Medicon Pharmaceutical Sciences
Volume 2 Issue 2 February 2022
Research Article

Stability-Indicating Assay Method for Estimation of Curcumin and its Degradants by RP-UHPLC Method

Vicky Anthony[1]*, Ritika Sahu[1], SK Jain[2], RamSingh Bishnoi[1] and Deepti Jain[1]

[1]School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Airport Road, Bhopal, MP, India

[2]Sagar Institute of Research and Technology- Pharmacy Opp. Sagar Estate, Ayodhya By Pass Road, Bhopal, MP, India

***Corresponding Author:** Vicky Anthony, School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Airport Road, Bhopal, MP, India.

Received: January 31, 2022; **Published:** February 05, 2022

Abstract

In this study stability-indicating assay method was developed and validated forestimation of curcumin and its degradation products as per ICH guidelines using RP-UHPLC.

For this the curcumin was subjected to forced degradation study under various stress conditions of hydrolysis (acidic, neutral and alkaline), oxidation, and thermal and photolytic as recommended by ICH Q1A (R2) guideline. A chromatographic method was developed for the estimation of curcumin in presence of its degradants. The chromatographic separation was obtained with Hypersil GOLD, C-18 reverse phase column (150mm×4.6mm×3µ) at flow rate 1 mL/min with mobile phase (Acetonitrile: Water:70:30). The pH of mobile phase was adjustedto 3.0 using formic acid. The peak was observed at 2.44 minutes at λ_{max} 425nmusingdiode array detector.

The method has been validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. The linearity obtained in the range of 2-10µg/ml. LOD was found to be 50ng/ml. Further it was concluded that the curcumin is highly susceptible to oxidation, alkaline and neutral hydrolysis, slightly prone to thermal and photolysis, whereas stable towards acidic hydrolysis.

Keywords: RP-UHPLC method; Stability Indicating Assay Method; Forced Degradation study; Determination of Curcumin; Stability of Curcumin; Analytical method development

Introduction

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione (Fig.1) is a yellow-coloured phenolic pigment which is obtained from the powdered rhizome of Curcumalonga Linn. (Family: Zingiberaceae) [1] Research over the last two decades has shown that curcumin is a potent antioxidant, anti-inflammatory, anti-proliferative, anti-metastatic, anti-angiogenic, anti-diabetic, hepatoprotective, anti-atherosclerotic, anti-thrombotic, and anti-arthritic agent [2]. It is a bis-α, β-unsaturated β-diketone. Curcumin exhibits both in keto-enol tautomeric forms. The keto form predominates in acidic and neutral aqueous solutions and it is shown that the same is stable in the cell membrane as compared to stability in blood [3].

The stability-indicating assay method is employed for the analysis of the stability of drug samples in the pharmaceutical industry. With the advent of International Conference on Harmonization (ICH) guidelines, there is a requirement for the establishment of stability-indicating assay method (SIAM) of drugs and formulations. The ICH Q1A (R2) describes various stress conditions to conduct the forced degradation study of drug substances such as oxidation, hydrolysis (alkaline, neutral, and acidic) photolysis and thermal to access the stability of drug products [4]. Literature study reveals that theliquid chromatographic (LC) method using spectrophotomet-

ric detection was designed to isolate and measure curcuminoids [5]. Gas chromatography (GC) was also applied for the quantification of the Curcumin [6]. Capillary electrophoresis was utilized for measurement of curcuminoids using photodiode array [7]. Methods for measurement in plasma using liquid-liquid extraction had also been developed with high-performance liquid chromatography (HPLC) with UV detection at 430 nm [8-9].

The current study aims at conducting the rapid, precise and accurate Degradation study of the curcumin and development of validated stability-indicating assay method for determination of curcumin and its degradants by RP-UHPLC method [10-11].

Experimental

Materials and Reagents

The standard drug Curcumin was purchased from Sigma Aldrich. HPLC grade Acetonitrile was obtained from Rankem, other chemicals such asformic acid, hydrochloric acid, sodium hydroxide and hydrogen peroxide were of analytical grade obtained from CDH chemical limited. The triple distilled water was obtained by Millipore (Milli-Q). The 0.45µ membrane filter was obtained from Rankem.

