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Research Article

STANDARDIZATION AND QUANTIFICATION OF CURCUMIN PRESENT IN *CURCUMA LONGA* EXTRACT USING HPLC

Shivarkar Rahul Shashikant [1*], S. B. Bhise [2] and V. Rama Mohan Gupta [3]

[1]Pharmacognosy Department, Sinhgad Institute of Pharmaceutical Sciences, Lonavala Kusgaon BK Lonavala Tal, Maval Dist, Pune 410401, Maharashtra, India.
[2]A202, Navakar Residency, Behind Bibvewadi Police Chowki, Pune 411037, Maharashtra, India.
[3]Pulla Reddy Institute of Pharmacy, Near Dundigal Air Force Academy Annaram (V), Jinnaram (M) Medak (Dt) 502313, Telangana, India.

ABSTRACT

Herbal product studies can be considered scientifically valid if the product tested was authenticated and characterized. Standardization of herbal drugs is generally based on one or more known and accepted active biochemical principle. Fingerprint analysis approach using high performance liquid chromatography has become the most potent tools for quality control of herbal medicines because of its simplicity and reliability. For the development of a botanical product, authentication of raw material is the basic starting point. However, the quality and consistency of a final product may dramatically affected due to harvesting, storing, processing and formulating methods by altering the desired active components or by increasing the possibility of unwanted contaminants. Thus, validated methods to ensure quality control in manufacturing and storage are required tools for optimal efficacy and safety of the products. In present work, *Curcuma longa* ethanolic extract was standardizing by quantifying curcumin using HPLC in this standard curcumin was used as biomarker. Standardization was done by considering retention time of curcumin peak of standard curcumin and that of the *Curcuma longa* extract. In both chromatograms curcumin peak has shown almost same retention time. By injecting various concentrations of curcumin (in methanol) in HPLC system, the linearity was observed in standard curcumin solutions ranging from 5 to 40 µg/ml. Using the data obtained from the linearity curve, the concentration of curcumin present in extract of *Curcuma longa* was calculated and it is found to be 57.4%.Thus HPLC is found to be suitable, sensitive and reliable method for the standardization and quantification of curcumin present in the *Curcuma longa* extract with respect to curcumin as a marker. Thus this developed HPLC method is a useful validation tool for the standardization and quality control analysis of *Curcuma longa*, either extract or formulation form.

Key words: Standardization, *Curcuma longa*, Curcumin, HPLC, Retention time.

Corresponding Author **Shivarkar Rahul Shashikant** Email: rahulshivarkar19@gmail.com

INTRODUCTION

Fingerprint analysis approach using high performance liquid chromatography has become the most potent tools for quality control of herbal medicines because of its simplicity and reliability (Dobrial R and Narayana D, 1998). For the development of a botanical product, authentication of raw material is the basic starting point. However, the quality and consistency of a final product may dramatically have affected due to harvesting, storing, processing and formulating methods by altering the desired active components or by increasing the possibility of unwanted contaminants. Thus, validated

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methods to ensure quality control in manufacturing and storage are required tools for optimal efficacy and safety of the products. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements.

Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized. Standardization of herbal drugs is generally based on one or more known and accepted active biochemical principle (Monfort *et al*, 1992). However, many a times where the active biochemical principle is not known, a characteristic compound is used as a "marker," which signifies the presence of the other biochemical compounds that give the herb its therapeutic properties. Besides the physical and chemical markers, genetic markers are also now recommended to be used to authenticate the basic ingredients of botanical and traditional drugs.

In general, one or two markers or pharmacologically active components in herbs and or herbal mixtures are currently employed for evaluating the quality and authenticity of herbal medicines, in the identification of the single herb or herbal preparations, and in assessing the quantitative herbal composition of an herbal product (Bhutani K, 2000). This kind of the determination, however, does not give a complete picture of a herbal product, because multiple constituents are usually responsible for its therapeutic effects. These multiple constituents may work 'synergistically' and could hardly be separated into active parts.

