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Stability-indicating methods for determination of vincamine in presence of its degradation product

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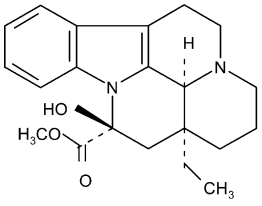
Abstract

Three different stability indicating assay methods are developed and validated for determination of vincamine in the presence of its degradation product (vincaminic acid). The first method is based on the derivative ratio zero crossing spectrophotometric technique using 0.1 N hydrochloric acid as a solvent. In the second method, measurements are based on spectro-densitometric technique using high performance thin-layer chromatography (HPTLC) plates with a developing system consisting of methanol–chloroform–ethyl acetate (2:1:1, v/v/v). The third method depends on high-performance liquid chromatography (HPLC). Separation of vincamine from vincaminic acid using Lichrocart RP-18 column (250 mm × 4.6 mm i.d.) with a mobile phase consisting of acetonitrile–ammonium carbonate (0.01 M) (7:3, v/v) is achieved. The methods showed high sensitivity with good linearity over the concentration ranges of 12 to 48 μg ml^{-1}, 3 to 17 μg/spot, and 2 to 20 μg ml^{-1} for derivative spectrophotometry, spectro-densitometry and HPLC methods, respectively. The developed methods were successfully applied to the analysis of pharmaceutical formulations containing vincamine with excellent recoveries.

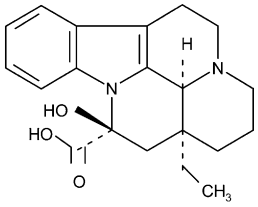
Keywords: Vincamine; Vincaminic acid; Derivative ratio spectrophotometry; Densitometry; Liquid chromatography

1. Introduction

Vincamine is claimed to have a selective vaso-regulatory action on cerebral circulation and adopting cerebral blood flow to metabolic needs [1].



Vincamine



Vincaminic acid

Vincamine (3α, 14β, 16α)-14,15-dihydro-14-hydroxy-eburnamienine-14-carboxylic acid methyl ester) is a white powder soluble in acid medium but insoluble in alkaline medium [2]. Various chromatographic methods have been developed for the separation and quantitation of vincamine in different matrices [3–9]. In Eburnane alkaloids, vincamine have been determined using HPLC with a pre-packed cyano-column [3], while micropak MCH-10 column, maintained at 25 °C, has been used for the analysis of drug in pharmaceutical formulations [4]. Reversed-phase RP-18 columns have been used extensively for analysis of drug in plasma [5] and Vinca extracts [6–8]. Determination of vincamine in plasma has been carried out by HPLC using RP-8 column [9]. RP-8 column has been also employed for the determination of vincamine in Catharanthus alkaloids using HPLC with a mobile phase consisting of acetonitrile–ammonium acetate (pH 7.2; 0.1 M) (29:21, v/v) and mass spectrometric (MS) detection [10]. Ion-exchange chromatography has been used for the

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determination of vincamine in Vinca alkaloids using a column contains α- or β-cyclodextrin polymer 80–125 μm and a mobile phase consisting of phosphate buffer (pH 5–5.5) with UV-detection [11]. Determination of vincamine in plasma by gas chromatography with mass spectrometric detection (GC–MS) using a column contains 1% of SE-30 has been reported [12]. Vincamine has been analysed in plasma by capillary gas chromatography using a wall coated open tubular column contains OV-1 [13]. The concentration of vincamine has been measured in blood plasma densitometrically using a solvent system composed of chloroform-methanol (9:1, v/v) and silica gel plates [14]. Spectrophotometric determination of vincamine in vinca major extract after separation on TLC silica gel LS 5/40 plates using benzene-ethyl acetate-methanol (1:1:1, v/v/v) as a developing system has been reported [15].

The present work aims to develop feasible, sensitive and specific analytical procedures for the analysis of vincamine in presence of its degradation product. Adaptation of the proposed procedures to the analysis of the available dosage forms is also an important task in order to solve problems encountered in quality control and analysis of expired samples. Moreover, kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceutical products necessitate such methods.

2. Experimental

2.1. Samples

- Vincamine powder batch number 1999091004 was kindly supplied by Glaxo-Smith-Klein (GSK) Egypt.
- Oxybral capsules (each capsule contains 30 mg vincamine), manufactured by Glaxo-Smith-Klein (GSK) Egypt, batch no. 012261A, 021554A and 011345A, were purchased from the Egyptian market.

