Annals of Phytomedicine 12(1): 601-605, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.70

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Analysis of phytoconstituents and its phytoformulation of curcumin chewable tablet as per ICH guidelines

R. Raghavi and M. Vijey Aanandhi and M. Sumithra◆

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India

Article Info

Article history
Received 6 April 2023
Revised 24 May 2023
Accepted 25 May 2023
Published Online 30 June-2023

Keywords
Curcuminoids
HPLC analysis
Chewable tablet
ICH Q2 R1
Validation

Abstract

A simple, selective, precise RP-HPLC method has been developed for the determination of curcumin in pharmaceutical formulation. Curcumin was analyzed by HPLC using C18 analytical column (250 mm x 4.6 mm, 5 µm) with UV detection at 420 nm. The mobile phase consisted of a mixture of 60 volumes of tetrahydrofuran and 40 volumes of citric acid. The chromatography was performed by isocratic elution at a flow rate of 1.0 ml/min. The detector response for the curcumin was linear over the selected concentration range from 1 to 10 µg/ml, with a correlation coefficient of 0.9999. The accuracy was between 98.0% to 102.0%. The precision (R.S.D) among the six sample preparations was 0.9%. The recovery of curcumin was about 100.92%. The method was validated according to International Conference of Harmonization Guidelines in terms of accuracy, precision, linearity, specificity, robustness, ruggedness, and solution stability method. The method development of curcumin was applied successfully for HPLC analysis of pharmaceutical dosage form.

1. Introduction

Curcuminoids are a partially purified natural complex of diarylheptanoid derivatives isolated from turmeric. The main source of the phenolic chemical curcumin is *curcuma longa* L., a member of the Zingiberaceae family (Kislay *et al.,* 2018). It contains more than 130 species of *Curcuma* worldwide. Most of them have common local names and are highly used because of their various medicinal properties (Sivakumar *et al*., 2022). Whole turmeric or the extracted curcuminoids appear to be active in many disease processes with specific reference to chronic ailments such as cardio-vascular, degenerative, infective, carcinogenesis, gastrointestinal effects, wound healing and also proved as hepatoprotective, anticoagulant, antifertility, antifungal, antibacterial, antitumor, antiparasitic, antispasmodic, antiinflammatory and inhibits cancer growth (Deepika *et al.,* 2014). Turmeric is typically used in smaller doses for therapeutic purposes, as contrast to being consumed in high amounts (up to about 1.5 g of curcumin per person per day) in some Southeast Asian countries (Aggarwal *et al.,* 2007). Furthermore, turmeric is widely used as a coloring agent and spice in many food items (Jayaprakasha *et al.,* 2005). The rhizome contains a yellow pigment, which is composed of curcumin (77%), demethoxy-curcumin (17%), and bisdemethoxycurcumin (3%) (Sharma *et al.,* 2020).

Corresponding author: Dr. M. Sumithra
Associate Professor, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India
E-mail: sumithra.sps@velsuniv.ac.in
Tel.: +91-9444788949

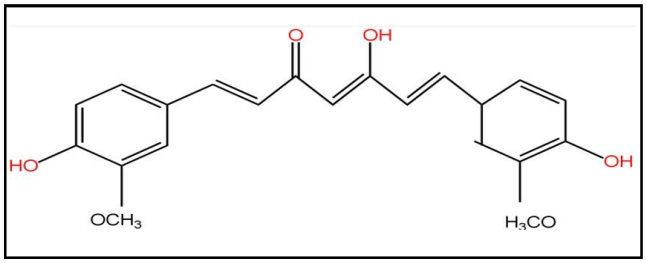

Figure 1: Structure of curcuminoids.

Curcumin ability to neutralize free radicals produced by oxygen is explained by the presence of phenolic groups in its structure. The hydroxyl radical, singlet oxygen, superoxide radical, and nitrogen dioxide are the free radicals that curcumin can neutralize (Rayess *et al.,* 2020). Although, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione is the substance that is typically referred to as "curcumin"(Rasheed *et al.,* 2017). In brief, curcumin is a diferuloylmethane that has the chemical formula $C_{21}H_{20}O_6$, a crystalline yellow-orange colour, a molecular weight of 368.39 g/mol, and a melting point of 183°C. Chemically, it shows keto-enol tautomerism, which means that, neutral and acidic solutions primarily contain the keto form. It is insoluble in water and ether but soluble in various other organic solvents, such as methanol, ethanol, glacial acetic acid, dimethylsulfoxide-acetone, and acetone, among others (Ganesh *et al.,* 2022). Curcumin has a low oral bioavailability in the body because of its poor absorption, rapid metabolism, and high rate of excretion. The therapeutic effects of curcumin have been significantly limited by its lower bioavailability (Devassy *et al.,* 2015). Chewable tablets are required to be broken and chewed in between the teeth before ingestion. These tablets are given to the children who have difficulty in swallowing and to the adults who dislike swallowing (Renu *et al.,* 2015). Patients who

