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Research Article

Pharmaceutical Analysis for Novel drug Formulation

Bioanalytical Method Development and Validation of Selinexor in Rat Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract: A simple, rapid, precise, sensitive, and reproducible reverse-phase high-performance liquid chromatography (RP-HPLC) LCMS/MS method has been developed for the bioanalytical method for Selinexor with D_6-Selinexor as Internal Standard in the pharmaceutical dosage form. Chromatographic separation of Selinexor was achieved on Waters Alliance-e2695, by using X-Bridge phenyl, 150x4.6mm, 3.5µm column, and the mobile phase containing 0.1% Formic acid & Acetonitrile in the ratio of 80:20% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 225nm using a photodiode array detector at ambient temperature. The method was validated to fulfill International Conference on Harmonization (ICH) requirements and this validation included specificity, selectivity, matrix effect, linearity, the limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. The proposed method was Bio-analytical validated according to USFDA guidelines. This method was found to be a very simple, economical, suitable, precise, accurate, and stable method for pharmacokinetic analysis of Selinexor and study of its stability. The calibration curve was linear over the concentration range from 0 to 40 ng/ml, and the lower limit of detection of 12.5 ng/ml. The accuracy and precision of the method were within the acceptable limit of ±20% at the lower limit of quantitation and ±15% at other concentrations. Selinexor was unstable at room temperature it showed more than 25% loss after 24 h. While, Selinexor is very stable at refrigerator 4^0C auto-sampler, freeze/thaw cycles, and 30 days storage in a freezer at 35 ± 2^0C. All results were acceptable and this confirmed that the method is suitable for its intended use in routine quality control and an assay of drugs.

Keywords: Reverse-phase high-performance liquid chromatography (RP-HPLC), HPLC LCMS/MS, Selinexor, Matrix effect, Precision, Linearity.

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generic drug development and quality control procedures. Quantitative determination of drugs from the dosage forms using in vitro methods by means of assay and dissolution techniques is important to assess the quality of the dosage forms. While bioanalytical methods are used for the qualitative and quantitative analysis of drug substances in biological fluids (mainly plasma, serum and urine) or tissue. Bioanalytical methods are essential for the bioavailability and bioequivalence studies and play a significant role in the evaluation and interpretation of pharmacokinetic data. When determining pharmacokinetic properties of a drug, plasma is commonly selected as matrix. Each step in the analytical or bioanalytical method must be investigated to determine the extent to which environment, matrix, or procedural variables can affect the estimation of analyte in the matrix from the time of collection up to the time of analysis. To validate bioanalytical HPLC LCMS/MS method for the estimation of Selinexor in bulk and pharmaceutical drugs in rat plasma. To develop a simple, rapid, and specific HPLC LCMS/MS bioanalytical method for the estimation of Selinexor in bulk and combined pharmaceutical dosage forms. To validate the proposed methods by the analytical parameters mentioned in the ICH guidelines, such as system suitability, accuracy, precision, specificity, linearity, recovery, matrix factor, stability, LOD, and LOQ.

1.1. Drug profile of Selinexor

Structure of Selinexor

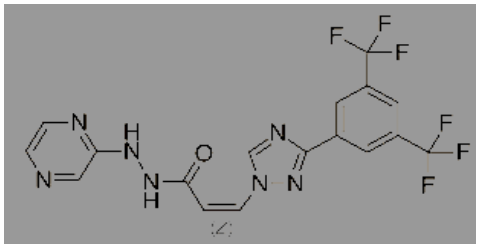

Fig 1.1: Structure of Selinexor

IUPAC Name: 2Z)-3-{3-[3,5-Bis(trifluoromethyl)phenyl]-1,2,4-triazol-1-yl}-N′ -pyrazin-2-ylprop-2-enehydrazide.
Molecular weight: 443.313 g·mol− 1
Molecular formula: $C_{17}H_{11}F_6N_7O$
Category: Selinexor is a first-in-class selective inhibitor of nuclear transport (SINE) compound.[1] It is currently approved for the treatment of multiple myeloma, cancer that forms from antibody-producing plasma cells.[2]

2. Mechanism of Action of Selinexor

Selinexor binds to and inhibits exportin-1 (XPO1). XPO1 is a nuclear exporter protein that contains a pocket to which nuclear proteins can bind.[3] When complexed with these proteins and ran, activated through guanosine triphosphate (GTP) binding, the XPO1-protein-Ran-GTP complex can exit the nucleus through a nuclear pore. Once outside, GTP is hydrolyzed and the complex dissociates.[4] The inhibition of this process in cancer cells allows the targets of XPO1, many of which are tumor suppressors, to collect in the nucleus and result in increased transcription of tumor suppressor genes. Tumor suppressor proteins known to be affected by XPO1 inhibition include p53, p73, adenomatous polyposis coli, retinoblastoma, forkhead box protein O, breast cancer 1, nucleophosmin, and merlin. Regulators of cell cycle progression are also affected, namely p21, p27, galectin-3, and Tob. Inhibitor of NFκ B also collects in the nucleus as a result leading to reduced activity of NFκ B, a known contributor to cancer.[4,5] XPO1 participates in the formation of a complex with eukaryotic initiation factor 4E and contributes to the transport of messenger RNA for several oncogenes including cell cycle promotors, cyclin D1, cyclin E, and CDK2/4/6, as well as antiapoptotic proteins, Mcl-1 and Bcl-xL.[6,7] These wide-ranging changes in protein expression and gene transcription culminate in cell cycle arrest and the promotion of apoptosis in cancer cells.[8,9]

3. Side Effects of Selinexor

- severe ongoing nausea, vomiting, or diarrhoea.[10]
- loss of appetite that prevents you from eating and causes weight loss.[11]
- confusion, dizziness, fainting, or changes in mental status.[12]
- easy bruising, unusual bleeding.[13]

4. Absorption

A single 80 mg dose of Selinexor produces a mean Cmax of 680 ng/mL and a mean AUC of 5386 ng/mL.[14] This relationship is dose proportion over the range of 3-85 mg/m_2 which encompasses the range of 0.06-1.8 times the approved dosage.[15] The official FDA labeling reports the Tmax as 4 hours but phase 1 studies have found a range of 2-4 hours.[16] Administering Selinexor with food, either a high or low-fat meal, increases the AUC of approximately 15-20% but this is not expected to be clinically significant.[17,18]

2. MATERIALS AND METHODS

All the chemicals and reagents used in the present work were obtained from Merck and Rankem companies.

