INDIAN JOURNAL OF NATURAL PRODUCTS

Official website: www.indiansp.com

A simple RP-HPLC method for the determination of curcumin in commercial turmeric herbal products

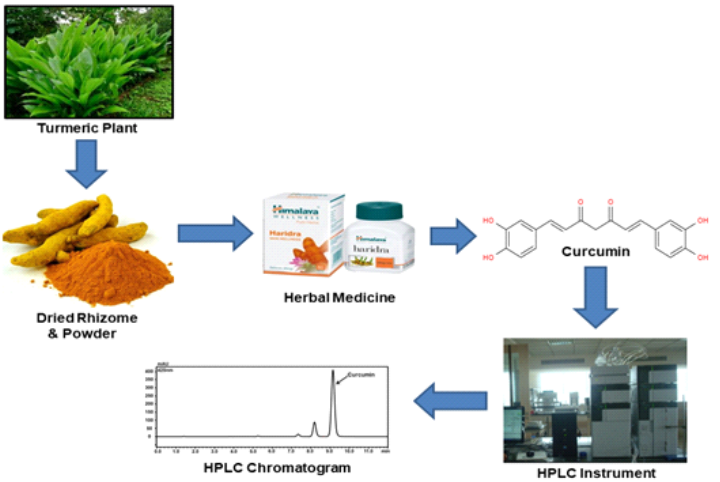
Malleswara Rao Peram[a,b], Sunil S Jalalpure*[a,b], Sumit A Joshi[b,c]

[a]*Department of Pharmacognosy,College of Pharmacy, KLE Academy of Higher Education and Research, Belagavi, India*

[b]*Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India*

[c]*Department of Pharmacology, KLE Society's College of Pharmacy, Nipani, Karnataka*

Graphical Abstract



Abstract

Background: Curcumin derived from the dried rhizomes of turmeric has been extensively studied due to its wide range of biological activities and pharmacological effects. Previously reported HPLC methods have several disadvantages such as long run time, high flow rate, higher limits of detection and quantification, complicated mobile phase compositions with gradient elution and very low pH conditions.

*Purpose:*To develop a simple, rapid, economic, precise and accurate reverse phase high performance liquid chromatographic method for the quantitative determination of curcumin in various commercially available turmeric/curcumin products and turmeric extracts.

*Method:*Chromatographic separation was achieved using a Phenomenex Luna C18 column (150 x 4.6 mm; 5 µm) at 40 °C, with a mobile phase consisting of acetonitrile: 0.1% formic acid (50:50), at a flow rate of 0.8 ml/min. The detection was performed at 425 nm using photodiode array detector.

*Results:*The developed method was validated in accordance with International Conference on Harmonization (ICH) guidelines. All the system suitability parameters were within the acceptable limits. Calibration curve was linear over the concentration range of 2-64 µg/mL with correlation coefficient values of 0.999. The % RSD values for intraday and interday precision were ranged from 0.06 to 1.67. The mean percentage recovery of curcumin was found be in the range between 99.83 and 103.97 which were within the acceptance limits. The content of curcumin in the commercial Haridra capsule was found to be 2.5 % w/w, which is in accordance with the literature

*Conclusion:*The developed RP-HPLC method is appropriate for the analysis of curcumin in wide range of turmeric products and used for quality control of products that contain curcumin as main ingredient.

Key words: Curcumin; Reversed-phase HPLC; Validation; Herbal formulation

Introduction

Turmeric (*Curcuma longa* L., Zingiberaceae) is a herbaceous perennial plant native to India and other parts of Southeast Asia.

The powdered dried rhizomes of turmeric have been used for centuries as spice, coloring agent, flavouring agent and food preservative due to its vibrant yellow color and pleasant aroma.

As a traditional Indian medicine, turmeric powder has been used for thousands of years in the treatment of various disease conditions such as rheumatism, skin diseases, cough, cold, fever, wounds, body aches, constipation, inflammation, stomach and liver disorders(Ishita and Khaushik, 2004).

