Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

Development and Validation of RP-HPLC Method for Determination of Selinexor in Bulk Drug and Pharmaceutical Dosage Form

Jayesh T. Nimbalkar[1*], Neelesh Chaubey[1] and Gautam P. Vadnere[2]

1, College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences (SSSUTMS), Sehore, Madhya Pradesh, India

2, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Jalgaon, Maharashtra, India

Abstract

The present work aimed to develop a new simple, accurate and reproducible RP-HPLC method for the analysis of Selinexor in bulk and tablets. The HPLC analysis was performed on the Phenomenex Gemini C_{18} (250 mm × 4.60 mm), 5µm particle size in isocratic mode, at 25 ^{0}C temperature using a mobile phase consisting of methanol: water (95:5, *v/v*) at a flow rate of 1.0 mL/min. The detection was carried out at 254 nm. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.933 % for SLN. Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.37-99.98. The % RSD below 2.0 shows the high precision of proposed method. Results of precision studies carried out intraday and interday by using different concentrations of SLN 6, 8 and 10 µg/mL and showed %RSD in range of 0.47-1.36 and 0.64-0.99 respectively. Therefore, the results confirmed the suitability of the method for quantifying Selinexor in their formulations.

Key-words: Selinexor, Validation, Tablets, Bulk, RP-HPLC

***Corresponding Author**

E.mail: jayeshpharma333@gmail.com

Introduction

Method validation is the way to authenticate that the analytical procedure applied for a specific test is appropriate for its intended purpose. Methods need to be validated or revalidated. The International Conference of Harmonization (ICH) of technical requirements for the registration of pharmaceutical for human use has developed and provided a consensus text on validation of analytical procedures.

Selinexor is a first-in-class selective inhibitor of nuclear transport (SINE) compound. Selinexor, in combination with bortezomib and dexamethasone, is currently approved for the treatment of multiple myeloma, a type of cancer formed from antibody-producing plasma cells. [1] This condition is typically treated with high dose bortezomib and dexamethasone chemotherapy followed by an autologous stem-cell transplant. Other chemotherapies for multiple myeloma include lenalidomide and dexamethasone, thalidomide, and may include melphalan if the patient is not eligible for transplant. Selinexor was also granted accelerated approval for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) that have gone through at least 2 lines of systemic therapy. [2] The aim of present works in to develop and validate the drug using RP-HPLC method in bulk and tablet formulation.

Methodology

Selection of chromatographic parameters

Development of RP-HPLC Method for the Determination of Selinexor (SLN) from Bulk and Injection: In this study, a precise, sensitive and robust gradient reversed-phase HPLC (RP-HPLC)

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

method was developed and validated for determination of SLN in API samples. The developed method was validated based on International Conference on Harmonization (ICH) guidelines and it was proved to be accurate, precise and robust. Additionally, the limit of detection (LOD) and limit of quantification (LOQ) were also determined. [3-4]

Table 1: Initial chromatographic conditions

Chromatographic mode	Chromatographic condition
Standard solution	100 µg/mL of SLN in methanol
HPLC System	Shimadzu HPLC system
Pump	LC-10AT VP solvent delivery system
Detector	SPDM-10AVPphoto diode array detector
Data processor	Class-M10 data station
Stationary phase	Phenomenex Gemini C_{18}column(250mm x4.6mm,5µ)
Mobile phase	Methanol : water (95:5,v/v)
Detection wavelength	254 nm
Flow rate	1mL/min
Sample size	10µL
Column temperature	40 ^{0}C

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of SLN in 100 mL methanol and sonicated to complete dissolve, which gives concentration of100 µg/mL of SLN.

Optimization of chromatographic parameters

Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimization of mobile phase strength

The mobile phase was chosen after several trials with methanol and water in various proportions. A mobile phase consisted of methanol: water: (95:5, v/v) was selected toachievesymmetricalpeakandsensitivity.Theeffectsofflowratesintherangesof
0.9 to 1.1 mL/min were examined. A flow rate of 1 mL /min gave good sensitivity, system suitability parameter and reasonable retention time; using reversed phase C_{18}column, the retention times of SLN was observed 17.88 min at 254 nm wavelength. The total time of analysis was less than20 min.

Optimization of detection wavelength

PDA detector was used, as it is reliable and easy to set at the correct wavelength. A fixed concentration of analyte was analyzed at different wavelengths. As per the response of analyte, 254 nm wavelength was selected. A spectrum of SLN was shown in Figure 1.

