DEVELOPMENT AND VALIDATION OF A NOVEL HPTLC METHOD FOR QUANTITATIVE ESTIMATION OF VINCAMINE FROM *CATHARANTHUS ROSEUS* LINN. LEAVES EXTRACT

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ABSTRACT

Catharanthus roseus (Apocynaceae), commonly known as periwinkle, is a perennial plant that is mostly found in Southern Asia. Periwinkle is rich in indole alkaloids. This research work consists of the development and validation of one of the indole alkaloids, vincamine, by HPTLC. The chromatographic separation was achieved by using eluent chloroform, acetone, and formic acid in the ratio of 5:1:0.5 V/V/V at wavelength 222 nm. With an R_f value of 0.33±0.02, this method showed good separation of vincamine in the extract. Vincamine in the range of 200-1000 ng spot^{-1} showed a satisfactory linear relationship according to the regression analysis data, and the correlation coefficient (R^2) was found to be 0.9957. ICH Q2 (R1) guidelines were followed for validation.

Keywords: High-performance thin layer chromatography (HPTLC), vinca leaves, vincamine, quantification, alkaloids

INTRODUCTION

Catharanthus roseus (Apocynaceae), commonly known as pink periwinkle, is an essential medicinal and attractive plant that has long been used in traditional medicine. It is native to Madagascar and Southern Asia. *C. roseus* can also be found in India, especially in the southern and northern hills[1]. It was once cultivated as a decorative plant, but when the medicinal utility of its extract was discovered, it took its essential place as a medicinal herb in traditional medicine. In India, the liquid from the leaves of the plant is used to cope with wasp stings[2]. Vinca plants are studied extensively due to their high alkaloidal content and diverse pharmacological effects, including anticancer, antidiabetic, antibacterial, antimalarial, antidysentric, purgative, diuretic and antioxidant properties[3].

The task of determining the presence of additional alkaloids is still ongoing. There are many vinca alkaloids in clinical use and, to name a few for example, they are, vincristine, vinblastine, vindesine, vinorelbine and vincamine[4]. Vincristine, one of the most used vinca alkaloids for cancer treatment, has been utilized in a number of poly-chemotherapy regimens for central nervous system tumors and acute lymphoblastic leukaemia (ALL)[5]. Vinblastine is used to treat breast cancer, testicular cancer, and neuroblastoma[6]. Vindesine is an alkaloid derived from vinblastine and has shown activity against Hodgkin's & non-Hodgkin's lymphomas, lung cancer and breast cancer[7]. Vinorelbine is used for the treatment of non-small cell lung cancer (NSCLC), that has progressed to surrounding tissues or to other regions of the body[8]. Vincamine is one of the few alkaloids that have positive effects on living cells, according to a recent study. By increasing blood flow and regional glucose uptake, it serves as a cerebral metabolic enhancer that is neuroprotective against ischemia and hypoxia, and possesses antioxidant and antiapoptotic characteristics. Vincamine has been suggested as a potential component in the treatment for sickle cell disease because it appears to serve as an oxygen carrier in living cells[9]. Vincamine is a monoterpenoid indole alkaloid (Fig. 1) present majorly in periwinkle leaves[10]. In the current study, HPTLC was established to quantify vincamine from the methanolic extract of *C. roseus* leaves in accordance with the recommendations of the International Conference on Harmonisation, ICH Q2 (R1) guidelines[11].

MATERIALS AND METHODS

Materials

Vincamine was purchased from Yucca Enterprises, Mumbai, Maharashtra, India as the reference standard.

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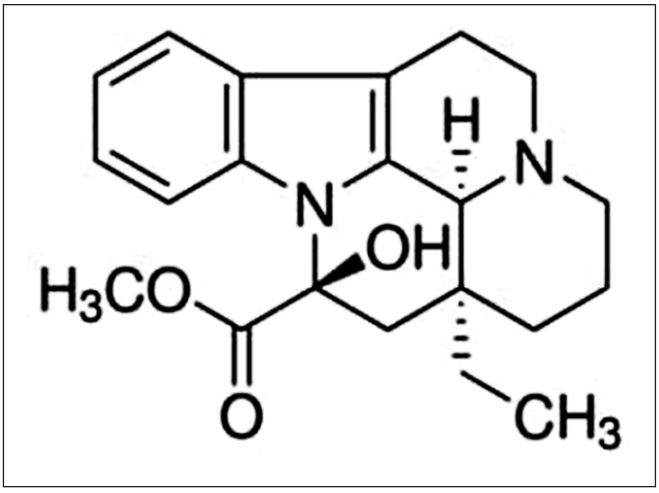

