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# Validated HPTLC Method Development for the Estimation of Curcumin with Respect to Crude Drugs, Extracts and Formulations

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**Abstract:**

Curcumin is a naturally occurring polyphenolic compound present in *C. longa* rhizomeswith a broad range of favourable biological functions, including anti-cancer, anti-oxidant and anti-inflammatory activities. The phytoconstituent curcumin was identified in different samples of C. longa Crude drug, Extracts and Formulations by TLC and quantified by HPTLC. The standard as curcumin was used with $R_f$ value approx. (0.37) whereas the mobile phase was used as Chloroform : Ethanol : Glacial acetic acid ( 90: 5 :1 ) and the plate was scanned at 425 nm. It is studiedthat highest Curcumin found in Crude drug 3 (3.3617%w/w), Extract 1(4.0463%w/w) and Formulation 1(0.01758 %w/w). Due to time of collection, geographical variation, genetic variation, growing conditions, timing and method of harvesting, exposure to air, light and moisture over time and type of preservations, there may be chances of variations in contents of Curcumin in different species.

**Key words:** Curcumin, Chromatography, TLC, HPTLC

## Introduction:

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. Depending upon whether the active principle of the plant is known or not, different concepts ("normalization vs. "standardization")[1]have to be applied in order to establish relevant criteria for uniformity. Reproducible efficacy and safety of phytopharmaceuticals is based on reproducible quality. Therefore, if phytopharmaceuticals want to be regarded as rational drugs, they need to be

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standardized and pharmaceutical quality must be approved. The world health organization (WHO) has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines.

Increasingly, the determination of low concentrations of active ingredients (either desired or undesired) in complex mixtures, sold for human consumption, has become more necessary. Federal regulations have imposed strict limits on the type and concentrations of a host of substances sold as foods or drugs. Such requirements demand analytical techniques that are fast and reliable and combine the separation (to alleviate interferences) and analysis steps in a single operation.

Chromatography is the most widely used technique for the analysis of non-inorganic mixtures. Gas chromatography (where the sample must be volatilized) and liquid chromatography (where the sample can be determined in the liquid state) are the most common methods in general use. High Performance Liquid Chromatography (HPLC) is the method of choice whenever the sample cannot easily be converted to the gas phase.

A chromatographic finger print profile represents qualitative and quantitative determination of various components present in a complex plant extract irrespective whether or not their exact identity is known. It is generally believed that the reported pharmacological action of a botanical is due to more than one constituent acting synergistically with other constituent present. From the pharmacopoeal perspective, a better quality control of raw material can be believed by specifying a quantitative test procedure for the determination of range or a minimum content of the marker substance or the "active" ingredient.[2] "Markers" are defined constitute of an herbal drug, which are of interest for control purpose. Markers may or may not have therapeutic activity. The markers can serve as a powerful tool in the finished form of herbal drug preparation, which depends upon the quantitative determination of the marker, when the starting material is selected.[3] Chemical analysis of the plant material is a critical factor for standardization. Techniques like HPLC, GC, and HPTLC are commonly used for chemical fingerprint analysis. In the recent years, Liquid chromatography-mass spectrometry (LCMS) technique has found increasing application in the analysis of medicinal plant material. It is an expensive technique but the cost may be justified by wealth of information it provides.

Turmeric is a spice that has received much interest from both the medical/scientific worlds as well as from the culinary world. Turmeric is a rhizomatous herbaceous perennial plant (*Curcuma*

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*longa*) of the ginger family [4]. The medicinal properties of turmeric, the source of curcumin, have been known for thousands of years; however, the ability to determine the exact mechanism(s) of action and to determine the bioactive components have only recently been investigated [5]. Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane, is the main natural polyphenol found in the rhizome of *Curcuma longa* (turmeric) and in others *Curcuma* spp. [6]. *Curcuma longa* has been traditionally used in Asian countries as a medical herb due to its antioxidant, anti-inflammatory[7], antimutagenic, antimicrobial [8,9] and anticancer properties [10,11].