Linearity studies

From stock solution a liquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mL were taken in 10 mL volumetric flasks and diluted up to the mark with methanol such that the final concentration of SLN in the range 2-14 µg/mL. Volume of 10 µL of each sample was injected with the help of syringe. All

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration.

Application of the proposed method to bulk sample:

Accurately weighed quantity 10 mg (SLN) was transferred to 100 mL volumetric flask. It was dissolved in methanol by sonication and volume was adjusted to mark and sonicated. The solution was further diluted to get concentration 5 µg/mL was subjected to proposed method and amount of SLN was determined. The procedure was repeated for six times.

Application of proposed method to tablet formulation: To determine the content of SLN in conventional tablets (Label claim80 mg Selinexor per tablet) The twenty tablets were weighed, their average weight determined and they were finely powered and powder equivalent 80 mg SLN was transferred into a 100 mL volumetric flask containing 50 mL methanol, sonicated for 30 min and diluted to 100 mL with methanol. The resulting solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted to get final concentration of 5 µg/mL was analysed by proposed method and amount of SLN was determined. The assay procedure was repeated for six times.

Validation proposed of RP-HPLC method for the determination of Selinexor (SLN) from bulk and formulation

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment. [5-7]

Accuracy

It was done by recovery study using standard addition method at 80, 100 and 120% level; known amount of standard SLN was added to pre-analyzed sample (5 µg/mL of SLN) and analyzed by the proposed HPLC method.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Repeatability

It is measured by multiple injections of a homogenous sample of 5 µg/mL of SLN that indicates the performance of the HPLC instrument under chromatographic conditions.

Intra-day and Inter-day precision

Intra-day precision was determined by analyzing, the three different concentrations 6 µg/mL, 8 µg/mL and 10 µg/mL of SLN, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week. This result shows reproducibility of the assay. The % RSD values are presented.

Robustness

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol using 5 µg/mL solution of SLN.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and Sis the slope of the line. LOD and LOQ were found to be 0.36µg and 1.10 µg for SLN, respectively.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

matrix.

The method is quite selective. There was no other interfering peak around the retention time of SLN; also the base line did not show any significant noise.

Ruggedness

From the stock solution, sample solution of SLN (5 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Results and Discussion

Development of RP-HPLC Method for Determination of Selinexor (SLN) from Bulk and Tablets

The HPLC analysis was performed on the Phenomenex Gemini C_{18} (250 mm × 4.60 mm), 5µm particle size in isocratic mode, at 25 ^{0}C temperature using a mobile phase consisting of methanol: water (95:5, *v/v*) at a flow rate of 1.0 mL/min. Table 1 represents the different concentration of mobile phase along with retention time in minutes of SLN. The detection was carried out at 254 nm. The spectra of SLN is depicted in Figure 1. Table 2 represents the final chromatographic conditions employed for the detection of SLN in bulk and in formulation. Linearity was observed in the concentration range from 2-14 µg/mL (r^2 = 0.9993) as shown in the Table 3. Figure 2 depicted the Linearity of SLN with Correlation Coefficient = 0.9993, Slope = 12494, Intercept = 1234.37. The average retention time for SLN was found to be 17.88 min as shown in Figure 3. The limit of detection and quantitation of SLN was 0.36µg and 1.10 µg, respectively. The method has been successively applied for the determination of SLN in bulk (Table 4). The method has been successively applied for the determination of SLN in tablets. There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.933 % for SLN (Table 5). Figure 4 represents the Chromatogram of SLN Tablet solution (5 µg/mL) can be interpreted that the retention time does not affected by the excipients of the formulation.

