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# Development and Validation of RP-HPLC And HPTLC Methods for the Quantification of Curcumin in Tablet Formulation.

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**KEYWORDS**

Curcumin, HPTLC, UHPLC

**ABSTRACT:**

**Introduction:**Curcumin tablets are herbal formulations based on Ayurveda that are used to treat arthritis and boost immunity. In this study, a novel and precise method for measuring curcumin in Curcumin tablet formulations was developed and validated.

**Objective:**The objective of the study was to create specific and accurate methods using RP-HPLC and HPTLC techniques to measure curcumin content in Curcumin tablet formulations according to ICH Q2(R1) guidelines.

**Methods:**Development of the methods engaged use of linomat IV sample spotter precoated TLC plate for HPTLC analysis. Toluene, ethanol, and formic acid were employed as mobile phase compositions with 8.6:0.7:0.7% v/v ratio for HPTLC while HPLC analysis used an acetonitrile, methanol, water (40:30:30) mobile phase at a flow rate of 1mL/min with maximum detection wavelength at 425 nm using C18 ODS column (250×4.6 mm, 5 µ). The linearity ranges for HPTLC and HPLC were found out as 600-1400 ng/spot and 10-50 µg/ml respectively. Rf values along with retention times were recorded.

**Results:**Accordingly, the Rf value was determined as 0.321±0.03 by hptlc while retition time was observed as9.287 by hplc method.The methods could accurately analyze the amount of curcumin in Curcumin tablet formulations within their respective linear ranges defined.

**Conclusion:**RP-HPLC and HPTLC methods are developed for quantification of curcumin in tablet formulation which are precise and can be used for routine analysis.

## 1. Introduction

Turmeric (Curcuma longa) is a rhizomatous herbaceous perennial plant of the Zingiberaceae family that has been used medicinally since antiquity [1]. Turmeric has been utilized medicinally in India's Vedic civilization for more than 4000 years. It served as a culinary spice and have a religious importance. Turmeric is used in herbal and traditional medicine for a variety of conditions, including Rheumatoid arthritis, retinochoroiditis, ophthalmitis, basal cell cancer, variola, varicella, tissue recovery, cystitis, and cirrhosis. Additionally it boosts energy, eliminates worms, regulates menstruation, dissolves gallstones, cleanses wounds, and treats digestive disorders [2]. Curcumin's antioxidant, anti-inflammatory, antibacterial, anticancer, and antimutagenic qualities make it an effective supplement for a variety of health disorders [3]. Curcumin is a brilliant yellow phytochemical that is polyphenolic in nature. Curcuma longa includes curcumin, desmethoxycurcumin, and bis-desmethoxycurcumin [4]. Curcumin is freey soluble within organic dissolvables

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like methanol, DMSO, acetone, and ethanol, but poorly soluble in water. Spectrophotometrically, it absorbs at 430 nm in methanol and 416 to 421 nm in acetone[5]. Curcumin (Figure 1), have another name called diferuloyl methane and it is a symmetric molecule. The IUPAC name of curcumin is (1E, 6E) 1,7-bis(4-hydroxy-3-methoxyphenyl). The molecular weight is 368.38 g/mole and its chemical formula for -1,6-heptadiene-3,5-dione is C21H20O6, and its [6]. Several analytical techniques, including UV-Visible Spectrophotometry, HPLC, and HPTLC, have recently been developed for measuring curcuminoids in Curcuma longa extracts. However, HPLC and UV spectrophotometric techniques are more suited. Curcumin concentrations in Curcuma longa preparations were measured using a variety of methods. As a result, for the quantitative analysis of curcumin in curcuma longa extract, a simple HPLC and HPTLC method was developed and validated as per ICHQ2R1 guideline in this study.

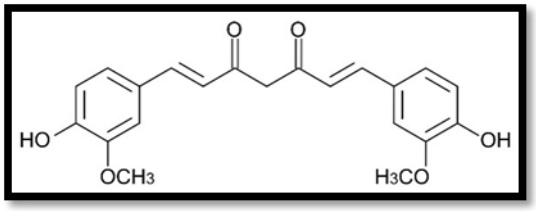


**Figure 1**: Curcumin structure

## 2. Objectives

To develop and validate novel UHPLC method for quantification of curcumin in tablet formulation as per ICH Q2R1 guidelines .

