Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1913-1918
Received: 19-07-2018
Accepted: 21-08-2018

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Standardization and quantification of curcumin from *Curcuma longa* extract using UV visible spectroscopy and HPLC

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Abstract
Curcuma longa (Turmeric) is used successfully in Ayurvedic formulations from ancient times. It is a rich source of bioactive phytoconstituents like curcuminoids, turmerone and many more. Curcuminoids is the group of chief dynamic components and has number of medicinal uses such as anti-inflammatory, anti – HIV, antitumour, antiviral, anticancer, antifungal and antiparasitic. Different analytical methods have been developed in recent year for the quality control analysis of curcuminoids in *Curcuma longa* extract including HPLC, HPTLC and UV-Visible Spectrophotometry. While the primary component curcumin from curcuminoids is still lacking for its analytical method development along with validation. Therefore, in the present study, a simple UV visible and HPLC method was developed and validated according to international conference harmonization (ICH) guidelines for the quantitative estimation of curcumin in *Curcuma longa* extract.

Keywords: Curcuminoids, curcumin, *curcuma longa*, HPLC, UV visible spectroscopy

Introduction
Natural plant products have been used for several medical treatments in humans [1]. Natural plant products are suitable for treating a wide range of infections and diseases. Plants of Zingiberaceae family have been frequently and widely used in traditional systems of medicine [2]. Turmeric (*Curcuma longa* Linn) is a member of the Zingiberaceae family and is cultivated in tropical and subtropical regions around the world and it is originates from India, Southeast Asia and Indonesia [3]. India is the largest producer of turmeric in the world (93.7% of total world production) and is cultivated in 150,000 hectares in India [4].For traditional Ayurvedics, turmeric plant was an excellent natural antiseptic, disinfectant and analgesic, while at the same time the plant has been often used to aid digestion, to improve intestinal flora, and to treat skin irritations [5]. Medicinally, it possess strong antimicrobial, anti-inflammatory, anti-tumour and immunological activities [2]. The pharmacological activity of turmeric has been attributed mainly to curcuminoids consists of curcumin (CUR) and two related compounds demethoxy Curcumin (DMC) and bisdemethoxycurcumin (BDMC) [3]. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric.[6] Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized. Standardization of herbal drugs is usually based on one or more known and accepted active biochemical compound. Many a times where the active biochemical compound is not known, a characteristic compound is used as a "marker," which signifies the presence of the other biochemical compounds that give the herb its therapeutic properties [7].
Different analytical methods have been developed in recent year for the quality control analysis of curcuminoids in *Curcuma longa* extract including; HPLC, HPTLC and UV-Visible Spectrophotometry. While UV-spectrophotometric and HPLC methods are more suitable methods to quantify the curcumin in *Curcuma longa* extract. Therefore, in the present study, a simple UV and HPLC method was developed and validated according to international conference harmonization (ICH) guidelines for the quantitative estimation of curcumin in *Curcuma longa* extract.

Material and Methods
Material
Preparation of standard solution of Curcumin for UV Visible Spectroscopy
Curcuma longa (curcuminoids 95%) extract has been procured

from Konark Herbals and Health care, Nani Daman while the Pure Curcumin (99%) has been received from Ari Healthcare Pvt. Ltd, Pune. All the other chemicals and reagents used were of analytical grade and HPLC grade quality.

Preparation of standard solution of Curcumin for UV Visible Spectroscopy

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 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 ml of solutions were withdrawn and diluted to 10ml with methanol to obtain concentrations of 1, 2, 3, 4, 5, 6, 7µg/ml, respectively [8, 9].

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 424nm.

Preparation of standard calibration curve of Curcumin by UV Visible Spectroscopy

The standard calibration curve of curcumin was obtained by measuring the absorbance of curcumin solution in concentration range (1-7µg/ml) prepared from stock solutions in methanol at 424 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 for UV Visible Spectroscopy

1 mg of *Curcuma longa* (curcuminoids 95%) 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.

Mobile phase composition for HPLC

Solvent A –15 mM Ammonium Acetate was prepared in water and pH was adjusted to 3 with Orthophosphoric acid solution.

