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Research Article

Analytical method development and validation for the simultaneous estimation of vinblastine and vincristine in combined dosage form by RP–HPLC method

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Abstract
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 Xterra C185µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with ortho phosphoric acid), detection wavelength was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-softwareversion-2. The retension times were found to be 2.399mins and 3.907mins. The %purity of Vinblastine and Vincristine was found to be 100.7% 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 Vinblastineand Vincristine was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient(r2)was found to be 0.999and 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 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLCmethod can be used for routine analysis of Vinblastine and Vincristine in API and Pharmaceutical dosage form.
Keywords: XterraC18, Vinblastine and Vincristine, RP-HPLC

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1. Introduction

Vinblastine is a vinca alkaloid used to treat breast cancer, testicular cancer, neuroblastoma, Hodgkin's and non-Hodgkin's lymphoma, mycosis fungoides, histiocytosis, and Kaposi's sarcoma.Vincristine is an antitumor vinca alkaloid isolated from VincaRosea. It is marketed under several

brand names, many of which have different formulations such as Marqibo (liposomal injection) and Vincasar. Vincristine is indicated for the treatment of acute leukaemia, malignant lymphoma, Hodgkin's disease, acute erythraemia, and acute panmyelosis. Vincristine sulfate is often chosen as part of polychemotherapy because of lack of significant bone–marrow suppression (at recommended doses) and of unique clinical toxicity (neuropathy).

Instrumentation

The instrumentused was HPLC Alliance Waters model No. 2695 separation module.2487 UV detector, Software-Empower. The stationaryphase used was XterraC185µm (4.6*250mm) column. Digital weighingbalance-Model number BSA224SC, Sonicator(Enertech)-SE60US, pHmeter Modelnumber Adwa–AD 1020, UV/VIS spectrophotometer UV3000 Lab India Software-UVWin5

2. Methodology

Vinblastine and Vincristine were gift samples supplied by Cipla labs Water, Methanol, Acetonitrile, Potassium dihydrogen orthophosphate were supplied by Merck.

Method development

Five trials were made by changing the mobile phase ratios and solvents Water: Methanol (40:60%v/v) Water: Methanol (40:60%v/v) Phosphate buffer (0.05m) pH 5.0: Methanol (50:50%v/v) Phosphate buffer (0.05M) pH 4.6: MeOH Phosphate buffer (0.05M) pH4.6: CAN (30:70%v/v). Finally, the mobile phase optimized was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v).

Chromatographic conditions: The chromatographic conditions were successfully developed for these parathion 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 orthophosphoricacid), detection wavelength was 255nm.

3. Results and Discussion

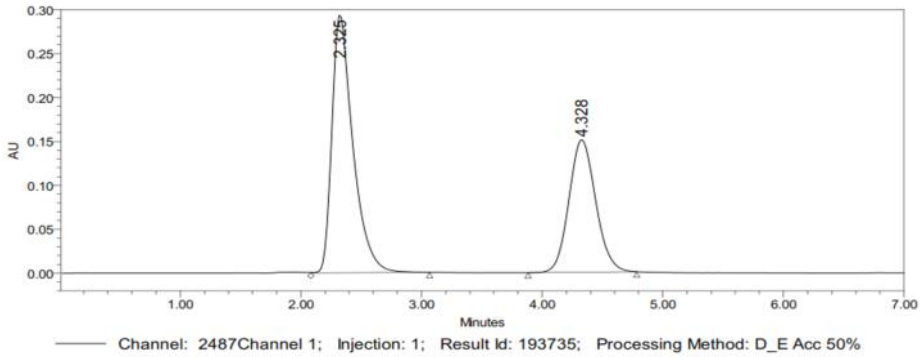

Figure 1: Chromatogram showing accuracy

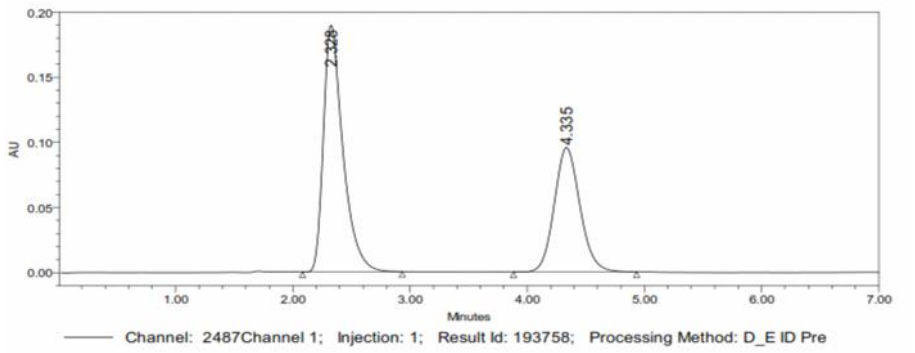

Figure 2: The results are summarized Vincristine

Table 1: Accuracy results of Vincristine

%Concentration (at specificationLevel)	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%	

Acceptance Criteria: The %Recovery for each level should be between 98.0 to 102.0%.