2.2. Reagents

The materials used were of analytical grade, concentrated hydrochloric acid, methanol, chloroform, ethyl acetate, acetonitrile for HPLC, ammonium carbonate and de-ionized water for HPLC are obtained from Merck (Germany).

2.3. Apparatus

- A double-beam UV–vis spectrophotometer (SHIMADZU, Japan) Model UV-1601 PC connected to IBM compatible computer and HP 680 inkjet printer. The bundled software was UVPC personal spectroscopy software version 3.7. The spectra bandwidth was 2 nm and wavelength-scanning speed was 2800 nm min^{-1}.
- Dual wavelength flying spot CS-9000 densitometer, Shimadzu (Japan).
- Pre-coated HPTLC plates, silica gel 60 F_{245} 20 × 20 cm, 0.2 nm thickness, Macheray-Nagel (Germany).
- Spot Applicator with a 10 μl syringe, Desaga.
- Liquid chromatograph consists of a "La-Chrom" HPLC instrument (Hitachi-Merck) Germany, pump model L-7150, connected with a photodiode array detector model L-7455. The injector was a manual Rheodyne injector (Model 7725i, Rohnert Park, California, USA) equipped with a 20 μl injector loop and a 100 μl Hamilton syringe. The instrument was connected to an IBM compatible PC bundled with Merck-Hitachi Model D-7000 chromatography Data Station software HPLC septum manager and an HP 800 inkjet printer. A Lichrocart RP-18 column (250 mm × 4.6 mm i.d.) particle size 5 μm (Merck, Germany) was used for the analysis.

2.4. Procedures

2.4.1. Degradation of vincamine

Accurately weighed amount of 100 mg vincamine was refluxed for 7 h in 100 ml of 2 N hydrochloric acid. During reflux, small portions were cooled and spotted on a TLC plate. Spotting was repeated at 30 min intervals to follow up time required for complete degradation. Solution was cooled, spotted and then developed using methanol–chloroform–ethyl acetate (2:1:1, v/v/v) as a developing system.

2.4.2. Derivative ratio method

2.4.2.1. Spectral characteristics of vincamine and its degradation product. Aliquot portions equivalent to 20 μg ml^{-1} vincamine and 20 μg ml^{-1} vincaminic acid in 0.1 N hydrochloric acid were transferred into two 25 ml volumetric flasks and completed to volume with the same solvent. The zero order and the first derivative spectra of the prepared solutions were recorded.

2.4.2.2. Linearity. Different aliquots of vincamine stock solution (250 μg ml^{-1} in 0.1 N hydrochloric acid) ranging from 1.2 to 4.8 ml at 0.4 ml intervals and 2.0 ml of the vincaminic acid stock solution (250 μg ml^{-1} in 0.1 N hydrochloric acid) were transferred into 25 ml volumetric flasks then completed to volume with 0.1 N hydrochloric acid. The spectra of the prepared standard solutions were scanned from 200 to 400 nm and stored in the computer. The stored spectra of vincamine were divided (amplitude at each wavelength) by the spectrum of 20 μg ml^{-1} standard vincaminic acid. The first derivative of the ratio spectra (^{1}DD) were obtained at $\Delta\lambda = 4$ nm. The amplitude of the first derivative peak of (vincamine/vincaminic acid) was measured at 293 nm and used to calculate the content of vincamine. Calibration graph was constructed relating the peak amplitude of $^1DD_{293}$ to the corresponding concentrations of vincamine over a range of 12 to 48 μg ml^{-1}.

2.4.3. Densitometric method

2.4.3.1. Linearity. Aliquot portions 3.0–15.0 μl at 2.0 μl intervals of vincamine stock solution (1000 μg ml^{-1} in chloroform) were spotted on HPTLC plates using a Desaga Applicator. Plates were developed by ascending chromatography to a distance 11.5 cm from the spots at room temperature with a solvent system consisting of methanol–chloroform–ethyl acetate (2:1:1, v/v/v). Plates were left to dry then the spots were detected under UV-lamp (254 nm). Area under the peak was measured at 281 nm using the reflection photo-mode and zigzag scan mode at 10 mm swing width. A calibration curve relating the area under the peak to the corresponding concentrations of vincamine was constructed over a range of 3 to 17 μg/spot.

