Analysis of Vincristine in Leaf and Callus Extract by Validated HPTLC Technique and Its Stress Degradation Studies

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAB and MM designed the study, wrote the protocol. Authors MAB and Harshita managed the literature searches, performed the analytical work and managed the experimental process. Authors MAB and MAR wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: An incipient, concrete, sensitive, selective, precise, and reproducible high-performance thin-layer chromatographic (HPTLC) method was established and validated for estimation of vincristine alkaloid in leaf extract as well as it's callus.
Study Design: Mention the design of the study here.
Place and Duration of Study: Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India between June 2010 and July 2011.
Methodology: HPTLC was performed on aluminium foil precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of 73:25:2, v/v (methanol: toluene: diethyl amine) as a mobile phase. The densitometric analysis was carried out at 254 nm utilizing Camag TLC scanner which engendered compact spots for vincristine (Rf =0.38±0.02).

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Results: The linear regression data for the calibration plots showed good linear relationship (r^2=0.998±0.003) between peak area and concentration in the range of 500-3000 ng spot-1. The method was validated for precision, recovery, robustness, specificity, and detection and quantification limits, in accordance with ICH guidelines. The limits of detection and quantification were 3.06 and 9.29 ng spot-1, respectively.

Conclusion: Statistical analysis showed that the method could be prosperously applied for the estimation of vincristine in leaf extract and in callus. As the method could efficaciously separate the drug from its degradation products, it can be employed as a stability indicating one. Moreover, the proposed HPTLC method was utilized to investigate the kinetics of acid degradation process.

1. INTRODUCTION

For thousands of years, we have been dependent on the medicinal properties of plant species. Antediluvian records feature Mother Nature as the pharmaceutical provider of thousands of drugs obtained from plant sources [1].

One of the most consequential medicinal plants is Vinca rosea (L.) G. Don and it is kenned as Madagascar periwinkle and withal as Kemuning Cina, in Malaysia [2]. Terpenoid indole alkaloid (TIA)-engendering plant is *Catharanthus roseus* (L.) G. Don (Madagascar periwinkle) that belongs to the Family Apocynaceae [3]. The source of the indolomonoterpenic alkaloids such as vincristine (leurocristine) and vinblastine (vincaleucoblastine) are leaves of the plant species, and has anticancer activity which characterizes potent therapeutics to many diseases, such as Hodgkin lymphoma [4].

Catharanthus roseus plant, biosynthesize more than 130 alkaloids is from the aerial and root components of the plant, after which prominent high value secondary metabolites vincristine and vinblastine are evaluated and are utilized in treatment of diverse cancer (chemotherapy), whereas other alkaloid like ajmalicine and serpentine are recommended for hypertension [5,2,6]. Therapeutic role is the paramountcy of this plant species as the source of the anticancerous alkaloids such as vincristine (VCR) and vinblastine (VLB) [3]. Other alkaloids obtained such as vindoline and catharanthine are precursors of the vinblastine and vincristine group of alkaloids [7]. Vincristine was introduced in 1963 and is an oxidized form of vinblastine. Vincristine is utilized in the treatment of acute lymphoblastic leukemia (ALL) and has consequential role in prosperous treatment of ALL in children. When children suffering from acute lymphoblastic leukemia (ALL), vincristine is integrated to the treatment course of therapy,and eighty percent survival rate reached [3]. The mechanism of anticancer activity of these alkaloids is to disrupt microtubules, causing the dissolution of mitotic spindles and metaphase apprehend in dividing cells [8,9].

The metabolic engineering and cell line cull shows consequential contributions in the amendment of analytical methods and most of the efforts have been placed on chromatographic technologies which now show to be highly efficient, expeditious, precise and high-throughput for the analysis of plant alkaloids [6].

For the standardization of drugs derived from plant in the modern years, a conventional analytical approach is high performance thin layer chromatography (HPTLC), due to its advantages like low operation cost, high sample throughput and desideratum for minimum sample clean up [10, 11]. HPTLC (high performance thin layer chromatography) is generally utilized for the assay identification, and the testing for purity, stability, dissolution or content uniformity of raw materials (herbal & animal extracts, fermentation coalescences, drugs and excipients) and formulated products [pharmaceuticals, cosmetics and nutrients]. HPTLC is different from the other conventional analytical method like high performance liquid chromatography (HPLC) in the way of its advantages like reducing the time and cost per analysis that many samples can be run simultaneously utilizing a minute volume of mobile phase. These flexible and cost-efficacious techniques present the advantage of the simultaneous processing of standards and samples with multifarious detection possibilities, including a great variety of post-chromatographic derivatization reagents [12].