3.1 Selection of the mobile phase

Based on the trial-and-error method ratio of the buffer and organic (acetonitrile) was optimized. Each of the buffer systems mentioned in the previous section was mixed with Acetonitrile. After equilibrating the system for at least 30minutes, Selinexor 20µg/ml was injected. The run time was set for 30 mins initially to confirm the peak followed by

minimum run time to avoid the unnecessary wastage of the mobile phase. The selection of the best mobile phase was based on peak shape, retention time, theoretical plate count, asymmetry factor, resolution, etc.

3.2 Chromatographic conditions

During the selection of chromatographic conditions, many trials were carried out and the best trial was selected for the optimized method.

3.3 Preparation of Selinexor stock solution

The stock solution of Selinexor used during the HPLC method development stage was prepared by dissolving the accurately weighed standard compound in acetonitrile. Concentration of Selinexor standard solution was 0.5 mg/ml. Appropriate dilutions with mobile phase were made from the stock solution to prepare the working standard solutions for method development, calibration curve, and quality control (QC) samples. The solution and working standard solutions were stored in polypropylene vials in a -20 °C freezer.

3.4 LCMS / MS method development of Selinexor:

A robust, selective, and sensitive HPLC & LCMS/MS Method with UV detection was developed to quantify Selinexor in rat plasma. It involves evaluation and optimization of the various parameters like sample preparation, chromatographic separation, detection, and quantification. Steps involved in method developments are mentioned below in the order they were followed.

3.5 λ_{max} determination of Selinexor

A stock solution containing 1 mg/ml of Selinexor was prepared by dissolving the drug in acetonitrile. This stock solution was further diluted to 10µg/ml with acetonitrile. Aliquots of this solution were taken HPLC vial and scanned for λ_{max} PDA Detector within the wavelength region of 200–400 nm. The absorption curve shows an isosbestic point at 225nm. Thus 225 nm was selected as the detector wavelength for the HPLC chromatographic method.

3.6 Selection of the Stationary phase (column) for the Selinexor

After the selection of the proper mobile phase, the HPLC column was selected again by the trial-and-error method. The bioanalytical HPLC method differs from the simple HPLC method meant for the analysis of raw drugs.[19] The matrix used in bioanalysis gives trouble to HPLC analysts as the matrix compounds also mostly co-elute with an analyte. To separate the interfering peak from the analyte, different stationary phases like C18, C8, Cyano, etc were tried. All other chromatographic conditions except the column were remained fixed during the entire procedure.[20]

3.7 Selection of the internal standard (IS) for Selinexor

One of the most important parts of analysis in a bioanalytical method is internal standard (IS). As a thumb rule, a compound with structural similarity with the analyte or with significant absorbance at the detection wavelength is selected as IS in the bioanalytical HPLC method. Good extraction recovery and or chromatographic behavior similar to the analyte would be added advantage. D_6 similarity to the analyte was tested as IS for the Selinexor HPLC method.[21]

3.8 Optimization of the final mobile phase

After selection of the internal standard, final tuning with mobile phase composition and buffer concentration was done based on the retention time of the Selinexor and IS. The final mobile phase was selected so that it could elute the Selinexor and IS with reasonable peak separation.[22]

3.9 Optimization of the flow rate

After optimization of mobile phase composition, different flow rates are experimented with to ensure proper RT, peak asymmetry, and resolution for both drug and IS. From this, the finalized flow rate is selected depending on the RT, proper peak asymmetry, and resolution.[23]

3.10 Extraction of Selinexor from plasma sample

Plasma samples as such cannot be injected onto the HPLC system to quantify the drug. Then it will block the HPLC column and make it unusable further. Before sample analysis, the drug has to extract in a suitable solvent followed by its evaporation to concentrate it before injection onto the HPLC system.

3.11 Selection of extraction solvent

Simple liquid-liquid extraction was not reported in the literature for Selinexor 200 µl blank rat plasma was taken and spiked with drug (20ng/ml) and IS (20ng/ml). Extracted with different organic solvents like dichloromethane; ethyl acetate, chloroform, chloroform: dichloromethane, Chloroform: isoamyl alcohol; Chloroform; isopropyl alcohol, Acetonitrile. Depending on the reproducibility and higher level of recovery, extraction solvent was selected.[24]

3.12 Estimating LOD and LLOQ (Signal-to-noise method)

By using the signal-to-noise method, the signal-to-noise ratio, around the analyte retention time was measured, and subsequently, the concentration of the analyte that would yield a signal equal to a certain value of noise to signal ratio was estimated. The noise value was calculated based on the peak height of the blank plasma around the retention time of Selinexor. The noise magnitude was measured either manually on the chromatogram printout. Generally, the analyte amount for which the signal-to-noise ratio was equal or more than 3 times was identified as LOD LLOQ was determined by the analyte amount for which the signal-to-noise ratio was equal or more than 5 times.[25]

3.13 Validation Bioanalytical method of Selinexor

The analytical method was validated to parameters such as specificity, selectivity, matrix effect, linearity, the limit of detection (LOD), the limit of quantification (LOQ), precision, and accuracy and was applied for forced degradation studies as per the ICH guidelines.

3.14 Validation of developed bioanalytical HPLC method for Selinexor

The HPLC method for Selinexor was validated to meet the acceptance criteria of industrial guidance for the bioanalytical method validation (Food and Drug Administration of the United States, 2001).

3. RESULTS AND DISCUSSIONS

3.1 Specificity and Selectivity

No interfering peaks were found in six different random blank rat plasma samples at the retention times of either Selinexor or ISTD (Fig.1, Fig.2, and Fig.3).