To develop and validate novel HPTLC method for quantification of curcumin in tablet formulation as per ICH Q2R1 guidelines .

## 3. Methods

Curcumin tablets from Bliss of Earth were acquired from a local medical shop in Nashik, Maharashtra. Curcumin standard powder was procured from Yucca Enterprises, Mumbai, Maharashtra.

Chemicals and Instruments:

The reagents used for this study are Water-HPLC grade and Methanol-HPLC grade, Acetonitrile, Toluene, Ethanol. HPLC system (SHIMADZU UHPLC Prominence- I LC2030C Plus ), HPTLC Spraylin Aetron linomat IV sample spotter.

Chromatographic conditions:

Chromatographic separation was done by Shimadzu UHPLC with Shimadzu, ODS (250×4.6mm), 5 µ column at 40°c temperature. The mobile phase was made up with acetonitrile, methanol, and water (40:30:30). The mode of elution isocratic with a flow rate of 1ml/minute and the wavelength of detection was 425 nm. Curcumin had a retention duration of 9.2 minutes in HPLC. Toluene, Ethanol, and Formic Acid (8.4:0.7:0.7) clear Curcumin Spot with a superior peak shape (Rf = 0.321 ± 0.03) in HPTLC.

Standard stock solution preperation:

A exact weight of 10 mg of standard curcumin was acquired and moved into a 100 mL volumetric flask. The volume was make up using methanol to reach 100 ug/ml for HPLC and ethanol for HPTLC, providing a concentration of 100 ng/µl.

Sample solution preperation:

10 number of tablets were weighed and triturated to get powder. The powder equal to 10 mg of curcumin was weighed and added to 25 ml methanol, and sonicated for fifteen minutes. This solution was then filtered by the using Whatman filter paper, and the residue was gently rinsed using methanol. The filtrate and washings were mixed together into100 ml volumetric flask and diluted with methanol to get a final concentration of 100 µg/ml of curcumin. To reach a concentration of around 100 ng/µl, 0.1 ml of the stock solution was taken to 100ml volumetric flask and volume make with methanol.

Specificity:

The method's specificity was determined by evaluating the interference of the primary peak with excipient peak in the formulation using HPTLC and HPLC, which were calculated using Curcumin RF and RT values, respectively [8].

Linearity:

The HPLC range of linearity for Curcumin was 10-50 ug/ml. To create solutions of 10, 20, 30, 40, and 50 µg/ml, an aliquot from stock solution was transferred and diluted using methanol. The range of linearity for HPTLC was 600-1400 ng/band. Various stock solution

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quantities (6, 8, 10, 12, and 14 µl) were tested on the HPTLC plate [7,8].

Precision:

The precision of the procedure is the degree of consistency among individual test findings when the test process is conducted repeatedly to several samples. [9].

LOD and LOQ:
The LOD and LOQ are the lowest concentrations of an analyte in a sample that can be detected and quantified with reasonable accuracy and precision. The LOD and LOQ of the developed method were computed using the SD of the response and the slope of the calibration curve [10].
Accuracy:
% Recovery: The method's accuracy was determined by calculating recovery studies after adding standard solution of curcumin to the sample solution at concentrations of 50%, 100%, and 150% for HPLC and 80%, 100%, and 120% for HPTLC (n = 3), all within the drug's linearity range [7,8].
Assay:
A 10mg of sample in a 10mL methanol was taken for analysis. The percent of curcumin in the sample solution was calculated by entering the area values of the curcumin-corresponding peaks into the equation of the line representing curcumin's calibration curve [7].

## 4. Results

HPLC analysis of the curcumin formulation:
Specificity:
The method's specificity was established by examining the interference between the principle and excipient peaks in the formulation. The RT was found to be 9.3387 using RP-HPLC.

