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STABILITY INDICATING RP-HPLC DETERMINATION OF CURCUMIN IN VICCO TURMERIC CREAM AND KASTURI TURMERIC CHURNA

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LOQ – Limit of Quantification.

ABSTRACT

A simple, specific, precise and stability-indicating RP-HPLC method was developed and validated for analysis of Curcumin in bulk drug, cream and churna formulations. Chromatographic separation were achieved using Agilent-TC C18 column (250 X 4.6 mm; 5µ) column at ambient temperature. Mixture of methanol, acetonitrile, and 5% acetic acid (35: 50: 15, v/v) was used as mobile phase at constant flow rate of 1.0 ml/min and 420 nm was selected as wave length for detection of method. The Curcumin peak was obtained at RT 4.92 min. The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.9993, in the concentration range 80–120 µg/ml. The method was validated for specificity, precision and recovery. LOD was found to be 1.67 µg/ml and LOQ was found to be 10.28 µg/ml. Curcumin was subjected to acid, neutral and alkali hydrolysis, oxidation, thermal, UV light and humidity degradation and indicates that the drug is not susceptible. As the developed method effectively separated the drug peak from its degradation products, shows that method is specific and stable. The developed method can be used in pharmaceutical industry for routine analysis of Curcumin in cream and churna based formulations.

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INTRODUCTION

Pharmaceutical Analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, micro-organisms, minerals and various synthetic products[(1)].

TYPES

There are mainly two types of chemical analysis.

- Qualitative (identification)
- Quantitative (estimation)

Qualitative Analysis: It is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not. Various qualitative tests are detection of evolved gas, formation of precipitates, limit tests, color change reactions, melting point and boiling point test etc.

Quantitative Analysis: They are mainly used to quantify any compound or substance in the sample. These techniques are based on

- The quantitative performance of suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained.
- The characteristic movement of a substance through a defined medium under controlled conditions.
- Electrical measurement.
- Measurement of some spectroscopic properties of the compound.

TYPES OF ANALYTICAL METHODS

The various methods of analysis can be grouped into two categories. They are:

- Chemical methods.
- Instrumental methods.

Instrumental Methods: These methods are based on the measurement of specific and non-specific physical properties (Sweetman et al., 2007; University of Alexandria; Journal of AOAC International, 2010; Czechowicz, 1969).

Table No: 1. List of instrumental methods

S.No.	Principle	Instrument method
1	Emission of radiation.	X – ray emission spectrometry. Fluorescence spectrometry.
2	Absorption of radiation.	UV / visible , I.R Spectrophotometry , NMR Spectroscopy , ESR spectroscopy , Atomic absorption spectrometry.
3	Mass to charge ratio.	Mass spectroscopy.
4	Refracting of radiation.	Refractometry .
5	Scattering of radiation.	Nephelometry.
6	Rotation of radiation.	Polarimetry.
7	Electrical potential.	Potentiometry.
8	Electric current.	Amperometry , polargraphy .
9	Electric resistance.	Conductometry.
10	Thermal properties	Differential thermal analysis , differential scanning calorimetry , Thermogravimetry .
11	Partition / Adsorption.	Chromatographic techniques.

Chemical Methods: In these methods, volume and mass are used as means of detection.

1. Titrimetric methods like acid-base, oxidation-reduction, non-aqueous, complexometric, precipitation titrations.
2. Gravimetric and thermo gravimetric methods.

ANALYTICAL METHOD VALIDATION (Pinkston, ?; Drug Testing and Analysis, 2013)

Definition: FDA defines validation as “Establishment of documented evidence which provides as high degree of assurance that a specific process will consistently produce a pre determined specification and quality attributes.”

Types of Analytical Procedures to be validated:

- Identification of test impurities .
- Quantitative test for impurities content.
- Limit test for control the of impurities.
- Quantitative test for active moiety of samples drug substance or drug products
- selected components in the drug product.
- Dissolution testing.

Types of Validation

Prospective Validation: These methods is employed when historical data of the product is not available or is not sufficient and in process and finished product testing are not adequate to ensure reproducibility or high degree of compliance.

Retrospective Validation: This provides trend of comparative and evaluation of existing information for comparison when historical data is sufficient and readily available.

Concurrent Validation: Based on information generated during implementation of a system.

Analytical Validation: Analytical validation of a pharmaceutical product or of specific ingredients within the products is necessary to ensure its safety/efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated/validated during the product development. Analytical validation ensures that the selective analytical method will give reproducible and reliable results adequate for intended purpose.

Analytical Validation Parameters

Characteristics, which should be considered, are listed below:

- Specificity
- Selectivity
- Precision
- Repeatability
- Intermediate precision
- Reproducibility
  - Accuracy
  - Linearity
  - Range
  - Limit of detection
  - Limit of quantification
  - Robustness
  - Ruggedness
  - System suitability

Assay

$$\% \text{ Assay [Anhydrous basis]} = \frac{A_{test} * W_{std} * \text{Std. Purity [100-LOD]} * P}{A_{std} * W_{test} * \text{Sample Purity [100 - LOD]}}$$

Where

A_{Test} = Average area of test solution
A_{Std} = Average area of Working Standard solution.
W_{Std} = Weight of Working standard in mg
W_{Test} = Weight of test sample in mg
LOD = Loss on drying
P = Purity of working Standard

Table No.2: Acceptance Criteria for Validation Parameter.

Validation parameters	Acceptance criteria
1) Specificity & System suitability	Specificity: There should be no interference of the diluents or impurities at the Rt of drug substance/internal standard. System Suitability: 1) Theoretical plates: NLT2000 2) Tailing factor: NMT2.0 3) Resolution: NLT 1.5 4) Capacity factor: NLT 2.0 5) Similarity factor: NLT 0.98 and NMT 1.02
2) Linearity & Range	1) Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. 2)The % y-intercept as obtained from the linearity data (without extrapolation through origin 0,0) should be within 2.0±
3) Precision	1) The RSD of the % assay values should. not be more than 2.00%
4) Accuracy	1) Recovery should be between 98 and 102% 2) %RSD of recovery should not be more than 2.00%.
5) Solution stability	1) The standard at initial and Predetermined time interval should meet the requirement of system suitability specified before. 2) % relative difference between initial value and the value at predetermined interval should not be more than 2.0.
6) Robustness	1) As per specificity and system suitability, 2) The RSD of assay values should not be more than 2.00%.

Aim and Objective

Literature survey reveals the availability of some methods for the estimation of curcumin using RP-HPLC. Only very few HPLC estimations have been reported in the literature for the determinations of curcumin present in cream and churna .
The objective of this experiment was to optimize the assay method for estimation of curcumin in marketed formulations based on the literature survey made.

Objective of the work: The experimental work has been planned as follows:

Step-1:

To develop simple and specific stability indicating RP-HPLC method for the quantitative estimation of curcumin in marketed formulations.

- Selection of stationary phase.
- Selection of mobile phase.
- Preparation of solutions.
- Optimizing the chromatographic conditions.

Step-2

To validate the developed methods in accordance with the analytical validation parameters mentioned as per ICH guidelines.

METHOD DEVELOPMENT

CHEMICALS AND REAGENTS

Curcumin (working standard) was purchased from Loba Chemicals, Bangalore, India. All chemicals (Methanol – AR Grade, Acetonitrile - HPLC Grade, Glacial acetic acid - AR Grade, Purified water - Milli Q) and regents used were of HPLC grade and were purchased from Merck Chemicals, India. Vicco turmeric skin cream-1g/15g pack (Vicco laboratories-Nagpur, India), Kasturi Turmeric Churna-100g/500g pack (Aalayam general stores, vijayawada,India).

INSTRUMENTS:

HPLC	Agilent Model A1100
Pump	Reciprocating - 510
Detector	UV/Visible detector
Software	Ezchrome elite
Column	Agilent-TC C18, (250×4.6 mm 5µ)
Analytical Balance	Metler-Toledo
pH Meter	Systronics, Hydrogen Electrode
Sonicator	Ultrasonic Bath Sonicator (PCI, Mumbai)
Filter	Nylon & PVDF 0.45µm (Millipore, Banglore)
Volumetric flasks	Class-A Glass ware
Centrifuge apparatus	Remi Equipments

All the glassware's used were of borosilicate glass and all the solvents and prepared solutions were filtered through Nylon filter 0.45µm.

METHOD DEVELOPMENT: Method development and optimization of chromatographic parameters for the estimation of curcumin in pharmaceutical formulations by RP-HPLC are discussed below.

Solubility studies: Solubility studies for curcumin revealed the solubility of drug in ethanol and methanol. Curcumin was insoluble in ether.

Trails undertaken for the estimation of curcumin

TRIAL1

Buffer preparation: 3.8 g of Ammonium acetate (0.5M) add 500 ml of water, sonicate and make up to 1000 ml with diluents and adjust pH to 5.2 with glacial acetic acid.

Diluents: Mobile phase

Mobile phase: mobile phase used was 50% Methanol and 50% Ammonium acetate buffer (0.5M), (pH-5.2adjusted with GAA)

Chromatographic conditions

Column: Agilent-TC C_{18} column (250×4.6 mm; 5µ)
Flow rate: 1.4 ml/min.
Wavelength: UV-400 nm
Column temperature: Ambient
Injection volume: 20 µL
Run time : 20 minutes.

