INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL.5 ISSUE 5, 2017; 193- – 197 ; http://ijpda.com; ISSN: 2348-8948

Research Article

Development and Validation of UV Spectrophotometric Method for the Estimation of Curcumin in an Ayurvedic Formulation Haridrakhand

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Date Received: 13th May 2017; Date accepted:

24th May 2017; Date Published: 25th May 2017

Abstract

A rapid, simple, selective and precise UV-Visible spectrophotometric method has been developed for the determination of curcumin in ayurvedic polyherbal formulation Haridrakhand. The intake formulation was subjected for maceration process using methanol as a solvent for the extraction of curcumin as a one of the ingredients in turmeric powder. The formulation containing 32 parts of turmeric powder out of total content. The spectrophotometric detection of curcumin was carried out at an absorption maximum of 421nm using methanol as a solvent. The method was validated according to ICH guidelines. The linearity range was found to be 1-25µg/ml with a correlation coefficient of 0.993. The accuracy was found to be within limit. The LOD and LOQ were found to be 1.29 and 4.3 µg/ml; respectively. The results demonstrated that the method can be conveniently employed for routine quality control analysis of Curcumin in Ayurvedic formulation of Haridrakhand.

Keywords: Haridrakhand, polyherbal formulation, Curcumin, UV spectrophotometry, Validation, ICH.

Introduction:

Haridrakhand is an important polyherbomineral Ayurvedic formulation. It is used in shitapitta, skin disorders, allergies, urticaria, psoriasis. The main and active ingredient of Haridrakhand formulation is turmeric [1].

The major ingredients of Haridrakhand are Haridra (curcuma longa), Haritaki *(terminaliachebula*), Nishottar (*Oprculina turpenthum*), Darve (*Berberis aristata*), Ajmoda (*Carum coxburghinanum*), Musta (*Cyperus rotundus*), Yavani (*Trachyspermum annani*), Chitrak (*Plubago zeylanica*), Katuka (*Picrorrhiza kurroo*), Jeerak (*Cuminum cyminum*), Pipali *(Piper ongum*), Ela (*Clettaria cardamomum*), Twak (*Cinnamomum zeyhnicum*), Tejpatra (*Cinnamomum tamala*), Vidanga (*Embelia ribes*), Guduchi (*Tinospora cardiofolia*), Kostha (*Sassurea lappa*), Triphala (*Terminalia chebulia, Terminalia belerica, Emblia officinalis*), Dhanyak (*Coriandrum sativum*), Loha bhasma (*Ash of iron*), Sharkara (*Sugar*) etc. [2].

Turmeric is one of the important components of Haridrakhand formulation. Turmeric power is showing very good results in skin disorders, psoriasis and related complications. Traditionally, the plant *Curcuma longa* widely used to impart flavor and color to the food and pharmaceuticals. Curcumin is the active ingredient of turmeric; turmeric contains 2.85% to 6.14%w/w. Curcumin has the ability to suppress both acute and chronic inflammation. Also helps to prevent the damage of the skin from UV rays of the sun [3, 4]. Curcumin is almost in soluble in water, but it is soluble in methanol, ethanol, DCM or DMSO [5].

Curcumin is widely extracted by means of maceration, digestion and infusion [6, 7]. Different analytical methods have been developed in recent year for the quality control analysis of herbs and their formulation including; HPLC, HPTLC and UV-Visible Spectrophotometry [8] As per the ICH guideline of drug analysis there are need for analytical method which is simple, sensitive, rapid and accurate for estimation of herbs and their formulation [9]. There-

fore, the aim of the present work was to develop and validate accurate, precise and robust method for the analysis of Haridrakhand containing curcumin as a major ingredient in form of turmeric powder by using UV Visible spectrophotometer. The same method will be acceptable for routine quality control testing of an ayurvedic formulation Haridrakhand.the chemical structure of curcumin shown in fig. 1.

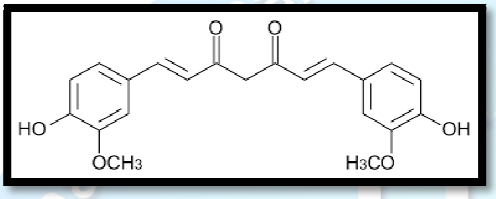

Fig. 1: Chemical structure of curcumin

MATERIAL AND METHODS:

Material:

Haridrakhand (Satyam Healthcare Pvt. Ltd., Vadodara, Gujarat, India) was purchased from local market at Baroda, Gujarat. Double beam UV/ Visible Spectrophotometric (SHIMADZU 1650 Tokyo, Japan) with 10mm quartz cuvettes were used for spectral measurements. All the other chemicals and reagents used were of analytical grade and quality.