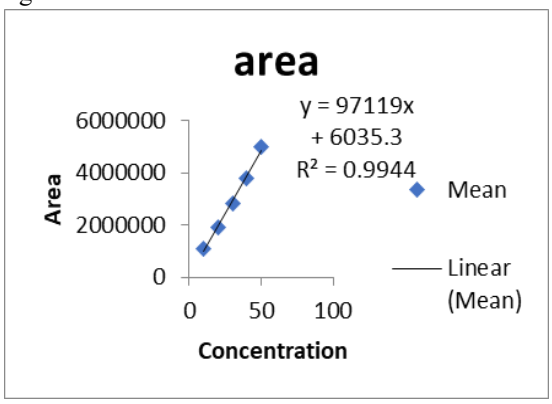


**Figure 2:** Specificity chromatogram Linearity:

The linear regression data for the calibration curves (N=5) are provided in Table 1. A linear relationship was found between peak height and peak area at concentrations ranging from 10 to 50µg/ml, with a slope of 0.994 and Y= 97119x +6035.3.

**Table 1:** Linearity by HPLC

| Sr.No | Concentration (ug/ml) | Area |
|---|---|---|
| 1 | 10 | 1089778 |
| 2 | 20 | 1898518 |
| 3 | 30 | 2817489 |
| 4 | 40 | 3794491 |
| 5 | 50 | 4997739 |

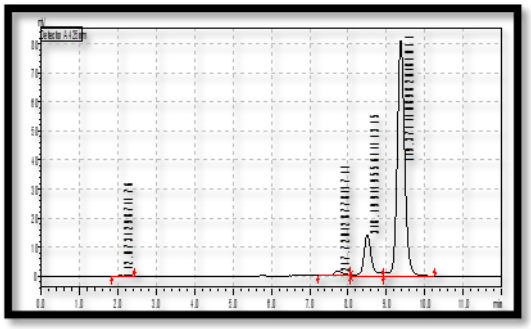

**Figure 3:** Curcumin calibration curve by HPLC

Precision:

Intra-day precision and inter-day precision:

Table 2 intra-day precision, and Table 3 shows the Interday precision results stated with %RSD, which depicts the intra-day and inter-day variance of the curcumin .

PRECISION (INTRADAY)

**Table 2:** Intraday precision by HPLC method
PRECISION (INTERDAY)

| PRECISION (INTERDAY) | | | | | | |
|---|---|---|---|---|---|---|
| SR | CONC | Area | | MEAN | STD | %RSD |
| | | 1 | 2 | | | |

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| NO. | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 10 | 1089822 | 1089824 | 1089823 | 1.4142136 | 0.0001298 |
| 2 | 30 | 2817317 | 2817316.3 | 2817316.7 | 0.7071068 | 1.76E-05 |
| 3 | 50 | 4997778 | 4997777 | 4997777.5 | 0.7071068 | 1.41E-05 |

**Table 3:** Interday precision by HPLC method

| PRECISION (INTRADAY) | | | | | | |
|---|---|---|---|---|---|---|
| SRNO. | CONC | Area | | MEAN | STD | %RSD |
| | | 1 | 2 | | | |
| 1 | 10 | 1089820 | 1089824 | 1089822 | 2.8284271 | 0.0002595 |
| 2 | 30 | 2817314 | 2817319 | 2817316.5 | 3.5355339 | 0.0001255 |
| 3 | 50 | 4997776 | 4997777 | 4997776.5 | 0.7071068 | 1.41E-05 |

LOD and LOQ::

The detection limit and quantification limit were obtained with the formulas LOD = 3.3 x σ/S and LOQ = 10 x σ/S. The method's sensitivity was demonstrated by LOD and LOQ values of 4.5 µg/ml and 13.7 µg/ml, respectively, where σ represents response's standard deviation and S represents the calibration curve slope.

Accuracy:

Accuracy of the method was done by using recovery studies, which involved determining percentage mean recovery of the sample at three different levels (50%-150%). The percentage mean recoveries was within a limit. All data were within the desired range, indicating acceptable values of recovery and thus confirming the accuracy of the suggested approach.

Assay:

Curcumin tablet assay was performed using the described approach. Sample solutions were made and then injected to the UHPLC apparatus. The sample solution was observed at 425 nm. The drug's percentage was 92.43%.