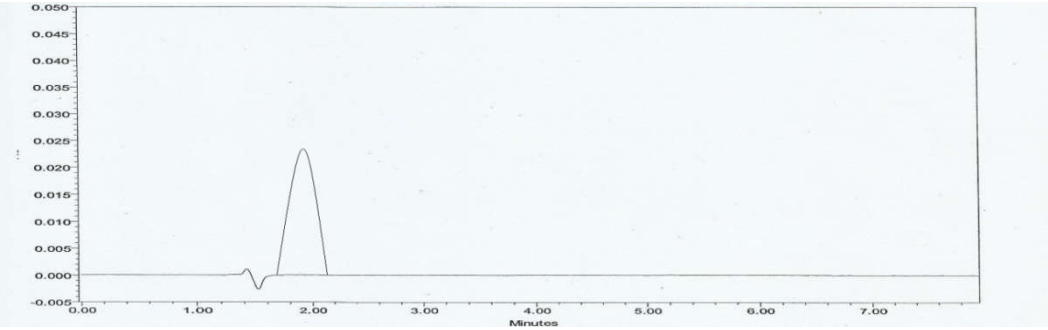

Fig.no.1. Chromatogram of Trial-1

Observation: Retention time at 1.9min broad peak was observed.

TRAIL- 2

Mobile phase: mobile phase used was methanol and HPLC grade water in the ratio 50:50%. v/v.

Chromatographic conditions

Column: Agilent-TC C_{18} column (250×4.6 mm; 5µ) Flow rate : 1.3 ml/min.
Wavelength: UV-410 nm
Column temperature: Ambient
Injection volume: 20 µL
Run time : 20 minutes

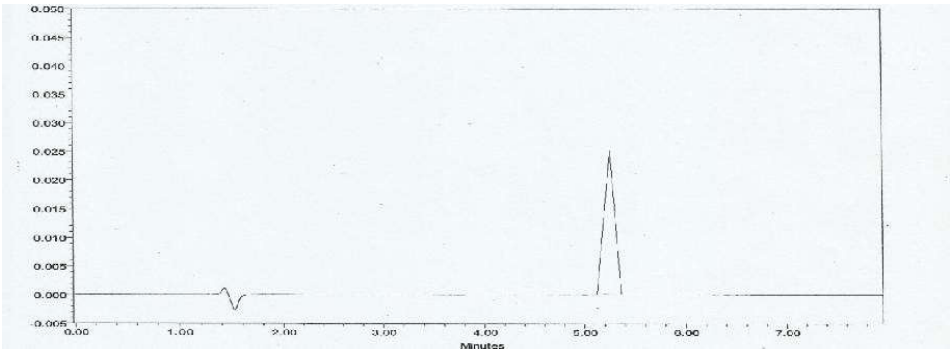

Fig.no.2. Chromatogram of Trial-2

Observation: Broad peak with tailing more than 2

TRIAL-3:

Mobile phase: mobile phase used was acetonitrile and HPLC grade water in ratio 50:50%v/v

Chromatographic conditions

Column: Agilent-TC C_{18} column (250×4.6 mm; 5µ) Flow rate : 1.2 ml/min.
Wavelength: UV-410 nm
Column temperature: Ambient
Injection volume: 20 µL
Run time: 20 minutes.

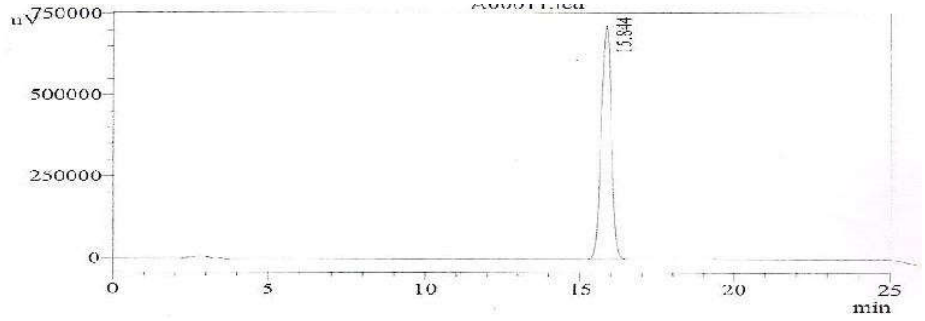

Fig.no.3 Chromatogram of Trial-3

Observation: peak is eluted at 15.8 more retention time

TRIAL-4:

Mobile phase: mobile phase used was methanol, acetonitrile, and 5% acetic acid (35: 50:15, v/v).

Chromatographic conditions

Column: Agilent-TC C_{18} column (250×4.6 mm; 5µ) Flow rate : 1.1 ml/min.
Wavelength: UV-410 nm
Column temperature: Ambient
Injection volume: 20 µL
Run time: 10 minutes.

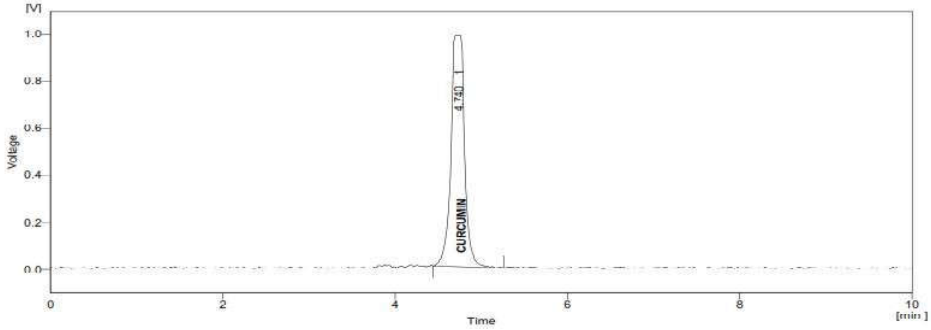

Fig.no.4. Chromatogram of Trial-4

Observation: good shape and tailing factor but low theoretical plates

OPTIMIZED METHOD

Mobile phase: Mobile phase used was methanol, acetonitrile, and 5% acetic acid (35: 50:15, v/v).
Diluent: Mobile phase

Chromatographic conditions

Column: Agilent-TC C_{18} column (250×4.6 mm; 5µ) Flow rate:1ml/min.
Wavelength: UV-420 nm
Column temperature: Ambient
Injection volume: 20 µL
Run time: 10 minutes.

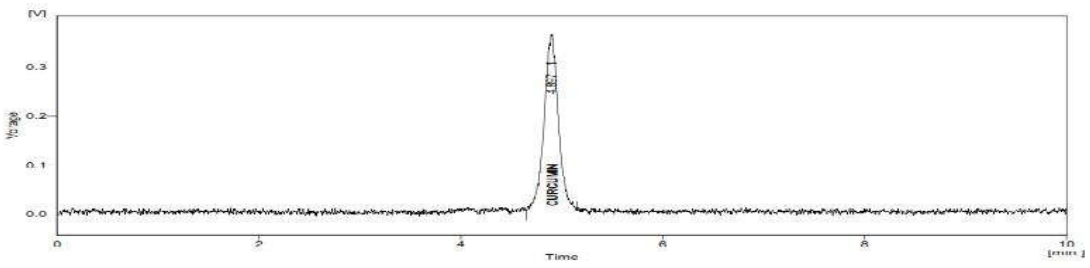

Fig.no.5 Chromatogram of Optimized method

Rt (min)	Peak area	Peak width	Tailing factor	Theoretical plates	Run time (min)
4.92	6739	0.16	1	5238	10

Observation: peak is eluted at 4.92 min in this trial

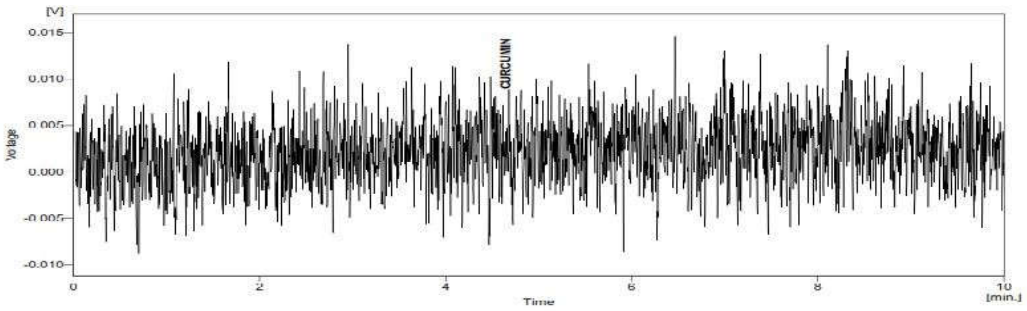

Fig.no.6. Chromatogram of blank

Observation: No peak was observed at drug retention time.

Standard stock preparation: The stock solutions were prepared by dissolving 50 mg of Curcumin was dissolved in 50 ml methanol to get a concentration of 1000 µg/ml. Analytical standard solutions for linearity were prepared by diluting the stock solution with methanol immediately prior to use. All the preparations were made in borosilicate glass wares.

Sample preparation for assay: Estimation of Curcumin in Vicco cream

Equalent to 100 mg VICCO TURMERIC SKIN CREAM was accurately weighted and transferred to a 100 ml volumetric flask directly with butter paper. 5 ml methanol was added to it and sonicated for 15 minutes to extract the curcumin from the cream. Then the volume was made up to the mark and centrifuged for 2 minutes at 2000 rpm. The centrifuged solution was filtered with 0.45 µm syringe filter (nylon) and 20 µl was injected in to RP-HPLC.

Estimation of Curcumin in Haridrakhand churna

Equalent to 100 mg KASTURI TURMERIC CHURNA was accurately weighted and transferred to a 100 ml volumetric flask and 5 ml methanol was added sonicated for 10 minutes to extract the Curcumin. Then the volume was made up to the mark and centrifuged for 2 minutes at 2000 rpm. The centrifuged solution was filtered with 0.45 µ syringe filter (nylon) and 20 µl was injected in to RP-HPLC.

