Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2016; 7(3); 51-54

ISSN 0975 9506

Research Article

Development and Validation of the UV-Spectrophotometric Method for Determination of Embelin

Shyam Baboo Prasad[1*], Meenu Bist[1], Suraj Pal Verma[2]

[1]*Department of Pharmacognosy, School of Pharmaceutical Science, Lovely professional university, Phagwara-144 411, Punjab, India.*
[2]*Department of Quality Assurance, School of Pharmaceutical Science, Lovely professional university, Phagwara-144 411, Punjab, India.*

Available Online:15th September, 2016

ABSTRACT
Embelin, chemically known as 2, 5-dihydroxy-3-undecyl-p-benzoquinone is one of the bioactive compound found in oldest herbal medicinal plant known as *Embelia ribes*. Embelin, having anti-cancer, anti-bacterial, anti-inflammatory, analgesic, hepatoprotective, antidepressant, anxiolytic, antioxidant, antifertility, antiulcer and wound healing activity. A double wavelength UV-spectrophotometric method was developed and validated for determination of embelin in *Embelia ribes* fruits. The standard solution of embelin in methanol shown maximum absorption at 289 nm. The response of embelin was linear over the range of 10-90 µg/ml with the correlation coefficient (R^2) value of 0.9991. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 50%, 100%, and 150%. The % recovery was found in the range of 98.96– 100.9%. The precision of the method was studied as an intraday; interday and repeatability. The % relative standard deviation (RSD) value < 2 indicate that the method was precise. The Limit of detection (LOD) and Limit of quantification (LOQ) of embelin were found 3.96 µg/mL and 12 µg/mL respectively. The method was validated using parameters provided as per International Conferences on Harmonization (ICH) guidelines.

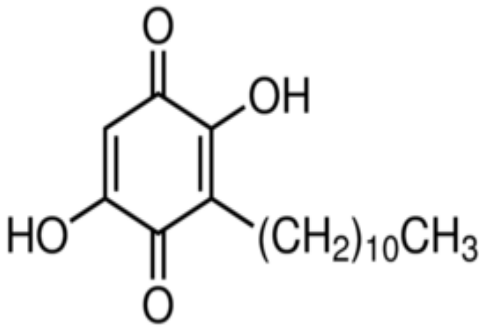
Keywords: *Embelia ribes*, Spectrophotometric method, LOD, LOQ, ICH.

INTRODUCTION

Myrsinaceae family consists almost 1000 species of trees and shrubs that are spread over 33 genera containing four genera specifically Myrsine, Maesa, Rapanea and Embelia, which are widely used from ancient time as herbal medicines. *Embelia ribes* is the most correlated species of Vidanga, a traditional herbal medicine used in Ayurveda, Siddha as well as in unani medicine system as Anthelmentic and to cure various skin diseases[1]. *Embelia ribes*, a natural source of Embelin has restricted distribution mainly in the Western Ghats and Eastern Himalayas. It is also a critically endangered species. Chemically, the embelin is 2, 5-dihydroxy-3-undecyl-1, 4 benzoquinone[2]. It is a promising anticancer agent[3]. Embelin shown others activities also such as antibacterial, antifungal, anticonvulsant, antioxidant, wound healing and anxiolytic activity. Embelin specially known for its Anthelmentic and contraceptive use. About 4.33% of the embelin content has been observed in the fruits of *Embelia ribes*[4-10]. Embelin is orange solid in appearance and is characteristics odour in nature. Molecular weight of embelin is 294.391 and melting point is 142.5^{0}c. Embelin is lipophilic in nature with log p of 4.34 (2). Few publications are available on method validation of analytical method on HPLC for embelin for determination in *Embelia ribes*[1]. There are two UV spectroscopic methods have been mentioned in the literature for the quantification of embelin in chloroform and methanol extract at 285 nm and 291 nm[2]. The aim of present work was to develop and validate analytical methods for the estimation of embelin in ether extract of *Embelia ribes* for development of topical gel formulation. Developed methods were validated as per ICH guidelines and validation data was evaluated with the aid of various statistical parameters.

MATERIALS AND METHOD

Method development for estimation of embelin
Determination of absorption maxima
Accurately weighed 10 mg of embelin was transferred to 100 ml volumetric flask. Volume of flask was made up to 100 ml by adding methanol in order to get the



**Author for Correspondence: b.shyam2@gmail.com*

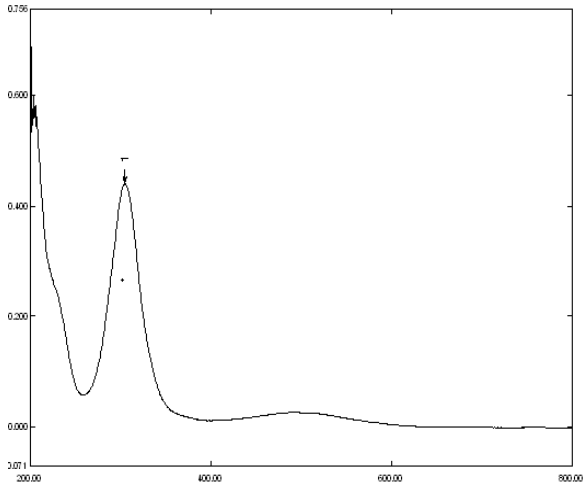


Figure 1: Scan of embelin (scanned between 200-800nm).