Fig. 1: Chemical structure of vincamine

Analytical grades of acetone, formic acid, methanol, and chloroform were purchased from S.D. Fine-Chem Ltd., Mumbai, India. By using TLC, FTIR, UV-visible spectroscopy and DSC, the purity of the vincamine was confirmed. The dried leaves of *C. roseus* used in this investigation were also obtained from Yucca Enterprises, Mumbai. The plant material was authenticated by Alarsin, Mumbai, Maharashtra, India (Reference no. ALA/PG/2022/071).

Instrumentation and software

A CAMAG HPTLC system (Muttenz, Switzerland) comprising of a CAMAG automatic TLC sampler 4 (ATS 4) applicator, CAMAG TLC Scanner 4, CAMAG TLC visualizer, flat bottom twin-trough developing chamber

Table I: Linearity study data

Concentration (ng spot^{-1})	Vincamine				
	200	400	600	800	1000
Mean area	2065.18	3938.90	5572.06	7123.29	8425.09
Linearity equation	y = 7.9521x + 653.65				
Correlation coefficient (R^2)	0.9957				

Table II: Accuracy study data

	Vincamine		
% Level	80	100	120
Initial amount (ng spot^{-1})	200	200	200
Spiked amount (ng spot^{-1})	160	200	240
Total amount (ng spot^{-1})	360	400	440
Average area	3396.62	3749.74	4303.49
Concentration found	344.93	389.34	458.97
Recovery (%)	95.81%	97.33 %	104.30 %

Table III: Precision study data

Vincamine	Concentration (ng spot^{-1})	Average peak area	Standard deviation	%RSD
Intra-day	600	5613.77	70.71	1.25
Inter-day	600	5620.00	72.10	1.27

Table IV: Results of the robustness of vincamine

Method parameters	Level of variation	Modified parameter	%RSD	
			200 ng spot^{-1}	800 ng spot^{-1}
Saturation time (20 mins)	+10	30 minutes	1.279	1.862
	-10	10 minutes	1.092	1.874

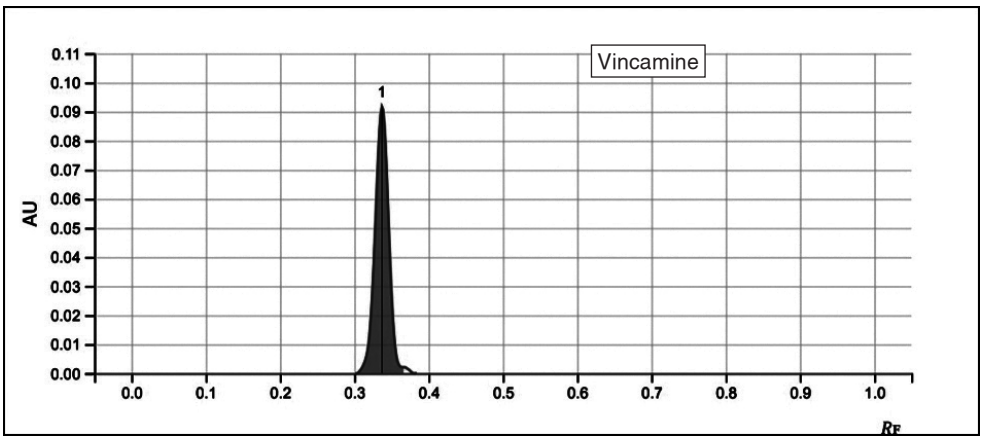

Fig. 2a: HPTLC densitogram of vincamine obtained using optimized chromatographic conditions