2.4.4. Liquid chromatographic method

2.4.4.1. Linearity. Aliquot portions 0.5–5.0 ml at 0.5 ml intervals of vincamine stock solution (100 μg ml^{-1} in 0.1 N hydrochloric acid) were transferred into a series of 25 ml volumetric flasks. The flasks were completed to volume with 0.1 N hydrochloric acid. Aliquots (20 μl) of the prepared solutions were injected into HPLC. The peak area was measured at 280 nm using a mobile phase consisting of acetonitrile–ammonium carbonate (0.01 M) (7:3, v/v) and a Lichrocart RP-18 (250 mm × 4.6 mm i.d.) column, particle size 5 μm at a flow rate 1.6 ml min^{-1}. A calibration graph representing the relative area under the peak of vincamine to that of vincamine external standard (10 μg ml^{-1}), versus the corresponding concentrations of vincamine over a range of 2 to 20 μg ml^{-1} was constructed.

2.4.5. Analysis of laboratory prepared mixtures containing different ratios from vincamine and its degradation product

2.4.5.1. Derivative ratio method. Accurately aliquot portions equivalent to 18–42 μg ml^{-1} of vincamine and vincaminic acid of 6–30 μg ml^{-1} from their corresponding stock solutions (250 μg ml^{-1} in 0.1 N hydrochloric acid) were transferred into a series of 25 ml volumetric flasks. The volumes were completed with 0.1 N hydrochloric acid. Vincamine concentration was calculated as mentioned under (Section 2.4.2.2).

2.4.5.2. Densitometric method. Aliquot portions equivalent to 300–900 μg ml^{-1} of vincamine and vincaminic acid of 100–700 μg ml^{-1} from their corresponding stock solutions (1000 μg ml^{-1} in chloroform and methanol respectively) were transferred into a series of 10 ml volumetric flasks. Aliquot portions 10 μl of the prepared mixtures were spotted on a HPTLC plate and developed as mentioned under (Section 2.4.3.1).

2.4.5.3. Liquid chromatographic method. Aliquot portions equivalent to 2–18 μg ml^{-1} of vincamine and vincaminic acid of 2–18 μg ml^{-1} from their stock solutions (100 μg ml^{-1} in 0.1 N hydrochloric acid) were transferred into a series of 25 ml volumetric flasks. Aliquot portions 20.0 μl of the prepared mixtures were injected into HPLC adopting the conditions under (Section 2.4.4.1).

2.4.6. Analysis of vincamine in oxybral capsules

2.4.6.1. Derivative ratio method. The powder of 20 capsules, after unpacking, was weighed. An amount of the powder equivalent to 25 mg vincamine was weighed into a 250 ml beaker. A volume of 50 ml 0.1 N hydrochloric acid was added. Mixture was stirred for 10 min using a magnetic stirrer then filtered into a 100 ml volumetric flask and completed to volume with 0.1 N hydrochloric acid. Accurately 3.2 ml of the filtrate was transferred into a 25 ml volumetric flask, the volume was completed with 0.1 N hydrochloric acid and subjected to analysis as mentioned under (Section 2.4.2.2).

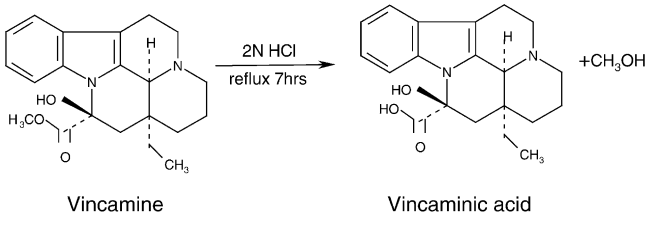
2.4.6.2. Densitometry. The powder of 20 capsules, after unpacking, was weighed. An amount of the powder equivalent to 25 mg vincamine was weighed into a 250 ml beaker. A volume of 10 ml chloroform was added and shaken well then filtered into 25 ml volumetric flask. The volume was completed to the mark with chloroform. On a HPTLC plate, aliquots of 10 μl of the prepared solution were spotted and the procedure under (Section 2.4.3.1) was followed.

