Analytical Method Development and Validation for Estimation of Curcumin Using Rp-Hplc Techniques

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***Abstract*— High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques. More than 85% of pharmaceuticals are analysed by HPLC.It has become mandatory to perform stability studies of a new drug moiety before filing in registration dossier. The stability studies include long term studies (12 months) and accelerated stability studies (25°C/60% RH or 40°C/75% RH, 6 months). But intermediate studies (6 months) can be performed at conditions milder than that used in accelerated studies. HPLC is essentially is a form of column chromatography in which the stationary phase consists of small particles (3-50µm) packing contain in column with small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). Linearity range was 20-120µg/mL. Five replicate sample from the same batch were analysed by proposed method has been shown in the result within acceptance limit indicating reproducibility of the method. The accuracy of the method was ascertained on the basis of recovery study performed by the standard addition method. Stress degradation study was carried out on all the three developed methods as per ICH Q1A (R2) guidelines The mobile phase of HPLC ACN: aqueous buffer pH adjusted to 3.0 with phosphoric acid was selected**

***Index Terms*— HPLC, stability studies, ICH Q1A (R2), CURCUMIN, Stress degradation**

I. INTRODUCTION

The most important technique used for the analytical investigation of raw drug materials, the intermediates, drug formulations, impurities, degradation products, and the biological samples containing the drugs and their metabolites is the separation technique based on High Performance Liquid Chromatography (HPLC). In order to develop a new method for estimation of a drug, the analyst must have an idea of the required sensitivity, accuracy, precision, range of analysis of the method. High Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques. More than 85% of pharmaceuticals are analyzed by HPLC. HPLC is essentially is a form of column chromatography in which the stationary phase consists of small particles (3-50µm) packing contain in column with small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). Chromatography is a process in which separation of solute by an effective differential migration process in a system containing of two or more phases. Essentially, the techniques of chromatography is based on the differences in the rate at which the components of the mixture move through a porous medium (called stationary phase) under the influence of same solvent or gas (called mobile phase).

Validation of analytical methods is required for the following

The method validation implies the process of evaluation to assure that the proposed analytical method provides required analytical result and meets the intended use. Validation of analytical methods is required for the following

- Assuring the quality of drugs
- Obtaining acceptance of products by the international agencies
- Mandatory requirement for accreditation as per ISO 17025 guidelines
- Mandatory requirement for registration of any pharmaceutical product or pesticide formulation.
- Validated methods are only acceptable for undertaking proficiency testing.
- Validated or evaluated methods undergo quality control procedures for further evaluation.

Table 1.: Separation goals for HPLC

Goal	Comment
Resolution	Precise and rugged quantitative analysis requires that the resolution be greater than 1.5
Separation time	< 5-10 min is desirable for routine procedures
Quantization	≤ 2% for assays ≤ 5% for less-demanding analyses ≤ 15% for trace analyses
Pressure	< 150 bar is desirable < 200 bar is usually essential (for a new column)
Peak height	Narrow peaks are desirable with large signal/noise ratio
Solvent consumption	Use of minimum mobile phase per run is desirable

II. FORCED DEGRADATION/ STRESS TESTING STUDY

Forced degradation or stress testing study is the process of subjecting drug compounds (i.e. drug substance, drug products and placebo) to extreme chemical and environmental conditions to determine product breakdown levels and preliminary degradants, ion kinetics, and to identify degradant species.

As compared to stability studies, forced degradation studies help in generating degradants in much shorter span of time, mostly a few weeks. The samples generated from forced degradation can be used to develop the stability indicating method which can be applied latter for the analysis of samples generated from accelerated and long-term stability studies.

Objective of forced degradation study

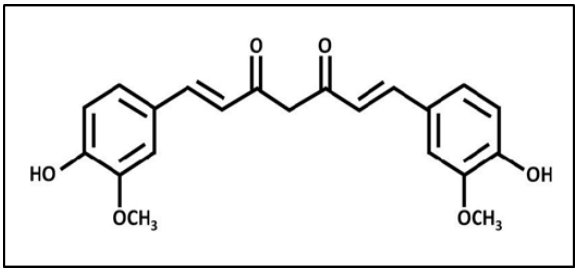
Forced degradation studies are carried out to achieve the following purposes:

1. To establish degradation pathways of drug substances and drug products.
2. To elucidate the structure of degradation products.
3. To determine the intrinsic stability of a drug substance in formulation.
4. To establish stability indicating nature of a developed method.
5. To understand the chemical properties of drug molecules.
6. To generate more stable formulations.

III. DRUG PROFILE

Drug profiles are scientifically sound descriptions of drugs in the form of 'drug profiles'. Presented in a standardized way.

