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A STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SELINEXOR AND BORTEZOMIB IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Keywords:

Selinexor and Bortezomib linearity range, Recovery, Precision

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ABSTRACT: Symmetric peak and effective peak resolution in chromatography were achieved by adjusting the mobile phase's pH and amount of organic Phase. The retention time of 2.127 and 4.145 minutes was observed after the elution of Bortezomib and Selinexor respectively. To accurately measure both Selinexor and Bortezomib at the same time, an approach was developed with an optimal wavelength of 236nm. Selinexor and Bortezomib were successfully separated by chromatography using a mobile phase of Acetonitrile: 0.1% Formic acid (30:70, v/v) and Luna Phenyl Hexyl (250×4.6mm, 5µ) column running at 1.0 ml/min. When looking at the linearity range, statistics correlating 0.999 indicates a very excellent correlation. For intermediate precision intervals, the relative standard deviation of Selinexor and Bortezomib, respectively, was 0.47 and 0.53, indicating the reproducibility of the analytical approach. The suggested analytical approach has a low LOD, which means that it is also extremely sensitive. This indicates that the suggested approach has sample accuracy by ICH requirements, as 99.9 and 100.2 percent of the spiking Selinexor and Bortezomib, respectively, were recovered. According to ICH recommendations, forced degradation of under twenty per cent is acceptable. Selinexor and Bortezomib showed degradation of under 20 percent using the suggested approach, which indicates stability.

INTRODUCTION: Xpovio, also known as Selinexor, is a medication used to treat cancer by selectively inhibiting nuclear export. This mechanism operates by inhibiting the function of exportin [1], thereby preventing the movement of various proteins responsible for promoting the growth of cancer cells from the nucleus to the cytoplasm. As a result, the cell cycle is halted, leading to programmed cell death [2]. This drug has a unique mechanism of action [3, 4].

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Bortezomib is a dipeptide boronic acid derivative and proteasome inhibitor that is commonly prescribed for the treatment of multiple myeloma and mantle cell lymphoma [5].

This protein complex, known as the 26S proteasome, plays a crucial role in breaking down ubiquitinated proteins through the ubiquitin-proteasome pathway.

It is believed that bortezomib primarily works by reversibly inhibiting the 26S proteasome, which leads to cell cycle arrest and apoptosis in cancer cells. There may be multiple mechanisms at play in the anticancer activity of bortezomib [6]. **Fig. 1** and **2** display the molecular structure of Selinexor and Bortezomib, respectively.

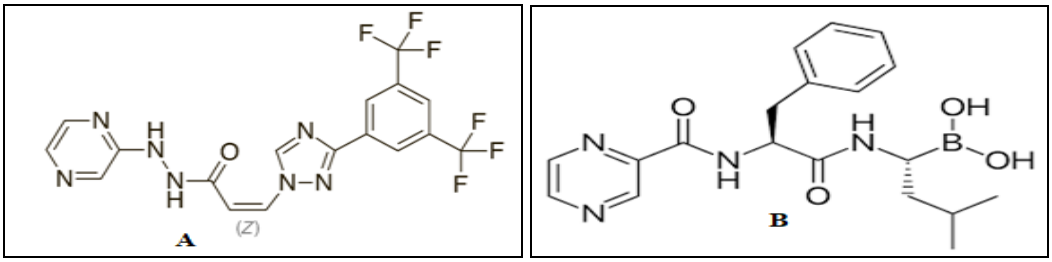

FIG. 1: STRUCTURE OF (A) SELINEXOR (B) BORTEZOMIB

MATERIALS AND METHOD:

Chemicals and Reagents: Samples of Selinexor and Bortezomib were supplied as the reference material by Supriya Labs in Mumbai. All compounds, including HPLC -grade acetonitrile, Formic acid, were procured from the chemical division of Merck located in Mumbai.

Instrumentation: The investigation was carried out utilizing a high-performance liquid chromatography (HPLC) apparatus comprised of a Waters e 2695 Shimadzu column, a photodiode array (PDA) detector, and Empower software version 2.0 as its driver. a UV detector, a Shimadzu UV-visible spectrophotometer, and a Phoenix 4.5 L digital ultrasonic cleaner was utilized.