2.4.6.3. Liquid chromatography. The powder of 20 capsules, after unpacking, was weighed. An amount of the powder equivalent to 25 mg vincamine was weighed into a 250 ml beaker. A volume of 50 ml 0.1 N hydrochloric acid was added and shaken well. The mixture was filtered into 100 ml volumetric flask, and then completed to the mark with 0.1 N hydrochloric acid. Aliquots portions 20 μl of the prepared solution was injected as under (Section 2.4.4.1).

3. Results and discussion

3.1. Degradation of vincamine

Vincaminic acid, the degradation product of vincamine, is one of the metabolites of vincamine in rats [16,17]. Vincamine is a stable drug, however, forced stability study under stress conditions revealed the instability of the drug in acid medium. The scheme for degradation of vincamine is proposed as follows: Scheme 1.



Scheme 1. Degradation of vincamine.

Vincaminic acid is soluble in 0.1 N hydrochloric acid as it still contains one basic nitrogen, being a tertiary amine. The molecular weight of vincaminic acid was confirmed by mass spectroscopy. It was noted that 7 h reflux using 0.1 N hydrochloric acid was enough to ensure complete degradation of vincamine; this was demonstrated by the use of thin layer chromatography. Furthermore, complete shift of vincamine's UV-spectrum in 0.1 N hydrochloric acid takes place (Fig. 1).

3.2. Derivative ratio spectrophotometric method

The zero and first order absorption spectra of vincamine and its degradation product Fig. 1 and Fig. 2, respectively show severe overlap that prevents the use of direct and derivative spectrophotometry for the analysis of vincamine in presence of vincaminic acid. The derivative ratio spectroscopy could be applied to resolve such a mixture and to determine the concentration of vincamine. The zero order of the derivative ratio spectra of vincamine and the first order of the derivative ratio spectra are presented in Figs. 3 and 4, respectively. The concentration of the devisor was also studied. It was found that upon dividing by 30 μg ml^{-1} the sensitivity of the method was decreased. Dividing by the spectrum of 20 μg ml^{-1} degradation product gave the best results in terms of sensitivity, repeatability and signal to noise ratio. The linearity was checked between the peak amplitude at the selected wavelength (293 nm) and the corresponding concentrations of vincamine. A linear response was obtained

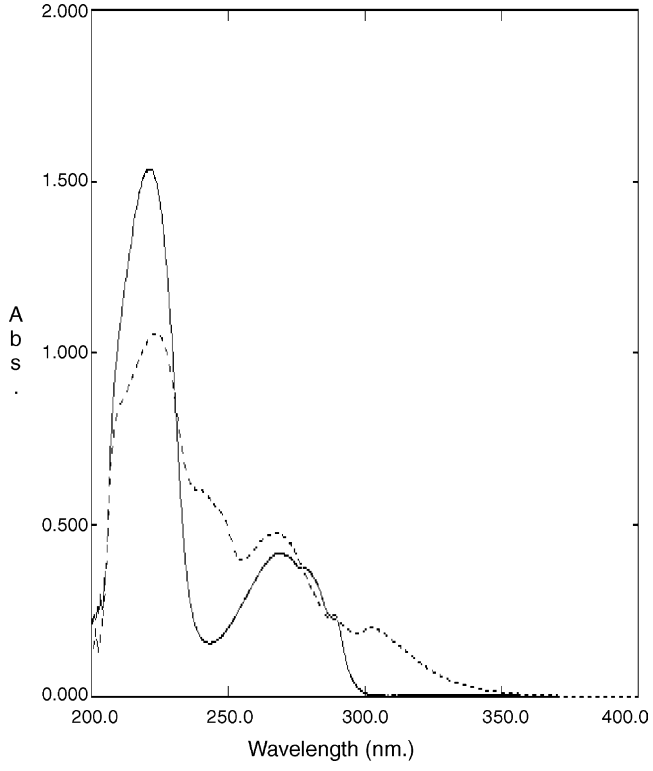


Fig. 1. Absorption spectra of vincamine 20 μg ml^{-1} (—) and degradation product 20 μg ml^{-1} (- - - -) in 0.1 N hydrochloric acid.