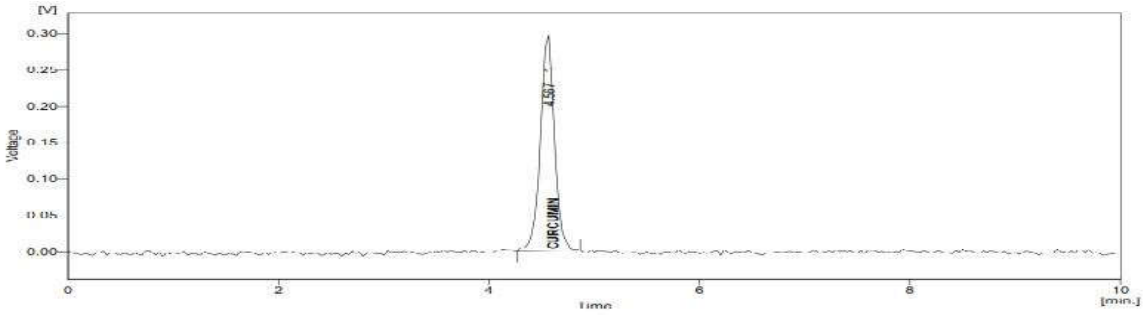

Fig.no.7. Standard Chromatogram of Curcumin

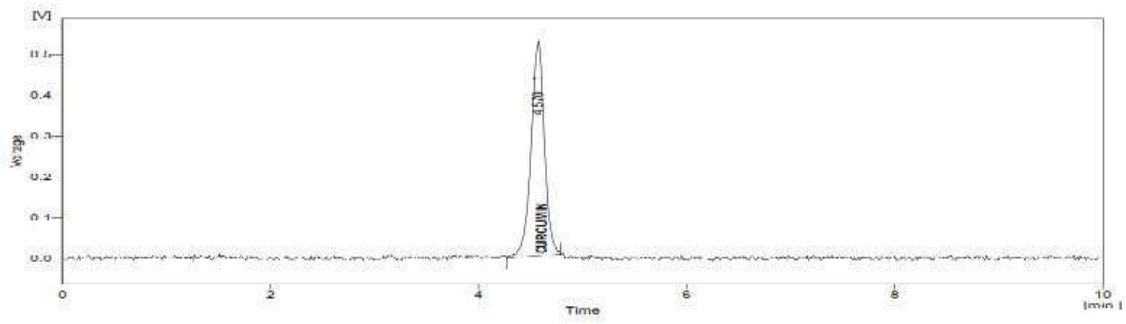

Fig.no.8. Test chromatogram of Kasturi turmeric churna

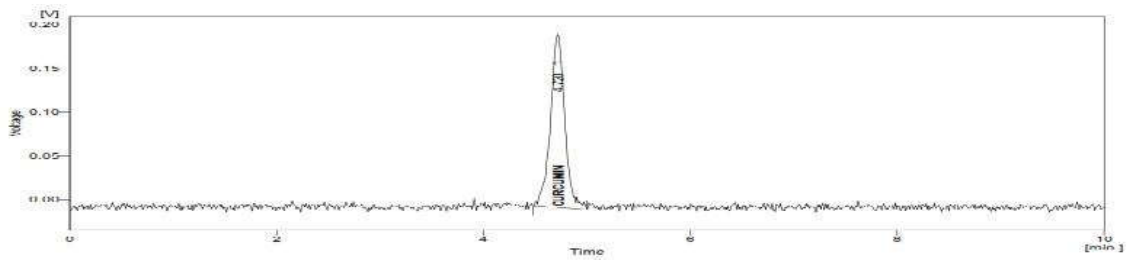

Fig.no.9. Test Chromatogram of Vicco turmeric skin cream

Assay calculations

Calculate the amount of Curcumin in mg/ml of solution using the following formula

% Content of Curcumin as per label claim (%Assay):

$$= \frac{Ps}{Pt} \times \frac{Ws}{100} \times \frac{5}{25} \times \frac{100}{P} \times \frac{25}{100} \times \frac{Wr}{5} \times \frac{100}{Lc} \times 100$$

Where;

Ru = Peak area of Curcumin in sample solution
Rs = Average peak area Curcumin in standard solution
Ws = Weight of Curcumin working standard taken in mg
Wr = Weight of sample taken in mg
P = Assay Curcumin working standard used on as basis (%Purity) Lc = Label claim.

Method validation

Validation is a process of establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce meeting, its predetermined specifications and quality attributes. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Validation parameters

- System suitability testing
- Specificity
- Accuracy
- Precision
- Linearity & Range
- Robustness
- LOD and LOQ
- Solution stability
- Filter compatibility

SYSTEM SUITABILITY

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Before performing any validation experiment, HPLC method and the procedure should be capable of providing data of acceptable quality. These tests are to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. It is based on the concept that equipment, electronics, analytical operations and sample constitute.

Procedure

The standard solution and sample solution are prepared as per the assay method of concentration 10µg/ml and filter it through Millipore filter and sonicate. Carried out the system suitability studies for Standard solution and sample solution with a minimum of six replicates of single preparation. Calculate %RSD for area and retention time of Mebeverine Hydrochloride, and record the tailing factor & theoretical plate count details were given in table below.

Acceptance Criteria: % RSD should not be more than 2.0% . Tailing factor should not be more than 2 and theoretical plates not less than 2000.

Table.no.3 Data for System Suitability Standard

S.NO.	Retention Time (min)	Area	Tailing Factor	Theoretical Plates
1	10.129	1544494	0.450	10053
2	10.148	1545311	0.563	10042
3	10.129	1549914	0.435	10050
4	10.125	1550421	0.421	10028
5	10.127	1550173	0.673	10031
% RSD		0.17		

DataInterpretation: It was observed from the data tabulated above; the method complies with the system suitability parameters. Hence it was concluded that the system suitability parameter met the requirement of method validation

SPECIFICITY (SELECTIVITY)

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. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Procedure

Specificity of the stability indicating method was established by separation of the principle peak with the excipients peak in the Vicco cream and that of the degradant peak in the Curcumin pure, Vicco turmeric skin cream and Kasturi turmeric churna after forced degradation.

Acidic hydrolysis

100 mg of curcumin was transferred to a 100 ml volumetric flask. 10 ml 0.1N HCl was added and kept for 3 days at 60°C, (in dark to avoid the possible degradation effects of light) cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC. Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask. To each flask,10 ml 0.1N HCl was added and kept for 3 days at 60°C (in dark to avoid the possible degradation effects of light), cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to10 ml volumetric flask and extracted as per assay procedure. Finally, test sample was diluted up to the mark with mobile phase to get 100 µg/ml and injected in to HPLC.

Alkali degradation

100 mg of curcumin was transferred to a 100 ml volumetric flask. 10 ml 0.1N NaOH was added and kept for 3 days at 60°C, (in dark to avoid the possible degradation effects of light) cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC. Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask. To each flask,10 ml 0.1N NaOH was added and kept for 3 days at 60°C (in dark to avoid the possible degradation effects of light), cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and extracted as per assay procedure. Finally, test sample was diluted up to the mark with mobile phase to get 100 µg/ml and injected in to HPLC.

Neutral degradation

100 mg of curcumin was transferred to a 100 ml volumetric flask. 10 ml distilled water was added and kept for 3 days at 60°C, (in dark to avoid the possible degradation effects of light) cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC. Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask. To each flask,10 ml distilled water was added and kept for 3 days at 60°C (in dark to avoid the possible degradation effects of light), cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and extracted as per assay procedure. Finally, test sample was diluted up to the mark with mobile phase to get 100 µg/ml and injected in to HPLC.

Direct sunlight

100 mg of curcumin was transferred to a 100 ml volumetric flask and exposed to direct sunlight for 3days. Sample further diluted to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to

10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC. Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask and exposed to direct sunlight for 3days cool. Sample further diluted to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC.

Oxidative degradation by peroxide

100 mg of curcumin was transferred to a 100 ml volumetric flask. 10 ml 3% H2O2 was added and kept for 3 days at 60°C, (in dark to avoid the possible degradation effects of light) cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC. Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask. To each flask,10 ml 3% H2O2 was added and kept for 3 days at 60°C (in dark to avoid the possible degradation effects of light), cooled, neutralised the solution and made up to 100ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to10 ml volumetric flask and extracted as per assay procedure. Finally, test sample was diluted up to the mark with mobile phase to get 100 µg/ml and injected in to HPLC.

Degradation by UV light

100 mg of curcumin was transferred to a 100 ml volumetric flask and exposed to UV chamber at 254 nm for 3days. Sample further diluted to 100 ml with methanol. The solution was filtered through 0.45µ membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC.
Equalent to 100 mg Vicco turmeric cream and kasturi turmeric churna were transferred to respective 100 ml volumetric flask and exposed to UV chamber at 254 nm for 3 days. Sample further diluted to 100ml with methanol. The solution was filtere membrane filter paper. From the filtrate 1 ml was transferred to 10 ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml, injected in to HPLC.

Thermal Degradation

The 100 mg of Curcumin, 100 mg Vicco turmeric cream and 100 mg Kasturi turmeric Churna were separately kept in porcelain dish at 90°C in hot air oven for 3 days. After degradation, samples were transferred to 100 ml volumetric flask and diluted to 100 ml with methanol. 1ml of the sample solution was transferred to respective 10 ml volumetric flask and extracted as per assay procedure. The resultant solutions were then further diluted with mobile phase to get final concentration 100 µg/ml and injected in to HPLC.

Humidity

The 100 mg of Curcumin, 100 mg Vicco turmeric cream and 100 mg Kasturi turmeric Churna were separately kept in porcelain dish at 90% relative humidity in humidity chamber for 3 days. After degradation, samples were transferred to 100 ml volumetric flask and diluted to 100 ml with methanol. 1ml of the sample solution was transferred to respective 10 ml volumetric flask and extracted as per assay procedure. The resultant solutions were then further diluted with mobile phase to get final concentration 100 µg/ml and injected in to HPLC.