need easy-to-swallow dosage forms, like chewable tablets, the most include geriatric and pediatric patients, as well as patients travelling who might not have ready access to water (Ray *et al.,* 2013*)*. The gum core of a chewable tablet, which may or may not be coated, makes up its composition. These are intended to be chewed in the mouth prior to swallowing and are not intended to be swallowed intact. Additionally, chewable tablets facilitate more rapid release, and hence more rapid absorption of active ingredients and provide quick onset of action. Hence, it was decided to formulate curcumin chewable tablet to improve the compliance in children. However, no analytical method was found for the determination of curcumin chewable tablets. Thus, the aim of this paper was to develop and validate an efficient, precise, accuracy, and robust HPLC-UV method for pharmaceutical formulation of curcumin chewable tablets.

2. Materials and Methods

2.1 Chemicals and reagents

Curcumin was obtained from Wacker Chemical Corporation (USA) Adrian Mich. Tetrahydrofuran used was of HPLC grade and was purchased from SRL PVT, LTD. Citric acid used was of HPLC grade from Thermo Fisher Scientific India. Methanol used was of HPLC grade and was purchased from Advent chembio PVT. LTD. Syringe filter (Rankem (0.45 λm)) and nylon filter (Thermo fisher QNN9797919) are used. The column used as hypertsil BDS C18 (250 mm × 4.6 mm, 5 µm) for the study.

2.2 Instruments and apparatus required

HPLC with UV detector Agilent Technologies (water 2695) by using the column hypertsil BDS C18 (250 mm × 4.6 mm, 5 µm) or equipment chromatograms were recorded using the chem station version B.04.03 Software Technologies installed on a personal computer. Balance-Mettler XS 205 and sonicator-chem labs/CHEM54.

2.3 Instrumentation and chromatographic conditions

HPLC analysis was carried out on hypertsil BDS C18 (250 mm x 4.6 mm, 5 µm), reversed phase column. The mobile phase consisting of citric acid: tetrahydrofuran 40:60 v/v. The flow rate of mobile phase was 1.0 ml/min. The detection was carried out by at 420 nm. All analysis was carried out at a temperature of 20°C under isocratic condition.

2.4 Preparation of mobile phase ml

Prepare a mixture of citric acid solution and tetrahydrofuran in the ratio of 1200:800 (% v/v), mix well and degas.

2.5 Preparation of standard

Accurately weigh about 130.21 mg of curcumin working standard and transfer it into a 50 ml volumetric flask, add 20 ml of diluent, sonicate to dissolve the contents and dilute to volume with diluent; mix well. Pipette out 5 ml of this solution into a 50 ml volumetric flask, dilute to volume with diluent and shake it well (40 µg/ml).

2.6 Preparation of sample

Weigh 20 tablets and calculate the average weight. Crush the tablets into fine powder by using mortar and pestle. Accurately weigh and transfer the tablet powder equipment to 228.71 mg of curcumin into a 50 ml volumetric flask add 20 ml of diluent and sonicate for 30 minu. Then dilute to volume up to the mark with diluent. Centrifuged the sample at 3000 rpm for 5 min. Pipetted out 5 ml centrifuged sample into a 50 ml volumetric flask and dilute to volume with diluent. Inject into HPLC system (40 µg/ml).

3. Results

3.1 Method validation

The analytical method was optimized and validated in accordance with the current ICH Q2 R1 guidelines and to accomplish the vision of specificity, accuracy, linearity, precision, robustness, filter validation, solution stability.

3.1.1 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.,

For the evaluation of specificity; blank solution, placebo solution, sample solution, standard solutions. No interference was observed from blank solution and placebo at the retention time of chromatographic peak of curcumin and internal standard. The typical chromatograms of various samples under optimized HPLC conditions was depicted Table 1.

