AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Method Development and Validation for Quantification of Betaine in *Achyranthes aspera* by HPLC

VB Krishna[1], Naira Nayeem[1*]

1.Krupanidhi College of Pharmacy, Bangalore.

ABSTRACT

A simple, accurate, rapid and precise isocratic High performance liquid chromatographic (HPLC) method was developed and validated for the quantification of betaine in *Achyranthes aspera*. The method employs Reversed Phase Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size system consisted of prominence LC-20AT gradient pumps, Prominence SIL-20A Auto sampler and a Prominence SPD-M20A Diode Array Detector (SHIMADZU), Japan and flow rate of 1.0 ml/min with a load of 20µl. Water and acetonitrile was used as mobile phase in the composition of 10:90. The detection was carried out at 242 nm. Linearity range was 0.32-0.48 mg/ml. Retention time of betaine was found to 8.42 min. The recovery studies 98-103%. This method was validated for accuracy, precision, linearity and Robustness as per ICH guidelines. The method was found to be specific, selective, and robust.

Keywords: *Achyranthes aspera*, Betaine, RP HPLC, validation

*Corresponding Author Email: krishnaboddiboyina@gmail.com

Received 06 July 2013, Accepted 10 July 2013

Please cite this article in press as: Nayeem M. *et al.*, Method development and validation for quantification of Betaine in *Achyranthes aspera* by HPLC. American Journal of PharmTech Research 2013.

INTRODUCTION

Achyranthes Aspera L is an important medicinal plant, an erect annual herb found throughout in India and other tropical countries[1-3]. It is a well recognized plant in the traditional medicine and was used by people in rural areas for cold, cough, colic, debility, dropsy, dog bite asthma, bleeding and gynaecological disorders [4-7]. The flowering spikes are ground with water and are used as external application for bites of poisonous snakes [8]. Traditionally, the plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc.

Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. Inhaling the fume of *Achyranthes aspera* mixed with *Smilax ovalifolia* roots is suggested to improve appetite and to cure various types of gastric disorders[9]. The crushed leaves rubbed on aching back to cure strained back. Paste of the roots in water is used in ophthalmic and opacities of the cornea. Paste of fresh leaves is used for allaying pain from bite of wasps [10].There are no methods reported for quantification of Betaine in *Achyranthes aspera* till now hence there was a need of development and validation of HPLC method in plant extract

MATERIAL AND METHODS:

Collection of plant material:

The dried & authenticated leaves of *Achyranthes aspera* were obtained from Green Chem. Private Limited, Bangalore. A voucher specimen was deposited for further reference.

Extraction of plant material:

The powdered Leaves of *Achyranthes aspera* (200 gm) was extracted by macerating with Methanol (200 ml) for 3 hr, filtrate was concentrated and % yield was calculated.

HPLC Analysis of Phytoconstituents:

Quantification of Betaine by HPLC includes the use of following materials, instruments and chemicals i.e. HPLC (Shimadzu), Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size, Diode array Detector, Sonicator (Labindia), Weighing balance, Methanol ,Acetonitrile, Water HPLC grade (Rankem), Betaine standard as obtained from Green Chem ltd.

Selection of Wavelength

Appropriate dilution was prepared using standard stock solution of 100µg/ml of Betaine. The solution was scanned over range of 400-200nm, using medium scan speed. The sampling wavelength for analysis includes, Absorption maxima (λ_{max}) of Betaine = 242 nm.

METHOD DEVELOPMENT:

Preparation of Mobile Phase

The mobile phase was prepared by mixing 10 volumes of water and 90 volumes of Acetonitrile and filtered through 0.45µ nylon filter.

Preparation of Stock Solutions

Accurately about 10 mg of Betaine pure compound was weighed and transferred into clean dry standard 25 ml volumetric flask, 10 ml Methanol was added to this and shaken for 5 min and the volume was made up with Methanol.