Solvent B – Acetonitrile (100%)

Preparation of Standard solution of Curcumin for HPLC

10 mg of standard Curcumin was accurately weighed and transferred into 20 mL volumetric flask. 250 mL of diluent (Methanol) was added and then sonicated in ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with diluent. Then filtered through 0.45 µ syringe filter. The resulting solution was used as standard solution.

Preparation of test solution for HPLC

10 mg of Extract was accurately weighed into 250 mL volumetric flask. 70 mL of diluent was added and sonicated in ultrasonic water bath for 30 minutes. The resulting solution was cooled and volume was made up to the mark with diluent. The content of volumetric flask was filtered through Whatman filter paper No. 41 and then 0.45 µ syringe filter. Resulting solution was used as test solution [10].

Result and Discussion

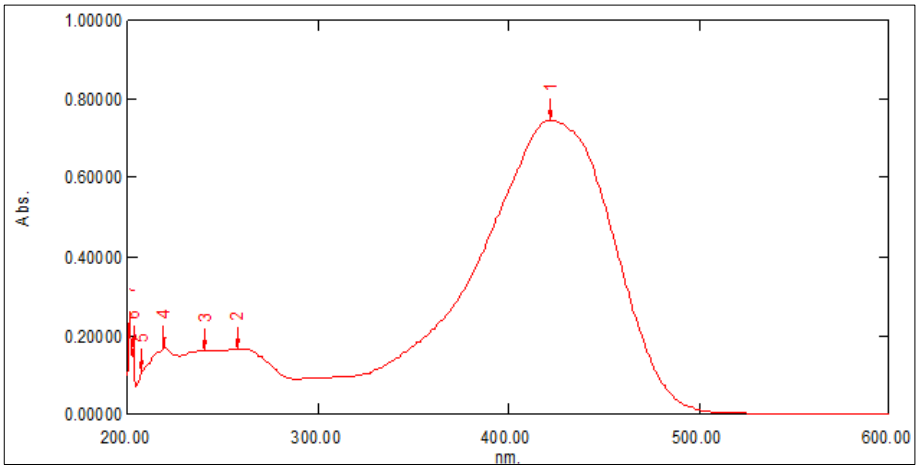

Fig 1: Determination of maximum wavelength of curcumin by UV

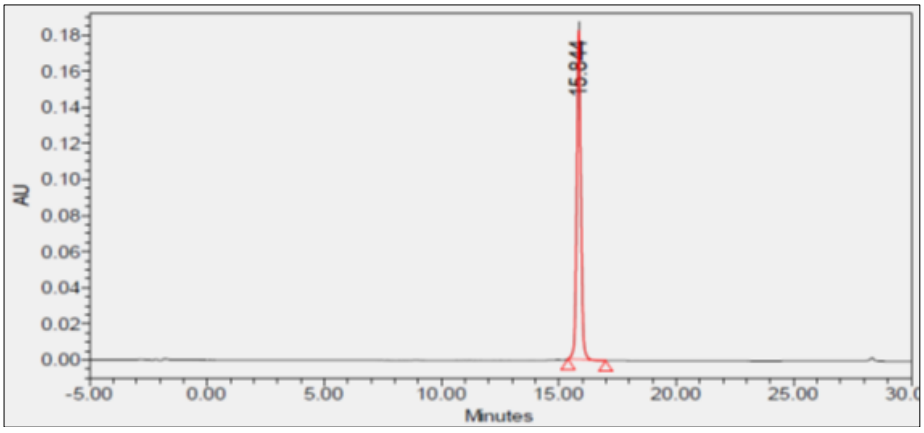

Fig 2: HPLC chromatogram of Standard Curcumin solution

Analysis of Extract

Estimation of Curcumin content present in extract by UV Visible Spectroscopy:

The absorbance of test solution was measured at 424 nm and percentage of Curcumin present in extract was calculated by calibration curve method. The results were reported in Table 1.

Estimation of Curcumin content present in extract by HPLC

Test solution was injected and peak area was reported. The percentage of Total curcumin was calculated. The results were reported in Table 1.