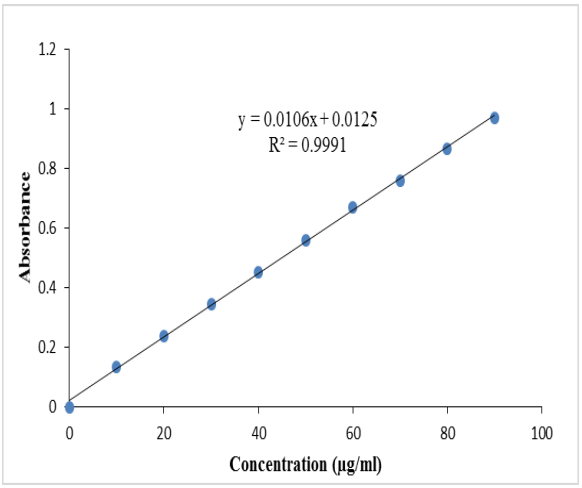


Figure 2: Calibration Plot of embelin in methanol at 289 nm

Table 1: UV absorptions of different concentration of embelin

S. No	Concentration (µg/ml)	Average absorbance
1.	10	0.135
2.	20	0.238
3.	30	0.336
4.	40	0.470
5.	50	0.567
6.	60	0.663
7.	70	0.761
8.	80	0.865
9.	90	0.972

concentration of 100µg/ml. The resultant solution (100 µg/ml) was scanned on double beamed UV spectrophotometer so as to observe the absorption maxima of embelin.

Construction of calibration plots for embelin

The standard solution was prepared by transferring 10 mg of isolated embelin in volumetric flask of 100 ml capacity containing methanol. The final volume was adjusted to the mark with methanol to form a concentration of 100 µg/ml of drug in solution. The absorption spectrum of the solution was recorded ranging from 200 to 800 nm using methanol as a blank. A series of dilutions were prepared from the standard stock solutions ranging from 10 to 90 µg/ml of embelin. From this standard solution 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml were withdrawn separately and poured into 10 ml of volumetric flasks and volumes were adjusted to the mark by using methanol. This results in formation of solutions having different concentrations of 10, 20, 30, 40, 50, 60, 70, 80 and 90µg/ml. Absorbance was recorded at 289 nm under UV spectrophotometer. All the measurements were taken three times to ensure accuracy of the results. A plot was constructed by taking concentration (µg/ml) on X- axis and absorbance on Y-axis. Subsequently linear regression equation and linear regression coefficient were calculated from the calibration plot.

Analytical method validation

Accuracy

Accuracy of developed method was checked by taking three different concentrations levels which are 50% (20µg/ml), 100% (40µg/ml), 150% (60µg/ml). All concentration was prepared using methanol and were determined (*n = 3*). Accuracy of the method was analyzed with the help of different methods like standard deviation (SD), recovery at each level of concentration and percent relative standard deviation (RSD).

Precision

Repeatability studies on the three same concentrations which were used to determine accuracy were carried out. RSD and recovery concentration were used to calculate precision of the method.

Linearity

A series of dilutions were made using methanol and then their absorbance was determined at 289 nm (n=3). This is the wavelength above which the Beer's law is followed by the light. Linearity measures were recorded using the linear regression coefficient.

Limit of Quantification (LOQ) and Limit of Detection (LOD)

For determination of Limit of Quantification and Limit of detection a minimum concentration was choosen for which calibration plot was made. Absorbance was measured six times (n = 3) using methanol as diluent until relative standard deviation of LOQ was less than 10% and LOD was less than 30% for the developed method [11].

RESULTS AND DISCUSSION

Determination of absorption maxima (λmax)

λmax was determine by scanning embelin between 200-800 nm, and λmax was found to be 289nm which are shown in fig. 1

Method Validation of embelin in methanol

Construction of calibration plots for embelin

Calibration plot was plotted by covering the entire Beer law range (0.2 to 0.8 absorbance level). Calibration plots of embelin are shown in (Fig.2 and Table 1). All the reading was recorded in replicate of three (n = 3). Result was in accordance of ICH Q2 (R1) guidelines. Linear

Table 2: Accuracy data of developed method of embelin in methanol

S.No	Concentration Level	Mean Absorbance of embelin (±S.D.)	Mean concentration of embelin(µg/ml) ± S.D.	RSD%	%Recovery
1	50%	0.267	24.00±0.005	1.87	98.96%
2	100%	0.401	40.25±0.009	2.16	99.68%
3	150%	0.678	62.78±0.004	0.58	100.9%

Table 3: Precision data (%RSD) (interaday)

