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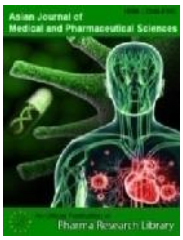

Research Article **Open Access**

Analytical Method Development and Validation For in Vinblastine and Vincristine Combine Dosage Forms by RP–HPLC Method

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Vinblastine and Vincristine by using XterraC185µm (4.6*250mm) column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module2695, PDA Detector 996, Empower-softwareversion-2. The retention times were found to be 2.399mins and 3.907mins. The %purity of Vinblastine and Vincristine wasfoundtobe100.7%and 101.4% respectively. The system suitability parameters for Vinblastine and Vincristine such as theoretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2(R1)). The linearity study for Vinblastine and Vincristine was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient(r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2and0.4,%RSDfor intermediate precision was 0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95and 3.04, and LOQ value was 9.87 and 10 respectively.

Keywords: Vinblastine and Vincristine, Empower-software, ICH etc.

A R T I C L E I N F O

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1. Introduction

Vinblastine is a chemotherapy medication, typically used with other medications, to treat a number of types of cancer. This includes Hodgkin's lymphoma, non-small cell lung cancer, bladder cancer, brain cancer, melanoma, and testicular cancer. It is given by injection into a vein.Most people experience some side effects.Commonly it causes a change in sensation, constipation, weakness, loss of appetite, and headaches. Severe side effects include low blood cell counts and shortness of breath.It should not be given to people who have a current bacterial infection.Use during pregnancy will likely harm the baby. Vinblastine works by blocking cell divisionVincristine, also known as leurocristine and marketed under the brandname Oncovin among others, is a chemotherapy medication used to treat a number of types of cancer.This includes acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin's disease, neuroblastoma, and small cell lung cancer among others. It is given intravenously. Most people experience some side effects from vincristine treatment.Commonly it causes a change in sensation, hair loss, constipation, difficulty walking, and headaches.Serious side effects may include neuropathic pain, lung damage, or low blood white cells.It will likely cause harm to the baby if given during pregnancy. It works by stopping cells from dividing properly.

2. Materials and Methods

Instrument: HPLC-auto sampler –UV detector, Separation module2695, UV.detector2487, Empower-software version-2, Waters, U.V double beam spectrometer, UV 3000+, U.V win software, Lab India. Digital weighing balance (sensitivity 5mg), pH meter, Sonicator.

Chemicals: Vinblastine and Vincristine Ortho phosphoric acid, Acetonitrile, Methanol, Water, KH_2PO_4, K_2HPO_4.

Chromatographic conditions:

Table 1: Optimized chromatographic conditions

Column	InertsilC_{18} 5µm (4.6*250mm)
Mobile phase	Phosphatebuffer(0.05M) pH4.6:CAN (30:70%v/v)
Detection wavelength	255 nm
Flow rate	1 ml/min
Injection volume	20µl
Column temperature	Ambient

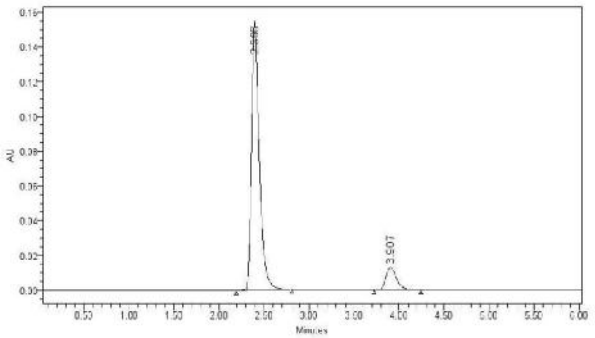

Figure 1: Optimized chromatogram for vincristine and vinblastine

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits.

Standard and sample Solution Preparation:

The required concentrations according to the optimized method were prepares and evaluated.

3. Results and Discussion

Assay Calculations for Vinblastine and Vincristine: The assay study was performed for the Vinblastine and Vincristine. Each three injections of sample and standard was inject into chromatographic system. The chromatograms are shown in Fig. 2 and results are tabulated in Tables 1.

