Research Article

SPECTROPHOTOMETRIC STANDARDIZATION OF BHUVNESVARA VATI USING GALLIC ACID AS MARKER COMPOUND

Biresh Kumar Sarkar¹, S.C.Verma², Palash Mandal¹, Ravi Kumar¹, M. Ramaiah³, Manish Devgan⁴

¹Assistant Director Pharmacy, Central Ayurveda Research Institute for Respiratory Disorders, Moti Bagh Road, Patiala, Punjab, India.
²Pharmacopoeia Commission for Indian Medicine & Homoeopathy (Ministry of AYUSH, Govt. of India), Ghaziabad-U.P., India.
³Department of Pharmacognosy, Hindu college of Pharmacy, Amaravathi Road, Guntur-522002, A.P., India.
⁴Faculty of Pharmacy, R.P. Educational Trust Group of Institutions, Karnal, Haryana, India.

ARTICLE INFO

ABSTRACT

Present study involves spectrophotometric standardization of bhuvnesvara vati using gallic acid as chief constituent. Gallic acid showed maximum absorbance at 274 nm. The analytical marker gallic acid was found to follow beer Lambert's law and linearity was observed in the concentration range of 5-25μg/ml. The method was developed and validated as per ICH guidelines and was found to be simple, accurate, precise and specific for the routine estimation of the gallic acid in formulation. Presence of gallic acid as chief constituent also confirmed when isolated fraction of sample was analyzed through FTIR.

Keywords:
Gallic acid, bhuvnesvara vati, UV/spectrophotometer, FTIR
INTRODUCTION:
The therapeutic importance of traditional herbal formulations are all well known from ancient time. There are many herbs having excellent therapeutic values for the treatment of various diseases. The quality standardization of ayurvedic herbal formulations also necessary to ensure their therapeutic value; since these formulations are mixture of more than one constituent thus their standardization is difficult as compared to single component medicine. The standardization of herbal formulation mainly depends upon the presence of specific marker which can be utilized for qualitative and quantitative detection using any sophisticated analytical technique like; HPLC, HPTLC and UV-Visible spectroscopy. In past few years many researchers successfully standardized various herbal formulations and explored area of herbal quality control analysis [1-4].

_Bhuvnesvara vati_ (BV) is an well known ayurvedic formulation official in Ayurvedic Formulary; containing _Emblica officinalis, Terminalia bellerica, Terminalia chebula, Trichyspermum ammi, Aegle marmelus_ and also contains minerals like; rock salt and Soot as main ingredients. The _Bhuvnesvara Vati_ known to have beneficial effects in diarrhoea and dysentery [5-6]. The study related to the quality standardization of _Bhuvnesvara Vati_ is not available exhaustively; thus the present investigation was aimed to standardize _Bhuvnesvara Vati_ by spectroscopic method using gallic-acid as analytical marker since formulation contains gallic acid chiefly.

Material and Methods

**Material:** Formulation was prepared as per standard procedure and all other chemicals and reagents used were of analytical grade.

**Apparatus:** A UV visible spectrophotometer (Shimadzu).

**PROCEDURES** [7]

**Preparation of stock solution and calibration curve:** The standard stock solution of gallic acid was prepared using methanol as solvent and the final volume was adjusted with the same solvent in 10ml volumetric flask to get final concentration 1000μg/ml. This stock solution was further diluted appropriately to prepare working standard solution. Gallic acid was scanned at 274 nm then regression equation was generated using calibration data.

**Analysis of herbal formulation:**
Accurately weighed quantity of powdered herbal _vati_ (100mg), was transferred in volumetric flask and dissolved in methanol using sonication that after sample solution was filtered using syringe filter (0.45μ). This solution was then diluted with appropriate quantity of methanol to get appropriate concentration of extracted gallic acid. The absorbance of the sample solution was measured at 274 nm against methanol as blank.

**VALIDATION OF THE DEVELOPED METHOD** [8]

**Linearity and range:** The standard stock solution containing 1000μg/ml of Gallic acid was further diluted to get concentration of 5-25 μg/ml. Each concentration was analyzed in triplicates. Calibration curve was plotted against concentration vs absorbance.

**Interday and Intraday precision:** The interday and intraday precision was determined by assay of sample solutions on the same day and on different days at different time intervals respectively (Six replicates).

**Limit of Detection:** The detection limit is determined by the analyzing the samples with known concentration of analyte and by establishing the minimum level at which analyte can be consistently detected; where, \( \sigma \) = the standard deviation of the response \( s \) = the slope of the calibration curve.

**Limit of Quantitation:** The limit of quantitation can be determined by analyzing samples with known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

**FTIR Analysis**

FTIR analysis of the isolated fraction of formulation was done and compared with the standard gallic acid the spectrum was recorded in the region of 4000 to 400cm\(^{-1}\). The dry sample was dispersed in 1:1 ratio in potassium bromide (KBr) and was compressed into discs by applying pressure in a hydraulic press to get a thin pellet which was then placed in the light path and spectrum was recorded.

**Results and Discussion**

Spectrophotometric standardization of _bhuvnesvara vati_ using gallic acid as marker was performed. The calibration curve was prepared using standard isolated gallic acid then sample solution was measured spectrophotometrically using standard calibration data, the presence of gallic acid in sample fraction was also confirmed using FTIR analysis. The developed method also validated as per ICH guideline (Table 1).

The linearity range and correlation coefficient for gallic acid were found to be 5-25 μg/ml and 0.996 respectively at 274 nm as showed in Figure 1 and Figure 2. The marker in formulation showed good regression values and equation was found to be linear. The results of interday and intraday precision study were also found within range (Table 2). The LOD and LOQ values were found to be 0.711 and 2.188 respectively which are approximately acceptable ratio of 1/3 from each other. The conform qualitative estimation for the presence of gallic-acid was performed by FTIR analysis and FTIR study confirmed presence of gallic acid as chief constituent in isolated fraction of sample (Figure 3 & Figure 4).
Table 1: Results of Validation Parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amax (nm)</td>
<td>274</td>
</tr>
<tr>
<td>2</td>
<td>Linearity Range (µg/ml)</td>
<td>5.25</td>
</tr>
<tr>
<td>3</td>
<td>R²</td>
<td>0.996</td>
</tr>
<tr>
<td>4</td>
<td>LOD (µg/ml)</td>
<td>0.711</td>
</tr>
<tr>
<td>5</td>
<td>LOQ (µg/ml)</td>
<td>2.188</td>
</tr>
<tr>
<td>6</td>
<td>Amount Found (µg/ml)</td>
<td>3.533</td>
</tr>
</tbody>
</table>

Figure 1. UV-Spectra of Gallic acid

Figure 2: Calibration curve of Gallic Acid

Table 2: Intraday and Interday Precision

<table>
<thead>
<tr>
<th>INTRADAY PRECISION</th>
<th>INTERDAY PRECISION</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Amount of Gallic acid Found</td>
<td>% RSD</td>
</tr>
<tr>
<td>100.0542</td>
<td>0.9118</td>
</tr>
</tbody>
</table>

Conclusion

The proposed spectrophotometric method was found to be rapid, simple, accurate, precise and economic. This method was validated as per ICH guidelines in terms of linearity, precision, and LOD/LOQ. This method can be successfully used for estimation of gallic acid in bhuvnesvara vati.

References