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A NEW RP-HPLC METHOD FOR THE ESTIMATION OF VINBLASTINE IN PHARMACEUTICAL FORMULATIONS

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Abstract

A new simple, precise and accurate reverse phase HPLC method was developed and validated for the estimation of vinblastine in pharmaceutical formulations using the mobile phase 90% methanol, 5% acetonitrile and 5% of 0.1% orthophosphoric acid and the pH was maintained at 3.5. The resultant chromatogram obtained has a high resolution and low tailing factor (1.29). The linearity curve showed a correlation coefficient (r^2) of 0.999 for a wide range of drug concentration of 3-15 ppm. The method was also validated in respect of precision, accuracy and specificity. The applicability of the method was also tested with commercial sample of Cytoblastin.

Keywords: Vinblastine, Estimation, RP-HPLC, Cytoblastin.

Introduction

Chemically Vinblastine (MF: $C_{46}H_{58}N_4O_9$; MW: 810.974) (Fig.1) is dimethyl(2β,3β,4β,5α,12β,19α)-15-[(5S,9S)-5-ethyl-5-hydroxy-9-(methoxycarbony)] and is soluble in ethanol, chloroform and acetone. Vinblastine is a vinka alkaloid and a chemical analogue of vincristine. Vinka alkaloids are generally used incombination therapy with synthetic molecules[1-3]. The indole alkaloids vinblastine and vincristine, initially extracted from the common Madagascarperiwinkle (*Catharanthus roseus* G.Don, an Apocynaceae) have been in use for 30 years as anticancer agents[4]. Vinblastine might be effective against cancers of the white blood cells such as lymphoma. A survey of literature revealed that there are some HPLC[5,6] methods, LC-MS[7] method, RP-HPLC[8] method and CE[9] methods for the estimation of vinblastine. In the present investigation an attempt was made to develop a new RP- HPLC method for the estimation of VIAL in pharmaceutical dosage forms.

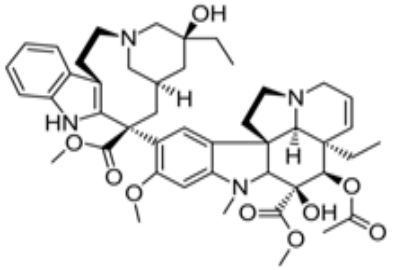


Fig-1: The structure of Vinblastine.

2. Materials and Methods

2.1. Equipment

Analysis was carried out using PEAK 7000 isocratic HPLC with rheodyne manual sample injector with switch (77251) and the column used was analytical column kromosil 100-5 C18 (250x4.6mm). Electronic balance used was ELB 300, DIGISUN pH meter was used for all pH measurements.

2.2. Chemicals and reagents

Vinblastine reference standard was a kind gift of V.V.MED Laboratories, Hyderabad and the tablet formulation (VIAL-10mg) used for testing the method was purchased from local market. The solvents used were methanol and Acetonitrile of HPLC grade and phosphoric acid (GR) of Merck manufactures.

2.3. Optimized Chromatographic Conditions

Chromatographic analysis of the vinblastine was done using a Kromasil C18, (250x4.6mm, 5µm) column. The mobile phase composition used was MeOH (HPLC grade) 90%, Acetonitrile(HPLC grade) 5% and 5% of 0.1% orthophosphoric acid(GR) was filtered through 0.5µ nylon membrane filter before use and the pH was at 3.5. The analysis was carried out in isocratic mode at a flow rate of 1mL/min. The detector wavelength is 268nm and the operating pressure is 30.2MPa at room temperature. The injecting volume is 20µL and the total run time is 6min.

2.4. Preparation of standard solutions

Pure standards of vinblastine were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. About 10mg of vinblastine drug transferred into a 10ml volumetric flask and made up to the mark by using methanol. The flask containing standard stock solution was sonicated for 10 minutes to degas it. The standard solution was then filtered with 0.45µm membrane filter paper. A series of different dilutions (3-15ppm) were prepared using above stock solution with mobile phase (Methanol,acetonitrile and orthophosphoric acid in the ratio 90:5:5 (v/v/v)).