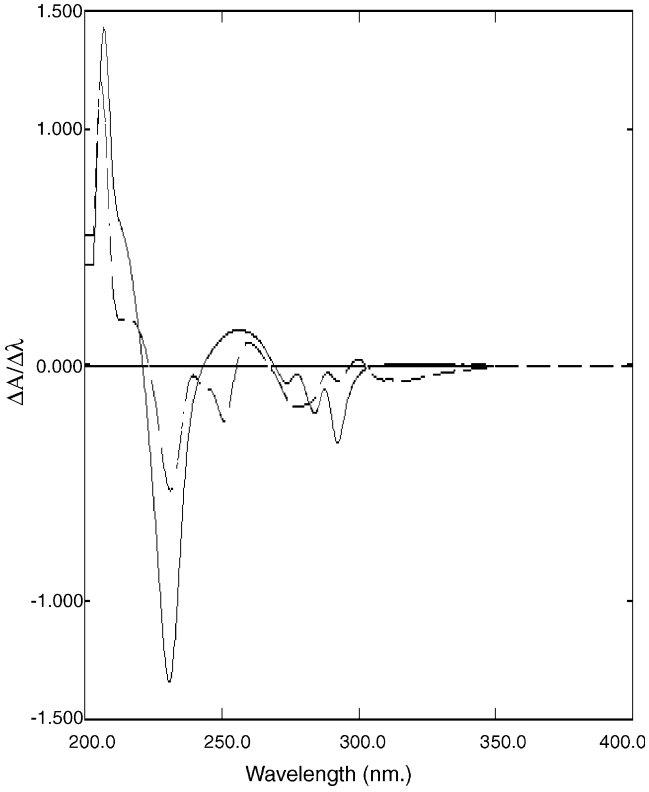


Fig. 2. First order spectra of vincamine 20 μg ml^{-1} (—), degradation product 20 μg ml^{-1} (- - -) in 0.1 N hydrochloric acid.

for concentration range from 12 to 48 μg ml^{-1}. The regression equation was found to be: $^{1}DD_{293} = -0.04\,C - 0.021$ ($r = 0.9992$) where C is the concentration of vincamine in μg ml^{-1}, ($^{1}DD_{293}$) is the first derivative curve of the peak amplitude for (vincamine/vincaminic acid) at 293 nm and r is the correlation coefficient.

3.3. Densitometric method

A densitometric method is described for the determination of vincamine in presence vincaminic acid without prior separation. Different solvent systems were tried for the separation of vincamine from its degradation product including methanol–chloroform (1:1, v/v) and methanol–chloroform–ethyl acetate (2:1:1, v/v/v). Satisfactory results were obtained by using the second system ($R_f = 0.69$, 0.47 for vincamine and vincaminic acid, respectively). The separation allows the determination of vincamine with no interference from vincaminic acid (Fig. 5). The linearity was confirmed by plotting the measured peak area versus the corresponding concentration at 281 nm over a range of 3 to 17 μg/spot where a linear response was obtained. The regression equation was found to be: $A = 0.3241\,C + 0.0958$, $r = 0.9998$ where A is the integrated area under the peak $\times 10^{-4}$ for vincamine, C the concentration in μg/spot for vincamine and r the regression coefficient.

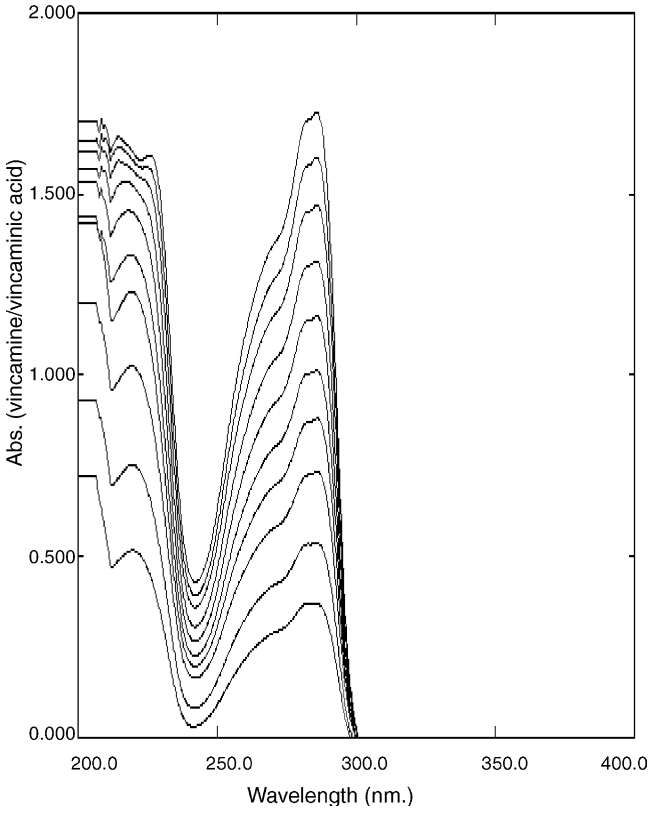


Fig. 3. Zero order of derivative ratio spectra of vincamine 12–48 μg ml^{-1} using 20 μg ml^{-1} of degradation product as a divisor in 0.1 N hydrochloric acid.