Table 1: Drug Content by UV Visible Spectroscopy and HPLC

UV		HPLC	
Sample No.	**% Assay**	**Sample No.**	**% Assay**
1	98.54	1	95.96
2	96.49	2	94.23
3	97.86	3	94.25
4	97.17	4	94.10
5	95.81	5	93.98
6	95.12	6	93.01
Mean	96.88	Mean	94.25
% RSD	1.32	% RSD	1.01

Analytical Method Validation

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for several parameters like Linearity, Accuracy, Precision, Ruggedness, Robustness, Limit of detection (LOD), Limit of quantification (LOQ) as per ICH guidelines (Q2) R1 [11].

Linearity

The linearity was determined by analyzing different concentrations from stock solution. Curcumin shows linearity in concentration range of 1-7µg/ml at 424 nm for UV Visible spectroscopy and concentration range of 20 to 60µg/ml for HPLC. The calibration curves were plotted as concentration against absorbance for UV and concentration against average peak area for HPLC. A regression equation and correlation coefficient were determined for curcumin standard concentrations.

Table 2: Linearity

UV Visible spectroscopy		HPLC	
Concentration in µg/ml	**Absorbance**	**Concentration in µg/ml**	**Average Peak area of Curcumin**
1	0.150	20	925656
2	0.299	24	1120212
3	0.448	32	1482569
4	0.596	40	1845812
5	0.745	48	2221254
6	0.886	56	2578985
7	1.026	60	2755658

