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Research Article

# DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ESTIMATION OF VINORELBINE IN PHARMACEUTICAL DOSAGE FORMS

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**ABSTRACT**

A simple, precise, accurate RP-HPLC method was developed and validated for the estimation of Vinorelbine in pharmaceutical dosage forms. An Inertsil ODS C18 column (150 mm x 4.6 mm), 5 µ particle size was used as stationary phase with mobile phase consisting of phosphate buffer and methanol in the ratio of 40:60 V/V. The flow rate was maintained at 1 mL/min and effluents were monitored at 269 nm. The retention time was 3.203 min. The linearity of the method was observed in the concentration range of 10-50 µg/mL with correlation coefficient of 0.999. The percentage assay of Vinorelbine was 100.13%. The method was validated for its accuracy, precision and system suitability. The results obtained in the study were within the limits of ICH guidelines and hence this method can be used for the estimation of Vinorelbine in pharmaceutical dosage forms.

**Keywords**: Vinorelbine, RP-HPLC, Phosphate buffer and Validation.

## INTRODUCTION

Vinorelbine tartrate (Fig. 1) is chemically 3',4'-didehydro-4'-deoxy-C'-norvincaleuko blastine[R-(R*,R*)-2,3-dihydroxybutanedioate(1:2)(salt)]. Vinorelbine is an anti-mitotic chemotherapy drug indicated for some types of cancer, including breast cancer and non-small cell lung cancer[1]. Vinorelbine is a vinca alkaloid that interferes with microtubule assembly. The antitumor activity of Vinorelbine is thought to be due primarily to inhibition of mitosis at metaphase through its interaction with tubulin[2].

Literature survey revealed that few analytical methods such as spectrophotometric[3], TLC[4], HPLC[5-7], capillary electrophoresis[8] and LC-MS[9-13] methods have been reported for the estimation of Vinorelbine. Hence a new sensitive and efficient HPLC method was developed and validated as per ICH guidelines[14] for the estimation of Vinorelbine in pharmaceutical formulations.

## MATERIALS AND METHODS

### Instrumentation

To develop a high pressure liquid chromatographic method for quantitative estimation of Vinorelbine using Waters HPLC system on Inertsil ODS C18 column (150 mm x 4.6 mm, 5µ) was used. The instrument is equipped with an auto sampler and DAD or UV detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower2 software.

### Chemicals and solvents

The working standard of Vinorelbine was provided as gift sample from Pharma Train, Hyderabad, India. The market formulation NAVELBINE capsules (Vinorelbine 20 mg) were procured from local market. HPLC grade water and methanol were purchased from E.Merck (India) Ltd, Mumbai, India. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade were obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

**Chromatographic conditions**
A mixture of phosphate buffer and methanol in the ratio of 40:60 V/V was found to be the most suitable mobile phase for ideal chromatographic separation of Vinorelbine. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 269 nm. The run time was set at 6 min.

**Preparation of phosphate buffer pH 2.5**
2.72 grams of potassium dihydrogen phosphate was weighed and transferred into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water. pH was adjusted to 2.5 with orthophosporic acid.

**Preparation of mobile phase and diluent**
400 mL of the phosphate buffer was mixed with 600 mL of methanol. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

**Preparation of standard solution**
10 mg of Vinorelbine was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 0.3 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentrations.

**Preparation of sample solution**
Twenty commercial capsule contents were weighed to obtain the average capsule content weight and the contents were mixed. A sample of the mixed capsule content of the powder equivalent to 10 mg of Vinorelbine was accurately weighed, transferred to 10 mL volumetric flask and is dissolved in 7 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume is made up to 10 mL with diluent to get a concentration of 1 mg/mL stock solution. Further pipetted 0.3 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent to obtain required concentrations of Vinorelbine in pharmaceutical dosage form.

**Linearity**
Several aliquots of standard solution of Vinorelbine was taken in different 10 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations of Vinorelbine were in the range of 10 to 50 µg/mL. Evaluation of the drug was performed with UV detector at 269 nm, peak area was recorded for all the peaks. The correlation coefficient value of Vinorelbine was 0.999. The results show that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated.

**Limit of detection and limit of quantification**
The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution using the developed HPLC method. The LOD for Vinorelbine was found to be 0.06 µg/mL. The LOQ for Vinorelbine was found to be 0.19 µg/mL.

**System suitability**
System suitability parameters like retention time, resolution, theoretical plates and tailing factor were calculated and compared with standard values.