HPTLC analysis of curcumin formulation :

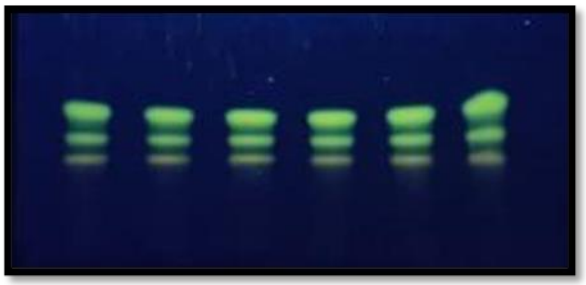

**Figure 5:** Chromatograph of curcumin shows separate spots in sample and standard

Specificity:

The chromatogram of the curcumin formulation prepared by use of the described approach revealed 1 peak with RF-value of 0.329 for the sample, which determined near around Rf (0.321) for the standard medication. It had not any disruption from the mobile phase or dilution agent over Curcumin RF levels, demonstrating the method's specificity in the presence of various excipients.

Linearity:

A curcumin calibration curve was created by graphing the mean peak volume versus the concentration throughout a range, that is 6- 14 µl/spot. The $r^2$ was calculated to be 0.993, with y = 174.4x + 997.2. (Figure 5).

**Table 5:** Linearity study by HPTLC

| Sr.No. | concentration (ng/ul) | vol |
|---|---|---|
| 1 | 6 | 1145.3 |
| 2 | 8 | 1365.3 |
| 3 | 10 | 1539 |
| 4 | 12 | 1706 |
| 5 | 14 | 1847.2 |

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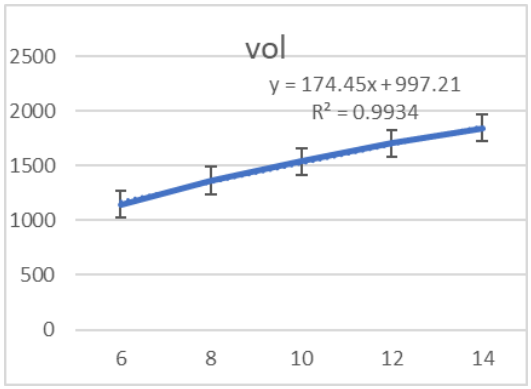


**Figure 5:** Calibration curve of curcumin by HPTLC

Precision:

The intra-day variance, as RSD, was 0.27%, while the inter-day variance, as RSD, was 0.13%, as seen in Tables 6 and 7. These low RSD levels indicate that the method's repeatability and precision were good.

PRECISION (INTRADAY)

**Table 6:** Intraday precision by HPTLC method

| PRECISION (INTRADAY) | | | | | | |
|---|---|---|---|---|---|---|
| SR NO. | CONC | VOLUME | | MEAN | STD | %RSD |
| | | 1 | 2 | | | |
| 1 | 6 | 403<br>0.15 | 401<br>0.34 | 402<br>0.245 | 1.2798<br>63274 | 0.318<br>80033 |
| 2 | 10 | 448<br>0.81 | 451<br>0.04 | 449<br>925 | 1.576<br>848122 | 0.3504<br>691095 |
| 3 | 14 | 464<br>0.76 | 465<br>0.84 | 465<br>0.3 | 0.7636<br>75324 | 0.1641<br>25365 |
| | | | | mean | 1.206 | 0.277 |

Precision (Interday)

**Table 7:** Intraday precision by HPTLC method

| PRECISION (INTERDAY) | | | | | | |
|---|---|---|---|---|---|---|
| SR NO. | CONC | VOLUME | | MEAN | STD | %RSD |
| | | 1 | 2 | | | |
| 1 | 6 | 403.15 | 402.26 | 402.705 | 0.629325 | 0.1562745 |
| 2 | 10 | 448.81 | 449.5 | 449.155 | 0.4879037 | 0.108627 |
| 3 | 14 | 468.7 | 469.67 | 469.185 | 0.6858936 | 0.1461883 |
| | | | | mean | 0.601 | 0.137 |

Accuracy:

The recovery trial outcomes varied from 99 to 102%the results of recovery trials are presented in Table 8.

Assay : Curcumin tablet assay was performed using the described approach. The test [Assay %] was 95% in tablet.