Method:

Preparation of extract from Haridrakhand formulation:

Maceration:

1 gram of dried powder of Haridrakhand with 30 ml of methanol (curcumin is soluble in methanol) in a shaker with 210 rpm at room temperature for 2 days. The extract was filtered through whatman1 filter paper. Other portions of the solvent were added to the solids and the extraction was repeated until the reactant was colorless. The extracts were combined and filtered. [10]

Preparation of standard stock solution

Curcumin (10mg) was accurately weighed and transferred in a 100ml volumetric flask. Methanol was added to obtain a concentration of 100µg/ml (Stock-I). From Stock-I 10 ml of solution was withdrawn and transferred to a 100ml volumetric flask and made up the volume with methanol to obtain a concentration of 10µg/ml (Stock-II). From the above stock solution-II aliquots of 2ml, 4ml, 6ml, 8ml and 10ml were withdrawn and transferred into 10 ml volumetric flasks and made up the final volume with methanol to obtain a concentration of 2µg/ml, 4µg/ml, 6µg/ml and 8µg/ml, respectively.

Determination of maximum wavelength

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

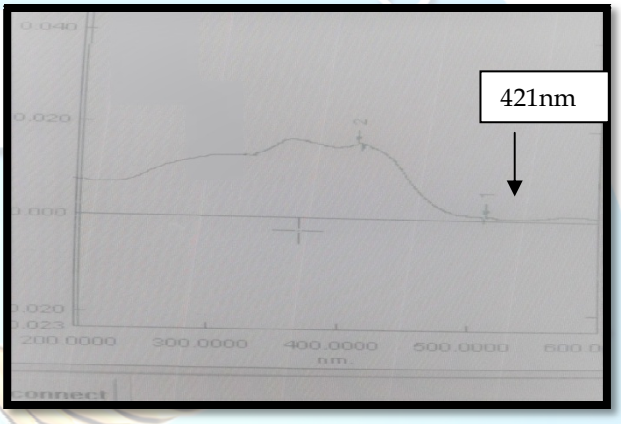

Fig.2 Determination of maximum wavelength

Preparation of standard calibration curve

The standard calibration curve of curcumin was obtained by measuring the absorbance of curcumin solution in concentration range (2-10µg/ml) prepared from stock solutions in methanol at 421nm in triplicate. Calibration curve of curcumin was then plotted with absorbance on y-axis and curcumin concentration on x-axis shown in fig. 3.

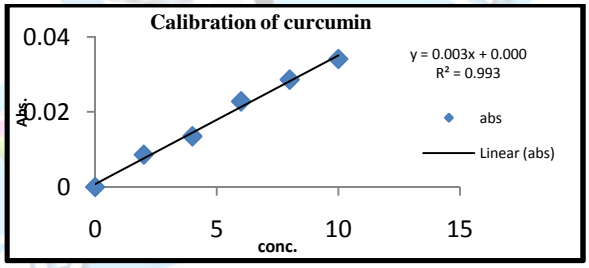

Fig. 3: Calibration curve of Curcumin

Analytical Method Validation

Validation can be defined as (ICH) Establishing documented evidence, which provides a high de-

gree 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 and range

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, 1-10 µg/ml of the standard solution of curcumin was prepared from stock solution and analyzed. All the measurements were performed in triplicate.

Table no. 1 - Linearity

Concentration in µg/ml	Absorbance (±SD)
2	0.0086± 0.004
4	0.0135± 0.008
6	0.0228± 0.002
8	0.0286± 0.003
10	0.0341±0.005

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed analytical method. Repeatability was determined by preparing six replicates of 6 µg/ml concentration of the sample and the absorbance was measured. Intraday precision study was carried out by preparing drug solution of 6µg/ml concentration and analyzing it at two different times in a day. The same procedure was followed for two different days to determine interday precision. The results were reported as %RSD. The precision result showed a good reproducibility (Table 2) with percent relative standard deviation less than 2. The results of intraday and interday precision studies are shown in (Table 3 and Table 4).

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure curcumin. The results are shown in (Table 5).

Ruggedness:

Ruggedness was determined by carrying out analyzing 6µg/ml concentration solution in methanol six times by two different analysts at 421nm. The results were indicated as %RSD (Table 6).

Robustness

Curcumin 5µg/ml solution was analyzed six times by change in instrument (UV- Visible spectrophotometer single beam; SHIMADZU) to determine robustness of the method. The results were indicated as %RSD (Table 7).