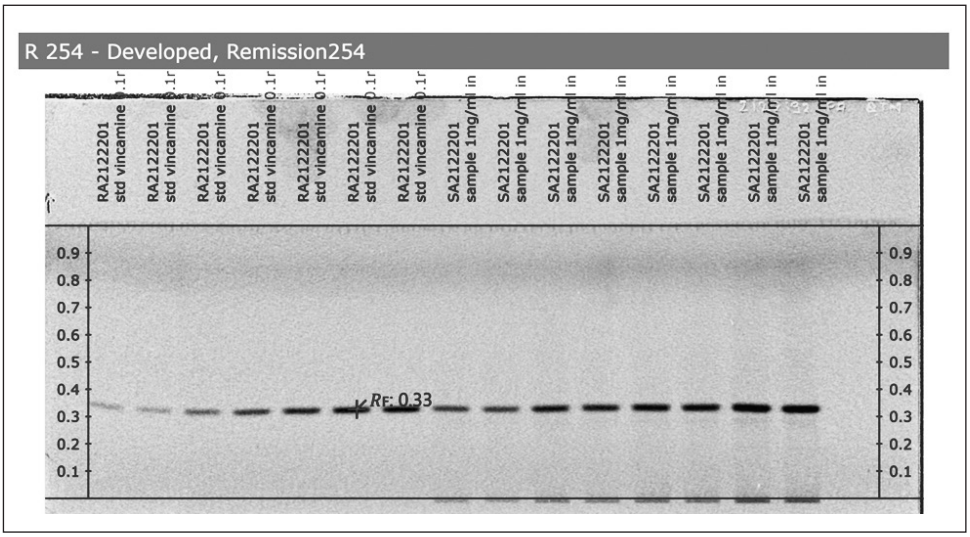

Fig. 2b: Developed HPTLC plate of vincamine standard (Track 1-7) and *C. roseus* extract (Track 8-15)

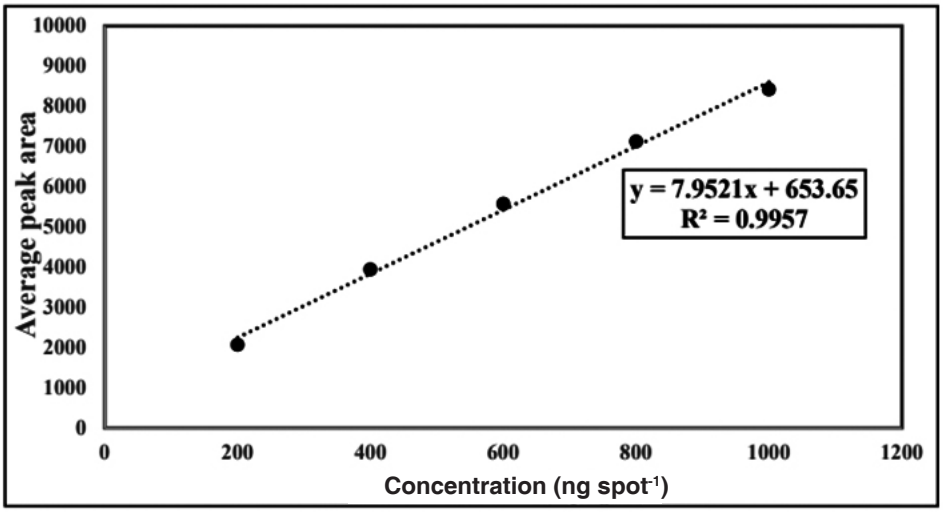

Fig. 3: Calibration plot of vincamine

(10 cm ×10 cm), UV cabinet with dual-wavelength UV lamp, Hamilton syringe (100 µL; Bonaduz, Switzerland), ultrasonic bath (Frontline FS-4, Mumbai, India) and CAMAG visionCATS software were employed in the study.

Preparation of stock and working standard solutions

5 mg of vincamine was solubilized in 5 mL of methyl alcohol to get a stock solution of 1000 ppm, which was diluted further to get a solution of 100 ppm. This diluted solution was used for analysis to achieve 200, 400, 600, 800, and 1000 ng of vincamine per spot on the TLC plate. Prior to use in chromatographic analysis, all solutions were brought to room temperature from a constant temperature of 4-6 °C.