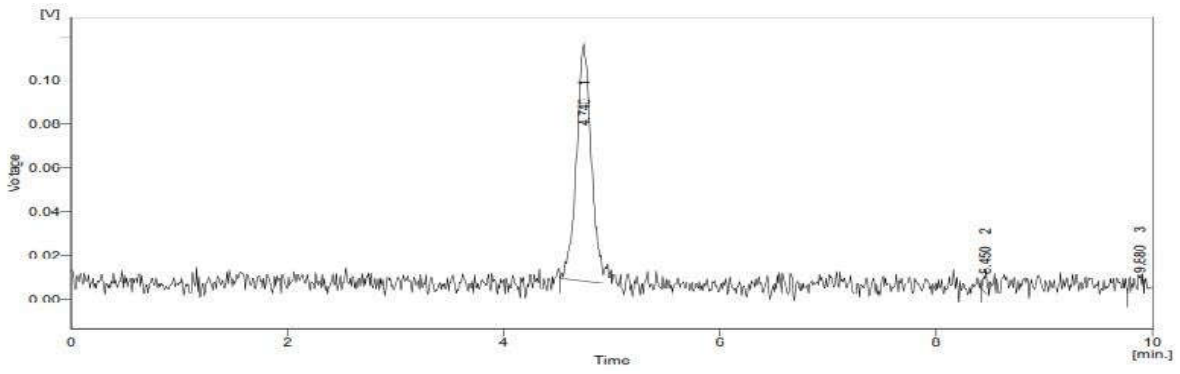

Fig.No.10.Chromatogram of curcumin Acid Degradation

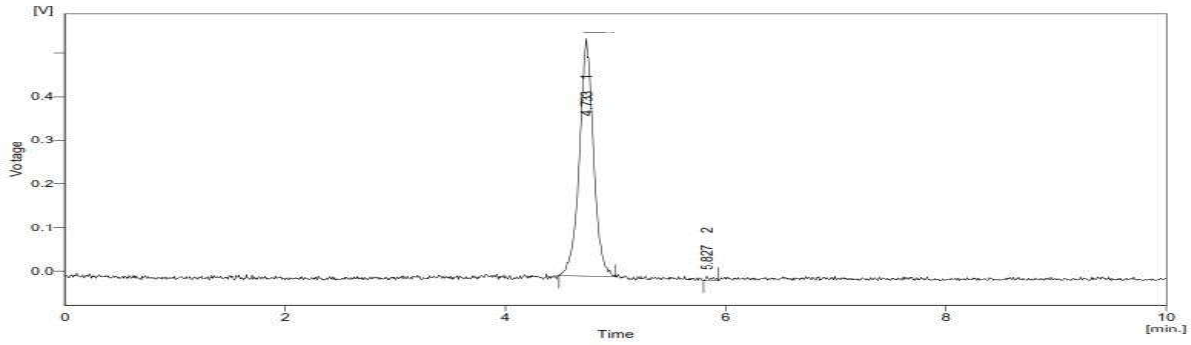

Fig.No.11.Chromatogram of curcumin Base Degradation

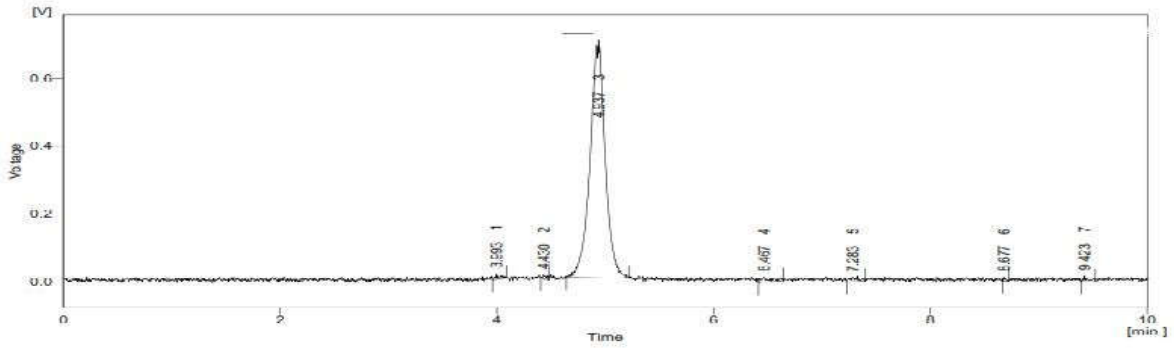

Fig.No.12.Chromatogram of curcumin Neutral Degradation

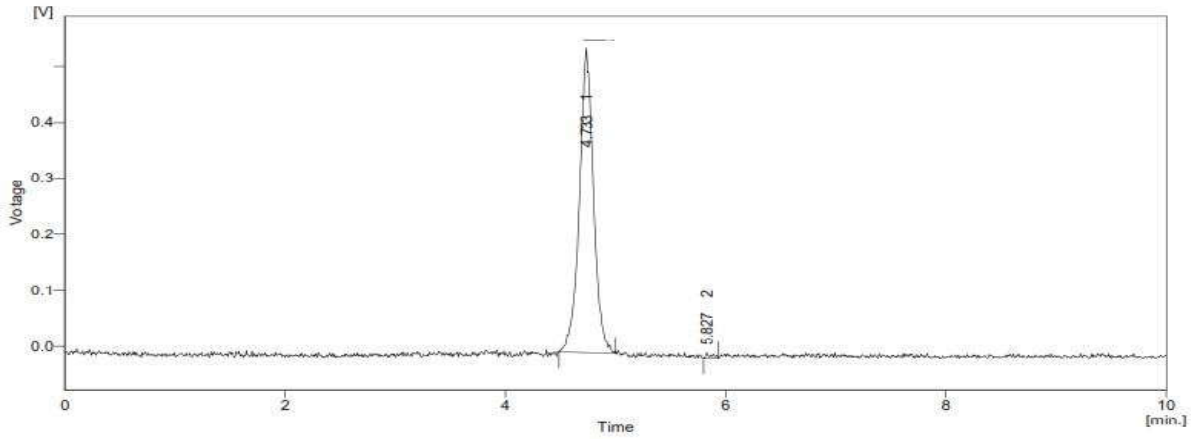

Fig.No.13.Chromatogram of curcumin Direct Sunlight Degradation

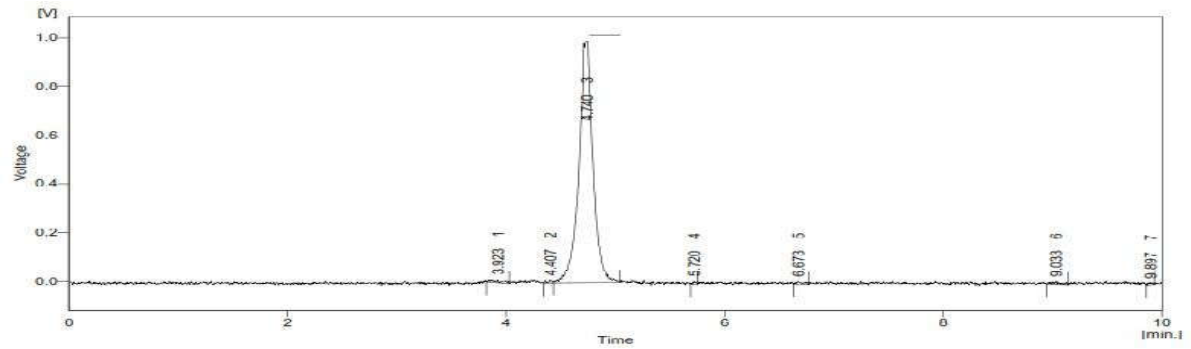

Fig.No.14.Chromatogram of curcumin Oxidative Degradation

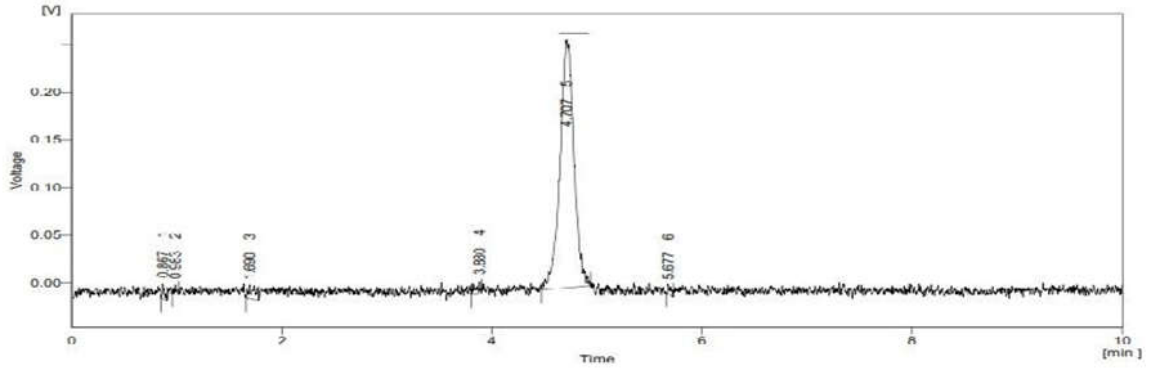

Fig.No.15.Chromatogram of curcumin Thermal Degradation

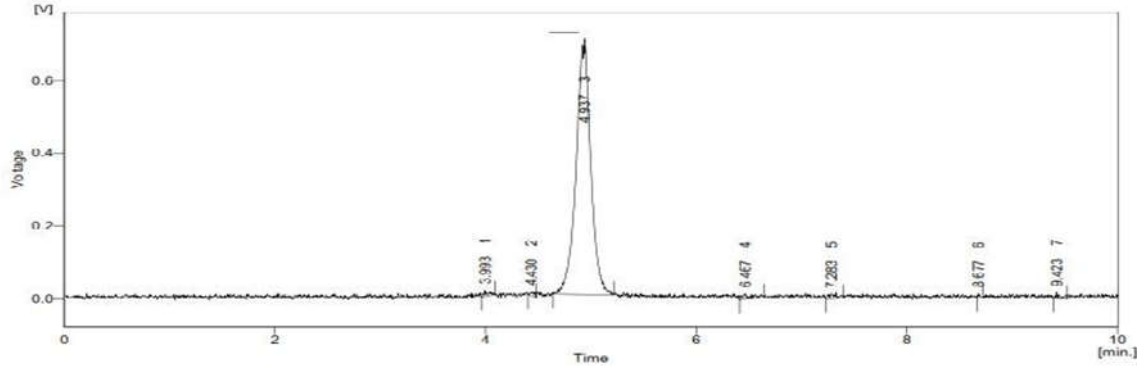

Fig.No.16.Chromatogram of curcumin UV Degradation

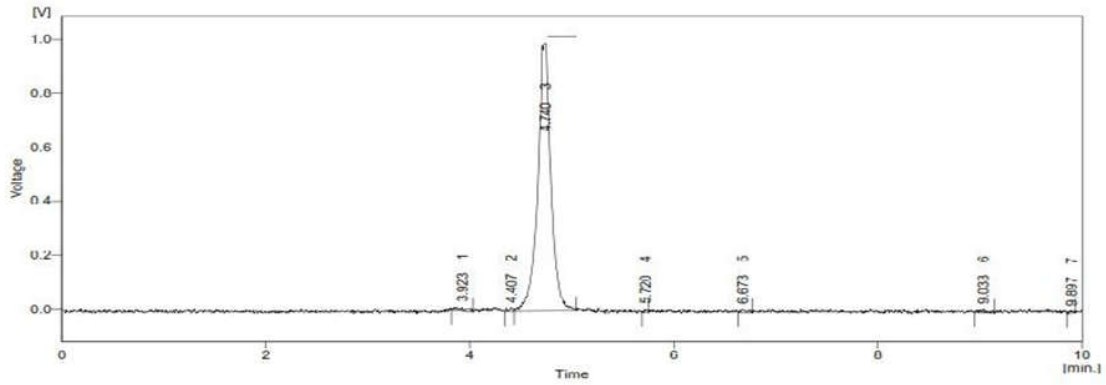

Fig.No.17.Chromatogram of curcumin Humidity Degradation

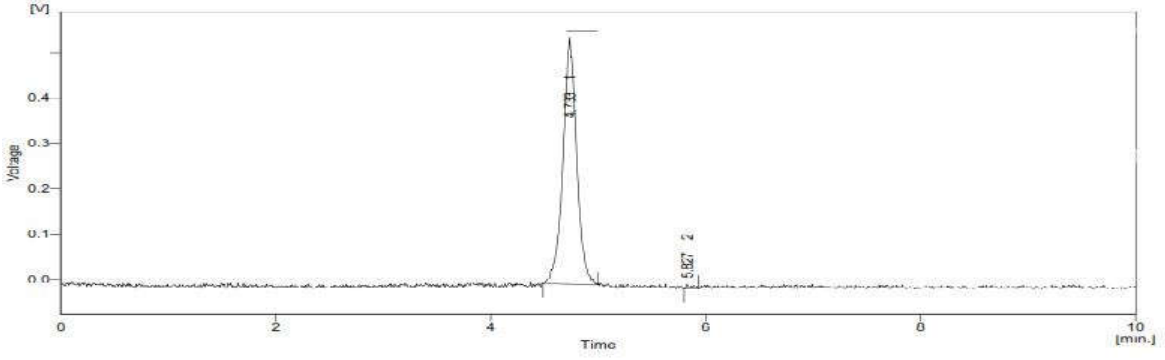

Fig.No.18.Chromatogram of Vicco cream Acid Degradation

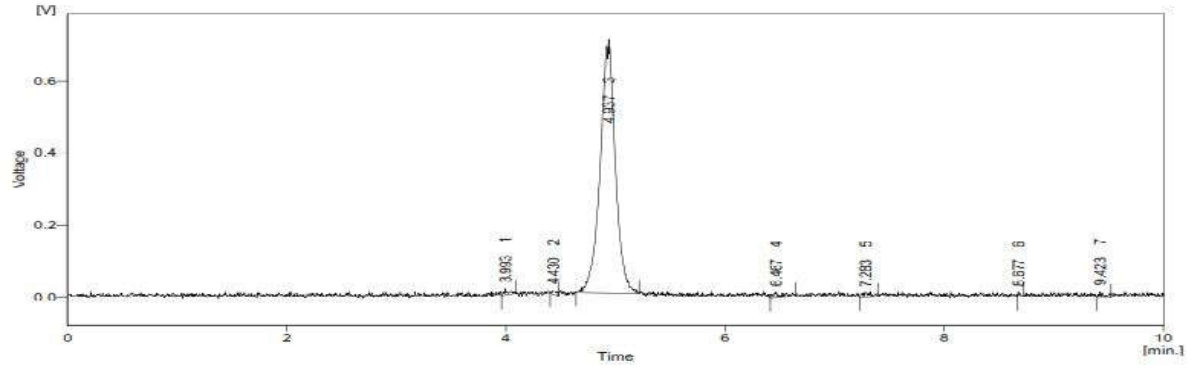

Fig.No.19.Chromatogram of Vicco cream Base Degradation

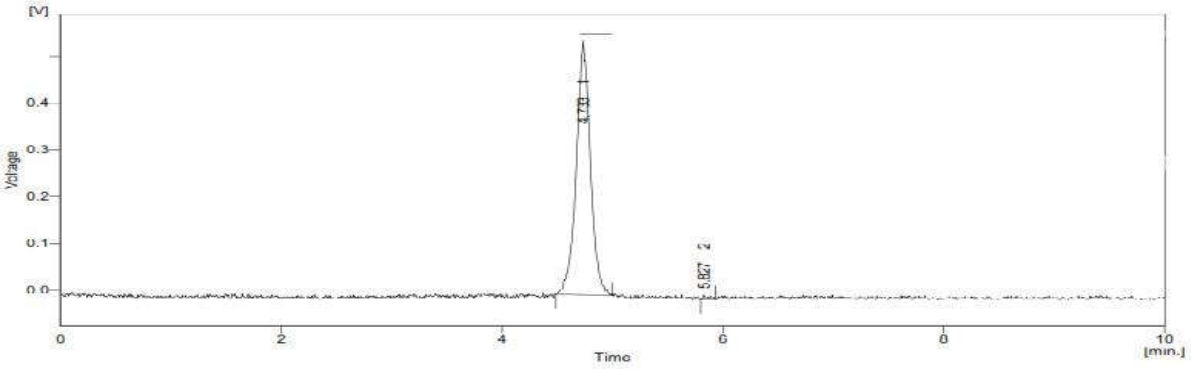

Fig.No.20.Chromatogram of Vicco cream Neutral Degradation

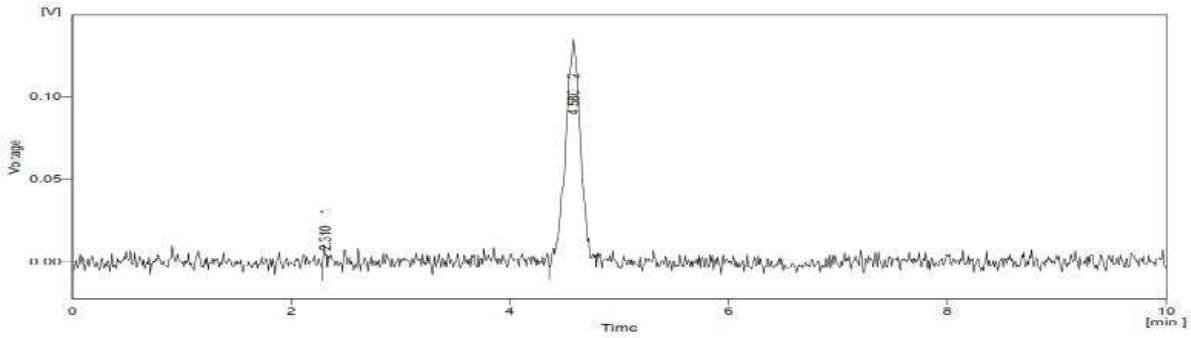

Fig.No.21.Chromatogram of Vicco cream Direct Sunlight Degradation

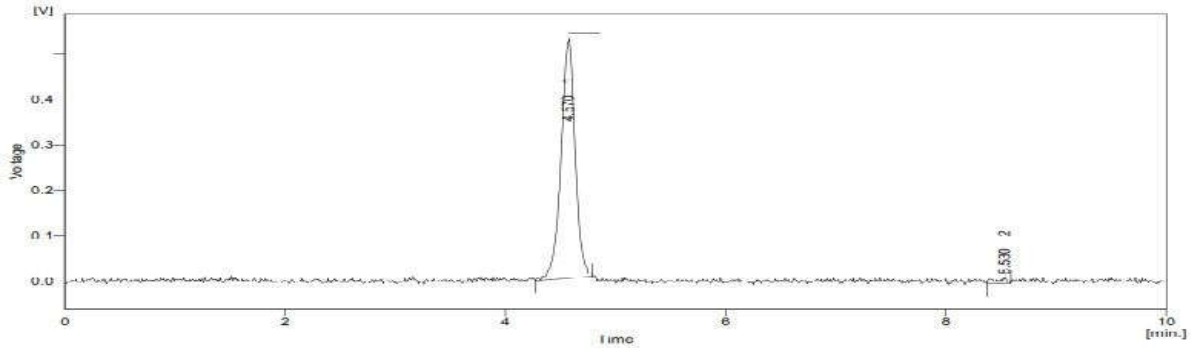

Fig.No.22.Chromatogram of Vicco cream Oxidative Degradation

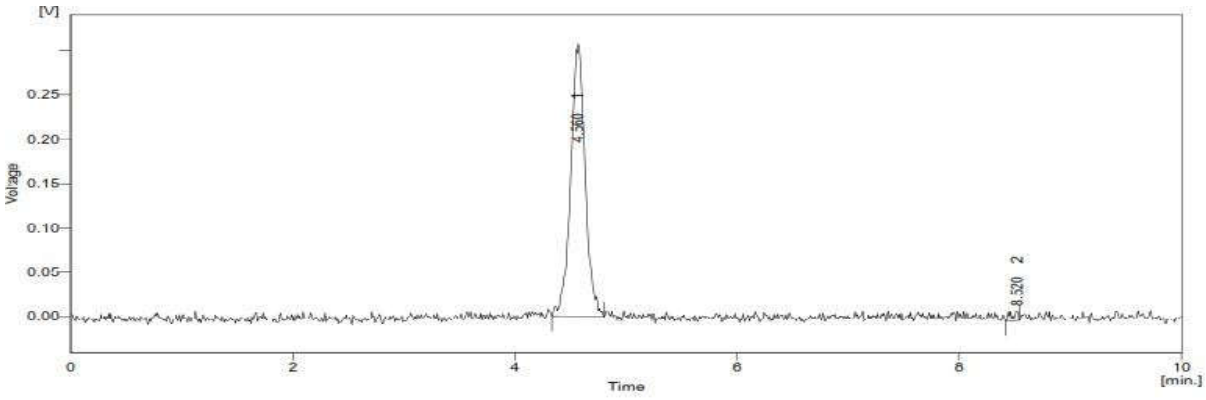

Fig.No.23. Chromatogram of Vicco cream Thermal Degradation

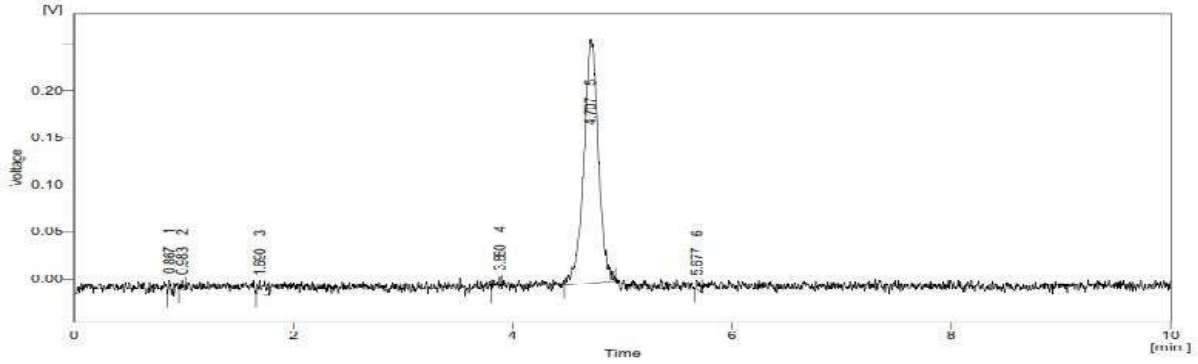

Fig.No.24.Chromatogram of Vicco cream UV Degradation

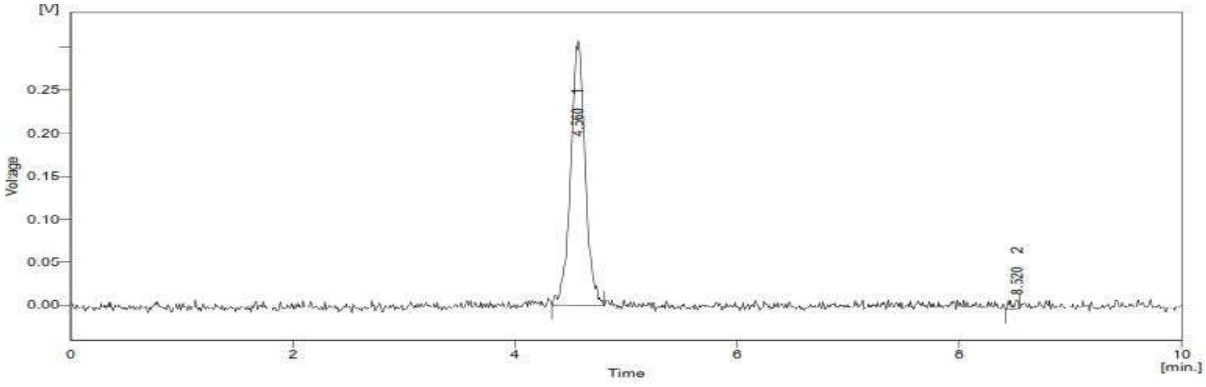

Fig.No.25.hromatogram of Vicco cream Humidity Degradation

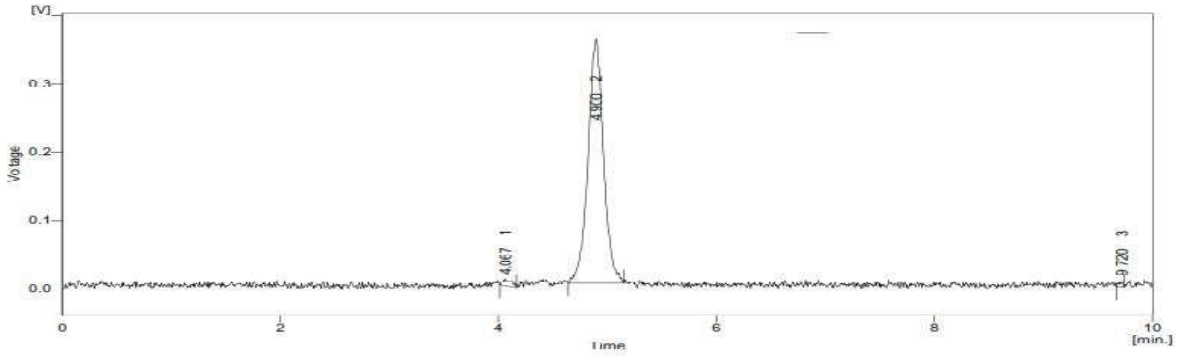

Fig.No.30.Chromatogram of Kasturi turmeric churna Peroxide Degradation

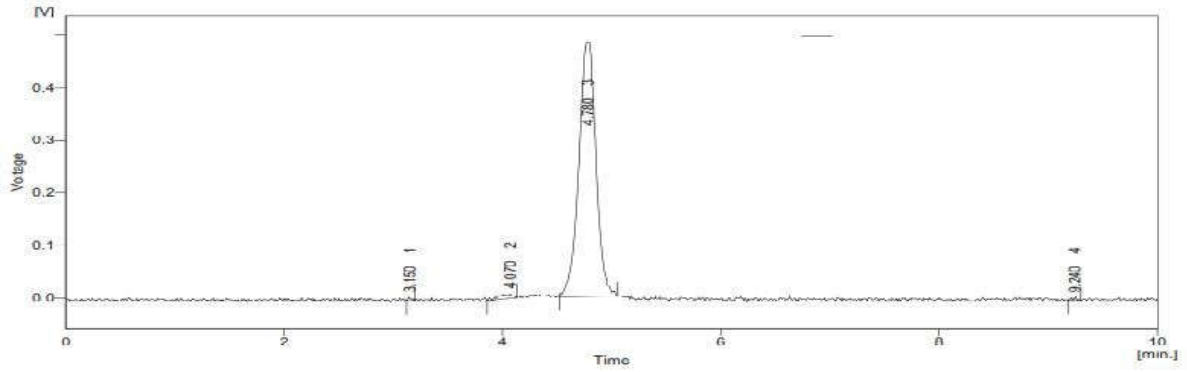

Fig.No.31.Chromatogram of Kasturi turmeric churna Thermal Degradation

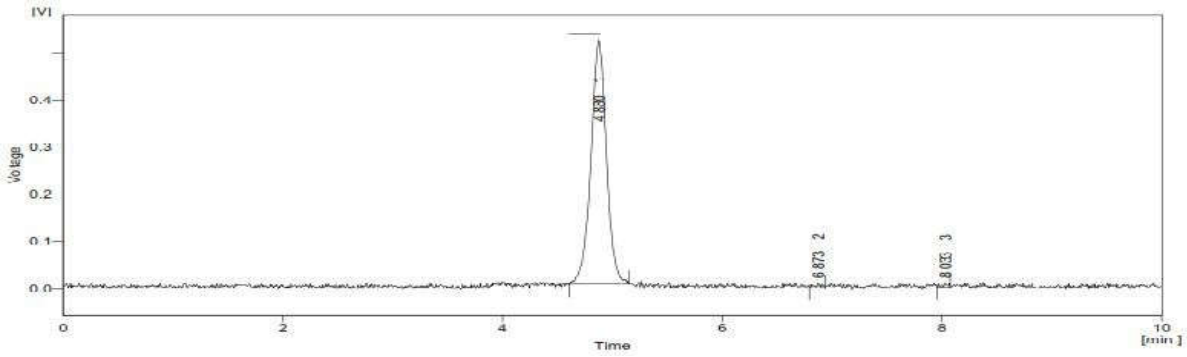

Fig.No.32.Chromatogram of Kasturi turmeric churna UV Degradation

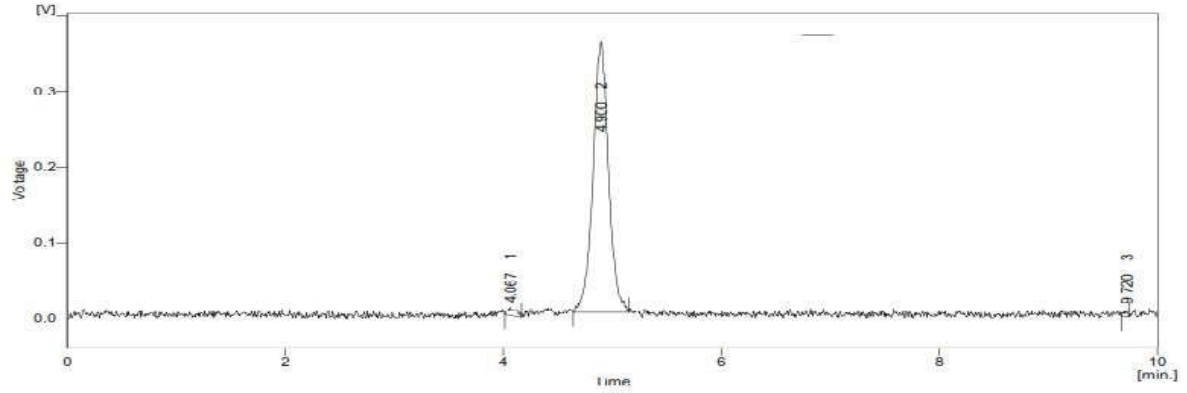

Fig.No.33.Chromatogram of Kasturi turmeric churna Humidity Degradation

Table 4 Data for Specificity

Injections	Analyte RT(min)			Sample Interference		
	Curcumin	Cream	Churna	Curcumin	Cream	Churna
Blank	-	-	-			
Sample Acid degradation Base degradation Neutral degradation UV degradation Thermal degradation Peroxide degradation Humidity degradation	4.91	4.92	4.92			
	4.82	4.91	4.92			
	4.92	4.91	4.90			