The aim of the present study was to develop a simple and economical analytical method for the quantitative estimation of vincristine from leaves and aseptically engendered callus of *C. roseus* var. *alba* for the routine analysis. In this perspective, a stability-betokening HPTLC method for the simultaneous estimation of vincristine was developed and validated in accordance with the International Conference on Harmonization (ICH) guidelines.

2. EXPERIMENTAL DETAILS

2.1 Materials and Methods

Vincristine, Sodium hypochlorite solution, 2,4-Dichlorophenoxyacetic acid, 1-Naphthaleneacetic acid and Kinetin were purchased from Sigma-Aldrich Chemicals Private Limited (Bangalore, India). Precoated aluminium backed silica gel 60F-254 plates (20 cm X 10 cm with 0.2 mm thickness) were purchased from E. Merck (Darmstadt, Germany). Methanol and all other chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals (Mumbai, India).

2.1.1 Plant material and aseptically produced callus

The fresh leaves of *C.roseus* var. *alba* were collected from the leaf garden of Jamia Hamdard campus and was taxonomically authenticated. A voucher specimen (JHCP137) was deposited in college herbarium. Callus of *C.roseus* var. *alba* were aseptically developed in plant tissue culture laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

2.1.2 Preparation of leaf and callus extract

The *C. roseus* var. *alba* leaves and aseptically produced callus were air dried, powdered, passed through 80 mesh sieve and stored in airtight container at 25°C. 5 g powder of leaf and callus was extracted with methanol (95%) separately for 1 hour. The methanolic extract were filtered and concentrated at 50°C, diluted with water (100 mL), acidified with 1N sulfuric acid (pH 2) and the mixture was centrifuged at 2000 rpm for 10 min and sediment was re-extracted with additional methanol (100 mL) for 30 min. The combined supernatant from two repeated extraction was filtered and extracted with petroleum ether (200 mL) to eliminate chlorophyll and other lipophillic compounds. The aqueous solution was adjusted to pH 6.4 using NH$_4$OH solution (25%) and the solution was filtered, evaporated to dryness under reduced pressure to give an alkaloid fraction. The content of the tube were filtered through Whatman filter paper (E. Merck, Mumbai, India). The clear supernatant was used for analysis. The yields of the leaf and callus extract were 19.6% w/w and 17.8% w/w, respectively.

2.1.3 Chromatographic condition

HPTLC was performed on 20 cm × 10 cm aluminium foil precoated with silica gel 60F-254 (E. Merck, Germany). The samples were applied as 5 mm bands by means of a Camag microlitre syringe (Switzerland) using Linomat V (CAMAG, Muttenz, Switzerland). A constant application or spraying rate of 150 nL s^{-1} and scanning speed 20 mm s^{-1} were employed. Linear ascending development with solvent (v/v) as mobile phase was performed in a twin-trough glass chamber (Camag) previously saturated with mobile phase vapour for 20 min (optimized chamber saturation time) at room temperature (25±2°C) and relative humidity 55±5%. The chromatogram was developed upto 80% height of plates by ascending linear techniques with mobile phase, methanol, toluene, diethylamine (73: 25: 2 v/v). After development, plates were dried in a current from an air-dryer. Densitometric scanning at 254nm was then performed with a Camag TLC scanner III in absorbance mode operated by win CATS software (version 1.2.0). The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190-400 nm. The slit dimensions were 5 mm × 0.45 mm and the scanning speed 20 mm s^{-1}.

2.1.4 Optimization of mobile phase

Various polarity natures of solvents (Chloroform, ethyl-acetate, toluene, methanol, acetonitrile, diethyl ether and diethyl amine) were used for optimization of mobile phase. Toluene and methanol were selected as one of the components of mobile phase as acceptable resolution was obtained. As the R$_f$ value was low, the solvent strength was increased by adding polar solvent. Good resolution and medium R$_f$ range were achieved with the ratio of 73: 25: 2, v/v (methanol: toluene: diethyl amine). Standard vincristine, leaf of *C. roseus* var. *alba* and callus extract solution were spotted on preparative TLC plates, developed chromatograms in saturated chamber using optimized mobile phase. The R$_f$ values were calculated for each chromatogram respectively.

2.1.5 Calibration curve of standard vincristine

A stock solution of vincristine (100 µg mL^{-1}) was prepared in mobile phase. The different volumes of stock solution (0.5,1,1.5,2,2.5 and 3µL) were applied to a plate to furnish 500, 1000, 1500, 2000, 2500 and 3000 ng spot^{-1}, respectively. Each amount was applied to the plate three times. Peak area data and the corresponding drug concentration were treated by linear least-square regression analysis.