Curcumin, a polyphenol, has been shown to target multiple signaling molecules while also demonstrating activity at the cellular level, which has helped to support its multiple health benefits. It has been shown to benefit inflammatory conditions [12], metabolic syndrome [13], pain [14], and to help in the management of inflammatory and degenerative eye conditions [15, 16]. In addition, it has been shown to benefit the kidneys[17]. While there appear to be countless therapeutic benefits to curcumin supplementation, most of these benefits are due to its antioxidant and anti-inflammatory effects. Despite its reported benefits via inflammatory and antioxidant mechanisms, one of the major problems with ingesting curcumin by itself is its poor bioavailability[18], which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination. Several agents have been tested to improve curcumin's bioavailability by addressing these various mechanisms. Most of them have been developed to block the metabolic pathway of curcumin in order to increase its bioavailability.

**Materials and Methods:**

**1. Collection and Sampling of Drug Materials**

i) The dried rhizomes of Curcuma longa (Raw 1, Raw 2, Raw 3) were collected from the market of the different places with batch no. CL/NG/06/06, CL/CO/07/06, CL/DL/08/06 respectively. All the samples of Raw 1, Raw 2, Raw 3 were weighed 50gm/each for the further study.

ii) The four extracts of Curcuma longa (Extract 1, Extract 2, extract 3 and Extract 4) were collected from the different vendors with the batch no. RD/1346, RD/1347 , CL 06020 , TACL-3005 and weighed as 50gm/each for experiment.

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iii) The two In house formulations(Formulation 1 and Formulation 2 ) containingCurcumin were prepared at laboratory with batch no. Mf/201, MF/202, MF/203 respectively. 5ml of each formulation was measured and used for further study.

iv) Standard Curcumin of purity 95%w/w was used and accurately weighed 4.088mg dissolved in 10ml of methanol by sonication.

**2. Identification of Curcumin by TLC in Crude drugs, Extracts and Formulations**

**a) Sample preparation:**

**i) Crude raw materials–** Accurately weighed powdered sample was macerated with methanol for about 15min in sonicator and filtered, then make up the volume with methanol.

**ii) Extracts –** Accurately weighed extract was dissolved in little quantity of methanol by sonication, then make up the volume with methanol.

**iii) Formulations –** Accurately weighed sample was extracted with 100ml of extraction media (mixture of Chloroform; Butanol& Methanol) for three times, then collected the combined extracts are filtered, evaporated to dryness. Then made up the volume with methanol.

**b) Standard preparation:**

Accurately weighed 4.088mg of standard Curcumin of purity 95%w/w was dissolved in 10ml of methanol by sonication.

**c) Stationary phase:**Precoated silica gel $60F_{254}$ TLC aluminum sheets.

**d) Mobile phase:** Chloroform: Ethanol: Glacial acetic acid (95: 5: 1)

**e) Procedure:**

Applied specific quantity of the sample and standard solution with the help of Linomat5 as bands on the TLC plate and developed with mobile phase up to about 70mm. The developed chromo-plate was dried by hair dryer. The spots were found to be visible without using spraying reagent. Then photo documented with the help of digital camera. $R_f$values were calculated.

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**3. Quantification of Curcumin by HPTLC technique in Crude drugs, Extracts and Formulations**

**a) Sample preparation**

**i) Crude raw materials–** Accurately weighed (100 mg) powdered sample is dissolved in methanol and kept for sonication for about 15 min. and filtered, then make up the volume with methanol.

**ii) Extracts –** Accurately weighed (100 mg) extract was dissolved in little quantity of methanol by sonication, then make up the volume with methanol.

**iii) Formulations -** Accurately weighed (5.0 ml) sample was extracted with 100 ml of extraction media (mixture of Chloroform, Butanol& Methanol) for three times, then collected the combined extracts are filtered, evaporated to dryness by buchi (vacuum evaporator) . Then the volume wasadjusted with methanol.

