Stability-indicating RP-HPLC determination of Curcumin in Vicco Turmeric cream and Haridrakhand churna.

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INTRODUCTION

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione (Fig. 1) is a yellow colored phenolic pigment obtained from powdered rhizome of Curcuma longa Linn. (Family: Zinziberaceae)[1], from ancient it was being used for relieving the pain and inflammation since ancient times in traditional medicine. Extensive researches have also revealed the potent anti-inflammatory effects of curcumin[2-4]. It blocks the synthesis of certain prostaglandins[3], reduces pro-inflammatory cytokine synthesis[5-7], inhibit pro-inflammatory arachidonic acid as well as neutrophils aggregation[8,9] when inflammatory conditions occurs. However the oxygen radical scavenging activity[10,11] of Curcumin has also been observed in its anti-inflammatory effects[12]. Curcumin is unstable at basic pH and undergoes alkaline hydrolysis in alkali/higher pH solution. Decomposition of Curcumin in Hydrolytic decomposition is reported in in vitro physiological condition (isotonic phosphate buffer, pH 7.2) [13-15]. It undergoes photodegradation while exposing to light in solution as well as in solid form[13]. There are already various methods developed for the analysis of Curcumin in the literature like UV[16], HPLC[17-19], TLC[20,21] and HPTLC[22], but there are very few reports on analytical methods for the estimation of curcumin in bulk and its dosage form. Stability-indicating method reported by Ansari et al.[23] and in this paper pure curcumin was brought under stress condition. The International Conference on Harmonization (ICH) guideline entitled ‘stability testing of new drug substances and products’ requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance[24]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability-indicating method quantifies the percent drug and also resolves its degradation product, it can be employed as a stability-indicating. The newly developed method can be used in pharmaceutical industry for routine analysis of Curcumin in cream and churna formulations.

Abstract

A simple, specific, precise and stability-indicating HPLC method of analysis of Curcumin both as a bulk drug and in cream and churna formulations was developed and validated. Chromatographic separation were achieved using Lachrom HPLC with Lichrospher, ODS, (250× 4.6) mm, 5 μ column at ambient temperature. Mixture of ACN: THF: 2% Aceticacid: Water (35: 30: 20:15) was used as mobile phase and delivered at constant flow rate of 0.5 ml/min. 429 nm was selected as wave length for detection of method. The Curcumin peak was obtained at RT 6.20. The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.996, in the concentration range 1–4 μg/ml. The value of slope, intercept and correlation coefficient were found to be 3E+06, 478941 and 0.9985. The method was validated for specificity, precision and recovery. The limits of detection and quantitation were 52.9 ng/ml and 160 ng/ml respectively. Curcumin was subjected to acid and alkali hydrolysis, oxidation and photodegradation. The drug undergoes degradation under acidic, basic, light and oxidation conditions. This indicates that the drug is susceptible to acid, base hydrolysis, oxidation and photo oxidation. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating. The newly developed method can be used in pharmaceutical industry for routine analysis of Curcumin in cream and churna formulations.
2. Experimental
2.1. Materials

Curcumin was purchased from Loba Chemicals Bangalore, India. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

2.2. HPLC instrumentation

The chromatographic system consists of a L-7110 solvent delivery system (Merk Hitachi), L-7400 double beam UV detector (Merk Hitachi), L-7500 integrator and a rheodyne injector valve bracket fitted with a 20 μl sample loop.

2.3. Chromatographic conditions

Chromatographic separation were performed on a stainless steel lichroCART ODS Column, (250×4.6 mm) packed with 5 μ particle diameter, LichroCART HPLC guard cartridge system and a winchrom software. Mobile phase consisting of a mixture ACN, THF, 2 %Acetic acid and Water (35:30:20:15), was delivered at a flow rate of 0.5 ml/ min with detection at 429 nm.

2.4. Sample 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 tubes.

2.5 Calibration curves of curcumin

A stock solution of curcumin (1 mg/ml) was prepared in methanol by transferring 50 mg of drug in 50 ml volumetric flask. 5 ml of this solution was transferred to 50 ml volumetric flask and volume was made up to the mark. From this solution concentration of 1, 1.5, 2.0, 2.5, 3, 3.5 and 4 μg/ml were prepared by diluting 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4 ml of previously prepared solution up to 10 ml with methanol in different 10 ml volumetric flask. The slope, intercept and correlation coefficient were found to by 3E+06, 478941 and 0.9985. Calibration curve of Curcumin has been shown in Figure 1.

No significance difference was observed between the slopes of the calibration curve (P>0.005).

