Standardization of *Adhatoda vasica* Nees Market Preparations by RP-HPLC Method

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ABSTRACT: In recent times, quality control of herbal and traditional medicines with modern scientific techniques and knowledge are of great concern. The present study reveals a simple and improved reversed phase HPLC method for qualitative analyses of *Adhatoda vasica* Nees market preparations via quantitation of its major metabolite, vasicine as a marker compound. Three market preparations, each of four different herbal and traditional manufacturers, were analyzed. The market preparations were extracted with chloroform and the residue obtained from extraction of each market preparation was analyzed for quantitation of vasicine by RP-HPLC method with ODS column using a mixture of water and methanol (60:40) as mobile phase at a flow rate of 0.5 ml/min. The estimated quantities of vasicine compared to reference standard for marketed products of four different manufacturers were found to be 1.502 ± 0.064 g, 1.590 ± 0.081 g, 1.761 ± 0.061 g and 1.627 ± 0.082 g, respectively per 100 ml of preparation.

Key words: Standardization, Quality control, *Adhatoda vasica* Nees, Vasicine, RP-HPLC

INTRODUCTION

Plants have been used in many countries for the healing and diseases preventing properties for thousands of years.[1] From the beginning of human civilization, the plant based treatments were familiar to people by different systems such as Ayurveda, Siddha, Unani, Yoga, Naturopathy, Homoeopathy and many more.[2] These systems are playing important role in providing health care to large section of population, especially in developing countries.[2] About 80% of world population rely chiefly on traditional medicines for their primary health care needs.[3] Plant based medicines provide first line and basic health services in remote areas where people have been living in poor health care services.[4] In the recent time, *Adhatoda vasica* Nees (Family: Acanthaceae) commonly named as basak has been used the Indian subcontinent for its prominent bronchodilatory activities.[5] The uses of *A. vasica* are growing fast in spite of modern H-1 receptor blockers to the world's people due to its less toxic effects and low cost. Beside this, *A. vasica* has been used for multitude of disorders including bronchitis, leprosy, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumors, mouth troubles, sore-eye, fever and gonorrhea.[6] Investigation showed that beside many more additional components, *A. vasica* leaves contain vasicine and vasicinone (pyrroloquinazoline alkaloids) which are responsible for bronchodilatory activities.[7] Vasicine is an optically active component in normal environment and get racemized during extraction.[8]

Now-a-days, the quality of herbal and traditional medicines is a raising concern due to the growing demand of traditional systems of medicines. According to the guideline published by the WHO, various macroscopic, microscopic, spectrophoto-

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metric (UV, FTIR, LCMS/MS, LC-NMR, GC-MS) and chromatographic methods (TLC, HPLC, HP-TLC, HPLC-MS, HPLC-NMR) are employed for qualitative analyses of *A. vasica* preparations.[9,10] Most of the macroscopic (organoleptic properties, moisture content, total ash determination and particle size), microscopic analyses (microbial load), assessment of other toxic materials (pesticide residues, heavy metal analysis and aflatoxins) and *p*H determination only fulfill the initial prerequisite of the preparations but not the final quality of the preparations like presence and quantity of active metabolites.[11] Thus, the developed countries are maintaining the spectrophotometric and chromatographic methods for quality control and standardization of traditional and herbal medicines besides macroscopic and microscopic analyses.[12] In the present study, an attempt has been taken to standardize the *A. vasica* market preparations via quantitation of its major metabolite vasicine by a simple, precise and cost effective RP-HPLC method.

MATERIALS AND METHODS

Market samples. Most of our local herbal and traditional manufacturers have started marketing of *A. vasica* preparation under various trade names. A total of twelve samples from four different manufacturers (three samples of each company) were purchased from local market ensuring that their batch numbers were not similar to each other. The preparations were stored at cool and dark place until use.