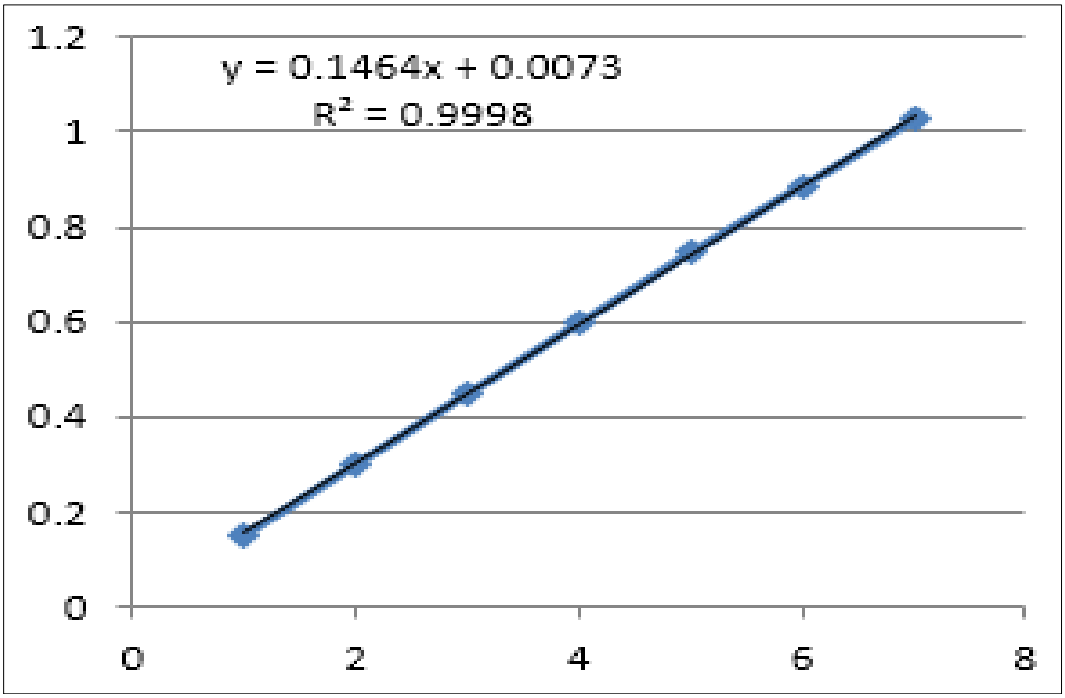

Fig 3: Linearity Graph for Curcumin by UV

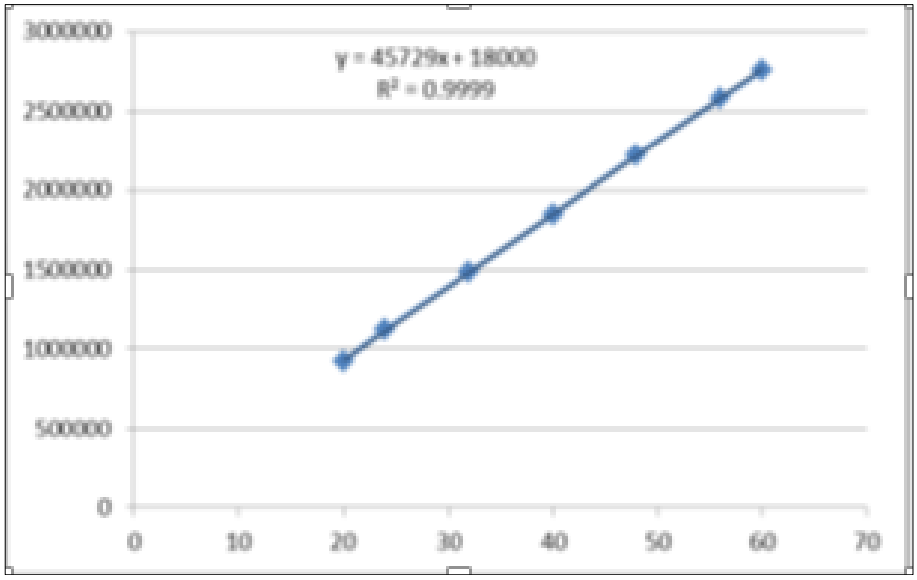

Fig 4: Linearity Graph for Curcumin by HPLC

Accuracy

The accuracy was determined from recovery studies. A known but varying amount of working standard Curcumin was spiked into pre-analyzed extract test solution at 80%, 100% and 120% recovery levels of working concentration in triplicate. The spiked test solution was analyzed according to the proposed procedure. The percentage recoveries were calculated against respective levels and mentioned in Table 3.

Table 3: Recovery for Curcumin

	Recovery Study by UV Visible spectroscopy			Recovery Study by HPLC		
Analyte	**Recovery level**	**% Recovery**	**Average % Recovery**	**Recovery level**	**% Recovery**	**Average % Recovery**
Total Curcumin	80% - 1	98.76	99.52	80% - 1	101.22	101.32
	80% - 2	100.28		80% - 2	101.61	
	80% - 3	99.52		80% - 3	101.12	
	100% - 1	97.42	97.63	100% - 1	100.81	101.17
	100% - 2	96.7		100% - 2	101.56	
	100% - 3	98.79		100% - 3	101.43	
	120% - 1	98.19	99.01	120% - 1	101.08	100.87
	120% - 2	100.05		120% - 2	100.62	
	120% - 3	98.81		120% - 3	100.91	

Precision

Precision was evaluated by using repeatability and intermediate precision. Repeatability was analyzed using different concentrations of curcumin for UV Visible spectroscopy and HPLC. The intermediate precision was analyzed using three different concentrations of curcumin for UV Visible spectroscopy and HPLC. Precision was evaluated by using repeatability and intermediate precision. Repeatability was analyzed using curcumin 5 µg/mL for UV Visible spectroscopy and 40µg/mL for HPLC six times in the same day (intra-day) for. The intermediate precision was analyzed using three different curcumin concentrations 1, 3 and 5 µg/mL for UV and 20, 40, 60µg/mL for HPLC for three times on three consecutive days (inter-day).

Table 4: Repeatability (Intra-Day Precision) of the Proposed UV Visible spectroscopy and HPLC Method.