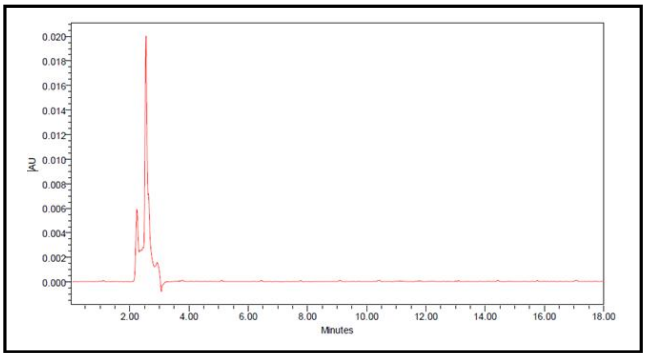

Figure 2: Chromatogram of blank.

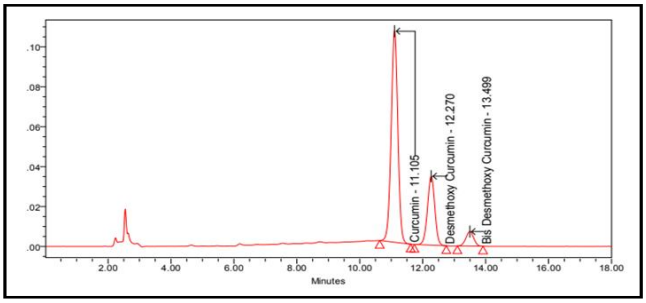

Figure 3: Chromatogram of sample.

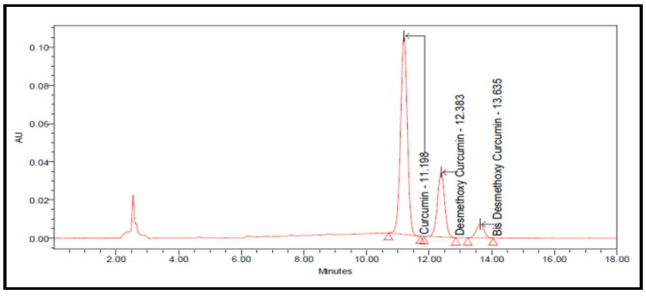

Figure 4: Chromatogram of standard.

Table 1: Specificity studies for curcumin

Peaks	Name of the phytoconstiutents	Retention time in min	Area
01	Curcumin	11.198	1553357
02	Demethoxy	12.383	551221
03	Bisdemethoxy	13.635	125147

3.1.2 Accuracy

Accuracy of the analytical method was evaluated at a known concentration of curcumin at about 50%, 100% and 150% of test concentration of sample solution. % accuracy at individual level and overall average of % recovery at all level for curcumin found to be in the range 98% to 102 % and % RSD for % assay of all levels found to be 0.9% as tabulated in Table 2.

Table 2: Accuracy studies for curcumin

Accuracy levels	Performed triplicate	% Recovery	Average % recovery (n=3)	% RSD (n=3)
50 %	Sample-1	101.77	101.36	0.3
	Sample-2	100.99		
	Sample-3	101.31		
100 %	Sample-1	100.67	100.92	0.8
	Sample-2	100.21		
	Sample-3	101.87		
150 %	Sample-1	101.46	101.24	0.5
	Sample-2	100.73		
	Sample-3	101.54		

3.1.3 Linearity

The analyte response was linear (r^2=0.9999) over the concentration range of 2-6 µg/ml of curcumin. The results were shown in table and figure shows the calibration curve. The curve shows the selected concentration gives acceptable accuracy and precision over a wide concentration range. The results demonstrate that an excellent correlation coefficient between the absorbance and concentration of curcumin drug substance. The correlation coefficient, intercept, and slope were calculated.

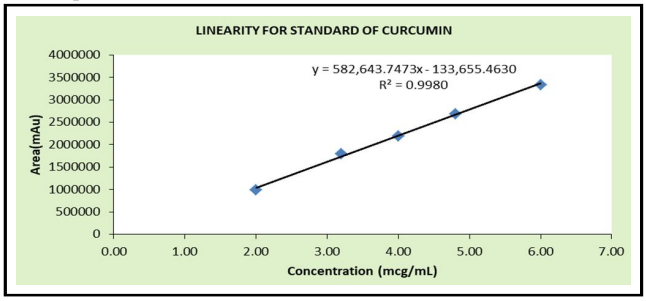

Figure 5: Curcumin linearity.

3.1.4 Method precision

For the evaluation of method precision of the analytical method, six samples from homogenous mixture of single batch were prepared as per the test procedure of methodology and analyzed on HPLC system. % RSD for % assay of curcumin of six samples found to be 0.9% as tabulated in Table 3.