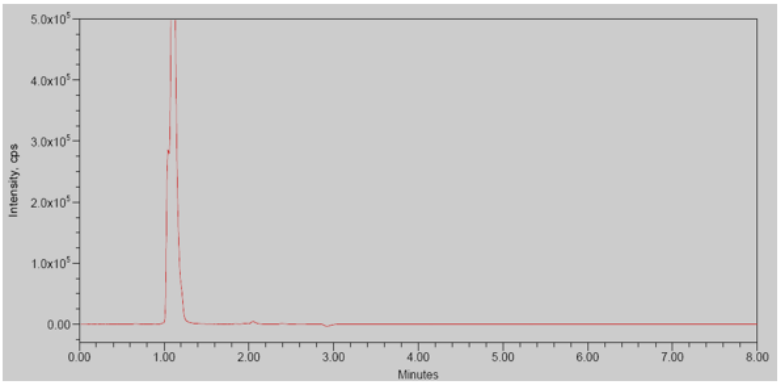

Fig1: Blank rat plasma

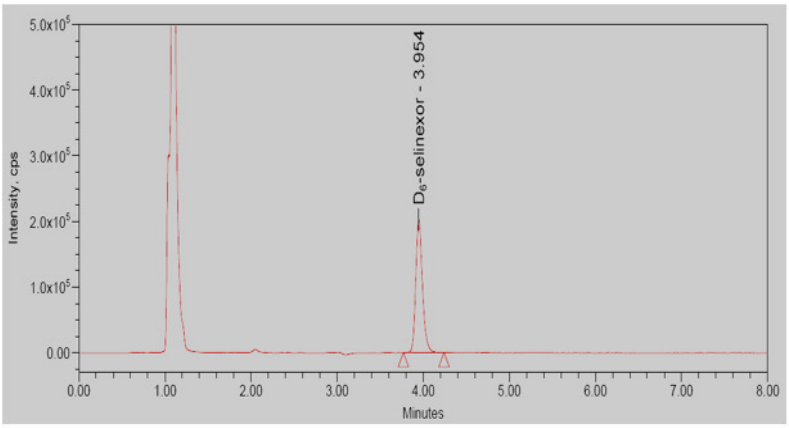

Fig 2: Blank rat plasma spiked with IS

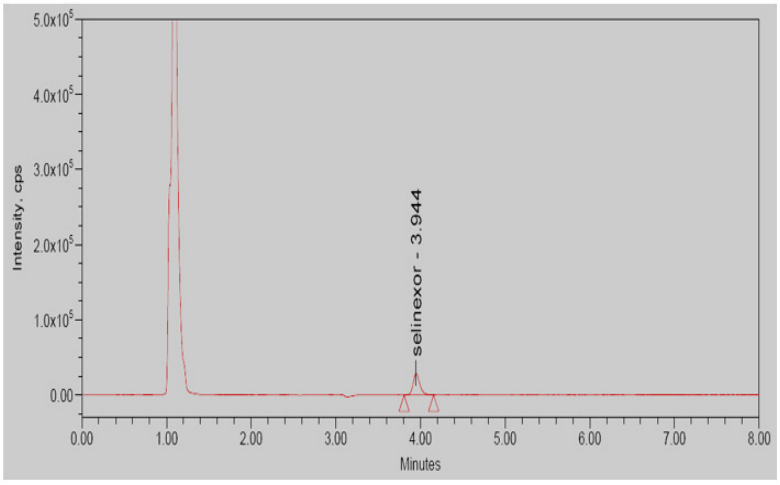

Fig 3: Blank rat plasma spiked with analyte at LLOQ and IS

As observed from the above chromatogram, the total run time was 8 min and the retention time of drugs is about 3.942min. For the blank plasma chromatogram, there were no interfering peaks near the peaks for Selinexor and IS. The same is observed in the case of the chromatogram of blank plasma spiked with IS.

3.2 System suitability

The %CV for Selinexor and ISTD area ratio was found to be 0.77%. Hence it passed the system suitability (Fig.4 and Table.1).

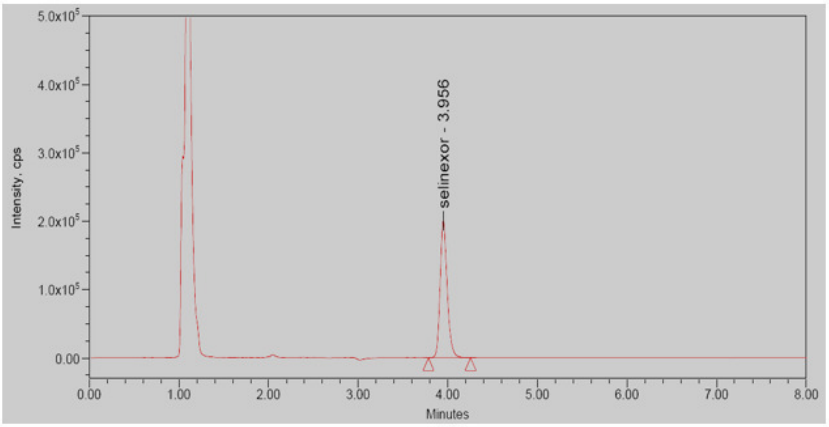

Fig 4: Chromatogram of system

Table 1: System suitability Results of Selinexor					
Sample Name	**Analyte Area**	**Analyte RT (min)**	**ISTD Area**	**ISTD RT (min)**	**Area Ratio**
MQC	$2.154x10^5$	3.952	$2.188x10^5$	3.954	0.9845
MQC	$2.172x10^5$	3.954	$2.196x10^5$	3.949	0.9891
MQC	$2.159x10^5$	3.957	$2.164x10^5$	3.951	0.9977
MQC	$2.122x10^5$	3.949	$2.169x10^5$	3.955	0.9783
MQC	$2.136x10^5$	3.950	$2.185x10^5$	3.946	0.9776
MQC	$2.148x10^5$	3.956	$2.172x10^5$	3.952	0.9890
Mean	$2.149x10^5$	3.953	$2.179x10^5$	3.951	0.9860
SD	0.01762	0.00322	0.01249	0.00331	0.00758
%CV	0.82	0.08	0.57	0.08	0.77

Table 1The table will provide the data of the system suitability of the Selinexor. After performing the six trials calculated the mean, standard deviation, and % CV for the Selinexor. Based on the obtained results, the present studies reveal that the Selinexor was passed the system suitability by acquiring the area ratio of 0.77%. The acceptance criteria for the selinexnor was found to be ≤ 5.00.