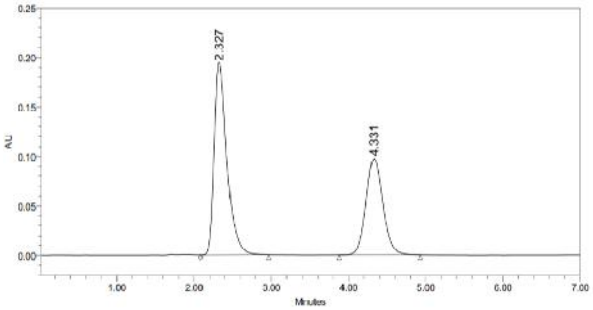

Figure 2: The chromatogram showing assay results

Observation: The system suitability parameters for vinblastine and Vincristine such as theoretical plates and tailing factor were found to be 5117.5, 1.3 and 3877.3, 1.4. Resolution was 8.1. The % purity of Vinblastine and Vincristine in pharmaceutical dosage form was found to be 100.7% and 101.4% respectively.

Validationresults:

Accuracy: The accuracy study was performed for 50%, 100% and 150 % for vinblastine and Vincristine. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. Chromatograms are shown in Figs.4.18-4.26 and results are tabulated in Tables 3,4.

Precision:

Repeatability: The precision study was performed for five injections of vinblastine and Vincristine. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of %RSD. The results are tabulated in Table 5, 6.

Intermediate precision/Ruggedness:

The intermediate precision study was performed for five injections of vinblastine and Vincristine. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD. The chromatograms are shown in Fig.4.32-4.36 and results are tabulated inTable4 7,8.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. The chromatograms are shown in Fig. 3 and 4.

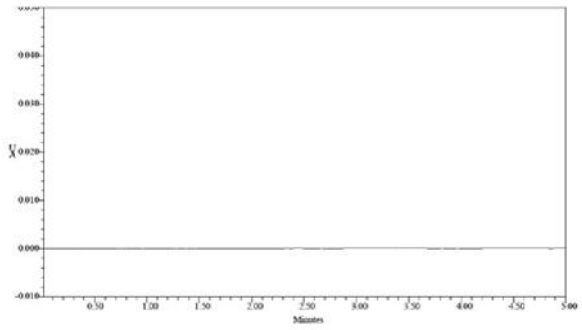

Figure 3: Chromatogram of blank

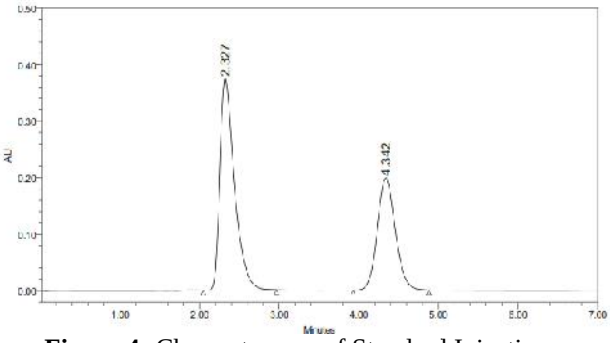

Figure 4: Chromatogram of Standard Injection

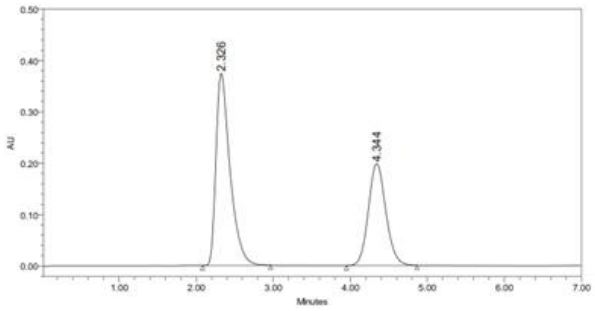

Figure 5: Chromatogram of Sample Injection

The specificity test was performed for vinblastine and Vincristine. It was found that there was no interference of impurities in retention time of analytical peak.