Selinexor and Bortezomib Stock Solution: Weigh out precisely 100 mg of Selinexor and 10 mg of Bortezomib for the working standard. Then, transfer the contents to a 100 mL volumetric flask that has been diluted with diluent to volume.

Selinexor and Bortezomib Standard Solution: Take 5 ml of the stock solution of Selinexor and Bortezomib and transfer it to a 50 ml volumetric flask. Next, dilute the solution with the appropriate diluent until the flask is filled to the desired volume.

Selinexor and Bortezomib Sample Solution: Accurately weigh 10 milligrams of Bortezomib and 42.5 mg of Selinexor, then transfer to a 10 ml volumetric flask. To dissolve, add around 7 ml of diluents and sonicate for 20 minutes. Then, dilute with diluent to volume.

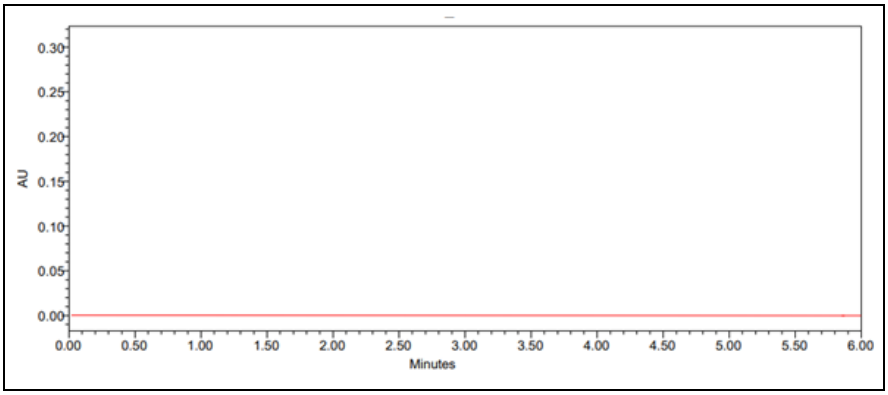

FIG. 2: BLANK CHROMATOGRAM

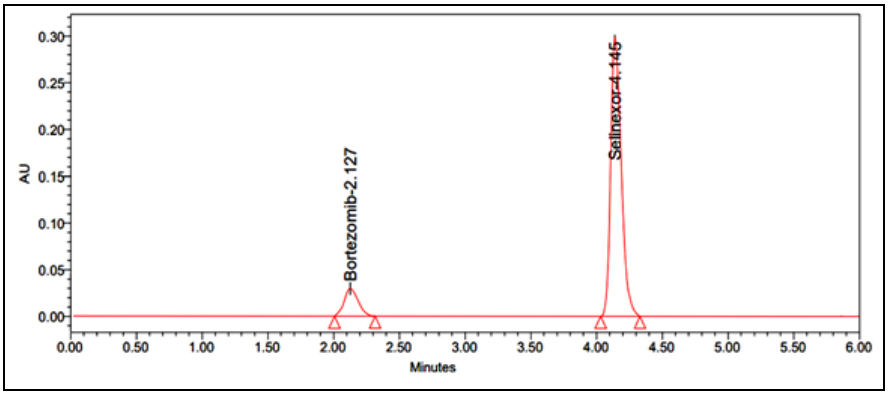

FIG. 3: STANDARD SAMPLE CHROMATOGRAM

Method Development: An in-depth analysis of chromatographic settings, including column type and temperature, mobile phase, and flowing velocity, is essential for optimizing chromatographic methodology, achieving symmetrical peak design, and enhancing resolution. The mobile phase was optimized by trying out numerous mixtures of appropriate solvents, and in the end, Acetonitrile: 0.1% Formic acid (30:70, v/v) was chosen as the optimal mobile phase at a rate of 1.0ml/min. **Table 1** displays the optimum chromatographic parameters. **Fig. 2** & **3** displayed the blank and optimized chromatogram, respectively.

