Research Article

Novel, Rapid, Isocratic RP-HPLC Method for Simultaneous Estimation of Piperine and Embelin in Herbal Formulation

Vandana Jain*, Revati Sonone, Leena Tandel
Department of Quality Assurance, Oriental College of Pharmacy, Sanpada - Navi Mumbai, Maharashtra, India.
***Corresponding author's E-mail:** vandana.jain@ocp.edu.in

Received: 10-06-2020; **Revised:** 04-09-2021; **Accepted:** 13-09-2021; **Published on:** 15-10-2021.

ABSTRACT

The objective of this paper was to develop and validate a novel, simple, rapid, precise and accurate, reverse-phase high-performance liquid chromatographic (RP-HPLC) method for simultaneous quantitative estimation of piperine and embelin in the herbal formulation as per the International Conference on Harmonization guidelines (ICH). Chromatographic separation was achieved using a Cosmosil C-18 (250*4.6mm) SH 5.0 µm column with a mobile phase consisting of methanol and 0.02 M phosphate buffer in ratio 98:2 v/v, (pH adjusted to 2.3 with ortho-phosphoric acid) at a flow rate of 1 mL/min and column temperature maintained at 28°C and ultraviolet (UV) detection at 288 nm. The retention time of piperine and embelin was found to be 4.15 and 5.69 min respectively. The linearity of piperine and embelin was tested in the range of 5-40 µg/mL. The correlation coefficient for piperine and embelin was found to be 0.997 and 0.995, respectively. The recovery values (98-102%) indicate a satisfactory accuracy. The method was found to be precise as the percentage relative standard deviation was found to be <2 %. The proposed novel isocratic RP-HPLC method is rapid (short run time below 10 min), precise, accurate and sensitive. The method was successfully applied for the simultaneous analysis of piperine and embelin in herbal formulation.

Keywords: Piperine, embelin, herbal formulation, Reverse-phase high-performance liquid chromatographic, Validation.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v70i02.001

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v70i02.001

INTRODUCTION

Herbal medicines widely used in health-care in both developed and developing countries.[1-2] Herbals are traditionally considered harmless and effective. To ensure the quantity, quality, safety, and therapeutic effect of ingredients in each dose, standardization of herbal drug is essential. Standardization minimizes batch to batch variation of the polyherbal formulation.[3]

The present study focuses on the standardization of an herbal formulation using high- performance liquid chromatography. Fattolin is a well-known marketed herbal formulation that is indicated mainly against obesity, high chlolesterol, supportive in cardiac diseases, arthritis.

The selected ayurvedic formulation consists of powder of Shunthi (*Zingiber officinale*), Marich (*Piper nigrum*), Pippali (*Piper longum*), Chitrak (*Plumbago zeylanica*), Vidanga (*Embelia ribes*), Shuddha Gandhak 20mg. each, Kanchnar (*Bauhinia variegata*), Gokshur (*Tribulus terrestris*) 40mg. each, Triphala, Shuddha Guggul (*Balsamodendron mukul*) 100 mg each, two chemical markers, one from each medicinal herb, were selected for the present work, namely, embelin from *Embelia ribes* and piperine from *Piper nigrum.*

Embelin exhibits diverse biological activities which mainly include anxiolytic, anticonvulsant, antidepressant, antidiabetic, wound healing, anthelmintic, antimicrobial, antitumor, chemopreventive, antioxidant, antifertility as reported in various literature.[4] Piperine is an alkaloid from *Piper nigrum,* commonly used as a spice due to the pungent and biting taste of piperine.[5]Piperine possesses antidepressant, antioxidant, hepatoprotective, anti-platelet, antithyroid, antitumor, antihypertensive, anti-inflammatory, bioenhancer effect.[6]

The literature survey reveals that various analytical methods for the estimation of embelin and piperine were reported alone and in combination with other drugs.[7-10] However, no isocratic high-performance liquid chromatography (HPLC) method has been reported for the simultaneous estimation of embelin and piperine. Therefore, an attempt has been taken to develop a novel isocratic reverse-phase HPLC method for the simultaneous estimation of embelin and piperine in the herbal formulation and validate the developed method in accordance with the International Council for Harmonization (ICH).[11]

MATERIALS AND METHODS

Instrument

HPLC chromatographic separation was performed on Shimadzu (LC 2030) model with Lab Solution software.

Ultraviolet (UV)-visible spectrometer was used for obtaining the maximum wavelength of compounds.

Standards and reagents

HPLC grade embelin and piperine (purity 99%) were purchased from Sigma-Aldrich, Mumbai, India. Herbal formulation of Fattolin tablet (Sharangdhar Pharmaceuticals Pvt. Ltd, Pune, India) used for analysis was purchased from the local market. HPLC grade solvents were purchased from Thermo Fisher Scientific India Pvt. Ltd.

