INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

APPLICATION OF RP-HPLC METHOD FOR THE DETERMINATION OF BOLDINE IN *ALPHONSEA SCLEROCARPA* THWAITES

Venkata Naga Anantha Sandhya Rani Nandyala [1]*, Kothapalli Bonnoth Chandrasekhar [2]
[1]Research Scholar, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu, Andhra Pradesh, India
[2]Department of Chemistry, Jawaharlal Nehru Technological University Anantapur (JNTUA), Ananthapuramu, Andhra Pradesh, India
*Corresponding Author Email: sandhyarani.nandyala@gmail.com

Article Received on: 11/05/18 Approved for publication: 12/06/18

DOI: 10.7897/2230-8407.09697

ABSTRACT

Determination of Boldine in *Alphonsea sclerocarpa* Thwaites was followed through by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Separation and determination of Boldine in chloroform, bark & leaf extracts of *Alphonsea sclerocarpa* was accomplished on an inertsil ODS(150mmX 4.6mm, 5µm i.d.) column system employing isocratic elution mode with a mobile phase consisting of Acetonitrile and 0.1% Octane sulphonic acid buffer (70:30) (v/v) at a constant flow rate of 1ml/min. Calibration graph of Boldine in the concentration range of 84-420µg.ml^{-1} was obtained by using the linear relationship between concentration and peak area measured at a wavelength of 302nm using UV detector. The entire chromatographic run was carried out at ambient temperature. The developed RP-HPLC method was successfully validated as per ICH guidelines (ICH, Q2 (R1)) The retention time of boldine was found to be 2.1 min. Percentage recovery was found to be 100.82, 100.04 &100.44 at 50, 100, 150 % levels respectively. The %RSD for intermediate precision and repeatability was found to be 0.9 and 0.8 respectively. Limit of Detection and Limit of Quantitation was found to be 5.22 and 15.82 µg.ml^{-1}. The method developed was validated and successfully applied for the quantitative analysis of Boldine in Chloroform-Bark and leaf extracts of *Alphonsea sclerocarpa* Thwaites.

Keywords: *Alphonsea sclerocarpa* Thwaites, Reverse Phase High Performance Liquid Chromatography (RP-HPLC), Inertsil ODS, UV Detector, Boldine, Isocratic elution.

INTRODUCTION

Species of Alphonsea belonging to the Dicotyledonous plant family Annonaceae are small trees and shrubs found mostly in India, Srilanka, Southeast Asia and Malaysian region [1]. *Alphonsea sclerocarpa* is a under storey tree with Oblong to Lanceolate or elliptic simple, alternate leaves ranging in size from 2.5-6.5 × 2-3 cm, fissured bark, flowers in cymes and fruits in the form of aggregated berries [2]. The genus Alphonsea contains about 30 species distributed in China and Indo-Malayan region [3] .Among several of the species *Alphonsea lutea* is a tree of Bangladesh, Orissa, Myanmar and Srilanka and the other species viz. *Alphonsea madraspertana* and *Alphonsea sclerocarpa* were the under storey trees found in hills of Cuddapah, Andhrapradesh, Thiruvannamalai district, Tamilnadu and Agasthyamalai hills, Kerala respectively [4,5]. Among the above mentioned species *Alphonsea sclerocarpa* is the least explored and the most widely available species at the place of our study and hence the plant was chosen and from the phytochemical studies conducted so far it is evident that the bark of *Alphonsea sclerocarpa* contains non-quaternary alkaloids such as Liriodenine (12%), Anonaine (5.5%) etc and the leaves of the plant were known to contain Crotsparine (25%), isoboldine etc [6] . The plant is proven to possess anti-fungal and anti-oxidant principles in its bark and leaves [7]. The activities shown may be attributed to the phytoconstituent Boldine present in it and hence a novel HPLC method is essential for the identification and quantification of Boldine present in *Alphonsea sclerocarpa* and hence the present study is undertaken to prove the presence of Boldine in *Alphonsea sclerocarpa* as well to quantify it in the extracts. From the past 60 years several approaches for the quantification of Boldine were utilised as densitometry, spectrofluorimetry, Micellar Electro kinetic chromatography and the first HPLC method was developed in this era of innovation considering the use of Boldine in Pharmaceuticals [8-12]. The published HPLC analytical method for the quantification of Boldine was found to have less sensitivity, reliability and even longer analysis time [13] and hence this study is undertaken to develop and validate a robust, sensitive and reliable method for the determination and quantification of Boldine in extracts as per ICHQ2 (R1)[14] guidelines. The phytoconstituent is of prime importance in pharmaceutical industry due to its pharmacological effects viz. Anti-inflammatory, Anti-tumor, Choleretic, Cholagogue etc [15]. Owing to its pharmacological importance a focused quest for determining its presence in *Alphonsea sclerocarpa* is made with RP-HPLC and the attempt was found to be successful. In this study a novel reliable, rapid and sensitive RP-HPLC method was developed and applied for the estimation of Boldine in leaf and bark extracts of *Alphonsea sclerocarpa.*

MATERIALS & METHODS

Plant Material

Aerial parts (Leaf & Stem bark) of the plant were collected from agasthyamalai hills in Kerala during the month of June 2016. Folia and bark of the plant were washed under tap water; air dried, homogenized and sealed in air tight bottles for future use.