Method Validation: System suitableness, linearity, accuracy, robustness, precision, and selectivity are only few of the factors that were considered while validating the suggested technique in accordance with ICH Q2R1 recommendations [7-9].

Linearity: According to ICH, linearity is the extent to which the method of analysis yields test findings that scale linearly with the quantity of analyte in the given sample [10, 11]. The spectrum of an analyte concentrations, defined as the difference between the highest and lowest concentrations, is an indicator of the analytical method's accuracy, precision, as well as linearity. In order to establish linearity, small portions were prepared and analyzed in threefold throughout an amount range of 25-150µg/ml for Selinexor and 2.50-15.00µg/ml for Bortezomib. By comparing the calibration graph to the linear regression formula, a correlation coefficient was calculated. **Fig. 4** and **5** displayed the linearity results.

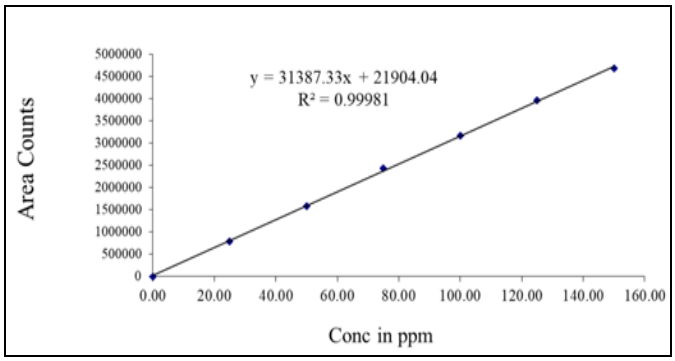

FIG. 4: CALIBRATION CURVE OF SELINEXOR

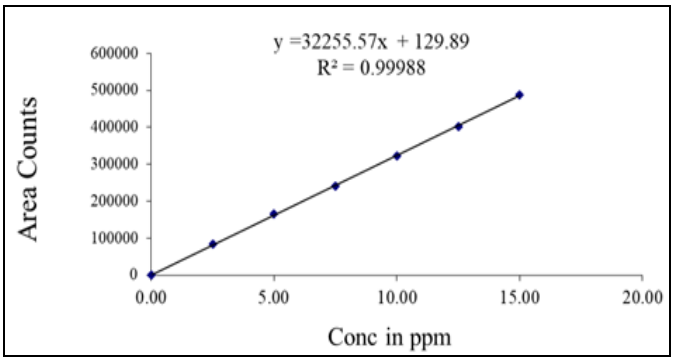

FIG. 5: CALIBRATION CURVE OF BORTEZOMIB

Precision: Analysis precision was calculated using both intra- and inter-day standards. The RSD is a measure of accuracy [12].

Accuracy: Accuracy is measured by how much data can be recovered. Pre-examined samples are assessed at three concentrations before being spiked with established quantities of standard Selinexor and Bortezomib [13].

LOD and LOQ: According to ICH, the minimum detectable concentration of an analyte (although it is not always its quantitative value) is known as the limit of detection. The minimal detectable concentration of a component in a sample using an appropriate analytical technique is called the LOQ. The LOD and LOQ were calculated through data from a calibration graph.

Forced Degradation Studies: Using forced degradation studies (FDS), the stability-indicating characteristic of the proposed approach was evaluated. A range of conditions were used for the experiments, including thermal degradation, oxidative hydrolysis, acid hydrolysis, alkaline hydrolysis, and photolysis (UV energy-days/dark control). The stability of Selinexor and Bortezomib was evaluated by exposing them to FDS under various stress conditions [14, 15].

RESULTS AND DISCUSSION:

Chromatographic Optimization: After carrying out a series of experimental protocols, it was noted that the mobile phase, which comprised 30:70 of acetonitrile and 0.1% Formic acid displayed a peak characterized by desirable theoretical plate count, resolution, and tailing factor. Therefore, this procedure was optimised and validated.

Specificity and Selectivity: The procedure's specificity and selectivity were assessed by looking for interference peaks in the chromatograms of placebo and blank samples. In the retention time

ranges, the HPLC chromatograms for the drug matrix (a mix of the Drug and placebos) revealed almost no interference peaks. As a result, the HPLC approach presented in this study was restricted.