Chromatographic conditions

RP-HPLC Shimadzu LC Prominence-i 2030 model with Lab Solution software was employed in this method. The separation of embelin and piperine was carried on Shimadzu Cosmosil C-18 (250*4.6mm) SH 5.0 µm column. The mobile phase used was methanol and 0.02 M phosphate buffer in the ratio (98:2 v/v) at a flow rate of 1 mL/min, injection volume was 20 µL, column temperature was 28°C, and standard solution of piperine and embelin were prepared and scanned separately in the range of 200-400 nm. The 288 nm wavelength was selected as detection wavelength for the detection of piperine and embelin.

Preparation of (0.02 M) phosphate buffer (pH 2.3)

About 2.72 g of potassium dihydrogen phosphate buffer was accurately weighed and then dissolved in 950 mL of water. The pH was adjusted to 2.3 with *ortho*-phosphoric acid, and the volume was made up to 1000 mL in a volumetric flask. The solution was then filtered

Preparation of standard stock solution

The standard stock solutions containing 100 mg each of piperine and embelin were prepared separately in 100 mL volumetric flask and then the volume was made up to methanol to obtain a stock solution of 1000 µg/mL. The stock solution was used for further analysis after suitable dilutions.

Preparation of sample solution

Ten tablets were triturated and 1 g of powder was accurately weighed and then taken for extraction. The powder was extracted with methanol for 30 min using the reflux assembly. The extract was made up to 100 mL with methanol. The solution was then filtered through Whatman filter paper to obtain a clear solution. The solution was injected after suitable dilutions.

RESULTS AND DISCUSSION

Method development

Different trials were carried out using methanol: phosphate buffer with varying concentrations. When a mixture of methanol: phosphate buffer (80:20 v/v) was used, the peak of piperine was obtained but embelin was not eluted. When a mobile phase consisting of methanol: phosphate buffer (90:10 v/v) was used, the peak of piperine was obtained but there was tailing in embelin peak. A satisfactory result was achieved at methanol and 0.02 M phosphate buffer (pH 2.3 adjusted with *ortho*-phosphoric acid) in a ratio of 98:2 v/v at flow rate 1 ml/min followed by detection at 288 nm respectively. The injection volume was kept 20 µL. The total run time were set at 7 minutes respectively. The column temperature was set at 28°C. The retention time of piperine and embelin obtained was 4.15 min and 5.69 min respectively. Chromatograms of mixed standard and sample solution are shown in Fig. 1-2.

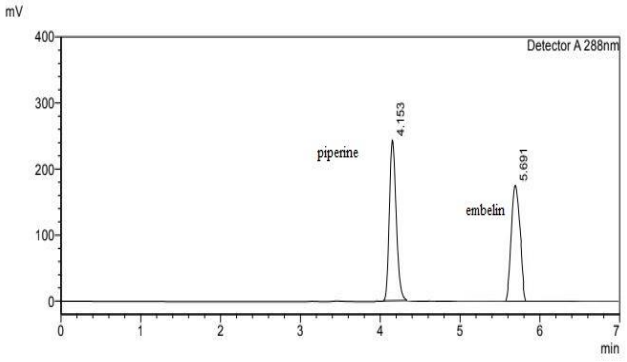

Figure 1: A typical RP-HPLC chromatogram of standard mixture of piperine and embelin obtained using optimized conditions

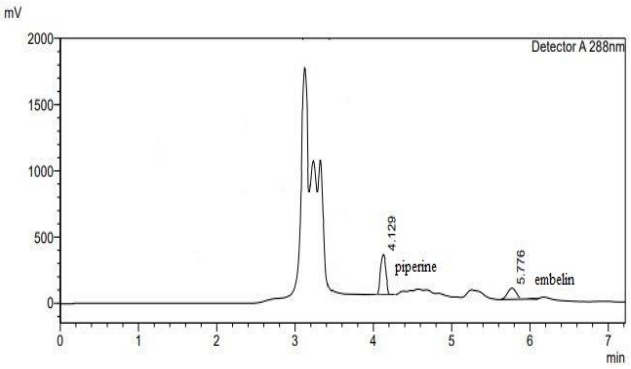

Figure 2: A typical RP-HPLC chromatogram of sample solution using optimized chromatographic conditions

Method validation

The developed method was validated for parameters such as specificity, precision, linearity, accuracy, robustness, limit of detection (LOD) and limit of quantification (LOQ) as per ICH guidelines.[11]

Specificity

Chromatograms of mixed standard and sample solution reveals that the peaks obtained are just because of the marker compound and blank has no peak at the retention time of piperine and embelin. Hence, the method is said to be specific.

