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HPLC method validation for quantification of tetrahydrocurcumin in bulk drug and formulation

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Abstract

Background: Tetrahydrocurcumin (THC), the active metabolite of curcumin, is gaining popularity amongst scientist due to its wide spectrum of pharmacological activities, better stability and colourless nature. The objective of this study was to develop a sensitive, cost-effective RP-HPLC method for the estimation of THC in bulk drug substance and formulation.

Results: Efficient chromatographic separation was achieved on Hypersil BDS, C18 column, 250 mm × 4.6 mm, 5 μm column by isocratic elution with mobile phase comprising of acetonitrile: methanol: water (40:23:37% V/V); adjusted to a pH of 3.0 ± 0.05. The flow rate of the mobile phase was 1.0 ml/min with a column temperature of 25 °C. UV detector was used for the analysis and detection was carried out at 280 nm. The developed method was validated according to ICH guidelines with respect to system suitability, linearity, accuracy, precision and robustness. The theoretical plates were found to be more than 5800. The method showed linearity over the range of 4 to 60 μg/ml with R^2 = 0.9998. The accuracy of the method in terms of recovery study was 98.23-99.99%. The %RSD for intra-day and inter-day precision were 0.272 and 0.275, respectively. The method was found to be robust with respect to change in wavelength, flow rate and column temperature.

Conclusion: The analytical method was found satisfactory on validation as per ICH guidelines. Hence, it can be routinely used for quantification of THC in bulk drug and formulation.

Keywords: Tetrahydrocurcumin, HPLC-UV, Quantification, Method validation

Background

Curcumin is a principal bioactive component of plant *Curcuma longa* (Linn). It is a potent antioxidant exhibiting various pharmacological activities [1]. Tetrahydrocurcumin (THC) is an active metabolite of it [2] where both the double bonds are reduced to single bonds (Fig. 1). Chemically, it is 1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane-3,5-dione with empirical formula $C_{21}H_{24}O_6$ and molecular weight 372.4. THC retains a range of therapeutic properties. It has been shown to ameliorate oxidative stress-induced renal injury [3] and was able to mitigate mitochondrial dysfunction in brain vasculature

during ischemic stroke [4]. It has shown antidiabetic [5], antiparkinson activity [6] and anticancer activities [7].

Excellent pharmacological effects with added advantage of colourless nature has made scientist to take note of THC. Different dosage forms like cream [8], porous scaffold for cartilage regeneration [9], self-emulsifying floating drug delivery systems [10] have been developed across the globe.

Various analytical methods HPLC, UHPLC, LC-MS/MS etc. have been developed for identification of metabolites of curcumin. THC being one of the metabolite of curcumin has been identified in biological samples like plasma and urine [11–13]. But reference for a simple reliable HPLC method for estimation of THC in the formulation is not available. In the present study, HPLC

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based analytical method has been developed and validated for quantitative determination of THC in the gel formulation.

Methods

Chemicals and reagents

All the solvents were of analytical grade and purchased from Qualigens (Thermo Fisher Scientific). Purified water was purchased from JK labs (Mumbai). The tetrahydrocurcumin (Sabiwhite) was obtained from Sami Labs, Bengaluru, Karnataka, India.

Method development

Preparation of standard solution

Ten milligrams of tetrahydrocurcumin was accurately weighed and transferred to a 50 ml volumetric flask. Thirty-five milliliters of methanol was added and with the aid of sonication, it was dissolved completely. Volume was made up and solution was mixed well. Ten milliliters of aliquot was withdrawn and diluted to 50 ml with diluent.

Preparation of sample solution

Five hundred milligrams of the 1% w/w tetrahydrocurcumin gel was weighed on a butter paper and transferred the gel along with butter paper to a 50 ml beaker containing 25 ml of diluent. The sample was sonicated for 5 min to form clear solution and the contents of the beaker were transferred to a 50 ml volumetric flask. The beaker was rinsed 2 times by 10 ml of methanol and transferred the same to the volumetric flask. The volume was made up to the mark with diluent and mixed well. The solution was filtered through a 0.45 μm Nylon syringe filter discarding 3-5 ml of sample. Ten milliliters aliquot was further diluted with methanol to 25 ml.