3. Validation of Proposed Method

3.1 Specificity

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 cream and Haridrakhand churna after degradation.

3.1.1 Acidic hydrolysis

1 ml of the stock solution of Curcumin (1 mg/ml) was transferred to a 10 ml volumetric flask. 1 ml 1 N HCl was added and kept for six hours at 90°C in dark to avoid the possible degradation effects of light. 1 ml of the solution was transferred in to 100 ml volumetric flask and diluted up to the mark with methanol to get 10 μg/ml. 2.5 ml of this solution was transferred to a 10 ml volumetric flask and diluted up to the mark with methanol to give 2.5 μg/ml solution.

500 mg Vicco cream was transferred to a 10 ml volumetric flask. 1 ml 0.05 N HCl was added to it and
kept for six hours at 90°C in dark to avoid the possible degradation effects of light.

500 mg Haridrakhand churna was transferred to a 10 ml volumetric flask. 1 ml 0.5 N HCl was added to it and kept for six hours at 90°C in dark to avoid the possible degradation effects of light.

3.1.2. Alkali degradation

1 ml of the standard stock solution of Curcumin (1 mg/ml) was transferred to a 10 ml volumetric flask. 1 ml 1 M NaOH was added to it and kept for six hours at 90°C in dark to avoid the possible degradation effects of light. 1 ml of the solution was transferred in to 100 ml volumetric flask and diluted up to the mark with mobile phase to get 10 μg/ml. 2.5 ml of this solution was transferred to a 10 ml volumetric flask and diluted up to the mark with mobile phase to get test concentration of 2.5 μg/ml.

500 mg Vicco cream was transferred to a 10 ml volumetric flask. 1 ml 0.01 M NaOH was added to it and kept for six hours at 90°C in dark to avoid the possible degradation effects of light.

3.1.3. Direct sunlight

The 50 mg of Curcumin, 500 mg Vicco cream and 500 mg Haridrakhand Churna were separately dissolved in 10 ml of methanol in 10 ml volumetric flask and exposed to direct sunlight and UV chamber at 254 nm for 24 h. The resultant solutions were then further diluted with methanol to get test concentration of 2.5 μg/ml.

3.1.4. Oxidative degradation by peroxide

1 ml of standard stock solution of Curcumin (1 mg/ml) was transferred in a 10 ml volumetric flask. 1 ml of 30 % H₂O₂ was added to it and kept for 6 hours on boiling water bath. Volume was made up to the mark with methanol. 1 ml was transferred to 10 ml volumetric flask and volume was made up to the mark. Further 2.5 ml was transferred to 10 ml volumetric flask and volume was made up to the mark and 20 μl was injected.

500 mg Vicco cream was weighed and transferred to a 10 ml volumetric flask. 1 ml 30 % H₂O₂ was added to it and kept on boiling water bath for 6 hours.

500 mg Haridrakhand churna was weighed and transferred to a 10 ml volumetric flask. 1 ml 30 % H₂O₂ was added to it and kept on boiling water bath for 6 hours. It was diluted to 10 ml with methanol.

3.1.5. Degradation by UV light

The 50 mg of Curcumin, 500 mg Vicco cream and 500 mg Haridrakhand Churna were separately dissolved in 10 ml of methanol in 10 ml volumetric flask and exposed to direct sunlight and UV chamber at 254 nm for 24 h. The resultant solutions were then further diluted with methanol to get test concentration of 2.5 μg/ml.

3.1.6. Thermal Degradation

The 50 mg of Curcumin, 500 mg Vicco cream and 500 mg Haridrakhand Churna were separately kept in porcelain dish at 90°C in hot air oven for 5 hours. It was dissolved in 10 ml of methanol in 10 ml volumetric flask and the resultant solutions were then further diluted with methanol to get test concentration of 2.5 μg/ml.

3.2. Linearity and Range

Linearity is accessed by visualizing the graph of calibration curve. The points in the calibration curve distributed equally above and below the trend line show linearity.

3.3. Precision

3.3.1 Repeatability

Repeatability was accessed by six replicate injections of 2.5 μg/ml solution of drug prepared for standard stock solution. 20 μl volume was injected.

3.3.2 Intraday

2 ml, 2.5 ml and 3 ml was taken out from the 10 μg/ml Curcumin solution and diluted to 10 ml to make 2 μg/ml, 2.5 μg/ml and 3 μg/ml respectively. Three replicates were injected three times a day.

3.3.3. Interday

Same procedure was followed and three replicates were injected in three days.