Table 2: Optimization of mobile phase strength for SLN

Sr. No.	Mobile Phase Strength [Methanol: Water v/v]	Flow rate [mL/min]	R_T of SLN [min]
1	90:10	1	20.10
2	95:5	1	17.88
3	100	1	15.82

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

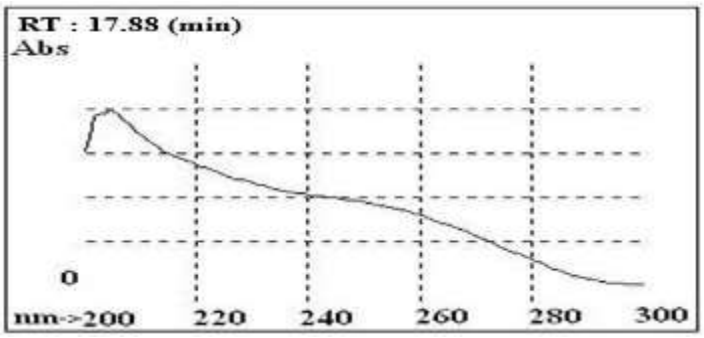

Figure 1: Spectra of SLN

Table 3: Final chromatographic conditions for SLN

Chromatographic mode	Chromatographic condition
Standard solution	100 µg/mL of SLN in methanol
HPLC System	Shimadzu HPLC system
Pump	LC-10 AT VP solvent delivery system
Detector	SPD M-10AVP photo diode array detector
Data processor	Class-M 10 data station
Stationary phase	Phenomenex Gemini C_{18} column (250 mm x 4.6mm,5 µ)
Mobile phase	Methanol: water (95:5, v/v)
Detection wavelength	254 nm
Flow rate	1 mL/min
Sample size	20 µL
Column temperature	25 ^{0}C

Table 4: Linearity study of SLN

Sr. No.	Concentration of SLN [µg/mL]	Mean peak area [n=5]	%RSD
1	2	138205.1	1. 36
2	4	244333.5	1. 26
3	6	372118.6	1.56
4	8	498641.2	1. 84
5	10	618460.4	1.61
6	12	750382.3	1.52
7	14	884836.8	1.23

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

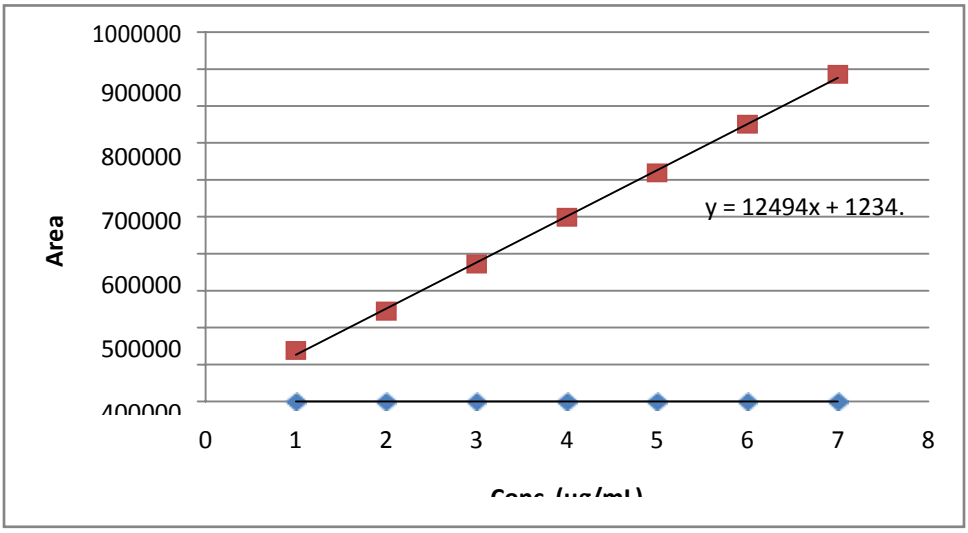

Figure 2: Linearity of SLN
Correlation Coefficient = 0.9993, Slope = 12494, Intercept = 1234.37

Table 5: Analysis of SLN in bulk sample

Component	Amount taken [µg/mL]	Amount Found [µg/mL]	Amount found [%]
SLN	5	5.01	100.2
	5	4.98	99.6
	5	5.03	100.6
	5	4.97	99.4
	5	4.98	99.6
	5	5.01	100.2
	Mean ± SD	4.997 ± 0.021	99.933 ± 0.427
	% RSD	0.468	0.468

Table 6: Assay of SLN tablet

Brand name: XPONIO (Karyopharm Therapeutics Inc.**)**
Batch no. LG1264 **Average wt = 249.6 mg**

Drugs	Label claim [mg]	Amount found [mg]	Amount found [%]
SLN	80	79.52	99.41
	80	79.60	99.51
	80	80.56	100.68
	80	80.32	100.40

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

	80	79.32	99.13
	80	78.92	98.63
	Mean □ SD	79.72 □ 0.15	99.63 □ 0.77
	%RSD	0.78	0.78