3.4 Acceptance Criteria

The % RSD of the retention time (RT) should be ≤ 2.00 %. The % RSD of the area ratio should be ≤ 5.00.

3.3 Sensitivity

The %CV for Selinexor was found to be 7.16%. Hence it passed the sensitivity (Fig.5 and Table.2).

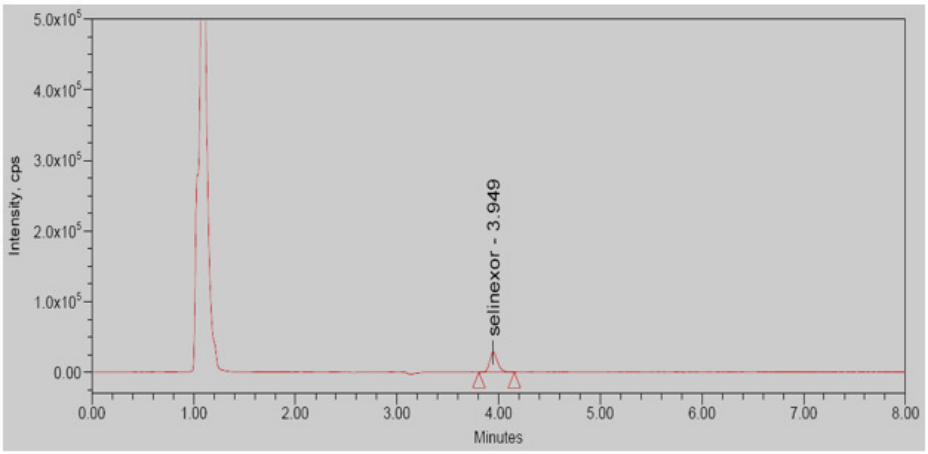

Fig 5: Sensitivity Chromatogram of LLQ

Table 2: Sensitivity Results of Selinexor	
Replicate Number	LLOQ
	Nominal Concentration(ng/ml)
	2.125
	Nominal Concentration Range(ng/ml)
	(2.135-2.368)
	Area of Analyte
1	0.234 x10^5
2	0.267 x10^5
3	0.239 x10^5
4	0.251 x10^5
5	0.228 x10^5
6	0.219 x10^5
n	6
Mean	0.239 x10^5
SD	0.01715
%CV	7.16
% Mean Accuracy	104.8%

Table 2 From the table-2 the sensitivity results of the selixnexor were obtained by considering the six trials of the selixnexor. After performing the six trials for the sensitivity of the selixnexor the results were tabulated in the given above table. The mean sensitivity of the selixnexor was found to be 0.239x10^5. The standard deviation was found to be 0.01715. The %CV for Selinexor was found to be 7.16%. Based upon this data Selinexor was passed the sensitivity. The % mean accuracy for the selixnexor was found to be 104.8% which reveals that the acceptance criteria for the Selinexor were within the range.

3.5 Acceptance Criteria

At least 67 % (4 out of 6) of samples should be within 80.00-120.00 %. Percentage Mean accuracy should be within 80.00-120.00 %. %RSD accuracy should be ≤ 20.00 %.

3.6 Matrix effect

The matrix of plasma constituents over the ionization of analyte was determined by comparing the response of post-extracted plasma standard QC samples (n = 6) with the response of analyte from neat samples at equivalent concentrations. The matrix effect intended method was assessed by using chromatographically screened rat plasma. Precision (%CV) is 0.53% and 1.75% for Selinexor at HQC and LQC (Fig.6, Fig.7, and Table.3).

