

ORIGINAL RESEARCH ARTICLE

Development and Validation of HPTLC Method for Simultaneous Quantitation of Embelin and Assay of Marketed Formulation

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ABSTRACT

Embelia ribes burm f., also known as Vidanga, is one of the oldest herbs in Indian traditional medicine. *Embelia ribes* have a long history of use in ayurvedic system of medicine in various forms like churna, asava, aristha and taila. It is used mainly as an anthelmintic, carminative and stimulant. A selective, precise and accurate High Performance Thin Layer Chromatography (HPTLC) method has been developed for the simultaneous quantitation of Embelin in Vidanga churna formulations. The method employed TLC aluminium plate (10 cm x 10cm x 250 µm) precoated with silica gel 60 F₂₅₄ as a stationary phase. The solvent system consists of Chloroform: Ethyl acetate: Formic acid (5:4:1 v/v/v). Densitometric analysis was carried out in the absorbance mode at 291 nm using Camag TLC scanner-III. Developed HPTLC method showed good regression ($r^2 = 0.9986 \pm 0.0020$) and the recovery of Embelin was in the range of 99.09 – 102.01%. The limit of detection and limit of quantitation were found to be 61.28ng/spot and 185.71ng/spot respectively. The method was validated for precision, recovery, limit of detection and limit of quantitation. The proposed HPTLC method was found to be simple, precised and accurate and can be used for the quality control of the raw materials as well as formulations.

KEY WORDS: Vidanga, Embelin, HPTLC, Quality control

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. But the scene has changed now; herbal medicines are being manufactured on a large scale in pharmaceutical units^[1]. Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant

in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants^[2, 3, 4]. Over the past decade, interest in drugs derived from higher plants, especially the phytotherapeutic ones, has increased expressively. In some particular cases, such as antitumoral and antimicrobial drugs, about 60% of the medicines currently available on the market and most of those in the late stages of clinical trials are derived from natural products, mainly from higher plants^[3].

Embelia ribes burm f. also known as Vidanga^[5], is one of the oldest herbs in Indian traditional medicine. *Embelia ribes* have a long history of use in ayurvedic system of medicine in various forms like churna, asava, aristha, lauha and taila. It is an Indo-Malaysian species, mainly found in India, Sri Lanka, Singapore, and Malaysia. In India it is

majority found in central and lower Himalayas, Arunachal Pradesh, Assam, Bengal, Orissa, Andhra Pradesh and Madhya Pradesh [6]. It is available throughout India up to an altitude of 5000 feet [7]. The main active component is Embelin, chemically 2,5-dihydroxy-3-undecyl-1,4-benzoquinone. Embelin is occur in golden yellow needles and is insoluble in water but soluble in alcohol, chloroform and benzene. Other components are christembine, qercitol, vilangin and resinoid [8]. It is mainly used as an anthelmintic, carminative and stimulant. It is also used in treatment of abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease and obesity [9].

In the present study, HPTLC method have been developed as chemical fingerprints for Vidanga churna for three different formulations using Embelin as an active chemical marker. The developed method were validated in terms of accuracy, specificity, precision, linearity range, robustness, limit of detection (LOD) and limit of quantitation (LOQ) as per ICH guidelines. The developed method can also be used as a quality control tool.

MATERIAL AND METHOD

Instrument

Analysis was performed on 10x10 cm precoated silica gel 60 F₂₅₄ TLC plates (E. Merck). Samples were applied to the plates by means of Linomat V automatic sample spotter with the aid of Hamilton 100 µl syringe. TLC plates were developed in flat bottom twin trough chamber. Densitometry was performed with a TLC scanner III linked to WinCATS software.

Material

Dried ripe fruits of *Embelia ribes* Burm. f., family- Myrsinaceae were procured from local market of Guwahati, Assam in the month of November. The collected fruits of *Embelia ribes* were authenticated at Botanical survey of India, Pune. All the chemicals used were of A.R. grade obtained from Merck chemicals, India.