IUPAC Name: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-5-dione



Molecular Formula: $C_1 H_{20}O_6$ Molecular Weight: 368.39 g/mol

Mode of action: Curcumin exerts its effects primarily through: Inhibition of nuclear factor-kappa B (NF-κB) signaling, reducing inflammation. Modulation of various enzymes (like cyclooxygenase-2) and transcription factors. Antioxidant action by scavenging free radicals. Inducing apoptosis in cancer cells by activating caspase pathways.

IV. EXPERIMENTAL WORK AND RESULTS

Chemicals and Reagents

HPLC grade Acetonitrile, Methanol and Milli-Q water was used for dilution of stock solution.

Table no.2: Reagents/ Materials

Sr. No.	Reagents/ Materials	Grade
1	Milli-Q water	HPLC grade
2	Methanol	HPLC grade
3	Acetonitrile	HPLC grade
4	Potassium dihydrogen orthophosphate	HPLC grade

Instrument

i. Agilent- 1100 series HPLC system comprising of Quaternary gradient pumps G1311A with on line Degasser G1322A, Variable wavelength UV detector G1314A, Autosampler G1313A, Zodiac 100 C18 column (250mm x 4.6 mm i.d, 5um).
ii. UV Spectrophotometer (PROLAB)
iii. Digital Balance (RADWAG)
iv. Ultra-sonicator

v. Digital pH meter (Prolab India).
vi. Digital Hot air oven

Preparation of Stock standard solution of Curcumin:
Accurately weighed quantity of 10.0 mg of Curcumin transferred to 10.0 ml volumetric flask. & the volume was made up to 10 ml using methanol.

Working standard solution of Curcumin
Dilute 5 ml of standard stock solution to 10 ml volumetric flask and make up the volume with mobile phase to get a concentration of 500 µg/ml.
Location of λmax Curcumin shows maximum absorbance at 425 nm.

Selection of mobile phase
The mobile phase containing mixture of ACN: aqueous buffer (phosphate buffer pH 3.0) (60:40) was found to be most satisfactory as it gave good resolution and sharp peaks. The detection wavelength selected was 425 nm Initial Chromatographic Conditio

V. OPTIMIZATION OF REPORT

Final Chromatographic Condition
Sample Preparation: 10mg curcumin taken in 10ml volumetric flask and make up the volume with Methanol.

Table3: Final Chromatographic Condition

Column	Zodiac 100 C18 Column		
Mobile phase	ACN: aqueous buffer (phosphate buffer pH 3.0) :(60:40)	Injection volume	10µL
Wavelength	425nm	Temperature	25°C
Flow rate	1ml/min	Run time	15min
Retention Time	9.01	Diluent	ACN:aqueous buffer (phosphate buffer pH 3.0)
pH	3.0		

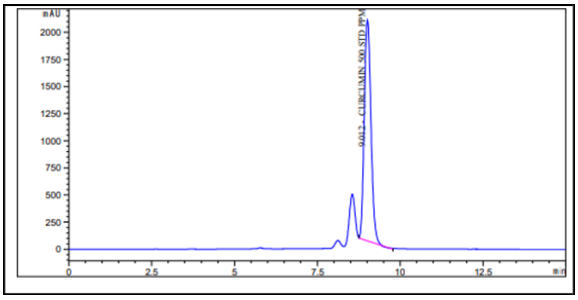


Fig. 1. HPLC Chromatogram of Curcumin

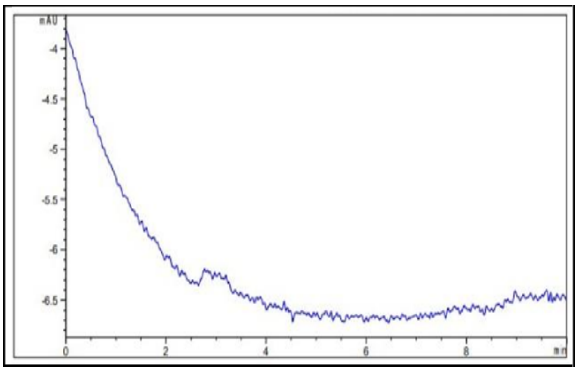


Fig. 2. HPLC Chromatogram of Blank

Validation of proposed method.
According to the guidelines of ICH Q2 (R1) all the parameters as discussed below were analyzed and validated accurately following the procedure of the proposed method.

System suitability test parameters of Curcumin
The system suitability is the pharmacopeial requirement and is used to verify the resolution and reproducibility of the chromatographic system. The test was performed by collecting the data from five replicate injection of standard solution.