	4.92	4.90	4.92			
	4.92	4.89	4.95			
	4.92	4.92	4.91	No interference at RT of Analyte Peak		
	4.91	4.89	4.92			
	4.92	4.92	4.92			

Data Interpretation: On the basis of chromatograms, there is no interference of matrix excipients with curcumin retention time.

ACCURACY (% RECOVERY)

Accuracy of test method was carried out by spiking known amount of drug substance to get concentration 80 %, 100 % and 120 % of target concentration in triplicate for each level. Each solution was injected in triplicate. The average % recovery was calculated.

Acceptance criteria: The mean % recovery at each level should be in the range of 98.0 –102.0%.

Table.no.5 Accuracy data for Vicco turmeric cream

Concentration level	Con. Found before Spiking (µg/ml)	Amount spiked (µg/ml)	Final conc. (µg/ml)	Conc. found after spiking	%Recovery	Mean ± SD
		80	158.63	157.62	99.98	
80%	78.63	80	159.98	157.04	99.36	
		80	158.63	157.87	99.15	99.49 ± 0.256
		100	201.38	200.42	99.49	
100%	101.28	100	202.56	201.87	99.11	
		100	201.29	200.46	99.74	99.44 ± 0. 311
		120	240.09	239.34	99.91	
120%	120.69	120	241.38	240.53	99.42	
		120	241.47	241.45	100.16	99.83 ± 0.366
%Cumulative Recovery						99.76

Table.no.6 Accuracy data for Kasturi Turmeric Churna

Concentration level	Con. Found before Spiking (µg/ml)	Amount spiked (µg/ml)	Final conc. (µg/ml)	Conc. found after spiking	%Recovery	Mean ± SD
		80	159.54	158.45	99.98	
		80	159.08	158.02	99.36	99.49 ± 0.154
80%	79.54	80	158.63	157.39	99.15	
		100	199.57	198.42	99.49	
100%	99.56	100	200.33	201.42	99.11	99.44 ± 0.179
		100	199.08	201.42	99.74	
		120	24178	240.53	99.91	
120%	121.78	120	241.22	240.53	99.42	99.83 ± 0.203
		120	240.59	240.53	100.16	
%Cumulative Recovery						99.84

Data Interpretation: The recovery results indicating that the test method has an acceptable level of accuracy.

PRECISION

It is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample.

METHOD PRECISION/ REPEATABILITY/INTRA DAY

The standard solution was prepared as per the assay method of concentration 0.05mg/ml and filter it through Millipore filter and sonicate.

Procedure

Carried out the system precision for Standard solution with a minimum of six replicates of single preparation. Calculate %RSD for peak area and retention time.

Acceptance Criteria: % RSD should not be more than 2.0% for Area.

Data Interpretation: % RSD of the was found to be 0.42.