Chromatographic conditions

The system comprised of a Jasco PU-2089 HPLC Pump equipped with Jasco-2070 UV/Vis detector. The Mobile phase composed of acetonitrile: methanol: water (40:23: 37% V/V); adjusted to a pH of 3.0 ± 0.05 was used for the study. The mobile phase was filtered through a 0.45 μm pore size membrane PVDF filter and degassed ultrasonically after mixing. Twenty microliters of the solution was injected. The flow rate was maintained at 1 ml/min. The column used for the study was Hypersil
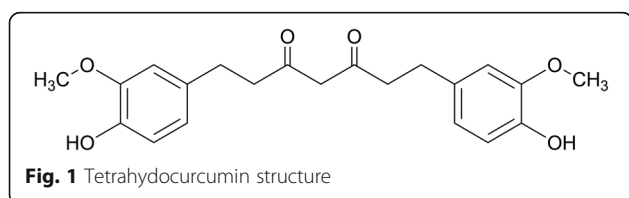

Fig. 1 Tetrahydrocurcumin structure

Table 1 System suitability study

Injection	Retention time	Peak area	Asymmetry factor	Theoretical plates
1	6.62	19186889	1.16	5943
2	6.62	19126526	1.15	5997
3	6.62	19234275	1.15	5985
4	6.62	19198526	1.15	5973
5	6.63	19183676	1.15	5956
6	6.63	19196342	1.16	5883
Mean	6.623	19187705.67	1.153	5956.17
SD	0.005	34986.36	0.005	40.76
%RSD	0.078	0.18	0.448	0.68

BDS, C18 column, 250 mm × 4.6 mm, 5 μm. The column oven temp was maintained at 25 °C. The detection was carried out at wavelength 280 nm. The run time for the analysis was 10 min.

Validation of method: quantitation of THC in bulk and pharmaceutical dosage form

The developed method was validated for system suitability, accuracy, precision, linearity and robustness in accordance with ICH guideline for validation of analytical procedures Q2(R1).

System suitability

System suitability testing helps to decide whether the developed chromatographic system is suitable for the analysis. %RSD of retention times, asymmetry factors and theoretical plates were the parameters for the study. Six replicate samples containing tetrahydrocurcumin were analyzed using the developed method.

Filter study is carried out to ensure that no drug is retained by the filter during sample preparation. The solution was collected after passing through a 0.45 μm PVDF filter and a 0.45 μm Nylon filter, by discarding 5 ml of solution. The results were compared to unfiltered centrifuged sample solution.

Specificity

The mobile phase or the excipients in the formulation should not interfere with the analysis. The developed method needs to be specific. The blank, placebo solution, THC solution and gel solution were injected and peak purity was determined.