3.4 Accuracy

3.4.1 Recovery of Vicco cream

300 mg cream was accurately weighed and transferred to a 10 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 μ syringe filter. Three replicate samples were prepared and spiked with 1 μg/ml, 1.5 μg/ml and 2 μg/ml pure Curcumin respectively. Three injections (20 μl) of each sample were injected. Data of recovery of Vicco cream has been shown in Table 1.

3.4.2 Recovery of Haridrakhand Churna

100 mg churna was accurately weighed and transferred to a 10 ml volumetric flask and 5 ml methanol was added and
Stability-indicating RP-HPLC determination of Curcumin in Vicco Turmeric cream and Haridrakhand churna

sonicated for 5 minutes to extract the Curcumin. Then the volume was made up to the mark and centrifuged for 1.5 minutes at 2000 rpm. The centrifuged solution was filtered with 0.45 μ syringe filter. 0.3 ml was taken and diluted to 10 ml. and 20 μl were injected. Three replicate samples were prepared and spiked with 1 μg/ml, 1.5 μg/ml and 2 μg/ml pure Curcumin respectively. Three injections (20 μl) of each sample were injected. Data of recovery of Vicco cream has been shown in Table 2.

3.5. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated through linear regression method.

3.6. System suitability testing

System suitability testing was performed by using six replicates of test concentrations. Variations in Tailing factor, asymmetry factor and R.T. were calculated. Number of theoretical plates (N) and HETP were calculated. Results have been shown in the Table 3.

4. Estimation of Curcumin in formulation

4.1 Optimization of extraction time for the method

Four samples of the Vicco cream were prepared according to the method given in the above section and sonicated for 5, 10, 15 and 20 minutes and 20 μl were injected. Centrifugation for 2 minutes at 2000 rpm was sufficient to settle the undissoved matter.

Four samples of the Haridrakhand churna were prepared in the mobile phase and sonicated for 5, 10, 15 and 20 minutes and 20 μl was injected immediately. Centrifugation for 1.5 minute at 2000 rpm was sufficient to settle the undissoved matter.

4.2 Estimation of Curcumin in Vicco cream

500 mg Vicco cream was accurately weighted and transferred to a 10 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 μ syringe filter and 20 μl was injected.

4.3 Estimation of Curcumin in Haridrakhand churna

500 mg Haridrakhand churna was accurately weighted and transferred to a 10 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 1.5 minutes at 2000 rpm. The centrifuged solution was filtered with 0.45 μ syringe filter.

**Table 1: Recovery study of Vicco cream**

<table>
<thead>
<tr>
<th>Conc. found before spiking (μg/ml) C₁</th>
<th>Conc. of Std. added (μg/ml) C₂</th>
<th>Conc. found after Spiking (μg/ml) C₃</th>
<th>% Recovery ((C₃/C₁) \times 100/C₂)</th>
<th>Mean ± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.68</td>
<td>0.49</td>
<td>2.18</td>
<td>102.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.17</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.19</td>
<td>104.08</td>
<td>100.49±0.023</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.69</td>
<td>98.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.68</td>
<td>1.024</td>
<td>2.71</td>
<td>100.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.68</td>
<td>97.65</td>
<td></td>
<td></td>
</tr>
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</table>

**Table 2: Recovery study of Haridrakhand churna**

<table>
<thead>
<tr>
<th>Conc. found before spiking (μg/ml) C₁</th>
<th>Conc. of Std. added (μg/ml) C₂</th>
<th>Conc. found after Spiking (μg/ml) C₃</th>
<th>% Recovery ((C₃/C₁) \times 100/C₂)</th>
<th>Mean ± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.86</td>
<td>0.48</td>
<td>2.35</td>
<td>102.08</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2.36</td>
<td>104.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.34</td>
<td>96</td>
<td>100.16±0.026</td>
<td>2.653</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.88</td>
<td>100</td>
<td></td>
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</tr>
<tr>
<td>1.86</td>
<td>1.035</td>
<td>2.89</td>
<td>98.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.86</td>
<td>96.62</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Summary of system suitability parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Number of theoretical plates (N)</td>
<td>17500</td>
</tr>
<tr>
<td>2.</td>
<td>Height equivalent to theoretical plate (HETP)</td>
<td>$1.42 \times 10^{-3}$</td>
</tr>
<tr>
<td>3.</td>
<td>Retention time</td>
<td>6.44</td>
</tr>
<tr>
<td>4.</td>
<td>Capacity factor</td>
<td>2.078</td>
</tr>
<tr>
<td>5.</td>
<td>Tailing factor</td>
<td>0.87–0.90</td>
</tr>
<tr>
<td>6.</td>
<td>Asymmetry factor</td>
<td>0.85–0.88</td>
</tr>
</tbody>
</table>

0.3 ml was taken and diluted to 10 ml and 20 μl were injected.