Voucher specimen of the species was deposited at Plant Anatomy Research Centre with the reference number PARC/2016/3289. Locality in which the species is available is tabulated and the yield of alkaloids from the investigated species is presented in Table 1.

Chemicals

Boldine used as a marker compound was procured from Sigma Aldrich (USA).Chromatographic grade double distilled water and Acetonitrile used were obtained from Merck Darmstadt, Germany. 1-Octane Sulphonic acid used was purchased from Sigma Aldrich (USA). All the other chemicals utilised in the study were of analytical grade.

Extraction

Twenty five grams of air dried leaf and bark powders were first defatted with petroleum ether and then extracted with chloroform in a Soxhlet Apparatus for 24 hours. The chloroform extract was concentrated by removing the solvent using Rotavapor and then evaporated on a water bath till dried and stored in airtight containers for future use. The extracts were dissolved in the mobile phase and were filtered through a 0.5µm PTFE filter. The filtered sample solutions were then injected into the RP-HPLC Column system.

Apparatus

A Waters Alliance 2695 separation module equipped with a 2487 UV detector was availed in the entire study. Separation was achieved on an Inertsil C18 Column (4.6mm X 150mm, 5.0µm i.d.) during the study. Detection responses in peak area terms were measured at 302nm using UV detector. Waters Empower software integrated the peak areas automatically. Mettler Toledo electronic balance was employed throughout the study. Solubility of the extracts was enhanced using Ultrasonicator (Remi).

Standard Solutions

Mobile Phase or Diluent is prepared by accurately weighing 2.5g of 1-Octane sulphonic acid and dissolving it in 250ml HPLC grade water. The final pH of the solution is adjusted to 3.0 using Sodium Hydroxide (0.1N). 30% of the above solution is mixed with 70% HPLC grade Acetonitrile, degassed and filtered through 0.5µm PTFE filter.

Stock solution (8.4mg $10mL^{-1}$) of Boldine was prepared in the above mobile phase. Standard series of Boldine in the concentration range of 84-420µg mL^{-1} were obtained by using the above stock solution. All the calibration and sample solutions were prepared by employing the mobile phase prepared during the study.

Chromatographic Conditions

An Inertsil ODS (4.6mm X 150mm, 5.0µm i.d.) column system together with the mobile phase consisting of Acetonitrile and 0.1% Octane sulphonic acid buffer (70:30) (v/v) at a constant flow rate of 1 ml min^{-1} was utilized for achieving HPLC separation and analysis. All the solvents employed for the study were first filtered through 0.5µm Millipore filter and further degassed using Ultrasonicator. Column temperature was maintained at 25 ºC. Injection volumes were 20µL for both the standard as well leaf and bark sample solutions. Quantification was carried out at 302nm employing 2487 UV detector.

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of Boldine in Leaf and Bark extracts of *Alphonsea sclerocarpa* was performed by RP-HPLC in this study. In this analysis, various mobile phase compositions and chromatographic conditions were tried to optimize chromatographic conditions. A mobile phase consisting of Acetonitrile and buffer (0.1% Octane Sulphonic acid) (70:30, v/v) and a constant flow rate at 1ml min^{-1} on an Inertsil C18 (4.6mm X 150 mm, 5.0µm i.d.) column using an isocratic elution mode and a temperature of 25º C were found to be the optimal conditions for the separation of Boldine from leaf and bark extracts of *Alphonsea sclerocarpa*. Baseline resolution was obtained, and good peak shapes were observed without tailing.

Chromatograms were obtained by using UV detector at 302nm and the responses were measured as peak areas. In this analysis 20µL of chloroform Leaf and Bark extracts were injected into the RP-HPLC Column system at the flow rate of 1ml min^{-1}. The retention time for standard Boldine and the extracts was observed to be 2.1min as shown in Fig 1 a, b, and c respectively.