Chemicals and instruments. The analytical grade solvents and reagents were used without further purification. HPLC grade methanol (Sigma Aldrich, Germany) and water purified by Millipore system were used for the analysis. RP-HPLC (Shimadzu, Japan) equipped with a manual injector, a vacuum degasser, a multiple-wavelengths UV/Visible detector (Shimadzu SPD 20A, Japan) and an ODS column (Luna C_{18} 100 Å, 250x4.6 mm I.D., 5.00±0.30 µm particle size) were used for the chromatographic analyses. Reference standard of vasicine (Potency 100%, Article no. 89821, Batch no. 3784, Report no. 10543241, Germany) was supplied as generous gift by Square Herbal and Neutraceuticals Limited, Pabna, Bangladesh.

Standardization of market preparations

Preparation of standard solution. The standard stock solution of 640 µg/ml was prepared by dissolving reference standard vasicine in HPLC grade methanol with occasional shaking and sonication. The standard solutions with the concentration of 320 µg/ml, 160 µg/ml, 80 µg/ml, 40 µg/ml, and 20 µg/ml were then prepared by dilution of stock solution with methanol. All the solutions were filtrated separately through 0.45 µm disc filter and preserved at below 4 ^{o}C.[13]

Preparation of market samples. A total of twelve market preparations from four different companies were collected and each product contained 100 ml of liquid preparation. For each sample, about 10 ml of market preparation was taken and shaken with 30 ml of methanol (95%) for about 10 mins and then treated with aqueous solution of citric acid (5%). The solution was extracted with chloroform at a ratio of 2:1 (chloroform:solution) for three times and the aqueous layer was collected. The combined collected aqueous layer was filtered and then basified with ammonia solution (25%) to pH 9.5 and further extracted with chloroform at ratio of 2:1 (chloroform:solution) for three times. The organic layer was collected, combined and dried at reduced pressure to get yellowish residue. Finally, an appropriate quantity of residue was dissolved in HPLC grade methanol with sonication to prepare sample solution with the concentration of 150 µg/ml and then filtered through 0.45 µm disk filter. All the samples were preserved at air tight vial bellow 4 ^{o}C.[13]

Chromatographic conditions. The quantity of vasicine in sample solution was estimated by using reversed phase HPLC equipped with C_{18} column. The isocratic elution was carried out with a binary solvent system of water and methanol (60:40) as mobile phase at a flow rate of 0.5 ml/min maintained at 19 ^{o}C temperature and 27% relative humidity. The

sample injection volume was 20 µl and the analyses were monitored with the UV-Vis detector at 298 nm.[13,14]

Quantitation of vasicine in market samples. The concentrations of vasicine in market samples were determined using the calibration curve for reference standard. The standard solutions of different concentrations were analyzed through RP-HPLC method and peak areas were recorded. A calibration curve was prepared by using peak areas versus concentrations of standard solutions. Sample solutions (150 µg/ml) were then analyzed with the RP-HPLC method maintaining similar chromatographic conditions and the peak areas were recorded at defined retention time and vasicine concentrations were determined using linear regression equation of the calibration curve. All determinations were conducted in triplicate.

RESULTS AND DISCUSSION

The quantitation of vasicine in market samples were carried out by RP-HPLC method. The retention time for reference standard vasicine was observed at 4.792 min (Figure 1). The linear regression equation for the calibration curve was Y=25533X+11560 (X= concentration and Y= peak area) with the correlation coefficient (R^2) of 0.998 (Figure 2, Table 1).

Table 1. Peak areas of RP-HPLC chromatogram for various concentrations of reference standard of vasicine.

No.	Concentration (µg/ml)	Peak area
1	20	520064
2	40	990128
3	80	2097316
4	160	4255907
5	320	8791634
6	640	16210732

Table 2. Estimation of vasicine concentration in market sample solutions (150 µg/ml).