Detection of limit: LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve(S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

Formula:

$$LOD = 3.3 \times \frac{\sigma}{S}$$

Where
σ-Standarddeviation(SD)
S–Slope

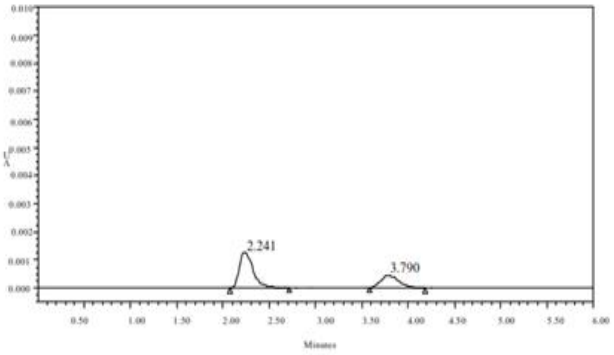

Figure 6: Results of LOD

Quantification Limit:

Vincristine

Calculation of S/N Ratio: Average Base line Noise obtained from Blank: 41µV
Signal Obtained from LOQ solution: 412µV
S/N= 412/41= 10.0

Acceptance Criteria:

S/N Ratio value shall be 10 for LOQ solution.

Vinblastine

Calculation of S/N Ratio:
Average Base line Noise obtained from Blank: 41µV
Signal Obtained from LOQ solution: 405µV
S/N= 405/41= 9.87

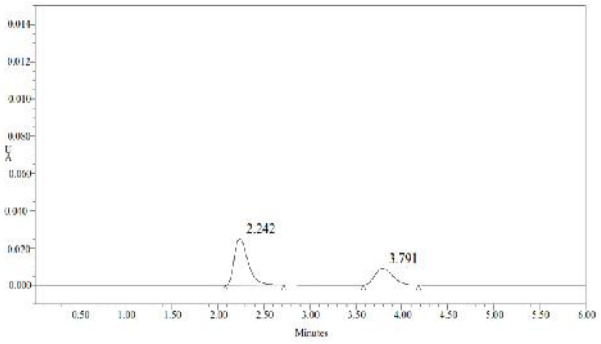

Figure 7: Results of LOQ

Table 2: Accuracy results of Vincristine

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	100.5%
100%	3132697	10	9.99	99.9%	
150%	3918997	15	14.9	99.1%	

Table 3: Accuracy results of vinblastine

%Concentration (at specification level)	Area	Amount Added(mg)	Amount Found (mg)	%Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	100.0%
100%	4735088	10	9.94	99.4%	
150%	5911798	15	14.8	99.2%	

Table 4: Repeatability results of vinblastine &Vincristine

Injection	Area	
	Vinblastine	Vincristine
Injection-1	1501417	2235319
Injection-2	1486940	2240678
Injection-3	1490656	2249490
Injection-4	1487329	2245822
Injection-5	1490384	2251694
Average	1491345	2244601
Standard Deviation	5881.4	6656.8
%RSD	0.39	0.32

Table 5: Ruggedness results of Vincristine &vinblastine

Injection	Area	
	Vinblastine	Vincristine
Injection-1	2194758	1456296
Injection-2	2195700	1457422
Injection-3	2196191	1456513
Injection-4	2195326	1454579
Injection-5	2200951	1451483
Average	2196585	1455259
Standard Deviation	2496.0	2347.6
%RSD	0.11	0.16

Table 6: System suitability results For Vincristine (Flowrate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 7: System suitability results for vinblastine (Flow rate)

S.No	FlowRate(ml/min)	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

Table 8: System suitability results for Vincristine (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10%More	1948.0	1.2

Table 9: System suitability results for Vinblastine (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10%Less	883.3	1.56
2	Actual	1234.0	1.1
3	10%More	969.2	1.6

4. Conclusion

A new method was established for simultaneous estimation of vinblastine and Vincristine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of vinblastine and Vincristine by using XterraC185µm(4.6*250mm)column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer(0.05M)pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector996, and Empower-softwareversion-2. The retention times were found to be 2.399 mins and 3.907mins. The % purity of vinblastine and Vincristine was foundtobe100.7%and 101.4% respectively. The system suitability parameters for vinblastine and Vincristine such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH,Q2(R1)).The linearity study for vinblastine and Vincristine was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient(r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of vinblastine and Vincristine in API and Pharmaceutical dosage form.

5. References

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