Table 3: Method precision studies for curcumin

No. of sample	%Assay(curcumin)
1	102.37
2	103.41
3	103.99
4	102.06
5	101.60
6	102.61
Mean	102.68
SD	0.680
% RSD	0.9%

3.1.4.1 System precision

Six replicates injections of standard solution were injected in to the HPLC system and the chromatograms and area ratio of curcumin recorded. For curcumin theoretical plates and tailing factor found to be 12980 and 1.0 respectively. % RSD for area ratio of curcumin of six replicate injections of standard solution found to be 0.15% implies that system is précises as tabulated in Table 4.

Table 4: System precision studies for curcumin

Name of the parameter	curcumin
Retention time (RT)	11.198
Area (% RSD NMT2.0)	0.9%
Tailing factor (NMT 1.8)	1.1
Theoretical plates (NLT 2000)	13072

3.1.4.2 Method ruggedness

The ruggedness was evaluated through analysis of six samples from a homogenous mixture of single batch by different analyst by using different column, different system and on different day. %RSD for % assay of ruggedness samples found to be 0.8% for curcumin as tabulated in Table 5.

Table 5: Method ruggedness studies for curcumin

No. of sample	%Assay(curcumin)
1	97.00
2	98.00
3	98.00
4	96.00
5	96.00
6	97.00
Mean	97.00
SD	0.550
% RSD	0.8%

3.1.5 Robustness

The effect of small but deliberate variations in method parameters such as flow rate, composition of the mobile phase, and wavelength was evaluated in this study. 2-6 µg/ml concentration of curcumin was used in six replicates to study robustness of the method. The standard deviation of peak areas and % relative standard deviation (RSD) was determined. The % RSD of peak areas was calculated for each parameter and was found to be <2%.

Table 6: Robustness studies for curcumin

Parameter	Parameter varied	% RSD
Mobile phase	640:360 600:400 560:440	0.8%
Flow rate in ml/min	0.8 ml/min 1.0 ml/min 1.2 ml/min	0.9%
Wavelength in nm	418 nm 420 nm 422 nm	0.9%
Column Temperature	35ºC 40ºC 45ºC	0.8%

3.1.6 Filter validation

The results and % difference between centrifuged samples, 0.45 µm PVDF filters and 0.45 µm nylon filter samples were found to be within the acceptance criteria.

Table 7: Filter validation studies for curcumin

Filter details	% Results	% Difference
Centrifuged (unfiltered)	98.00	-
0.45 µ nylon Filter 1 ml discarded	98.00	0
0.45 µ nylon Filter 2 ml discarded	96.00	-2.0
0.45 µ nylon Filter 4 ml discarded	96.00	-2.0

3.1.7 Solution stability of analytical solutions

Solution stability standard and sample solutions was established at various conditions such as bench top at room temperature and in refrigerator 2-8ºC. The stability of standard and sample solutions was determined by comparison of initial prepared and sample solutions with freshly prepared standard solutions. The data obtained was given in Table 8.

Table 8: Solution stability studies for curcumin

Time interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA

Results for solution stability of standard at room temperature.

Table 9: Solution stability studies for standard

Time point	% Recovery refrigerator
0 h	NA
6 h	100.4
20 h	99.9
24 h	99.7
36 h	100.6
48 h	99.4

Results for solution stability of sample at room temperature.

Table 10: Solution stability studies for sample

Time point	% Recovery refrigerator
0 h	NA
6 h	100.7
20 h	99.6
24 h	99.7
36 h	100.9
48 h	99.4

4. Discussion

A simple HPLC method was developed and applied in the analysis of curcumin chewable tablets. The method was fully validated, and the assay parameters are presented in the following section. The specificity of the method is indicated from chromatograms obtained in the analysis of standard, sample, and placebo solutions (Figures 1, 2 and 3). There was no interfering peak with the same retention time as that of main curcumin peak. Moreover, peak purity data (not shown) proves the specificity of the proposed method. The proposed method is linear, according to the results obtained by means of the calibration curve in the concentration range from 20 ìg/ml to 60 ìg/ml. The results obtained in the curcumin analysis presented a linear correlation between the injected concentration and its peak area with a correlation coefficient (r^2) greater than 0.9980 and a line equation of y =582,643.7473x + 133,655,4630. The RSD values obtained in intra- and inter-day analysis of curcumin, by proposed method, were below 2%. This indicates an excellent precision of the proposed method (Tables 3, 4 and 5).The average recovery of standard from sample matrix was between 98.0% to 102.0%, which indicates acceptable accuracy of the proposed method. Moreover, the standard variation among the obtained results was below 2% (Table 2). The results obtained in the evaluation of robustness (Table 6) showed that a small variation in the composition of the mobile phase, its pH, flow rate, column temperature, and C18 column packing has an insignificant impact on the curcumin chromatograms. The filter validation studies is the critical part of QC tests samples were filtered by using syringe filters and filtrate were collected and analyzed by HPLC for the quantification of API. Centrifuged samples were used as controls for recovery of 100% and to calculate the analyte binding in a syringe filter. The solution stability of standard and samples are stable upto 24 h on bench top and refrigerator (2-8°C). However, no analytical techniques for curcumin chewable tablet. In this study, we developed a new formulation of curcumin combining an orally