**Accuracy**
The accuracy of the method was assessed by recovery study of Vinorelbine in the dosage form at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels. Each level was repeated three times. The content of Vinorelbine per capsule was calculated. The mean recovery of Vinorelbine was in the range of 100.77% that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

**Precision**
The precision was determined for Vinorelbine in terms of intra-day and inter-day precision. For intra-day precision evaluation, a standard solution of fixed concentration was injected at various time intervals and %RSD for Vinorelbine was 0.84% (limit %RSD < 2.0%). In addition, the inter-day precision was studied by injecting the same concentration of standard solution on consecutive days and the

%RSD for Vinorelbine was 0.70% (limit %RSD < 2.0%).

**Ruggedness and robustness**

The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the HPLC method so developed is rugged and robust.

**Assay**

20 µL of each standard and sample solution were injected and from the peak area of Vinorelbine, amount of each drug in samples were computed. The result of assay undertaken yielded 100.13% of label claim of Vinorelbine.

**RESULTS AND DISCUSSION**

The HPLC procedure was optimized with a view to develop an accurate method in capsule dosage form using Inertsil ODS C18 column (150 x 4.6 mm, 5 µ) in isocratic mode with mobile phase composition of phosphate buffer: methanol (40:60 V/V) and pH adjusted to 2.5 with orthophosphoric acid. The use of phosphate buffer and methanol in the ratio of 40:60 V/V resulted in peak with good shape and resolution. The flow rate was 1.0 mL/min and the drug component was measured with UV detector at 269 nm. The results of optimized HPLC conditions were shown in Table 1. The method was linear in the range of 10 to 50 µg/mL for Vinorelbine with correlation coefficient of 0.999. The linearity results were shown in Table 2 and the linearity curve was shown in Fig. 2. The % recovery was found to be 100.77% for Vinorelbine, which indicate the method is accurate. The results of recovery studies were shown in Table 3. The %RSD for intra-day precision and inter-day precision for Vinorelbine were found to be 0.84 and 0.70, which indicate the method is precise. The results of precision studies were shown in Table 4.

The retention time of Vinorelbine was 3.203 min. The number of theoretical plates was 3586 and tailing factor was 0.98 for Vinorelbine, which indicates efficient performance of the column. The limit of detection and limit of quantification for Vinorelbine were found to be 0.06 µg/mL and 0.19 µg/mL, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 5. Validated method was applied for the determination of Vinorelbine in commercial formulations. The %assay was found to be 100.13% for Vinorelbine and the assay results were shown in Table 6.

Typical chromatogram of drug Vinorelbine was shown in Fig. 3. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in the formulation did not interfere with the estimation of the drug by the proposed HPLC method.

**CONCLUSION**

A simple, precise, selective and sensitive RP-HPLC method with UV detection for Vinorelbine was developed and validated. This method will be useful for the easy and quick estimation of Vinorelbine with almost no interferences in bulk and pharmaceutical dosage forms.