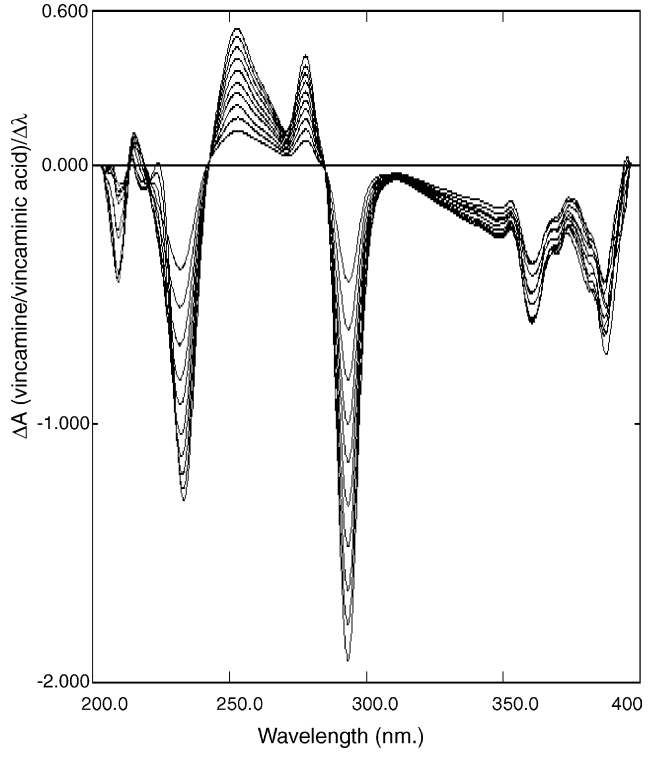


Fig. 4. First order of derivative ratio spectra of vincamine 12–48 μg ml^{-1} using 20 μg ml^{-1} of degradation product as a divisor in 0.1 N hydrochloric acid.

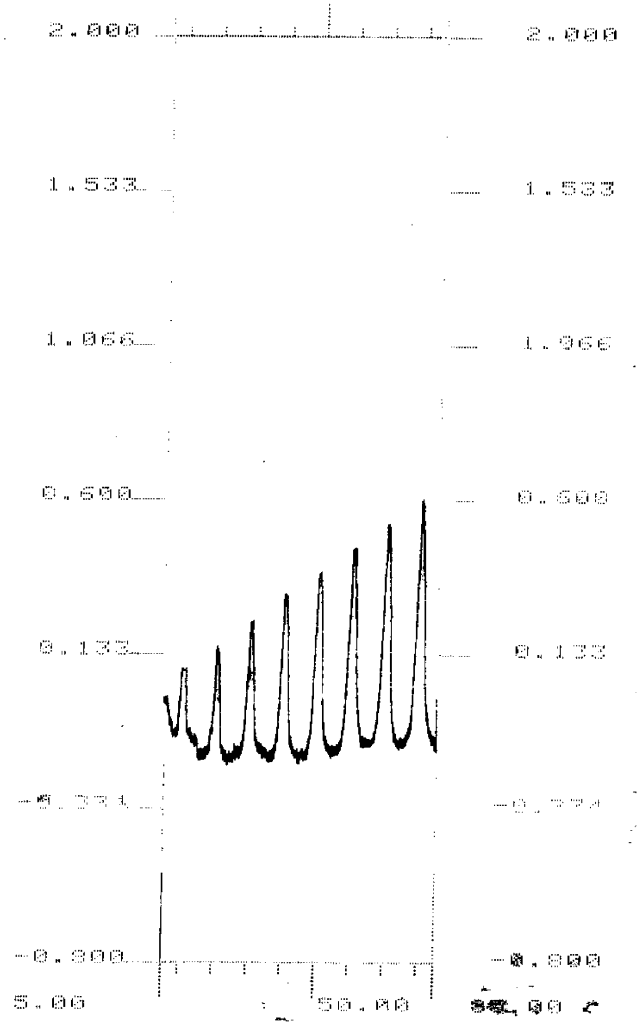


Fig. 5. Scanning profile of the TLC chromatogram of vincamine at 281 nm.

