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SPECTROPHOTOMETRIC DETERMINATION OF EMBELIN IN BULK AND PHARMACEUTICAL FORMULATION BY FIRST ORDER DERIVATIVE METHOD

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ABSTRACT

The aim of present work was to develop an accurate, precise, reproducible and economical First order derivative Spectrophotometric method for estimation of Embelin. The Embelin standard solution was scanned in UV range of 400-200nm in 1cm quartz cell in double beam spectrophotometer. The absorbance at λ_{min}=282.1nm, λ_{max}=320.8nm and zero cross=310.40nm was measured. The standard and sample solutions of embelin were prepared in methanol. The accuracy and precision of the method was determined and validated statistically. The method shows good recovery and reproducibility with % RSD less than 2. The method was found to be rapid, specific, precise and accurate and hence can be employed for routine analysis of embelin in bulk and its pharmaceutical dosage form.

KEYWORDS: first order derivative, Embelin, UV Spectrophotometry, Quantitative estimation.

INTRODUCTION

Embelia ribes Burm f, family: Myrsinaceae Know as vidanga[1] is one of the oldest herb most widely used in traditional medicine and currently is an endangered species in India. Vidang is well known home medicinal product used as home remedy and in Sanskrit it is named as "jantanasa".[2] The name suggests its fine action as Germicidal and fungicidal. Embelia ribes fruits are most commonly used in ayurvedic system of medicine by various routes of administration like oral, topical and by inhalation.[3] The fruits mainly contains

benzoquinione derivatives such as Embelin (Figure 1) chemically 2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadine-1,4-benzoquinone and vilangin.[4] *Embelia ribes Burm f* is mainly used as an anthelmintic, carminative and stimulant as well as in treatment of abdominal disorders, lung diseases, constipation, indigestion, and headache. It is used in cases of toothache, mouth ulcers, sore throat, pneumonia, heart diseases, haemorrhoids and obesity.[5] Embelin's activity against cancer is being widely studied, embelin as a fairly potent, nonpeptidic, cell-permeable, small-molecule inhibitor of XIAP and represents a promising lead compound for entirely new class of anticancer agents that target the BIR3 domain of XIAP[6], also it prevents the development of chemical carcinogen induced hepatocarcinogenesis in rats.[7,8]

Only a few analytical methods have been reported in the literature for the assay of Embelin. They include UV spectrophotometric, VIS spectrophotometric[9], HPLC[10,11], HPTLC[12] method, but no first order derivative UV spectrophotometric method was found for the embelin. The purpose of this work was to develop a simple, accurate, precise, reproducible and economical UV spectrophotometric First order derivative method for estimation of Embelin according to guidelines given by International Conference on Harmonisation (ICH).[13]

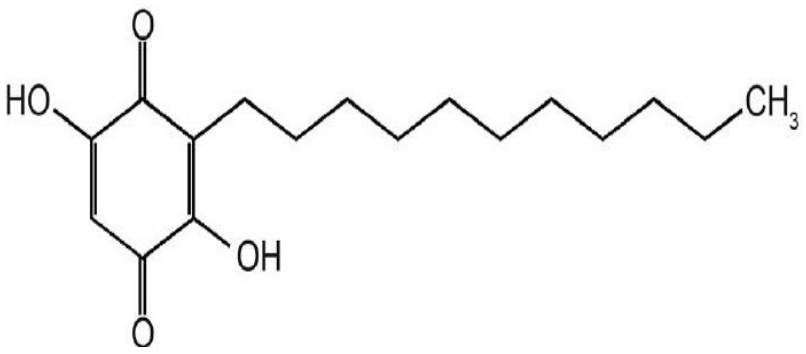

Figure 1: Chemical Structure of Embelin

MATERIALS AND METHODS

INSTRUMENTATION AND APPARATUS

Study was carried out using Shimadzu 1800 double beam UV - Visible spectrophotometer with UV probe software, spectral band width of 1 cm matched pair quartz cells. Single pan electronic balance (Shimadzu ATY 224) was used for weighing purpose. Ultra sonicator (Spectra lab UCB 40, India) was used to carry out sonication of the solution. Calibrated volumetric glass wares (Borosil) were used in this study.