Thus, it seems to be necessary to determine most of the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control (Jain S et al., 2007). Several chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), thin layer chromatography (TLC) and high-performance thin layer chromatography (HPTLC) can be applied for the standardization of herbal medicines.

Curcuma is genus of about 70 spices of rhizomatous herbs distributed in South East Asia and especially India, China, Thailand, Italy, Malaysia, Archipelago and N. Australia. Commercially *C. amada, C. angustifolia, C. aromatica, C. caesia, C. zedoria* and *C. longa* is more important due to its uses like spice, condiment, antiseptic in bruises, as anti-inflammatory and in sprains (Kokate et al., 2003).

Curcuma longa, a perennial herb, is a member of the Zingiberaceae (ginger) family. Externally, the drug is yellowish-brown in color with characteristic odor and slightly bitter in taste. Round turmeric rhizome are oblong, while long variety is cylindrical and short branched. Root scars and annulations are present. The fracture is horny and internal surface is orange. Rhizomes are 5 to 10 x 2 to 4 cm in size. It has oblong, pointed leaves and bears funnel-shaped yellow flowers (Leung, 1980).

HPLC has become the most versatile, safest, dependable fastest and sensitive chromatographic technique for quality control of drug components. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines.

It is necessary to notice that the optimal separation condition for the HPLC involves many factors, such as the different compositions of the mobile phases, their pH adjustment, pump pressures, etc. Thus, a good experimental design for the optimal separation seems in general necessary. It can serve as a tool for identification, authentication and quality control of herbal drugs. Determination of total curcumin is very important for standardization of *C. longa*.

MATERIALS AND METHODS

Extraction:

Curcuma longa rhizomes (Turmeric) collected from market and dried in oven at 50 ˚C Extraction of the powdered plant material (rhizome of *Curcuma longa*) was subjected to Soxhlet extraction using ethanol as a solvent. The extract material was evaporated on water bath and *Curcuma longa* dried whole ethanolic extract was collected. This extract was further used for all phytochemical and pharmacological evaluation.

Rotary extraction can be preferentially be used for efficient and economic extraction. The turmeric, after crushing to a coarse powder, is loaded to the extractor and solvent (about 4-5 times of raw material) is charged. The contents are heated to boiling and then for refluxing 2-3 hours under the mild turbulence given by forced circulation or rotation. Three extractions are enough in rotary extractors for complete exhaustion of turmeric while it may take 4-5 extractions in vertical extractors. The extraction solvent after concentration under vacuum at maximum temperature of 55 ºC to an appropriate density is transferred to crystallizers, maintained at temperature 8-10 ˚C. The crystals obtained are filtered, washed and dried in vacuum tray dryer or fluid bed dry (Yogeshwaran, 2005).

Reference compound and reagents

Reference curcumin was procured from Loba Chemie, Mumbai. All HPLC grade solvents were obtained from Merck (Mumbai, India).

Preparation of standard solutions

10 mg of standard curcumin was accurately weighed and was dissolved in 10 ml HPLC grade methanol (Stock solution 1mg/ml). This stock solution was further

diluted with HPLC grade methanol to get different concentrations of solution ranging from 5, 10, 20, 30, and 40 µg/ml using HPLC grade methanol. The λ_{max} of curcumin in methanol was found to be 425 nm. Hence the absorbance of the above mentioned standard solutions were recorded at 425 nm. Before use, the mobile phase was degassed by an ultrasonic bath. Separation was performed at room temperature.

Preparation of sample solution

10 mg of extract of *Curcuma longa* was accurately weighed and dissolved in 10 ml HPLC grade methanol to get solution of 1 mg/ml. This solution was further diluted to get concentration of50 µg/ml. This solution was then filtered using membrane filter before injecting into the HPLC system. The separation system consisted of a C_{18} reversed-phase column, an isocratic elution system of methanol: water (70:30 % v/v) and an UV detector.