**b) Standard preparation**

Accurately weighed 4.088 mg of standard curcumin of purity 95 %w/w was dissolved in 10 ml of methanol by sonication. From the stock solution 2 ml taken and diluted to 25 ml with methanol.

**c) Stationary phase**

Precoated silica gel $60F_{254}$ TLC aluminum sheets.

**d) Mobile phase**

Chloroform : Ethanol : Glacial acetic acid ( 90: 5 :1 )

**e) Procedure**

Applied specific quantity of the sample and standard solution with the help of Linomat 5 as bands on the TLC plate. And developed with mobile phase up to about 70mm. The developed chromo-plate was dried by hair dryer. The plate was scanned at 425nm by using the Camag TLC plate Scanner3. The content of curcumine was calculated by comparing the peak areas of sample and standard spots.

**Results and Discussion:**

**1. Identification of Curcumin by TLC in Crude drugs, Extracts and Formulations**

The phytoconstituent curcumin present in rhizomes, Extracts and Formulation of *Curcuma longa* Linn was identified by TLC on the basis of the marker compound. The mobile phase was

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used as Chloroform : Ethanol : Glacial acetic acid ( 90: 5 :1 ) and the plate was scanned at 425 nm.The standard used was curcumin with $R_f$ value approx. 0.37 and Rf values for different samples were also found at 0.37 Rf which confirms the presence of Curcumin and good composition of mobie phase. The results showed in Fig. 1-3 and Table 1.

**2.Quantification of Curcumin by HPTLC technique in Crude drugs, Extracts and Formulations**

Curcumin content in Curcuma longa samples of Crude drugs, Extracts and Formulations was quantified and studied by 3D spectrum. The content of Curcumin in Raw 1, Raw 2, Raw 3 were foundas 2.5918%w/w, 1.9003%w/w, 3.3617%w/w respectively. In Extract 1, extract 2, extract 3 and Extract 4 showed 4.0463%w/w, 3.8461 %w/w, 3.0961%w/w, 2.5960 %w/w respectively, whereas in Formulation 1 and Formulation 2 showed as 0.01758 %w/w and 0.00856 %w/w respectively. Results showed in Fig. 4- 6and Table 2- 4.