CHEMICALS AND REAGENTS

Embelin pure drug was obtained from yucca enterprises, Mumbai. Marketed Ayurvedic churna formulation of *Embelia ribes* were procured from local market of Pune, India. AR grade of methanol was purchased from Merck India Ltd., Mumbai, India.

METHOD DEVELOPMENT

Preparation of standard solution: The standard stock solution of Embelin was prepared by dissolving accurately weighed 100 mg of Embelin in 60 ml methanol with the help of sonication then it is transferred to 100 ml volumetric flask. Then volume was made up to the mark by using methanol to give concentration of 1000 µg/ml which is standard stock solution and it is further diluted with Methanol to get concentration 5-17.5 µg/ml.

Determination of absorption maxima

The Methanol was used as blank for baseline correction and then the working standard solution of 10µg/ml was scanned between 400 nm to 200 nm in UV spectrophotometer against Methanol. Wavelength was selected on wavelength maxima at 303 nm. (Figure 2).

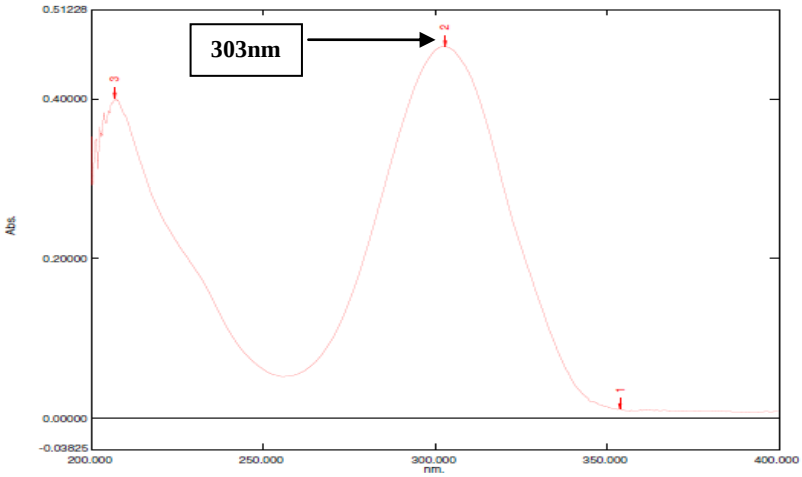

Figure 2: 10µg/ml Embelin solution.

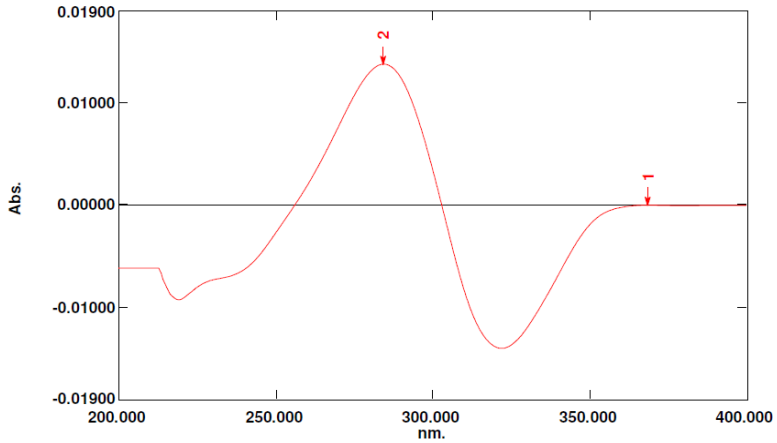

Figure 3: 10µg/ml Embelin solution (First order derivative spectra)

First Order Derivative Method: In this method first different working standards of embelin were prepared between 5-17.5µg/ml by appropriate dilutions from standard stock solution and scanned in spectrum mode from 400 to 200 nm. The absorption spectra obtained were derivatives from first to fourth order. The First order derivative spectra were selected for analysis of drug. From the spectra of drug (figure 3), the absorbance was measured at λ_{min}=282.1nm, λ_{max}=320.8nm and zero cross=310.40nm, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentration of standard and was plotted against Concentrations and regression equation was calculated.