2.1.6 Method validation

2.1.6.1 Linearity

From the stock solution of vincristine 0.5, 1, 1.5, 2, 2.5 and 3µl were spotted on HPTLC plates giving a concentration range of 500, 1000, 1500, 2000, 2500 and 3000 ng spot^{-1}, respectively. The linearity of response was assessed in the range of 500-3000 ng spot^{-1} for standard drug.

2.1.6.2 Accuracy, as recovery

The analyzed samples were spiked with extra 50, 100 and 150% of the standard vincristine and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the leaf extract as well as in the callus extract.

2.1.6.3 Precision

The precision of the system was determined by measuring repeatability of sample application and measurement of peak areas for three replicates of the band (500, 750, 1000 and 1250 ng spot^{-1}). Intra and inter-day variation for the determination of vincristine was carried out. The inter-day precision was studied by comparing assays performed on three different days. The precision of the system and method were expressed as relative standard deviation (% RSD) and standard error of mean (SEM).

2.1.6.4 Robustness of the method

Robustness was studied in triplicate at 500 and 1000 ng spot-1by making small changes to mobile phase composition, mobile phase volume, and duration of mobile phase saturation and activation of TLC plates. The effect on the results was examined by calculation of %RSD and SEM of peak areas. Mobile phases prepared from methanol, toluene and diethyl amine with ratio of 73: 25: 2, v/v were used for chromatography. Mobile phase volume and duration of saturation investigated were 15±2 mL and 20±10 min, respectively. The plates were activated at 55±5°C for 2, 5, and 7 min before chromatography.

2.1.6.5 Limits of Detection and Quantification

To estimate the limits of detection (LOD) and quantification (LOQ), methanol was used as blank and the standard deviation (σ) of the analytical response was determined. The LOD was expressed as 3.3σ/slope of the calibration plot for vincristine and LOQ was expressed as 10σ/slope of the calibration plot.

2.1.6.6 Specificity

The specificity of the method was assessed by analyzing and comparing the R_f values and spectra of the vincristine band from a sample with that from a standard. The peak purity of the vincristine bands was assessed by comparing spectra acquired at three different positions on the band - thepeak start (S), peak apex, (M), and peak end (E).

2.1.7 Analysis of vincristine in *C.roseus* var. *alba* leaves and callus extract

The filtered solution (2,4 and 6 µL) was applied to a TLC plate followed by development and scanning as described in the instrumentation section. The analysis was repeated in triplicate. The possibility of interference from other components of the extract in the analysis was studied. Vincristine content in extracts from *C. roseus* var. *alba* leaves and callus sources were also studied.

2.1.8 Detection of related impurities

The related unknown impurities were determined by spotting higher concentrations of the vincristine. Vincristine solution was prepared at a concentration of 2000 µg mL^{-1} in methanol, and this solution was termed as sample solution. One milliliter of the sample solution was diluted to 40mL with methanol and this solution was termed as standard solution (50 µg mL^{-1}). Two microlitres of both the standard (100 ng spot^{-1}) and the sample solution (4000 ng spot^{-1}) were applied on HPTLC plate and the chromatograms were run as described in instrumentation section.

2.1.9 Stability studies

To evaluate the stability indicating properties of the developed HPTLC method, forced degradation studies were carried out in accordance to the ICH guidelines. The standard drugs were subjected to acid, base, oxidation, wet heat, dry heat, and photo-degradation studies using methods in literature [13].

2.1.10 Study of acid induced degradation kinetics

Accurately weighed 100 mg of drug was dissolved in 100 mL methanol. Twenty milliliter of this standard solution was transferred into 100 mL of double neck round bottom flask. To it 20 mL of 10N HCl was added and refluxed at different temperatures (50, 60, 70, 80 and 90 °C). At a specified time intervals the contents of the flask were quantitatively transferred to 10 mL volumetric flasks and estimated by HPTLC method by one point standardization using external standard. The experiment was carried out in triplicate. The concentration of the remaining drug was calculated for each temperature and time interval. Data was further processed and degradation kinetics constants were calculated.

3. RESULTS AND DISCUSSION

3.1 Development of the Optimized Mobile Phase

The *C. roseus* var. *alba* leaves and callus extract was subjected to thin layer chromatography (TLC) to find out the concentration of vincristine alkaloid in it, to optimize the different ratio of mobile phase, methanol: toluene: diethyl amine and to quantify the marker compound. The solvent system consisting of methanol: toluene: diethyl amine (73: 25: 2) used in the investigation gave compact spots for vincristine in different concentration levels at R_f value 0.38 (Fig. 1).

Densitometric analysis was carried out in the absorption mode at λ_{max}.

3.2 Validation of the Method

3.2.1 Linearity

A good linear relationship (correlation coefficient $r^2=0.998\pm0.003$) was obtained between vincristine peak area and concentration in the range 500–3000 ng spot^{-1}. The mean values (±SD) of the slope and intercept were 2.8908 ±0.24 and 425.02±2.372, respectively. There was no major difference was observed in the slopes of standard plots (ANOVA, $P < 0.05$).