Table 2: Accuracy results of Vinblastine

%Concentration (at specificationlevel)	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 3: Repeatability results of Vinblastine

Injection	Area
Injection-1	1501417
Injection-2	1486940
Injection-3	1490656
Injection-4	1487329
Injection-5	1490384
Average	1491345
Standard Deviation	5881.4
%RSD	0.39

Table 4:Repeatability results of Vincristine

Injection	Area
Injection-1	2235319
Injection-2	2240678
Injection-3	2249490
Injection-4	2245822
Injection-5	2251694
Average	2244601
Standard Deviation	6656.8
%RSD	0.32

Table 5: Ruggedness results of Vincristine

Injection	Area
Injection-1	2194758
Injection-2	2195700
Injection-3	2196191
Injection-4	2195326
Injection-5	2200951
Average	2196585
Standard Deviation	2496.0
%RSD	0.11

Table 6: Ruggedness results of Vinblastine

Injection	Area
Injection-1	1456296
Injection-2	1457422
Injection-3	1456513
Injection-4	1454579
Injection-5	1451483
Average	1455259
Standard Deviation	2347.6
%RSD	0.16

Table 7 Showing results of standard injection

S.No	Peakname	Rt	Area	Height	USP Plate count	USP Tailin	USP Resolution
1	vinblastine	2.23	**791379**	39418	2632	1.8	
2	Vincristine	4.34	**185338**	16275	2614	1.6	5.23

Table 8 Showing results of sample injection

S.No	Peakname	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Vinblastine	2.3	47263	3764	2455	1.60	
2	Vincristine	4.3	31225	19841	2614	1.11	5.52

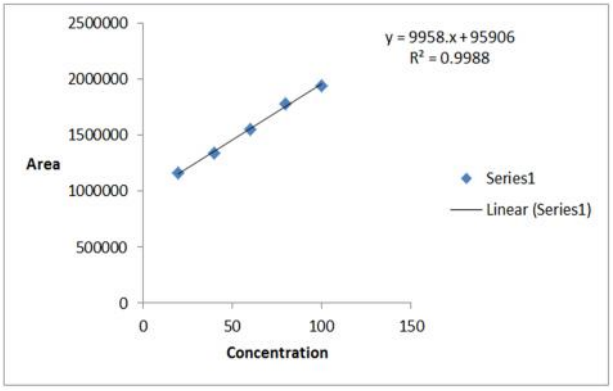

Figure 3:Calibration curve of Vincristine

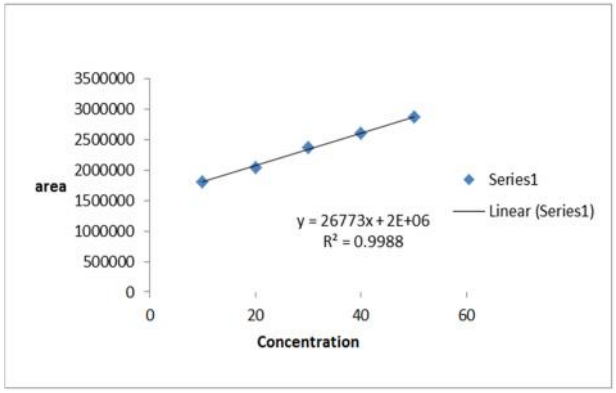

Figure 4: Calibration curve of Vinblastine

Table 9 System suitability results for Vincristine (Mobile phase)

S.No	Change in Organic Composition in the MobilePhase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
1	10%Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10%More	1948.0	1.2

Table 10 System suitability results for Vinblastine (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	Systemsuitabilityresults	
		USPPlatecount	USPTailing
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 byRP-HPLCmethod.The chromatographic conditions were successfully developed for the separation of Vinblastine and Vincristine by using XterraC185µm(4.6*250mm) column, flowrate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M)pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoricacid), detection wavelength was 255nm.The instrument used was WATERS HPLC Auto Sampler ,Separation module 2695, PDA Detector 996, Empower-software version-2. The retention times were found to be 2.399 mins and 3.907mins. The %purity of Vinblastine and Vincristine wasfound to be 100.7% and 101.4% respectively. The system suitability parameters for Vinblastine and Vincristine such as theoretical plates and tailing factor were found to be1.3, 5117.5, 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 0.999and 0.999,%mean recovery was found to be100% and 100.5%, %RSD for repeatability was 0.2 and 0.4,% RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, andLOQ value was 9.87 and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Vinblastine and Vincristine inAPI and Pharmaceutical dosage form.

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