Repeatability (n=6) for UV Visible spectroscopy			**Repeatability (n=6) for HPLC**		
Curcumin (µg/mL)	Absorbance at 424 nm (Mean±SD)	%RSD	Curcumin (µg/mL)	Peak Area of Curcumin (Mean±SD)	%RSD
5	0.745±0.001211	0.1625	40	1852371.±30980.53	1.6724

Table 5: Repeatability (Intra-day precision) of the proposed UV Visible spectroscopy method for extract

Repeatability (n=6) for UV Visible spectroscopy		
Curcumin (µg/mL)	Absorbance at 424 nm (Mean±SD)	%RSD
5	0.739±0.005033	0.681

Table 6: Intermediate precision (Inter-day precision) of the proposed UV Visible spectroscopy and HPLC method

Intermediate precision for UV Visible spectroscopy (n=3)			**Intermediate precision for HPLC (n=3)**		
Curcumin (µg/mL)	Absorbance at 424 n (Mean±SD)	%RSD	Curcumin (µg/mL)	Peak Area of Curcumin (Mean±SD)	%RSD
1	0.150± 0.000577	0.3849	20	943573.3±16688.60	1.7686
3	0.448±0.001	0.2232	40	1856577±11076.14	0.5965
5	0.745±0.003	0.4026	60	2758486±6091.984	0.220

Table 7: Intermediate precision (Inter-day precision) of the proposed UV Visible spectroscopy method

Intermediate precision for UV Visible spectroscopy (n=3)			
Curcumin (µg/mL)	Absorbance at 424 nm(Mean±SD)	%RSD	Percentage
1	0.145± 0.00251	1.7355	94.28
3	0.445±0.00251	0.5655	99.73
5	0.742±0.00351	0.4026	99.95

System precision

System precision was evaluated from five replicate injections of standard as per proposed method. The Peak area, average and % RSD were calculated and tabulated in the Table 8.

Table 8: Peak Area of Curcumin by HPLC

Injection No	Peak Area of Curcumin
1	1861464
2	1852753
3	1845812
4	1899230
5	1802598
Mean	1852371
% RSD	1.92

Robustness for HPLC

The influence of slightly changed parameters of the chromatographic conditions was tested according to ICH guidelines to demonstrate sufficient robustness of the method. The tests are carried out by injecting Standard solution by varying each of the parameters of chromatography.

Table 9: Robustness for Curcumin by HPLC

Robustness parameter		% RSD	Peak tailing	Theoretical Plates	Remark
Curcumin					
Wavelength (nm)	420	1.41	1.32	32760	Pass
	425	0.60	1.28	34338	Pass
	430	1.73	1.31	32811	Pass
Column Temperature (°C)	27	0.42	1.26	35139	Pass
	32	0.83	1.38	26276	Pass
	37	0.73	1.30	33692	Pass
Flow (mL/min)	1.0	0.38	1.25	37031	Pass
	1.1	0.52	1.21	44453	Pass
	1.2	0.45	1.27	30454	Pass

LOQ and LOD

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy.

LOQ and LOD were determined using the following equation:

LOQ = 10 * S.D. / Slope

LOD = 3.3 * S.D. / Slope

Table 9: LOQ and LOD by UV Visible spectroscopy and HPLC

	UV Visible spectroscopy	HPLC
LOD	0.0617	2.23
LOQ	0.127	6.774

Stability in standard and test solution

The standard and test solutions were prepared as per the proposed method and kept at room temperature. The standard and test solutions were analyzed at initial and at different time intervals. As the Percentage Relative changes of Curcumin and Extract were within limit, Standard solution and Test solution is stable up to 24 hours at Room temperature.

Conclusion

The Specificity of the UV Visible spectroscopy and HPLC test for Assay of Curcumin was proven by spectroscopic and chromatographic comparison. Both the methods were found to be specific. The linearity of the proposed method was determined from the correlation coefficient and the method was found to be linear and within the broad range of working concentration. The accuracy of the methods was calculated by recovery study and the proposed methods were found to be accurate as all the parameter of the method complies as per the acceptance criteria. Standard and test solutions were found to be stable up to 24 hours at Room temperature. The present validation proves that the UV Visible spectroscopy and HPLC-methods were suitable for the determination of Assay of curcumin from *Curcuma longa* (Curcuminoids 95%) Extract at prescribed conditions.

Acknowledgement

Authors are grateful to Marathwada Mitra Mandal's College of Pharmacy, Thergaon – Pune, for providing necessary facility to carry out study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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