Turmeric contains a wide range of phytochemicals, including the most important phenolic compounds known as

* Corresponding author. Tel: +91 831 2444122
E-mail address: jalalpuresunil@rediffmail.com

curcuminoids which include curcumin (CUR), demethoxy curcumin (DMC) and bisdemethoxy curcumin (BDMC) (Amalraj et al., 2016). In addition to these, there are some important volatile oils (turmerone, atlantone, and zingiberene), sugars, proteins and resins. The bright yellow color and therapeutic effects of turmeric are mainly due to the presence of curcuminoids, which constitute approximately 3-5% of turmeric preparations. Commercially available curcumin used in research contains a mixture of three curcuminods namely curcumin (77%), demethoxycurcumin (17%) and bisdemethoxy curcumin (3%) (Ahmed and Gilani, 2014). Although, these three curcuminoids differ in their chemical structure only with respect to methoxy substitution on the aromatic ring, they exhibit significantly different antioxidant, anti-inflammatory and antitumor activities(Anand et al., 2008).

Of the three curcuminoids, curcumin is the most abundant, potent and extensively studied phytochemical due to its wide range of biological activities and pharmacological effects. These include, antioxidant, anti-inflammatory, antitumor, anti-coagulant, anti-diabetic, anti-bacterial, anti-viral, anti-fungal, hyperlipidemic and hepatoprotective activities (Anand et al., 2007). Curcumin is a potential candidate for the prevention and treatment of cancer because of its strong chemopreventive, chemotherapeutic and chemosensitizing activities. It inhibits every stage in tumour progression including the initiation step, promotion, angiogenesis and metastatis. Apart from all these activities, curcumin is "Generally Recognized as Safe (GRAS)" by Food and Drug Administration (FDA) and extremely safe even after administration of very high doses makes it a potential compound to explore its use in various disorders (Salem et al., 2014). Because of its easy availability, extreme safety profile, low cost and unique ability to work through mutiple pathways, many pharmaceutical companies are producing various turmeric/curcumin products in the form of tablets, capsules, ointments, gels, drinks and soaps for daily and medical use (Goel et al., 2008). Hence, it is important to develop and validate a method for quantitative estimation of curcumin from various turmeric products.

Various methods has been reported for the estimation of curcumin in the literature which include high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), ultra performance liquid chromatography (UPLC), capillary electrophoresis and liquid chromatography mass spectrometry (LCMS). Most of these previously reported HPLC methods have several disadvantages such as long run time, high flow rate, poor resolution, inadequate separation of peaks, higher limits of detection and quantification, complicated mobile phase compositions with gradient elution and very low pH conditions (Wichitnithad et al., 2009). Therefore, the objective of the present study was to develop a simple, rapid, accurate, precise and sensitive RP-HPLC method and validate as per International Conference on Harmonization (ICH) guidelines. Furthermore, the developed method was used for the quantification of curcumin in various commercially available turmeric/curcumin products as well as turmeric extracts.

Experimental

Chemicals

Standard sample of curcuminoids (total curcuminoids content 99.5% determined by HPLC) was gifted by Green Chem Herbal Extracts & Formulatons (Bangalore, India). Commercially available turmeric capsules (haridra) containing curcumin was purchased from the Himalaya Drug Company (Bangalore, India). HPLC grade acetonitrile and formic acid were purchased from Fisher Scientific (Mumbai, India). All other chemicals and reagents were of analytical grade. Purified water was prepared by using Millipore Direct-Q® 3 water purification system (Millipore, Moisheim, France).

HPLC instrumentation

The HPLC system consisted of Shimadzu LC-20AD prominence system (Kyoto, Japan) equipped with LC-20AD pump, SPD-M20A Diode array detector, DGU-20A5 online degasser, SIL-20AC HT autosampler, Rheodyne injection valve with 20 µL loop and CTO-10AS VP colum oven. Integration and processing of chromatograms was done by using a Shimadzu LC solution (version 1.25) software program.

Chromatographic conditions

The chromatographic seperations were achieved using a Phenomenex Luna C18 column (150 x 4.6 mm, 5µm, Phenomenex Inc,) equipped with a phenomenex guard column. The mobile phase consisted of acetonitrile: 0.1% formic acid (50:50 v/v) at a flow rate of 0.8 mL/min. Before use, the mobile phase was filtered through 0.45µm membrane filters (Millipore, Bedford, USA) and degassed. The column temperature was 40 °C, injection volume was 10 µL and detection wavelength was 425 nm. The column was equilibrated for 60 min with the mobile phase prior to the HPLC injection. The quantitative analysis of curcumin was carried out using the peak areas and retention time. The total chromatographic run time was 12 min for each sample.