Sample	Batch	Peak area (average)	Vasicine concentration (µg/ml)	Average concentration (µg/ml) ± S.D.
A	1	627813	22.655	23.667±0.849
	2	682101	24.732	
	3	652914	23.615	
B	1	798801	29.197	30.724±1.088
	2	862955	31.652	
	3	854331	31.322	
C	1	881722	32.370	33.390±0.723
	2	923303	33.961	
	3	920127	33.839	
D	1	790352	28.874	27.769±1.152
	2	774120	28.253	
	3	719925	26.179	

The sample solutions (150 µg/ml) of all batches were analyzed and chromatograms showed different peak areas with the retention time at 4.450 min, 4.767 min, 4.833 min and 4.542 min for samples A, B, C and D, respectively (Figure 3 A-D). The average concentrations of vasicine in each sample solution of four different manufacturers were found to be 23.667±0.849 µg/ml, 30.724±1.088 µg/ml, 33.390±0.723 µg/ml and 27.769±1.152 µg/ml (Table 2). The quantities of vasicine in market preparations of four different manufacturers were estimated as 1.502±0.064 g, 1.590±0.081 g, 1.761±0.061 g and

1.627±0.082 g per 100 ml preparations (Table 3). Standardization of *A. vasica* market preparations were accomplished through quantitative analyses of major metabolite vasicine. The results showed that market preparations contained significant quantity of vasicine when compared to reference standard. The method has the advantages of being simple, precise, less time consuming and more convenient especially for routine analyses, and may be used for evaluating the quality of herbal and traditional medicines beside regular macroscopic and microscopic analyses.

Table 3. Estimation of vasicine in market preparations.

Sample	Batch	Extracted residue obtained from 10 ml of market preparation (mg)	Vasicine concentration in 150 µg/ml of extracted residue solution (µg/ml)	Vasicine content in 10 ml of market preparation (mg)	Vasicine content in 100 ml of market preparation (g)	Average content (g) ± S.D.
A	1	950.18	22.655	143.509	1.435	1.502 ± 0.064
	2	947.72	24.732	156.260	1.563	
	3	958.56	23.615	150.909	1.509	
B	1	768.94	29.197	149.672	1.497	1.590 ± 0.081
	2	780.15	31.652	164.622	1.646	
	3	778.75	31.322	162.613	1.626	
C	1	783.27	32.370	169.030	1.690	1.761 ± 0.061
	2	790.33	33.961	178.936	1.789	
	3	798.72	33.839	180.186	1.802	
D	1	872.67	28.874	167.983	1.680	1.627 ± 0.082
	2	886.38	28.253	166.953	1.670	
	3	878.41	26.179	153.306	1.533	

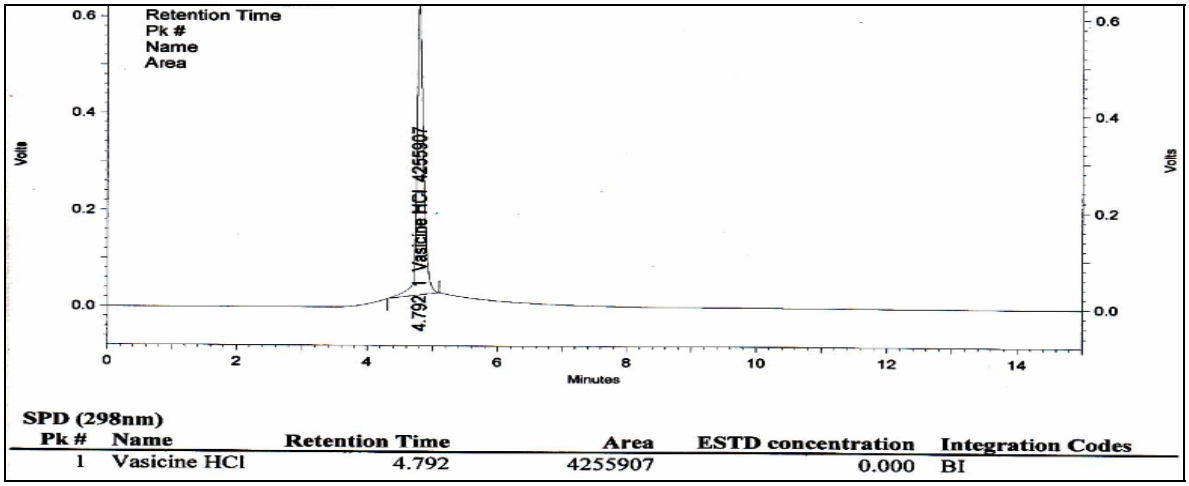


Figure 1. Chromatogram for reference standard of vasicine observed by RP-HPLC.