Instrument and Chromatographic Conditions

Linearity and Range

Identification was based on comparing retention times and UV–Vis spectral data of the peaks detected to those of original reference standard compound. The calibration curve was prepared by injecting various concentrations of the calibrator solutions. The calibration plot was found to be linear in the range 5µg/ml to 40 µg/ml. With a correlation coefficient was found to be 0.990. Retention time and peak area of standard curcumin at various concentrations and *Curcuma longa* extract are shown in table 1.2 and 2.3 respectively. The calibration curve of standard curcumin is shown in Fig 1.

Table 1. Optimized chromatographic conditions for the standardization of curcumin using HPLC

Chromatographic Mode	Chromatographic Condition
Standard solution	1 mg/ml in methanol
HPLC System	Jasco HPLC system
Pump	Jasco PU 1580 Intelligent HPLC Pump
Detector	Jasco UV-VIS 1520 Detector
Data processor	Borwin Chromatographic Software
Stationary phase	C18 column (4.6 x 250 mm, 5 micron)
Mobile phase	methanol: water (70:30 % v/v)
Detection wavelength	425 nm
Flow rate	1 ml/min
Injection volume	20 µl

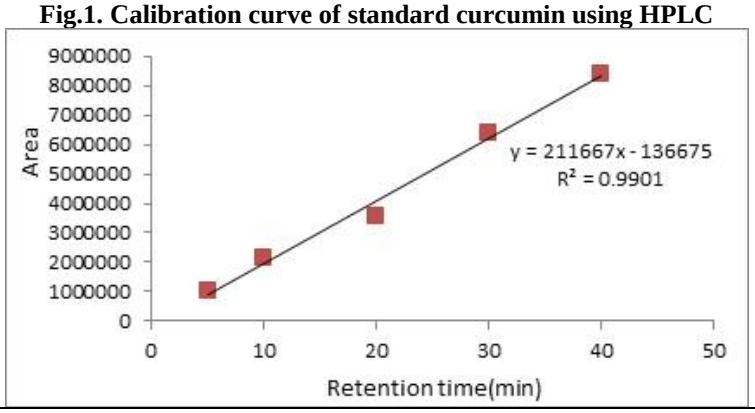
Table 2. Retention time and peak area of standard curcumin at various concentrations

Sr. No	Standard curcumin conc. (µg/ml)	Retention time (Mins)	Peak Area
1	5	9.69	1037805
2	10	9.80	2134553
3	20	9.78	3560845
4	30	9.79	6414809
5	40	9.78	8393662

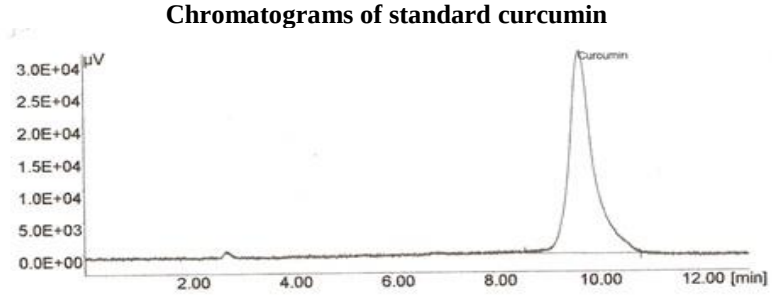
Table 3. Retention time and peak areas of extract of *Curcuma longa*

