Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2009; 1(3): 115-118

Research Article ISSN 0975 1556

A Quantitative Estimation of Aeridin in *Wattakaka volubilis* by HPTLC

G Mridula[1*], CS Shreedhara[1], K Katkar[2], A Suthar[2], VS Chauhan[2]

[1]*Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India*
[2]*Piramal Life Sciences Ltd, 1, Nirlon Complex, Off. Western Express Highway, Goregaon (East), Mumbai – 400 063. Maharashtra, India*

ABSTRACT
The present paper deals with development and standardization of HPTLC method used for quantification of Aeridin in *Wattakaka volubilis* commonly known as Green milkweed climber, a plant of the family *Asclepiadacea*. Aeridin is a new phenanthropyran derivative 2, 7– dihydroxy - l, 3-dimethoxy – 9, 10-dihydrophenanthropyran, isolated from the whole plant of *W. volubilis*, which possesses anti-inflammatory activity. Hence, there is a need to develop and standardize a chromatographic method for quantification of Aeridin, for standardization of *W. volubilis*. An attempt has been made to quantify Aeridin in *W. volubilis* extracts by HPTLC method. The lowest detectable limit of Aeridin was found up to 2 ng with good resolution and separation of Aeridin from other constituents of *W. volubilis*. Further, the recovery value of Aeridin was found to be about 101 %, which shows the reliability and suitability of the method. The validated HPTLC method was found to be simple, reproducible, accurate and precise. The structure of isolated Aeridin was characterized and confirmed by various advanced spectroscopic methods.

Keywords: HPTLC, *Wattakaka volubilis*, Aeridin.

INTRODUCTION

Wattakaka volubilis is a medicinal plant, which is traditionally used for several diseases and human requirements. [1] The young leaves are eaten in curries and are used in the treatment of pyodermas and fevers in children. The roots are used as an emetic. The alcoholic extract of the plant *W. volubilis* is widely used in India as a traditional medicine in the application for boils and abscesses. [2] It is also frequently used as a remedy for cough, fever, severe cold, rheumatic pain, diabetes, eye diseases, snake bite [3-4] etc. The alcoholic extract of the plant is reported to show activity on the central nervous system, as well as anticancer activity against sarcoma 180 in the mice. [5] The literature survey revealed that among the various saponins obtained from the stem and flower of *W. volubilis*, two compounds are active against Ehrlich's ascites carcinoma. [6-7]

There are no chromatographic methods available for quantitation of Aeridin in *W. volubilis*. Hence, the present work focuses on development and validation of high-performance thin layer chromatographic method for the determination of this major constituent, Aeridin, in *W. volubilis* extract. The proposed HPTLC method was attempted for fast, precise, sensitive and reproducible method with good recoveries for standardization of extracts of *Wattakaka volubilis*.

***Corresponding author: Ms. G Mridula,**
Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India; **Tel:** 09020219459
E-mail: inform.mridu@gmail.com

MATERIALS AND METHODS

Wattakaka volubilis L. f. (*Asclepiadaceae*) whole plant collected from Sawantwadi, Konkan region of Maharashtra, India in April 2008 and used as samples for quantification of Aeridin.

Preparation of the extract

Pure Aeridin was isolated from the dried aerial parts of *W. volubilis*. Purity and structure of isolated compound (Aeridin) was confirmed by HPTLC, HPLC and spectral analysis like NMR, IR and MS. [8] This isolated compound Aeridin was used as working standard for quantification in extracts.

Equipment

A Camag HPTLC system equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner III and Reprostar, Wincats and integration software 4.02 (Switzerland).

Sample preparation

Accurately weighed 20-50 mg of aqueous extract and methanol extract of *wattakaka* and extracted with methanol (10 ml) by sonicating the solution for 5 min. and allowed to stand for 5 min. at room temperature. The methanol extract was then filtered through Whatmann no.42 filter paper; extracts were pooled and concentrated to dryness under

vacuum. Final volume was made to 10 ml with methanol in volumetric flask. Aeridin content were then analyzed after subjecting to HPTLC.