Linearity: Both Selinexor (y = 31387.33x + 21904.04) and Bortezomib (y = 32255.90x + 1300.61), when tested across the dosage ranges of 25-150 µg/ml and 2.50-15.00 µg/ml, correspondingly, were found to have linear concentration-response relationships.

Table 1 displays the linearity information for Selinexor and Bortezomib.

TABLE 1: CALIBRATION CURVES FOR LINEAR REGRESSION WITH A SAMPLE SIZE OF 6

Selinexor		Bortezomib	
µg/mL	**Area Response**	**µg/mL**	**Area Response**
0.00	0	0.00	0
25.00	793381	2.50	83235
50.00	1587739	5.00	166394
75.00	2432045	7.50	240481
100.00	3172849	10.00	322216
125.00	3964202	12.50	402078
150.00	4681459	15.00	488135
Correl Coeff	0.99981	Correl Coeff	0.99988
Slope	31387.33	Slope	32255.90
Intercept	21904.04	Intercept	1300.61

LOD and LOQ: The LOD and LOQ values for Selinexor were found to be 0.30 and 1.0 µg/ml, respectively, while for Bortezomib, they were determined to be 0.300 and 1.0 µg/ml.

Precision: Intermediate precision findings for Selinexor and Bortezomib, respectively, reveal a %RSD of 0.47 and 0.53, respectively, demonstrating the approach's accuracy.

Table 2 respectively highlight the outcomes of accuracy.

TABLE 2: INTERMEDIATE PRECISION OF SELINEXOR AND BORTEZOMIB

Drug	Conc.	Mean	%RSD
Selinexor	42.5µg	99.6	0.47
Bortezomib	10µg	100.2	0.53

Accuracy: The reliability of the procedure was tested by analysing recoveries after 50%, 100%, and 150% spiking. Using %RSD, we calculated that the mean recovery rates for Selinexor were 99.6, 99.9, and 100.2, while those for Bortezomib were 100.4, 100.2, and 100.1. The results were 0.20, 0.17, 0.14 for Selinexor and 0.37, 0.46, 0.17 for Bortezomib. **Table 3** shows the results of the Selinexor and Bortezomib accuracy values.

TABLE 3: ACCURACY FOR SELINEXOR AND BORTEZOMIB

	Selinexor			Bortezomib		
Levels	**Mean**	**% RSD**	**% recovery**	**Mean±SD**	**% RSD**	**% recovery**
50	99.6±0.20	0.200	99.6	100.4±0.37	0.370	100.4
100	99.9±0.17	0.170	99.9	100.2±0.46	0.460	100.2
150	100.2±0.14	0.140	100.2	100.1±0.17	0.170	100.1

Robustness: In terms of robustness, there is not a significant variance in peak area or resolution comparing Selinexor and Bortezomib after making minor and intentional modifications to mobile phase proportion, column temperatures, and rate of flow. Information on robustness may be found in **Table 4.**

TABLE 4: ROBUSTNESS OF SELINEXOR AND BORTEZOMIB

Parameter	Selinexor		Bortezomib	
	Mean±SD	**%RSD**	**Mean±SD**	**%RSD**
Flow rate (-)	99.7±0.1	0.1	100.08±0.379	0.38
Flow rate (+)	100±0.611	0.61	100.2±0.833	0.83

ORG minus	99.6±0.404	0.41	100.6±0.721	0.72
ORG plus	99.7±0.702	0.70	100.0±0.058	0.58

Assay: With % relative standard deviations (RSDs) of 0.221 for Selinexor and 0.276 for Bortezomib, the analysis of the drug product showed that there was not any peak interference by degradants, additives, or impurities, at retention duration of Selinexor and Bortezomib. **Fig. 6** shows chromatogram of Marketed formulation.