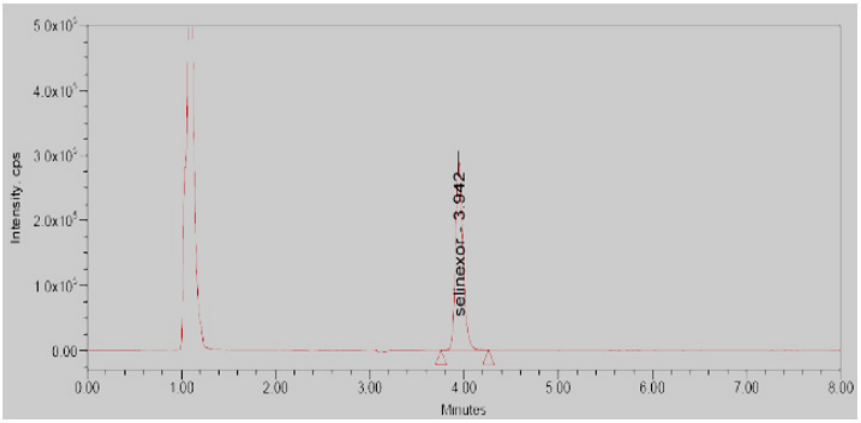

Fig 6: Matrix Effect Chromatogram of HQC

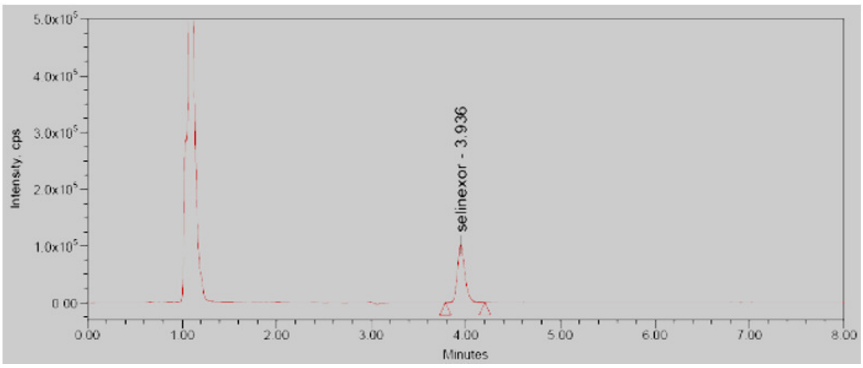

Fig 7: Matrix Effect Chromatogram of LQC

Table 3: Matrix effect Results of Selinexor

S.No.	Plasma Lot No.	HQC	LQC
		Nominal Concentration(ng/ml)	
		30.569	10.349
		Nominal Concentration Range(ng/ml)	
		(30.234-30.751)	(10.157-10.535)
		Area of Analyte	
1.	Lot 1	3.021 x10^5	1.052 x10^5
		3.036 x10^5	1.041 x10^5
		3.047 x10^5	1.072 x10^5
2.	Lot 2	3.055 x10^5	1.035 x10^5
		3.018 x10^5	1.042 x10^5
		3.025 x10^5	1.084 x10^5
3.	Lot 3	3.033 x10^5	1.079 x10^5
		3.052 x10^5	1.045 x10^5
		3.031 x10^5	1.039 x10^5
4.	Lot 4	3.057 x10^5	1.056 x10^5
		3.046 x10^5	1.047 x10^5
		3.029 x10^5	1.018 x10^5
5.	Lot 5	3.022 x10^5	1.022 x10^5
		3.058 x10^5	1.037 x10^5
		3.041 x10^5	1.034 x10^5
6.	Lot 6	3.037 x10^5	1.067 x10^5
		3.065 x10^5	1.052 x10^5
		3.074 x10^5	1.034 x10^5
n		18	18
Mean		3.042 x10^5	1.048 x10^5
SD		0.01623	0.01834
%CV		0.53	1.75
% Mean Accuracy		92.6%	94.2%
No. of QC Failed		0	0

Table 3 The above table will provide the data related to the matrix effect of selixnexor. The six trials were performed for the Selinexor and the results were given in the above table. The mean, standard deviation, % CV, and % mean accuracy for both the HQC and LQC were calculated. At least 67 % (2 out of 3) of samples at each level should be within 85.00-115.00 %. At least 80 % (5 out of 6) of the matrix lot should be within the acceptance criteria. The % mean accuracy of the back-calculated concentration of LQC and HQC samples prepared from different biological matrix lots should be within 85.00-115.00 %.

3.7 Acceptance Criteria

At least 67 % (2 out of 3) of samples at each level should be within 85.00-115.00 %. At least 80 % (5 out of 6) of the matrix lot should be within the acceptance criteria. The % mean accuracy of the back-calculated concentration of LQC and HQC samples prepared from different biological matrix lots should be within 85.00-115.00 %.

3.8 Linearity

The standard curves were linear over the concentration range of 2.0-40.00 ng/ml of Selinexor. The mean correlation coefficient was 0.999. Samples were quantified using the ratio of peak area of the analyte to that of IS. Peak area ratios were plotted against plasma concentrations (Fig.8, Fig.9 and Table.4, Table.5).

3.9 Acceptance Criteria

The Linearity Regression coefficient should be $R^2 = 0.999$

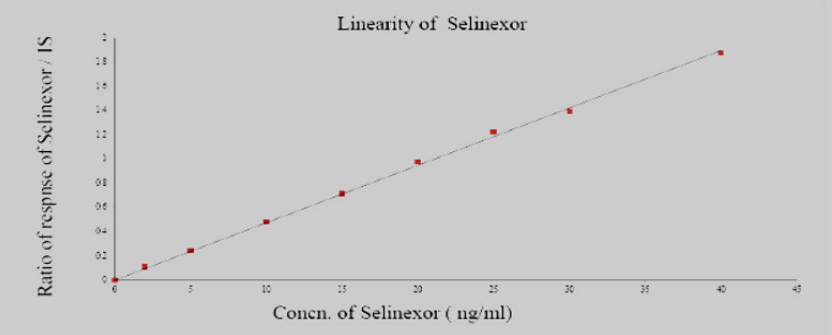

Fig 8: Calibration plot for concentration

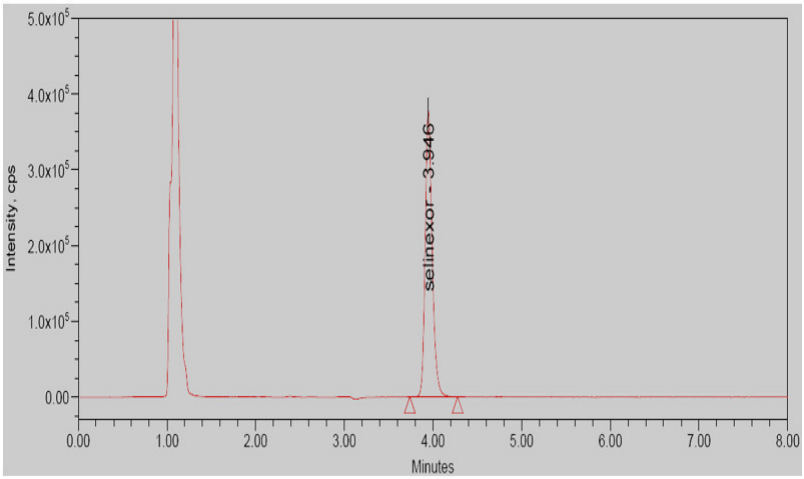

Fig 9: Chromatogram for Linearity-8 v/s Area ratio of Selinexor

Table.4: Preparation of stock solution					
Standard	**Drug taken**	**Acetonitrile adds**	**Further diluted**	**Further diluted**	**Final Conc.**
Selinexor	5 mg	10 ml	0.4ml/50ml	0.2ml/10ml	80 ng/ml
Internal Standard	5 mg	10 ml	0.4ml/50ml	0.2ml/10ml	80 ng/ml

Table 4 An aqueous stock solution containing 20 ng/ml Selinexor. and 20 ng/ml IS was prepared in a diluent. The solution was divided into three containers, the first one stored at room temperature, the second one stored at deep freezer (mention the temperature), and the last one stored at -20 °C (assumed stable as a freshly prepared solution). The solutions of drug and IS from each storage condition were taken out at predetermined time intervals (0, 12, 24 hrs) and were injected onto the HPLC. The peak area from the chromatogram of each sample was compared with that of freshly prepared samples.

