isolated curcumin were taken and dissolved it in 25 ml of methanol. After complete dissolution, Filter through Whatman no.41 filter paper. Wash the filter paper with methanol and transfer the filtrate to 100 ml volumetric flask and make up the volume with methanol. From the resulting solution pipette out 10 ml, transfer it to 100 ml volumetric flask and make up the volume with methanol. Make a final concentration of three stock solutions to 5 μg/ml. Measure the absorbance at 422 nm in 1 cm cell using methanol as blank (Table 3).

$$\text{Percent of Curcumin in test sample} = a \times 100/l \times A \times W$$

where, a = absorbance at 422 nm, l = Cell length in cm, A = Absorptivity, W = Weight of sample in g.

Table 3
Estimation of curcumin in standard curcumin cream, isolated curcumin.

Sr. No	Formulation	Concentration (μg/ml)	Absorbance (nm)	Practical yield	% Purity
1	Standard cream	5	0.813	5.043	100.86
2	Isolated curcumin	5	0.161	1.011	20.22
3	Isolated curcumin cream	5	0.159	1.004	20.08

3.4. Percent drug content

To determine the content of curcumin in cream, 5 g of cream was dissolved in 50 ml methanol, transfer it into a 100 ml volumetric flask, sonicated for 30 min and volume was adjusted to 100 ml using methanol. Then cool the solution and filter it through Whatman filter paper. The final concentration was made 5 μg/ml. The absorbance was measured at 422 nm. The same procedure was followed for making the dilution of placebo cream and it is used as blank. The absorbance was mentioned in Table 3. The analysis was repeated three times. The possibility of interference was studied. The same procedure was followed for standard curcumin cream.

4. Result and discussion

The Curcumin was found to be soluble in methanol. The λ_{max} of drug was found to be 422 nm as shown in Fig. 1. From the result obtained from Table 1, it was found that Curcumin obeys linearity within the concentration range of 1 μg/ml–7 μg/ml and coefficient correlation was found to be 0.999. The regression of the curve was $y = 0.166x - 0.010$ as shown in Fig. 2. The detection and quantitation limits as LOD ($k = 3.3$) and LOQ ($k = 10$) were calculated and these were found to be 0.28 μg/ml and 0.87 μg/ml respectively. The precision (measurements of intra-day and inter-day) results showed (Table 2) significant reproducibility with percent relative standard deviation (% RSD) is below 2.0. This indicated that method is highly precised. The percent recovery value (Table 2), which was higher than 100%, indicates the accuracy of the method. The estimation of Curcumin in extract and in cream formulation was found to be 20%.

5. Conclusion

The developed method was found to be simple, sensitive, accurate, precise, reproducible & the most important cost effective and can be used for routine quality control analysis Curcumin. The proposed method is specific while estimating commercial formulations without interference of excipients.

Conflicts of interest

All authors have none to declare.

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