Dilutions of Boldine were prepared in the mobile phase (Acetonitrile: 0.1% Octane sulphonic acid 70:30, v/v) in the concentration range of 84-420µg mL^{-1}. The calibration graph was obtained by plotting concentration on abscissa and peak areas on ordinate. Linear regression analysis and its results were summarized in Table II. Content of Boldine in the samples was calculated from the linear regression equation obtained above.

Method Validation

Validation of the above developed RP-HPLC method was done as per International Conference on Harmonization (ICH) monograph [16]. For the quantitative analysis of Boldine at 302nm, linearity of the RP-HPLC detector response was observed in the concentration range of 84-420µg mL^{-1} at five different concentration levels. Analysis of each concentration was done in triplicate to observe peak area difference between samples of same concentration. The linearity of the linear regression equation of Boldine at 302nm was checked by the high value of the correlation coefficient. Repeatability was characterized by RSD%. Linear regression equation for the developed method was given in Table 2.

The precision of the developed RP-HPLC procedure was tested by injecting the samples in six replicates within the working concentration range. Relative standard deviation was found to be 0.9%. The accuracy of the developed method was tested at three different levels (50,100&150%) and the mean recovery value was found to be 100.43%. The working standard and the sample solutions were stored at room temperature and all the experiments were performed on the same day. The above developed method has shown good agreement between the analysis results. Systematical error, interference was not observed during the analysis.

According to the ICH [16-18], the Limit of detection (LOD) and Limit of Quantization (LOQ) were calculated by using standard deviation of the response and the slope of linear regression line (Table 2).

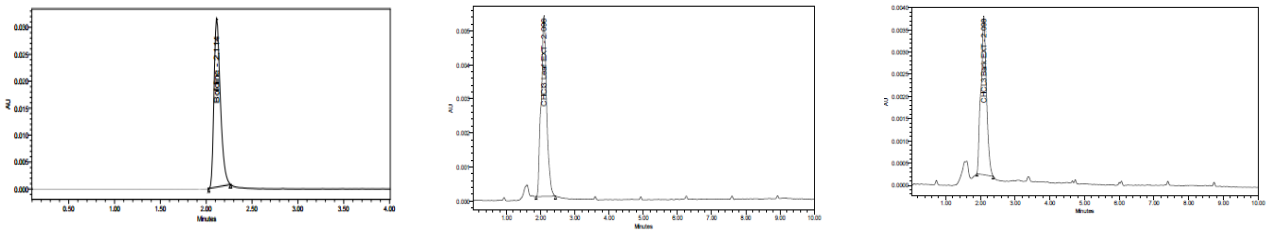
Table 1. Experimental results for boldine content in the chloroform extracts of *Alphonsea sclerocarpa* by RP-HPLC Method

Species and Extracts	Locality	% Boldine mg/mg[a]
Alphonsea sclerocarpa	Agasthyamalai Hills, Kerala, 14.06.2016. 500m (PARC/2016/ 3289)	----
Alphonsea sclerocarpa Chloroform Leaf Extract	In-house	0.67
Alphonsea sclerocarpa Chloroform Bark Extract	In-house	0.44

[a] Each Value is the mean result of measurements made in triplicate

Table 2. Statistical data for the calibration graphs of boldine in mobile phase

Statistical Parameter	**Experimental Values Obtained**
Linearity range µg mL $^{-1}$	84-420
Slope (m)	222366
Intercept (c)	8380.5
Correlation coefficient of the regression function (r)	0.999
Limit of Detection (LOD µg mL $^{-1}$)	5.22
Limit of Quantitation (LOQ µg mL $^{-1}$)	15.82
Repeatability (RSD %)	0.8
Accuracy (Mean Recovery %)	100.43



a **b** **c**

Fig 1. Chromatograms of 84µgmL^{-1} standard Boldine (a); *Alphonsea sclerocarpa* Leaf Chloroform Extract (b); & *Alphonsea sclerocarpa* Bark Chloroform extract (c)

Sample Analysis

The proposed RP-HPLC method was applied for the determination of Boldine in Leaf and bark extracts of *Alphonsea sclerocarpa*. The assay results were provided in Table 1. It was noticed that the content of boldine was more in chloroform leaf extract than that of Chloroform Bark extract. The content of boldine in Chloroform Leaf and Bark extracts were found to be 0.67 and 0.44% respectively.

SUMMARY AND CONCLUSION

The developed RP-HPLC Method was elaborated for the determination of boldine in Chloroform Leaf & Bark extracts of *Alphonsea sclerocarpa*. Upon comparing the proposed RP-HPLC method with those reported in the literature the developed method needs short analysis time, Low flow rates to provide higher chromatographic resolutions. It was observed that the total retention time for boldine analysis in our case is much shorter than in the previously reported studies. This method was found to be very economical owing to its very short run time and less solvent consumption. Our method gave us the shortest retention time of 2.1 min. Moreover, a very narrow chromatographic peak was obtained for boldine. The proposed RP-HPLC method is useful for boldine analysis in *Alphonsea sclerocarpa*.