Table.5: Preparation of Selinexor working stock solution for standard curve								
Linearity	**Plasma (µl)**	**ACN (µl)**	**Std Stock (µl)**	**IS (µl)**	**MP added (µl)**	**Selinexor Conc. (ng/ml)**	**Selinexor response**	**Area res ratio**
Linearity-1	200	300	50	500	950	2.00	0.239	0.109
Linearity-2	200	300	125	500	875	5.00	0.542	0.247
Linearity-3	200	300	250	500	750	10.00	1.037	0.479
Linearity-4	200	300	375	500	625	15.00	1.546	0.713
Linearity-5	200	300	500	500	500	20.00	2.135	0.977
Linearity-6	200	300	625	500	375	25.00	2.652	1.221
Linearity-7	200	300	750	500	250	30.00	3.018	1.394
Linearity-8	200	300	1000	500	0	40.00	4.035	1.873
SLOPE							0.0471	
INTERCEPT							0.01442	
R^2 square							0.99912	

Table 5 An 8-point calibration curve was prepared by spiking appropriate amounts of working solution into the blank plasma to obtain final concentrations of 2, 5, 10, 15, 20, 25, 30, and 40ng/ml for the Selinexor. The calibration curve was prepared by plotting the peak area ratio of the transition pair of Selinexor. to that of IS against the nominal concentration of calibration standards. The results were fitted to linear regression analysis. The acceptance criterion for each back-calculated standard concentration was ±15% deviation (SD) from the nominal value, except at LLOQ, which was set at ±20% (Food and Drug Administration of the United States, 2001)

3.10 LOD and LOQ

LOD and LOQ were separately determined by the calibration curve method. LOD and LOQ of the compound were determined by injecting progressively lower concentrations of standard solutions using the developed RP-HPLC method. The LOD concentrations for Selinexor are 0.02 ng/ml their s/n values are 5. The LOQ concentration for Selinexor is 0.2ng/ml their s/n value is 25(Fig.10, Fig.11, and Table.6).

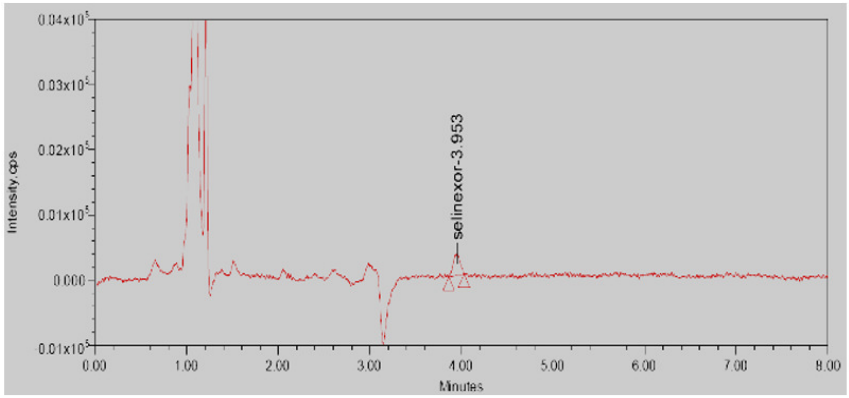

Fig10: Chromatogram for LOD

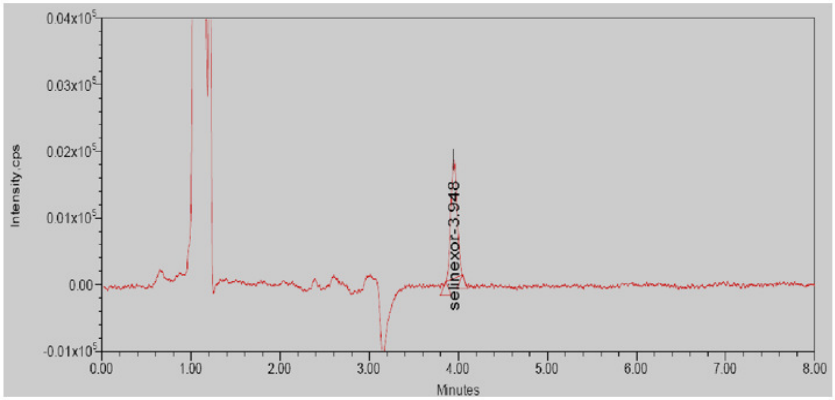

Fig 11: Chromatogram for LOQ

Table 6: LOD and LOQ data for Selinexor

Name	LOD		LOQ	
	Concentration (ng/ml)	s/n	Concentration (ng/ml)	s/n
Selinexor	0.02	5	0.2	25

Table 6 By using the signal-to-noise method, the signal-to-noise ratio, around the analyte retention time was measured, and subsequently, the concentration of the analyte that would yield a signal equal to a certain value of noise to signal ratio was estimated. The noise value was calculated based on the peak height of the blank plasma around the retention time of Selinexor. The noise magnitude was measured either manually on the chromatogram printout. Generally, the analyte amount for which the signal-to-noise ratio was equal or more than 3 times was identified as LOD, LOQ was determined by the analyte amount for which the signal-to-noise ratio was equal or more than 5 times.