3.2.2 Accuracy, as recovery

The recovery of the method was determined by spiking an aforetime analyzed test solution with additional drug standard solution, was 99.89-100.28%. The relative standard deviation (% RSD), standard error of mean (SEM) and recovery (%) values are listed in Table 1 designate the method was accurate.
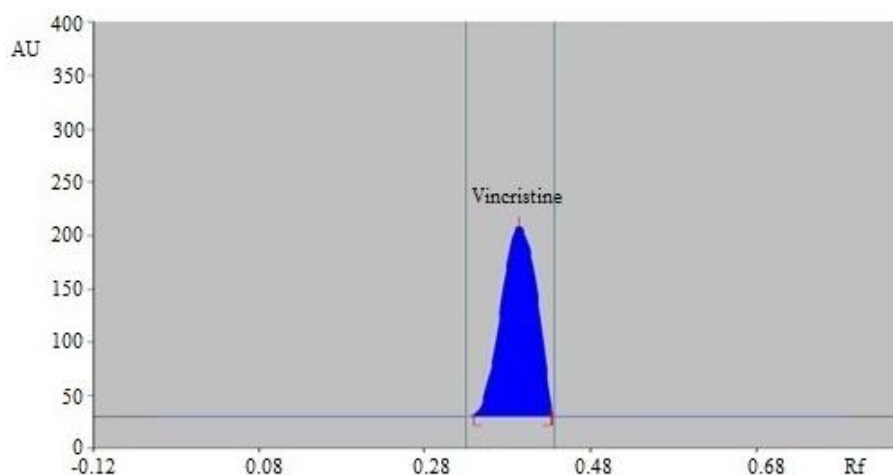
3.2.3 Precision

Repeatability of sample application was tenacious as intra-day variation whereas intermediate precision was tenacious by carrying out inter-day variation at four different concentration levels in triplicates. RSD for the repeatability of sample application and peak area at four different concentrations resulted in low values of SEM and RSD (<1%) for inter and intra-day variation (Table 2), which suggested the precision of the method was excellent.

3.2.4 Robustness of the method

The standard deviation of peak areas was calculated for each parameter and % R.S.D. was found to be less than 2%. The low values of % RSD as shown in Table 3 designated robustness of the method.

Table 1. Accuracy, as recovery of the proposed method (n=3)

Excess drug added to analyte (%)	Theoretical content (ng spot^{-1})	Conc. found (ng spot^{-1}) ±SD	% Recovery	% RSD	SEM
0	500	499.47±1.26	99.89	0.25	0.73
50	750	752.1±2.62	100.28	0.35	1.51
100	1000	999.33±2.36	99.93	0.24	1.36
150	1250	1249.7±2.64	99.97	0.21	1.52

Fig. 1. Chromatogram of standard vincristine (250 ng spot^{-1}), Peak 1: R_f: 0.38±0.02, mobile phase-73: 25: 2, v/v (methanol: toluene: diethyl amine) at 254 nm

Table 2. Precision of the method (Intra-day and Inter-day)

Conc. (ng spot^{-1})	Repeatability precision (intra-day)		Intermediate precision (inter-day)	
	Mean area ± SD	% RSD	Mean area±SD	% RSD
500	942.34±6.48	0.68	964.24±6.24	0.64
750	1548.63±6.92	0.45	1622.47±7.31	0.45
1000	2144.62±11.66	0.54	2216.75±11.59	0.52
1250	3274.73±12.85	0.39	3449.52±18.86	0.55

3.2.5 Limits of detection and quantification

The LOD and LOQ were 3.06 and 9.29 ng spot^{-1}, respectively, indicating the sensitivity of the method was adequate.

3.2.6 Specificity

The peak purity of vincristine was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of the spot i.e., r (start, middle) = 0.9996 and r (middle, end) = 0.9994. Good correlation (r=0.9998) was withal obtained between standard and sample spectra of vincristine. The data of summary of validation parameters are listed in Table 4.

3.3 Stability in Sample Solution

Two different concentrations (500 and 1000 ng/spot) were prepared from sample solution and put away at room temperature for 6.0, 12.0, 24.0, 48.0 and 72.0 hours, respectively. The solutions were put away in firmly topped volumetric cups shielded from light on a lab seat. They were then applied on the same TLC plate, after development the chromatogram was assessed as recorded in Table 5 for extra spots if any. The

% RSD for the samples analyzed at different elapsed assay times was found to be <2%. Thus, the drug was stable in solution state. There was no evidence of compound instability in the sample solution.