RUGEDNESS/ INTERMEDIATE PRECISION / INTER DAY

Intermediate precision expresses within laboratory variation as on different days or with different analysts or equipment within the same laboratory. It is not considered necessary to study these effects individually.

Acceptance criteria

For assay: the difference between the average assay results obtained by both analysts should not be more than 3%.

Table.no.7. Intra Day Precision data

Sample. No	Vicco turmeric skin cream	Kasturi Turmeric Churna
	Day 1, Analyst 1, Inst (Agilent)-1	Day 1, Analyst 1, Inst (Agilent)-1
	% Assay	%Assay
1	98.63	99.44
2	98.13	98.35
3	98.43	98.87
4	98.37	99.88
5	98.40	9821
6	98.62	98.62
Average	98.46	98.63
%RSD	0.34	0.45
Cumulative % RSD	0.42	

The different analyst using the different equipment on different day tested the same batch samples tested in method repeatability containing the analyte through the complete analytical procedure from sample preparation to final result, in the same laboratory. The comparative results are given below:

Day-II Name of the instrument 1: Schimadzu, Name of the instrument 2:Agilent

Table no.8 Interday precision data Vicco turmeric skin cream

Sample. No	Vicco turmeric skin cream	
	Day 1, Analyst 1, Inst (Agilent)-1	Day 2, Analyst 2, Inst (Shimadzu)-2
	% Assay	%Assay
1	98.6	98.5
2	98.1	98.1
3	98.4	98.9
4	98.3	98.4
5	98.4	98.7
6	98.6	98.7
Average	98.4	98.6
%RSD	0.34	0.45
Cumulative % RSD	0.47	

Table no.9 Intrerday Precision for Kasturi Turmeric Churna

Sample no	Kasturi Turmeric Churna	
	Day 1, Analyst 1, Inst (Agilent)-1	Day 2, Analyst 2, Inst (Shimadzu)-2
	% Assay	%Assay
1	99.44	99.34
2	98.35	99.34
3	98.87	99.23
4	99.88	98.26
5	9821	98.89
6	98.62	99.12
Average	98.63	98.78
%RSD	0.67	0.53
Cumulative % RSD	0.58	

Data Interpretation*:* From the above data, it was concluded that the method is rugged.

LINEARITY AND RANGE

The linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. A minimum of 5 standard solutions in the range of 80% to 120% of the test concentration required for assay procedure should be prepared and analysed. It should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. Linear regression is calculated by the method of least squares. The squared correlation coefficient (r2), y-intercept and slope of the regression line should be calculated and reported.

Method