3.11 Precision and accuracy

The intra-assay precision and accuracy were estimated by analyzing six replicates containing Selinexor at six different QC levels. The inter-assay precision was determined by analyzing the four levels of QC samples on four different runs. The criteria for acceptability of the data include accuracy within 85–115% from the actual values and precision of within ±15% relative standard deviation (RSD) except for LLQC, where it should be within 80–120% for accuracy and <20% of RSD (Fig.12, Fig.13, Fig.14, Fig.15 and Table.7

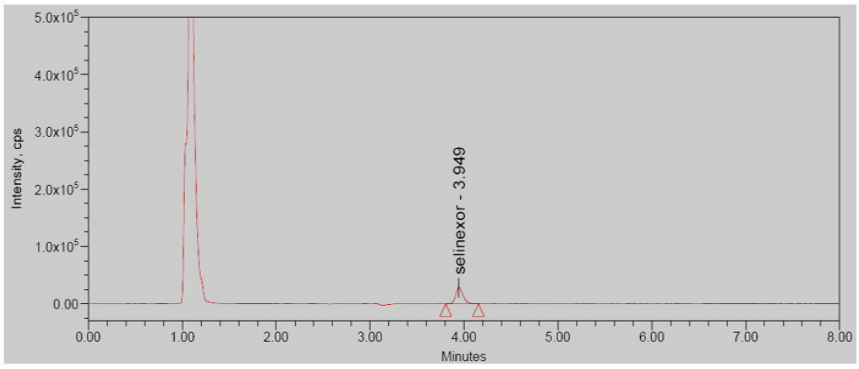

Fig 12: Chromatogram for Accuracy & Precision LLOQ

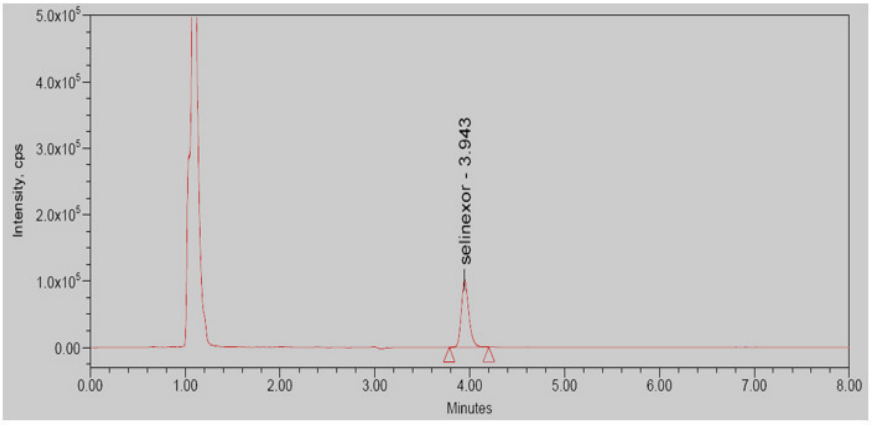

Fig 13: Chromatogram for Accuracy & Precision_LQC

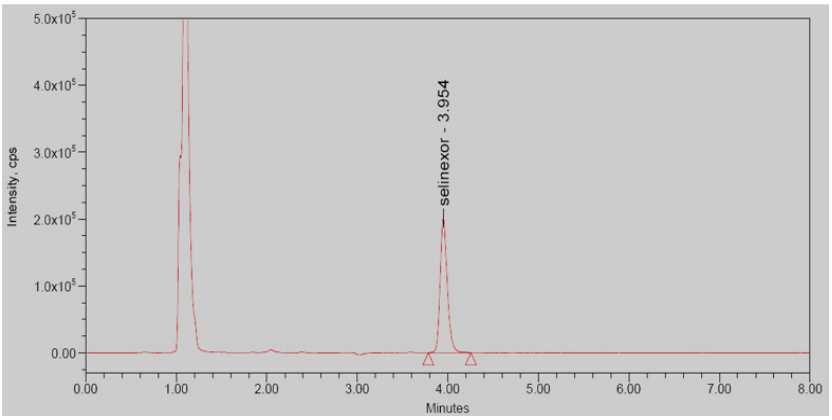

Fig 14: Chromatogram for Accuracy & Precision MQC

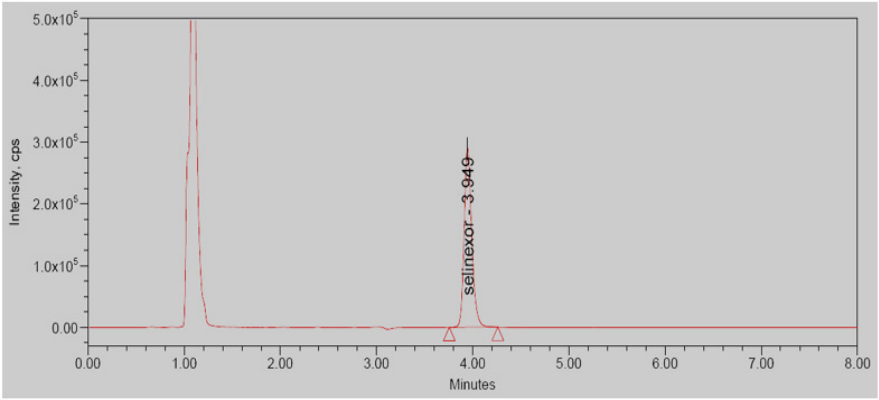

Fig15: Chromatogram for Accuracy

Table 7: Accuracy and precision of data of the Selinexor (n= 6)

Quality control sample	Area of Analyte	Mean Area	SD	Accuracy (%)	RSD (%)
Intra-day					
LLOQ	0.2428 x10^5	0.2552 x105	0.00452	118.7	1.04
LQC	1.0622 x10^5	1.0759 x105	0.00596	100.2	0.41
MQC	2.1562 x10^5	2.1699 x105	0.00741	96.3	0.62
HQC	3.0251 x10^5	3.0297 x105	0.00238	94	0.75
Inter-day					
LLOQ	0.2269 x10^5	0.2321 x105	0.00524	108	0.47
LQC	1.0485 x10^5	1.0523 x105	0.00356	98	0.53
MQC	2.1462 x10^5	2.1596 x105	0.00528	95.9	0.36
HQC	3.0567 x10^5	3.0217 x105	0.00462	93.7	0.48

Table 7 Intraday precision was determined by analyzing six replicates of LLOQ, LQC, MQC, and HQC samples. Whereas reproducibility (day-to-day variation i.e., inter-day precision) of the method was validated using six sets of LLOQ, LQC, MQC, and HQC samples on three different days. Intra and inter-day assay precision were determined as % of co-efficient of variance (%CV), i.e., the ratios of standard deviation (SD) to the mean and expressed as a percentage.

$$\text{Precision (CV \%)} = \frac{\text{Standard deviation (SD)}}{\text{Mean}} \times 100$$

Intra and inter-assay accuracy was determined by analyzing six replicates at four QC levels (LQC, MQC, HQC including LLOQ) on the same day and three different days respectively. Accuracy was determined by the ratio of determined concentration and actual concentration multiplied by 100%.

$$\text{Accuracy (\%)} = \frac{\text{Found concentration}}{\text{Theoretical concentration}} \times 100$$

The criteria for acceptability of the data included accuracy within ±15% deviation (SD) from the nominal values and a precision of within ±15% relative standard deviation (RSD), except for LLOQ, where it should not exceed ±20% of SD (Food and Drug Administration of the United States, 2001).