Preparation of extracts of marketed formulations

Marketed formulations (F-I, F-II and F-III) were macerated with chloroform at room temperature for 48 hrs in dark. The extract was filtered and concentrated over water bath till a dry residue was obtained. This extract was further used for HPTLC analysis.

Preparation of standard solution

10 mg of Embelin was dissolved in sufficient volume of methanol and sonicated for 2 min. Then volume was made up to 10 ml with methanol to get the concentration of 1000 ng/µl. Appropriate aliquots from above stock solutions was spotted on HPTLC plate to get concentrations 2000 ng/spot.

Preparation of sample solution

Dried chloroform extract equivalent to 10 mg of Embelin of F-I, F-II and F-III respectively was dissolved in sufficient volume of methanol and sonicated for 10 min. After sonication the solution was filtered through Whatman filter paper no.41 and then volume was made up to 10 ml with methanol. Appropriate aliquots from these stock solutions were further diluted with same solvent to obtain 2000 ng/spot of Embelin.

Preparation of Mobile Phase

The mobile phase i.e. chloroform: ethyl acetate: formic acid in the proportion of 5:4:1 v/v/v was prepared in Camag twin-trough chamber by mixing and chamber was saturated for 10 minutes

Validation

The developed method has been validated in terms of linearity, precision, specificity, robustness and accuracy as per ICH guidelines [10]

Calibration

Analysis was performed on 10x10 cm precoated silica gel 60 F₂₅₄ TLC plate with 250 µm thickness. The linear ascending development was carried out in glass twin trough chamber (10 x 10cm). Standard stock solutions of Embelin (1000ng/spot) in methanol were applied on TLC plates in concentrations of 400-2400 ng/spot using Camag Linomat 5 sample applicator under nitrogen stream. The TLC plate was dried in air with the help of drier and densitometric scan was performed at 291nm with Camag TLC scanner III. Each amount was analyzed six times and peak areas were recorded. Calibration curves were constructed by plotting average peak area versus concentrations and regression equations was computed for Embelin.

Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined using following formulae.

$$\text{LOD} = 3.3(\text{SD})/\text{S}$$

$$\text{LOQ} = 10 (\text{SD})/\text{S}$$

Where, SD = Standard Deviation of response

S = avg. of the slope of the calibration curve

Precision

Precision of the method was verified by repeatability (intra-day) and intermediate (inter-day) precision studies. Intra-day and inter-day precision were performed by analyzing sample solution of 2000 ng/spot of embelin six times on the same day and three different days respectively. Measurement of peak area for active compound was expressed in terms of % Relative Standard Deviation (% RSD)

Accuracy

Accuracy of the method was tested by carrying out recovery studies at different spiked level by standard addition method. Standard Embelin solution was added at three different levels (80,100 and 120%). At each level three determinations were performed and results were calculated by the difference between the spiked and un-spiked sample analyzed under the same conditions.

Robustness

To determine the robustness of the developed method, few parameters such as variation in composition of mobile phase, time of chamber saturation and volume of mobile phase were deliberately varied.

Quantitative analysis of embelin in marketed formulations

For assay of marketed formulations F-I, F-II and F-III, extracts were prepared as mentioned in the above section and subject to optimized HPTLC conditions.

RESULT AND DISCUSSION

Method Development and Optimization

Initially many solvent systems were tried. Different system parameters such as composition of mobile phase, method of sample preparation, detection wavelength were modified to obtain well resolved densitogram.

A solvent system that would give dense and compact spots with appropriate R_f value was desired for quantitation of Embelin in marketed formulation. Various solvent systems like chloroform: ethyl acetate, chloroform: ethyl acetate: acetic acid, chloroform: ethyl acetate: formic acid in different proportions was tried. It was found that in the chloroform: ethyl acetate: acetic acid the peaks were not symmetrical and sharp but in chloroform: ethyl acetate: formic acid (5:4:1 v/v/v) could show a sharp and symmetrical peak with R_f 0.75.