Preparation of sample

The dried vinca leaves were coarsely powdered using a domestic mixer. 500 mL of methanol was used to extract 50 g of powdered leaves using the Soxhlet apparatus over a 3-day period. The semisolid extract was obtained by evaporating methanol extract in a water bath. The solution of 50 mg of extract in 5 mL methanol was subjected to centrifugation at three thousand rotations per minute for 15 minutes. Under ideal chromatographic circumstances, the supernatant was collected and subjected to HPTLC analysis.

Chromatographic conditions

The sample was spotted as a series of narrow 8 mm

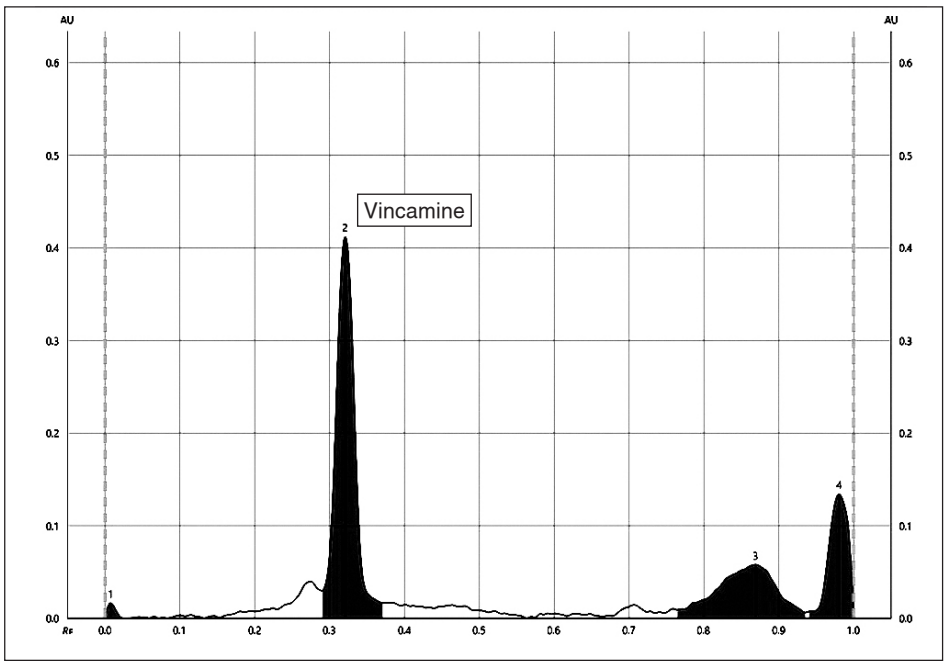

Fig. 4: HPTLC densitogram of methanolic extract of *C. roseus* leaves

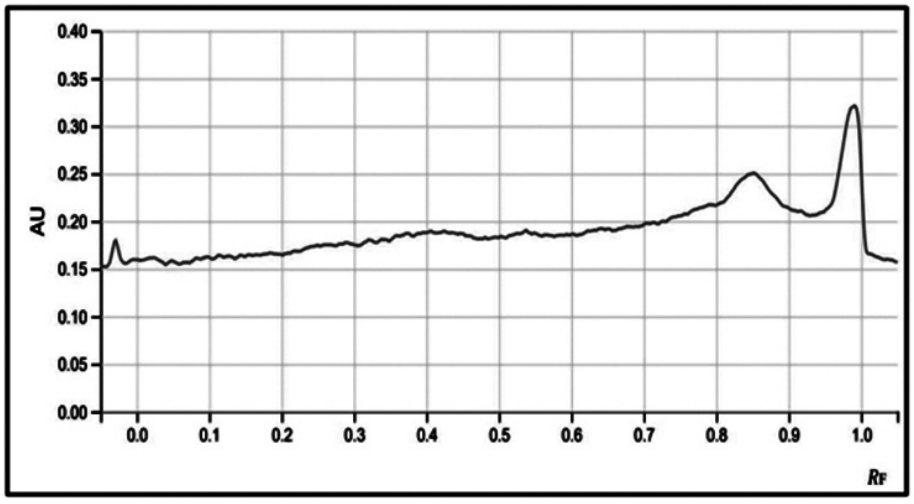

Fig. 5: HPTLC densitogram of eluent

bands on pre-coated silica gel TLC plate $60F_{254}$. The eluent used was a combination of chloroform, acetone and formic acid in the ratio of 5:1:0.5 (V/V/V). The chamber was allowed to saturate for 20 minutes. The distance from the origin spot to the solvent front was 7 cm. The densitometric scan was performed at 222 nm. The parameters for the slit dimensions were adjusted to 6 mm × 0.45 mm, 20 mm/s for scanning, and 100 µm/step for data resolution. The results were analysed to ensure separation reproducibility by achieving an optimal separation between spots and migration of spots.

