Validated method for estimation of curcumin in turmeric powder

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Curcumin, the active molecule present in Curcuma longa is known for its antitumour, antioxidant, antiarthritic, anti-amyloid, anti-ischemic, anti-inflammatory activities. In addition, it may also be effective in treating malaria, prevention of cervical cancer, etc. An efficient, sensitive, and precise high-performance thin-layer chromatography (HPTLC) method has been developed for determination of curcumin in several marketed spices sample of turmeric powder and has been compared with an in-house turmeric powder. The HPTLC separation was performed on precoated aluminium backed HPTLC plates of 0.2 mm layer thickness with silica gel 60 F254 with dichloromethane and methanol (99:1) combination as mobile phase. The plate was developed up to 80 mm at temperature of 20 ± 4°C with 10 min. of chamber saturation. Under this condition the retardation factor (Rf) of curcumin is 0.43 and the compound was quantified at its absorbance maxima (λmax) at 427 nm. The limit of detection and limit of quantification were found to be 49 ng and 148 ng per spot, respectively. The response of curcumin was linear over the range of 0.8 µg to 1.3 µg per spot with correlation coefficient 0.99395 indicates good relationship between peak area versus concentration. Recovery values from 99.60 to 99.73 % showed that the reliability and reproducibility of the method were excellent. The proposed method may be useful as an accurate, simple, cost effective and sensitive for quantitative estimation of curcumin.

Keywords: HPTLC, Curcumin, spices, Curcuma longa, Turmeric powder

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Curcuma longa Linn. (turmeric) belongs to the family Zingiberaceae. The plant has a lot of potential in terms of medicinal properties. Literature reveals its anti-inflammatory, cholagogue, hepatoprotective, blood-purifier, antioxidant, detoxifier and regenerator of liver tissue, antiasthmatic, anti-tumour, antiprotozoal, stomachic, carminative properties. It reduces high level of cholesterol in plasma. Its antiplatelet activity offers protection to heart and vessels. It also prevents DNA damage in lymphocytes. Several constituents present in this plant include curcumin (a flavonoid) (Fig.1); demethoxycurcumin, bisdemethoxycurcumin, volatile oils like turmerone, atlantone, zingiberene, sesquiphellandrene, terpinolene, phellandrene, p-cymene, cineol, caryophyllene, nerolidol, curlene, dehydrozingerone, zerumbone, germacrene, sesquiterpenes etc. Curcumin has a molecular formula C21H20O6, molecular weight: 368.91 and melting point 183ºC. It’s the main active constituent of Curcuma longa. The molecule has a broad spectrum of activities including antioxidant, anti-inflammatory, anticarcinogenic, hypocholesterolemic, antibacterial, wound healing, antispasmodic, anticoagulant, antitumor and hepatoprotective. It is also a potent free radical scavenger, having superoxide anions, singlet oxygen, hydroxyl radicals scavenging and lipid peroxidation inhibitory activities. HPTLC shows advantages of low operating cost, high sample throughput and need for minimum sample clean-up. Another major advantage is simultaneous application of several samples using small quantity of mobile phase. HPTLC makes scanning in situ and repeated detection of the chromatogram with the same or different parameters possible. HPTLC analysis of many plants used in Indian Systems of Medicine has been performed. Thus, the aim of the present work

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Fig.1 — Structure of curcumin
was to develop accurate, specific, repeatable HPTLC method for the determination of curcumin in plant extracts.

**Methodology**

**Chemicals and reagents**

Standard curcumin was obtained from Sigma Chemicals, Germany. All other chemicals and reagents used were from E-Merck and of analytical grade.

**Preparation of standard stock solution**

Stock standard solutions containing 1 mg/10ml of curcumin in methanol were freshly prepared before use. This same solution was used for making the calibration curve by applying different volume of standard to get different amount of standard per spot.

**Preparation of sample solution**

Five different brands of turmeric powder were purchased from the local market and the in-house turmeric powder was prepared from dried rhizome of turmeric. They were weighed accurately and extracted with methanol (20 ml X 5 times). Percentage yield of all of them were recorded (Table 1). Sample solutions were prepared from these extracts and the concentrations of these were as follows:

- Brand 1 (B1): 10.4 mg/10 ml
- Brand 2 (B2): 10.6 mg/10 ml
- Brand 3 (B3): 10.0 mg/10 ml
- Brand 4 (B4): 10.0 mg/10 ml
- Brand 5 (B5): 10.3 mg/10 ml

**Instrumentation and chromatographic conditions**

The spots were applied as bands with a band length of 5 mm and the distance between the tracks as 10 mm with the help of Camag HPTLC applicator Linomat IV. Stationary phase used was precoated silica gel G F<sub>254</sub> plates (20cm X 10cm) from E-Merck and the mobile phase composition was optimized to dichloromethane: methanol::99:1. The plate was developed in a Camag twin trough chamber after a chamber saturation time (10 minutes) for mobile phase. The length of chromatogram run was 8 cm. After developing, the TLC plates were dried by air blowing. The densitometric analysis was performed on a Camag TLC scanner in the absorbance mode at 427 nm. Chromatogram of standard curcumin and the in-house sample has been presented (Figs. 2 & 3).

**Preparation of calibration graphs**

Calibration curves for the standard curcumin were prepared by applying a series of spots of standard with different volume so as to get different amount of curcumin per spot. They were prepared with respect to height and area vs amount per spot.