HPTLC method

Silica gel 60 F_{254} precoated plates (20×10 cm) were used with Toluene: Ethyl acetate (80:20) as solvent system. 1-10 µl of working standard of Aeridin as well as test samples was spotted on pre-coated HPTLC plates. The bandwidth applied on plate was 6 mm and ascending mode was used for development of thin layer chromatography. Saturation time was 20 min along with humidity level – 65 % ± 5 % RH and room temperature - 25°C ± 2°C. TLC plates were developed up to 8 cm. The TLC plates were scanned at 308 nm for quantification purpose.

Procedure: 1 **(Calibration curve of standard Aeridin)**

10 milligram of working standard Aeridin was dissolved in 10 ml of methanol and further diluted to obtain the concentration of stock solutions in the range of 0.01 mg/ml. Calibration curve was obtained by spotting 5 ng to 80 ng of Aeridin per spot. Reproducibility, linearity and validation of the proposed method was checked along with correlation coefficient, coefficient of variance and the linearity curve for Aeridin.

Procedure: 2 **(Calibration curve using extract spiked with Aeridin)**

The content of Aeridin in aqueous and methanolic extracts was determined by comparing with the calibration curve of the working standard of Aeridin. The aqueous extract, which showed lowest content of Aeridin, was then used as blank. This blank was then used to spike extract with the working standard of Aeridin. Different samples with varying amount of standard Aeridin in range of 20 µg / ml to 60 µg / ml were spiked in 1 mg of blank extract with pre-determined Aeridin. Procedure for sample preparation was followed as mentioned above. In each sample preparation, 10 µl of spiked solution was then subjected to HPTLC with 10 µl of blank solution for comparison. The percent recovery of Aeridin standard was calculated. Reproducibility, precision and validation of the method were achieved by analyzing six replicate spiked sample solutions. Correlation coefficient, coefficient of variance was calculated.

Linearity along with limit of detection and limit of quantitation

For a long-term use of the analytical method, a rigorous validation is indicated and requires the following procedures. For the preparation of calibration curve, the stock solution was diluted freshly with methanol to obtain a set of 9 calibration standards. These standards were measured and the integrated peak areas were plotted against the corresponding concentrations of the standards. The complete procedure was repeated on three consecutive days. The so obtained three calibration curves were used to calculate a mean calibration graph. The limit of detection was obtained by analyzing signal to noise ratio and limit of quantification was defined as the lowest concentration of linear range.

Intraday and interday analysis using Aeridin

Three different concentrations using a different stock solution of Aeridin (20, 40 and 60 ng / spot) were spotted. For the determination of the intraday precision and accuracy, three replicates of the standard solutions were analyzed at the same day in triplicate. The precision and the accuracy of the interday analysis were determined by analyzing the standard solution on 3 different days in triplicate.

Stability

Aeridin (1 mg) and *W. volubilis* methanolic extract (10 mg) were extracted in 10 ml methanol as per the method described in sample preparation. The sample solutions were kept at 4°C in dark and analyzed on consecutive days (24, 48, 72 and 96 h) to observe the stability of standard as well as sample solution.

Robustness and Ruggedness studies

Robustness and ruggedness parameters were applied by making small deliberate changes of the conditions (mobile phase composition, mobile phase volume, saturation time, time from application to chromatography, time from chromatography to scanning and analyst) to validate the method.

RESULTS & DISCUSSION

Working standard Aeridin showed single peak in HPTLC chromatogram along with its UV spectrum (Fig I). The calibration curve of working standard Aeridin was obtained by spotting standard Aeridin on HPTLC plate after scanning at 308 nm. Spot of Aeridin is visible with Rf ≈ 0.33 - 0.36 at 254 nm. Two different extracts of *W. volubilis* were analysed by the proposed method and the data are recorded in Table I.