2.5. Sample preparation

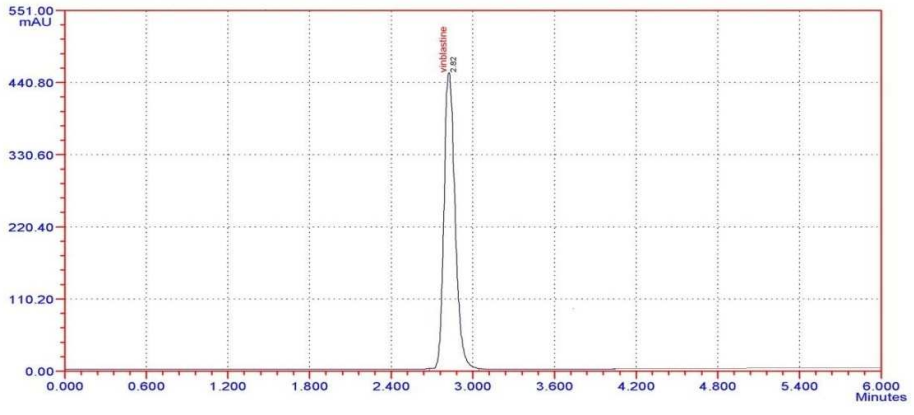
15ppm of sample solution was prepared by accurately weighing the required amount of the drug and transferring it into a 100ml volumetric flask and added mobile phase. The sample solution was then filtered with 0.45µm membrane sample filter.

2.6. Procedure for analysis

With the optimized chromatographic conditions set for vinblastine a study base line was recorded and stabilized for abut 30min. After the stabilization of base line successive aliquots of the sample solution were recorded, until the reproducibility of the peak areas was adequate. The sample was injected into the column at flow rate of 1mL/min.

3. Results and Discussion

A survey of literature revealed that VIAL was estimated by using HPLC[5,6] methods, LC-MS[7] method, RP-HPLC[8,9] method and CE[10] methods. However, in the present investigation, an efficient alternative RP-HPLC method was developed for estimation of VIAL in pharmaceutical formulations. The method was in good agreement in respect of linearity (0.999), accuracy (99.33%), run time (6min) when compared with above mentioned HPLC methods[5-10]. The change in the polarity of the mobile phase (Methanol: Acetonitrile: 0.1% orthophosphoric acid(GR)= 90%:5%:5%) compared to earlier reported method[8](MeOH: Acetonitrile: 0.005M Ammonium acetate: triethylamine: 1:2:2.5:002v/v) and the corresponding pH change may given the good separation of the VIAL at RT 2.82min. The method was developed according to ICH guide lines. The standard chromatogram obtained for the vinblastine was given in Fig.2.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	vinblastine	2.822	48244	339836.3	100.000	1.29	3199
	Sum:		48244	339836.3	100.0000		

Fig 2: standard chromatogram of the vinblastine.

3.1. Method Validation

After the completion of HPLC method development the method was validated in terms of different parameters like specificity, linearity, precision, accuracy, LOD and LOQ.

3.1.1. Evaluation of linearity

The Linearity of the method was evaluated by analyzing different concentrations of the standard solutions. Vinblastine solutions of 3ppm, 6ppm, 9ppm, 12ppm, 15ppm with standard 100% pure vinblastine in the mobile phase was prepared and analyzed. After analysis the area of peaks were recorded and were reported in Table-1. It was found that there were no notable changes in the chromatograms for flow rate variation, column temperature variation and mobile phase variation for a wide range of drug concentration of 3-15ppm. A plot was drawn by taking concentration on x-axis and area of the peaks on y-axis. It was fount that a straight line satisfying linearity condition i.e. the correlation coefficient (r^2=0.999, Fig. 3) of regression was found almost equal to 1.

Table-1: Linearity data of Vinblastine for the developed method.

TEST-2	LINEARITY			
	S.NO	CONC ppm	AREA	INTERCEPT =11044.06
	1	3	123248.6	SLOPE = 37953.37
	2	6	236255.0	C.C = 0.999
	3	9	358181.3	
	4	12	469521.0	
	5	15	575916.2	