Preparation of calibration curve

Working solutions were prepared from standard stock solution by further dilution with methanol to obtain the concentration of 5, 7.5, 10, 12.5, 15 and 17.5µg/ml respectively. These solutions were scanned from 400 to 200 nm and the values of dA/dλ were calculated. The calibration curve was plotted between dA/dλ against concentration (Figure 4).

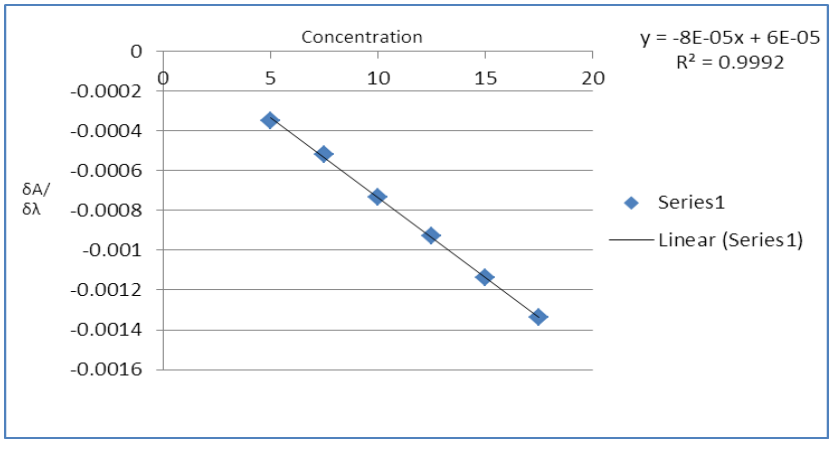

Figure 4: Calibration curve of Embelin.

METHOD VALIDATION[13]

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity & Range, Precision, Accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.

Linearity and Range: The linearity was determined by using working standard solutions between 5-17.5µg/ml. The spectrums of these solutions were recorded and dA/dλ values calculated (Table 1). Calibration curve of dA/dλ *v/s* Concentration was plotted after suitable calculation and simple linear regression was performed (Figure 4). Regression equation and correlation coefficient were obtained. The range of solution has been decided according to statistical parameters of generated equation.

Table 1. Linearity and range of Embelin.

Concentration µg/ml	dA/dλ
5	-0.00035
7.5	-0.00052
10	-0.00073
12.5	-0.00093
15	-0.00114
17.5	-0.00134

Method Precision: Precision studies were carried out to ascertain the reproducibility of the proposed method. This study divided into repeatability and Reproducibility which further contains intraday and interday.

Repeatability: The precision of the method was checked by repeatedly injecting (n= 6) standard solutions of Embelin (10µg/ml). The absorbance was measured at λ_{min}, λ_{max} and zero cross, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentrations.

Table2. Repeatability results for Embelin

Concentration	dA/dλ	Mean	SD	%RSD
10	-0.00073	-0.0007	0.000131	1.790
10	-0.00074881			
10	-0.00071986			
10	-0.00073651			
10	-0.0007512			
10	-0.0007223			

**n=6, SD = Standard Deviation, % RSD = % Relative Standard Deviation.*

Reproducibility: The reproducibility of the proposed methods was determined by performing the assay on same day (Intraday assay precision) at different time intervals (morning, afternoon and evening) and on three different days (Interday precision).

Table3. Precision results for Embelin.