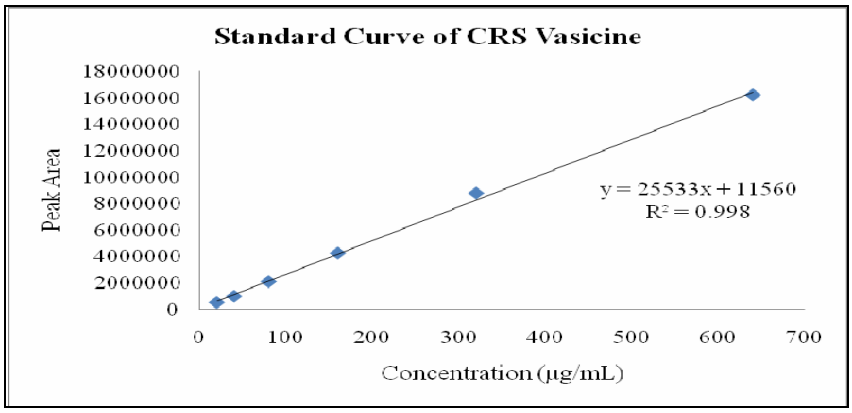
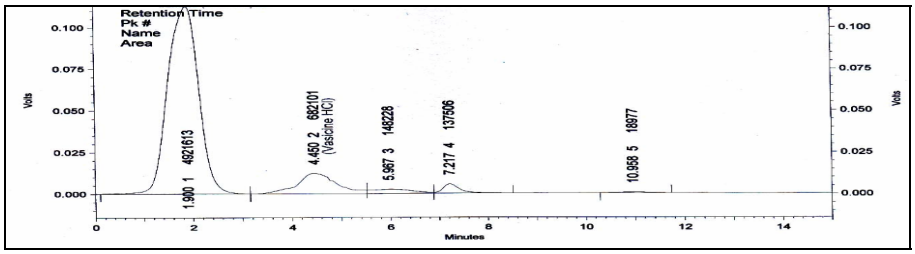
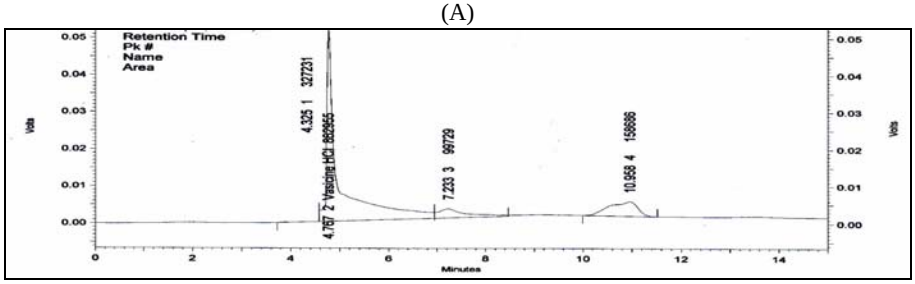


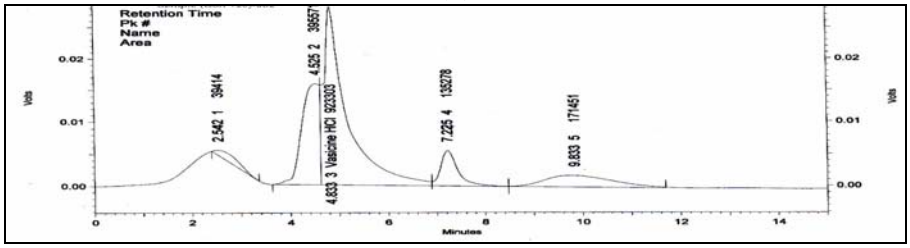
Figure 2. Calibration curve for reference standard of vasicine.