Table I: Percentage of Aeridin in different samples of *Wattakaka volubilis* commercial extracts by measuring area in HPTLC method

Sr.	Sample name	Aeridin content (% w/w)
1	Aqueous extract	-
2	Methanolic extract	0.75

Table II: Precision & accuracy of the method applied to spiked Aeridin samples

Amount added (µg / spot)	Amount found (µg /spot) (Mean ± S.D., n=6)	Precision / Reproducibility (C.V.)	Mean Recovery (%)
20	20.23 ± 0.58	2.98	101.19 %
40	40.86 ± 0.72	1.74	102.15 %
60	59.98 ± 1.47	2.46	99.97 %

Table III: Intraday and interday precision & accuracy of the method applied to Aeridin

Amount added (µg / spot)	Intraday		Interday	
	Precision (R.S.D., %)	Accuracy (%)	Precision (R.S.D., %)	Accuracy (%)
20	3.39	98.74	3.31	98.88
40	0.96	96.09	1.03	96.17
60	1.48	97.12	1.60	95.72

Chromatographic precision and recoveries from spike sample solution

Specificity

It was observed that the other phytoconstituents present in the extracts did not interfere with the peak of Aeridin. Therefore the method was specific and helps in separation of Aeridin from other constituents of herb and hence, helps to get the exact content of Aeridin. Test sample of methanolic extract of *W. volubilis* showed separated peak of Aeridin along with other phytoconstituents as obtained in HPTLC chromatogram (Fig II).

Limit of Detection

By applying the proposed method, the minimum detectable limit of Aeridin was found to be 2 nanogram / spot at 308 nm.

Limit of Quantification

By applying the proposed method, the minimum quantification limit of Aeridin was found to be 5 nanogram / spot at 308 nm.