## 5. Conclusion

**Table 9:**Methods specifications for validating the HPLC and HPTLC protocols

| **Parameter** | **HPLC** | **HPTLC** |
|---|---|---|
| Linearity – coefficient correlation | 0.994 | 0.993 |
| Intraday Precision(%RSD) | 0.0001 | 0.27% |
| Interday precision (%RSD) | 1.05% | 0.13% |
| Accuracy-%Recovery | 100.90% | 100.60% |
| LOD | 4.5µg/ml | 10.46 ng/spot |
| LOQ | 13.7µg/ml | 31.75 ng/spot |

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| | | |
|---|---|---|
| Assay(%PracticalYield) | 92% | 95% |

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**Refrences**

1. Kumar B, Aggarwal R, Prakash U, Sahoo PK. Emerging therapeutic potential of curcumin in the management of dermatological diseases: an extensive review of drug and pharmacological activities. Future Journal of Pharmaceutical Sciences. 2023 May 22;9(1):42
2. Salehi B, Stojanović-Radić Z, Matejić J, Sharifi-Rad M, Kumar NV, Martins N, Sharifi-Rad J. The therapeutic potential of curcumin: A review of clinical trials. European journal of medicinal chemistry. 2019 Feb 1;163:527-45.
3. Kaur K, Al-Khazaleh AK, Bhuyan DJ, Li F, Li CG. A Review of Recent Curcumin Analogues and Their Antioxidant, Anti-inflammatory and Anticancer Activities.
4. Oglah MK, Mustafa YF, Bashir MK, Jasim MH, Mustafa YF. Curcumin and its derivatives: A review of their biological activities. Syst. Rev. Pharm. 2020 Mar 1;11(3):472-81.
5. Jankun J, Wyganowska-Świątkowska M, Dettlaff K, Jelińska A, Surdacka A, Wątróbska-Świetlikowska D, Skrzypczak-Jankun E. Determining whether curcumin degradation/condensation is actually bioactivation. International journal of molecular medicine. 2016 May 1;37(5):1151-8.
6. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin: miniperspective. Journal of medicinal chemistry. 2017 Mar 9;60(5):1620-37.
7. Gosavi S, Kasar S, Warule P, Pawar S. Development and validation of RP-HPLC and HPTLC method for the estimation of curcumin in haridrakhand polyherbal tablet formulation. Journal of Pharmacognosy and Phytochemistry. 2019;8(3):2562-
8. Kadam PV, Yadav KN, Bhingare CL, Patil MJ. Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. Journal of Pharmacognosy and Phytochemistry. 2018;7(5):1913-8.
9. Purohit PJ, Kapupara PP, Shah KV. Development and validation of analytical method for simultaneous estimation of curcumin and gallic acid in different polyherbal formulations by HPLC. Research Journal of Pharmacy and Technology. 2014;7(7):749-53.
10. Ravikishore KV. Method Devolopment and Validation for Estimation of Curcumin in Poly Herbal Formulation by Rp-Hplc (Doctoral dissertation, KM College of Pharmacy, Madurai).
11. Kamran Ashraf et al. Validated HPTLC analysis method for quantification of variability in content of curcumin in Curcuma longa L. (Turmeric) collected from different geographical region of India. Asian Pacific Journal of Tropical Biomedicine. 2012, S584-S587.
12. Daily JW, Yang M, Park S. Efficacy of turmeric extracts and curcumin for alleviating the symptoms of joint arthritis: a systematic review and meta-analysis of randomized clinical trials. Journal of medicinal food. 2016 Aug 1;19(8):717-29.
13. Jadhav BK, Mahadik KR, Paradkar AR. Development and validation of improved reversed phase-HPLC method for simultaneous determination of curcumin, demethoxycurcumin and bis-demethoxycurcumin. Chromatographia. 2007 Apr;65:483-8.
14. Borman P, Elder D. Q2 (R1) validation of analytical procedures: text and methodology. ICH quality guidelines: an implementation guide. 2017 Sep 27:127-66.
15. Sonia K, Lakshmi KS. HPTLC method development and validation: An overview. Journal of Pharmaceutical Sciences and Research. 2017 May 1;9(5):652.