Instrumentation

The instrument used for all the analytical development purpose was Thermo Scientific Dionex UltiMate® 3000 UHPLC system with Hypersil GOLD, C-18 reverse phase column (150mm, 4.6mm,3µ). The UHPLC system having Integrated Vacuum degasser solvent rack SRD-3600. Dionex HPG-3400RSpump, WPS-3000RTSsplit-loop (size 20µl) sampler,2.5µl SSTflow cell and DAD-3000RSdetector. The system was operated using Chromeleon® chromatography data system (CDS) software version 6.0 SR7.

Calibration curve of Curcumin

The standard calibration curve of curcumin was plotted the aliquots prepared in the linearity range of 2-10µg/ml using acetonitrile as the solvent.

Sample Preparation

100mg of curcumin was dissolved in 100 ml of ACN to get 1000µg/ml stock solution then sub-stock of 100µg/ml prepared, from the above solution, concentration of 2,4,6,8,10 µg/ml of dilutions were prepared.

Chromatography

A chromatographic method was developed for the estimation of curcumin in presence of its degradants. The chromatographic separation was obtained with Hypersil GOLD, C-18 reverse phase column (150mm×4.6mm×3µ) at flow rate 1 mL/min with mobile phase (Acetonitrile: Water:70:30). The pH of mobile phase was adjusted to 3.0 using formic acid. The peak was observed at 2.44 minutes atλ_{max} 425nm using Diode array detector.

All the solvents used were filtered through nylon-66, 0.45µm membrane filter and degassed before using for the analysis. The sample (volume 20µl) was injected for drug and all stressed samples, peak of the standard curcumin was obtained at 2.447 min at a flow rate of 1ml/min.

Chromatographic method development

Chromatographic method development was carried out to optimize the system suitability parameters. A standard drug solution of 10µg/ml was injected to check the column performance by analysing various parameters like theoretical plates, height equivalent to theoretical plates (HETP), tailing factor and retention time. For the preparation of the calibration curve, dilutions in range of 2-10µg/ml was analysed using the same mobile phase The robustness of the method was determined by injecting the dilutions 2-10µg/ml at variation of ± 2% mobile phase composition,± 0.1 pH and ± 0.1 ml flow rate.

Forced Degradation studies

The forced degradation study of curcumin was carried out at alkaline, neutral and acidic hydrolysis, oxidation, thermal degradation and photo-degradation. The initial concentration used for degradation was 20µg/ml.

Acid/alkali hydrolysis

Curcumin was subjected to acidic and basic hydrolysis with 50 ml 0.1N HCl and 0.1N NaOH respectively at room temperature for 24 hrs and repeated at elevated temperature by refluxing at 80°C for 8hrs, 1 ml of sample was withdrawn allowed to cool and neutralized with an equal volume of 0.1N NaOH for acidic hydrolysis and with an equal amount HCl for basic hydrolysis. Neutralized sample diluted to 10µg/ml with acetonitrile. The sample was filtered with 0.45µ and injected for UHPLC analysis.

Neutral hydrolysis

Neutral hydrolysis was performed with 50ml millipore Water at room temperature for 24 hrs and repeated at elevated temperature by refluxing at 80°C for 8hrs, 1 ml of sample was withdrawn, allowed to cool and diluted with acetonitrile to produce 10µg/ml of sample The sample was filtered with 0.45µ and injected for UHPLC analysis.

Oxidation

Oxidative degradation of curcumin was performed with 3% of H_2O_2 at room temperature for 24 hrs and repeated at elevated temperature by refluxing for 8hrs at 80°C,1 ml of sample was withdrawn, allowed to cool and diluted with acetonitrile to produce 10µg/ml of sample The sample was filtered with 0.45µ and injected for UHPLC analysis.

Photo-degradation

100 mg of the powdered drug was exposed to direct sunlight for 7 days and same amount was kept in a UV chamber for about 24 hrs. The sample of 10µg/ml was withdrawn at intervals and prepared with acetonitrile, filtered with 0.45µ and injected for UHPLC analysis.