Method validation as per ICH guidelines Q2 (R1)

The linearity was determined at five concentration levels from 200-1000 ng spot^{-1} for vincamine by employing the linear regression method. The coefficient of regression, slope, and y-intercept were estimated for the vincamine to determine linearity. LOD and LOQ were calculated from the slope and standard deviation obtained from the graph of linearity. Nine replicates of a concentration of 600 ng spot^{-1} were used for the determination of intra-day and inter-day precision. Accuracy studies were performed at 80%, 100%, and 120% concentration levels. By deliberately making small changes in chromatographic conditions, robustness was performed in triplicate at 200 ng spot^{-1} and 800 ng spot^{-1} concentrations. Specificity was determined by checking the R_f of both the standard and the sample extract.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

A standard vincamine solution was used for the chromatographic separation process. The spots of vincamine were applied on the TLC plates. Several trials were conducted employing numerous solvents in various ratios by the linear ascending development approach for the development of the eluent. The optimized eluent showed satisfactory resolution at R_f 0.33 for vincamine (Fig. 2a). The developed HPTLC plate is shown in Fig. 2b.

METHOD VALIDATION

Linearity

Vincamine solution of 0.01% was applied on a TLC plate in the range of 200-1000 ng spot^{-1}. By graphing

the drug concentration (x) against the peak area (y), linearity was determined. The linearity was confirmed by the interpretation of the regression line, and the corresponding data are shown in Table I. The linearity graph obtained for the marker is shown in Fig. 3. The standard deviation and slope were used to calculate LOD and LOQ. The estimated values for LOD and LOQ for vincamine were 68.14 ng spot^{-1} and 208.01 ng spot^{-1}, respectively. This demonstrated the method's sensitivity, even at low concentrations.

Accuracy

Recovery studies at different concentrations of samples by triplicate analysis were used to verify the accuracy of the method. The samples were added to a known amount of drug solution at three concentration levels i.e., 80%, 100% and 120%, and recovery was calculated. The findings, which are presented in Table II, show that the method was effective for determining vincamine from a methanolic leaf extract of *C. roseus*, with all statistical values falling within an acceptable range and being linked with satisfactory recovery at each added concentration.

Precision

Through the analysis of replicate quality control samples of the marker, the repeatability or intra-day precision was investigated. By examining the same sample over three days, inter-day precision was investigated. The % RSD of the obtained assay values was determined. Results that are within the range are reported in Table III, which demonstrates that the method is sufficiently precise.

Robustness

To examine the robustness, small adjustments were made to the chromatographic settings, such as changing the chamber saturation time (±10 min) in triplicate at 200 ng spot^{-1} and 800 ng spot^{-1} concentrations. By measuring % RSD, the effects of these adjustments on peak area were assessed. Data from robustness studies are shown in Table IV, and no significant deviations are shown, demonstrating the robustness of the method.

Specificity

The densitogram shown in Fig. 4 represents specificity, which shows the same R_f value for vincamine in the extract. A mixture of eluent solvents was also applied on TLC plate in order to check any presence of a peak at the standard R_f, but there was no peak found at R_f 0.33, which also indicated that the applied analytical method was found to be specific (Fig. 5).

Quantification of vincamine from *C. roseus* leaves extract

The quantification of vincamine from *C. roseus* was accomplished using the developed and validated HPTLC method. The quantification of vincamine was carried out in triplicate. The methanolic extract of leaves contained 1.03 % w/w vincamine in total.

CONCLUSION

For the quantification of an alkaloid, vincamine, the planar chromatographic HPTLC method was developed and validated. The method was shown to be specific, simple, rapid, precise, accurate and robust. This developed HPTLC method can be utilized by any laboratory, to quantify vincamine in various vinca species and for the quality control of vinca species and formulations containing vincamine.

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