Development and Validation of UV Visible Spectrophotometric Method for Estimation of Gallic acid in Tagetes Erecta Extract

Rajashri B. Sumbe1*, Ashwini R. Gawade1, Albhar Shubhangi2, Barwal Surekha2

1School of Pharmacy, Dr.Vishwanath Karad MIT WPU, Kothrud, Pune
2Dr.N.J. Paulbudhe college of Pharmacy, Ahmednagar

*Corresponding author: Rajashri B. Sumbe, School of Pharmacy, Dr.Vishwanath Karad MIT WPU, Kothrud, Pune. E-mail: sumberrajashri450@gmail.com

ABSTRACT

A rapid simple and precise UV visible spectrophotometric method has been developed for the determination of Gallic acid in methanolic extract of Tagetes erecta. The spectrophotometric detection was carried out at an absorption maximum of 273 nm using ethanol as solvent. The method was validated using parameters as linearity, accuracy and precision, limit of detection and limit of quantitation as per ICH guidelines. The concentration of Gallic acid present in Tagetes erecta extract was found to be 3.2 ± 0.036 w/w. The samples were prepared in ethanol and methods obeyed the beer Lambert law in concentration ranges employed for evaluation. The result of analysis has been validated statistically; hence the proposed method can be used for the reliable quantification of active marker compounds in crude drugs and its herbal formulation.

Keywords: Extract, ICH guideline, Gallic acid, Tagetes erecta, UV spectrophotometer.

INTRODUCTION

The plant Tagetes erecta locally known as GendaPhul (Marigold) belongs to the family Asteraceae (composite). It is a stout, branching herb, native to Mexico and other warmer parts of America and naturalized elsewhere in the tropics and sub-tropics including India and Bangladesh (Nikkon F 2009). Tagetes erecta has been shown to contain quercetagetin, a glucoside of quercetagetin, phenolics, gallic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallat. Different parts of this plant including flowers are used in folk medicine to cure various diseases (Kiranmai M and Ibrahim M, 2012). Four phenolic derivatives have been isolated from the species of Tagetes L (XU Li-wei et al 2012). Among them Gallic acid is a tri hydroxy benzoic acid, type of phenolic acid, a type of organic acid, also known as 3,4,5- tri hydroxybenzoic acid (Gallic acid - Wikipedia, the free encyclopedia.html). It is found in the form of free acids, esters, catechin derivative and hydrolysable tannins. The interest in this compound is due to its pharmacological activity as radical scavengers. It has been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including antioxidant, antimicrobial. Antiviral, anticarcinogenic, radio protective neurodegenerative disorders and anti-purgative (Vallapudas H et al, 2013).
Study was undertaken for quantitative estimation of Gallic acid in Tagetes erecta extract. The literature revealed that UV Spectrophotometric method is not yet reported for the estimation gallic acid in Tagetes erecta. The method is developed in solvent Ethanol. Method validation is done as per ICH guidelines.

MATERIALS AND METHODS

Plant material

The fresh flower plant of Tagetes erecta was collected in the month of October from Wakad, Pune District, Maharashtra state, India. The plant was identified and authenticated by botanical survey of India, western Regional centre 7, Pune and a voucher specimen was deposited with a voucher specimen sample No. BSI/WRC/Tech/2012/431.

Apparatus

Instrument used was an UV Visible Spectrophotometer, SHIMADZU model 1800 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 nm matched quartz cells was used to measure absorbance of all the solutions. An electronic analytical balance was used for weighing the sample.

Reagent and Materials

All the chemicals and solvents were used by A. R. Grade. Standard Gallic acid was procured as a gift sample from Yucca enterprises, Mumbai.

Preparation of Crude extracts

The fresh flower part of the plant was dried in shade for 20 days. About a significant amount of dry flower petals was extracted with methanol (40-60°C) by continuous hot percolation using soxhlet apparatus. The methanol extract was filtered and concentrated to a dry mass by using vacuum distillation. A deep reddish brown viscous residue obtained having pungent spicy odor. Further the solvents were evaporated to dryness. The dried extract thus obtained was used for formulation.

Preparation of Standard stock solution of Gallic acid

The stock solution (100ug/ml) was prepared by dissolving accurately about 100 mg of standard gallic acid in sufficient quantity of ethanol and then volume was adjusted to 100 ml with ethanol. From standard stock solution, aliquots portion were suitably taken and diluted to different concentration using ethanol to get final concentration of 4 µg/ml, 8 µg/ml, 12 µg/ml respectively and were scanned in the wavelength range of 200-800 nm to determine λ max (Kumar R et al, 2010; Thakker V et al 2011).

Calibration Curve of Gallic acid

A series of calibrated 10 ml volumetric flask were taken and appropriate dilutions (2 to 12 µg/ml) of the working standard solution of Gallic acid were withdrawn and diluted up to 10 ml with ethanol. The absorbance was measured at absorption maxima 273 nm against the reagent blank prepared in similar manner without the Gallic acid. Absorption maxima and Beer’s law limit were recorded and data that prove the linearity and obeys Beer’s law; limits were noted. The linear correlation between these concentrations (X-axis) and absorbance (Y-axis) were graphically presented and slope (m), intercept (b) and correlation (R2) were calculated for linear equation (y=mx-b) by regression (Pawar N P and Salunkhev V R, 2013).