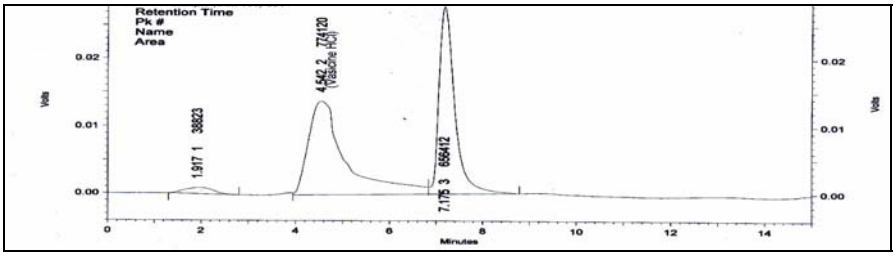
(A)



(B)



(C)



(D)

Figure 3. Chromatograms of *A. vasica* market preparations (sample A-D) obtained during RP-HPLC analyses.

CONCLUSION

Recently, most of the herbal and traditional manufacturers have been marketing *A. vasica* preparations for its potential bronchodilatory activity but due to lack of proper instrumentations and knowledge, manufacturers cannot maintain the desired quality requirements of the preparations. The described method will be supportive to herbal and traditional manufactures for producing *A. vasica* preparations maintaining quality requirements imposed by the regulatory authorities.

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REFERENCES

1. Awang, D.V.C. 1997. Quality control and good manufacturing practices: Safety and efficacy of commercial herbals. *Food Drug Law Inst*. **52**, 341-344.
2. Ravishankar, B. and Shukla, V.J. 2007. Indian systems of medicine: A brief profile. *Afr. J. Trad. Cam.* **4**, 319-337.
3. Farnsworth, N.R., Akerele, O. and Bingel, A.S. 1985. Medicinal plants in therapy. *Bull. World Health Org*. **63**, 965-981.
4. World Health Organization, 1998. Essential medicines and health products information portal: Guidelines for the appropriate uses of herbal medicines- A World Health Organization Resource, Regional Office for the Western Pacific, Manila.
5. Nadkarni, K.M. 1976. Indian Materia Medica, Popular Prakashan, Bombay, India, Chapter 1, pp. 40-41.
6. Atal, C.K. 1980. Chemistry and pharmacology of vasicine: A new oxytocic and abortifacient, New Delhi Press, New Delhi, India, Chapter 5, pp. 58-59.
7. Gupta, O.P., Sharma, M.L. and Ghatak, B.J.R. 1977. Pharmacological investigations of vasicine and vasicinone: The alkaloids of *Adhatoda vasica*. *Indian J. Med. Res*. **66**, 680-691.
8. Rachana, Sujata, B., Mamta, P., Priyanka, K.M. and Sonam, S. 2011. Review & future perspectives of using vasicine, and related compounds. *Indo-Global J. Pharmaceut. Sci.* **1**, 85-98.
9. Nikam, P.H., Kareparamban, J., Jadhav, A. and Kadam, V. 2012. Future trends in standardization of herbal drugs. *J. Appl. Pharmaceut. Sci.* **6**, 38-44.
10. Kunle, Folashade, O., Omoregie, E.H. and Ochogu, A.P. 2012. Standardization of herbal medicines: A review. *Int. J. Biodiver. Conserv*. **4**, 101-112.
11. Dixit, V.K. and Yadav, N.P. 2008. Recent approaches in herbal drugs standardization. *Integr. Biol*. **2**, 195-203.
12. Jianga, Y., David, B., Tu, P. and Barbin, Y. 2010. Recent analytical approaches in quality control of traditional Chinese medicines- A review. *Anal. Chim. Acta*. **657**, 9-18.
13. Sajeeb, B.K., Kumar, U., Rouf, A.S.S., Uddin, M.A. and Bachar, S.C. 2015. Evaluation of *Justicia adhatoda* L. syn. *Adhatoda vasica* Nees extract by major metabolite analysis. *Indian J. Trad. Knowl.* **14**, 637-642.
14. Ravishankara, M.N., Shrivastava, N., Mahendru, N., Padh, H. and Rajani, M. 2001. Spectrophotometric method for the estimation of alkaloids from *Cinchona officinalis*'s stems, bark and its formulations. *Indian J. Pharm. Sci.* **63**, 76-78.