HPTLC fingerprint analysis of Embelia ribes Burm f formulations

(Figure 1) showed characteristic peak of embelin at R_f value of 0.75. To obtain the fingerprints of three marketed formulations (F-I, F-II and F-III), densitogram of all three formulations were overlaid with the densitogram of isolated chemical marker embelin as shown in (Figure 2) which clearly indicates common peak in all the formulations. And it is evident that marketed formulations show peaks at same R_f value as that of Embelin (0.75), and hence can be said to contain same chemical component.

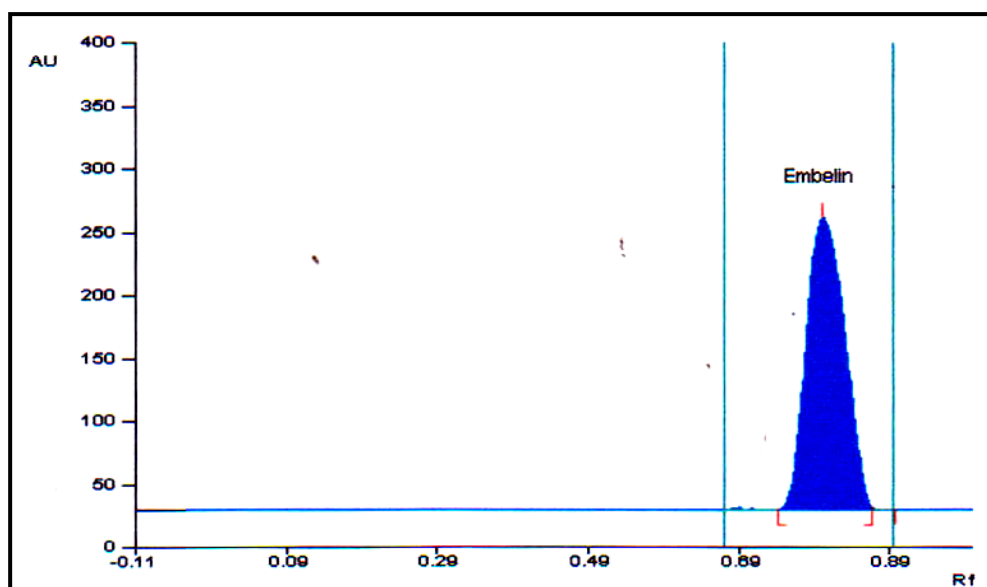


Figure 1: HPTLC densitogram of Embelin as an active chemical marker

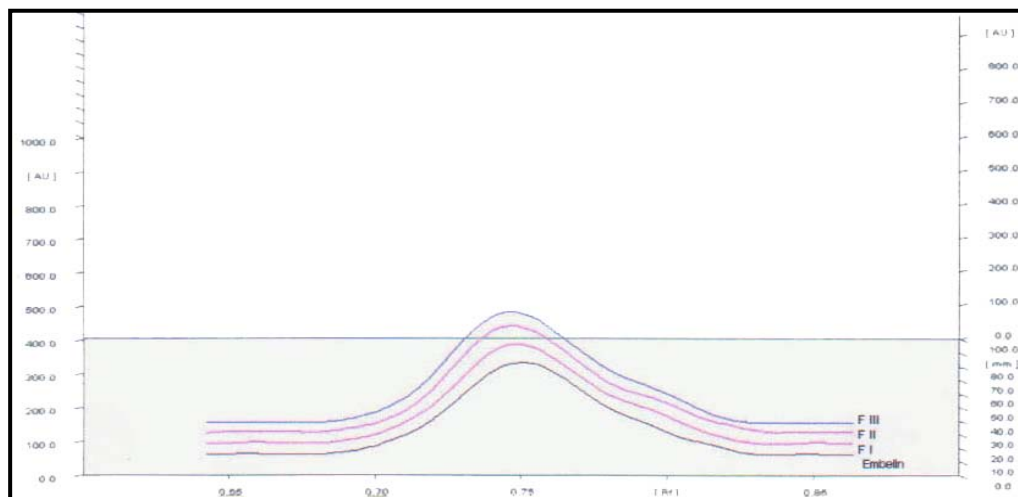


Figure 2: Overlain HPTLC densitogram of embelin and marketed formulations

Validation of method

Linearity plots and detection limits

The linearity for embelin was established by plotting the peak area versus concentration. The linearity of calibration curves was verified by

correlation study and the correlation coefficients were found to be 0.9986. The LOD and LOQ for embelin were found to be 61.28 ng/spot and 185.7 ng/spot respectively as shown in (Table 1).