***Curcuma longa*extract**	Concentration of extract	Retention time(mins)	Peak Area
Ethanolic	50 µg/ml	9.81	6131723

Fig.1. Calibration curve of standard curcumin using HPLC

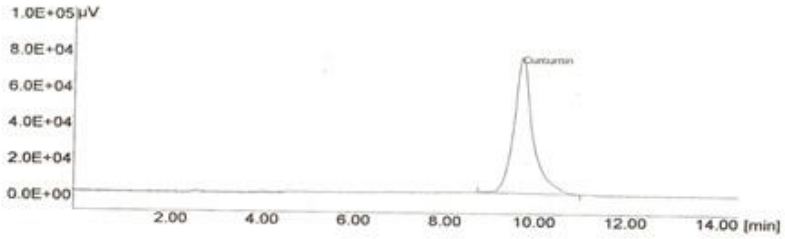


Chromatograms of standard curcumin

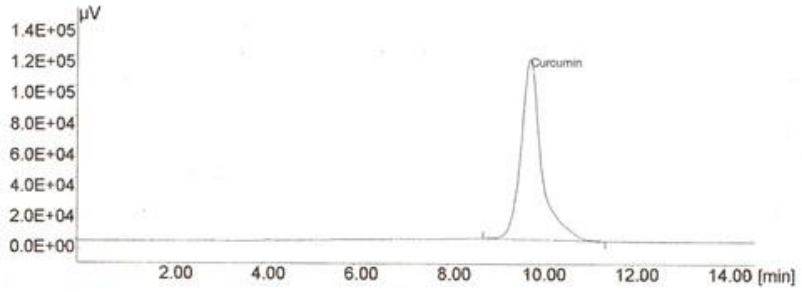


Sr no.	Curcumin concentration (µg/ml)	Retention time(mins)	Peak area
1.	05	9.69	1037805

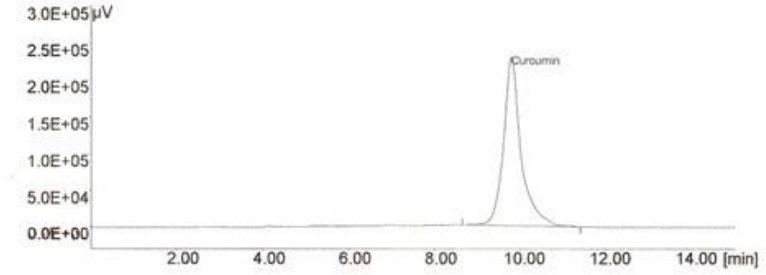
Chromatograms of *Curcuma longa* extract



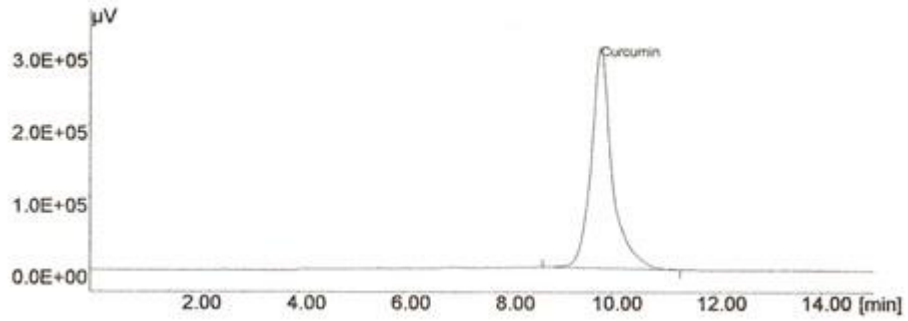
Sr no.	Curcumin concentration (µg/ml)	Retention time(mins)	Peak area
1.	10	9.80	2134553



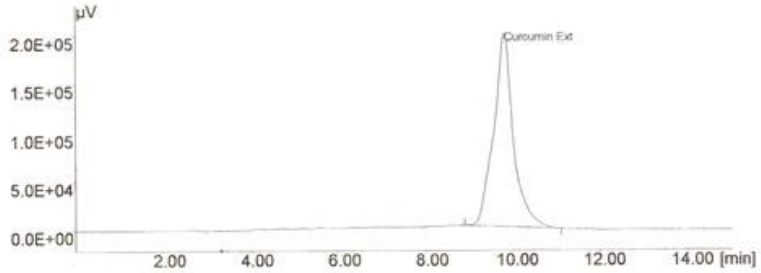
Sr no.	Curcumin concentration (µg/ml)	Retention time(mins)	Peak area
1.	20	9.78	3560845