Precision

Precision includes system precision and method precision. The system precision was carried out by injecting six injections of mixed standard of piperine and embelin. Method precision was performed by injecting the sample

solution of the same concentration six times. The percentage relative standard deviation (% RSD) was found to be less than 2. Therefore, the method is found to be precise. The system and method precision data are tabulated in table 1 and 2.

Table 1: Result of system precision

S. No	Peak area of piperine (10 µg/mL)	Peak area of embelin (10 µg/mL)
1.	1441961	1754696
2.	1428698	1746752
3.	1467324	1763746
4.	1446276	1764732
5.	1434760	1753821
6.	1485436	1746849
Mean ± SD	1450743 ± 21527	1755099 ± 7837
% RSD	1.48	0.45

SD: standard deviation, # %RSD: percent relative standard deviation

Table 2: Result of method precision

S. No	Peak area of piperine	Peak area of embelin
1.	1269051	884436
2.	1246278	882614
3.	1272763	874269
4.	1223792	886107
5.	1250287	879462
6.	1236273	887598
Mean ± SD	1249741 ± 18814	882414 ± 4893
% RSD	1.51	0.55

SD: standard deviation, # %RSD: percent relative standard deviation

Linearity

The linearity between peak area and concentration was analyzed using calibration curves obtained with standard solutions of piperine and embelin with different concentrations of each standard. The proposed method was found to be linear over a wide range of concentrations 5-40 µg/ml for piperine and embelin with a regression coefficient of 0.997 and 0.995, respectively. Hence, the method was found to be linear. The plots obtained from linear regression are shown in fig. 3 and fig. 4.

Quantification of marker in the formulation

Quantification of the markers was done by performing HPLC analysis of sample solutions. The amount of piperine and embelin present in the formulation was calculated using linear regression analysis. The % content of piperine and embelin was found to be 0.05 and 0.02 % w/w respectively.

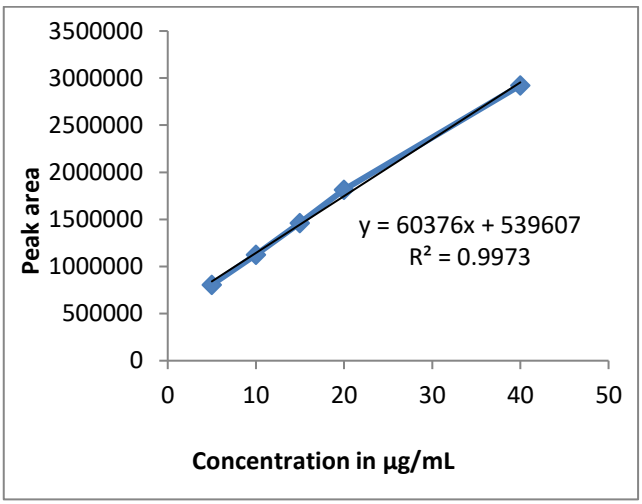

Figure 3: Calibration curve of piperine

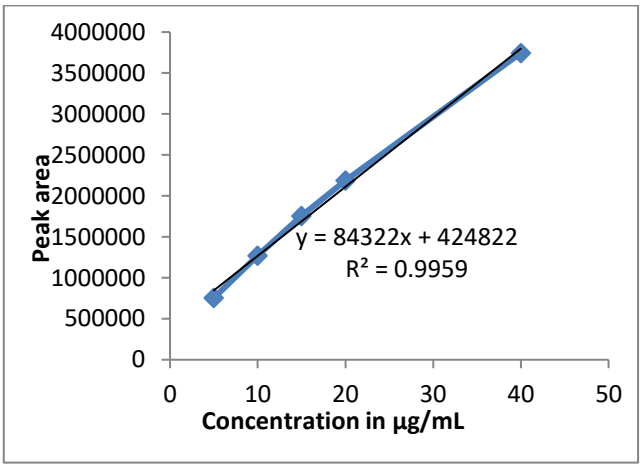

Figure 4: Calibration curve of embelin

Accuracy

Recovery of piperine and embelin from formulation was checked by spiking a known quantity of standards at three concentration levels (i.e. 80%, 100% and 120% of the quantified amount) to the test samples in triplicate using HPLC. This way, accuracy was performed and calculated. The % recovery was observed to be within the acceptance criterion of 98-102 % and the results are tabulated in table 3 for piperine and embelin respectively.

Robustness

The developed method was evaluated for robustness by small deliberate changes in optimized method parameters which were carried out with sample solution by making deliberate changes in flow rate (± 0.1 ml/min), column temperature (± 1°C), and wavelength (± 1 nm). The % RSD of peak area response of the test solution was found to be less than 2.0, hence the robustness parameters were found to be acceptable. The robustness results are tabulated in table 4.