3.12 Acceptance Criteria

The within and between batch precision for LQC, MQC and HQC samples should be ≤ 15.00 % and for the LLOQ QC, it should be ≤ 20.00 %.

3.13 Assay

(Fig.16, Fig.17, and Table.8).

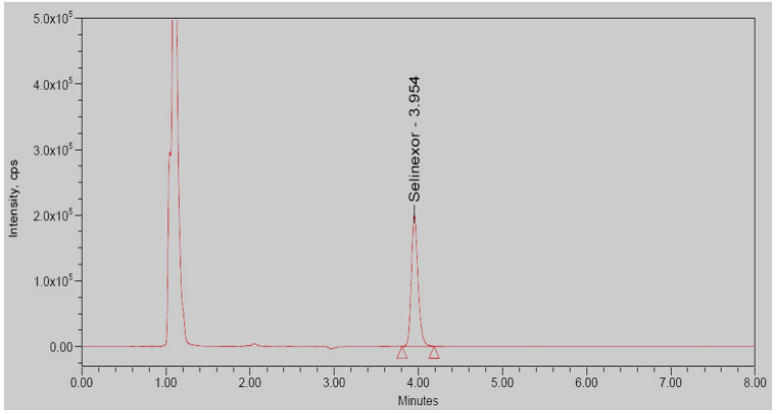

Fig16: Chromatogram of Assay-1

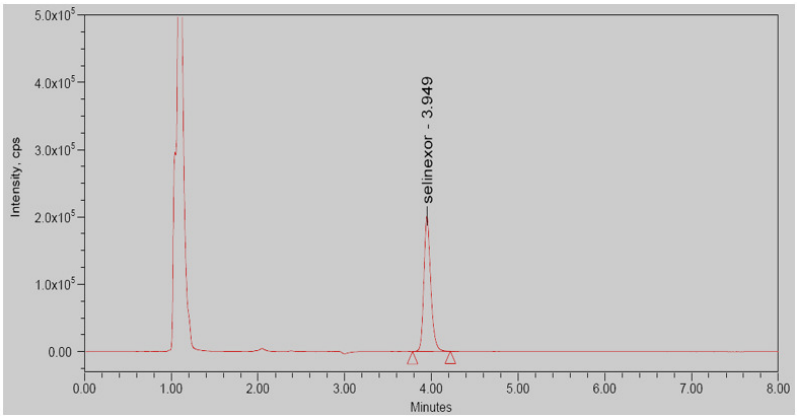

Fig17: Chromatogram of Assay-2

Table No.8: Assay of Selinexor							
Drug	Avg sample area (n=5)	Std. wt (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	Amount found (ng/ml)	% Assay
Selinexor	2.157x105	5	7	80	99.7	20	99.82
	2.163x105						98.96

Table 8 The table describes the % assay of Selinexor and it was found to be 99.82 and 98.96.

4. DISCUSSION

An attempt has been made to develop validated stability-indicating RP-HPLC[26] for the estimation of Selinexor. Literature survey revealed that there are no analytical methods have been reported individually or in combination with other drugs. However, no method was reported for bio analytical[27] for the estimation of these two drugs by the HPLC method.[28] The general information on RP-HPLC and method development, General information on bioanalytical validation. The stability-indicating RP-HPLC&LCMS/MS Method Development and Bio-analytical Method Validation for Simultaneous Selinexor in Bulk and their Pharmaceutical dosage form.[29] Using Waters alliance HPLC system, Quaternary gradient pump of e2695 series equipped with an autosampler injector with 20ng/ml is injected eluted with the mobile phase containing 0.1% Formic acid and Acetonitrile in the ratio of 80:20 v/v which is pumped at a flow rate of 1ml/min and detected by UV detector at 225nm.[30] The peak of Selinexor was eluted at retention times of 3.9 min. This proposed HPLC method for the selected drugs showed good linearity. Results for the recoveries of selected drugs were found to be within limits (98 – 102 %). These indicate that the proposed method was accurate for the analysis.

5. CONCLUSION

In these present studies, experiments were performed on Selinexor. The instrument used in these experiments is HPLC&LCMS/MS coupled with a PDA detector. HPLC LCMS/MS is mostly available in all analytical laboratories due to its low cost. Though in the published literature protein precipitation and solid extraction methods were adopted we have developed liquid-liquid extraction for sample preparation with increased sensitivity as well as the increased column life in comparison to the protein precipitation method. The solid-phase extraction method was avoided because of its high economic rate. The different parameters used in these techniques are selected after proper justification and various trials and errors. The HPLC method described for the analysis of Selinexor in plasma is very specific and sensitive. The methods developed in our laboratory are very simple utilizing a liquid-liquid extraction procedure, which makes the method high throughput for analysis. All the validation data were met the range acceptance criteria of the USFDA guideline.

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7. AUTHORS CONTRIBUTION STATEMENT

M Sreekanth, Syed Sadaq Valli conceptualized, designed, and executed the study. Murthaeti Yaswanth has curated the data and prepared the original draft. D Venkata Narayana, V Sreedhar, Kanuma Venkataramana, K E Pravallika discussed the methodology, analyzed the data, and provided valuable inputs. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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