Table 1: Calibration curves, Limit of detection and Limit of quantitation of Embelin

Linear range*	Correlation coefficient	LOD*	LOQ*
400-2400 ng/spot	0.9986	61.28 ng/spot	185.71 ng/spot

* Results are mean of six determinations

Precision, accuracy and robustness

As shown in (Table 2), % RSD value for inter-day and intra-day precision based on peak measurement was found to less than 2 (n=6), hence the methods were found to be precise. The average recoveries of the embelin were in the range of 99.09-102.01%. Satisfactory recoveries with small % relative standard deviations (less

than 2) were obtained, which indicate the accuracy of the method. The robustness study revealed that there were no significant differences in peak areas, retention time and R_f values after small, deliberate variation of the analytical conditions, which evaluated the robustness of the proposed method.

Table 2: Precision and Accuracy for Embelin

Formulation	Precision (%RSD)		Recovery*					
			80%		100%		120%	
	Interday	Intraday	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
F-I	0.92	0.301	99.09%	0.65	99.93%	0.16	100.03%	0.54
F-II	0.2	1.16	99.94%	0.1	102.01%	0.079	99.82%	0.105
F-III	0.108	0.153	99.71%	0.46	99.08%	0.11	99.88%	0.11

* Results are mean of three determinations

Quantitative analysis of embelin in marketed formulations

After chromatographic development the peak areas of the bands from samples were measured and the amount of embelin in each formulation

was determined from the respective calibration plots. The analytical procedure was repeated three times in each case. Results are shown in (Table 3).

Table 3: Quantitative analysis of Embelin from Marketed formulations

Formulation	Concentration	% Purity*	Embelin content* (w/w)
F-I	2000 ng/spot	99.76%	2.19%
F-II	2000 ng/spot	99.84%	2.19%
F-III	2000 ng/spot	100.08%	2.20%

* Results are mean of three determinations

CONCLUSION

The proposed HPTLC method was developed and validated for simultaneous determination of

Embelin. The method was found to be simple, sensitive, accurate, rugged, robust, rapid and precise. Hence, the above said method can be

successfully applied for routine quality control analysis and quantitative determination of

Embelin.

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REFERENCE

1. <http://www.Pharmainfo.net/reviews/chromatographic-fingerprint-analysis-herbal-medicines-quality-control-tool>, 2008, 6 (2) Accessed September 2008
2. Farnsworth NR & Morris RW (1976). Higher plants - the sleeping giant of drug development. *American Journal of Pharmaceutical Education*, 148: 46-52.
3. De Smet PAGM (1997). The role of plant-derived drugs and herbal medicines in healthcare. *Drugs*, 54: 801-840.
4. Cragg GM, Newman DJ & Snader KM (1997). Natural products in drug discovery and development. *Journal of Natural Products*, 60: 52-60.
5. Quality standards of Indian Medicinal Plants, 2003. Vol. IV, Indian council of Medical Research, New Delhi, P. 130-136
6. Jha N. K, Pandey I.K. Embelia ribes: Vidanga (Feature Article). *Phytopharm.* 2008 April: 3-14
7. http://www.himalayahealthcare.com/herbfinder/h_embeli.htm Accessed September 2008
8. Jarald E. E. Textbook of Pharmacognosy and Phytochemistry. New Delhi: CBS Publishers and distributors; 2006. p. 41
9. Pharmacopoeia of India, 2nd ed, Delhi: Manager of Publication, 1966. P. 796
10. ICH Harmonized-Tripartite Guidelines, Validation of analytical Procedures: Text and Methodology Q2 (R1), November; 2005