disintegrating system as a chewable tablet to enhance the dissolution rate of curcumin. The developed methods were validated as per ICH guidelines which specific, simple and reliable which successfully applied for the quantification of curcumin without any excipient interference.

5. Conclusion

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The results obtained were within the acceptance criteria. So, it can be concluded that the developed method is simple, precise, cost-effective, eco-friendly, safe and can be successfully employed for the routine analysis of curcumin in bulk and pharmaceutical dosage forms.

Acknowledgements

The authors thank the management and staffs, Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS). Micro Therapeutic Research Labs (MTRL) Pvt Ltd, Chennai for providing us the raw material to carry out this work,

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Aggarwal, B.B.; Surh, Y.J and Shishodia, S. (2007). The molecular targets and therapeutic uses of curcumin in health and disease. AEMB., **595**.https://doi.org/10.1007/978-0-387-46401-5-20.

Deepika; Kuldeep Singh Yadav; Preeti Maan; Sanjana Bhatia and Narayan Prasad Yadav (2014). Exploration of anti-inflammatory activity of turmeric and onion combination on phorbol ester induced ear inflammation in mice. Ann. Phytomed., **3**(2):50-54.

Devassy, J.G; Nwachukwu, I.D and Jones, P.J.(2015). Curcumin and cancer: Barriers to obtaining a health claim. Nutr. Rev., **73**(3):155-165.

Ganesh Bharskar; Someshwar Mankar and Suhas Siddheshwar (2022). Analytical methods for estimation of curcumin in bulk, pharmaceutical formulation and in biological samples. Asian J. Pharm. Anal., **12**(2):142-8. doi: 10.52711/2231- 5675.2022.00025.

Jayaprakasha Jagan; Mohan; Rao, L and Sakariah, K.K. (2005). Chemistry and biological activities of *C.longa*. Trends Food Sci Technol., **16**:533-548. doi: 10.1016/j.tifs.2005.08.006.

Kislay Baranwal; Hina Khan and Himanshu Kashudhan (2018). Development and evaluation of nano-encapsulated curcumin chewable tablets. IJARIIT., pp:866-872.

Rasheed, N.M.A.; Sowmya Srividya, G and Nagaiah.K. (2017). HPTLC method development and quantification of curcumin content in different extracts of rhizomes of *Curcuma longa* L. Ann. Phytomed., **6**(2):74-81. DOI: 10.21276/ap.2017.6.2.6

Ray,C.; Arora,V and Sharma,V. (2013). Fast dissolving tablets-A novel drug delivery system for pediatric and geriatric patient. Int. J. Drug Dev. Res., **1**(2):55-70.

Renu; Jyoti Dahiya and Pawan Jalwal (2015). Chewable tablets: A comprehensive review. J. Pharm. Innov., **4**(5):100-105.

Sharifi-Rad,J.; Rayess, Y.E and Rizk, A.A. (2020). Turmeric and its major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. Front Pharmacol., **15**(11):01021. doi: 10.3389/fphar.2020.01021.

Sharma, K.S and Sahoo,J. (2020). Chromatographic determination of curcumin in the presence of its degradation products by HPLC. Int. J. Pharm. Sci. and Res., **11**(5):2342-49. doi: 10.13040/IJPSR. 0975-8232.z**11**(5).2342-49.

Sivakumar, P.; Monisha, S.; Vijai Selvaraj, K.S.; Chitra, M.; Prabha, T.; Santhakumar, M.; Bharathi, A and Velayutham, A. (2022). Nutritional value, phytochemistry, pharmacological and *in vitro* regeneration of turmeric (*Curcuma longa* L.): An updated review. Ann. Phytomed., **11**(1):236-246. DOI: http://dx.doi.org/10.54085/ap.2022.11.1.23.

Citation **R. Raghavi and M. Vijey Aanandhi and M. Sumithra (2023). Analysis of phytoconstituents and its phytoformulation of curcumin chewable tablet as per ICH guidelines. Ann. Phytomed., 12(1):601-605. http://dx.doi.org/10.54085/ap.2023.12.1.70.**