Table 3: Percentage recovery results for piperine and embelin

Compound	Level %	Sample (µg/mL)	Standard added (µg/mL)	Total amount	Recovery	% Recovery
Piperine	**80**	60.4	48.3	108.7	108.5	99.8
	100	60.4	60.4	120.8	121.2	100.3
	120	60.4	72.4	132.8	131.9	99.3
Embelin	**80**	27.2	21.7	48.9	48.8	99.7
	100	27.2	27.2	54.4	54.5	100.1
	120	27.2	32.6	59.8	59.7	99.8

Table 4: Robustness results of piperine and embelin

Parameter (n=3)	Deviation	% RSD	
		Piperine	Embelin
Flow rate	0.9 mL/min	1.69	1.24
	1.1 mL/ min	1.36	0.79
Column temperature	27 ˚C	1.49	0.48
	29 ˚C	1.49	1.03
Wavelength	287	1.37	0.86
	289	1.04	1.17

n: number of injections

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ are expressed as follows:

LOD = 3 σ/S

LOQ=10 σ/S

Where, σ is the standard deviation of the responses and S is the slope of the calibration curve.

LOD and LOQ of piperine were found to be 1.20 and 3.56 µg/mL,

respectively, and that of embelin were found to be 0.31 and 0.92 µg/mL, respectively.

A low LOD and LOQ indicate that the method is sensitive.

CONCLUSION

The present paper describes a novel rapid RP-HPLC method for the simultaneous estimation of phenylephrine and embelin in the herbal formulation. This developed method was validated as per the ICH guidelines and results found to be linear, accurate, precise, repeatable, sensitive for the detection and quantification of both drugs. Hence, the proposed method was found to be satisfactory and can be applied for routine qualitative and quantitative analysis of piperine and embelin in an herbal formulation containing these markers as one of the ingredients.

Acknowledgment: The authors are thankful to the Oriental College of Pharmacy for providing necessary research facilities.

Authors' Contributions

All authors have equally contributed toward the preparation of the manuscript.

Conflicts of Interest

Authors declare that no conflicts of interest exist in this research work.

REFERENCES

1. Subramani Parasuraman, Gan Thing, Sokkalingam Dhanaraj; Polyherbal formulation: Concept of Ayurveda; Pharmacognosy review. 2014;8(16):73-80. Doi: 10.4103/0973-7847.134229, PMID: 25125878
2. Bhattacharjee Abhishek; An overview on phytosome as a novel drug delivery system for herbal drugs, International journal of innovative pharmaceutical sciences and research, 2015;3(6):730-739. ISSN: 2347-2154
3. Kumari Rajesh, Kotecha Mita; A review on the standardization of herbal medicines; International journal of pharma sciences and research, 2016;7(2):25-29. ISSN: 0975-9492
4. Kumar Sakesh, Deshmukh Rahul; Embelin as a potential drug molecule: a review; Journal Pharmacognosy and natural Products. 2017;3(3):2472-0992. DOI:10.4172/2472-0992.1000144
5. Dogra RKS, Khanna Santosh, Shanker Ravi; Immunotoxicological effects of piperine in mice;Toxicology. 2004;196(3):229-236. doi:10.1016/j.tox.2003.10.006, PMID:15036749
6. Gorgani Leila, Mohammadi Maedeh, Najafpour Ghasem D, Nikzad Maryam; Piperine- the bioactive compound of black pepper: from isolation to medicinal formulation; Comprehensive reviews in food science and food safety. 2017;16:124-140. Doi: 10.1111/1541-4337.12246.
7. Rastogi Shruti, Bhatia Amandeep K, Kushwaha Ashish, Pandey Manoj K, Sharma Alok, Prakash Jai, Singh GN; Development and validation of a liquid chromatography method for determination of embelin in crude extract of *Embelia ribes*; Asian Journal of

Biomedical and Pharmaceutical Sciences. 201;04(36): 9-13. Doi: 10.15272/ajbps.v4i36.554, ISSN: 2249-622X

8. Pathan Imran, Patel Rakesh K, Bhandhari Anil; Standardization, development, validation of spectrophotometric method for simultaneous estimation of embelin and gallic acid as individual and in combination in ayurvedic churna formulation; Asian Journal of Pharmaceutical and Clinical research. 2013;6(9):170-175. ISSN: 0974-2441.
9. Jain Vandana, Shaikh Sultana; Development and validation of a RP-HPLC method for the simultaneous determination of curcumin, piperine and camphor in an Ayurvedic formulation; International Journal of Pharmacy and Pharmaceutical Science. 2018;10(4): 115-21. Doi: 10.22159/ijpps.218v10i4.25216
10. Shaikh Sultana, Jain Vandana; A novel RP-HPLC method for simultaneous estimation of ellagic, quercetin and piperine in ayurvedic formulations; Asian journal of pharmaceutical and clinical research. 2018;11(6):312-317.
DOI: https://doi.org/10.22159/ajpcr.2018.v11i6.25627
11. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1); 2005.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.