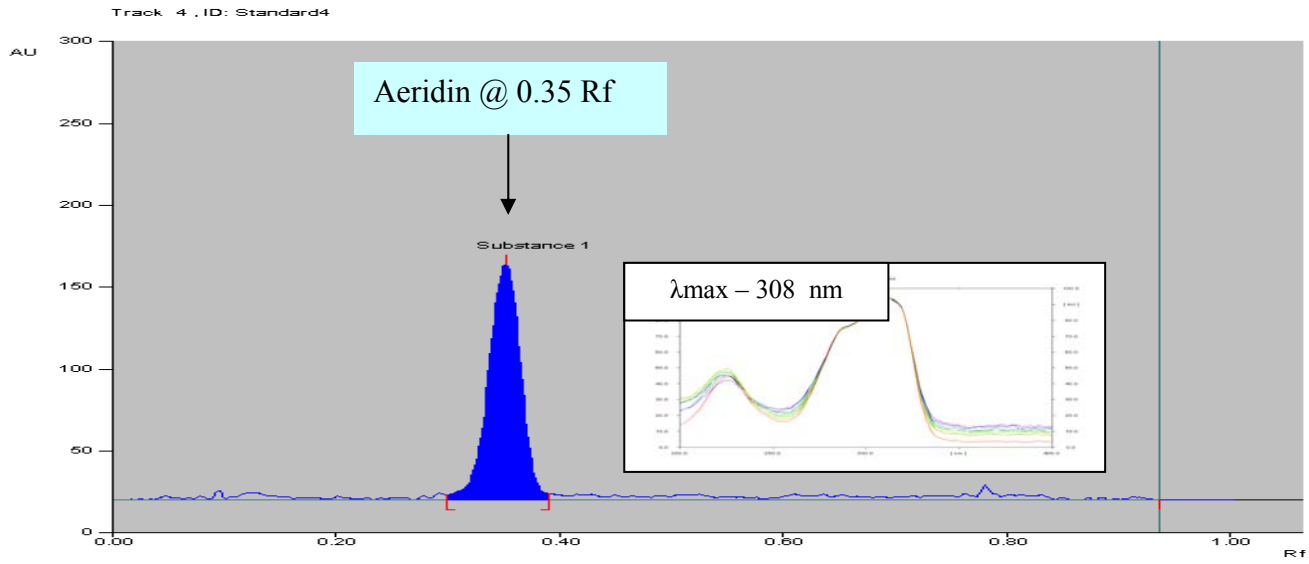

Fig. I: TLC Chromatogram of standard Aeridin along with UV spectra

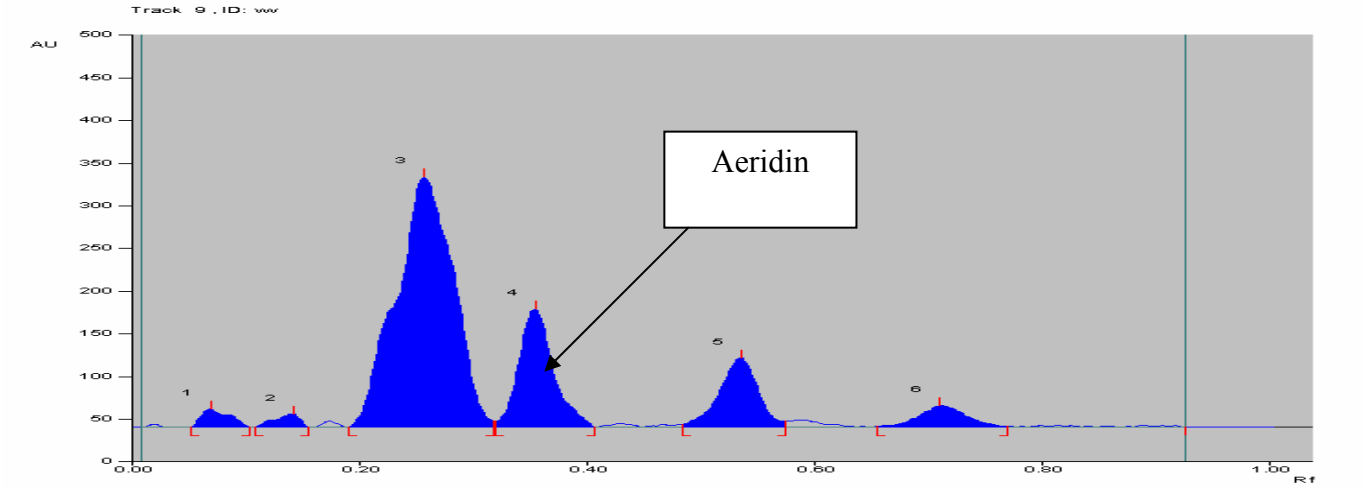

Fig. II: TLC Chromatogram of Aeridin in *W. volubilis* methanolic extract

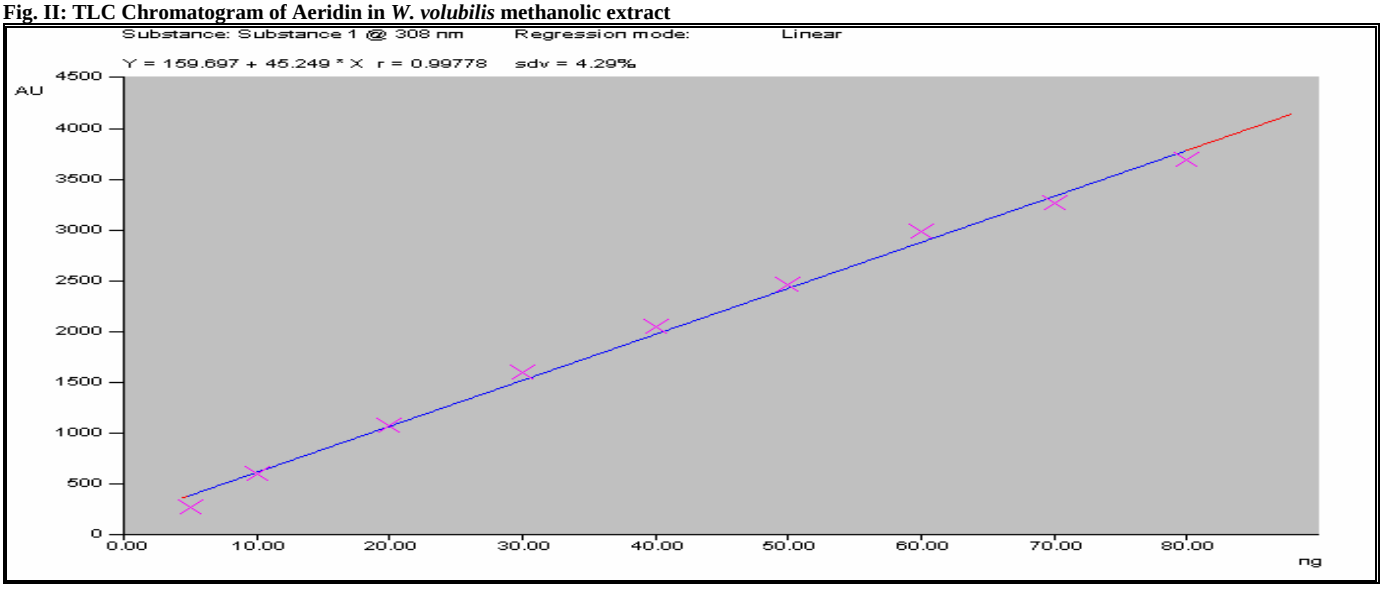

Fig. III: Calibration curve of working standard Aeridin with respect to the area under curve at various concentrations.

Linearity

The linearity of the method was checked with working standard Aeridin with the calibration curve in the concentration range of 5 – 80 ng / spot based on a 0.5 - 10 µl sample volume. The regression equations (Y = 47.698* X + 188.6) and correlation coefficient were obtained with 6 replicate analysis for each concentration. Correlation coefficients were obtained in the range of 0.9916-0.9933 indicated excellent linearity of the procedure for working standard Aeridin analysed. Calibration curve of working standard Aeridin is shown in Fig. III.

Accuracy and precision

The method was applied to determine concentration of spiked Aeridin in test sample with the range of 20 – 60 ng / spot for

assessing the accuracy & precision of the procedure. Table II represents the mean values and coefficient variance (C.V.) results indicate the levels in the above range can be estimated with accuracy and precision.

Intraday and interday analysis using Aeridin

Furthermore the precision and accuracy of the intraday and interday analysis were investigated on the basis of a set of standard solution. The results given in Table III stands for a quite good trueness of the proposed method particularly considering interday and intraday analysis.

Stability

In the current assay, analyses of stability samples in methanol on consecutive days (24, 48, 72 and 96 h) revealed that the constituent, Aeridin either in standard solution or in the methanolic extract of *Wattakaka volubilis* are stable in solution form with relative standard deviation (RSD (%) 1.03 (n = 3) and for Aeridin at 4°C.