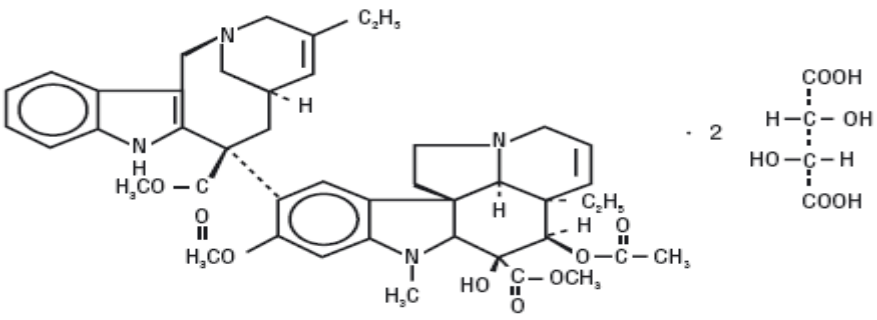

**Fig. 1: Chemical structure of Vinorelbine tartrate**

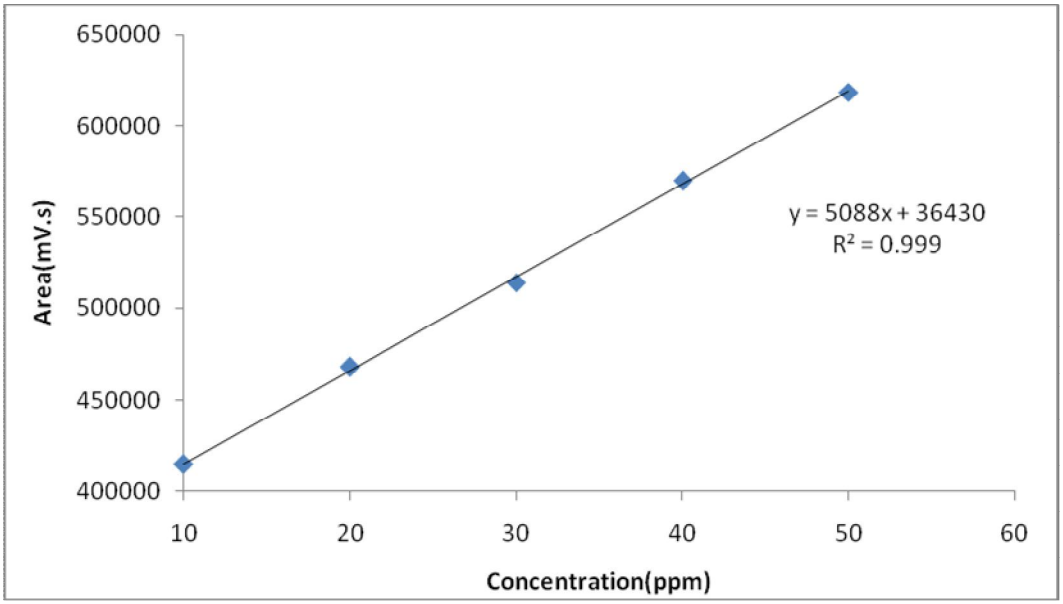


**Fig. 2: Linearity curve of Vinorelbine**

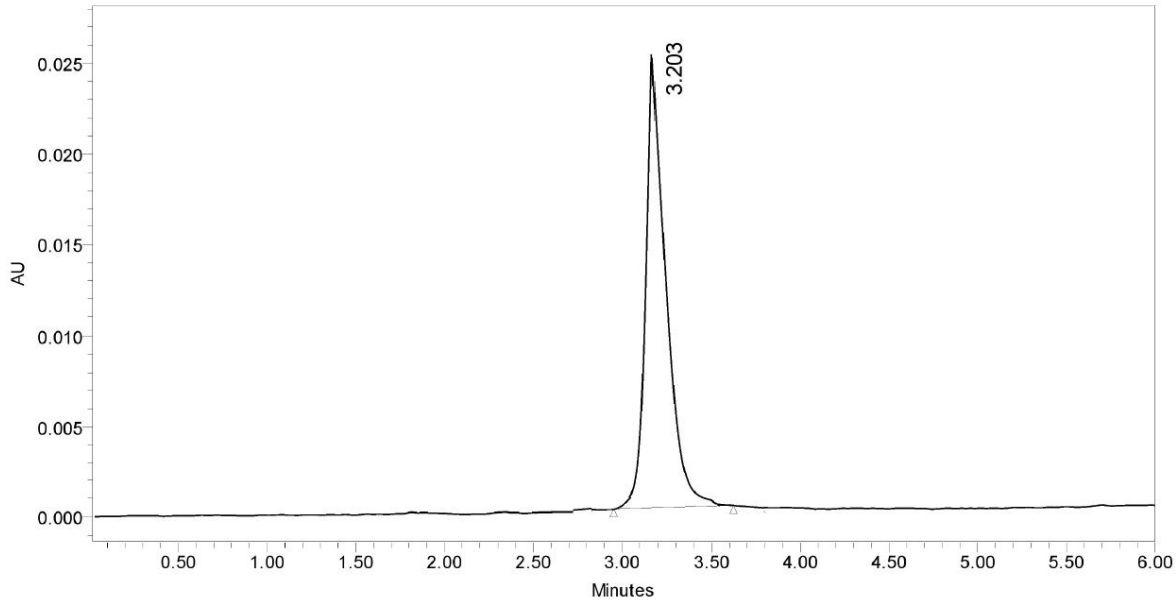


**Fig. 3: Typical chromatogram of Vinorelbine**

**Table 1: Optimized chromatographic conditions of Vinorelbine**

| Parameter | Condition |
|---|---|
| Mobile phase | Phosphate buffer:methanol (40:60, V/V) |
| pH | 2.5 |
| Diluent | Mobile phase |
| Column | Inertsil ODS C18 column (150 mm x4.6 mm, 5µ) |
| Column temperature | Ambient |
| Wave length | 269 nm |
| Injection volume | 20 µL |
| Flow rate | 1.0 mL/min |
| Run time | 6 min |
| Retention time | 3.203 min |

**Table 2: Linearity results of Vinorelbine**

| Concentration in µg/mL | Area |
|---|---|
| 10 | 414634 |
| 20 | 468186 |
| 30 | 513940 |
| 40 | 569651 |
| 50 | 618300 |

**Table 3: Recovery results of Vinorelbine**

| Level | Amount added | Amount found | % Recovery | Mean recovery |
|---|---|---|---|---|
| 50% | 5.0 | 4.97 | 99.48% | 100.77% |
| 100% | 10.0 | 10.14 | 101.44% | |
| 150% | 15.0 | 15.21 | 101.39% | |