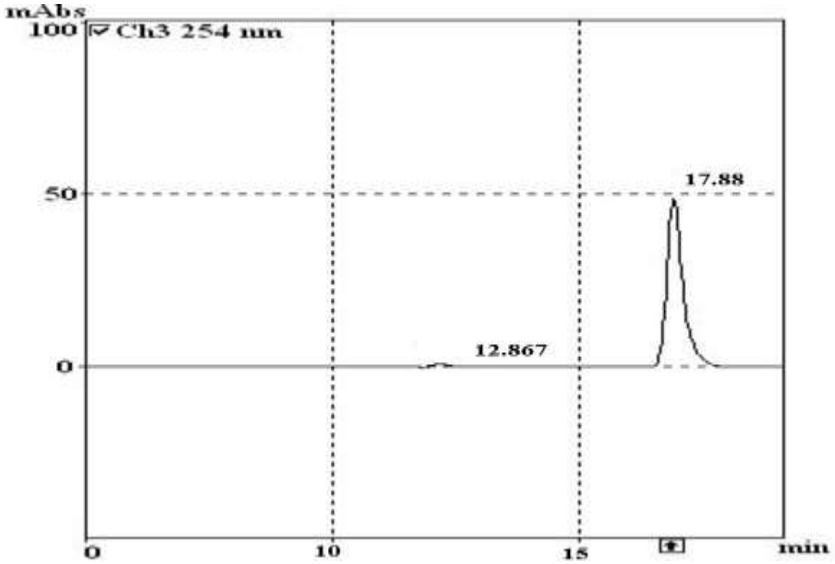

Figure 3: Chromatogram of standard SLN (5 µg/mL)

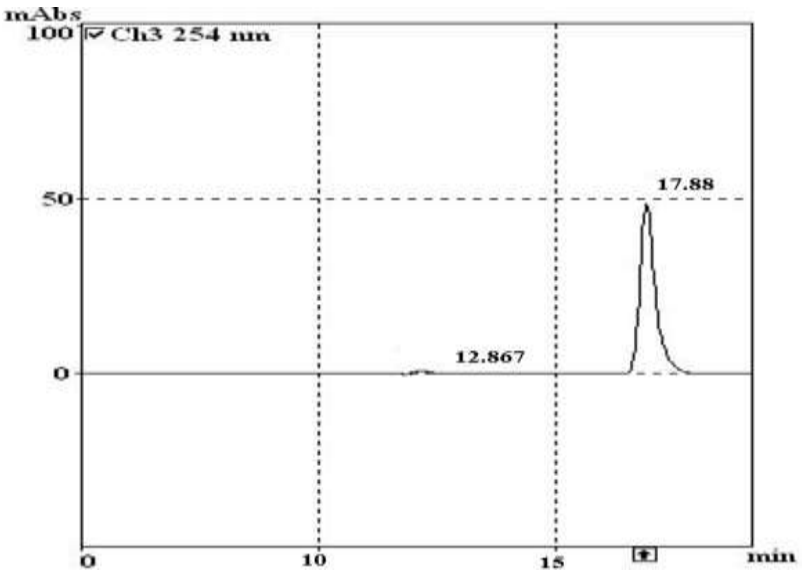

Figure 4: Chromatogram of SLN Tablet solution (5 µg/mL)

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

Validation of Proposed RP-HPLC Method for Determination of Selinexor (SLN) from Bulk and Tablets

Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 % level. The results of recovery studies are presented in Table 6. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.37-99.98. The % RSD below 2.0 shows the high precision of proposed method. According to USP (621), system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. Results of precision studies carried out intraday and interday by using different concentrations of SLN 6, 8 and 10 □g/mL and showed %RSD in range of 0.47-1.36 and 0.64-0.99 respectively; the detailed results are summarized in table 8. Robustness evaluation of the HPLC method was determined by different chromatographic conditions i.e. varying in flow rate and change in concentration of mobile phase. The study was performed in triplicate.