Thermal degradation

The powdered drug was exposed to dry heat at 60°C for 48 hrs. The sample of 10µg/ml was prepared and withdrawn at intervals then filtered with 0.45µ and injected for UHPLC analysis.

Results and Discussion

The mobile phase was optimized by examining the effect of pH, ratio of mobile phase acetonitrile and water (80:20; 75:25; 70:30; 65:35; 60:40; 55:45; 50:50). During optimization pH 3.0 with a flow rate of 1.0ml/min and the ratio of 70:30 (acetonitrile:water) was considered as the best mobile phase for the detection and separation of curcumins with its degradants. Various system suitability parameters were analysed such as theoretical plates, height equivalent to theoretical plates (HETP), tailing factor, retention time and resolution were analysed. Peak area and concentration were subjected to linear least-squares regression analysis to calculate the calibration equation and the correlation coefficient of the analysis. The curcumin showed good linearity between the range of 2-10µg/ml. The linearity was confirmed by the correlation coefficient which was found to be 0.991.

For Neutral Hydrolysis

The drug was degraded completely in about 12 hours with water at room temperature and on refluxing at elevated temperature it gets degraded about 95.63 % in2 hours and formed three degradants which observed at 1.903min, 2.067min and 4.337min respectively.

For Oxidative Hydrolysis

The drug was degraded completely in about 16 hrs with 3% H_2O_2 at room temperature whereas on refluxing it for 2 hrs at 60°C leads

to 79.40% degradation, obtained degradants were observed at 2.230 min and 2.907 min.

For Alkaline Hydrolysis

The drug was degraded 84.25 % in about 8 hours with0.1 N NaOH on refluxing at 60°C temperature it gets degraded, Thus, the drug is susceptible to alkaline pH and degrades rapidly on alkaline hydrolysis.

For Thermal Degradation

The drug is sensitive towards heat, it get degraded about 83.77 % on heating at 60°C for 24 hours forming two degradants were observed at 1.903min and 2.300 min.

For photolytic Degradation

The drug was exposed towards sunlight and UV, it gets degraded about 22.31 % in 24 hours forming one degradant which was observed at 2.073min.

The drug gradually decreases in concentration with alkaline degradation at 60°C at 0.1N NaOH and the degradation product was formed 2.050 min. However, in neural hydrolysis the drug degraded about 95.63% producing three degradants at 1.903min, 2.067min and 4.337min. With oxidation with 3% H2O2 the drug degraded about 79.40% producing degradants at 2.230, 2.907. On heating, the drug at 60°C for 24 hrs the drug degraded about 83.77% producing two degradants at 1.903min, 2.300min. On exposing the drug to UV rays and sunlight the drug remained about 77.69% producing a degradant at 2.073. The degradation in 0.1N HCl (acidic hydrolysis) was negligible, thus the drug was stable in acidic conditions.

For mixture of degradants

Figure shows the UHPLC Chromatogram of a mixture of stressed samples of curcumin at various degradation conditions, depicting the formation of three major degradants at Rt 1.947, 2.780 and 4.137. Figure shows the UV spectra of the mentioned degradants respectively.

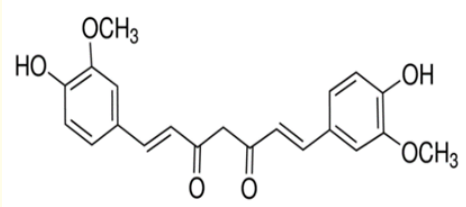

Figure 1: Chemical Structure of Curcumin.

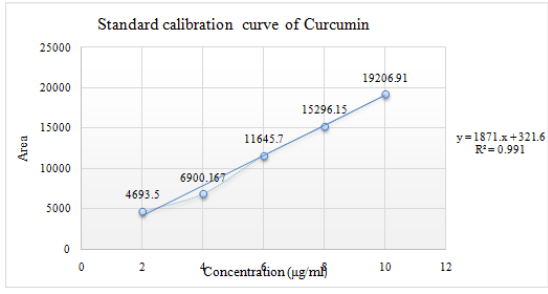

Figure 2: Calibration Curve of Curcumin.

