DEVELOPMENT AND STANDARDIZATION OF TURMERIC CREAM BY HPTLC

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**Abstract**

The aim of this study was to formulate turmeric cream and to standardize it by HPTLC using pure curcumin as a bioactive chemical marker. It was developed by incorporating extract of *Curcuma longa* (rhizome) in a cream base by a w/o emulsification technique. Preformulation and drug-excipients interactions were studied by HPTLC finger printing. Chromatography was performed on silica gel 60 F₂₅₄ TLC plate using chloroform: ethanol: acetic acid (48:2:0.1 v/v/v) as solvent system. Densitometry scanning was performed under reflectance-absorbance mode at 300 nm to quantify the spots. The content of curcuminoids in formulated cream was found to be 4.27 mg/g. The HPTLC method was validated as per the ICH guidelines. The proposed method is simple and sensitive and can be used for the routine assay of curcuminoids in phytomedicines containing Turmeric as an ingredient.

**Keywords:** *Curcuma longa*, curcuminoids, formulation and estimation.

**Introduction**

Turmeric (the rhizome of *Curcuma longa* L., Zingiberaceae) commonly called as Haldi in Hindi, is a well known plant drug in Ayurveda and Unani medicines¹. It has been widely used as a dietary pigment and spice in the Indian subcontinent. Traditionally, it is used for the treatment of a wide variety of diseases and conditions including those of the skin, pulmonary and gastrointestinal systems, aches, wounds, sprains and liver. During last half century, extensive research has proved that most of the activities associated with turmeric, are due to curcumin².³. Phytochemical investigation of the rhizomes of *curcuma longa* has led to the isolation of pharmaceutically active curcuminoids viz mixtures of curcumin (77-90%), demethoxycurcumin (6-17%), bis-demethoxycurcumin (2-4%) as well as volatile oils (termerone and zingiberone), sugars, proteins and resins⁴,⁵. High Performance Thin Layer Chromatography (HPTLC) is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean-up. The major advantages of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. It is an important tool used for the identification, estimation, purity testing, stability testing, dissolution and content uniformity testing of raw materials (herbal and animal extracts, fermentation mixtures, drug and
excipients) and formulated product (pharmaceutical, cosmeceuticals, nutraceuticals)\textsuperscript{6,7}. The aim of the present work was to develop a turmeric cream in a w/o cream base and to standardize both, the extract and the finished product by HPTLC to standard curcuminoids.

**Materials and Methods**

**Plant Material**

Rhizomes of *C. longa* were collected from local market of Manipal, Karnataka, India and authenticated by Dr. Gopalkrishna Bhat, Professor, Department of botany, Poorna Prajna College Udupi, Karnataka, India. A voucher specimen No. PP 532 has been deposited in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

**Chemicals**

Authentic standards of curcumin, demethoxycurcumin and bisdemethoxycurcumin were purchased from Sigma Aldrich, Bangalore, India. Graded methanol was obtained from Merck, India. Before use, solvents were filtered through a 0.45 µm Millipore membrane after sonication for 15 min.

**Extraction of plant material for analysis**

Air dried (35-50°C) rhizomes of *C. longa* (100 gm) was extracted with 70% methanol (250 ml) by soxhlet apparatus for 12 hours, filtered through Whatman filter paper after extraction. Extract was concentrated under vacuum to a dry mass. The extract was stored in an air tight dry labelled container, until further use.

**Preparation of Cream**

Cream was prepared using fusion method by taking 16% cream base (beeswax) and 50% softening agent (liquid paraffin), 0.8% emulsifying agent (borax) in 33.2% of distilled water. Both oil and aqueous phases were mixed at 70°C in the mortar and stir continuously until a homogenous product was formed and then 0.5% of turmeric extract was added to it with continuous trituration. Reaction mixture was homogenized by mixer into a cream form. About 0.2% of perfumes were added at the end.

**Evaluation of cream**

Physiochemical parameters of formulation i.e. determination of phase separation (stability testing), grittiness of particles, washability, stainability, pH of cream, viscosity testing, drug-excipients were studied.

**Preparation of stock solution**

Stock solutions of references compounds were prepared separately in methanol at a concentration of 1 mg/ml. Studies on the stability of the analysts in stock solutions showed that there were no decomposition products in the densitogram or differences in the measured areas during the analytical procedure, even after storage for 7 days at +4°C.

**Chromatographic Condition**

Chromatography was performed on Aluminium-backed silica gel 60 GF\textsubscript{254} HPTLC layers (20×10 cm, 300µm layer thickness). Methanol solutions of samples and standard curcumin, demethoxycurcumin, bisdemethoxycurcumin of known concentrations were applied to the layers as 7 mm-wide bands positioned 15 mm from the bottom and 20 mm form the side of the plate, using a Camag Linomat V automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nL/s from the syringe. The parameters were kept constant were kept constant throughout the analysis of samples.