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 was determined using the following equation:

LOQ = 10 * S.D. / Slope = 10 * 0.00129/0.003 = 4.3 µg/ml

LOD = 3.3 * S.D./ Slope = 3 * 0.00129 /0.003 = 1.29 µg/ml

Table no. 2 - Precision

Concentration in µg/ml	Absorbance
6	0.0218
6	0.0232
6	0.0298
6	0.0221
6	0.0212
6	0.0230
S.D.= 0.00129 R.S.D. = 0.054 % R.S.D. = 5.49%	

Table no. 3 -Intraday precision

Concentration in µg/ml	Absorbance at 0 hr		Absorbance after 5 hrs	
6	0.0228	S.D.= 0.00025	0.0216	S.D.= 0.0006
6	0.0218	R.S.D. = 0.011	0.0222	R.S.D. = 0.026
6	0.0219	% R.S.D. =1.138%	0.0230	% R.S.D. = 2.64%
6	0.0227		0.0215	
6	0.0226		0.0221	
6	0.0211		0.0228	
Average % R. S. D. = 1.138 + 2.64 = 3.54 /2 = 1.88 %				

Table no. 4 - Interday precision

Concentration in µg/ml	Absorbance at 1st day		Absorbance at next day	
6	0.0229	S.D. = 0.0003	0.0221	S.D. = 0.00045
6	0.0221	R.S.D. = 0.0155	0.0231	R.S.D. = 0.01
6	0.0225	%R.S.D.= 1.55%	0.0232	%R.S.D.= 1.99%
6	0.0230		0.0228	
6	0.0226		0.0232	
6	0.0232		0.0222	
Average % R. S. D. = 1.55 + 1.99 = 3.54 /2 = 1.77%				

Table no. 5 - % Recovery Study

	Concentration in µg/ml		Absorbance		% Recovery
	Standard	Test	Standard	Test	
50 %	5	2.5	0.0101	0.0095	94.05%
	5	2.5	0.0108	0.0098	90.74%
	5	2.5	0.0105	0.0097	92.38%
100 %	5	5	0.0110	0.0112	101.81%
	5	5	0.0109	0.0114	104.54%
	5	5	0.0106	0.0112	105.66%
150 %	5	7.5	0.0191	0.0201	105.17%
	5	7.5	0.0194	0.0203	104.46%
	5	7.5	0.0199	0.0210	105.46%
Mean= 100.47%					

Table no. 6 - Ruggedness by change in analyst

Concentration (µg/ml)	Absorbance by analyst 1		Absorbance by analyst 2	
6	0.0216	S.D. = 0.00039 R.S.D. = 0.017 **%R.S.D.= 1.76%**	0.0221	S.D. = 0.00031 R.S.D. = 0.014 %R.S.D.= 1.41%
6	0.0237		0.0227	
6	0.0228		0.0230	
6	0.0217		0.0222	
6	0.0219		0.0232	
6	0.0210		0.0211	
Average % R. S. D. = 1.76 + 1.41 = 3.17 /2 = 1.585%				

Table no. 7 - Robustness by change in instrument

Concentration in µg/ml	Single beam		Double beam	
6	0.0218	S.D. = 0.00032 R.S.D. = 0.015 %R.S.D.=1.50%	0.0229	S.D.=0.000408 R.S.D. = 0.022 %R.S.D.=2.01%
6	0.0221		0.0230	
6	0.0217		0.0216	
6	0.0201		0.0204	
6	0.0222		0.0226	
6	0.0217		0.0216	
Average % R. S. D. = 1.50 + 2.01 = 3.51 /2 = 1.75%				

RESULST AND DISCUSSION

The proposed method provides a accurate, simple, rapid, linear, economical and convenient method for the analysis of Curcumin using UV spectrophotometry. The wavelength corresponding to maximum absorbance in methanol was found at 421nm. Beers law was obeyed in the concentration range of 1-25 µg/ml and correlation coefficient found to be 0.993. Accuracy of the proposed method was determined by the recovery studies, and %recovery (100.47%) obtained indicates that the method is accurate. The method was found to be precise as %RSD values for interday and intraday was found to be 1.77% and 1.88% respectively. The method was also found to be rugged and robust as the % RSD values were found to be 1.41% and 2.01% respectively. The limit of detection and limit of quantification of the proposed method was found to be 1.29 and 4.3 µg/ml respectively, indicating that the method developed is sensitive.

CONCLUSION

Based on above results, the proposed developed method for estimation of curcumin in presence of other plant constituents was found to be simple, rapid, linear, accurate, precise, reliable, robust and economical for laboratory scale determination of curcumin in Haridrakhand formulation. The proposed method is specific without and interference of other ingredients and hence can be applied for routine quality control analysis and estimation of Curcumin in polyherbal Ayurvedic formulation like Haridrakhand.

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