	Concentratio (µg/ml)	dA/dλ			(Mean ± SD)*	%RSD
		1	2	3		
Intraday	7.5	-0.000496	-0.000514	-0.000521	-0.0005 ± 0.00014	1.89
	10	-0.00075	-0.00072	-0.00074	-0.0007 ± 0.00017	1.94
	12.5	-0.000931	-0.000897	-0.000922	-0.0009 ± 0.00015	1.97
Interday	7.5	-0.000453	-0.000449	-0.000460	-0.0005 ± 0.000041	1.2
	10	-0.000685	-0.000690	-0.000691	-0.0007± 0.00005	0.473
	12.5	-0.0008813	-0.000893	-0.000886	-0.0009± 0.000046	0.67

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

Six sets of known concentrations (5-17.5µg/ml) were prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation and following formulae as

LOD = 3.3 * SD/S

LOQ = 10 * SD/S

Where,

SD is standard deviation of y-intercept of the calibration curves

S is mean slope of six calibration curves.

Accuracy

The accuracy for the analytical procedure was determined at 80%, 100% and 120% levels of standard solution. The absorbance was measured at λ_{min}, λ_{max} and zero cross, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentrations and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated. The results were tabulated in Table 4.

Table 4. Accuracy results for Embelin

Test (µg/ml)	Accuracy level	Amount of standard drug added (µg/mL)	% Recovery	Standard deviation	%RSD
10 µg/ml	80%	8	99.51	0.74888	0.7509
	100%	10	99.11	0.34239	0.3440
	120%	12	98.56	0.31511	0.3144

**n=3*

ASSAY OF CHURNA FORMULATION

The churna powder equivalent to 50 mg of embelin was accurately weighed, dissolved it in 25ml of methanol and transferred it in 50ml volumetric flask and make up volume up to the mark. The solution was filtered with whatmann filter paper No. 41 and the first 3 ml of filtrate was discarded. This solution was further diluted to obtain 10 µg/ml solutions with same solvent and the amplitude difference (dA) with respect to wavelength difference (dλ) was recorded. This procedure was repeated three times and the mean reading was taking into consideration.(Table 5)

Table 5: Assay studies of Embelin

Churna formulation	Amount taken(Equivalent Weight)	Amount found	Assay %	Mean % Assay
Vavdinga churna.	50 mg	49.65 mg	99.30	99.273
	50 mg	49.60 mg	99.20	
	50 mg	49. 65 mg	99.32	

RESULTS AND DISCUSSION

An attempt was made to develop a simple and specific First order derivative method for the determination of embelin in bulk and its pharmaceutical dosage form. The obtained regression equation was, $\frac{dA}{d\lambda} = -0.00008x + 0.00006$ with correlation coefficient (R^2) of 0.999, where dA/dλ is amplitude difference and 'x' is concentration. The R^2 indicates the method was linear. The proposed method was found to be precise as %RSD values for interday as well as intraday precision were satisfactory. The drug at each level i.e. 80%, 100%, 120% showed good recovery in range of 99.1-99.6%, hence it could be said the method was accurate. The LOD and LOQ were found to be 0.65 and 1.974 respectively. The assay of pharmaceutical dosage form was found to be 99.273%.Hence, the method can used for the routine analysis of Embelin in bulk and its dosage form. The validation parameters were summarised in Table 6.

Table 6. Summary of Validation parameters.

Parameters	Results
λ_{max}	λ_{min}=282.1nm λ_{max} =320.8 nm
Linearity range	5 – 17.5 µg/ml
Regression Equation(y=mx+c)	y = -0.00008x + 0.00006
Correlation Coefficient (R^2)	R^2 = 0.999

Precision (%RSD)	
Repeatability (*n=6)	1.790
Intraday (*n=3)	1.92
Interday (*n=3)	0.745
Accuracy	80% = 99.30 100% =99.20 120% =99.32
LOD	0.65142µg/ml
LOQ	1.974µg/ml
Assay	99.273 ± 1.93

CONCLUSION

It can be concluded from the results that the proposed method was accurate, precise and consistent for the determination of Embelin in bulk and its pharmaceutical dosage form. This method was validated as per ICH guideline Q2 (R1). Results suggest that this method can be used for routine estimation of Embelin in bulk and tablet dosage forms.

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