3.3.1 Spot stability

The time the sample is left to remain on the solvent prior to chromatographic development can impact the stability of isolated spots and are obliged to be researched for validation [14]. Two-dimensional chromatography utilizing same solvent system was used to figure out any decay happening amid spotting and development. In the event that, if deterioration happens during development, peak(s) of decomposition product(s) shall be obtained for the analyte both in the first and second course of the run. No decomposition was seen during spotting and development.

3.4 Analysis of Vincristine in *C. roseus* var. *alba* Leaves and Callus Extract

A solitary spot at Rf 0.38 was seen in both the chromatogram of leaf and callus extract

(Fig. 2 & 3). The percentage of vincristine obtained was 0.67 and 0.59, respectively with a %RSD of 0.54, 0.63 and SEM of 0.38, 0.45, respectively. It might be, consequently, reasoned that there was no connection with the chemicals utilized for extraction. The low %RSD and SEM further demonstrates the suitability of the method for routine examination of the drug.

Table 3. Robustness of the method (n=3)

Parameters	500 ng spot^{-1}				1000 ng spot^{-1}			
	SD of peak area	% RSD	SEM	R_f value	SD of peak area	% RSD	SEM	R_f value
Mobile phase composition								
75:23:2, v/v/v	1.23	0.15	0.71	0.37	2.62	0.25	1.51	0.38
71: 27:2, v/v/v	1.21	0.32	0.70	0.38	3.54	0.26	2.04	0.39
Mobile phase volume	1.42	0.17	0.82	0.37	2.86	0.22	1.65	0.39
(13,15 and 17 mL)	1.33	0.14	0.77	0.39	1.64	0.19	0.95	0.37
	1.36	0.12	0.78	0.37	2.35	0.43	1.36	0.37
Duration of saturation	1.44	0.16	0.83	0.37	3.34	0.18	1.92	0.37
(10, 20 and 30 min)	1.51	0.12	0.87	0.36	2.64	0.15	1.52	0.38
	1.16	0.23	0.70	0.37	2.32	0.12	1.34	0.38
Activation of TLC plates	1.13	0.44	0.65	0.37	1.22	0.11	0.70	0.37
(2, 5 and 7 min)	1.43	0.15	1.03	0.38	1.54	0.09	0.89	0.38
	1.36	0.11	0.75	0.39	1.68	0.07	0.97	0.38

Table 4. Summary of validation parameters

Parameter	Data
Linearity range (ng spot^{-1})	500-3000
Correlation coefficient	0.998±0.003
Limit of detection (ng spot^{-1})	3.06
Limit of quantitation (ng spot^{-1})	9.29
Recovery (n = 3)	100.02±0.45
Precision (% RSD)	
Inter-day (n = 3)	0.54
Intra-day (n = 3)	0.51
Robustness	Robust
Specificity	Specific
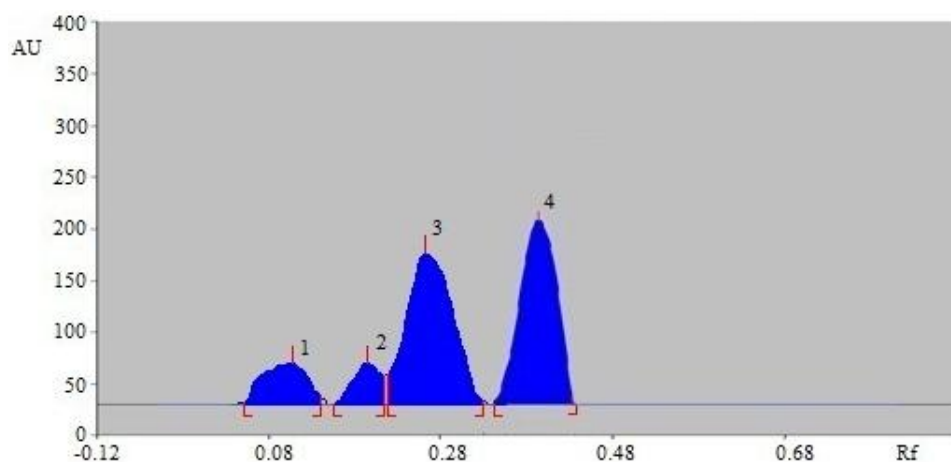
Fig. 2. HPTLC chromatogram of methanolic extract of *Catharanthus roseus* var. *alba* leaves at 254 nm (peak 4: vincristine, R_f; 0.38)

3.5 Detection of Related Impurities

The spots other than the principal spot and the spot of the beginning stage from the sample solution were not intense than the spot from the standard solution. The standard solution demonstrated one extra spots at Rf 0.23 (Fig. 4). However the region of the extra spots was discovered to be significantly less when compared with the standard solution of vincristine.