Preparation of sample solution: Accurately about 100mg of *Achyranthes aspera* extract was weighed and transferred into clean dry standard 25ml volumetric flask , to this 25 ml of Methanol was added and shaken for 5 min and then the volume was made up with Methanol and the solution was filtered. The optimized condition for the chromatographic run is as follows:

Table 1: Chromatographic Condition for Optimized Method:

Parameters	Description
Column	Phenomenex (US) C18 Column 250 x 4.6mm I.D, 5µ Particle Size
Mobile phase	Water :Acetonitrile (10:90)
Injection volume	20µl
Flow rate	1.0ml/min
Detector wave length	Diode array detector 242nm
Column Temperature	Ambient
Run time	25 min

RESULTS AND DISCUSSION

The results of the Method development and validation of Betaine are as show in the following tables and chromatograms.

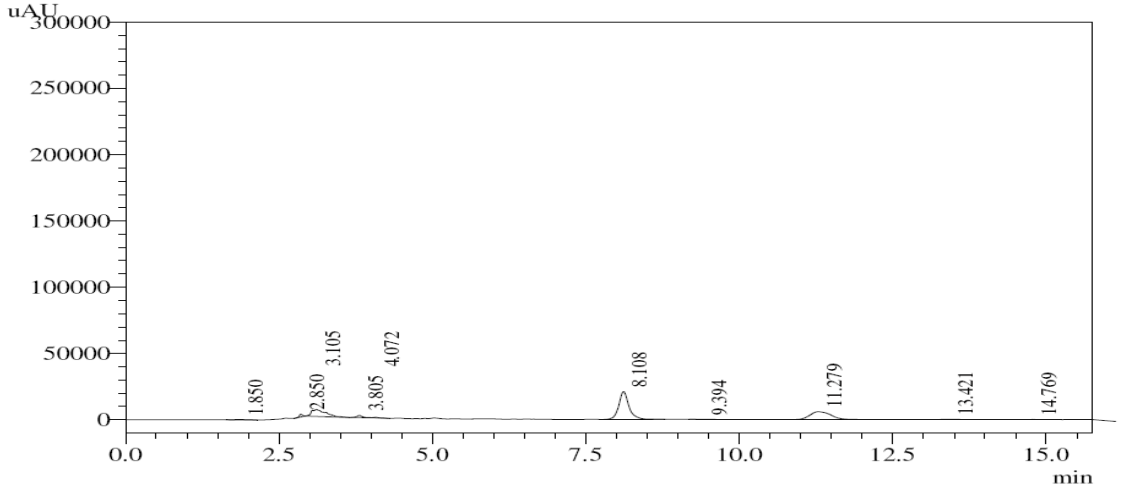

Figure: 1 Chromatogram of *Achyranthes aspera* for methanol extract sample

Table 2: Retention times methanolic extract sample

Peak	Ret. Time	Area	height	Area %	Height %
1	1.850	3616	278	0.720	0.751
2	2.850	7958	2120	1.585	5.722
3	3.105	83152	5159	16.557	13.922
4	3.805	10076	1649	2.006	4.450
5	4.072	1701	342	0.339	0.9222
6	8.108	245372	20988	48.858	56.634
7	9.394	2467	154	0.491	0.416
8	13.421	4549	262	0.906	0.708
9	14.769	2752	149	0.548	0.403
Total		502212	37053	100	