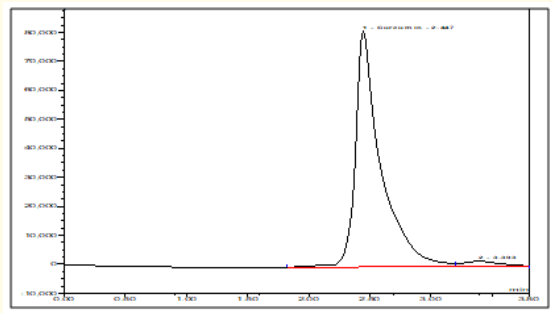

Figure 3: UHPLC Chromatogram of Standard Drug Curcumin.

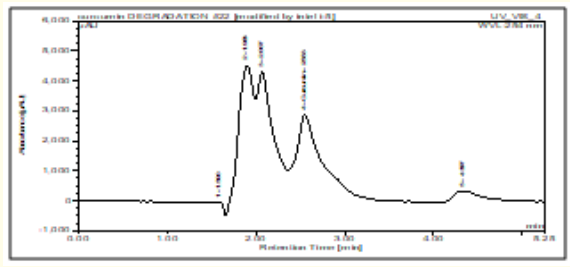

Figure 4: Chromatogram of Curcumin at Rt 2.553 and degradants at Rt (1.159, 1.903, 2.067, 4.337) in Water.

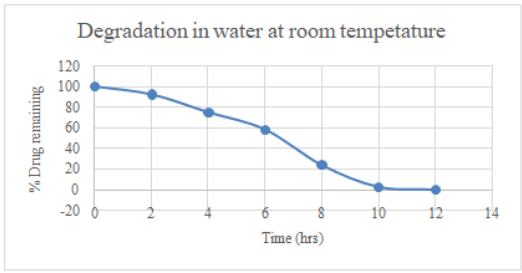

Figure 5: Graph representing degradation of curcumin in water at room temperature.

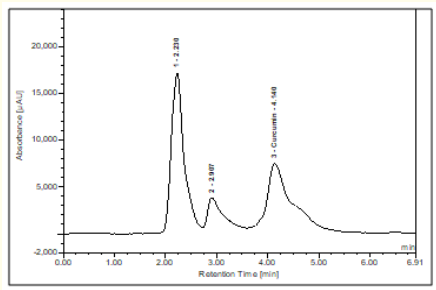

Figure 6: Chromatogram of oxidative Hydrolysis with 3% H_2O_2 at 80°C for 2 hrs.

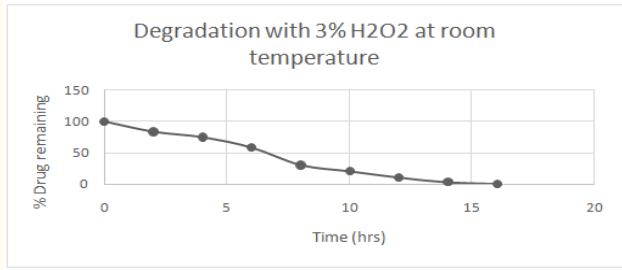

Figure 7: Graph representing degradation of curcumin in 3% H_2O_2 at room temperature.

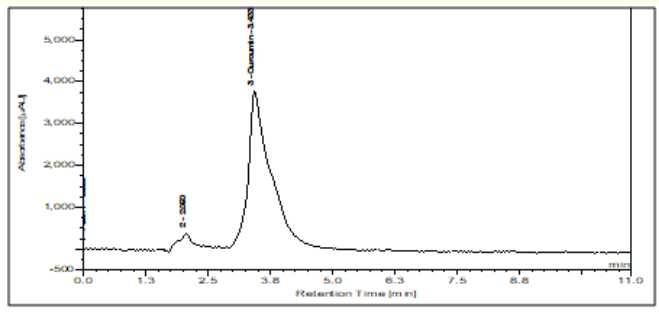

Figure 8: Alkaline Hydrolysis with 0.1 N NaOH at 80°C for 2 hrs.