Robustness and Ruggedness studies

The method was found to be re-producible from one analyst to another. The low values of R.S.D. (2.897 % - 3.214 %) obtained after small deliberate changes of the conditions (mobile phase composition, mobile phase volume, saturation time, time from application to chromatography, and time from chromatography to scanning) used for the method indicated its robustness.

CONCLUSION

The lowest detectable limit of Aeridin was found upto 2 ng / spot provides good resolution and separation of Aeridin from other constituents of *Wattakaka volubilis*. Further, recovery values of Aeridin were found to be about 101%, which shows the reliability and suitability of the method. The proposed HPTLC method is simple, rapid, reproducible, accurate and precise for quantitative monitoring of Aeridin in *Wattakaka volubilis* samples.

REFERENCES

1. Pullaiah T. In: Medicinal plants in India, *Wattakaka volubilis* (L.F) stapf 2002. p. 535-6.
2. Anonymous. The Useful Plants of India; New Delhi-India; Council of Scientific & Industrial Research. 1992; 183.
3. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. J Ethnobiol Ethnomed 2006; 2: 43.
4. Prasad PRC, Reddy CS, Raza SH, Dutt CBS. Fitoterapia 2008; 79: 458.
5. Anonymous. The Wealth of India, Raw Materials, New Delhi-India; Council of Scientific & Industrial Research, 1976; 6: 564.
6. Yoshimura S, Narita H, Hayashi K, Mitsuhashi H. Chem Pharm Bull (Tokyo) 1983; 31: 3971-83
7. Sahu N, Panda N, Mandal NB, Banerjee S, Koike K, Nikaido T. Phytochem 2002; 61: 383-8.
8. Katkar K, Mridula G, Shreedhara C, Patil P, Naik V, Bhamare S, Vasani J, Suthar A, Chauhan VS. Research Journal of Pharmacognosy and Phytochemistry 2009; 1 (3): 232 - 233.