**Validation procedures**

**Linearity range**

For determining the linearity range of standard curcumin, a series of spots of standard with different volume (1 µl-15 µl) were applied so as to get different

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Table 1 — Percentage yield of methanolic extract of the marketed turmeric powder

<table>
<thead>
<tr>
<th>Weight taken</th>
<th>Extractive Weight</th>
<th>% yield [w/w]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 1</td>
<td>5 gm</td>
<td>0.4914 gm</td>
</tr>
<tr>
<td>B 2</td>
<td>5 gm</td>
<td>0.4558 gm</td>
</tr>
<tr>
<td>B3</td>
<td>5 gm</td>
<td>0.5016 gm</td>
</tr>
<tr>
<td>B 4</td>
<td>5 gm</td>
<td>0.4120 gm</td>
</tr>
<tr>
<td>B5</td>
<td>5 gm</td>
<td>0.4250 gm</td>
</tr>
<tr>
<td>In-house</td>
<td>5 gm</td>
<td>0.5579 gm</td>
</tr>
</tbody>
</table>

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Fig. 2 — HPTLC chromatogram of standard curcumin

Fig. 3 — HPTLC chromatogram of methanolic extract of turmeric powder
amount of curcumin per spot. The plate was scanned and a curve was prepared with respect to height and area vs amount per spot.

Limit of detection and limit of quantitation

Limit of detection (LOD) and quantitation (LOQ) were determined based on the standard deviation of the response and the slope as per ICH guideline\(^\text{15}\). They were calculated based on the following:

\[
\text{Detection Limit} = \frac{3.3\sigma}{S}
\]

\[
\text{Quantification Limit} = \frac{10\sigma}{S}
\]

Where \(\sigma\) = standard deviation of the response 
\(S\) = slope of the calibration curve
\(\sigma\) was determined from the responses of a number of blank samples.

Assay

Standard curcumin and test solutions were spotted on HPTLC plate. The percentage of curcumin present in each test solution was determined by measuring area for the standard and test solutions. Thereby the % of curcumin was calculated for each brand of turmeric powder and compared with the in-house powder.

Recovery studies

Recovery studies were performed by the method of standard addition. B3 test solution was used as *Curcuma longa* extract. 20 µl of the same was used for this purpose. Each time 100 ng standard curcumin were added to that extract and the % recovery was determined (Table 2).

Results and discussion

The linearity range for curcumin was found to be between 0.8 µg and 1.3 µg/spot. Calibration curve was described by the equation \(Y = 22664.828 + 22.046 \times X\) with \(r = 0.99395\) and a standard deviation of 1.11%. LOD and LOQ were found to be 49 ng and 148 ng, respectively. The different percentages of curcumin present in the different brands of turmeric powder have been presented (Table 3) while results of recovery study have been presented in Table 3. Recovery values ranged from 99.60 to 99.73 % with an average of 99.67 %. Using this simple and yet rapid HPTLC method, the methanol extract of turmeric powder obtained from market, was found to contain curcumin in the range of 21.69-31.97 % w/w and with respect to the raw powder, it is 1.82-3.20 % w/w. The in-house standard powder showed curcumin as 34.50 % w/w in methanolic extract and 3.85 % w/w with respect to the raw powder.

Conclusion

Thus the developed method for the estimation of curcumin in marketed turmeric powder can be used routinely with good reliability and reproducibility and it showed presence of lesser amount of curcumin in the marketed turmeric powder.

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**Table 2** — Recovery study of curcumin

<table>
<thead>
<tr>
<th>Excess curcumin added to extract (ng)</th>
<th>Measured content of curcumin in mixture (ng)</th>
<th>Average amount of curcumin found (ng)</th>
<th>Average recovery (%)</th>
<th>Overall average recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>639</td>
<td>636.76</td>
<td>99.65</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>739</td>
<td>736.78</td>
<td>99.70</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>839</td>
<td>836.73</td>
<td>99.73</td>
<td></td>
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<tr>
<td>300</td>
<td>939</td>
<td>935.24</td>
<td>99.60</td>
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</tr>
<tr>
<td>400</td>
<td>1039</td>
<td>1035.77</td>
<td>99.69</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** — Percentages of curcumin present in different brands of turmeric powder

<table>
<thead>
<tr>
<th>Brands</th>
<th>% yield of MeOH extract</th>
<th>Weight of extract taken (mg)</th>
<th>Concentrations prepared (µg/µl)</th>
<th>Volume applied (µl)</th>
<th>Curcumin determined (ng/spot)</th>
<th>% of curcumin in extract</th>
<th>% of curcumin in raw powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>9.83</td>
<td>10.4</td>
<td>1.04</td>
<td>10</td>
<td>225.6</td>
<td>21.69</td>
<td>2.13</td>
</tr>
<tr>
<td>B2</td>
<td>9.12</td>
<td>10.6</td>
<td>1.06</td>
<td>10</td>
<td>279.4</td>
<td>26.35</td>
<td>2.40</td>
</tr>
<tr>
<td>B3</td>
<td>10.03</td>
<td>10.0</td>
<td>1.00</td>
<td>10</td>
<td>319.7</td>
<td>31.97</td>
<td>3.20</td>
</tr>
<tr>
<td>B4</td>
<td>8.24</td>
<td>10.0</td>
<td>1.00</td>
<td>10</td>
<td>220.6</td>
<td>22.06</td>
<td>1.82</td>
</tr>
<tr>
<td>B5</td>
<td>8.50</td>
<td>10.3</td>
<td>1.03</td>
<td>10</td>
<td>302.4</td>
<td>29.35</td>
<td>2.50</td>
</tr>
<tr>
<td>In-house</td>
<td>11.16</td>
<td>10.0</td>
<td>1.00</td>
<td>10</td>
<td>345.0</td>
<td>34.50</td>
<td>3.85</td>
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</table>
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References