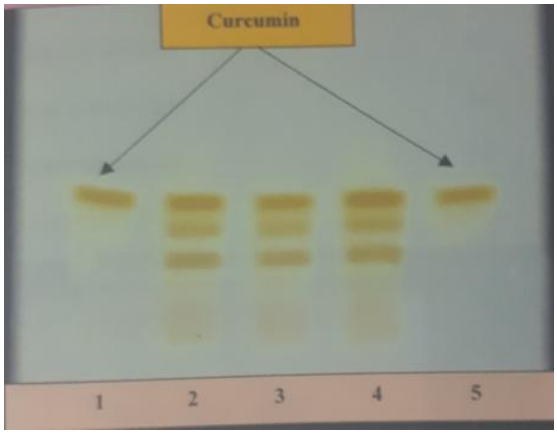


**Fig 1.: TLC of C. longa Raw samples**

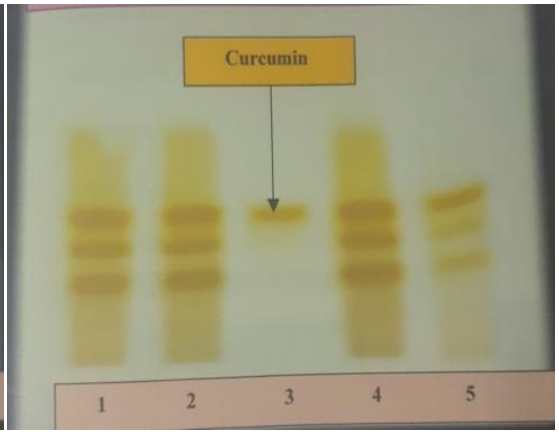


**Fig. 2.: TLC of C. longa Extracts**

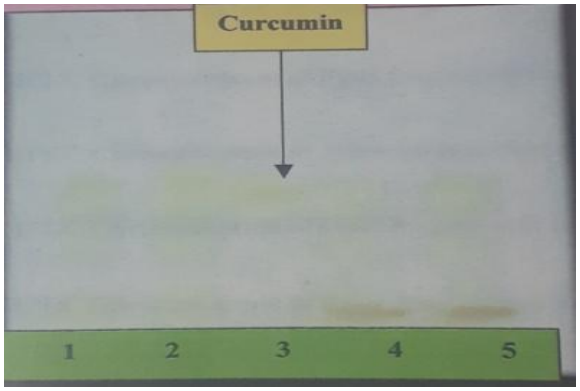


**Fig. 3.: TLC of C. longa Formulations**

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**Table 1: TLC Profile of Raw sample , Extracts and Formulations**

| | | C. longa Raw | Rf | C. longa Extracts | Rf | C.longa Formulation | Rf |
|---|---|---|---|---|---|---|---|
| Distance travelled by solvent front | | 87mm | | 89mm | | 90mm | |
| Distance travelled by sample | Standard | 33mm (Spot 1& 5) | **0.37** | 33mm (Spot 3) | **0.37** | 33mm (Spot 3) | **0.36** |
| | Sample 1 | 33mm (Spot 2) | **0.37** | 33mm (Spot 1) | **0.37** | 34mm (Spot 1 & 2) | **0.37** |
| | Sample 2 | 33mm (Spot 3) | **0.37** | 33mm (Spot 2) | **0.37** | 34mm (Spot 3 & 4) | **0.37** |
| | Sample 3 | 33mm (Spot 4) | **0.37** | 33mm (Spot 4) | **0.37** | - | - |
| | Sample 4 | - | - | 33mm (Spot 5) | **0.37** | - | - |

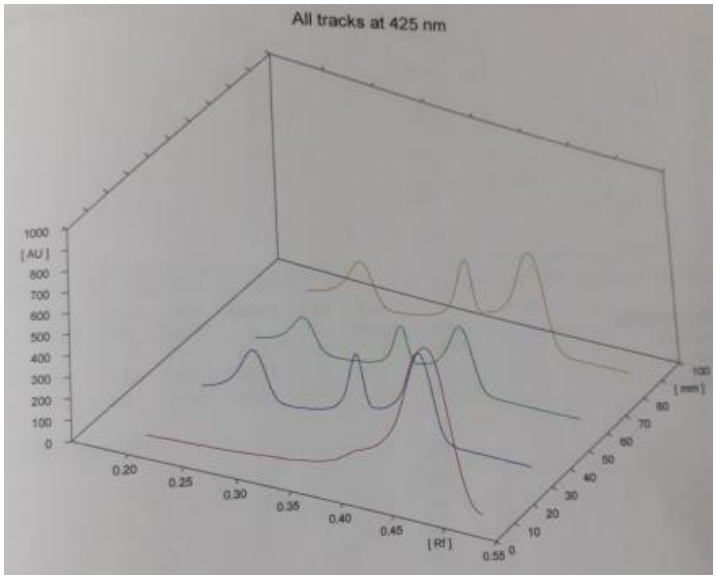


**Fig4.: HPTLC 3D spectra of Raw samples**

**Table 2: HPTLC Profile of Raw samples**

| | Raw – 1 | | Raw – 2 | | Raw – 3 | |
|---|---|---|---|---|---|---|
| **Sample Avg. peak area** | 13326.3 | | 9524.4 | | 16833.4 | |
| **Standard Avg. peak area** | 44684.5 | | 44684.5 | | 44684.5 | |
| **Sample dilution** | 111.7 mg | In100 ml | 108.9 mg | In 100 ml | 108.8 mg | In 100 ml |
| **Sample dilution factor** | 1.117 | | 1.089 | | 1.088 | |
| **Standard dilution** | 4.088 mg | In 10 ml | 4.088 mg | In 10 ml | 4.088 mg | In 10 ml |