Table 2 Filter study

Sample	% Absolute difference
0.45 μm PVDF filter	0.10
0.45 μm Nylon filter	0.08
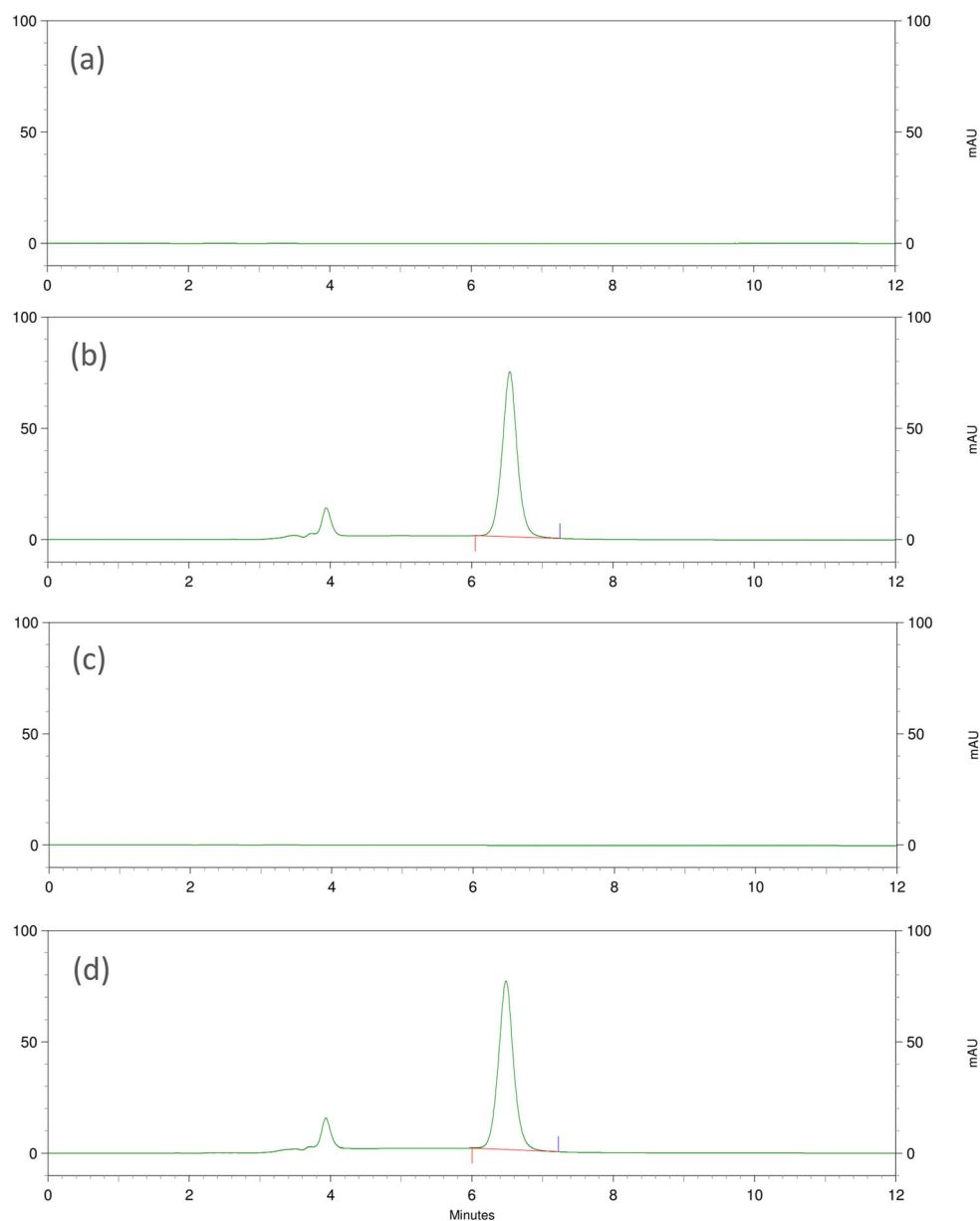
Fig. 2 Chromatograms of (**a**) blank (**b**) tetrahydrocurcumin standard (**c**) placebo (**d**) tetrahydrocurcumin sample

Linearity

THC solutions at 5 levels were prepared from 10 to 150% of working concentration. At each level, analysis was carried out in triplicate. The peak areas versus concentrations data was evaluated by linear regression analysis. Three data set corresponding to triplicate analysis were constructed. This data was analyzed by the test one-way ANOVA with post test Tukey using a significant level of $\alpha = 0.05$ (95% of confidence interval).

Accuracy

An accuracy study was performed by adding known amounts of THC to the placebo preparation. The actual and measured concentrations were compared and recovery was calculated. Recovery of the method was evaluated at three different concentration levels (corresponding to 50, 100 and 150% of test preparation concentration). For each concentration level, three sets were prepared and measured.

Precision

The precision of the assay method was evaluated in terms of repeatability by performing six independent assays (intra-day) of THC. Under the same experimental conditions, intermediate precision of the method was checked by another person performing the same

Table 3 Specificity study

Blank	No interference at RT of Tetrahydrocurcumin in blank
Standard solution	Peak purity was 0.997
Placebo	No interference at RT of Tetrahydrocurcumin in placebo
Sample solution	Peak purity was 0.996

procedure on a different day (inter-day). %RSD not more than 2 was taken as the limit.

Robustness

The factors chosen for this study were the change in wavelength (+, −3 nm), flow rate (+,−10%) and column temperature (+,−2 °C). The appropriate amounts of THC, bulk drug and formulation were weighed and diluted with methanol. The effect of changed parameters on analysis of THC in bulk drug was evaluated in terms of RT, asymmetry factor, theoretical plates and assay.