S. No	Concentration Level	Mean Absorbance of embelin (±S.D.)	Mean concentration of embelin (µg/ml) ± S.D.	RSD%	%Recovery
1	50%	0.261	24.9±0.001	0.583	98.196%
2	100%	0.408	39.6±0.003	0.926	96.041%
3	150%	0.665	65.3±0.002	1.147	98.281%

Table 4: Precision data (%RSD) (interday)

S. No.	Concentration Level	Mean Absorbance of embelin (±S.D.)	Mean concentration of embelin (µg/ml) ± S.D.	RSD%	%Recovery
1	50%	0.267	25.5±0.001	2.183	97.45%
2	100%	0.415	40.3±0.009	1.921	95.97%
3	150%	0.618	60.6±0.004	0.612	97.94%

Table 5: Linearity data for developed method of embelin in methanol (n=3)

Slope	Intercept	Correlation coefficient (R^2)	Range
0.0106	+0.0125	0.9991	10-90µg/ml

Table 6: LOQ and LOD (µg/ml) data of developed method of embelin (n=3)

LOD	LOQ
3.96	12

regression coefficient and linear regression equation were also established. *Analytical Method Validation*

Accuracy

Accuracy of developed method was calculated by RSD (%) which was found to be less than 2. The method shown to be accurate as percent recovery was in range of 98.96 – 100.9%. Data associated with accuracy measurements is listed in Table 2

Precision

In both types of precision studies (repeatability and intermediate precision) %RSD was found to be less than two which show compliance with ICH guidelines. Percent recovery was found in range of 98.196 to 98.281 in case of intermediate precision studies (inter day) and 97.45 to 97.94 in case of repeatability (intraday). Data associated with these measurements is shown in Table 3 and Table 4

Linearity

Linearity was found to be in range of 10-90 µg/ml which confirmed that the developed method was follows Beer's law. The values of slope, intercept, and regression coefficient (R^2) associated with linearity plots are listed in Table 5

LOQ and LOD

LOQ and LOD for embelin were found to be 12µg/mL and 3.96*µ*g/mL, respectively. It was calculated by equation. Data associated with LOD and LOQ analysis is mentioned in Table 6

CONCLUSION

The method was developed using standard embelin and validated as per ICH guideline [Q2 (A)]. Linearity, accuracy, precision, LOD, and LOQ were measured to validate. Linearity for embelin was found to be in the range between 10–90 µg/ml. Accuracy was confirmed by recovery studies. Mean percentage recovery for embelin was found to be 99.84%. In precision studies % RSD was found to be less than 2 for embelin. This method is more reliable and reproducible as compared to existing method.

REFERENCES

1. Asadulla, S. and Ramandang, R., 2011. Pharmacognosy of Embelia ribes burm F. *Int J Res Pharm Chem*, *1*(4), pp.1236-1251.
2. Bist M. Prasad SB., 2016. Embelia ribes: A valuable medicinal plant. Journal of Chemical and Pharmaceutical Research, 8(4):1229-1233.
3. Kaur V, Hallan SS, Kalia AN, Mishra N., 2015. Isolation of embelin from Embelia ribes and evaluation of its anti-cancer potential in breast cancer. 1(1):33–9.
4. Chitra M, Devi CSS, Sukumar E. Antibacterial activity of embelin. 2003;74(03):401–403.
5. Radhakrishnan, N., Gnanamani, A., & Mandal, A. B. 2011. A potential antibacterial agent Embelin, a natural benzoquinone extracted from Embelia ribes. *Biology and medicine*, *3*(2), 1-7.
6. Suthar, M., Patel, R., Hapani, K., & Patel, A. (2009). Screening of Embelia ribes for antifungal activity. *Int J Pharma Sci Drug Res*, *1*(1), 203-206.

7. Mahendran, S., Thippeswamy, B. S., Veerapur, V. P., & Badami, S. (2011). Anticonvulsant activity of embelin isolated from Embelia ribes. *Phytomedicine*, *18*(2), 186-188.
8. Singh, D., Singh, R., Singh, P., & Gupta, R. S. (2009). Effects of embelin on lipid peroxidation and free radical scavenging activity against liver damage in rats. *Basic & clinical pharmacology & toxicology*, *105*(4), 243-248.
9. Swamy, H. K., Krishna, V., Shankarmurthy, K., Rahiman, B. A., Mankani, K. L., Mahadevan, K. M., ... & Naika, H. R. (2007). Wound healing activity of embelin isolated from the ethanol extract of leaves of Embelia ribes Burm. *Journal of ethnopharmacology*, *109*(3), 529-534.
10. Afzal, M., Gupta, G., Kazmi, I., Rahman, M., Upadhyay, G., Ahmad, K., ... & Anwar, F. (2012). Evaluation of anxiolytic activity of embelin isolated from Embelia ribes. *Biomedicine & Aging Pathology*, *2*(2), 45-47.
11. Verma, H., Verma, S., & Singh, H. (2013). A stability indicating assay method development and validation for the frovatriptan succinate monohydrate by using UV: a spectrophotometric technique. *ISRN Spectroscopy*, *2013*.