5. RESULTS AND DISCUSSION

5.1 Validation of the proposed method

5.1.1 Specificity

The method separates the peak of excipients in the Vicco cream with resolution 2.4 and Haridrakhand churna shows no extra peak except Curcumin peak. Also the method separates the peaks of potential degradants formed after forced degradation studies with the resolution more than 2. Hence it can be concluded that the method is specific in nature. The results of the forced degradation study.

5.1.2 Linearity

Two points exists above the calibration curve, two points exists below the calibration curve and one point on calibration curve shows the linearity.

5.1.3 Range

Linearity range: 1 – 4 μg/ml.  
Target range: 2, 2.5, 3 μg/ml.  
Working range: 0.16–4 μg/ml.  
Target concentration: 2.5 μg/ml.

5.1.4 Precision

5.1.4.1 Repeatability

RSD of six replicates injection of test concentration (2.5 μg/ml) was 0.438, hence method is repeatable because the calculated RSD is less then one.

5.1.4.2 Intraday and interday

The mean RSD for intraday and interday precision was 0.704 and 0.672 respectively which is less than two concluding method is precise.

5.1.5 Accuracy

5.1.5.1 Recovery study of Vicco cream (Table 2)

5.1.5.2 Recovery study of Haridrakhand churna (Table 3)

5.1.6 Limit of quantification (LOQ) and limit of detection (LOD)

LOD and LOQ of the method were found to be 52.9 ng/ml and 160 ng/ml respectively.

5.1.7 System suitability

Summary of system suitability parameters is given in Table 3.

Table 4: Summary of validation parameters

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Specificity</td>
<td>Resolution of degradants and excipients with drug peak &gt; 1.5,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hence method is specific.</td>
</tr>
<tr>
<td>2.</td>
<td>Linearity</td>
<td>Method shows linearity between 1–4 μg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linearity range: 1–4 μg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Target range: 2, 2.5, 3 μg/ml</td>
</tr>
<tr>
<td>3.</td>
<td>Range</td>
<td>Working range: 0.16–4 μg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Target concentration: 2.5 μg/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cream: 97.65–104.08 %</td>
</tr>
<tr>
<td>4.</td>
<td>Accuracy (%recovery)</td>
<td>Churna: 96–104.17 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Repeatability: 0.438</td>
</tr>
<tr>
<td>5.</td>
<td>Precision (RSD)</td>
<td>Intraday: 0.704</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interday: 0.672</td>
</tr>
<tr>
<td>6.</td>
<td>LOD and LOQ</td>
<td>52.9 and 160 ng/ml respectively</td>
</tr>
</tbody>
</table>
Figure 2 Chromatogram obtained after acidic hydrolysis of A) Pure Curcumin B) Vicco cream and C) Hridrakhand churna
Figure 3 Chromatogram obtained after alkali degradation of A) Pure Curcumin B) Vicco cream and C) Hridrakhand churna
Figure 4 Chromatogram obtained after degradation from direct sunlight of A) Pure Curcumin B) Vicco cream and C) Hridrakhand churna
Figure 5 Chromatogram obtained after oxidative degradation of A) Pure Curcumin B) Vicco cream and C) Hridrakhand churna
Figure 6 Chromatogram obtained after degradation by UV of A) Pure Curcumin B) Vicco cream and C) Haridrakhand churna
Summary of validation parameters has been given in the Table 4

5.2 Estimation of Curcumin in formulation

Extraction time was optimized to 12 min. for cream and 10 min for churna and content of Curcumin was found to be 5.608 and 18.59 μg/ml for cream and churna formulation respectively.

5.3 stability indicating property

The chromatograms of the samples degraded with acid, base, hydrogen peroxide and light showed well-separated spots of pure Curcumin as well as some additional peaks at different RT values. The spots of degraded product were well resolved from the drug spot as shown in Figure 2 – 6. The number of degradation products with their RT values, resolution with Curcumin peak remained and percentage recovery were calculated and listed in Table 5.

5.4 CONCLUSIONS

In the present work the RP-HPLC method for the estimation of Curcumin in Vicco cream and Haridrakhand churna has been developed. The method is simple, precise, accurate and 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 newly developed method can be used in pharmaceutical industry for routine analysis of Curcumin in cream and churna formulations.

REFERENCES:

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