Estimation of Berberine in Ayurvedic Formulations Containing *Berberis aristata*

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A sensitive, simple, rapid, and efficient high-performance thin-layer chromatographic (HPTLC) method has been developed and validated for the analysis of berberine in marketed Ayurvedic formulations containing *Berberis aristata* DC for regulatory purposes. Chromatography of methanolic extracts of these formulations was performed on silica gel 60 F254 aluminum-backed TLC plates of 0.2 mm layer thickness. The plate was developed up to 66 mm with the ternary-mobile phase butanol–acetic acid–water (8 + 1 + 1, v/v/v) at 33 ± 5°C with 5 min of tank saturation. The marker, berberine, was quantified at its maximum absorbance of 350 nm. The limit of detection and limit of quantitation values were found to be 5 and 10 ng/spot. The linear regression analysis data for the calibration plot showed a good linear relationship with correlation coefficient = 0.9994 in the concentration range of 10 to 50 ng/spot for berberine with respect to peak area. The instrumental precision was found to be 0.49% coefficient of variation (CV), and repeatability of the method was 0.73% CV. Recovery values from 98.27 to 99.11% indicate excellent accuracy of the method. The developed HPTLC method is very accurate, precise, and cost-effective, and it has been successfully applied to the assay of marketed formulations containing *B. aristata* for determination of berberine.

*Berberis aristata* DC, locally known as Daruharidra (Family Berberidaceae), is a very important medicinal plant. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. Its stem, root, and fruit are used in many Ayurvedic preparations and have always intrigued pharmacologists and clinicians. Traditionally, its root bark is very useful in the treatment of different types of skin diseases, menorrhagia, diarrhea, malaria, jaundice, and eye troubles (1, 2). The decoction of root bark, mixed with an equal quantity of milk, is used to cure piles (3). Berberine is the active constituent of *B. aristata*, which is chiefly found in outer barks of stem and root of the plant. It has a wide range of pharmacological (4) and biochemical (5, 6) effects. It has demonstrated a significant antimicrobial activity against a variety of organisms, including several bacteria, fungi, protozoans, and viruses (7–12). It can also be used as an antidiarrheal, antihypertensive, antiarrhythmic, and anti-inflammatory agent (13–20). The pharmacological action of berberine includes antioxidant (21), antiproliferation (22–24), antibiotic (25, 26), antitumor (27–32), and antimotility (33, 34) properties, and it is also useful in the treatment of type 2 diabetes (35, 36).

The Ayurvedic formulations like Leucoforte (S1) and Piloguard (S2) contain *B. aristata* as one of the ingredients, and these are used for the treatment of leucorrhea and piles, respectively. In the search for a reproducible method for standardization of Ayurvedic formulations containing *B. aristata*, the marker compound, berberine, has been isolated from the root and quantified in the above-mentioned formulations. Today, quantification of bioactive principles through modern analytical tools is essential for establishing the authenticity and creditability of the product. The present study aimed to develop an analytical method for standardization of these formulations based on the bioactive marker.

**Experimental**

**Equipment**

The CAMAG (Muttenz, Switzerland) high-performance thin-layer chromatographic (HPTLC) system included a Linomat 5 sample applicator (100 µL syringe), TLC scanner 3 with winCATS software, and twin-trough plate development glass chamber (20 × 10 × 4 cm). Merck KGaA (Darmstadt,
Figure 1. Densitogram of (A) Leucoforte (S1), (B) Piloguard (S2), and (C) standard berberine at 350 nm.
Germany) aluminium foil-backed TLC plates were precoated with silica gel 60 F254 (20 × 10 cm, 0.2 mm thickness).

**Materials**

The roots of *B. aristata* were collected from a local market and authenticated at the Regional Plant Resource Centre, Bhubaneswar, Orissa, India. A voucher specimen was deposited in the herbarium. The formulations such as Leucoforte and Piloguard were procured from a local market and authenticated at the Central Research Institute (Ayurveda), Bhubaneswar, Orissa, India.