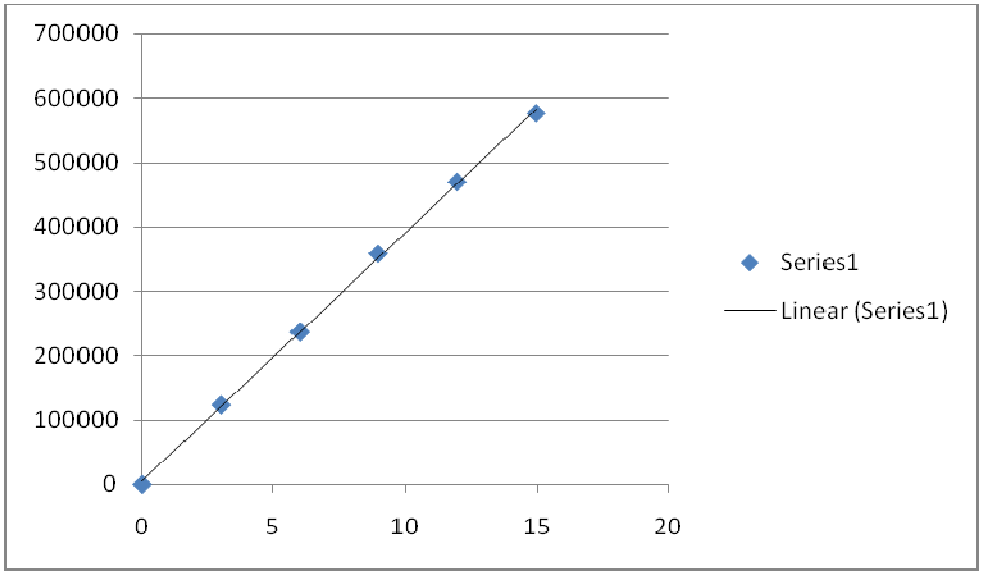


Fig 3: Linearity of Vinblastine.

3.1.2. Accuracy (% Recovery)

To study of the reliability, validity, suitability and accuracy of the method, recovery experiments were carried out for vinblastine. For this, the recovery studies were performed using standard addition method i.e. a known quantity of pure drug was added to the pre-analysed sample formulation. The results of the experiments were given in Table 2. The % recovery of the drug is calculated by using the formula given below. The recovery of the vinblastine is 97.92%.

Table-2: Recovery from linearity curve.

S.NO	CONC	AREA	Th.PLATES
1 TARGET	6ppm	219499.1	5202.97
2 ADDED	3ppm	128266.0	8583.53
3 TOTAL	9ppm	345103.9	3058.86

RECOVERY = 97.92%

% recovery = [(b-a)/c] X100

Where a- The amount of drug found before the addition of standard drug.

b- The amount of drug found after the addition of standard drug.

c- The amount of standard drug added.

The values obtained (Table-2) above are more reliable and in good agreement in terms reliability, suitability and accuracy when compared to earlier methods[5-10]. The % of vinblastine found in cytoblastin by using the developed method was 4.12%.

3.1.3. Precision

A standard solution (6ppm) of drug substance was injected six times and corresponding peak areas were recorded. The % RSD found were less than 1%. The value of the %RSD obtained in intraday precision is0.926 (Table 3&4) and the inter day precision is 0.608. The values of %RSD within a day, day to day variation (<1%) proves that the method is precise.

Table-3: Intraday precision.

TEST.1	CONC 6ppm			PRECISSION
Intraday	INJECTION	AREA	TH.P	%R.S.D = 0.926
	1	227491.7	4903.63	
	2	224066.6	5027.48	
	3	222747.4	5087.73	
	4	221220.0	5135.29	
	5	224222.0	5021.28	
	6	223878.2	5035.95	

Table-4: Inter-day precision.

TEST.1	CONC 6ppm			PRECISSION
Inter-day	INJECTION	AREA	TH.P	%R.S.D = 0.608
	1	225898.6	4984.32	
	2	221815.0	5118.68	
	3	223321.3	5067.23	
	4	224639.5	5034.26	
	5	223734.7	5042.41	
	6	224084.6	5027.19	

3.1.4. Specificity of the method

The specificity of the method was determined by observing any interference encountered from the ingredients present in the formulations. The test results obtained were compared with that of test results those obtained for standard drug. In the present study, it was shown that those ingredients are not interfering with the proposed method.

3.1.5. Ruggedness

Inter-day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on two different days over a period of one week.

3.1.6. Robustness and system suitability

Robustness of the method was carried out by varying two parameters slightly from the Optimized chromatographic conditions, such as flow rate, column temperature and mobile phase. It was found that there were no notable

changes in the chromatograms for flow rate variation, column temperature variation and mobile phase variation. The robustness limit for the above parameter variations was well within the acceptable limit and is less than 2%. This shows that the method is having good system suitability under the given set of conditions.

3.1.7. Limit of Detection and limit of Quantification (LOD and LOQ)

The limit of Detection (LOD) and limit of Quantification (LOQ) (Table 5) of the developed method were determined by injecting increasingly low concentrations of the standard solutions by following the developed HPLC method. The LOD is the smallest concentration of the analyte which gives a measurable response. The LOD for vinblastine was found to be 0.05ppm. The LOQ is the smallest concentration of the analyte, which gives response that can be absolutely quantified. The LOQ for vinblatine was found to be 0.165ppm. The results of LOD and LOQ supported the sensitivity of the developed method.

Table-5: LOD and LOQ.

Limit of detection (L. O. D.)	0.05ppm
Limit of quantification (L. O. Q)	0.165ppm

Conclusion

A more accurate, precise and convenient RP-HPLC method was developed and validated interms of linearity, accuracy, precision,etc. The proposed method is also applicable to the analysis of vinblastine in pharmaceutical formulations.

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