Estimation of Gallic acid in Tagetes Erecta extract

A total 0.1 gm of methanolic extract was dissolved in sufficient quantity of ethanol and shaken several times. Filter through whatman no.41 filter paper in 100l volumetric flask. Make up the volume with ethanol to get the final concentration of 100 µg/ml. The corresponding concentration (5 µg/ml) of Gallic acid against respective absorbance value was determined using
gallic acid calibration curve. The statistical analysis for checking the uniformity was performed (Pawar N P and Salunkhev V R, 2013).

(Sumbe R B, Gawade A R, 2021)

a) Linearity and range

The standard stock solution containing 100μg/ml each of Gallic acid; was further diluted to get linearity concentration of 2-12μg/ml for Gallic acid. Each concentration was analyzed in triplicates. Calibration curve was plotted by taking concentration on x-axis and absorbance on y-axis. The relation between drug and its absorbance was expressed by equation y = mx+b, where m=slope, and b= intercept.

b) Limit of detection and limit of quantitation

LOD and LOQ of the drug were derived by calculating the signal -to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ) using the following equation designated by ICH guideline. The residual standard deviation of regression line or standard deviation of y intercept of regression lines was used to calculate LOD and LOQ.

LOD=3.3× \frac{D}{S}

LOQ=10× \frac{D}{S}

Where, D=Standard deviation of y intercept of regression lines & S=Slope of calibration curve.

c) Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was performed three times at each level. In standard analysis method, concentrations (1μg/ml) of the standard Gallic acid in ethanol were prepared from independent stock solutions and their strengths were estimated by the standard curve.

d) Precision

The precision of the method was assessed by repeatability (intra-day) and intermediate precision (inter-day). Intra-day precision was determined by analyzing particular concentration (6μg/ml) of Gallic acid for three times within the day and average % RSD was calculated. Inter-day precision was determined by analyzing the same concentration of solutions for three days and average % RSD was calculated.

RESULT AND DISCUSSION

The method was validated as per ICH guideline. Method discussed in present work provides convenient way for simultaneous analysis of Gallic acid (Figure1). It obeys Beer Lambert’s law in concentration range 2-12μg/ml at the λ max 273 nm (Table 1) (Figure 2 and 3). The correlation coefficient (R2) was calculated, where the (R2) value 0.999 for Gallic acid indicates the good linearity between the concentration and absorbance. The estimation of Gallic acid in Tagetes erecta extract was carried out. The concentration of Gallic acid present in Tagetes erecta was found to be, 3.2±0.036w/w (Table 2). The % relative standard deviation (%RSD) value was found to be interday precision 0.48±0.015 and intraday precision 0.32±0.03 for Gallic acid. The low value of standard deviation showed that, the method is precise (Table 3). From the data; it is obvious that the present method of UV Spectrophotometric method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine estimation of Gallic acid in Tagetes erecta extract.

Table 1: Data for standard curve of gallic acid.
<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance (273 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.4973</td>
</tr>
<tr>
<td>4</td>
<td>0.9983</td>
</tr>
<tr>
<td>6</td>
<td>1.4895</td>
</tr>
<tr>
<td>8</td>
<td>1.9902</td>
</tr>
<tr>
<td>10</td>
<td>2.4665</td>
</tr>
<tr>
<td>12</td>
<td>2.9888</td>
</tr>
</tbody>
</table>

| Tagetes erecta extract | Gallic acid content %w/w | 3.2 ± 0.036 |

Table 2: Content of gallic acid with SD in Tagetes erecta extract.

Table 3: Validated parameters of gallic acid.

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Result obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Gallic acid</td>
</tr>
<tr>
<td>Wavelength range (nm)</td>
<td>273</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>02-Dec</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.248x + 0.002</td>
</tr>
<tr>
<td>y = mx - b (m = slope, b = intercept)</td>
<td>m = 0.248, b = 0.002</td>
</tr>
<tr>
<td>correlation coefficient ($r^2$)</td>
<td>$r^2 = 0.999$</td>
</tr>
<tr>
<td>Accuracy (Recovery)</td>
<td>99.73 ± 0.025</td>
</tr>
<tr>
<td>(n=3)</td>
<td>100.10 ± 0.011</td>
</tr>
<tr>
<td>Precision (% RSD) Intraday</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>(n=3) Interday</td>
<td>0.48 ± 0.015</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.079</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Recovery was calculated with ±SD, where I, II, III are amount drug taken, i.e; 80, 100 and 120% respectively.

Figure 1: Structure of gallic acid.
Figure 2: Maximum absorption of gallic acid on U.V spectrophotometer.

Figure 3: Calibration curve of gallic acid on U.V spectrophotometer.
CONCLUSION

The analytical method developed on UV Visible Spectrophotometer was simple, reliable, accurate and reproducible. Low cost, faster speed, satisfactory precision and good specificity, to assess unequivocally the analyte in the presence of components, which may be expected to be present, are the main features of this method. Method was successfully validated as per ICH guidelines and can be conveniently employed for routine quality control analysis of Gallic acid in extract without any interference from any phytocconstituent. UV spectrophotometric estimation of active marker compound highlights assurance of batch uniformity and integrity of the product manufactured. UV analysis is most useful for quantitative estimation of target molecules in herbal products. UV detection of such compound is primary screening for further analysis of same by chromatographically technique.

REFERENCES