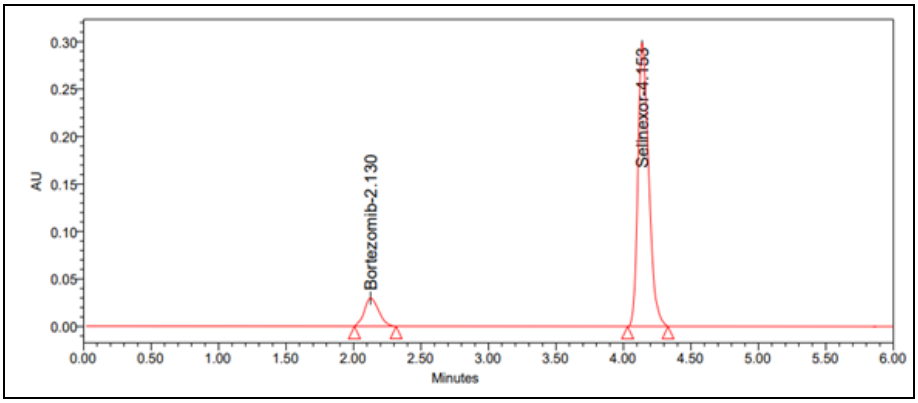

FIG. 6: CHROMATOGRAM OF MARKETED FORMULATION SAMPLE

Forced Degradation Studies: Sample solutions were subjected to a variety of stresses in order to conduct accelerated degradation investigations. Selinexor and Bortezomib were found to degrade under acidic, alkaline and peroxide, according to the results of degradation experiments. Chromatograms depicting degradation as a function of pH, acidity, peroxide concentration, temperature, and light intensity are presented in **Fig. 7, 8, 9, 10**, and **11. Fig. 12** displays that neither Selinexor and Bortezomib showed any degradation peaks under, Reduction, Thermal, Photolytic hydrolytic conditions. **Table 5** is a summary of the findings we collected on degradation.