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| | | | |
|---|---|---|---|
| **Standard dilution factor** | 0.4088 | 0.4088 | 0.4088 |
| **Sample applied** | 20 µl | 20 µl | 20 µl |
| **Standard applied** | 5 µl | 5 µl | 5 µl |
| **Standard purity** | 95 %w/w | 95 %w/w | 95 %w/w |
| **% Curcumin** | **2.5918%w/w** | **1.9003%w/w** | **3.3617%w/w** |

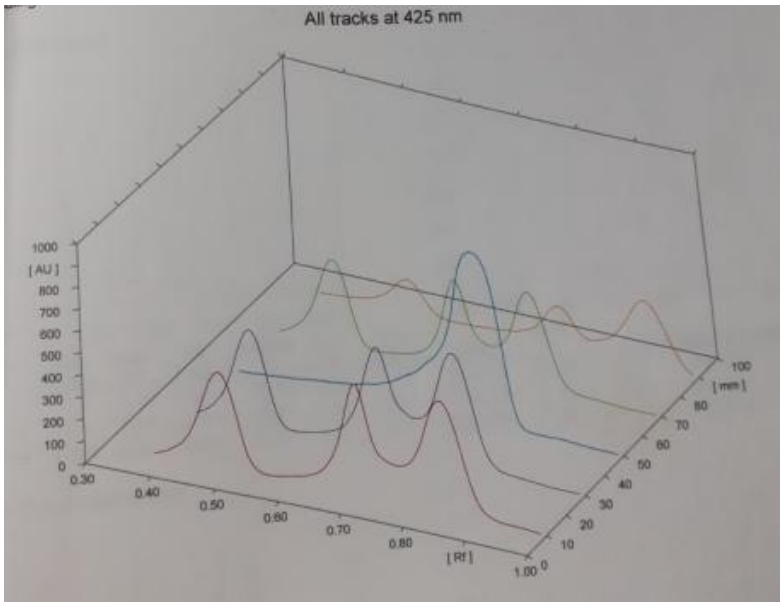


**Fig 5.:HPTLC 3D graph of Extracts**

**Table 3: HPTLC Profile of Extracts**

| | **Extract – 1** | **Extract – 2** | **Extract – 3** | **Extract – 4** |
|---|---|---|---|---|
| **Sample Avg. peak area** | 16692.0 | 15985.5 | 13168.2 | 10578.7 |
| **Standard Avg. peak area** | 37607.2 | 37607.2 | 37607.2 | 37607.2 |
| **Sample dilution** | 106.5 mg In 50 ml | 107.3 mg In 50 ml | 109.8 mg In 50 ml | 105.2mg In 50 ml |
| **Sample dilution factor** | 2.13 | 2.146 | 2.196 | 2.104 |
| **Standard dilution** | 4.088 mg In 10 ml | 4.088 mg In 10 ml | 4.088 mg In 10 ml | 4.088mg In 10 ml |
| **Standard dilution factor** | 0.4088 | 0.4088 | 0.4008 | 0.4088 |
| **Sample applied** | 10 µl | 10 µl | 10 µl | 10 µl |
| **Standard applied** | 5 µl | 5 µl | 5 µl | 5 µl |
| **Standard purity** | 95 %w/w | 95 %w/w | 95 %w/w | 95 %w/w |
| **% Curcumin** | **4.0463 %w/w** | **3.8461%w/w** | **3.0961 %w/w** | **2.5960 %w/w** |