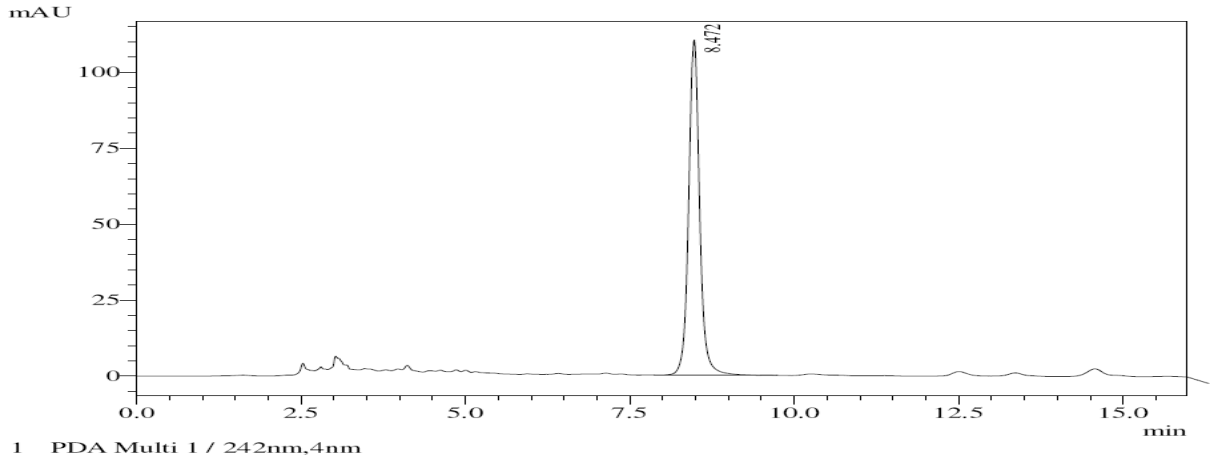

Figure 2 Chromatogram of Betaine Standard

Table 3: Retention time of Betaine standard

Peak	Ret Time	Area	Height	Area %
1	8.472	1263258	110299	100.00
Total		1263258	110299	100.00

The amount of Betaine in sample was found to be 10.37 mg/gm

VALIDATION:

The method was performed and validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines.

Specificity and selectivity:

It is the extent to which the procedure applies to analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of blank and any other excipients. The figure shows that drug was clearly separated from blank and its excipients. Thus the proposed HPLC method is selective.

Linearity:

The concentration, peak area and retention time for linearity of betaine and the regression line relating standard concentrations of drug using regression analysis, the calibration curves were linear in the studied range and equations of the regression analysis were obtained R^2 =0.9995 for betaine. Linearity concentration was in the range of 0.32mg/ml-0.48mg/ml.

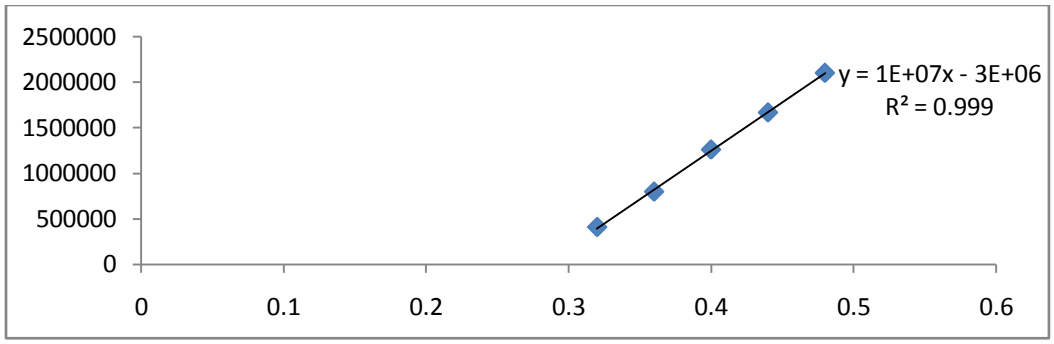

Figure : 3 Linearity of betaine

Precision:

Precision of the method was studied as repeatability, intra-day and inter day variations. The intra-day precision was determined by analyzing Betaine six times each on same day(intra-day study). This was repeated on the second day(inter-day study) and results were shown in table 4.