**Table 4: Precision studies of Vinorelbine**

| Concentration (µg/mL) | Intra-day precision (%RSD) | Inter-day precision (%RSD) |
|---|---|---|
| 30 | 0.83 | 0.70 |

**Table 5: Summary of system suitability and validation parameters of Vinorelbine**

| Parameter | Results |
|---|---|
| Linearity range (µg/mL) | 10-50 |
| Correlation coefficient | 0.999 |
| Theoretical plates (N) | 3586 |
| Tailing factor | 0.98 |
| LOD (µg/mL) | 0.06 |
| LOQ (µg/mL) | 0.19 |

**Table 6: Assay results of Vinorelbine**

| Formulation | Label claim | Amount found | %Assay |
|---|---|---|---|
| NAVELBINE | 20 mg | 20.02 mg | 100.13% |

## REFERENCES


1. Marty M, Fumoleau P, Adenis A, Rousseau Y, Merrouche Y, Robinet G, Senac I and Puozzo C. Oral Vinorelbine pharmacokinetics and absolute bioavailability study in patients with solid tumors. Ann Oncol. 2001;12(11):1643-1649.
2. Casanova M, Ferrari A, Spreafico F, Terenziani M, Massimino M, Luksch R, Cefalo G and Polastri D. Vinorelbine in previously treated advanced childhood sarcomas: evidence of activity in rhabdomyosarcoma. Cancer. 2002; 94(12):3263-3268.
3. Saini PK, Singh RM, Jain CL, Mathur SC and Singh GN. Quantification of Vinorelbine in bulk and injection dosage form by spectrophotometric method. Pharmbit. 2010;22(2):105-109.
4. Chunfang Z, Yin X, Longjiang Y, Shuo L and Zeqiang W. TLC and HPLC methods to follow the synthesis of Vinorelbine. J Chromatogr Sci. 2010; 48(8):685-689.
5. Van Belle SJ, De Smet M, Monsaert C, Geerts F, Storme GA and Massart DL. High-performance liquid chromatographic determination of Navelbine in MO4 mouse fibrosarcoma cells and biological fluids. J Chromatogr. 1992;576(2): 351-357.
6. Puozzo C, Ung HL and Zorza G. A high performance liquid chromatography method for Vinorelbine and 4-O-deacetyl Vinorelbine: a decade of routine analysis in human blood. J Pharm Biomed Anal. 2007;44(1):144-149.
7. Sidorova AA, Yaroshenko DV, Murashko EA and Grigorev AV. Development of chromatographic and electrophoretic methods for determining Vinblastine in blood plasma and prostate gland tissue. J Anal Chem. 2013;68(3):265-271.

8. Qing-Wei Z, Di W, Qin M, Hai-Bo T, Zheng-Ren W, Ye K, Jian-Yuan Y and Jia-Jun C. Rapid and sensitive detection of Vinorelbine in the urine of tumor patients by capillary electrophoresis with tris(2,2′-bipyridyl)ruthenium(II)-based electrochemiluminescence assay. Anal Sci. 2013;29(7):757-760.
9. Ramirez J, Ogan K and Ratain MJ. Determination of Vinka alkaloids in human plasma by liquid chromatography/ atmospheric pressure chemical ionization mass spectrometry. Cancer Chemother Pharmacol. 1997;39(4):286-290.
10. Ragot S, Sauvage FL, Rousseau A, Genet D, Dupuy JL, Tubiana-Mathieu N and Marquet P. Sensitive determination of Vinorelbine and its metabolites in human serum using liquid chromatography-electrospray mass spectrometry. J Chromatogr B. 2001;753(2):167-178.
11. Van Heugen JC, De Graeve J, Zorza G and Puozzo C. New sensitive liquid chromatography method coupled with tandem mass spectrometric detection for the clinical analysis of Vinorelbine and its metabolites in blood, plasma, urine and faeces. J Chromatogr A. 2001;926(1): 11-20.
12. Cheng T, Si D and Liu C. Rapid and sensitive LC-MS method for pharmacokinetic study of Vinorelbine in rats. Biomed Chromatogr. 2009; 23(9):909-911.
13. Qian J, Wang Y, Chang J, Zhang J, Wang J and Hu X. Rapid and sensitive determination of Vinorelbine in human plasma by liquid chromatography-tandem mass spectrometry and its pharmacokinetic application. J Chromatogr B. 2011;879(9-10):662-668.
14. ICH Harmonised Tripartite Guideline, Q2(R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, Geneva. 2005;1-13.