**Chemicals**

Analytical grade solvents used during the course of analysis were obtained from S.D. Fine Chemical Ltd. The standard berberine was isolated from a 50% aqueous ethanolic extract of root by a gravity column chromatographic method followed by recrystallization from ethanol, and characterized by UV, infrared, nuclear magnetic resonance, and mass spectral studies (37–39).

**Standard Solution**

A standard solution of pure isolated berberine was prepared by dissolving 1 mg in 50 mL (20 ng/μL) of analytical grade methanol in a volumetric flask (stock solution). For determination of the limit of detection (LOD) and limit of quantitation (LOQ), 5 mL of the stock solution was diluted to 50 mL (2 ng/μL), and 25 mL of the same solution was diluted to 100 mL (5 ng/μL) for the linearity study.

**Preparation of Sample Solutions**

All of the samples were taken in powdered form (accurately weighed 4 g of each) in a thimble and extracted exhaustively with methanol in a Soxhlet apparatus for 24 h. The soluble portion was filtered under reduced pressure and dried completely. The sample solutions for S1 and S2 were prepared by dissolving 50 mg of each extract in 25 mL methanol.

**Chromatography**

Chromatography was performed on 20 × 10 cm HPTLC plates precoated with 0.2 mm layers of silica gel 60 F254. The

![Figure 2. Overlay of in situ absorption spectra of berberine in sample and standard tracks scanned over the range of 250–400 nm.](image)

![Figure 3. Linear calibration plot for berberine.](image)
plates were prewashed with methanol and activated at 55°C for 30 min prior to chromatography. Sample and standard solutions were applied onto the plate as 6 mm bands using a Linomat 5 applicator with 100 mL syringe under nitrogen flow. The bands were located 10 mm from the bottom of the plate with 12 mm between them. The dosage speed of the syringe was 130 nL/s. Volumes of sample solutions applied (in triplicate) were 8 mL for S1 and 10 mL for S2. For determination of the LOD and LOQ of berberine, the standard solution with a concentration of 2 ng/L was applied on separate plates, and the standard solution, having a concentration of 5 ng/L, was used for calibration of the method. The plate was developed for a distance of 66 mm in a vapor-saturated twin-trough chamber at 33 ± 5°C with 57 ± 3% relative humidity using butanol–acetic acid–water (8 + 1 + 1, v/v/v) as the mobile phase. After development, the mobile phase was removed completely with a stream of hot air, and the developed HPTLC plates were scanned by using a TLC scanner 3 with winCATS software (Version 1.3.4) in absorbance-reflectance scan mode. The slit dimension was set at 5.0 × 0.45 mm (Micro), and 20 mm/s scanning speed with 100 μm/step data resolution was used. The maximum absorbance of berberine was measured at 350 nm using the deuterium lamp source. Concentration of the compound chromatographed was determined from the intensity of diffusely reflected light. Evaluation was done via peak area with linear calibration.

### Results and Discussion

The HPTLC method development required optimization of the mobile phase not only to enable satisfactory separation of the accompanying components from the extracts but also to obtain symmetrical, well-shaped spots. Among the mobile phases investigated for accurate measurement of berberine, the 3-component mobile phase, butanol–acetic acid–water (8 + 1 + 1, v/v/v), was found to be optimal. It gave dense, compact spots with well-resolved densitometric peaks (Figure 1A–C); the Rf value of berberine was 0.32. The peak purity of the separated berberine was assessed by comparing its UV absorption spectra recorded at start to middle and middle to end of the peak at 350 nm, i.e., r (start, middle) = 0.9994 and r (middle, end) = 0.9994. The identity of berberine peak was confirmed by comparing the UV absorption spectrum of the spot from the standard with the corresponding spot from the sample and finding they were superimposable (Figure 2). Chromatography was repeated as described above for the determination of the LOD and LOQ of berberine. The lowest amount of berberine detected on a chromatogram was 5 ng/spot, and the lowest amount for quantification was 10 ng/spot. The calibration plot for the compound was linear over the range of 10–50 ng/spot (Figure 3) and had the linear equation:

\[ y = 43.16x + 186.18 \]

where \( y \) is the peak area response and \( x \) is the amount of berberine. The correlation coefficient (r) was found to be 0.9994. International Conference on Harmonization (ICH) guidelines (CPMP/ICH/381/95, CPMP/ICH/281/95) were followed for the validation of the HPTLC method for accuracy, precision, and repeatability (Tables 1 and 2). The instrument precision was studied by scanning the same spot of berberine 6 times, and a coefficient of variation (CV) of 0.49% was calculated. The repeatability of the method was tested by analyzing 6 applications of the sample solution to the TLC plate; the CV was 0.73%. Variability of the method was

### Table 1. Method validation data for estimation of berberine by HPTLC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument precision (CV, %, n = 6)(^a)</td>
<td>0.49</td>
</tr>
<tr>
<td>Repeatability (CV, %, n = 6)</td>
<td>0.73</td>
</tr>
<tr>
<td>Limit of detection, ng/spot</td>
<td>5</td>
</tr>
<tr>
<td>Limit of quantitation, ng/spot</td>
<td>10</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9994</td>
</tr>
<tr>
<td>Linearity range, ng/spot</td>
<td>10–50</td>
</tr>
<tr>
<td>Standard deviation, %</td>
<td>1.85</td>
</tr>
<tr>
<td>Number of data points</td>
<td>5</td>
</tr>
<tr>
<td>RSD(^b) of slope</td>
<td>0.82</td>
</tr>
<tr>
<td>RSD of intercept</td>
<td>1.31</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
</tbody>
</table>

\(^a\) n = Number of replicates.  
\(^b\) RSD = Relative standard deviation.

### Table 2. Intraday and interday precision study

<table>
<thead>
<tr>
<th>Marker compound</th>
<th>Conc., ng/spot</th>
<th>Intraday precision</th>
<th>Interday precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>15</td>
<td>1.11</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.25</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.95</td>
<td>1.34</td>
</tr>
</tbody>
</table>

### Table 3. Results from the recovery study of berberine by HPTLC

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount of sample, μL</th>
<th>Amount of berberine present, ng</th>
<th>Amount of berberine added, ng</th>
<th>Total amount, ng</th>
<th>Total amount found, ng</th>
<th>Recovery, %</th>
<th>Average recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>16</td>
<td>10</td>
<td>26</td>
<td>25.55</td>
<td>98.27</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>16</td>
<td>20</td>
<td>36</td>
<td>35.43</td>
<td>98.42</td>
<td>98.60</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>16</td>
<td>30</td>
<td>46</td>
<td>45.59</td>
<td>99.11</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Content of berberine in different formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Berberine amount, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucoforte (S1)</td>
<td>$1.582 \times 10^{-2}$</td>
</tr>
<tr>
<td>Piloguard (S2)</td>
<td>$0.889 \times 10^{-2}$</td>
</tr>
</tbody>
</table>

studied by analyzing aliquots of a standard solution of berberine (15, 30, and 45 ng/spot) on the same day (intraday precision) and on different days (interday precision), and the results were expressed as CV. The accuracy of the method was confirmed by a recovery experiment. The recoveries were calculated on the basis of determination of analyte spiked at different levels (10, 20, and 30 ng) to a sample containing a known amount of berberine. The recovery was in the range of 98.27–99.11%, with an average value of 98.60% (Table 3), showing the accuracy of the method. The content of berberine in different formulations is presented in Table 4.

Conclusions

The developed HPTLC technique represents a specific, sensitive, accurate, and rapid method for quantitative determination of the biologically active component, berberine, in different Ayurvedic formulations. It was found that the Leucoforte formulation contained a higher percentage of berberine than Piloguard. The method can be used as a valuable analytical tool in the routine standardization of these formulations.

Acknowledgments

We wish to thank the Head of the University Department of Pharmaceutical Sciences, Utkal University, for providing the necessary facilities for carrying out this research work, and Om Prakash Rout for his valuable suggestions.

References

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