Preparation of standard solutions

Stock solution of curcumin containing 1mg/ml was prepared by dissolving accurately weighed quantity of curcumin in methanol. Standard solutions were prepared by diluting the stock solution with mobile phase to obtain concentrations of 2, 4, 8, 16, 32 and 64 µg/ml. All the solutions were stored in light resistant amber colored tightly stoppered volumetric flask.

Sample Preparation

Commercial Haridra capsules containing 100 mg of *curcuma longa* rhizome extract was weighed and transferred to 100 ml amber colored volumetric flask. Approximately 70 ml of

mobile phase was added to the above extract and sonicated for about 15 min and diluted with mobile phase to obtain a stock solution of 1mg/ml. The resulting solution was filtered through 0.45 µm membrane filter and 10 µL of the filtered solution was injected into HPLC system to analyze the curcumin content.

Validation of the RP-HPLC method

Method validation was carried out to demonstrate the suitability of the proposed analytical procedure for intended purpose. The developed method was validated in accordance with Internation Conference on Harmonization (ICH) guidelines for various parameters such as system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness (ICH, 2005). Microsoft excel was used to calculate mean, standard deviation, % relative standard deviation (%RSD), slope and correlation coefficient of the experimental data.

System suitability test

Six replicate injections of standard curcumin were injected and various parameters such as theoretical plates (N), % RSD of peak area and tailing factor (T) were determined. The theoretical plates more than 2000, % RSD of the peak area less than 2% and tailing factor less than 2 were set as acceptance criterion.

Linearity (calibration curve)

Six different standard solutions containing 2-64 µg/mL of curcumin were analysed in triplicate for each concentration. Calibration curve was obtained by plotting the concentration of the curcumin versus peak area (average of three runs). The linearity was determined by calculating the slope, y-intercept and correlation coefficient (r^2) using the least squre regression analysis.

Precision

It was evaluated with respect to repeatability and intermediate precision. The repeatability (intraday precision) of the method was evaluated by assaying three replicate injections of three different concentrations on the same day at different times and percentage relative standard deviation (%RSD) of the peak area was calculated. Intermediate precision (interday precision) was carried out by following the same procedure for three different days under the same experimental conditions. The % RSD of the peak area less than 2.0 % was set as acceptance criteria.

Accuracy (recovery studies)

To test the accuracy of the developed method, recovery studies were performed in triplicate by standard addition method at three different levels (50, 100 and 150%). Known quantities of curcumin were added to the pre-analyzed sample and its percentage recovery was estimated by comparing the actual and measured concentrations. Percentage recovery within 80-120% and % RSD less than 2% were set as acceptance criteria.

Limit of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) is the lowest concentration of the analyte in a sample that can be detected but not necessarily quantitated, under the defined experimental conditions. The limit of quantification (LOQ) is the lowest amout of analyte in a sample that can be quantitatively determined with adequate precision and accuracy, under the defined experimental circumstances. The LOD and LOQ were calculated based on the standard deviation (SD) of the y-intercept and slope (S) values (LOD = 3.3 (SD/S) and LOQ = 10 (SD/S).

Robustness

The robustness of the developed analytical method refers to its capability to remain unaffected by small but deliberate changes in the method parameters (flow rate) and provides an indication of its reliability during normal usage. The effect of these changes on the % RSD of the peak area, tailing factor, and theoretical plates were evaluated to ensure that robustness of analytical method is maintained whenever is used.

Results and Discussion

A variety of mobile phases and chromatographic conditions were investigated for the development of the HPLC method suitable for the analysis of curcumin in commerical turmeric herbal products. A mobile phase containing Acetonitrile: 0.1% Formic Acid (50:50 V/V), Phenomenex Luna C18 column (150x4.6 mm, 5µm), 0.8 ml/min flow rate, 10 µl injection volume, 40 °C column oven temperature, 425 nm wavelength and 12 min run time was selected as best chromatographic condition as peaks were well defined and resolved without any tailing. The retention time obtained for curcumin was 9.16 min. The HPLC chromatogram of standard curcuminoid mixture containing curcumin is shown in Figure 1A.

Validation of RP-HPLC method

System suitability

System suitability tests represent an integral part of the method development of many analytical procedures. These are used to ensure the validity of the analytical method and adequate performance of the chosen chromatographic system at all times. The % RSD of the peak area, number of theoretical plates and tailing factor were found to be 0.04%, 12311 and 1.08 respectively. The % RSD of the peak area (<2), theoretical plates (N>2000) and tailing factor (T>2) were within the acceptable limits confirming the validity of the developed method.