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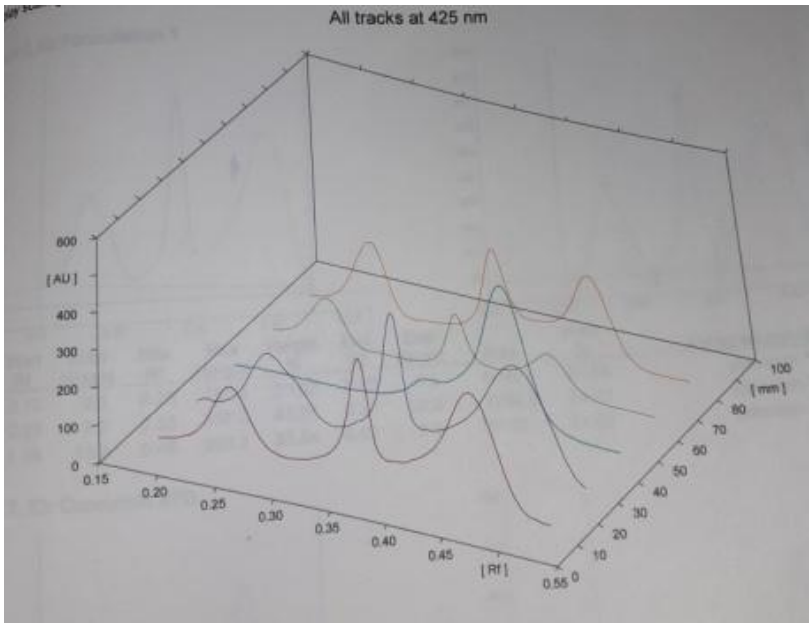


**Fig 6.: HPTLC 3D Spectra of Formulations**

**Table 4: HPTLC Profile of Formulations**

| | **Curcuma longa Formulation 1** | | **Curcuma longa Formulation 2** | |
|---|---|---|---|---|
| **Sample Avg. peak area** | 14167.4 | | 8847.3 | |
| **Standard Avg. peak area** | 17646.2 | | 17646.2 | |
| **Sample dilution** | 5.9164 gm | In 25 ml | 5.6814 gm | In 25 ml |
| **Sample dilution factor** | 236.656 | | 227.256 | |
| **Standard dilution** | 4.088mg | In 10 ml | 4.088mg | In 10 ml |
| **Standard dilution factor** | 0.4088 | | 0.4088 | |
| **Sample applied** | 15 µl | | 20 µl | |
| **Standard applied** | 2 µl | | 2 µl | |
| **Standard purity** | 95 %w/w | | 95 %w/w | |
| **% Curcumin** | **0.01758 %w/w** | | **0.00856 %w/w** | |

**Discussion:**

The phytoconstituents curcumin present in rhizomes of *Curcuma longa* Linn was identified by TLC and quantified by HPTLC on the basis of the marker compound. The standard used was curcumin with $R_f$ value approx. (0.37) whereas the mobile phase was used as Chloroform : Ethanol : Glacial acetic acid ( 90: 5 :1 ) and the plate was scanned at 425 nm.

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On the basis of study it is evaluated that the Curcumin content in Curcuma longa Raw3showed the highest amount of Curcumin of 3.3617%w/wwere complied the Indian pharmacopoeial limits of curcumin content (NLT 1.5%w/w).The highest content of curcumin was quantified in Extracts 1was found to be 4.0463%w/w which is found to be in specifications provided by Indian pharmacopoeial limits of curcumin content (NLT 1.5%w/w).The highest content of Curcumin in formulation 1 was found to be 0.01758 %w/w.

**Conclusion:**

The good content of curcumine in crude drugs, extracts and formulation obtained only with the good mobile phase i.e. Chloroform : Ethanol : Glacial acetic acid ( 90: 5 :1 ).

Due to time of collection, geographical variation, genetic variation, growing conditions, timing and method of harvesting, exposure to air, light and moisture over time and type of preservations, there may be chances of variations in contents of curcumine in different species.

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