**Detection and Quantification**
Following sample application, plates were developed in a Camag twin through glass tank pre-saturated with the mobile phase, chloroform: ethanol: acetic acid (48:2:0.1 v/v/v) for one hour, up to a height of 7 cm, under laboratory conditions of 25 ± 5°C and 70% relative humidity. The spots were visualized under Camag UV cabinet (254 and 366 nm) after drying. Quantitative analysis of the compounds was done by scanning the plates using Camag TLC scanner model III equipped with Wincats software (Camag) applying the following conditions: slit width 6×0.45 mm, wavelength (λ max) 300 nm, absorption-reflection scan mode. The identification of curcumin, demethoxycurcumin and bis-demethoxycurcumin in rhizomes were confirmed by superimposing the UV spectra of samples and standards within the same Rf window.

Calibration curves of standard curcuminoids
Serial dilutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml of curcuminoids in methanol were prepared from a stock solution of curcuminoids (1 mg/ml). Solution was then applied on the HPTLC marks and developed and area under the peak was recorded. A standard curve was prepared by plotting concentration of curcuminoids on x axis against the area under the peaks of curcuminoids on y axis.

Estimation of curcuminoids in turmeric extract
To determine the content of curcuminoids in extract, 10 mg/ml was transferred into a 100 ml volumetric flask containing 50 ml methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant was analyzed for drug content. Five microlitres of the filtered solution was applied on the TLC plate followed by development and scanning as described previously. The analysis was repeated six times. The possibility of interference from other components of extract in the analysis was studied.

Analysis of the formulated cream
To determine the content of curcuminoids in cream, 1 gm of cream was transferred into a 100 ml volumetric flask 50 ml methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant was analyzed for drug content. Five microlitres of the filtered solution was applied on the TLC plate followed by development and scanning as described previously. The analysis was repeated six times. The possibility of interference from other components of extract in the analysis was studied.

Method validation

The method was validated according to the ICH guidelines on the validation of analytical methods. Linearity graph, precision viz. intraday and interday variations, limit of detection and limit of quantification, accuracy (% recovery) and analysis of curcuminoids in prepared formulations were carried out. Limit of detection and limit of quantification were estimated on a statistical evaluation base for six determinations of the samples using ICH guidelines.

Result and Discussion
Curcuminoids are naturally occurring polyphenolic phytochemicals. There has been considerable public and scientific interest in the use of phytochemicals derived from the diet to reduce risk and
progression of major chronic diseases. It is established that polyphenols are potent antioxidative phytochemicals. Phytochemical investigation of the rhizomes of *Curcuma longa* leads to the isolation of pharmacologically active curcuminoids viz curcumin, demethoxycurcumin and bisdemethoxycurcumin. These were isolated from turmeric rhizomes by soxhlet extraction using 70% methanol. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. This article reports on standardization of *Curcuma longa* cream, an herbal medicine used in skin, pulmonary and liver disorder etc. A simple, accurate and precise HPTLC method has been developed for standardization of cream formulations. The solvent system, chloroform: methanol: acetic acid (48:2:0.1 v/v/v) was suitable for analysis of curcuminoids, gave sharp and symmetrical peaks. The 3-D graphical presentation chromatogram (fingerprints) and that of standard curcuminoids, gave sharp and symmetrical peaks. The 3-D graphical presentation chromatogram (fingerprints) and that of standard curcuminoids, gave sharp and symmetrical peaks. The Rf value of different samples shown in Table 1. Physico-chemical parameters of formulation were found to be in the appreciable range. The results were shown in Table 2. There was no interference from the excipients present in the formulations. The method was found to comply with ICH guidelines and the validation parameters. 4.27 mg/g of curcuminoids was determined in formulated cream.

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Table 1: \( R_f \) value of different samples

| Compounds                        | Standard curcumin | Turmeric extract | Formulated cream |
|----------------------------------|-------------------|------------------|------------------|
| Curcumin                         | 0.40              | 0.39             | 0.38             |
| Demethoxycurcumin                | 0.23              | 0.23             | 0.23             |
| Bis-demethoxycurcumin            | 0.19              | 0.17             | 0.16             |

Table 2: Physico-chemical parameter of formulated cream

| Physico-chemical parameter       | Observation                                |
|----------------------------------|--------------------------------------------|
| Grittiness                       | No gritty particle found                   |
| Appearance                       | Yellow orange colour, No phase separation observed |
| Washability                      | Easily washable                            |
| pH                               | 7.0-8.0                                    |
| Stainability                     | Does not leave any stain after washing the cream |
| Viscosity                        | Appreciable range                          |
Fig 1: 3-D Graphical representation of curcuminoids

Fig 2: Chromatogram of standard curcuminoids of *C. longa*.
Fig 3: Chromatogram of turmeric extract

![Chromatogram of turmeric extract](image1)

Fig 4: Chromatogram of turmeric cream formulation

![Chromatogram of turmeric cream formulation](image2)
Fig 5: Thin Layer Chromatography of formulated cream