Linearity

Linearity is the capability of the developed method to obtain the results which are directly proportional to the concentration of the analyte in the sample. It was evaluated by analyzing different concentrations of the standard curcumin. The regression data for the calibration curve (n=3) of curcumin showed a good linear relationship over a concentration range of 2-64 µg/ml with respect to the peak area. The regression equation for curcumin was y = 78246x - 22170 (where y is the

peak area and x is the concentraion of curcumin) with excellent correlation coefficient value 0.999 (**Figure 2**). The higher value of correlation coefficient (r) indicates that developed method have high degree of correlation and good linearity.

Precision

The precision of the analytical method assess how close the data values are to each other for a number of measurements under the similar conditions. The results of intra-day and inter-day precision study are shown in **Table 1**. The % RSD values for intraday and interday precision were ranged from 0.06 to 1.67. The low values of %RSD (<2%) suggested the highly precision of developed method. **Accuracy**

The accuracy of the analytical procedure signifies the closeness of agreement between the true value and experimental value. The results indicated that the mean percentage recovery of curcumin was found be in the range between 99.83 and 103.97 which were within the acceptance limits (**Table 2**). The high recovery and low % RSD values obtained by the developed method indicates that the method is accurate.

Limit of detection (LOD) and quantification (LOQ)

LOD and LOQ were calculated using the standard deviation of y-intercept and slope of the calibration curve and were found to be 0.68 µg/ml and 2.064 µg/ml respectively. The lowest values

Table 1. Intraday and interday precision of curcumin

Compound	Content µg/ml	Intraday (n = 3)		Inter day (n = 3)					
				Day 1		Day 2		Day 3	
		Found (µg/ml)	% RSD	Found (µg/ml)	% RSD	Found (µg/ml)	% RSD	Found (µg/ml)	% RSD
CUR	4.00	4.02	0.06	4.07	0.12	3.79	1.60	3.87	0.51
	8.00	8.10	0.60	7.86	0.21	8.01	0.90	7.97	1.61
	16.00	15.92	0.95	15.40	0.27	15.71	1.34	16.02	1.67

Table 2. Determination of accuracy by percent recovery method

Compound	Contents µg/ml	Quantity added (µg)	Theoretical amount (µg)	Recovered amount (µg)	Recovery (%)	RSD %
CUR	7.94	12.10	20.04	20.00	99.83	0.21
	7.94	16.33	24.27	24.59	101.35	0.06
	7.94	20.30	28.24	29.36	103.97	0.81

Table 3. Analysis of marketed formulation

Brand Name	Drug	Label Claim (mg)	Amount found (mg)	% Label claim
Himalaya Haridra (400mg)	Turmeric extract	10 mg	10.08 ± 0.32	100.80

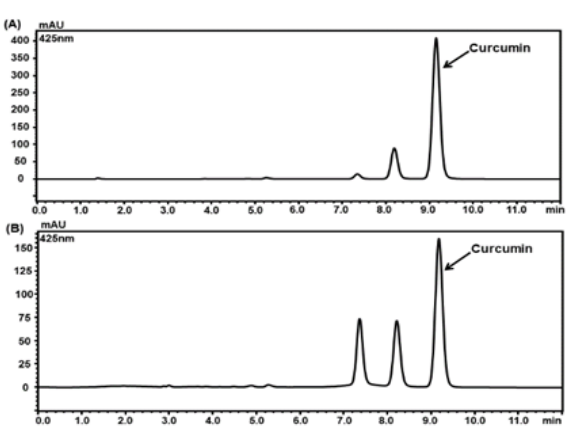


Figure 1. HPLC chromatogram of (A) standard curcuminoids mixture containing curcumin and (B) commercial Haridra capsule

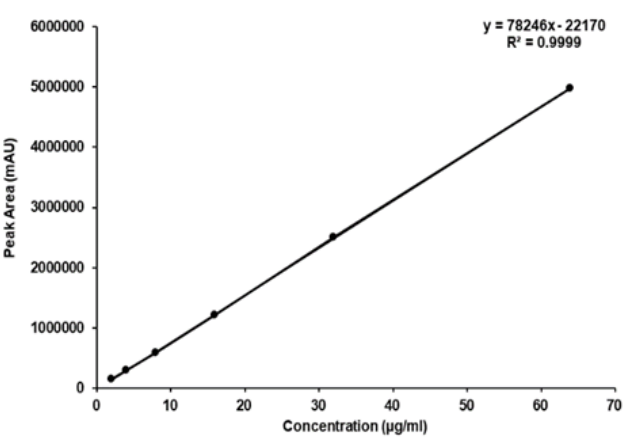


Figure 2. Calibration curve of curcumin

of LOD and LOQ as obtained by the developed method indicate the adequate sensitivity of the method.