Table 4: System suitability test parameters of Curcumin

Sr.No	Retention time	Peak area	Theoretical plates	Tailing factor
1	9.01	28679	9291	0.97
2	9.02	28680	9296	0.95
3	9.02	28676	9299	0.95
4	9.01	28666	9297	0.98
5	9.01	28788	9299	0.97
Statistics				
Mean	9.01	28678	9296	0.96
±SD	0.00	1.414	2.939	0.01
%RSD	0.05	0.005	0.032	1.24

Preparation of Calibration Curves for Curcumin
Each of the standard solution was injected separately. The aliquot portion of stock solution A mobile phase to get concentration of 20, 40, 60, 100, 120 µg/ml for Curcumin. The chromatogram was recorded. The graph is plotted as concentration versus response area (peak area) depicted in Fig. 6.5

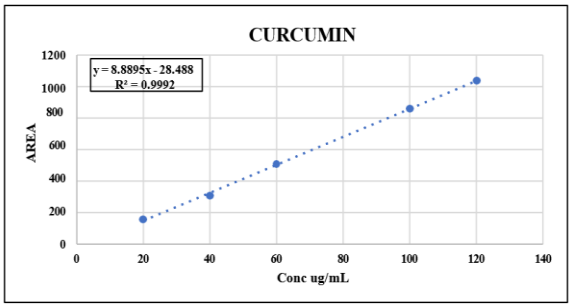


Fig. 3: Calibration curve for Curcumin

Linearity: The series of solution of curve were analyzed in 10 to 120 µg/ml, results are shown in Fig. 10

Table 5: Linearity

Sr. No.	Parameters	Curcumin
1	Linear dynamic range (µg/ml)	10-120
2	Slope	8.8895
3	Y-intercept	28.488
4	Correlation coefficient (R^2)	0.9992

Accuracy: It was determined on the basis of recovery study performed by standard addition method.

Table 6: Accuracy

Drug Name: Curcumin							
Std. conc. (%)	Std. (ppm)	Peak area	Drug (%)	Drug (ppm)	Peak area	Avg. peak area	Drug Rec. (%)
100%	500 ppm	28679	50	250	14320	14270	99.66
				250	14221		
			100	500	28681	28639	100.15
				500	28597		
			150	750	42559	42586	99.94
				750	42612		
Drug recovery Range (%) as per ICH = 100±10%							99.66%-100.15 %

VI. SPECIFICITY STUDY

The stress degradation study of curcumin was carried out as per ICH Q1A and photostability as per ICH Q1B guidelines. Forced degradation study of curcumin was carried out under acidic, alkaline, neutral and thermal stress conditions. All chromatographic parameters were kept constant as in method development.

Acidic hydrolysis study: It was performed by using 0.1 Molar HCl solution at room temperature for 24 hours

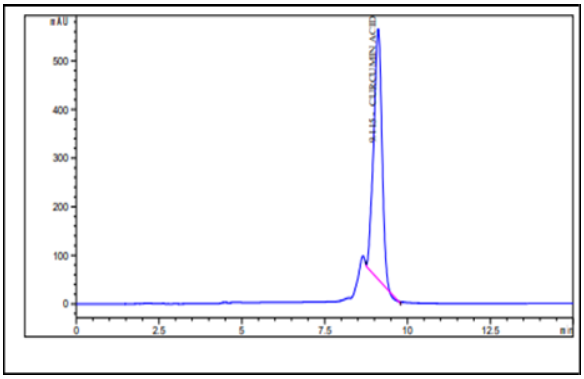


Fig.4: HPLC Chromatogram of specificity study of Curcumin of 0.1 M HCL (At room temp. for 24hrs.

Neutral degradation study

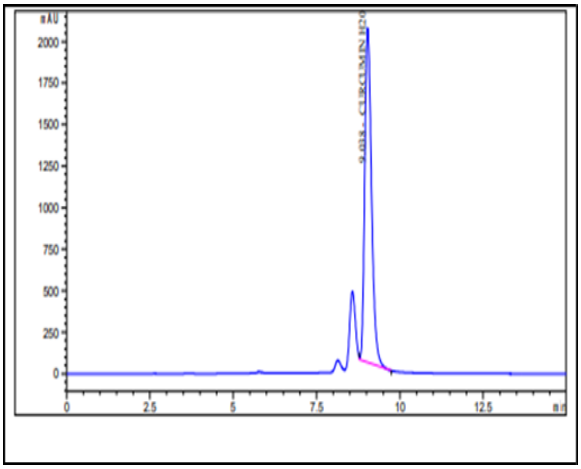


Fig. 5: HPLC Chromatogram of specificity study of Curcumin of water (neutral solvent at room temperature for 24hrs.)