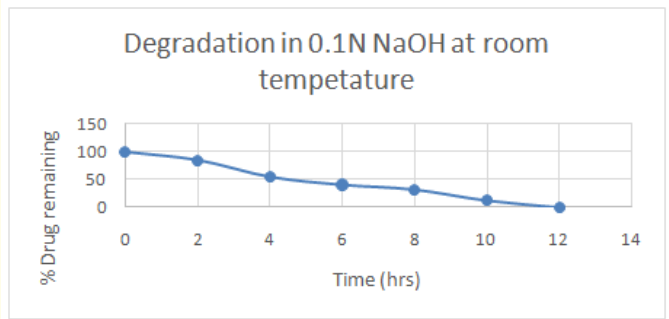

Figure 9: Graph representing degradation of curcumin in 0.1 N NaOH at room temperature.

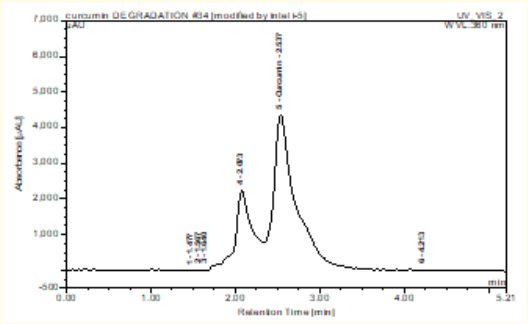

Figure 10: Chromatogram of Curcumin at Rt 2.537 and degradant at Rt (2.073) on exposure to UV-Visiblelight.

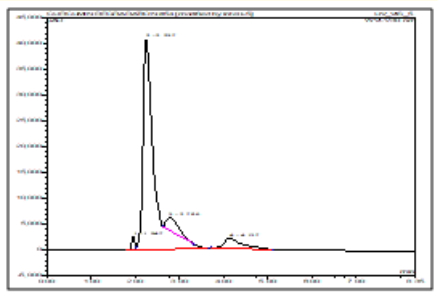

Figure 11: Chromatogram of Mixture of Degradants.

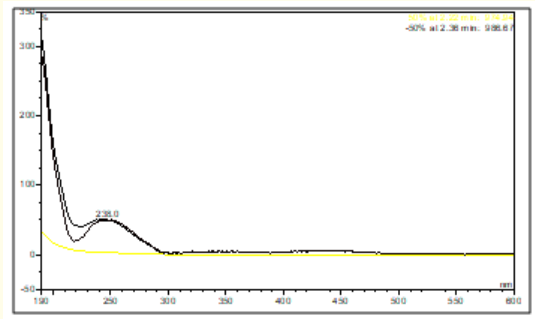

Figure 12: UV Spectra of 1st Degradant.

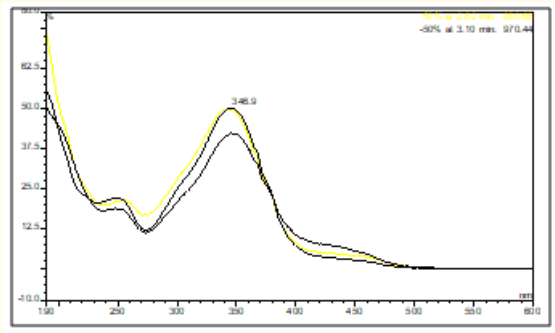

Figure 13: UV Spectra of 2nd Degradant.

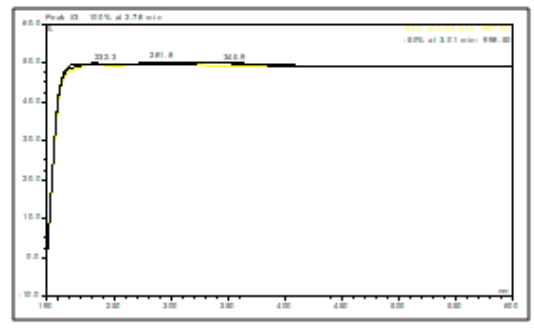

Figure 14: UV Spectra of 3rd Degradant.