Table 4: Precision results for betaine:

Drug	%RSD (intra-day)	%RSD (inter-day)
Betaine	1.3	1.4

Accuracy:

The accuracy of the method was determined by recovery studies. The recovery studies were performed by standard addition method at 80% for three times, 90% for three times,100% for three times, 110% for three times, 120% for three times and summarized in table 5.

Table 5: Accuracy results for betaine:

Sample No.	Spike Level	Amount (mg) added	% Recovery	% Mean Recovery	%RSD
1.	80 %	8	100.11	100.02	1.3
	80 %	8	100.03		
	80 %	8	99.91		
2.	100 %	10	99.78	99.74	1.4
	100 %	10	99.71		
	100 %	10	99.73		
3.	120 %	12	99.76	99.77	1.3
	120 %	12	99.76		
	120 %	12	99.80		

Robustness:

Robustness of the method was determined by making slight changes in the flow rate and column

temperature. It was observed that there were no marked changes in the retention time and area of the chromatograms which demonstrated that the RP HPLC method developed was robust and data are summarized in table 6.

Table 6: Robustness results

S. No	Parameters	Normal Range	Changes	%RSD
1	Flow Rate	1.0ml/min	±0.1ml	1.3
2	Temperature	25^0C	±0.5°C	1.4

Table 7: System suitability parameters for HPLC:

Validation Parameters	BETAINE
Mobile Phase	10:90 (water: acetonitrile)
Flow Rate	1 ml/min
Detection wave Length	242
Rt	8.4
Run Time	15 min
Linearity	R^2=0.999
Precision	% RSD < 2

CONCLUSION

Achyranthes aspera is an important medicinal plant and is used from ancient time in traditional system of medicine. A novel and new HPLC method was developed and validated according to ICH guidelines for the quantitative determination of betaine with new solvent system by which we can estimate amount present in sample. The proposed HPLC method can be applied for quality control of several traditional drugs and formulations in which Betaine is a component.

ACKNOWLEDGEMENT:

The authors are thankful to Dr Rajendran and Mr. Vijaya Raghavan ,Green Chem Pvt, Ltd Bangalore for their encouragement, support and for giving an opportunity to carry out the project in their R&D center.

REFERENCES:

1. Gokhale AB, Damre AS, Kulkarni KR, Saraf MN. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of S. lappa, A. speciosa and A. aspera. Phytomedicine . 2002;9(5):433-37.
2. Khan AV. Ethnobotanical studies of plants with medicinal and antibacterial properties. Ph.D. Thesis, Aligarh Muslim University,2002; Aligarh, India.
3. Khan AV, Alam MM. *Achyranthes aspera* L. in the health-care of the rural population of Uttar Pradesh, India. Hamdard Medicus . 2003;52 (1): 44-47.
4. Khan AV, Khan AA Medico-ethnobotanical uses of *Phyllanthus fraternus* Webst. (Family-Euphorbiaceae) from western Uttar Pradesh, India. J Natural Remedies .2004;

4(1): 73-76.

5. Nath D, Sethi N, Srivastava S, Jain A K. Survey in indigenous medicinal plants used for abortion in some districts of Uttar Pradesh. Fitoterapia.1997; 68 (3): 223-225.
6. Pakrashi A Bhattacharya N. Abortifacient principles of *Achyranthes aspera* L. Indian Journal of Experimental Biology.1977; 15 (10): 856-85.
7. Pakrashi A, Mookerji N, Basak B. Effect of chromatographic fractions of the plant *Achyranthes aspera* L. on fertility in female albino mice. Journal of Reproduction and Fertility .1975;43 (1): 127-129.
8. Singh VK, Ali ZA, Zaidi STH. Ethnomedicinal uses of plants of Gonda district forests of Uttar Pradesh, India. Fitoterapia. 1996; 67(2):129-39.
9. Khare CP. Indian medicinal plants. Springer. 2007; 11-3.
10. Anonymous. The Wealth of India - Raw Materials, Council of Scientific & Industrial Research (CSIR), New Delhi, 2007; 17-18.