Robustness

The robustness of the developed method was evaluated by deliberately varying the chromatographic conditions. The results showed that slight variations in the flow rate (0.8 ± 0.2 ml/min) have insignificant effect on the chromatographic parameters. The parameters such as % RSD of peak area (<2), tailing factor (T<2) and theoretical plates (N>2000) showed adherence to the acceptance criteria.This signify the robustness of the developed method.

Analysis of commercial formulation

The newly developed and validated RP-HPLC method was used for the determination of curcumin content in commercial turmeric herbal products (Haridra). The chromatogram of the curcumin in the turmeric product is shown in the Figure 1B. The peak areas of the triplicate samples were analyzed by linear regression equation obtained from the calibraton curve to determine the curcumin content. There was no interference with other formulation additives present in the commercial samples at the retention time of curcumin (**Figure 1B**). The content of curcumin in the commercial tumeric product (Haridra capsule) was found to be 2.5 % w/w, which is in accordance with the literature (**Table 3**) (Haridra, 2017).

Conclusion

A simple, fast, accurate and precise RP-HPLC method has been developed and validated for the determination of curcumin in standard and commercial turmeric herbal formulations. The developed method showed excellent precision, accuracy and linearity with shorter retention time for curcumin. The low LOD values indicate the excellent sensitivity of the method. The shorter retention time (9.16 min) and reduced run time (12 min) will allow rapid quantification of many samples in the routine and quality control analysis. The low flow rate (0.8ml/min) used in the present method compared to other methods will save solvent and thus reduce the cost. The developed HPLC method provides good resolution of curcumin from other exciepients and can be used for quantification of curcumin present in the turmeric herbal products and commercially available curcumin. The developed method has clear base line seperation, excellent peak shape and absence of peak tailing for all the curcuminoids. Apart from the analysis of curcumin content alone the potential of the developed method for the simultaneous determination of all the curcuminoids present in different turmeric herbal products needs to be explored.

Acknowledgements

The authors appreciatively acknowledge Green Chem Herbal Extracts and Formulation, India for providing the gift samples of standard curcuminoids mixture and Dr. Prabhakar Kore Basic Science Research Centre, KLE University, Belagavi, India for providing the necessary facilities to carry out the research work.

References

Ahmed, T., Gilani, A., 2014. Therapeutic Potential of Turmeric in Alzheimer â€™s Disease : Curcumin or Curcuminoids ? Phytother. Res. 525, 517–525.

Amalraj A, Pius A, Gopi S, Gopi S., 2016. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives - A review. *J Tradit Complement Med*.;7(2):205-233. doi:10.1016/j.jtcme.2016.05.005

Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB., 2007. Bioavailability of curcumin: problems and promises. Mol Pharm.;4(6):807-18. DOI: 10.1021/mp700113r

Anand, P., Thomas, S.G., Kunnumakkara, A.B., Sundaram, C., Harikumar, K.B., Sung, B., Tharakan, S.T., Misra, K., Priyadarsini, I.K., Rajasekharan, K.N., Aggarwal, B.B., 2008. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem. Pharmacol. 76, 1590–1611. https://doi.org/10.1016/j.bcp.2008.08.008

ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, 2005, 1-13.

Chattopadhyay, Ishita ; Biswas, Kaushik ; Bandyopadhyay, Uday ; Banerjee, Ranajit K (2004) *Turmeric and curcumin: biological actions and medicinal applications* Current science, 87 (1). pp. 44-53. ISSN 0011-3891

Salem, M., Rohani, S., Gillies, E.R., 2014. Curcumin, a promising anti-cancer therapeutic: a review of its chemical properties, bioactivity and approaches to cancer cell delivery. RSC Adv. 4, 10815-10829. doi:10.1039/c3ra46396f.

Wichitnithad, W., Jongaroonngamsang, N., Pummangura, S., Rojsitthisak, P., 2009. A simple isocratic HPLC method for the simultaneous determination of curcuminoids in commercial turmeric extracts. Phytochem. Anal. 20, 314–319. https://www.himalayastore.com/pureherbs/haridra.htm.(accessed 14 November 2014