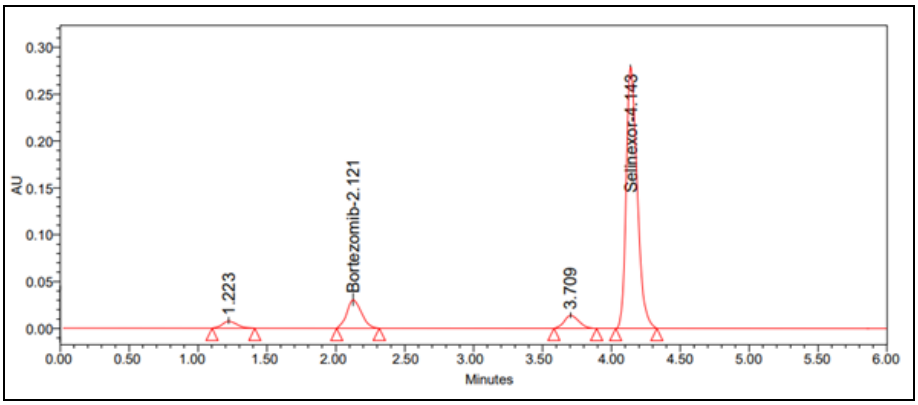

FIG. 7: CHROMATOGRAM OF ACID DEGRADATION

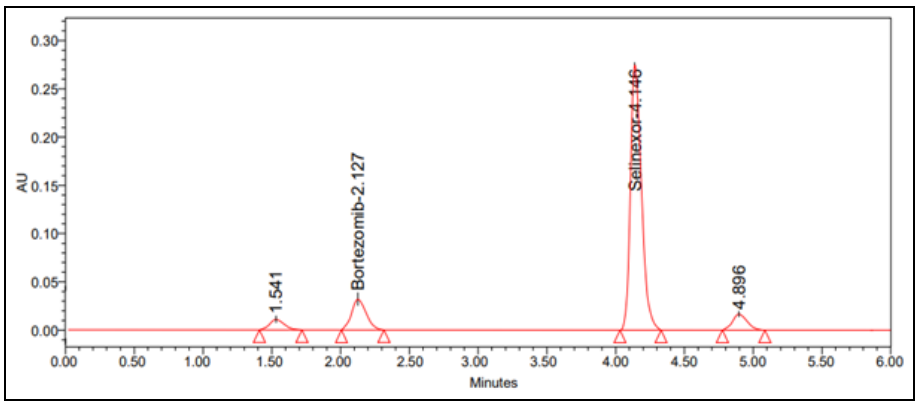

FIG. 8: CHROMATOGRAM OF ALKALI DEGRADATION

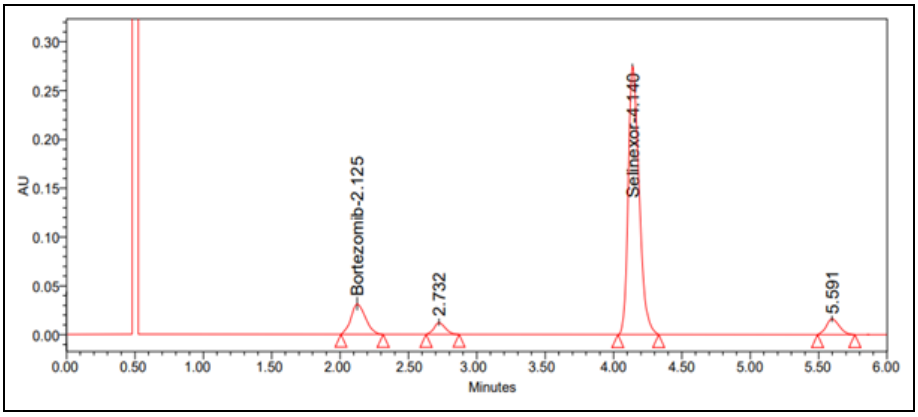

FIG. 9: CHROMATOGRAM OF PEROXIDE DEGRADATION

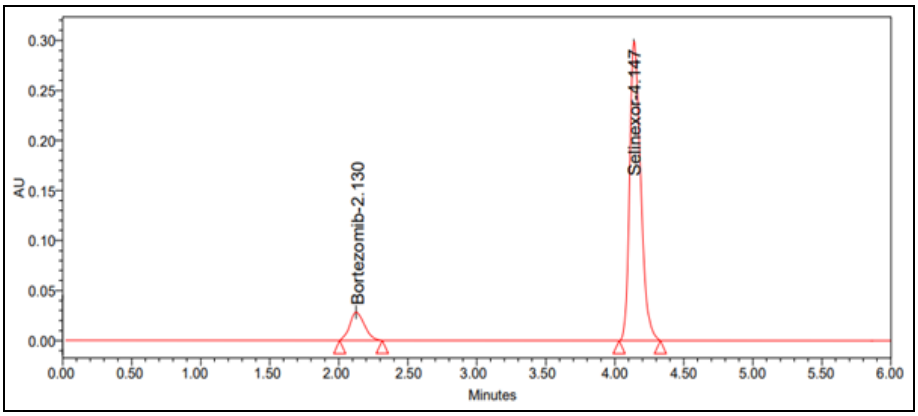

FIG. 10: CHROMATOGRAM OF REDUCTION DEGRADATION

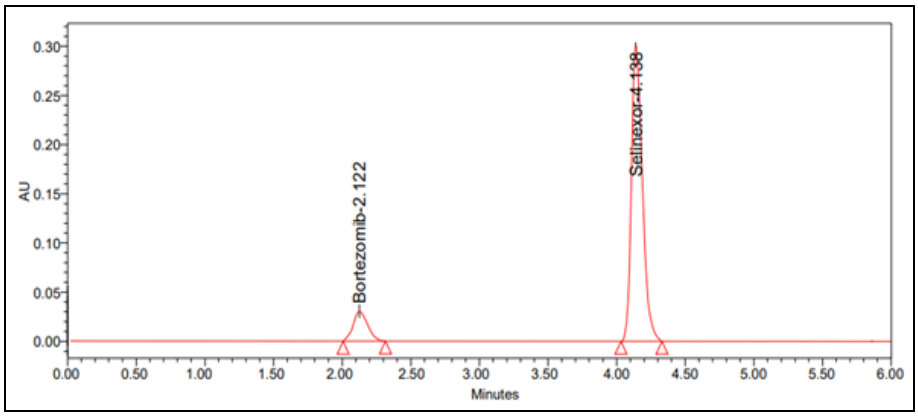

FIG. 11: CHROMATOGRAM OF THERMAL DEGRADATION (105°C FOR 72HRS)