3.6 Stability Studies

The results of the forced degradation investigation of vincristine utilizing 73: 25: 2, v/v (methanol: toluene: diethyl amine) as the mobile phase system are summarized in Table 6.

3.7 Degradation Kinetics

In corrosive medium, a decrease in the concentration of drug with increasing time was observed. The impact of temperatures on the degradation process in corrosive medium is demonstrated in Fig. 5. At the chose temperatures (50, 60, 70, 80 and 90°C), the degradation process followed pseudo first-order kinetics. Apparent first order degradation rate constant and half-life were gotten from the slopes of the straight lines at every temperature. Data obtained from first order kinetics treatment was further subjected to fitting in Arrhenius mathematical statement; $[K = Ae^{-Ea/RT}]$, where K is rate constant, A the frequency factor, Ea is energy of activation (kcal mole^{-1}), R the gas constant (1.987 cal/degmole) and T is absolute temperature (°C). A plot of $(2 + \log K_{obs})$ values versus $(1/T \times 10^3)$, the Arrhenius plot was obtained, which was found to be linear in the temp range 50-90°C. The degradation rate constant at room temperature $(K_{25}°)$ is obtained by extrapolating the resulting line in Arrhenius plot to 25°C (Fig. 6) and was found to be 13× 10^{-2} h^{-1} and calculated $t_{1/2}$ is 5.33 h [15].

Table 5. Stability of vincristine in sample solutions [a]

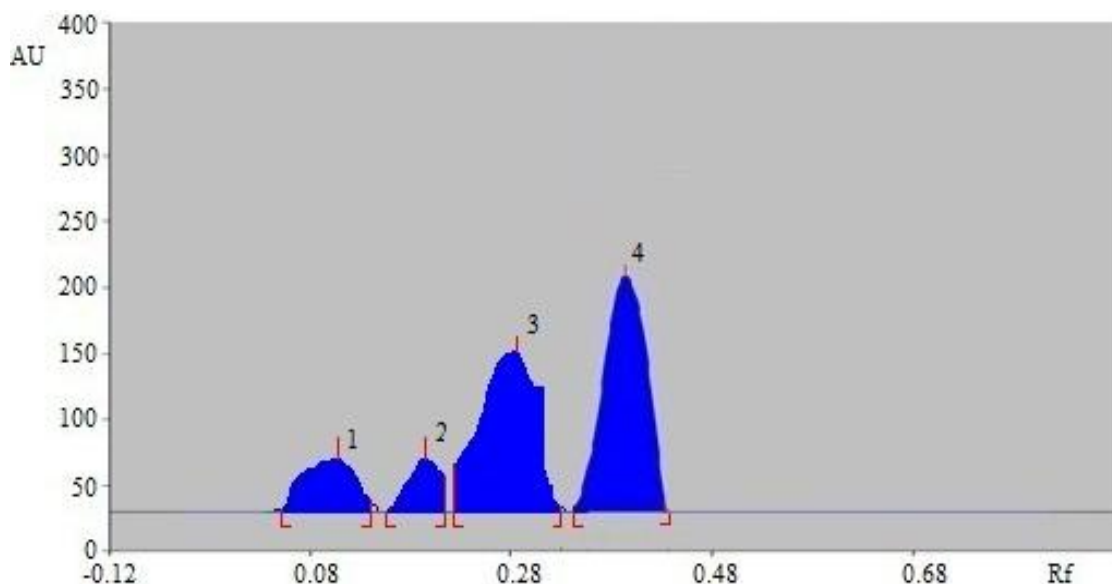| Actual (ng)	Area Mean ± SD	Area range	% RSD	SEM
500	964.46 ± 2.03	964.46-970.54	0.21	1.17
1000	2026.24 ± 2.46	2021.24-2038.32	0.12	1.42

[a] $n = 6$

Fig. 3. HPTLC chromatogram of methanolic extract of callus of *Catharanthus roseus var. alba* at 254 nm (peak 4: vincristine, R_f; 0.38)