Results

System suitability

In system suitability study, the peak showed good symmetry (average 1.15) and sufficient theoretical plates (average 5956). The average retention time was 6.62 min. %RSD of retention times, asymmetry factors and theoretical plates was well below 1% (Table 1). PVDF and nylon both filters were found to be suitable as maximum % absolute difference was 0.10% (Table 2).

Specificity

The method proved to be specific with no interference of mobile phase or excipients. (Fig. 2a-d). The peak

purity for the standard solution and sample solution was 0.997 and 0.996 respectively (Table 3).

Linearity

For linearity range, correlation coefficient was 0.9998, *F* value was less than 0.00002 and *P* value was 0.99999 (Fig. 3 and Table 4).

Accuracy

The method was accurate at 3 levels with %RSD in the range of 0.02 to 0.194 (Table 5).

Precision

Repeatability and intermediate precision studies showed %RSD, 0.272 and 0.275 respectively (Table 6).

Robustness

Change in wavelength, flow rate and column temperature caused not more than 2% difference in the assay value (Table 7).

Discussion

In HPLC, solvent under pressure moves through the stationary phase packed in the column. The components of a mixture carried by the solvent are separated from each other due to their different degrees of interaction with the stationary particles. This leads to the separation of the components as they flow out of the column and pass over the detector, where they are detected. The right choice of the stationary phase, mobile phase, detection technique, etc. can be done based on guidelines issued by regulatory agencies [14, 15].

Under ideal conditions, chromatographic peak has a Gaussian shape. Asymmetric peak is obtained in case of
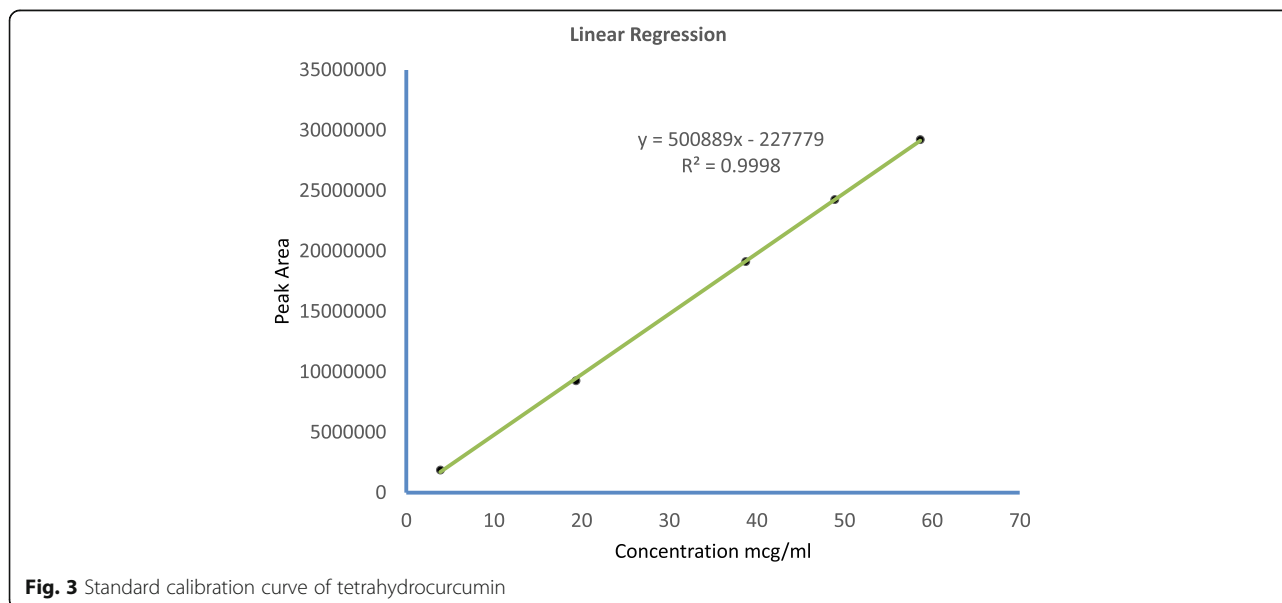
Fig. 3 Standard calibration curve of tetrahydrocurcumin

Table 4 Analysis of variance for linearity

Source of variation	SS	df	MS	F	P value	F crit
Between groups	3421080113	2	1710540056	1.38172E −05	0.99999	3.88529
Within groups	1.48557E + 15	12	1.23798E + 14			
Total	1.48558E + 15	14				