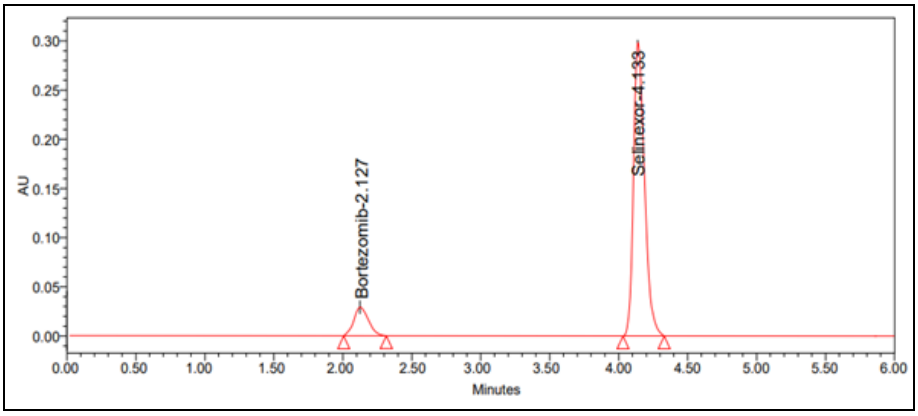

FIG. 12: CHROMATOGRAM OF PHOTO DEGRADATION (1.2 MILLXH AND 200WH/M2 LIGHT)

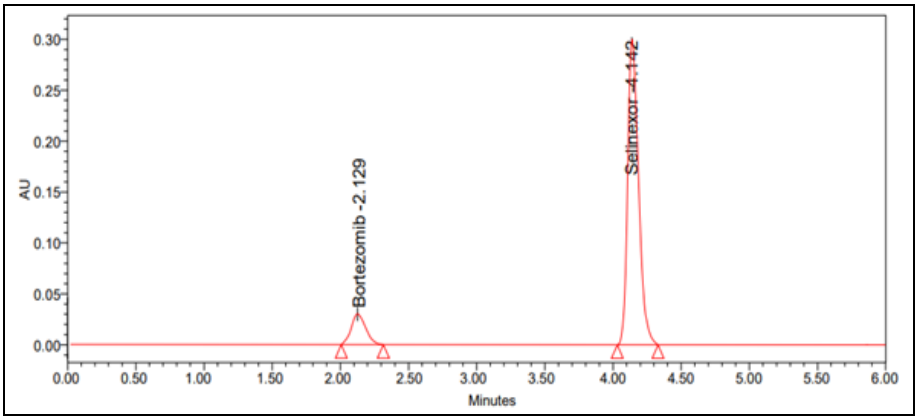

FIG. 13: CHROMATOGRAM OF HYDROLYSIS DEGRADATION (HEAT ON WATER BATH 60°C FOR 30MIN)

TABLE 5: SUMMARY OF DEGRADATION DATA

Stress condition	% Degradation			
	Selinexor		Bortezomib	
	Peak Area	% Degraded	Peak Area	% Degraded
Acidic	2831623	10.6	291561	9.3
Base	2814598	11.2	288123	10.3
Oxidative	2774562	12.4	286268	10.9
Reduction	3084335	2.7	317415	1.2
Thermal	3058572	3.5	314156	2.2
Photolytic	3041069	4	310237	3.5
Hydrolytic	3114451	1.7	316774	1.4

CONCLUSION: The current HPLC technique used for the measurement of bulk and dosage forms adopts great sensitivity and reliability. The present procedure was validated using the ICH Q2R1 recommendations. When compared to other approaches, the suggested approach stood out with regard to validation criteria and stability-indicating investigations. Findings for limits of detection, limits of quantification, and accuracy were all within the permitted limits during validation, demonstrating that the instrument can provide reliable findings down to very low concentrations. Peaks in degradation may be identified in research.

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CONFLICT OF INTEREST: The authors confirm that this article content has no conflict of interest.

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