S.No	*Concentration (µg/ml)*	*Retention Time (min)*	*Area ± SD*	*Theoretical Plates*
1	2.0	2.430	4693.5 ± 44.96	8526
2	4.0	2.428	6900.2 ±11.418	8526
3	6.0	2.427	11646 ± 58.990	8526
4	8.0	2.430	15296 ± 158.85	8526
5	10.0	2.440	19207 ± 417.25	8526

Table 1: AUC of Different Allequates of Standard Curcumin.

Parameters	***Values ± SD, % RSD***
System suitability	
Retention time	2.44 m
No. of Theoretical Plates	8526
Tailing Factor	1.055
HETP (mm)	0.017
Linearity	2-10 µg/ml
Correlation coefficient	0.991
Precision	
Inter-day	99.72 ± 0.266, 0.267
Intra-day	99.72 ± 0.329, 0.330
Accuracy	99.35 ± 0.854, 0.859
LOQ	200 ng/ml ± 2.08, 1.56
LOD	10 ng/ml ± 2.24, 1.52
Robustness	
Mobile phase composition (±2%)	98.04 ± 1.001, 1.001
Specificity	determined by analyzing the standard solution & blank mobile phase

Table 2: Various Parameters for Method Validation.

Conclusion

A new, sensitive and stability-indicating UHPLC method was developed for the estimation of curcumin and its degradants. The simple and easy elution was obtained using isocratic elution which saves time and cost-effective. The developed method was found sensitive, precise and accurate for curcumin in the presence of its degradants. The drug gets degraded rapidly in alkaline and neutral hydrolysis as compared to oxidation, thermal and photolytic degradation, whereas it was stable towards acidic hydrolysis. This stability-indicating method could be effective for the detection and isolation of curcumin and its degradants.

Acknowledgement

The author is thankful to (Head and prof.) School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal and my Co-guide for their guidance and support throughout the study. The author is also grateful to Director of Sagar Institute of Research & Technology- Pharmacy, Bhopal for providing facilities to carry out the research work.

References

1. Cooper TH., et al. Food Phytochemicals II: Teas, Spices, and Herbs. ACS, Washington, DC, USA (1994).
2. Anand P., et al. "Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature". Biochemical pharmacology 76.11 (2008): 1590-611.
3. Jovanovic SV., et al. "H-atom transfer is a preferred antioxidant mechanism of curcumin". Journal of the American Chemical Society 121.41 (1999): 9677-81.
4. ICH, Stability Testing of New Drug Substances and Products Q1A (R2), International Conference onHarmonization, IFPMA, Geneva (2003).
5. Srinivasan KR. "A chromatographic study of the curcuminoids in Curcuma longa, L". Journal of Pharmacy and pharmacology 5.7 (1953): 448-57.
6. Sanagi MM, Ahmad UK and Smith RM. "Application of supercritical fluid extraction and chromatography to the analysis of tur-

meric". Journal of chromatographic science 31.1 (1993): 20-5.

7. Lechtenberg M, Quandt B and Nahrstedt A. "Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of Curcuma domestica Val. and Curcuma xanthorrhiza Roxb. by capillary electrophoresis". Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques 15.3 (2004): 152-8.
8. Heath DD., et al. "Curcumin in plasma and urine: quantitation by high-performance liquid chromatography". Journal of Chromatography B 783.1 (2003): 287-95.
9. Basniwal PK., et al. "Stability-indicating HPLC assay method and degradation profile of tamsulosin". American-Eurasian Journal of Scientific Research 7.5 (2012): 193-8.
10. Singh S and Bakshi M. "Stress test to determine inherent stability of drugs". Pharm Technol 4 (2000): 1-4.
11. Wang L., et al. "Characterization of stress degradation products of curcumin and its two derivatives by UPLC–DAD–MS/MS". Arabian Journal of Chemistry (2016).

Volume 2 Issue 2 February 2022