Sr no.	Curcumin concentration (µg/ml)	Retention time(mins)	Peak area
1.	30	9.79	6414809

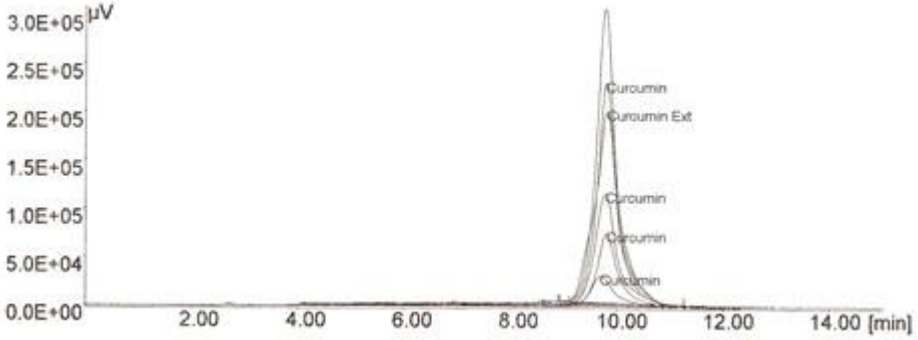


Sr no.	Curcumin concentration (µg/ml)	Retention time(mins)	Peak area
1.	40	9.78	8393662



Sr.No	Curcumin concentration (µg/ml)	Retention time(min)	Peak area
1.	50	9.81	6131723

Chromatograms of standard curcumin with curcumin present in *Curcuma longa* extract by HPLC



RESULTS AND DISCUSSION

Standard curcumin was used as biomarker for the standardization of *Curcuma longa* extract. Standardization was done by considering retention time of curcumin peak of standard curcumin and that of the *Curcuma longa* extract. In both chromatograms curcumin peak has shown almost same retention time. By injecting various concentrations of curcumin (in methanol) in HPLC system, the linearity was observed in standard curcumin solutions ranging from 5 to 40 µg/ml. Using the data obtained from the linearity curve, the concentration of curcumin present in extract of *Curcuma longa* was calculated. Thus by calculation, extract of *Curcuma longa* contains 57.4% of curcumin.

CONCLUSION

It can thus be concluded that the *Curcuma longa* can be standardized with curcumin as marker compound. HPLC is found to be suitable method for the standardization and quantification of curcumin present in the *Curcuma longa* extract with respect to curcumin as a marker. It is more sensitive and reliable method of

standardization and hence gives more accurate results than other methods of standardization. Thus this developed HPLC method is a useful validation tool for the standardization and quality control analysis of *Curcuma longa*, either extract or formulation form.

REFERENCES

Bhutani K. Fingerprinting of Ayurvedic Drugs, *The Eastern Pharmacist*, 50, 2000, 21-26.
Dobrial R, Narayana D. Ayurvedic Herbal Raw material, *The Eastern Pharmacist*, 48,1998, 31-35.
Jain S, Menghani E, Jain R. Biomarkers as a tool for validation of herbs and spices, *International Journal of Food Safety*, 9, 2007, 1-6.
Kokate C, Purohit A, Gokhale S. Terpenoids, Pharmacognosy, Nirali, New Dehli, India, 43, 2003**,** 11.110.
Leung A. Drugs and Cosmetics, Encyclopedia of Common Natural Ingredients Used in Food, New York, 1980, 313-314.
Monfort G, Ayora T, Maldonado M, Loyhola I. *Phytochemistry and. Analysis*., 3, 1992, 117.
Rajpal V. *Curcuma longa*, Standardization of Botanicals, Eastern, New Dehli, India, 2**,** 2005, 120-132.
Yogeshwaran. Turmeric: Traditional herb par excellence. *Herbal Tech Industry*, 2**,** 2005, 12-18.

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