A stock solution of curcumin (1 mg/ml) was prepared in methanol by transferring 50 mg of drug in 50 ml volumetric flask. From this solution concentration of 80, 90, 100,110 and 120 µg/ml were prepared by diluting with mobile phase in different 10 ml volumetric flask.

Acceptance criteria: Squared correlation coefficient (r^2) should not be not less than 0.990 for impurities/degradants. Squared correlation coefficient (r2) should not be less than 0.995 for dissolution.

Squared correlation coefficient (r2) should not be less than 0.999 for assay.

Table.no.10.Linearity Data

% level	Concentration(µg/mL)	Peak area
80	80	1208873
90	90	1397064
100	100	1572211
110	110	1710688
120	120	1869844
Squared correlation coefficient(r^2)		0.9993
Slope		31068
Y- intercept		421.10

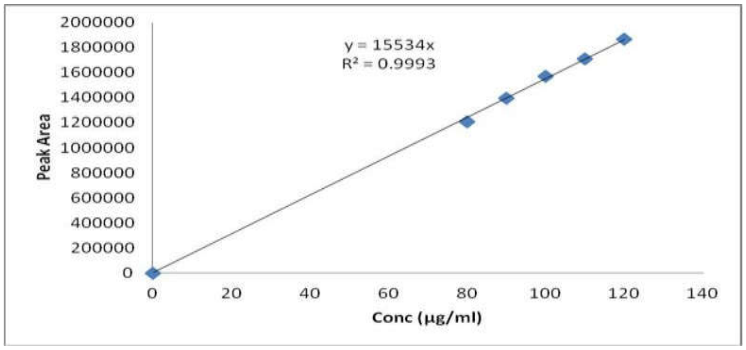

Fig.No.34 Curcumin Linearity

Data Interpretation: The correlation coefficient was found to be 0.9993. From the above study it was established that the linearity of test method is from 80 µg/ml to 120µg/ml of target concentration shown in linearity plots.

ROBUSTNESS

It is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and indication of its suitability during normal usage. Examples of typical variations in assay, impurities and dissolution method validation by HPLC are Influence of variation in buffer composition Different columns Mobile phase flow rate

Acceptance criteria: The system suitability parameters such as % RSD of peak area, tailing factor and theoretical plates of replicate injections of standard preparation should not vary with method parameters during the robustness study**.**

Influence on flow rate variation: robustness of assay method is demonstrated by changing the flow rate for 0.9ml/min and 1.1ml/min instead of specified flow rate (1ml/min). By injecting the 6 replicate injections of standard in 0.9ml/min and 1.1ml/min flow rate and found that system suitability parameters are passed. The % RSD, tailing factor and theoretical plates of Mebeverine HCl standard are within the limits

Table no. 11 Robutness data on flow rate variation

S.NO	System Suitability Parameters	Results		
		Low flow (0.9ml)	High flow (1.1ml)	Criteria
1	% RSD of peak area	0.17	0.32	NMT 2
2	Tailing factor	1.46	1.42	NMT 2
3	Theoretical plate count	9167	8715	NLT 2000

Data Interpretation: Results are within the limit. The analytical method was found to be robust with respect to change in flow rate.