Table 6 Precision study-repeatability and intermediate precision

	Repeatability	Intermediate-precision
Sample	Peak area	Peak area
Sample 1	19851008	19863257
Sample 2	19848253	19852637
Sample 3	19752637	19752634
Sample 4	19863415	19752639
Sample 5	19752635	19752633
Sample 6	19863128	19752489
Mean	**19821846**	**19787715**
SD	**53962.92**	**54505.19**
%RSD	**0.2722**	**0.2754**

non-uniform migration and non-uniform distribution of drug in the HPLC column. Lesser the deviation, the value of asymmetry factor will be closer to 1. The sharper the peak, the better is the column efficiency. It is measured in terms of the number of theoretical plates per column. Variation in peak area indicates non-suitability of the method. The method showed good system suitability with an average asymmetry factor as 1.15, %RSD for peak area less than 2 and theoretical plates more than 5900 (Table 1). The base-deactivated silica used in the column had blocked −OH groups. Because of reduced silanol activity, tailing was less, and good peak symmetry was observed. THC is insoluble in water and soluble in organic solvents like alcohol and acetone. Hence, methanol was chosen as a diluent for sample preparation. THC was adequately extracted in the methanol, and excipients did not cause interference in the analysis. The mobile phase was composed of acetonitrile: methanol: water (40:23:37% V/V), adjusted to a pH of 3.0 ± 0.05. Presence of methanol in the mobile phase ensured no plug formation after injection of sample solution. The analysis was carried out using a UV detector at a wavelength of 280 nm where THC showed maximum absorbance. High peak purity with no interference from solvent system or excipient indicated results specific to the THC (Fig. 2 and Table 3). The specifications for the column used in this study (i.e., C18 column, 250 mm × 4.6 mm, 5 μm) are frequently found

in columns used in laboratories and are commonly available in the market. Use of acidic pH for mobile phase and carefully selected composition of organic solvents ensured early elution of THC. The low retention time of 6.62 min ensured quick analysis. With short run time, more number of samples could be analyzed in the given time, making the process cost-effective. With correlation coefficient of 0.9998, linearity between peak area and analyte concentration was evident. *F* value lower than the F_{crit} and $P > 0.05$ confirmed that there is no significant difference between the slope values of the three analytical curves (Fig. 3 and Table 4). With difference less than 2%, the method was found to be accurate with test results close to the true values (Table 5). The method was precise with respect to intra-day and inter-day precision (Table 6). Change in wavelength, flow rate and column temperature did not affect the analysis significantly (Table 7).

Conclusion
A new HPLC method has been developed for the identification and quantification of THC. The method has satisfactory sensitivity, specificity and repeatability. Method

Table 5 Accuracy study

Level	% Recovery	Mean % recovery	%RSD
50%	98.24	98.23	0.194
	98.42		
	98.04		
100%	98.31	98.32	0.016
	98.32		
	98.34		
150%	100.01	99.99	0.021
	99.97		
	100.00		

Table 7 Robustness study

Parameter		RT	Asymmetry factor	Theoretical plates	% Assay
Change in wavelength	**+3 nm**	6.61	1.15	5671	98.56
	−3 nm	6.62	1.17	5661	100.36
Flow rate	**+10% ml/min**	6.64	1.18	5680	100.17
	−10% ml/min	6.66	1.18	5652	99.09
Column temperature	**+2 °C**	6.56	1.16	5627	100.38
	−2 °C	6.56	1.16	5641	100.33

was successfully validated as per ICH guidelines and can be conveniently employed for routine quality control analysis of THC as bulk drug and in pharmaceutical dosage form.

Abbreviations
THC: Tetrahydrocurcumin; HPLC: High-performance liquid chromatography; UHPLC: Ultra high-performance liquid chromatography; LC-MS: Liquid chromatography-mass spectroscopy; SD: Standard deviation; RSD: Relative standard deviation; SS: Sum of squares; df: Degrees of freedom; MS: Mean sum of squares

Authors' contributions
RR carried out the laboratory work, collected and analyzed the data and drafted the manuscript. JS supervised the work and assisted in the data analysis. All authors read and approved the final manuscript.

Availability of data and materials
All data is available upon request.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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