3.4. Liquid chromatographic method

A simple isocratic high-performance liquid chromatographic method is described for the determination of vincamine in presence of its acid degradation product. System suitability parameters were tested by calculating the capacity factor, tailing factor, the selectivity factor and resolution. The peak shape improved dramatically with the decrease in the percentage of ammonium carbonate (0.01 M) to acetonitrile. Best peak shape was obtained with acetonitrile–ammonium carbonate (0.01 M) (7:3, v/v) with retention times of 3.67 ± 0.02 and 6.61 ± 0.02 min for vincamine and vincaminic acid, respectively (Fig. 6). A linear response was obtained between the relative peak area and the corresponding concentrations of the vincamine in the range of 2 to 20 μg ml^{-1}. The regression equation was found to be: $A = 0.3643\,C - 0.2196$, $r = 0.9994$, where A is the relative area under the peak $\times 10^{-3}$, C the concentration in μg ml^{-1} and r the regression coefficient.

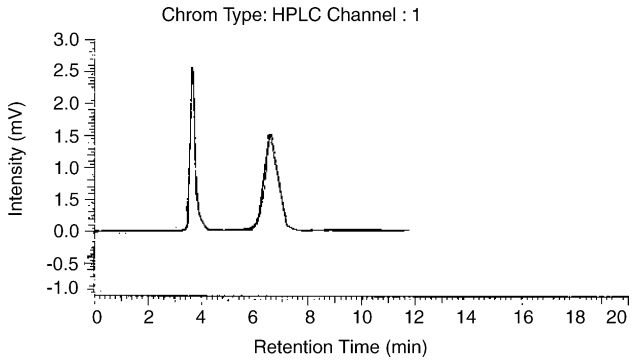


Fig. 6. Liquid chromatographic separation of vincamine (50 μg ml^{-1}) and its degradation product (50 μg ml^{-1}) using assay conditions mentioned in the text.

3.5. Method validation

The selectivity and specificity of the proposed methods were verified by determination of vincamine in laboratory prepared mixture containing different ratios of the drug and its degradation product. The analysis was valid up to 62.5, 70 and 90% of the degradation product for the (^{1}DD) method, densitometry and liquid chromatography, respectively (Tables 1–3).

To ascertain the accuracy of the proposed procedures, they were successfully applied for the determination of vincamine in oxybral capsules as presented in Table 4. The validity of

Table 1
Determination of vincamine in laboratory prepared mixtures by the proposed derivative ratio method

Concentration (μg ml^{-1})		Percentage		Recovery (%)
Vincamine	Degradation product	Vincamine	Degradation product	Vincamine
42	6	87.5	12.5	99.96
36	12	75	25	99.19
30	18	62.5	37.5	101.40
24	24	50	50	100.75
18	30	37.5	62.5	101.30
Mean				100.52
S.D.				0.937

Table 2
Determination of vincamine in laboratory prepared mixtures by the proposed densitometric method

Concentration (μg/spot)		Percentage		Recovery (%)
Vincamine	Vincaminic acid	Vincamine	Vincaminic acid	Vincamine
9	1	90	10	98.18
8	2	80	20	99.40
7	3	70	30	100.04
6	4	60	40	99.13
5	5	50	50	100.01
4	6	40	60	101.36
3	7	30	70	99.00
Mean				99.58
S.D.				1.007

Table 3
Determination of vincamine in laboratory prepared mixtures by the proposed HPLC procedure

Concentration (μg ml^{-1})		Percentage		Recovery (%)
Vincamine	Vincaminic acid	Vincamine	Vincaminic acid	Vincamine
18	2	90	10	99.13
16	4	80	20	98.45
14	6	70	30	100.89
12	8	60	40	98.85
10	10	50	50	101.33
8	12	40	60	100.24
6	14	30	70	99.76
4	16	20	80	98.63
2	18	10	90	99.75
Mean				99.67
S.D.				1.007

Table 4
Determination of vincamine in oxybral capsules by the proposed procedures

Batch number	Derivative ratio method	Densitometric method	HPLC method
	Recovery % ± S.D.[a]		
012261A	99.02 ± 0.811	99.96 ± 1.187	98.38 ± 1.021
011345A	98.98 ± 0.534	100.31 ± 0.197	99.36 ± 0.458
021554A	99.52 ± 0.471	99.57 ± 0.405	100.95 ± 0.359

[a] Average of four determinations.