Influence on variation of buffer composition

The robustness of assay method is demonstrated by changing the composition of buffer composition as 0.01% and 0.2% instead of 0.1%.standard solutions was prepared as per test procedure and injected 6 replicates into the chromatograph and evaluated the system suitability parameters. The %RSD, tailing factor and theoretical plates was found to be within limits.

Table no.12. Robustness data on variation of Buffer Composition

S.No	System Suitability Parameters	Results		Acceptance criteria
		Low Buffer (0.01%)	High Buffer (0.2%)	
1	% RSD of peak area	0.24	0.51	NMT 2
2	Tailing factor	0.67	1.02	NMT 2
3	Theoretical plate count	8900	8577	NLT 2000

Data Interpretation: Results are within the limits. The analytical method was found to be robust with respect to change in buffer composition.

Influence on variation columns

The robustness of assay method is demonstrated by changing column manufactures (different brands) instead of Agilent TC- C18 250 X 4.6; 5µ. Standard solutions were prepared as per test procedure and injected 6 replicates into the chromatograph and evaluated the system suitability parameters.

Table no.13. Robustness results on variation of column brands

S.NO	System Suitability Parameters	Results		Acceptance criteria
		Column brand-I (Agilent-C18)	Column brand-II (Equisil-C18)	
1	% RSD of peak area	0.92	0.65	NMT 2
2	Tailing factor	0.38	0.98	NMT 2
3	Theoretical plate count	8345	94567	NLT 2000

Data Interpretation: Results are within the limits. The analytical method was found to be robust with respect to change in buffer composition.

LIMIT OF DETECTION (LOD)

Definition: The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. It is a limit test that specifies whether or not an analyte is above or below a certain value.
It is expressed as a concentration at a specified signal-to-noise ratio, usually two- or three-to-one. Typically acceptable signal-to-noise ratios are 2:1 or 3:1.

Determination

LOD's may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD = 3.3 X S.D/S

Where, = S.D of the response of the response , S = Slope of the calibration curve

Data Interpretation: LOQ was found to be 1.67 µg/ml.

LIMIT OF QUANTITATION (LOQ)

Definition: The quantitation limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals.

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample. Typically acceptable signal-to- noise ratios are 10:1.

Determination

The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula: LOQ = 10 X S.D/S

Where, = S.D of the response of the response , S = Slope of the calibration curve

Data Interpretation: LOQ was found to be 10.28 µg/ml.

SOLUTION STABILITY

Sample and standard solutions should be tested over 24-48hrs period under normal laboratory conditions and refrigerated conditions (2°-8°C) and potency of solution should be determined by comparison to freshly prepared standards. Data should also be generated to establish the use before date and storage conditions of the standard solutions.

Method: a study to establish the stability of standard and test preparation at controlled room temperature (25±5°C) and refrigerated (2-8°C) condition was conducted over a period of 48hrs. the difference in percentage assay of standard and test preparations a initial, after 24hrs and after 48hrs of room temperature and refrigerator conditions were found within the limits.

Table.no.14 Stock solution stability data at room temperature (25±5°C)

Time	At controlled room temperature (25±5°C)	
	Assay (%)	Difference (%)
Initial	99.7	NA
After 24hrs	99.9	0.2
After 48hrs	100.3	0.6

Table.no.15. Stock solution stability data at refrigerated temperature (2-8°C)

Time	At refrigerator (2-8°C)	
	Assay (%)	Difference (%)
Initial	99.7	NA
After 24hrs	100.1	0.4
After 48hrs	100.3	0.6

Table.no.16. Sample solution stability at room temperature (25±5°C)

Time	Sample 1 (Vicco turmeric cream)		Sample 2 (Kasturi Turmeric Churna)	
	Assay %	Difference %	Assay %	Difference %
Initial	100.8	NA	100.9	NA
After 24hrs	101.5	0.7	101.2	0.3
After 48hrs	101.3	0.5	101.5	0.6

Table.no.17 Sample solution stability data at refrigerated temperature (2-8°C)

Time	Sample 1(Vicco turmeric cream)		Sample 2 (Kasturi Turmeric Churna)	
	Assay %	Difference %	Assay %	Difference %
Initial	100.8	NA	100.9	NA
After 24hrs	101.9	1.1	101.2	0.3
After 48hrs	101.2	0.4	101.4	0.5

Acceptance criteria: The solutions are considered 'STABLE', if the difference in % assay results from initial to 24hrs and 48hrs is NMT 2%.

Data Interpretation: Results are within the limits. Standard and test preparations are stable for a period of 48hrs at room temperature (25±2°C) and at refrigerator condition (2-8°C).

[

FILTER COMPATIBILITY

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Centrifuged and filtered different portions of the test preparation separately injected and analysed.

Acceptance criteria

The difference between the centrifuged and filtered sample solutions should be within ± 2% for assay and dissolution methods. The difference between the centrifuged and filtered sample solutions should be within ± 2% for impurities determination. A study was conducted to determine the effect of filter on the assay of Vicco turmeric cream and Kasturi Turmeric Churna test preparations were prepared as per test procedure. Centrifuged and filtered different portions of the test preparation separately through nylon and PVDF (polyvinyl idene di fluoride) and analysed for % assay.

Table.no18: Filter compatibility data for Vicco turmeric cream through nylon filter

Sample no	Centrifuged sample (%Assay)-A		Filtered through 0.45µm, 47mm PVDF filter (%Assay)-B		%Difference (C=A-B)	
	Cream	Churna	Cream	Churna	Cream	Churna
1	101.6	99.77	101.1	99.43	0.50	0.34
2	99.69	100.21	99.64	98.67	0.05	1.54

Table.no.19. Filter compatibility data for Kasturi Turmeric Churna through PVDF filter

Sample no	Centrifuged sample (%Assay)-A		Filtered through 0.45µm, 47mm Nylon filter (%Assay)-B		%Difference (C=A-B)	
	Cream	Churna	Cream	Churna	Cream	Churna
1	99.78	98.34	98.76	98.22	1.02	0.12
2	101.33	100.32	98.46	99.33	2.87	0.99

Data Interpretation: Results are within the limits. The analytical method was found to be FREE from filter interferences

SUMMARY

Validation Parameter	Acceptance Criteria	Results
SYSTEM SUITABILITY	The RSD Should be NMT2% for each peak	0.17%
SPECIFICITY	The interference of the diluents/placebo is considered insignificant, if the chromatogram of the placebo shows no peak, at the retention time of analyte peak	The method separates the peaks of potential degradants formed after forced degradation studies with the resolution more than 1.5. Hence it can be concluded that the method is specific in nature. The results of the forced degradation study.
PRECISION		
1.INTRA DAY	The %RSD of 5 replicate injections should be NMT 2.0%	%RSD is 0.42
2.INTER DAY	The% RSD calculated on 6 determinations of assay value should be NMT 2%	%RSD is 0.55
LINEARITY	The correlation coefficient should be NLT 0.9998	0.9993
ACCURACY	The method is considered accurate, if average recovery is NLT 98% AND nmt 102%.	Vicco turmeric cream- 99.76 Kasturi Turmeric Churna - 99.84
LOD & LOQ	-	LOD was found to be 1.67 µg/ml and LOQ was found to be 10.28 µg/ml.
ROBUSTNESS	The system suitability parameters should not vary with method parameters during robustness study.	Test results are within the limits.
SOLUTION STABILITY	Solutions are considered stable, when difference in % assay results from initial to 24hrs and 48hrs is NMT 2.0%	Results are within the limits.
FILTER COMPATIBILITY	1.For assay and dissolution methods-±2% of 2.For impurities determination-±5%	% assay was found to be within the limits.

Summary of degradation studies

S.No	Degradation Parameters	Sample type	Degradants Products RT (min)	Resolution	% Assay	% Degradation	Remarks
1	Acid hydrolysis	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 98.87 98.92	 2.2%	
2	Acid hydrolysis	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 96.87 97.87	 4.1% 3.5%	
3	Neutral Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			99.87 99.23	 1.4%	
4	Direct Sunlight Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM	2.21, 3.45, 4.5, 5.24, 6.56, 8.93, 9.12	2.19, 1.8, 2.4, 2.5, 2.9, 3.1, 4.6	- 97.95 98.43	 2.9% 2.1%	Un-Known degradation products were observed during forced degradation
5	Oxidative Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 96.76 97.83	 3.8% 2.8%	
6	UV Light Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 97.91 98.56	 2.8% 2.2%	
7	Thermal Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 95.87 96.14	 4.8% 3.9%	
8	Humidity Degradation	PURE CURCUMIN KASTURI PASUPU VICCO TERMIRIC CREAM			- 98.38 97.05	 2.2% 3.1%	

Conclusion

In the present research work, the RP-HPLC method for the estimation of Curcumin in Vicco cream and Kasturi turmeric churna was developed specific. The method doesn't suffer any interference due to common ingredients of the cream and churna formulations. Forced degradation studies also show that degradants doesn't interfere with the drug peak. The mobile phase used consists of methanol, acetonitrile, and 5% acetic acid (35: 50:15, v/v). A Agilent-TC C_{18} column (250 X 4.6 mm; 5µ) was used as the stationary phase. The detection was carried out using UV detector set at 420 nm. The solutions are chromatographed at a constant flow rate of 1.0 ml/ min. The retention time for curcumin was 4.92 min. The quantitative estimation (Assay) was carried out on Vicco turmeric cream and Kasturi Turmeric Churna using RP-HPLC and the results obtained are subjected to the statistical validation. Correlation co-efficient was greater than 0.999 and %RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries vary from 98.0 – 102.0%. The results obtained on the validation parameters met the ICH and USP requirements. It is inferred that the method was found to be *simple, specific, precise, accurate and linear*. The developed method can be used in pharmaceutical industry for routine analysis of curcumin in cream and churna based formulation.

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