Table 6. Stability studies for the developed method

Degradation condition	No. of degradation products	*Rf* value	Area of degradation product (%)
5N HCl (at 70 °C for 2 h)	4	0.28,0.42, 0.50, 0.60	7.50, 3.78, 12.04, 6.07
10 N NaOH (at 70 °C for 2 h)	1	0.27	5.84
7.4 Phosphate Buffer	1	0.27	10.46
Peroxide-induced degradation	2	0.25, 0.53	4.38, 7.65
Dry heat-wet heat (100 °C)	3	0.19, 0.33, 0.55	4.35, 1.43, 9.05
Photochemical degradation	1	0.18	0.93
UV degradation	1	0.53	0.86
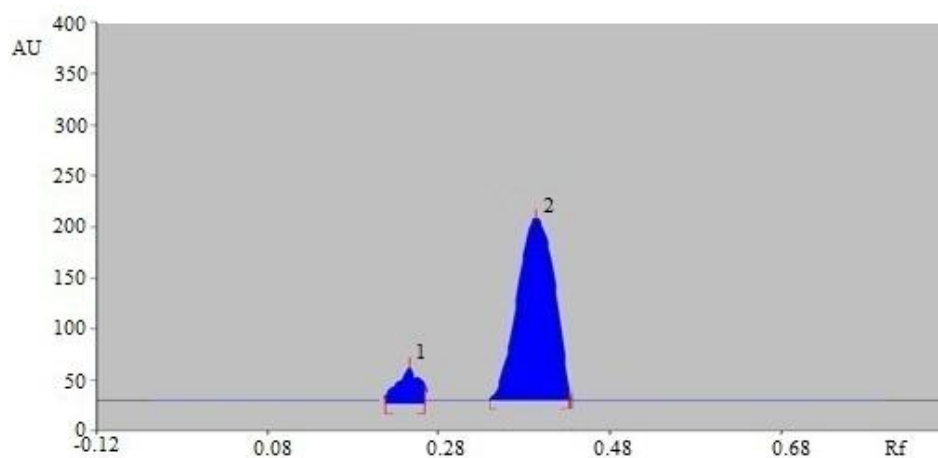
Fig. 4. Chromatogram of vincristine and its impurity (peak 1: impurity, Rf: 0.23; peak 2: vincristine, R_f: 0.38)
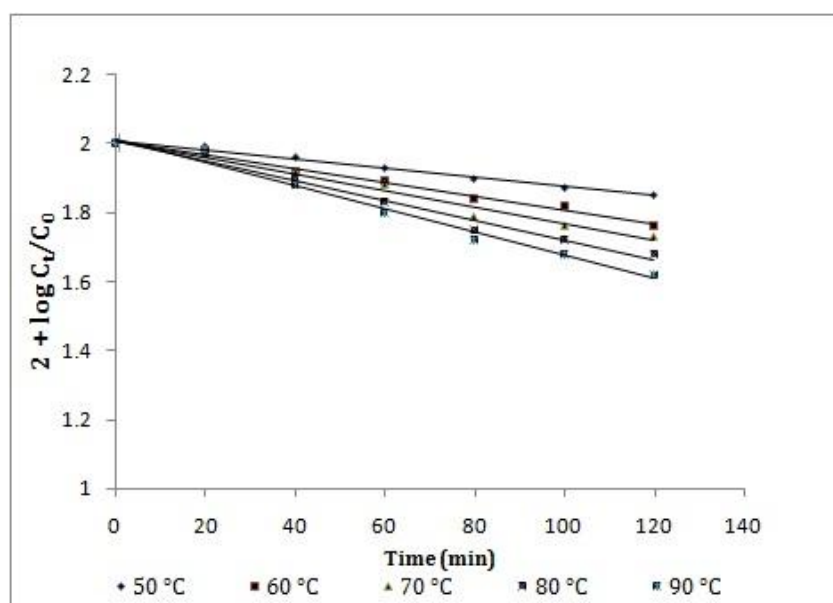
Fig. 5. Pseudo first-order plots for the degradation of vincristine with 5N HCl at various temperatures; Ct = concentration at time t; C0 = concentration at time zero
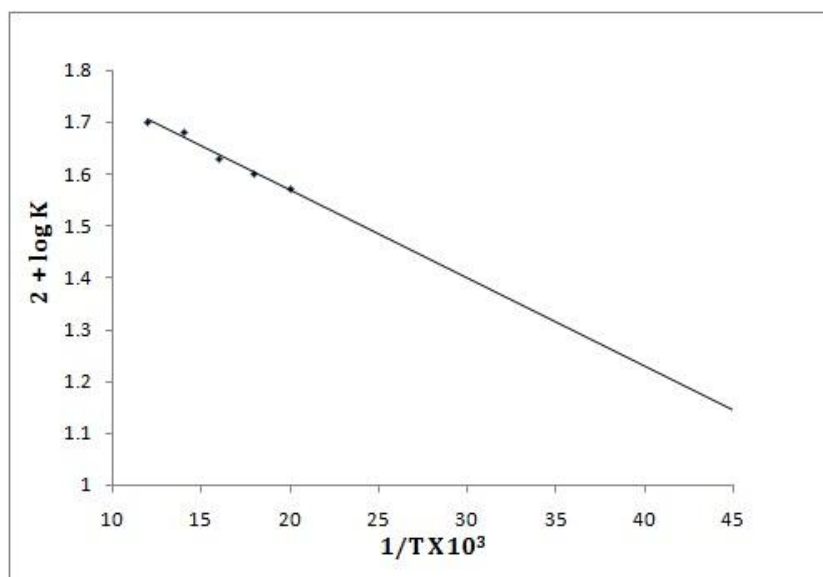
Fig. 6. Arrhenius plot for the degradation of vincristine in presence of 5N HCl and its extrapolation for predicting the degradation of vincristine at room temperature (25 °C) [15]