Table 7: Results of recovery studies

Drug	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered µ SD [µg/mL, n = 3]	% Recovery	% RSD
SLN	5	0	4.99 ± 0.03	99.78	0.51
	5	4	3.96 ± 0.03	99.98	0.86
	5	5	4.98 ± 0.08	99.50	1.61
	5	6	5.96 ± 0.07	99.37	1.10

Table 8: Results of repeatability

Sr. No.	Concentration [µg/mL]	Peak area
1	5	309372
2	5	302872
3	5	302639
4	5	309177
5	5	310687
6	5	625298
Mean ± SD		360007.5 ± 118684
% RSD		36.11

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

Table 9: Results of precision studies (Intra-day and inter-day)

Drug	Conc. [µg/mL]	Intra-day Amount found [µg/mL]		Inter day Amount found [µg/mL]	
		Mean ± SD	% RSD[n=3]	Mean ± SD	% RSD[n=3]
SLN	6	5.93 ± 0.03	0.54	5.95 ± 0.05	0.85
	8	7.83 ±0.10	1.36	7.91 ± 0.08	0.99
	10	9.99 ± 0.04	0.47	10.09 ± 0.06	0.64

Table 10: Robustness evaluation of the HPLC method

Chromatographic conditions	R_T	K'	T
A: Flow rate (mL/min)			
0.90	5.37	0.68	1.32
1.00	4.88	0.76	1.33
1.10	4.31	0.68	1.33
Mean ± SD	4.85 ± 0.53	0.71 ± 0.05	1.33 ± 0.01
B: Percentage methanol inmobile phase (v/v)			
94	5.19	0.81	1.41
95	4.88	0.76	1.33
96	4.64	0.67	1.38
Mean ± SD	4.91 ± 0.27	0.75 ± 0.07	1.37 ± 0.04

Table 5.11: Results of ruggedness

Analyst	Amount found of SLN [%]	% RSD [n=3]
I	99.62	0.57
II	100.18	0.64

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

Table 5.12: System suitability test

System suitability parameters	Proposed method
Retention time (T_R)	17.88
Capacity factor ($K^{'}$)	0.76
Theoretical plate (N)	3655
Tailing factor (T)	1.33

Table 5.13: Summary of validation parameters

Parameters	Observation
Linearity range (µg/mL)	2 - 14
Regression equation	Y = 12494X + 1234.37
LOD (µg)	0.36
LOQ (µg)	1.10
Recovery (%)	99.28
Precision (% RSD)	
Intra- day (n = 3)	0.47 – 1.36
Inter-day (n = 3)	0.64 – 0.99
Repeatability (n = 5)	0.57
Ruggedness (% RSD)	
Analyst I (n = 6)	0.57
Analyst II (n = 6)	0.64
Robustness	Robust
Specificity	Specific

Conclusion

From the present study it was concluded that and confirmed the suitability of the method for quantifying Selinexor in their formulations. Also, the RP-HPLC method was developed and validated.

References

1. Gandhi UH, Senapedis W, Baloglu E, Unger TJ, Chari A, Vogl D, Cornell RF: Clinical Implications of Targeting XPO1-mediated Nuclear Export in Multiple Myeloma. Clin Lymphoma Myeloma Leuk. 2018 May;18(5):335-345. doi: 10.1016/j.clml.2018.03.003. Epub 2018 Mar 14.
2. Xia Y, Shen S, Verma IM: NF-kappaB, an active player in human cancers. Cancer

ISSN:0975 -3583,0976-2833 VOL12, ISSUE 07, 2021

Immunol Res. 2014 Sep;2(9):823-30. doi: 10.1158/2326-6066.CIR-14-0112

3. Ravichandran V, Shalini S, Sundram KM and Harish Rajak. Validation of Analytical Methods-Strategies & Importance. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2010; 2(Suppl3):18-22.
4. Sharma Ajay and Sharma Rohit. Validation of Analytical Procedures: A comparison of ICH Vs Pharmacopoeia (USP) Vs FDA. *International Research Journal of Pharmacy*. 2012; 3(6): 39-42
5. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures, ICH-Q2A, Geneva; 1995.
6. Shuang-long Li "UPLC-MS/MS Measurement of the Effect of Isavuconazole, Itraconazole and Fluconazole on the Pharmacokinetics of Selinexor in Rats", Dove Press journal: Infection and Drug Resistance, Issue No.13, pp. 3153–3161, 2020.
7. Ponnilavarasan Ilankovan "Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Sparfloxacin and Dexamethasone in Bulk and Liquid Dosage Form", J. Pharm. Sci. & Res. Volume 11, Issue No. 6, pp. 2127-2132, 2019.