Alkaline hydrolysis study

It was performed by using 1 M NaOH solution at room temperature for 24 hours

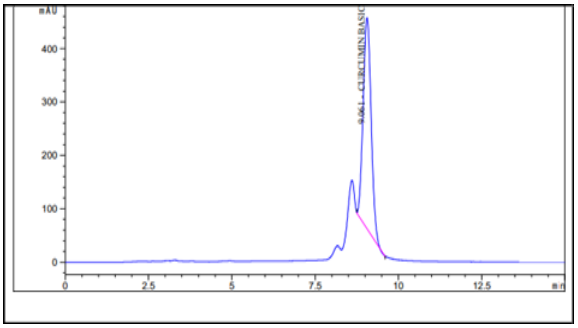


Fig. 6: HPLC Chromatogram of specificity study of Curcumin of 1 M NaOH (At room temp. for 24hrs.)

Thermal Degradation study- Carried out by exposing the solid form of drug to the temperature of 60ºC for 24 hrs

Table 7: Specificity study of Curcumin by HPLC

Medium	Area	% Area	Percent Recovery (%)
Standard	28722	NA	NA
Heat (at 60ºC))	28679	99.9	0.1
Alkaline Hydrolysis (0.1 M NaOH)	26349	91.74	8.27
Acid Hydrolysis (0.1 M HCl	25951	90.35	9.65
Neutral	28446	99.0	1.0

VII. PRECISION STUDY

The precision of HPLC method reflects its closeness to the agreement among the series of repetitive results, derived after multiple sampling of the same homogenous mixture of selected drugs under the given conditions. As displayed in Table 10; for intermediate variability for precision studies, this method is significantly precise over selected tested range of Curcumin. Moreover, the peak area of the studied samples was also correlated with selected concentration; where the % RSDs were <2%. The RSDs were observed well below 2% that reflects an acceptable precision with minimum variations of the proposed method.

Table 8: A) Data of intraday study

Conc. of Standard (µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Curcumin				
500	237.3	28678	28681	99.99
			28659	99.93
			28651	99.91

Table 9: - B) Data of Interday Day

Conc. of Standard (µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Curcumin				
500	237.1	28678	28691	99.95
			28571	99.63
			28664	99.95

Table 10: - C) Data of different analyst

Conc. of Standard (µg/ml)	Weight of tablet powder taken (mg)	AUC of standard solution	AUC of Sample	% Drug estimated
Drug Name: Curcumin				
500	237.3	28678	28641	100.13
			28671	99.98
			28690	100.04

Table 11: - D) Statistics

Sr.No.	Parameter	% of labeled claim		
		Intermediate precision		
		Intraday	Interday	Different analyst
		Drug Name: Curcumin		
1	Mean	99.94	99.84	100.05
2	± S. D	0.035	0.154	0.063
3	%RSD	0.035	0.154	0.063

VIII. SUMMARY AND DISCUSSION

Curcumin is often used alone or in combination with other therapeutic agents due to its antioxidant, anti-inflammatory, and anticancer properties. Analytical methods for the estimation of curcumin with other compounds are developed to enhance its therapeutic efficacy, considering its low bioavailability using RPHPLC technique are commonly used for the determination of curcumin in formulations. These methods are optimized for accuracy, precision, and cost-effectiveness, aiming to ensure reliable quantification in complex matrices.

The simple, precise and accurate HPLC method was developed for estimation of Curcumin using C18 column. The mobile phase of HPLC ACN: aqueous buffer pH adjusted to 3.0 with phosphoric acid was selected after several permutations and combination.

The flow rate was kept at 1.0 mL/min and 425 nm was selected as detection wavelength.

To validate chromatographic parameters various system suitability test were performed and based on these studies the HPLC method was developed for Curcumin. Linearity range was 20-120µg/mL. Five replicate sample from the same batch were analysed by proposed method has been shown in the result within acceptance limit indicating reproducibility of the method. The accuracy of the method was ascertained on the basis of recovery study performed by the standard addition method. The summarized results of proposed chromatographic method developed are given in Table.

Table: 12 Summarized results of proposed HPLC method

Parameters	ROS
Linearity range (µg/mL)	20-120
Correlation coefficient	0.9992
Retention time (min)	9.01
Tailing factor	0.97
Theoretical plates	9296
% Recovery	99.30%-100.95 %
Specificity	90.35-99.99
Intraday Precision (%RSD)	0.035
Interday Precision (%RSD)	0.154
Different Analyst (%RSD)	0.063

IX. CONCLUSION

The proposed HPLC methods were developed for the determination of Curcumin in pharmaceutical formulation were simple, accurate, sensitive and reproducible. Statistical analysis proves that the methods were repeatable and selective for the analysis of Curcumin. The methods were completely validated as per ICH Q2 (R1) guidelines showing satisfactory data for all the parameter tested. The proposed methods could be applied for routine analysis in quality control laboratories.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

Availability of data and materials Supplementary material that is available on the publisher's website along with the published article contains spectroscopic data of all compounds.

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