ACKNOWLEDGEMENTS

We are grateful to Jawaharlal Nehru Technological University Anantapur (JNTUA) and Pharma Train –Hyderabad for providing all the support during the experiment.

REFERENCES

1. Gaurav Srivastava, Rakesh C M. First Fossil Record of *Alphonsea* Hk. f. & T. (Annonaceae) from the Late Oligocene Sediments of Assam, India and Comments on Its Phytogeography. PLoS ONE. 2013; 8:1-6.
2. VNA Sandhya Rani N, Chandrasekhar KB. Pharmacognostical Standardization & Phytochemical Evaluation *of Alphonsea sclerocarpa* Thwaites Bark & Leaves.Pharmacognosy Journal.2017; 9(2):196-200.
3. Mabberley DJ. The Plant Book, a portable dictionary of the vascular Plants. Cambridge: Cambridge University Press; 1997. (05 Jan 2018)
4. Kessler PJA. Studies on the tribe Saccopetaleae (Annonaceae) - IV Revision of the genus Alphonsea Hook. f. Thomson. Botanische Jahrbücher. 1996; 118: 81-112.
5. Gamble JS .A manual of Indian Timbers. Dehradun: Bishen Singh Mahendra Pal Singh; 1972. (05 Jan 2018 Availablefrom:https://ia800303.us.archive.org/12/items/manualofindiant00gamb/manualofindiant00gamb_bw.pdf
6. Dragana Tadic, Percy Wannigama G. Alkaloids of *Alphonsea sclerocarpa*. Journal of Natural Products. 1987; 50 (3): 518-519.
7. Narendra Prasad D. Antioxidant Activity of *Alphonsea sclerocarpa* Bark. Research Journal of Pharmacology and Pharmacodynamics. 2009; 1 (2): 66-69.
8. Malcangio AM. Colorimetric determination of boldine. Farmaco Edizione Scientifica. 1955; 10(5):262-267.
9. Di Renzo N. Densitometric Determination of Boldine in Boldo Extracts. Bollettino Chimico Farmaceutico.1972; 111 (7):450-453.
10. Gurkan T. Fluorimetric analysis of alkaloids. Determination of serpentine, yohimbine, and boldine .Mikrochim Acta. 1976 1:173-180.

11. Vilchez JL, Sanchezpalencia G, Avidad R, Navalon A. Determination of boldine in drug formulations by first-derivative synchronous spectrofluorimetry. Journal of Pharmaceutical and Biomedical Analysis.. 1994; 12:313-317.
12. Sun SW, Lee SS, Chen LY, Chen CK. Separation of Aporphine alkaloids by Micellar Electrokinetic chromatography. Journal of Chromatography A. 1997; 767 :277-284.
13. Cristiane C. Study of quality assurance for *Peumus boldus* M Products by Botanic Profiling, Extraction optimization, HPLC Quantification and Antioxidant Assay. Pharmacognosy Journal. 2016; 8:264-272.
14. Masato K, Shinji U, Naoto T et.al, Validation of analytical procedures by high−performance liquid chromatography for pharmaceutical analysis. Chromatography. 2012; 33(2) :65-73.
15. Peter O Brien, Catalina Carrasco et.al, Boldine and its antioxidant or health-promoting properties. Chemico-Biological Interactions.2006; 159 (1) :1-17.
16. ICH harmonized tripartite guideline. Validation of analytical procedures: text and methodology, Q2-R1.2005.(05Jan2018)Availablefrom:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
17. Milos H, Stanislav M, Jolana C et.al, Development of an HPLC fluorescence method for determination of boldine in plasma, bile and urine of rats and identification of its major metabolites by LC–MS/MS.Journal of Chromatography B. 2013; 936 (1):48-56.
18. Camara CI, Bornancini CA, Cabrera JL et al, Quantitative analysis of boldine alkaloid in natural extracts by cyclic voltammetry at a liquid–liquid interface and validation of the method by comparison with high performance liquid chromatography. Talanta. 2010; 83 (2):623-630.

Cite this article as:

Venkata Naga Anantha Sandhya Rani Nandyala & Kothapalli Bonnoth Chandrasekhar. Application of RP-HPLC method for the determination of boldine in *Alphonsea sclerocarpa* Thwaites. Int. Res. J. Pharm. 2018;9(6):100-103 http://dx.doi.org/10.7897/2230-8407.09697

Source of support: Nil, Conflict of interest: None Declared