ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CURCUMIN IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT
A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of curcumin in tablet dosage form. Chromatography was carried out on a pre-packed Lichrocart Lichrosphere 250*4.0mm, 5um using filtered and degassed mixture of Sodium acetate Buffer: Acetonitrile as mobile phase at a flow rate of 1.0ml/min with gradient elution method and effluent was monitored at 250nm. The method was validated in terms of linearity, precision, accuracy, and specificity, robustness and solution stability. The method does require only 6 minutes as run time for analysis which prove the adoptability of the method for the routine analysis of the drug.

KEYWORDS: Curcumin, Method development, Gradient elution, Validation

INTRODUCTION
Curcumin5 is chemically described as (1E,6E)-1,7-bis (4 hydroxy- 3 methoxyphenyl) – 1,6 Heptadiene – 3,5-dione. Curcumin incorporates several functional groups. The aromatic ring systems, which are polyphenols are connected by two α,β-unsaturated carbonyl groups. The two carbonyl groups form a diketone. The diketone form stable enols or are easily deprotonated and form enolates, while the α,β-unsaturated carbonyl is a good Michael acceptor and undergoes nucleophilic addition. Curcumin is known for its anti tumor, antioxidant, anti arthritis, anti amyloid and anti inflammatory properties. In addition it may be effective in treating malaria, prevention of cervical cancer, and may with the replication of the HIV virus Interfere.

The object of the present study is to develop and validate a simple, accurate and rapid new method for estimation of Curcumin in tablet dosage form by RP-HPLC1-4.

MATERIALS AND METHODS
Working standards of Curcumin was obtained from well reputed research laboratories. HPLC grade Methanol, Acetonitrile, Merck grade Tetrahydrofuran, Sodium acetate trihydrate and Milli-Q water were procured from the market. The separation was carried out on Gradient HPLC system Shimadzu 2010A HT (Class vp6.13v) with pre-packed Lichrocart Lichrosphere 250*4.0mm, 5um column using filtered and degassed mixture of Buffer:Acetonitrile as mobile phase.

Preparation of Solvent A
Weighted and dissolved 6.8 gm of sodium acetate trihydrate in 1000 ml of water. Adjusted the pH 4.5 with glacial acetic acid and filtered the solution through 0.45 µm membrane filter.

Preparation of Solvent B
Mixed Acetonitrile 1000ml and 50 ml of Tetrahydrofuran and sonicated both the mixture A and B in the ultrasonic bath for 15 to 20 minutes.
Preparation of Standard solution
50mg of Curcumin working standard was accurately weighed and transferred it into a 50ml volumetric flask. 5 ml of standard solution A and 30 ml of methanol were added in it and sonicated the mixture in the ultrasonic bath for 10 to 15 minutes and made up the volume with methanol and Mixed well.

Chromatographic conditions
Flow rate 1.0ml/min; detection wavelength 250nm; injection volume 20µl; column used Lichrocart Lichrosphere 250*4.0mm, 5um; column temperature: 25°C; mobile phase: Buffer : Acetonitrile.
Filled the HPLC vials with the standard and placed the vials in the vial tray in HPLC. Gradient programme was choosen for optimized method which is given in Table 1. Run the mobile phase to find the peaks. Results are shown in the graph.

Method development
Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation
1.250gm of tablet sample (equivalent to 50mg of Curcumin) was accurately weighed and transferred it into a 25ml volumetric flask and 15 ml of methanol was added and sonicated in the ultrasonic bath for 30 minutes with occasional swirling. Made up the volume with methanol and filtered the solution through 0.45 um Teflon membrane filter.

Procedure
20µl of the standard preparation and assay preparation were separately injected and chromatographed (Fig-1). The results obtained for Curcumin was shown in Table 2.

RESULT AND DISCUSSION
Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of Curcumin. The assay was linear over the concentration range of 50mcg-150mcg/ml for Curcumin. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the excipients of Curcumin and was found to be 98.1% -100.2% and 99.1%-102.79% within precision RSD of 0.821%. The ruggedness and robustness %RSD were found within the limits.

CONCLUSION
The developed RP-HPLC method is simple and selective for estimation of Curcumin in tablet dosage form was found to be accurate, rapid and sensitive. The values of coefficient of variance were satisfactory low and recovery was close to 100% indicating reproducibility of the method. The linearity was observed within limit hence method is linear.

REFERENCES
Table 1: Gradient program used

<table>
<thead>
<tr>
<th>Time</th>
<th>Solvent A</th>
<th>Solvent B</th>
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<tbody>
<tr>
<td>0.01</td>
<td>90</td>
<td>10</td>
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<tr>
<td>10</td>
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<td>40</td>
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<tr>
<td>45</td>
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<td>10</td>
</tr>
<tr>
<td>50</td>
<td>90</td>
<td>10</td>
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Table 2: Validation Summary

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Curcumin</th>
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<td>System Suitability*</td>
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<td>Theoretical Plates (N)</td>
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<tr>
<td>Asymmetry</td>
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<tr>
<td>Retention Time</td>
<td>4.476</td>
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<tr>
<td>Precision* (% RSD)</td>
<td>0.821%</td>
</tr>
</tbody>
</table>

Ruggedness* Day to Day (% RSD) 0.87%

Robustness* Changing pH (%RSD) 0.42%, 1.180%
Changing flow rate (%RSD) 0.527%, 0.769%
Changing Temperature (%RSD) 0.427%, 0.679%

| Linearity                   | 50 mcg/ml – 150mcg/ml |
| Correlation co-efficient (R²) | 0.999            |
| Accuracy (%)                | 98.1% - 100.2%     |

*Average of six determinations

Figure 1: Chromatogram of Curcumin
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