Table 5
Results of application of standard addition to the determination of vincamine in oxybral capsules by the proposed methods

Batch number	Standard added (μg ml^{-1})	Derivative ratio	Standard added (μg ml^{-1})	Densitometry	HPLC
	32.00	99.73	1.00	97.90	99.90
012261A	48.00	101.50	1.50	97.40	98.40
	64.00	102.80	2.00	99.80	101.36
Mean ± S.D.		101.34 ± 1.541		98.36 ± 1.266	99.89 ± 1.480
	32.00	99.64	1.00	99.60	98.54
011345A	48.00	100.09	1.50	99.10	101.56
	64.00	99.19	2.00	99.59	99.79
Mean ± S.D.		99.64 ± 0.450		99.43 ± 0.285	99.96 ± 1.517
	32.00	98.15	1.00	100.09	97.96
021554A	48.00	98.98	1.50	100.68	102.32
	64.00	99.23	2.00	100.30	100.87
Mean ± S.D.		98.78 ± 0.565		100.35 ± 0.299	100.38 ± 2.220

Table 6
Assay parameters and validation sheet

Parameter	Derivative ratio method	Densitometric method	HPLC method
Range ($\mu g\,ml^{-1}$)	12–48	3–17 μg/spot	2–20
Slope	−0.04	0.324	0.3643
Intercept	−0.021	0.0958	−0.2196
Mean	99.90	100.09	100.16
S.D.	1.0413	0.761	1.026
Variance	1.084	0.579	1.054
Coefficient of variation	1.042	0.760	1.024
Correlation Coefficient (*r*)	0.9992	0.9998	0.9994
RSD (%)[a]	0.189–0.311	0.176–0.211	0.254–0.378
RSD (%)[b]	0.312–0.275	0.286–0.301	0.421–0.573

RSD % of concentrations 20.0–32.0 $\mu g\,ml^{-1}$ for derivative ratio; 9.0–11.0 μg/spot for densitometric method; and 10.0–14.0 $\mu g\,ml^{-1}$ for HPLC method.
[a] The intra-day precision ($n = 5$).
[b] The inter-day precision ($n = 5$).

Table 7
Statistical comparison for the results obtained by the proposed methods and the compendial method for the analysis of vincamine in bulk powder

	Derivative ratio method	Densitometry	HPLC	Reported method[a]
Mean	99.90	100.09	100.16	99.58
S.D.	1.041	0.761	1.026	1.011
Variance	1.084	0.579	1.054	1.022
N	10	8	10	6
F-test	1.060 (4.77)[b]	1.765 (4.362)[b]	1.031 (4.77)[b]	
Student's *t*-test	0.427 (2.145)[b]	0.746 (2.179)[b]	0.779 (2.145)[b]	

[a] Ref. no. [18].
[b] The figures in parenthesis are the corresponding tabulated values at $P = 0.05$.

the proposed procedures was further assessed by application of the standard addition technique. The small relative standard deviations indicate that the methods are accurate (Table 5).

The precision of the suggested methods was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis results (Table 6).

The results obtained by the proposed methods for determination of vincamine in bulk powder were statistically compared with those obtained by applying the compendial method [18], and it revealed insignificant difference (Table 7). The individual methods were also checked for its robustness by minor changes in assay conditions, the methods proved robust. Changes in instruments or personnel did not alter the results, which indicate the ruggedness of the proposed methods. The obtained assay parameters and a validation sheet are presented in Table 6.

4. Conclusions

The proposed procedures are simple, sensitive, selective and stability indicating. The methods can be used for the routine analysis of vincamine either in bulk powder or in pharmaceutical dosage forms. The proposed methods can be applied in laboratories lacking sophisticated instruments such as GC–MS or LC–MS. The suggested methods can be simply applied to kinetic studies and accelerated stability experiments to predict expiry dates of pharmaceuticals.

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