4. CONCLUSION

A simple and sensitive HPTLC method for the estimation of vincristine from methanolic extract of fresh leaves and aseptically produced callus of *Catharanthus roseus* var. *alba* was successfully developed. The Statistical assessment of methods proved that the developed HPTLC technique is precise, specific, accurate, stability indicating. The statistical analysis proved that the method is repeatable and selective for the analysis of vincristine as bulk drug and in leaf and callus extract without any interference. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities. Acid induced degradation studies disclose the suitability of method for the quantification of drugs in the presence of degradents. The above results showed the suitability of proposed method for acid induced degradation kinetic study of vincristine. The degradation rate constant and half-life of vincristine can be predicted. As the method separates the drug from its degradation products, it can be employed as a stability indicating one [15].

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem. 2005;77(1):7-24.
2. Siddiqui MA, Ismail Z, Saidan NH. Simultaneous determination of secondary metabolites from *Vinca rosea* plant extractives by reverse phase high performance liquid chromatography. Phcog Mag. 2011;7:92-96.
3. Fansong Mu, Liuqing Yang, Wei Wang, Meng Luo, Yujie Fu, Xiaorui Guo, Yuangang Zu. Negative-pressure cavitation extraction of four main vinca alkaloids from *Catharanthus roseus* leaves. Molecules. 2012;17:8742-8752.
4. Federico Ferreres, David M. Pereira, Patrícia Valentao, Jorge MA Oliveira, Joana Faria, Luís Gaspar, Mariana Sottomayor, Paula B Andrade. Simple and reproducible HPLC–DAD–ESI-MS/MS analysis of alkaloids in *Catharanthus roseus* roots. Journal of Pharmaceutical and Biomedical Analysis.2010;51(1):65-69.
5. Van Der Heijden R, Jacobs DI, Snoeijer W, Hallared D, Verpoorte R. The Catharanthus alkaloids: Pharmacognosy and bio-

technology. Curr Med Chem. 2004;11:607-628.

6. Steve Hisiger, Mario Jolicoeur. Analysis of *Catharanthus roseus* alkaloids by HPLC. Phytochem Rev. 2007;6:207-234.

7. Gupta MM, Singh DV, Tripathi AK, Pandey R, Verma RK, Singh S, Shasany AK, SPS Khanuja. Simultaneous determination of vincristine, vinblastine, catharanthine, and vindoline in leaves of *Catharanthus roseus* by high-performance liquid chromatography. Journal of Chromatographic Science. 2005;43:450-53.

8. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev. Cancer. 2004;4:253–265.

9. Zu YG, Fu YJ, Luo M, Mu FS. Structure modification for reduced toxicity derivatives and anticancer activity of vinblastine (in Chinese). Chem. Online. 2004;67:27.

10. Kaul N, Agrawal H, Patil B, Kakad A, Dhaneshwar SR. Application of stability-indicating HPTLC method for quantitative determination of metadoxine in pharmaceutical dosage form. IL Farmaco. 2005;60:351-360.

11. Alqasoumi SI, Alam P, Alrehaily AJ, Shakeel F, Abdel-Kader MS. Stability-indicating densitometric HPTLC method for qualitative and quantitative analysis of hydroquinone in commercial whitening creams. Journal of Planar Chromatography. 2011;24:48-52.

12. Mona Salih Mohammed, et al. Chromatographic finger print analysis of anti-inflammatory active extract fractions of aerial parts of *Tribulus terrestris* by HPTLC technique. Asian Pac J Trop Biomed. 2014; 4(1):930-35.

13. Hussain A, Rahman MA, Hussain MS, Mirza MA, Iqbal Z, Harwansh R, Singh LR. HPTLC method for analysis of sertraline in pure bulk drug and lipidic nano delivery system: A stress degradation studies. J Liq Chromatogr Relat Tech. 2013:36:700-16.

14. PD Sethi, High performance thin layer chromatography, quantitative analysis of pharmaceutical formulations, CBS Publishers and distributors, New Delhi; 1996.

15. Akhtar J, Fareed S, Aqil M. Stability-indicating assay of repaglinide in bulk and optimized nanoemulsion by validated high performance thin layer chromatography technique. J Pharm Bioall Sci. 2013;5:184-90.

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